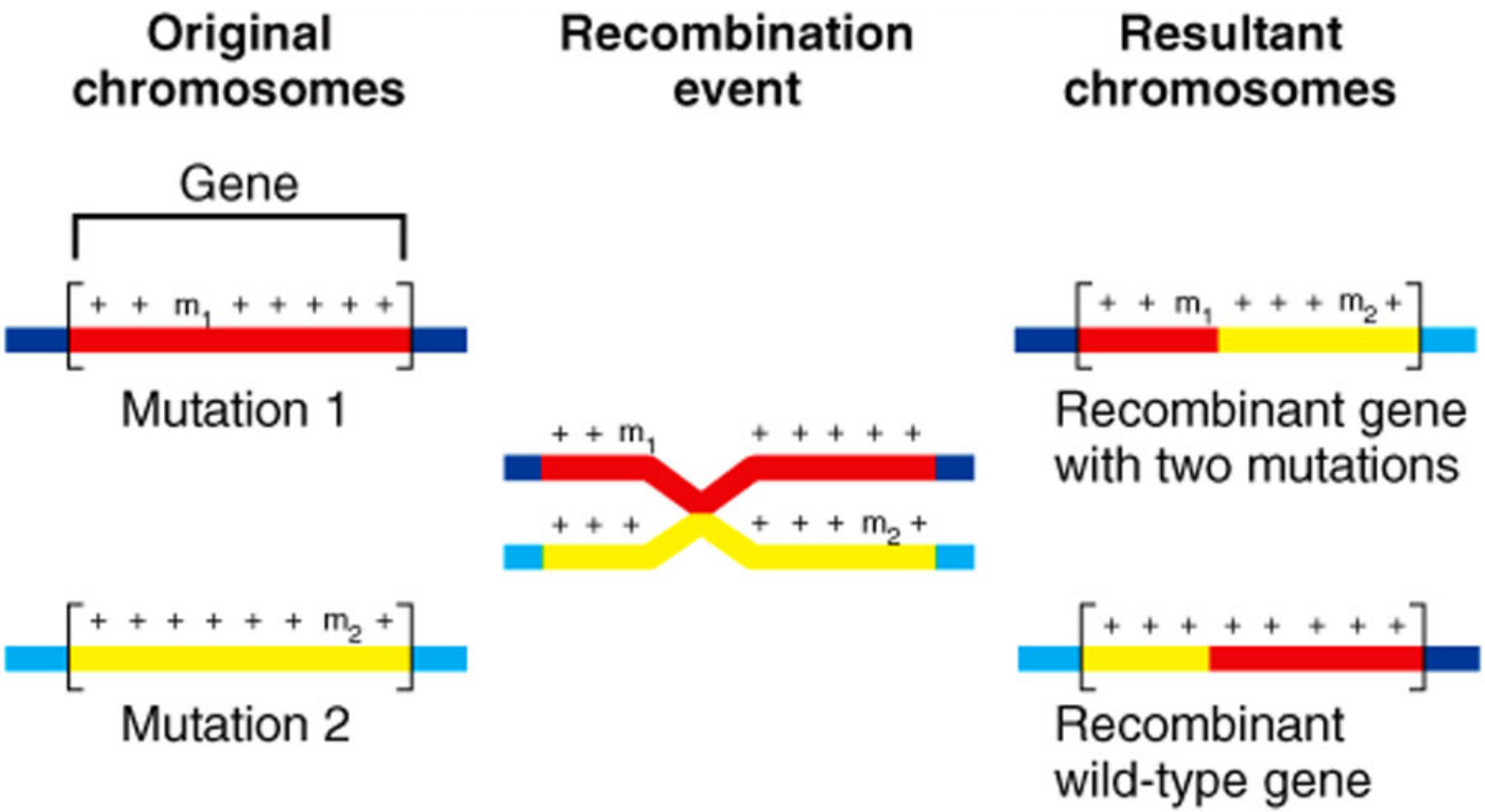
The gist of Benzer's work: Intragenic recombination can restore wild-type function to a gene.

