Molecular Biology:
Reducing Mendelian Genetics or Revising Our Concepts of Life?

"And gradually there is coming into being a new branch of science—molecular biology—which is beginning to uncover many secrets concerning the ultimate units of the living cell….in which delicate modern techniques are being used to investigate ever more minute details of certain life processes.
- Warren Weaver, Rockefeller Foundation, 1938

Making traits molecular

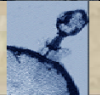
- Archibald Garrod (1909): "inborn errors of metabolism"
  - Alkaptonuria - an inherited condition in which the urine is colored dark red by alkaptons
  - Results from a single recessive gene, which causes a deficiency in the **enzyme** that normally breaks down alkapton

- Beadle and Tatum (1941): one gene=one enzyme
  - Strategy – find genetic mutants unable to carry out specific enzymatic reactions
  - Exposed Neurospora crassa (a bread mold) spores to X-rays or UV radiation and studied the resulting mutations.
  - Mutants required additions to their diets that their normal counterparts did not—e.g., thiamine or chlorine

- Revision: one gene, one polypetide chain

How To Study the Molecular

- Using X-rays to alter genes: Hermann Muller
- The physicists come to biology
  - In 1940s: Max Delbrück and Salvador Luria began working with bacteriophage, which consist of a protein coat surrounding DNA which invade a bacterium, causing it to make new phage
    - First established exclusion principle: only one strain will infect a bacterium
  - Erwin Schrödinger's *What is Life?* (1944)
    - Gene might be an aperiodic crystal
    - Thermodynamic considerations



Identifying DNA as the Genetic Material

- Although DNA was discovered in the 1869, through the 1940s it was generally discounted as too simply to be the genetic material
- Research by Oswald Avery (1944) and Alfred Hershey and Martha Chase (1952) began to point to its genetic role
- Linus Pauling discovered the helical structure of proteins and thought they might be the genetic material
  - After Hershey and Chase turned his attention to DNA and in December 1952 thought he had figured out the structure

Crick and Watson: 1953

- James Watson, learning of the evidence for DNA as genetic material, took a position at the Cavendish Laboratory at Cambridge to learn x-ray crystallography
  - There he was assigned to share an office with Francis Crick who was using x-ray crystallography to study hemoglobin structure
- Although directed not to work on DNA, the two set out to try to figure out the structure aided by
  - Access to Rosalind Franklin's crystallography results
  - Learning from Chargaff of nucleotide pairing: adenine with thymine and guanine with cytosine
  - Knowing the flaw in Pauling's model
- Early in 1953 Crick and Watson identify the double helix structure and beat Pauling to the prize.

OK, but what does DNA do?

- Watson and Crick conclude: "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material."
- Besides copying, DNA must do more if it is the genetic material
  - It must code for traits
  - There must be a mechanism by which it gets expressed as traits

Cracking the Code

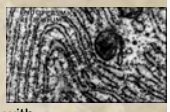
- One base pair could not specify an amino acid—4 base pairs and 20 amino acids
  - Two base pairs not enough—only 16 possibilities
  - Three base pairs more than enough—64 possibilities
- Crick discovers that addition or deletion of one or two nucleotides results in abnormal phenotype, but addition or deletion of three near to one another often results in normal phenotype
- Marshall Nirenberg discovered that synthetic polyuridylic acid (UUUU…) produced sequences of phenylaline (PHE-PHE-PHE-PHE-…)
- By 1964 he and Har Gorbind Khorana figured out the whole code by using radioactively labeled RNA

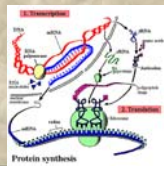
The Genetic Code

UUU, UUC phenylalanine UUA, UUG leucine	UCU, UCC, UCA, UCG serine	UAU, UAC tyrosine UAA, UAG stop	UGU, UGC cysteine UGA stop UGG tryptophan
CUU, CUC, CUA, CUG leucine	CCU, CCC, CCA, CCG proline	CAU, CAC histidine CAA, CAG glutamine	CGU, CGC, CGA, CGG arginine
AUU, AUC, AUA isoleucine AUG methionine	ACU, ACC, ACA, ACG threonine	AAU, AAC asparagine AAA, AAG lysine	AGU, AGC serine AGA, AGG arginine
GUU, GUC, GUA, GUG valine	GCU, GCC, GCA, GCG alanine	GAU, GAC aspartic acid GAA, GAG glutamic acid	GGU, GGC, GGA, GGG glycine

