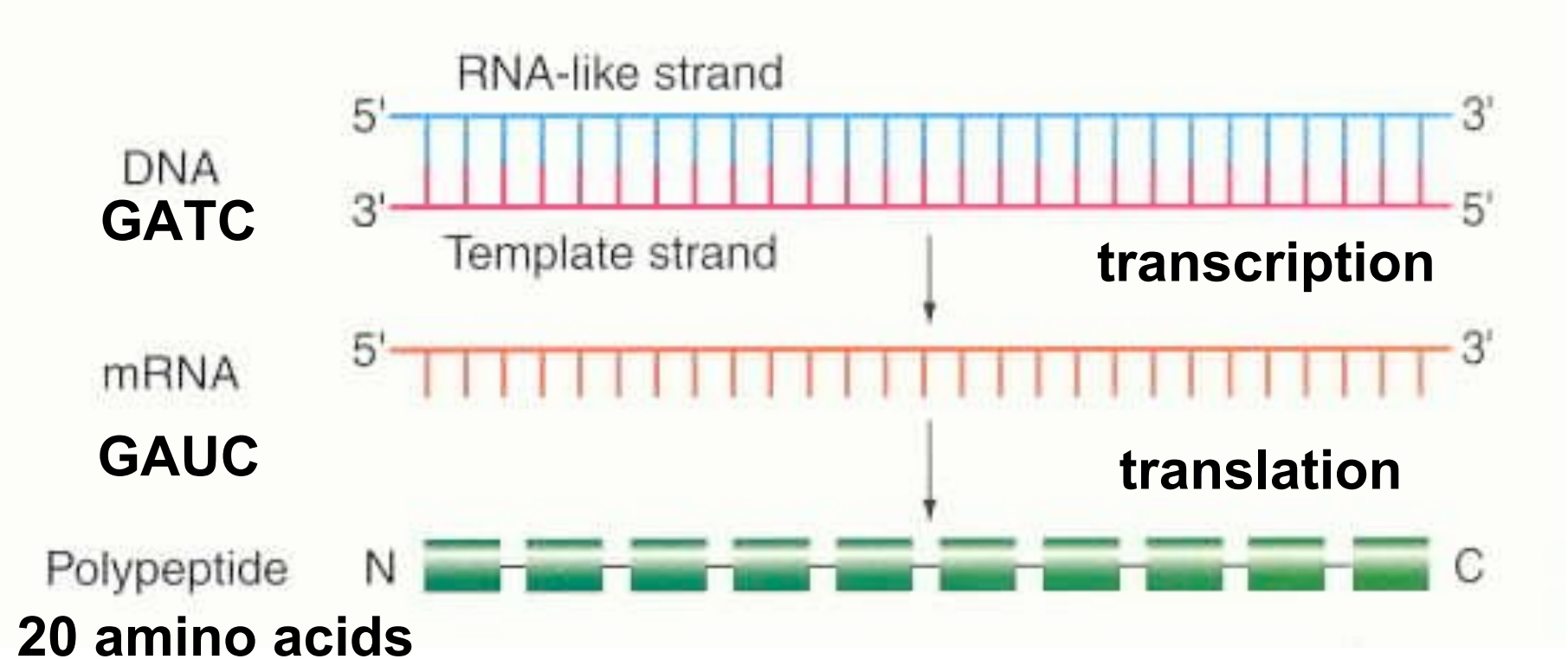
Gene Function

Chapter 12

The Central Dogma of Biology



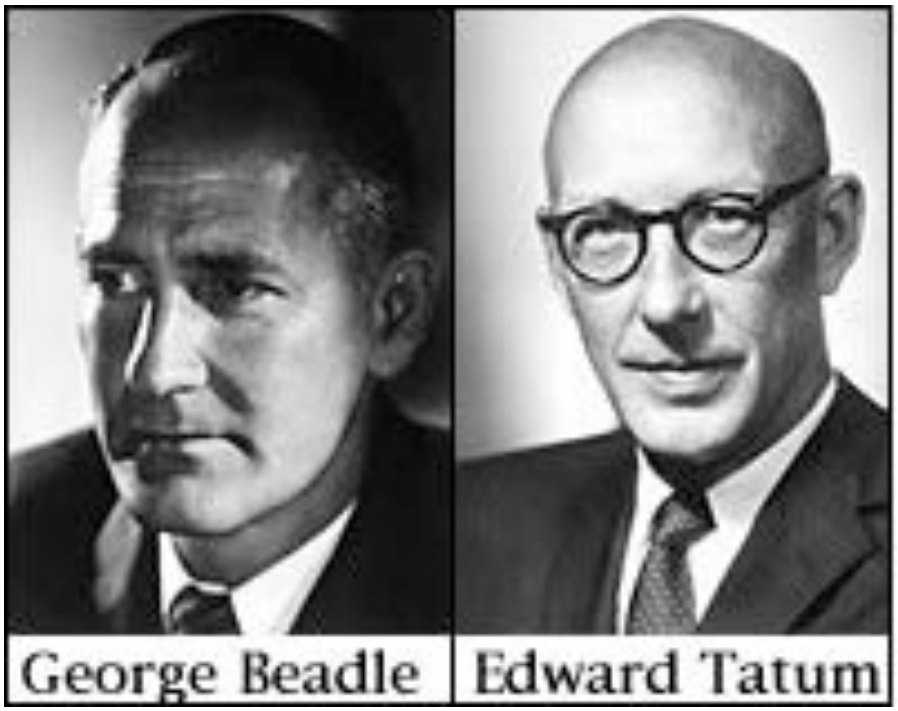
20 amino acids

Gene Control of Enzyme Structure

- Genes encode proteins, including enzymes.
- Genes work in sets to accomplish biochemical pathways.
- Genes often work in cooperation with other genes.
- These discoveries are the foundation of modern molecular genetics.