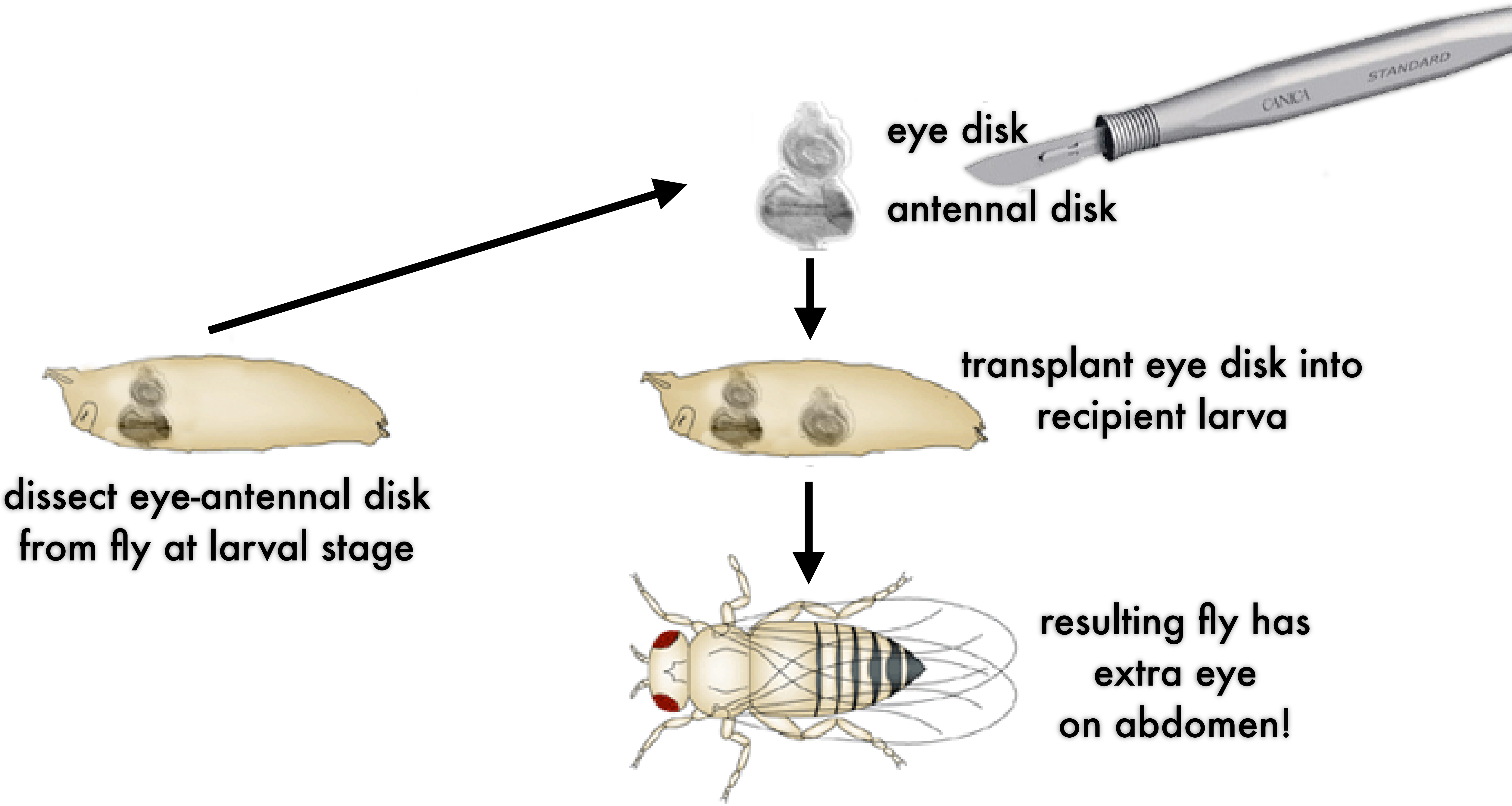


This implies that genes are linear strings of mutable elements.

Beadle and Ephrussi carrying out an eye disk transplantation experiment

Goal: understand the link between the genetic and biochemical basis of eye color specification in Drosophila

