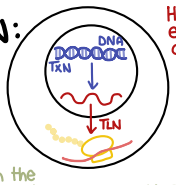
Chapter 7 ~ The Central Dogma - Mutations & Biochemical Pathways

Introduction: "Mutation"



- we think
  - Negative / Detrimental → But
    - It is a CHANGE in the DNA sequence, may have ONE or MORE effects on an organism depending on the what it is & which gene it occurs
- While some can have detrimental effects some mutations that can create new features
  - Mutations give us a tool whith which to investigate the gene & the biological processes in which it is involved

How is the genetic information in DNA (genes) expressed as biological traits, such as the colour of Mendel's peas?

↳ **Central Dogma**

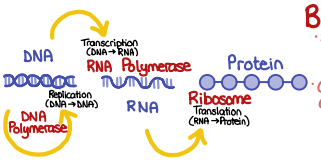
- Describes the concept that genetic info is encoded in DNA in the form of genes
  - This information is transferred as needed in a process called "TRANSCRIPTION" into mRNA sequence
  - This information is then transferred again in a process called "TRANSLATION" into a polypeptide sequence (protein)
  - The sequence of bases in DNA directly dictates the sequence of bases in the RNA which in turn dictates the sequence of amino acids that make up a polypeptide

Original Core

- Genetic information is NEVER transferred from protein back to nuclei acids.
- In certain circumstances, the information in RNA may be converted back to DNA through a process called "REVERSE TRANSCRIPTION"
  - This information can be replicated

Proteins

- Do most of the work in a cell
  1. Catalyze the formation & breakdown of most molecules w/in an organism
  2. Form their structural components
  3. Regulate the expression of genes
- By dictating the sequence & thus structure of each protein, DNA directs the function of that protein, which can thereby affect the potential form or "PHENOTYPE" of the organism
  - Environment can also influence phenotype



Mendel's Peas

- Purple-flowered plants have a gene that encodes an enzyme that produces a purple pigment molecule
- White-Flowered plants (pigment-less mutant), the DNA for this gene has been changed, or mutated, so that it no longer encodes a functional protein

Example of a spontaneous, natural mutation in a gene coding for an enzyme in a biochemical pathway

Biochemistry ← Life depends on it

- Supplys energy to produce the molecules that construct & regulate cells
- Genetic defects lead to the lack of an enzyme in a biochemical paths & causes a disease
  - Huge impact to modern genetics as it attempted to explain the biochemical mechanism behind the genes proposed in Mendelian genetics

The Beadle & Tatum Experiments

1940s... 30yrs post Garrods Discovery

- Beadle & Tatum built on this connection b/w genes & metabolic pathways
  - This research led to the "one gene, one enzyme" hypothesis
    - ↳States that each enzyme that acts in a biochemical pathway is encoded by a different gene

*We NOW know of MANY exceptions it is GENERALLY TRUE that each gene produces a protein with a distinct catalytic, regulatory or structural function

Beadle & Tatum used a Fungus *Neurospora crassa*

- It possesed practical advantages as a labratory model organism
  - Prototrophic → Could grow on minimal media (MM)
    - → Can synthesize the amino acids, vitamins, etc. necessary for normal growth

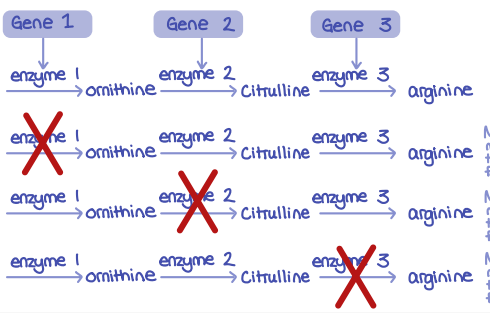
- They isolated a series of mutations known to interrupt the synthesis of arginine, an amino acid necessary for mould growth
  - Hypothesis: "Individual mutations inhibited discrete steps in the pathway used by the mould to synthesize arginine from precursors in their environmental medium"