Genetic Approach to Studying the Gene – Enzyme Connection

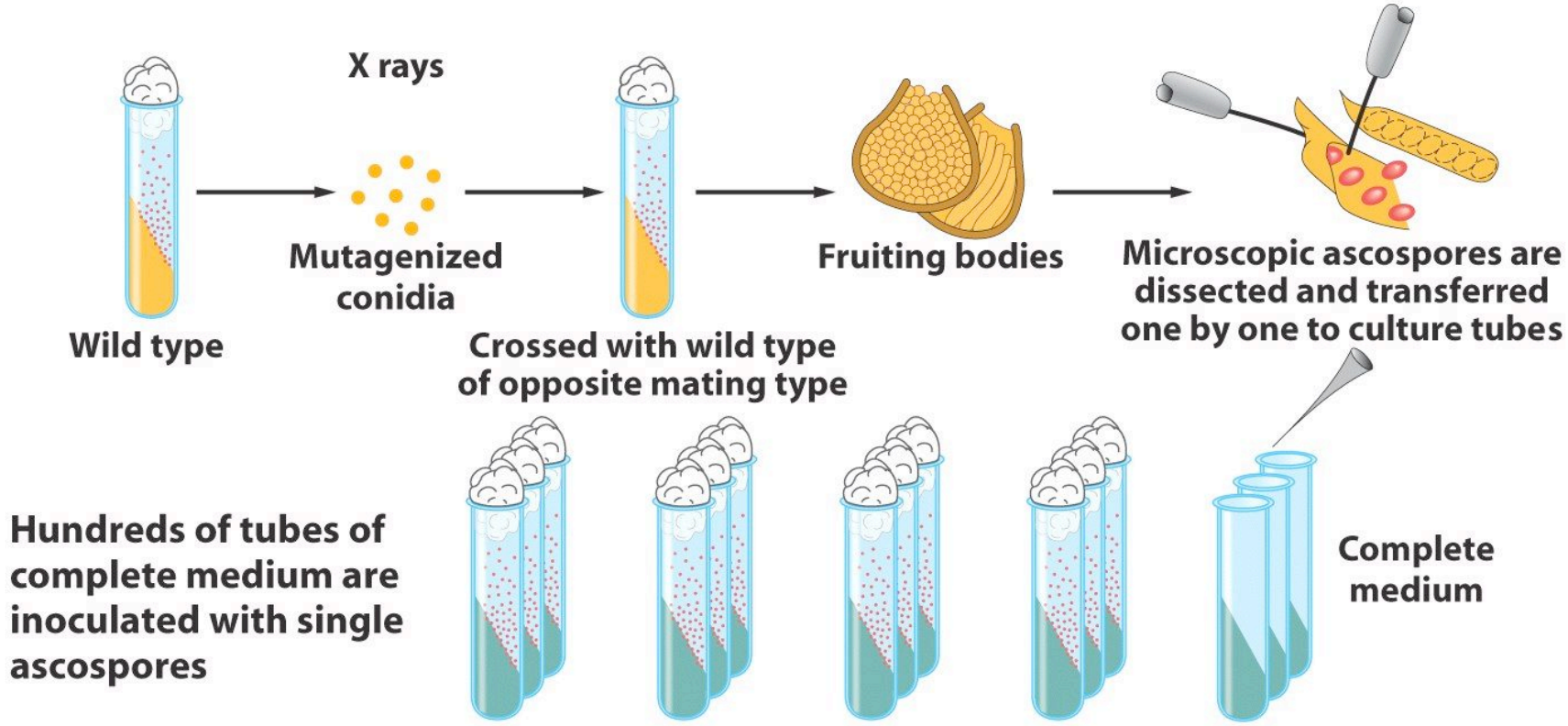
Beadle (*Drosophila* geneticist) and Tatum (biochemist), 1940's