Beadle and Ephrussi carrying out an eye disk transplantation experiment



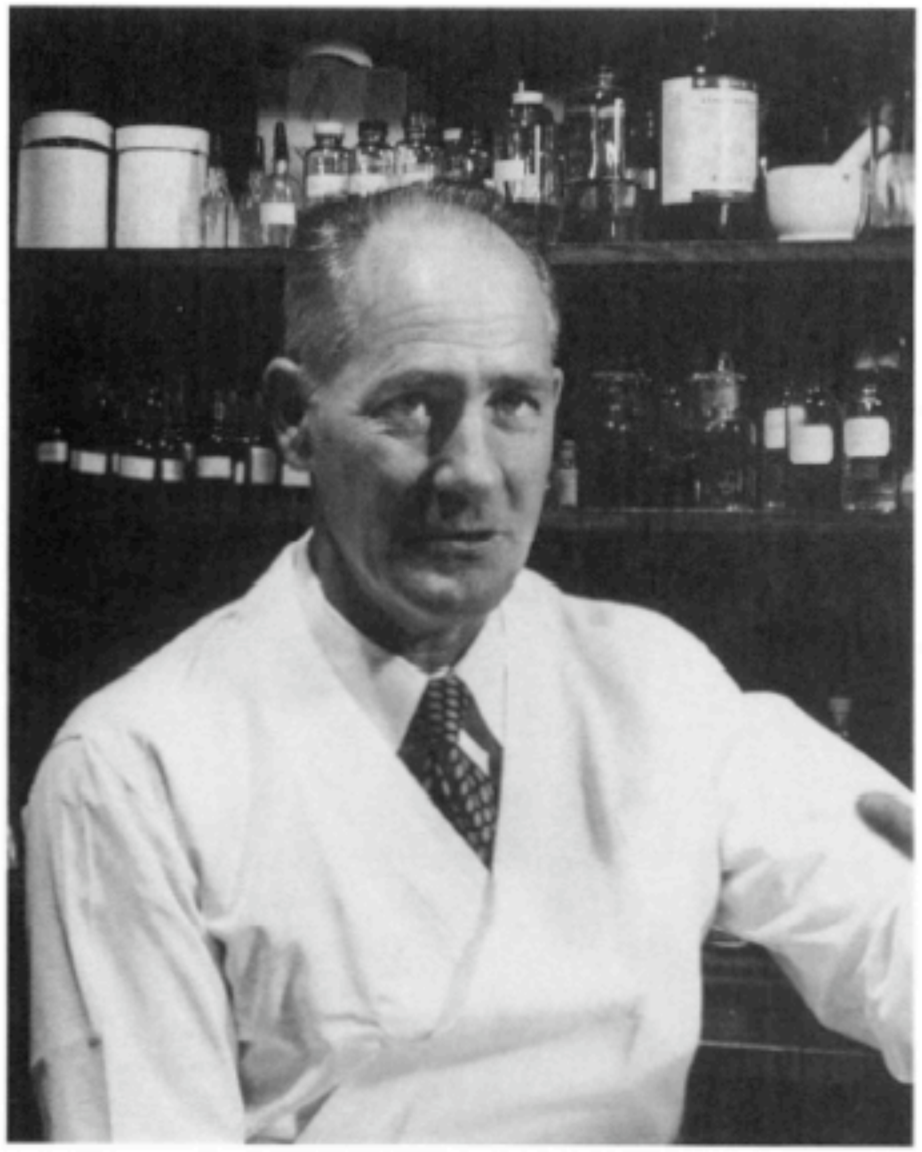
"Curiously, transplantation of vermilion eye discs into cinnabar larvae yielded normal colour eyes in the abdomen, but transplanting cinnabar eye discs into vermilion larvae still yielded cinnabar coloured eyes. Beadle and Ephrussi concluded that the formation of eye colour occurred through a pathway that has at least two steps: one led to the synthesis of the vermilion substance and the other to that of the cinnabar substance. From the asymmetrical outcomes of the reciprocal transplants of vermilion and cinnabar eye discs, they surmised that the vermilion gene acted before the cinnabar gene. The lasting achievement of the Beadle–Ephrussi collaboration was the realization that genes controlled individual steps in a metabolic pathway" (Singer and Berg (2004), *Nature Reviews Genetics*)

	vermilion		*cinnabar*	
Substance A (vermilion colored)	→	**Substance B** (cinnabar colored)	→	**Substance C** (wild-type colored)