Beadle & Tatum Experiments

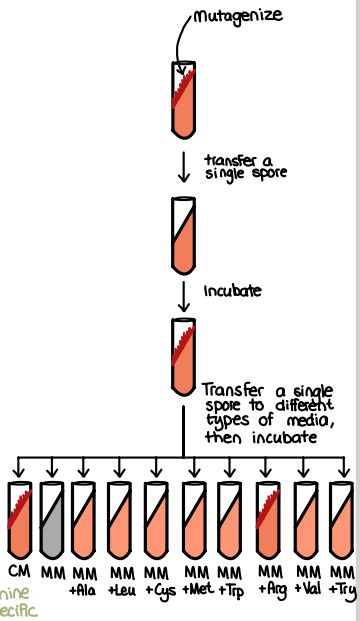
Bread Mold	Minimal Media (MM)	MM + Ornithine	MM + Citrulline	MM + Arginine
Wild Type	Grew	Grew	Grew	Grew
Mutant 1	Did not grow	Grew	Grew	Grew
Mutant 2	Did not grow	Did not grow	Grew	Grew
Mutant 3	Did not grow	Did not grow	Did not grow	Grew

*Minimal media lack most nutrients with the exception of a few minerals like simple sugars & biotin (a vitamin)

- They knew that by exposing *Neurospora* spores to x-rays, they could randomly induce mutations in genes
  - This is now known as damage to the DNA leading to DNA sequence change
- Each spore exposed to x-rays potentially contained a mutation in a different gene
  - Most mutagenized spores were still able to grow (prototrophic) SOME spores had mutations that changed their phenotype from prototroph into an AUXOTROPHIC strain (can still grow on complete medium)
- Instead these auxotrophs could grow on complete medium, which was minimal media supplemented w/ nutrients such as amino acids, vitamins, etc...
- Some auxotrophic mutations could grow on minimal media, w/ only one, single nutrients supplied, such as the amino acid arginine
  - Implies that each auxotrophic mutant was blocked at a specific step in a biochemical pathway & that by adding an essential compound, such as arginine, that block could be circumvented



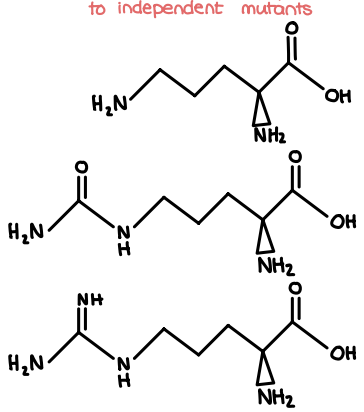
Beadle & Tatum linked MANY nutritional mutants to specific amino acids & vitamin biochemical pathways. This work demonstrated that individual genes are connected to specific enzymes. This inital discovery which made the link b/w genes & enzymes was called the "One gene-one enzyme" hypothesis



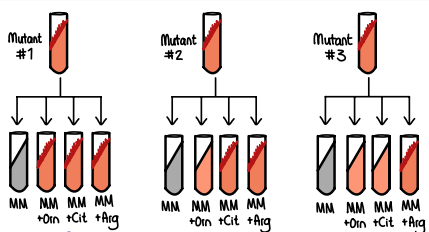
The "One Gene : One Enzyme" Hypothesis

NOTE: Beadle & Tatum's experiments are important not only for their conceptual advances in understanding genes, but also because they demonstrate the utility of "SCREENING FOR GENETIC MUTATIONS" to investigate a biological process - This is called GENETIC ANALYSIS

- Was useful to investigate biological processes, specifically the metabolic pathways that produce amino acids.
- ex: Srb & Horowitz (1944) tested the ability of the amino acids to RESCUE auxotrophic strains. They added one of each of the amino acids to minimal medium & recorded which of these restored growth to independent mutants



Precursors
↓ Gene A
Orn
↓ Gene B
Cit
↓ Gene C
Arg



Mutants in	MM + Orn	MM + Cit	MM + Arg
Gene A	Yes	Yes	Yes
Gene B	No	Yes	Yes
Gene C	No	No	Yes

Ex: ARGININE

- If progeny of mutagenized spore could grow on minimal media only when it was supplemented by ARGININE (Arg), then auxotroph must bear a mutation in the Arg biosynthetic pathway & was called an "argineless" strain (arg-)
- Synthesis of even a relatively simple molecule, such as arginine, requires many steps - each w/ a diff enz me
- Each enzyme work works sequentially on a different intermediate in the pathway
  - ex: For Arg, two biochemical intermediates = Org & Cit thus mutations of any one enzymes in this pathway could turn *Neurospora* into an Arg auxotroph (arg-)
    - ↳Extension on the analysis of Arg auxotrophs was done by testing the intermediates of amino acid biosynthesis for the ability to restore growth to the mutants