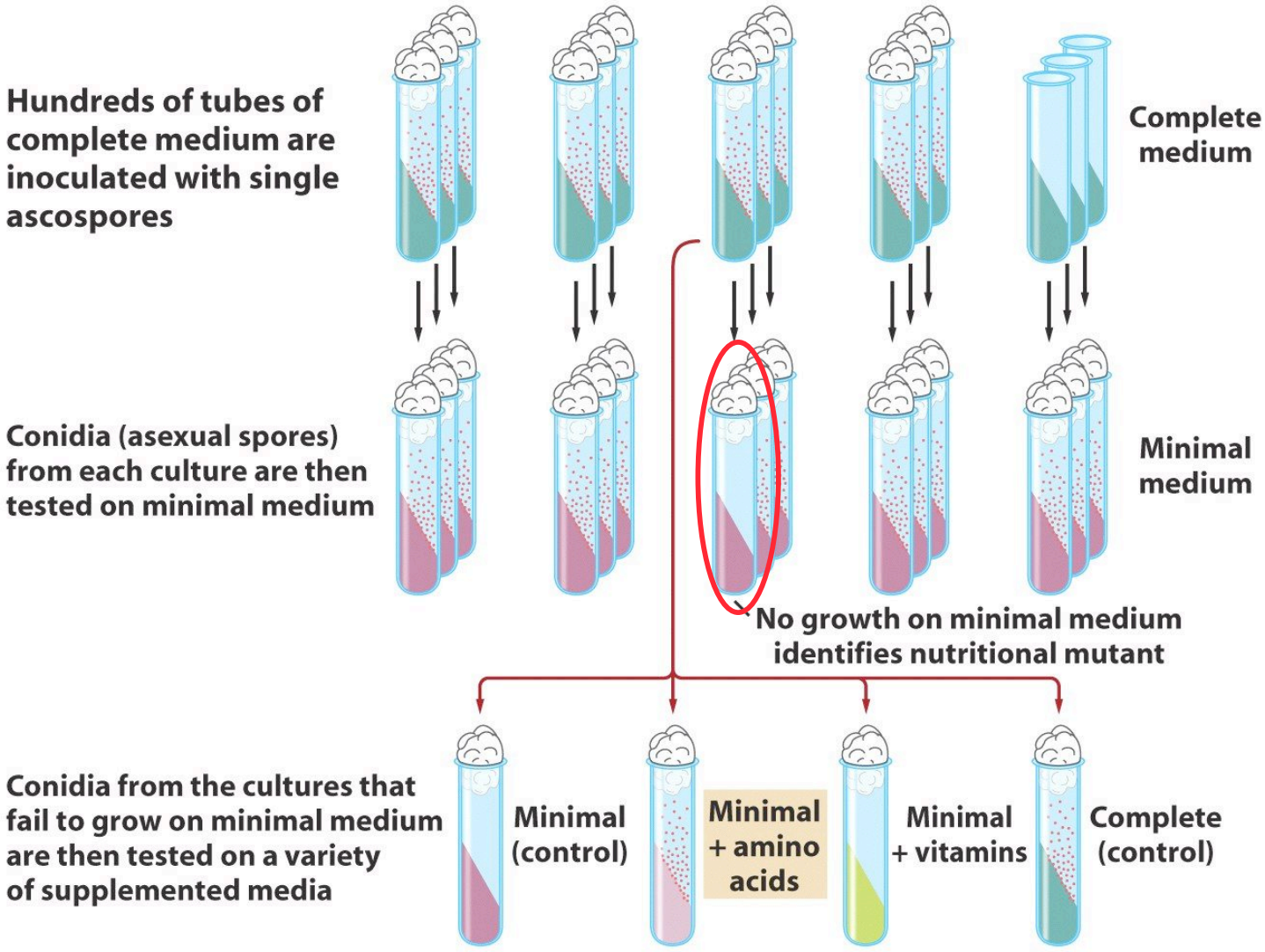
- Tried for 6 years (1935-1941) to link genes to chemical reactions in *Drosophila.*
- Switched to a simpler organism: *Neurospora crassa*
- Irradiated and isolated many arginine auxotrophs.

Beadle and Tatum and *Neurospora* mutants

- Mutagenized normal *Neurospora* cells; undergo meiosis…
- Isolated individual cells (ascospores) into separate tubes with complete media (growth media that is rich with amino acids, nucleotides, etc… opposite of minimal media).
- Tested each for the ability to grow on minimal media.