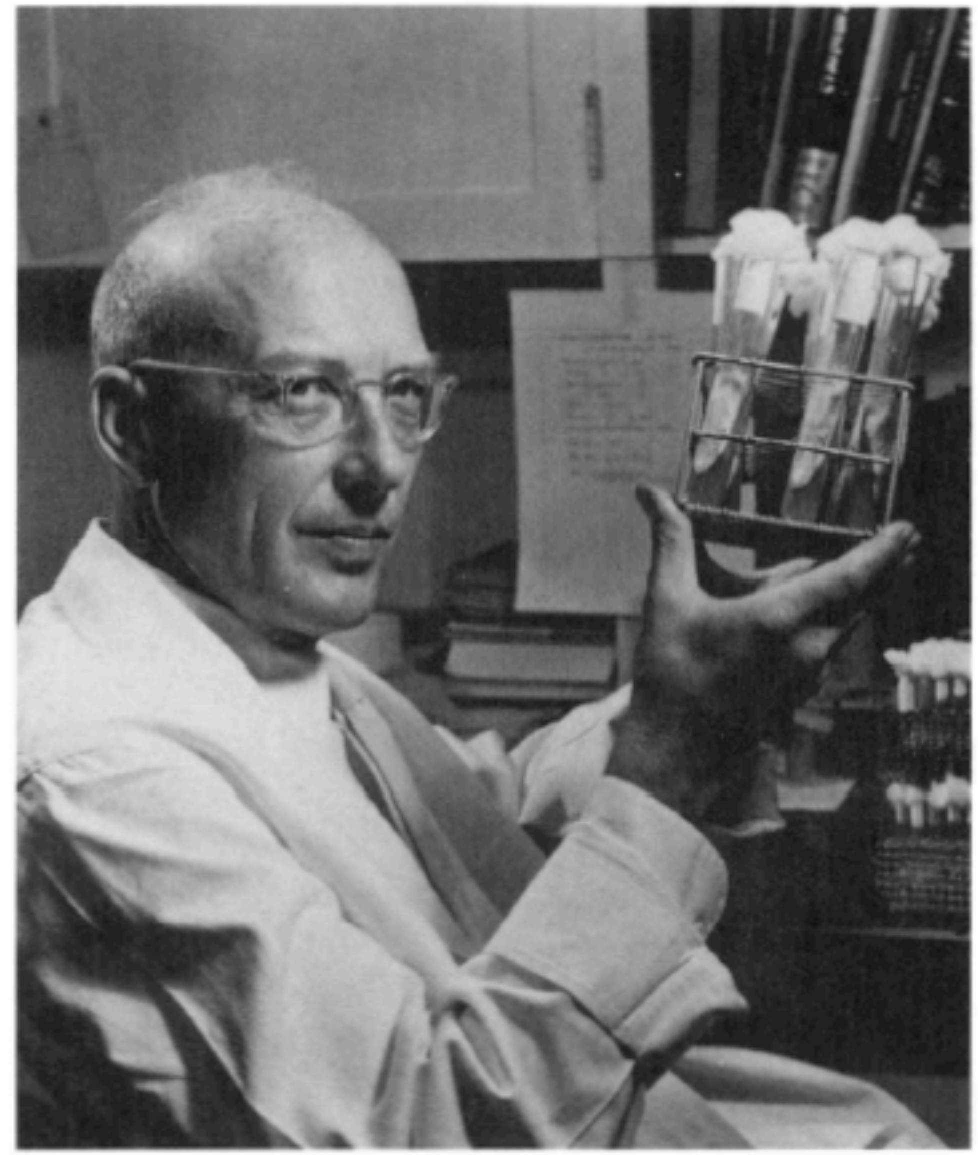
Limitation: the chemical basis for the different colors was unknown. Thus, it was not possible to show that the *vermilion* and *cinnabar* genes encoded single enzymes that could convert one to the other.

Eye colors (clockwise):
brown, cinnabar, sepia, vermilion, white, wild.
Also, the white-eyed fly has a yellow body,
the sepia-eyed fly has a black body,
and the brown-eyed fly has an ebony body.
source: Wikipedia

Beadle and Tatum turned to *Neurospora* to test the idea that genes specify protein function