It was found that ONLY Arg could rescue all the Arg auxotrophs, while either Arg or Cit could rescue some

Results allowed for the locations of each mutation in the Arg biochemical pathway to be found

Genetic Screening & Biochemical Pathways

Using many other mutations and the "one gene: one enzyme model" permits the genetic dissection of many other biochemical and developmental pathways

The general strategy for a genetic screen for mutations is to expose a population to a mutagen, then look for individuals among the progeny with defects in the biological process of interest.

There are many details that must be considered when designing a genetic screen
ex: How can recesive alleles be made homozygous

Nevertheless, mutational analysis has been an extremely powerful & efficient tool in identifying and characterizing the genes involved in a wide variety of biological processes, including many genetic diseases in humans

Genetic Screens

- Forward genetic screening refers to the process of finding the gene or genes responsible for a certain phenotype or biochemical process.
  - One way to identify genes that affect a particular biological process is to induce random mutation in a large population, & then look for mutants w/ phenotypes that might be cause by a disruption of a particular biochemical pathway
  - This is the strategy of mutant screening, which is used effectively to identify & understand the molecular components of hundreds of different biological processes.
  - To find the basic biological processes of memory & learning researchers have screened mutagenized populations of *Drosophila* to recover flies (or larva) that lack the normal ability to learn
    - Mutants lack the ability to associate a particular order w/ an electrical shock.
    - Because of the similarities of biology among all organisms, some genes identified by this mutant screen of a model organism may be relevant to learning & memory in humans
      - ex: Conditions like Alzheimers Disease
- On the other hand, reverse genetic screening refers to the process of creating a mutation in a gene, then identifying the phenotypic consequences of that specific mutant gene on the organism.
  - This method is becoming more useful w/ the advent of the whole genome sequencing
    - Here, we have identified the gene sequences, but are unsure of what each gene does
    - In a typical mutant screen, researchers treat a parental population with a mutagen.
    - This may involve soaking seeds in EMS, or mixing a mutagen w/ the food fed to flies.
  - Usually, no phenotypes are visible among the individuals directly exposed to the mutagen, because in all the cells, every strand of DNA will be affected independently.
    - Thus, the induced mutations will be heterozygous and limited to single cells
    - However, what is most important to geneticists are the mutations in the germline of the mutagenized individuals.
    - The germline is defined as the gametes & any of their developmental precursors, and is therefore distinct from the somatic cells (ex: non-reproductive cells) of the body.
    - Because most induced mutations are recessive, the progeny of mutagenized individuals must be mated in a way that allows the newly induced mutations to become homozygous (or hemizygous). Strategies for doing this vary b/w organisms.
  - In any case, the generation in which induced mutations are expected to show a phenotype can be examined for the prescence of novel traits.
  - Once a relevant mutant has been identified, genetisist can begin to make inferences about the normal function of the mutated gene based on its mutant phenotype.
  - This can be further investigated with molecular genetic techniques, to connect the gene function w/ the external appearance

Mutations without Detectable Phenotypes

Silent Changes

- After mutagen treatment, the vast majority of base pair changes (especially substitutions) have no obvious effect on the phenotype.
  - This is often because the change occurs in the DNA sequence of a non-coding region of the DNA, such as in INTERGENIC REGIONS (b/w genes) or within an intron where the sequence does not code for protein & is NOT essential for proper mRNA splicing.
  - Also, even if the change affects the coding region, it may not alter the amino acid sequence (recall that the genetic code is degenerate;
    - ↳ex: GCT, GCC, GCA & GCG all encode alanine and is referred to as a SILENT mutation.
  - Additionally the base substitution may change an amino acid, but this does NOT quantitatively or qualitatively alter the function of the product, so no phenotypic change would occur