From DNA to Protein

- One of the discoveries with the new electron microscope was the occurrence of a membrane structure (endoplasmic reticulum) extending throughout the cell cytoplasm
  - Parts of the endoplasmic reticulum coated with particles—ribosomes—which were partly constituted by RNA (rRNA)
  - rRNA could not explain the variability of proteins, and another RNA, messenger RNA, hypothesize to carry the message from the DNA to the ribosome
  - Third form of RNA, transfer RNA, binds amino acids and deposits them onto developing polypeptide chain





Relation of Molecular Genetics to Mendelian/Transmission Genetics

- In explaining the molecular structure of genes, molecular genetics seems to provide a ***reduction*** of Mendelian genetics
  - Mendelian factors/genes are pieces of DNA
- The theory-reduction model: Logically derive the reduced theory from the reducing theory
  - Deduction requires a common vocabulary or rules for relating vocabularies (bridge laws)

    Theory at lower level (molecular genetics)

    Bridge principles (relate classical genes with DNA strands)

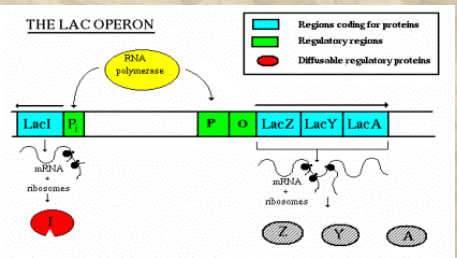
    Boundary conditions

    ∴Theory at higher level (classical genetics)

Control Genes: The Lac Operon

- In 1900, F. Dienert discovered that the enzymes needed for galactose metabolism were found in yeast only when the yeast used galactose as a carbon source
  - the presence of galactose had called forth or *induced* the specific enzymes (e.g., β-galactosidase) necessary to metabolize galactose
- Joshua Lederberg developed three mutant strains (*lacZ*$^-$, *lacY*$^-$, and *lacA*$^-$) that each lacked an enzyme needed to metabolize lactose and these were all mapped to the same region on the chromosome
  - This suggested the induction occurred at the level of the chromosome
- Lederberg produced a different mutant (*lacI*$^-$) which always produced the enzymes, and it was located nearby

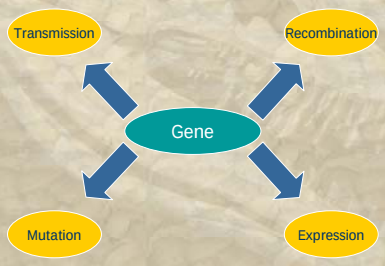
Basics of the Lac Operon: Jacob and Monod (1961)



The Molecular Gene Gets Messier

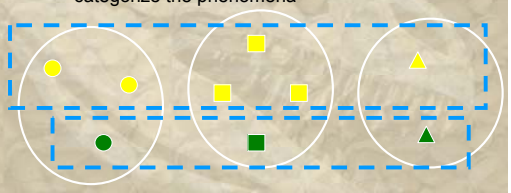
- Different amino acid chains could be derived from the same DNA chain depending on starting point (*overlapping genes*, Barrell et al., 1976)
- The portions of DNA that coded for amino acids (exons) were often split with other DNA inserted (introns) (*split genes*, Berget et al. 1977; Chow et al. 1977)
- Different procedures were employed to construct mRNA strands from DNA (*alternative splicing*, (Berk and Sharp 1978)
- What is a molecular gene?

Tensions on the Mendelian Conception of the Gene



The challenge for bridge laws

- The categories of two theories may cross-categorize the phenomena



Even worse, what if the only thing that the items that fit the higher-level category share is that they fit the higher-level category?

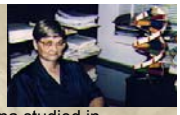
Going Genomic: The Human Genome Project

- With the development of tools first to sequence the amino acids in proteins and then the nucleotide sequence in DNA, interest grew in mapping the genome
- The Human Genome Project developed as a large-scale endeavor of the U.S. Department of Energy and NIH
- The Race: Between publicly funded Consortium of international sequencing centers and private corporation Celera
- Publication of the “rough draft” of the Human Genome, February 2001
- Also of many other species

But What to DNA Sequences Do?