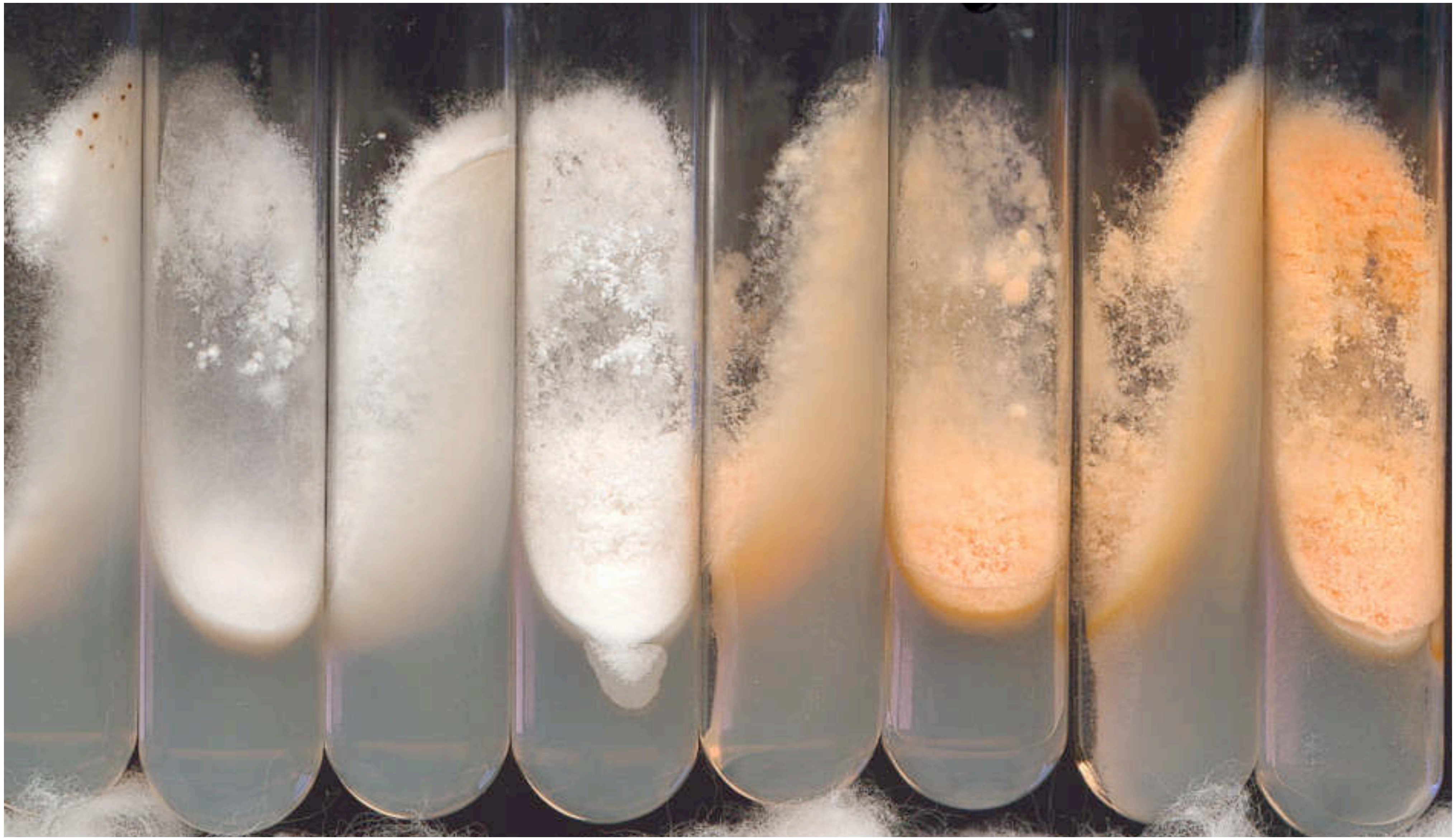
George Beadle



Edward Tatum

Neurospora can synthesize virtually all of the complex chemicals required for cell function from simple nutrients

(Sugar, nitrogen, phosphorus, minerals, and biotin)



In other words, *Neurospora* is *prototrophic* (self-sufficient) for almost every nutrient you can think of (amino acids, most vitamins, etc.)

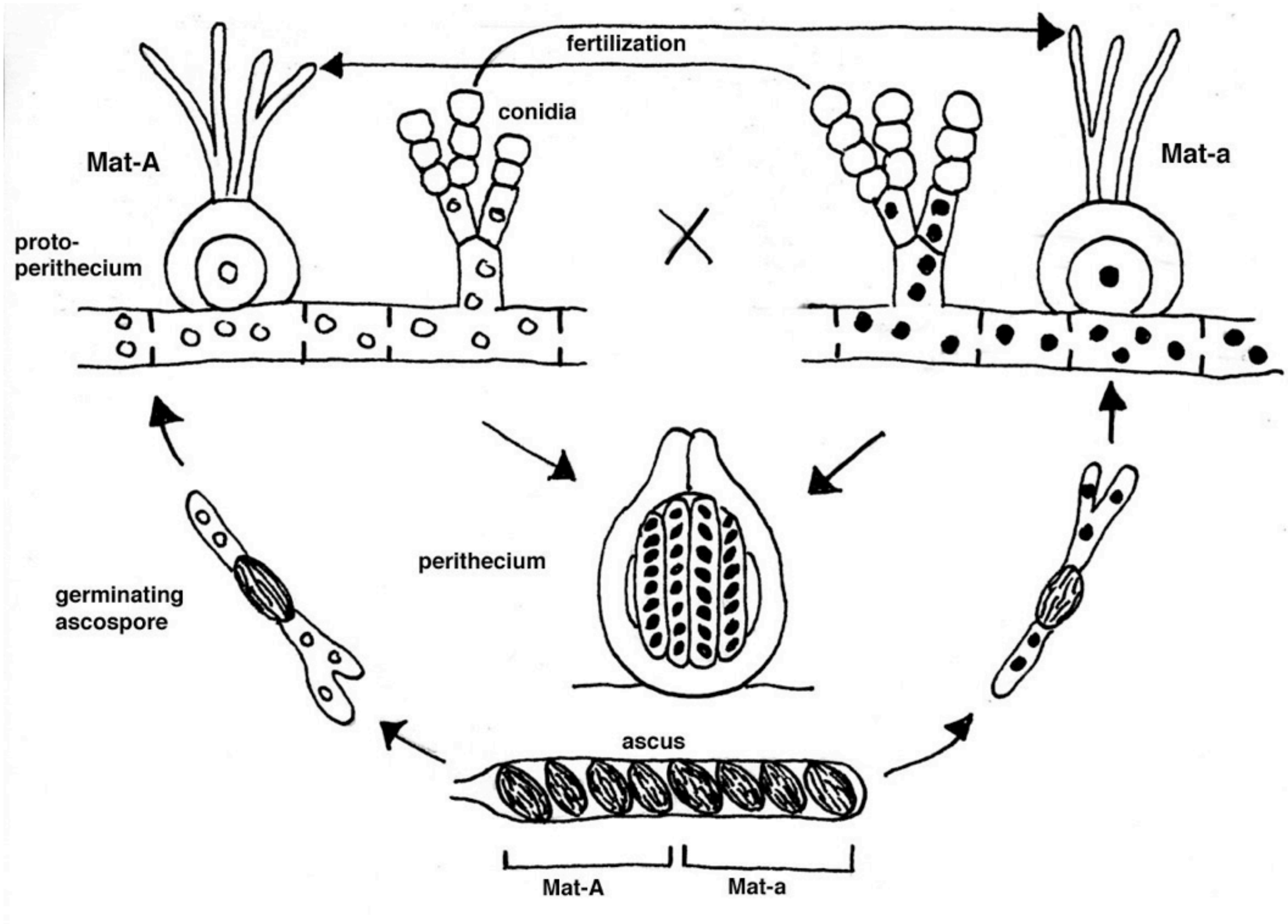
Edward Tatum and George Beadle: One-gene-one-enzyme