Environmental & Genetic Redundancy

- There are situations where a mutation can cause a complete loss-of-function of a gene, yet not produce a change in the phenotype, even when the mutant allele is homozygous.
- The lack of a visible phenotypic change can be due to ENVIRONMENTAL EFFECTS:
  - The loss of that gene product may NOT be apparent in that specific environment, but might be in another.
    - ↳ex: auxotrophic mutant on complete medium.
  - Conversely, researchers can alter the environment to reveal such mutants
    - ↳ex: auxotrophs on minimal media
- Alternatively, the lack of a phenotype might be attributed to genetic REDUNDANCY.
  - That is the mutant gene's lost function is compensated by another gene, at another locus, encoding a similarly functioning product.
  - Thus, the loss of one gene is compensated by the presence of another.
  - The concept of genetic redundancy is an important consideration in genetic screens
- A gene whose function can be compensated for my another gene, cannot be easily identified in a genetic screen for loss of function mutations

Essential Genes & Leathal Alleles

- Some mutant maybe required to reach a particular developmental stage before the phenotype can be seen or scored.
  - For example: flower color can only be scored in plants that are mature enough to make flowers, and eye color can only be scored in flies that have developed to the adult stage.
- However, some mutant organisms may not develop sufficiently to reach a stage that can be scored for a particular phenotype.
- Mutations in ESSENTIAL GENES create recessive lethal alleles that arrest/derail the development of an individual at an immature (embryonic, larval or pupal) stage.
  - This type of mutation may, therefore, go unnoticed in a typical mutant screen b/c they are absent from the progeny being screened.
- Furthermore, the progeny of a monohybrid cross involving an embryonic lethal recessive allele may all be of a single phenotypic class; giving a phenotypic ratio of 1:0 (which is the same as 3:0) → the mutation may not be detected.
- Nevertheless, the study of recessive lethal mutations (those in essential genes) has elucidated many important biochemical pathways.
- The identification of whole classes of genes involved in early embryonic development, is one example.

Three Drosophila geneticists:

① Eric Wieschaus
② Edward Lewis
③ Christiane Nüsslein-Volhard

were awarded a Nobel Prize in Physiology or Medicine in 1995

↓

identified pair-rule, gap, and segment polarity genes that have corresponding homologs in all segments organisms including humans

Naming Genes

- Many genes are first identified in mutant screens & so they tend to be named after their mutant phenotypes – NOT the normal function or phenotype.
  - This can cause some confusion for their student of genetics.
    - ↳ex: We have already encountered an X-linked gene named *white* in fruit flies
      Null mutants of the *white* gene have white eyes, but the normal *white* + allele has red eyes.

      This tells us that the *wild type* (normal) function of the gene is required to make red eyes

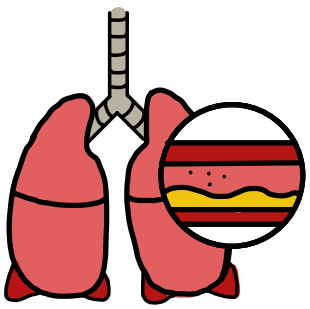
      We now know its product is a protein that imports a colourless pigment precursor into developing cells of the eye
- Why don't we call it the "Red" gene, since that is what its product does?
  - B/c there are more than one-dozen genes that, when mutant, alter the eye colour:

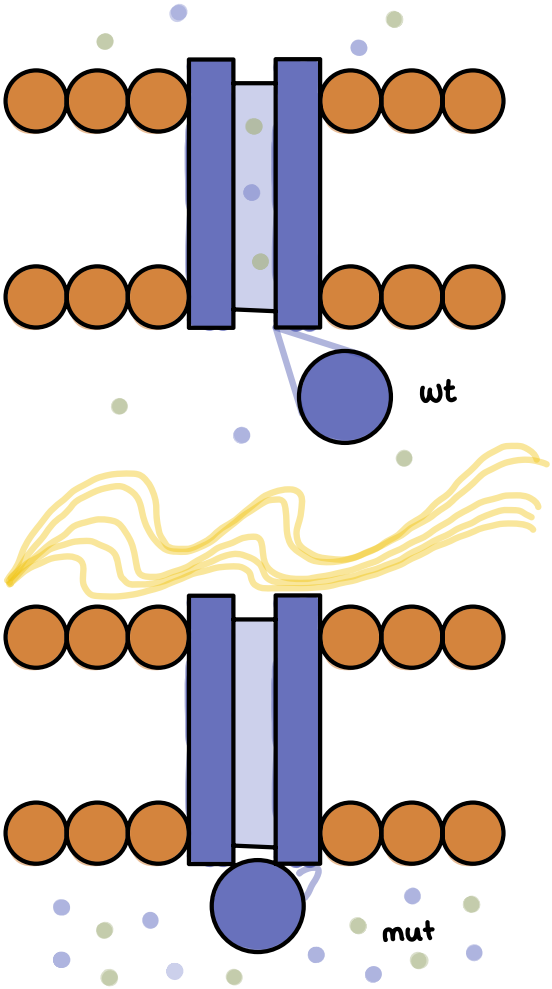
- For all of these genes their function is also needed to make the eye wild-type red & NOT the mutant colour.
- If we used the name "Red" for all these genes it would be confusing.
  - So we use the distinctive mutant phenotype as the gene name
- However, this CAN be problematic as w/ "lethal" mutations described above.
- This problem is usually handled by giving numbers or locations to the gene name / making up names that describe how they die
  - ex: even-skipped
    hunchback
    hairy
    runt