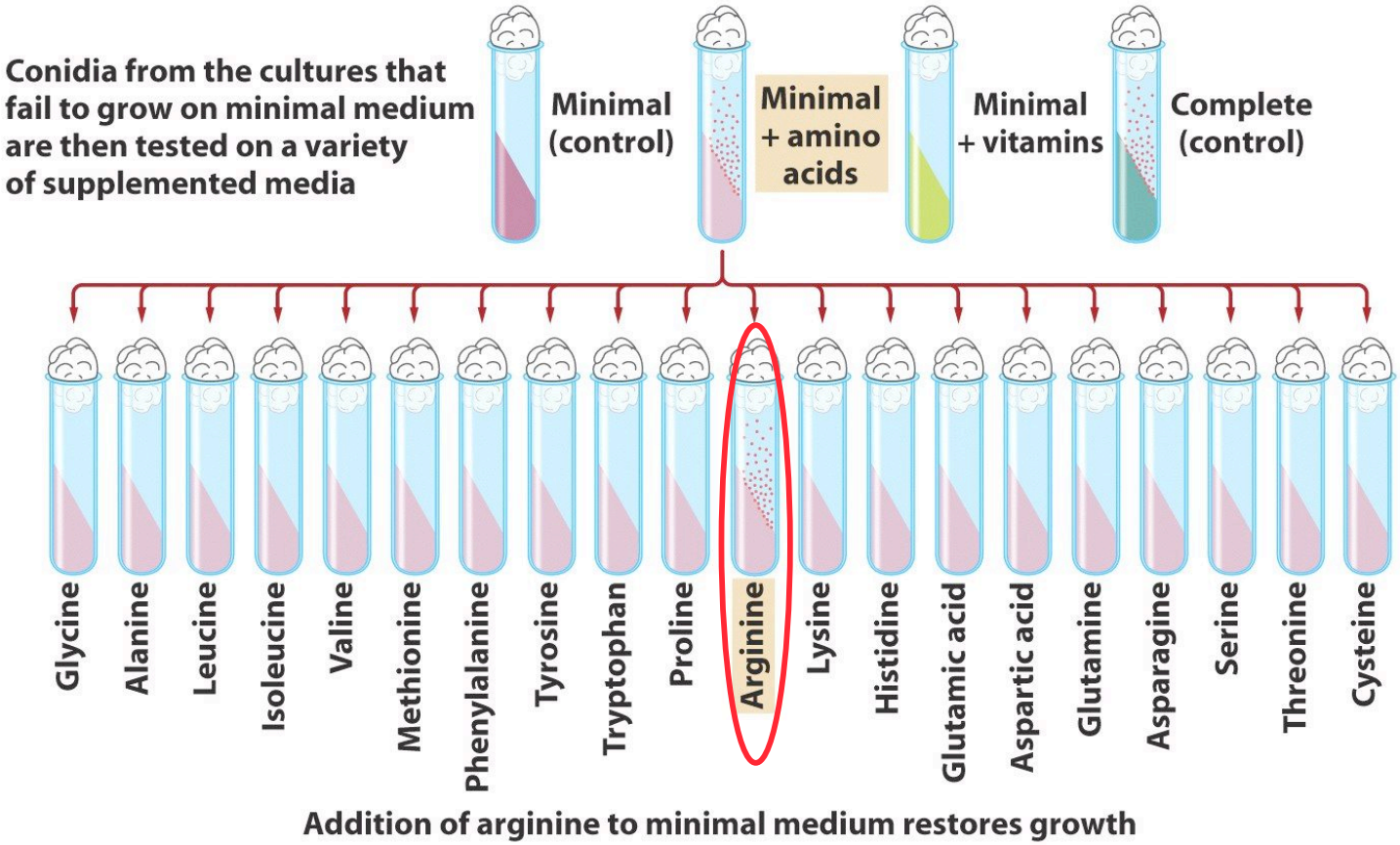
Neurospora Mutants



Certain cells did not grow on minimal medium.

The type of auxotrophy was tested on media with various supplements.

Arginine Mutants Identified



- After isolating mutants deficient in amino acid production, specific amino acid deficiencies were identified.
- For the purpose of our discussion, we will focus on the arginine mutants.
- Several independent arginine mutants were isolated.

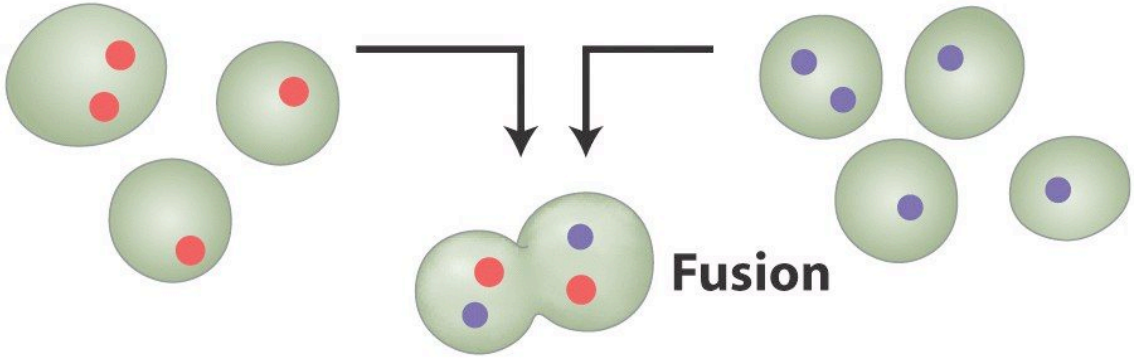
arg mutant 1

X

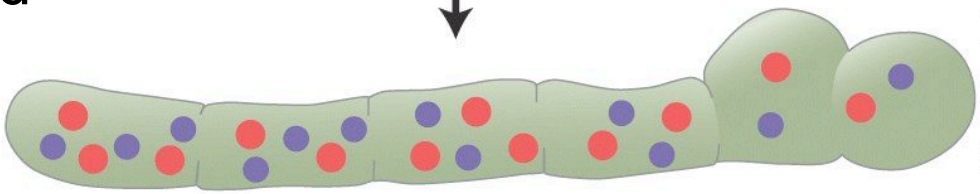
arg mutant 2

***arg-1* cells, defective for one specific enzyme in arginine synthetic pathway**

***arg-2* cells, defective for a different enzyme in arginine synthetic pathway**



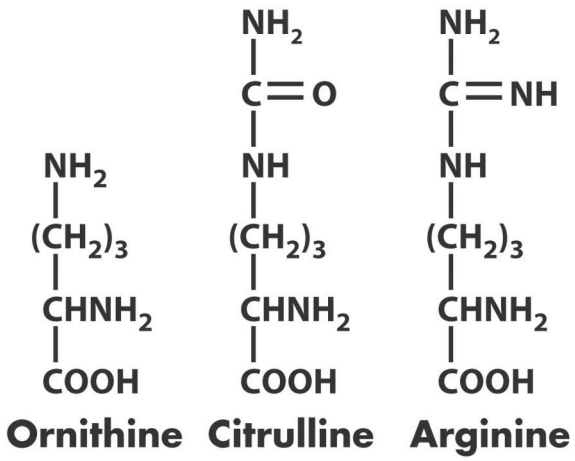
heterokaryon: a transient diploid



Heterokaryon grows without arginine

Only if strains are mutant for different genes

How Do We Figure Out The Pathway?



ornithine

↓

citrulline

↓

arginine

Each complementation group responded differently to supplements which were thought to be intermediates in the biochemical synthesis pathway leading to arginine.