- The grand hope was that knowing the genetic sequence for humans would allow for rapid advances in medicine (new drugs, gene therapy, etc.)
- For the most part, these have not occurred. Why?
  - One still needs to find out what genes do
  - They do many more things than code for amino acid sequences
  - Complex, interactive, dynamic mechanism in which genes are a major players, but don’t operate alone

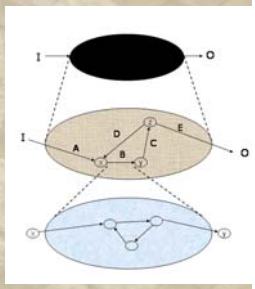
Darden and Maull’s Interfield Theories



- Identify relationships between phenomena studied in different fields
  - Identifying the physical location of a process
  - Provide physical characterization of functional entity
  - Locate the cause or effect of a phenomena
- Example: chromosomal theory of Mendelian heredity
  - Led to new problem-solving research—explain patterns of joint heritability of traits in terms of linkage on chromosomes
- No need to derive one theory from the other
- **Develop a theory that spans fields**, not a relationship between two complete theories

Mechanisms and reduction

- Parts of mechanisms are themselves mechanisms
- Can be decomposed into the operations of their parts and the organization imposed on them
- Allows for relating levels without deriving laws
  - Or giving priority to lower levels



DNA and UCSD Classes

- Some strands of DNA have been in close proximity to each other twice a week for the past nine weeks
- Why?
  - The relevant causal interactions are not at the DNA level
  - For whatever reasons, I taught this class and you took it
  - When we all come to class, so does our DNA
    - It doesn't have any choice
    - It comes along for the ride
- Mechanisms allow causality at multiple levels

Molecular Understanding of Life Itself

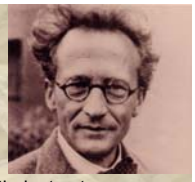
- For the most part, living organisms stand out against their environments as enduring structured entities
  - Also true of individual cells, the fundamental living units
- Living organisms, including cells, are not just passive systems but active ones
  - Metabolic systems: changing chemical compounds into other chemical compounds
  - Doing things that alter their environments in ways that help maintain themselves
- Focal issue: how do living systems maintain themselves?

Frederic Gowland Hopkins' Vision for Biochemistry

A living cell is "not a mass of matter composed of a congregation of like molecules, but a highly differentiated system: the cell, in the modern phraseology of physical chemistry, is a system of co-existing phases of different constitutions" (Hopkins 1913 [1949] p. 151)

"It is important to remember that changes in any one of these constituent phases ... must affect the equilibrium of the whole cell-system, and because of this necessary equilibrium-relation it is difficult to say that any one of the constituent phases ... is less essential than any other to the "life" of the cell ... Certain of the phases may be separated, mechanically or otherwise, as when we squeeze out the cell juices, and find that chemical processes still go on in them; but "life", as we instinctively define it, is a property of the cell as a whole, because it depends upon the organisation of processes, upon the equilibrium displayed by the totality of the co-existing phases."

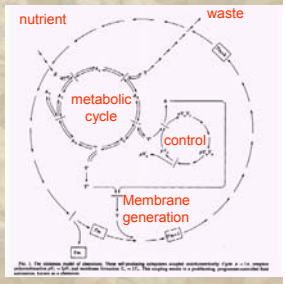
Erwin Schrödinger: *What is Life?*



- The entropy problem: How could order be maintained in the face of the 2nd Law of Thermodynamics
  - Problem 1: how could genes retain their structure in the face of mutation?
  - Problem 2: how could metabolism enable organisms to maintain themselves in a non-equilibrium state
    - Negentropy: Organisms route energy through themselves in a manner that builds structure (reduces entropy) and increases entropy outside of the self

Tibor Gánti's Chemoton

- The Hungarian chemist Tibor Gánti posed the question: what is the simplest chemical system exhibiting the features of life?
- Required a metabolic cycle that both extracted matter and energy and used it to rebuild itself
- Also need to build a containment vesicle
- And possess a way of controlling its own activities



Varela: Autopoietic Mechanisms

- Following a related line of thinking, Chilean biologist Francesco Varela (together with Humberto Maturana) introduced the notion of autopoiesis—self construction
- "An autopoietic system is organized (defined as a unity) as a network of processes of production (transformation and destruction) of components that produces the components that: (1) through their interactions and transformations continuously regenerate and realize the network of processes (relations) that produce them; and (2) constitute it (the machine) as a concrete unity in the space in which they exist by specifying the topological domain of its realization as such a network." (1979, p. 13).

From Autopoiesis to Autonomy

- "Autopoietic machines are autonomous: that is, they subordinate all changes to the maintenance of their own organization, independently of how profoundly they may be otherwise transformed in the process. Other machines, henceforth called *allopoietic* machines, have as the product of their functioning something different from themselves" (Varela, 1979, p. 15)