It was thought that genes somehow controlled the function of enzymes, but the nature of this link was still unclear. The major obstacle to demonstrating a systematic relationship between genes and enzymes was the difficulty of working out the biochemical basis for many genes' functions - e.g., the *white* gene in Drosophila melanogaster turns out to encode a protein that transports guanine and tryptophan, precursors of the red and brown eye color pigments.

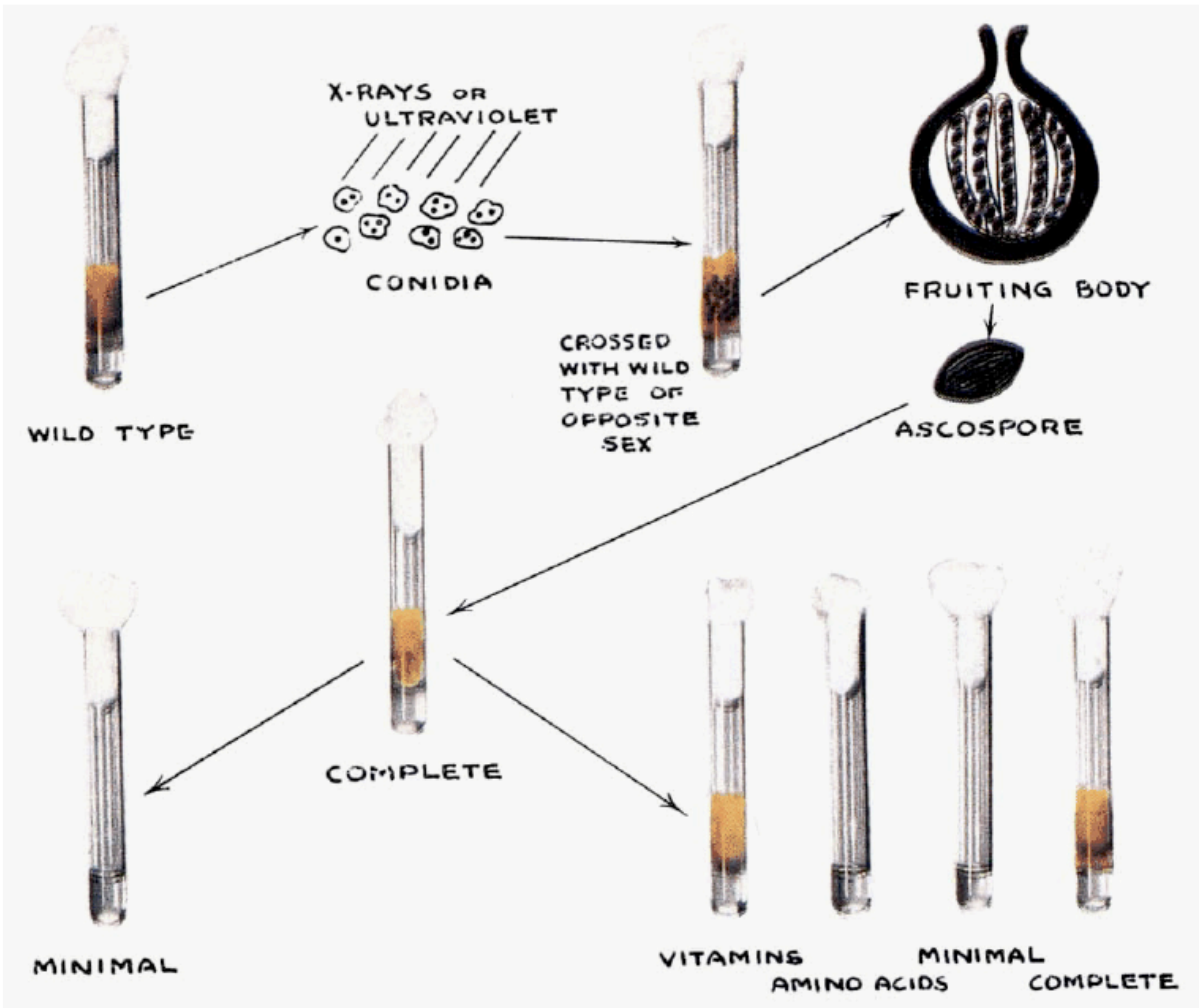
"Considerations such as those just outlined have led us to investigate the general problem of the genetic control of developmental and metabolic reactions by reversing the ordinary procedure and, instead of attempting to work out the chemical bases of known genetic characters, to set out to determine if and how genes control known biochemical reactions. The ascomycete *Neurospora* offers many advantages for such an approach and is well suited to genetic studies."