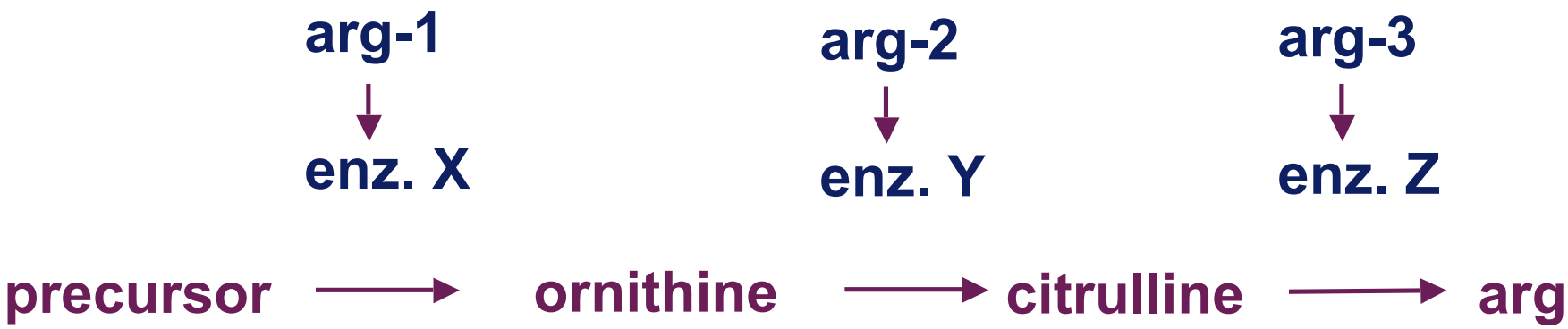
Table 6-1 Growth of *arg* Mutants in Response to Supplements

	minimal	Supplement		
Mutant		Ornithine	Citrulline	Arginine
arg-1	-	+	+	+
arg-2	-	−	+	+
arg-3	-	−	−	+

Note: A plus sign means growth; a minus sign means no growth.

Next, figure out at which step in the pathway each complementation group (gene) acts…

Mutant	minimal	citrulline	ornithine	arginine
arg-1	-	+	+	+
arg-2	-	+	-	+
arg-3	-	-	-	+



Practice Problem

Mutation #				
	A	B	C	D
134	+	+	-	+
276	+	+	+	+
987	-	-	-	+
773	+	+	+	+
772	-	-	-	+
146	+	+	-	+
333	+	+	-	+
123	-	+	-	+

Problem

- In what order do the compounds go?
- Look for compound that allows least mutants to grow?
  - C
  - Next?
    - A
  - Next?
    - B
    - D
- Then rewrite order of compounds from least growers to most or all growers….
- C → A → B → D

Problem

Mutation # & Group #	com pounds			
	C	A	B	D
276 GR 1	+	+	+	+
773 GR 1	+	+	+	+
134 GR 2	-	+	+	+
146 GR 2	-	+	+	+
333 GR2	-	+	+	+
123 GR 3	-	-	+	+
987 GR 4	-	-	-	+
772 GR 4	-	-	-	+

Genetically Based Enzyme Deficiencies in Humans

- Single gene mutations are responsible for many human genetic diseases.
  - Alkaptonuria
  - Phenylketonuria
  - Albinism
  - Lesch-Nyhan Syndrome
  - Tay-Sachs Disease

- Some mutations create a simple phenotype, while others are pleiotropic (Table 4.2).

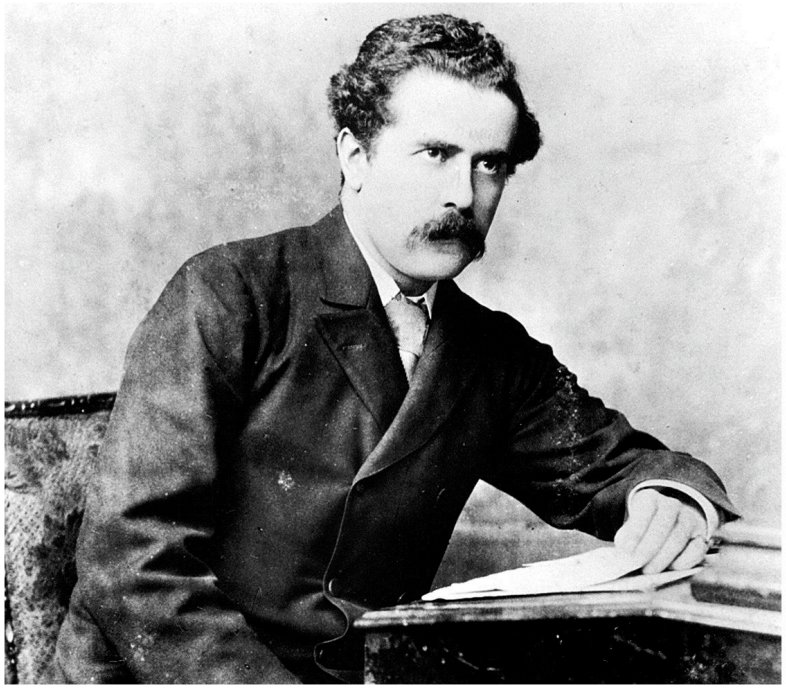
Garrod's Hypothesis of Inborn Errors of Metabolism

- Alkaptonuria is a human trait characterized by urine blackening on exposure to air and arthritis in later life.

- Archibald Garrod and William Bateson (1902) concluded alkaptonuria is genetically determined because:

  - Families with alkaptonuria often have several affected members.

  - Alkaptonuria is much more common in first cousin marriages than marriages with unrelated partners.



Garrod showed that alkaptonuria results from homogentisic acid (HA) in the urine

- HA is absent from normal urine.
- Garrod reasoned that normal people metabolize HA, but those with alkaptonuria do not because they lack the necessary enzyme.
  - He termed this an *inborn error of metabolism* (Figure 4.1).
- The responsible mutation is recessive.
  - The gene was later shown to be on chromosome 3.
- Garrod's work was the first evidence of a specific relationship between genes and enzymes.
- Garrod proved a mutation can block a human metabolic pathway by damaging an enzyme, causing a detectable buildup of that enzyme's substrate
  - he found a similar relationship in three other human diseases.