Cystic Fibrosis in Humans

Cystic Fibrosis – Autosomal Recessive

- Cystic fibrosis (CF) is one of many diseases that geneticists have shown to be primarily caused by mutation in a single, well-characterized gene.
- Cystic fibrosis is the most common ($\frac{1}{2500}$) life-limiting autosomal recessive disease among people of European heritage, with ~1 in 25 people being carriers.
  - The frequency varies in different populations
  - Most of the deaths caused by CF are the result of lung disease, but many CF patients also suffer from other disorders including infertility & gastrointestinal disease.
  - The disease is due to a mutation in the CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) gene, which was first identified by Lap-chee Tsui's group at the University of Toronto
  - Lap-Chee Tsui was inducted into the Canadian Medical Hall of Fame in March 2012 and is still a leader in CF research
- Epithelial tissues in some organs rely on the CFTR protein to transport ions (especially Cl^-) across their cell membranes.
  - The passage of ions through a six-sided channel is gated by another part of the CFTR protein, which binds to ATP
  - If there is insufficient activity of CFTR, an imbalance in ion concentration results, which disrupts the properties of the liquid layer that normally forms on the epithelial surface
- In the lungs, this causes mucus to accumulate and can lead to infection.
  - Defects in CFTR also affect pancreas, liver, intestines, and sweat glands ~all of which need this ion transport.
  - CFTR is also expressed at high levels in the salivary gland and bladder, but defects in CFTR function do not cause problems in these organs, probably because other ion transporters are able to compensate
- Over one thousand different mutant alleles of CFTR have been described
- Any mutation that prevents CFTR from sufficiently transporting ions can lead to cystic fibrosis (CF)
- Worldwide, the most common CFTR allele among CF patients is called ΔF508 which is a deletion of three nucleotides that eliminates a phenylalanine from position 508 of the 1480 aa wild-type protein.
  - Mutation ΔF508 causes CFTR to be folded improperly in the endoplasmic reticulum (ER), which then prevents CFTR from reaching the cell membrane.
- ΔF508 accounts for approximately 70% of CF cases in North America, with ~ 1/25 people of European descent being carriers.
  - The high frequency of the ΔF508 allele has led to speculation that it may confer some selective advantage to heterozygotes, perhaps by reducing dehydration during cholera epidemics, or by reducing susceptibility to certain pathogens that bind to epithelial membranes





CFTR is also notable because it is one of the well-characterized genetic diseases for which a drug has been developed that compensates for the effects of a specific mutation

The drug, Kalydeco (Ivacaftor) was approved by the FDA & Health Canada in 2012, decades after the CFTR gene was first mapped to DNA markers (in 1985) and cloned (in 1989).

Kalydeco is effective on only some CFTR mutations, most notably G551D
ex: where glycine is substituted by aspartic acid at position 551 of the protein; GLY551ASP

This mutation is found in less than 5% of CF patients.

The G551D mutation affects the ability of ATP to bind to CFTR & open the channel it for transport.

Kalydeco compensates for this mutation by binding to CFTR and holding it in an open conformation.

Kalydeco is expected to cost approximately $250,000 per patient per year