Beadle and Tatum (1941) *Proceedings of the National Academy of Sciences*

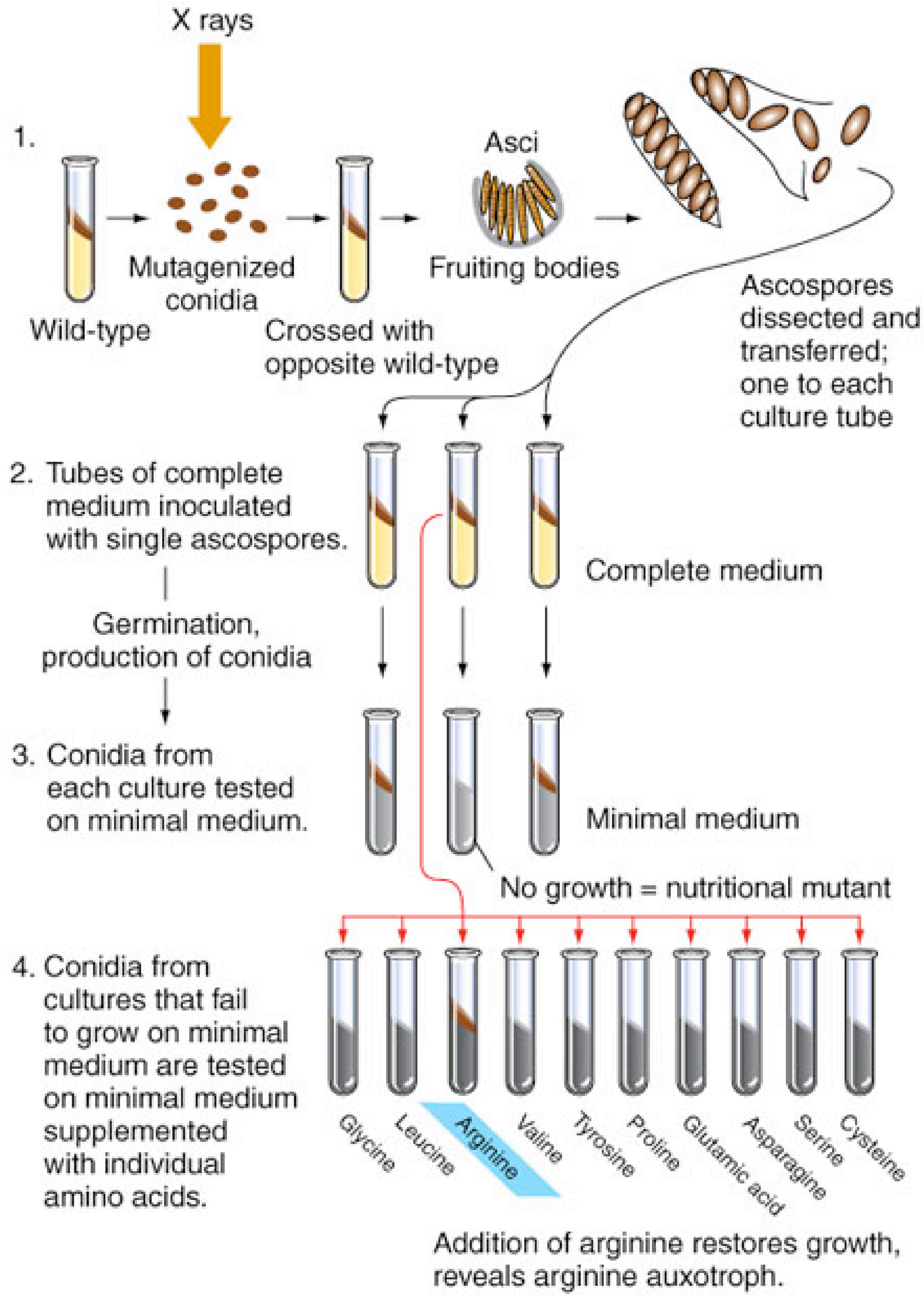
Also, Neurospora genetics tools are the bomb (once you get your head around the life cycle).