Phenylketonuria (PKU)

- commonly caused by a mutation on chromosome 12 in the phenylalanine hydrolase gene
  - Prevens the conversion of phenylalanine into tyrosine
- Phenylalanine is an essential amino acid
  - but in excess it is harmful
  - and so it is normally converted to tyrosine.
- Excess phenylalanine affects the CNS causing
  - mental retardation, slow growth, and early death.
- PKU's effect is pleiotropic.
  - Some symptoms result from excess phenylalanine.
  - Others result from inability to make tyrosine;
    - these include fair skin and blue eyes (even with brown-eye genes)
    - and low adrenaline levels.

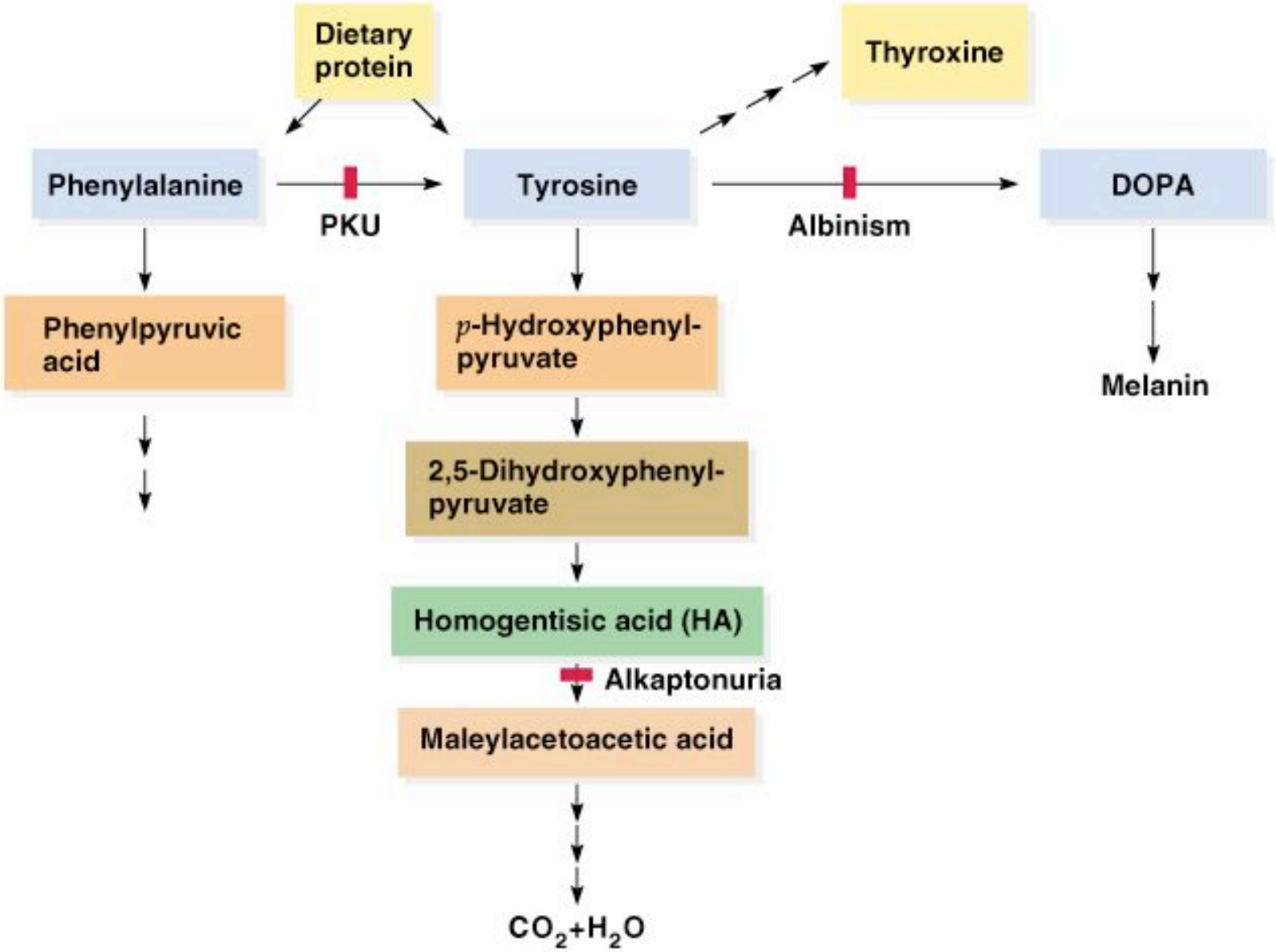
Albinism

- Classic albinism results from an autosomal recessive mutation in the gene for tyrosinase.

- Tyrosinase is used to convert tyrosine to DOPA in the melanin pathway.
  - Without melanin, individuals have white skin and hair, and red eyes due to lack of pigmentation in the iris.

- Two other forms of albinism are known, resulting from defects in other genes in the melanin pathway.
  - A cross between parents with different forms of albinism can produce normal children.

Fig. 12.1 Phenylalanine-tyrosine metabolic pathways



Lesch-Nyhan Syndrome

- results from a recessive mutation on the X chromosome
  - in the gene for hypoxanthine-guanine phosphoribosyl transferase (HGPRT).
    1. The fatal disease is found in males.
    2. Heterozygous (carrier) females may show symptoms when lyonization (inactivation) of the normal X chromosome leaves the X chromosome with the defective HGPRT gene in control of cells.
- HGPRT is an enzyme essential to purine utilization. In Lesch-Nyhan syndrome this pathway is highly impaired.
  - results in the accumulation of purines
    - which are eventually converted to uric acid.
  - individuals with HGPRT have high levels of uric acid in their bodies.
- The defect in a single enzyme, HGPRT, has very pleiotropic effects
  - giving rise to uremia, kidney failure, mental deficiency, and (so far inexplicably) self-mutilation.

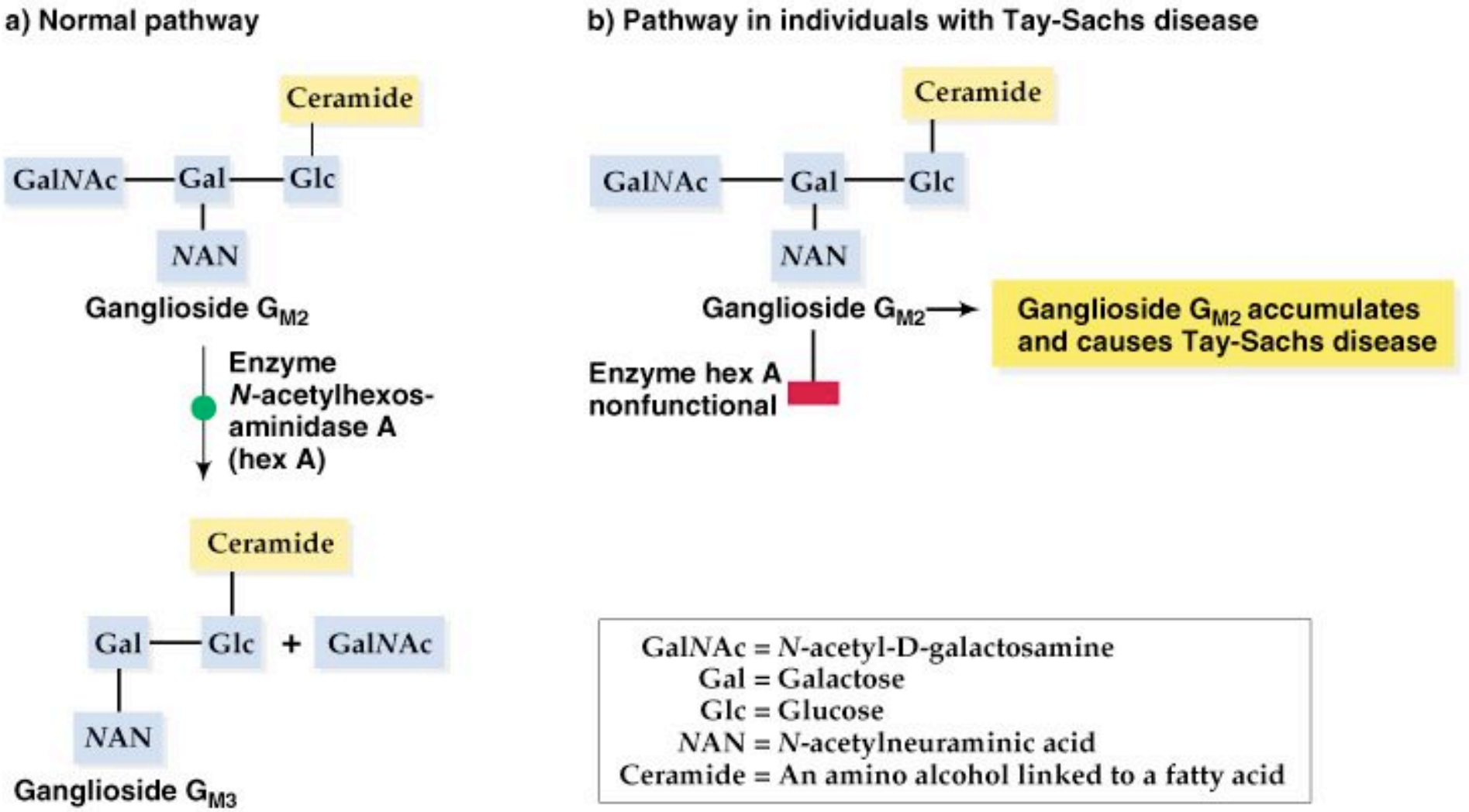
Tay-Sachs Disease

- Tay-Sachs is one of a group of diseases called *lysosomal-storage diseases.*
  - Generally caused by recessive mutations
  - result from mutations in genes encoding lysosomal enzymes.

- Tay-Sachs disease (infantile amaurotic idiocy) results from a recessive mutation in the gene *hexA*
  - which encodes the enzyme *N*-acetylhexosaminidase *A*.
  - The HexA enzyme cleaves a terminal *N*-acetylgalactosamine group from a brain ganglioside

- Infants homozygous recessive for this gene will have nonfunctional HexA enzyme.
  - Unprocessed ganglioside accumulates in brain cells, and causes various clinical symptoms:
    - Infants have enhanced reaction to sharp sounds.
    - A cherry-colored spot surrounded by a white halo may be visible on the retina.
    - Rapid neurological degeneration begins about age 1
      - brain loses control of normal functions due to accumulation of unprocessed ganglioside.

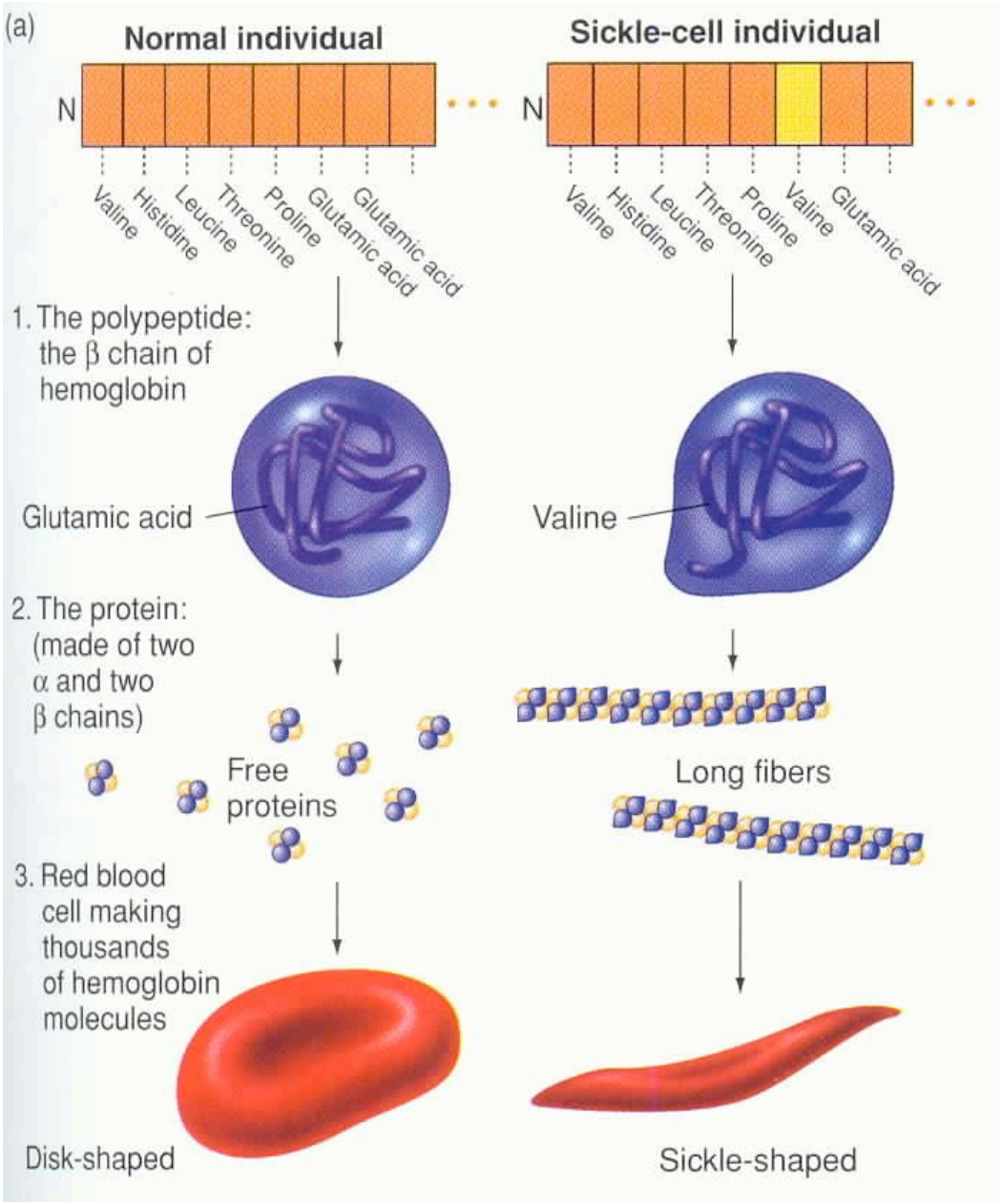
Fig. 12.6 The biochemical step for the conversion of the brain ganglioside G_{M2} to the ganglioside G_{M3}, catalyzed by the enzyme *N*-acetylhexosaminidase A (hex A)



Gene Control of Protein Structure

- Genes also make proteins that are not enzymes.
  - Such proteins are called Structural Proteins.

- These proteins are often abundant, making them easier to isolate, purify, and analyze.
  - such as hemoglobin



Gene Sequence Controls Protein Structure

- The sickle mutation is in the beta chain of hemoglobin.
- HbS has a valine in place of glutamic acid, which causes the proteins to aggregate, and this structural change results in the sickled-shape cell.

Sickle-Cell Anemia

- J. Herrick (1910) first described sickle-cell anemia, finding that red blood cells (RBCs) change shape (form a sickle) under low O_2 concentrations
  - Sickled RBCs are fragile, hence the anemia.
  - They are less flexible than normal RBCs, and form blocks in capillaries
    - resulting in tissue damage downstream.
  - Effects are pleiotropic, including
    - damage to extremities, heart, lungs, brain, kidneys, GI tract, muscles, and joints. Results include heart failure, pneumonia, paralysis, kidney failure, and abdominal pain.
  - Heterozygous individuals have sickle-cell trait, a much milder form of the disease.

Sickle-Cell Anemia

- The genetics and gene products involved in sickle-cell anemia and trait are as follows:
  - Wild-type β chain allele is β**A,** which is codominant with mutant β**S** allele**.**
  - Hemoglobin of β**A**/β**A** individuals has normal β subunits, while hemoglobin of those with the genotype β**S**/β**S** has β subunits that sickle at low O_2 tension.
  - Hemoglobin of β**A**/β**S** individuals is ½ normal, and ½ sickling form. These heterozygotes may experience sickle-cell symptoms after a sharp drop in the oxygen content of their environment.

- http://www.pbs.org/wgbh/evolution/library/01/2/l_012_02.html

Homework Problems

- Chapter 12
- # 12, 18, 34

- DON'T forget to take the online QUIZ!!
- DON'T forget to submit the online iActivity
  - "Pathways"