The experimental approach to find nutritional mutants (*auxotrophs*) in *Neurospora crassa* (as diagrammed by George Beadle)



The experimental approach to find nutritional mutants (*auxotrophs*) in *Neurospora crassa*

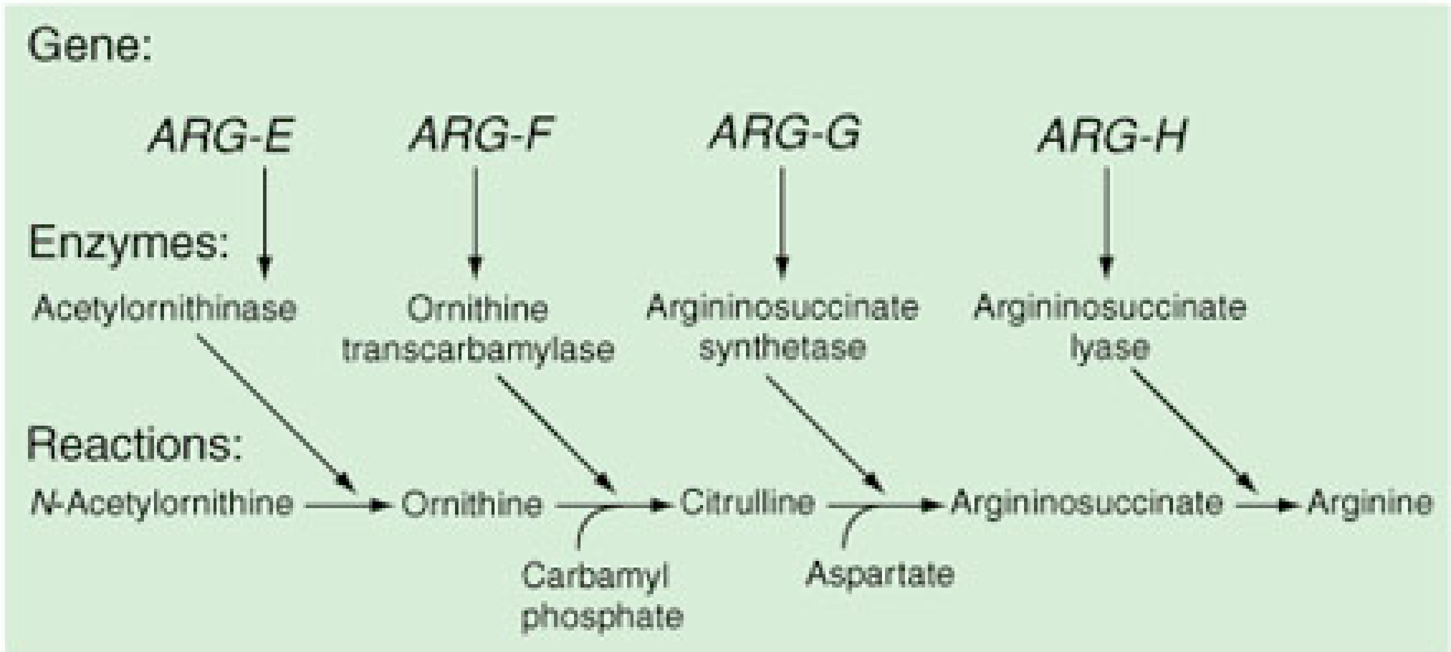


Complementation testing and nutritional dependence allowed Tatum and Beadle to put the individual genes into an ordered biochemical pathway

(b) Growth response if nutrient is added to minimal medium

Mutant strain	Supplements: Nothing	Ornithine	Citrulline	Arginino-succinate	Arginine
Wildtype: *Arg*$^+$	+	+	+	+	+
Arg-E$^-$	−	+	+	+	+
Arg-F$^-$	−	−	+	+	+
Arg-G$^-$	−	−	−	+	+
Arg-H$^-$	−	−	−	−	+

(c) Inferred biochemical pathway



Knowledge of the chemical structures of arginine and its precursors indicated a 1:1 correspondence between genes and enzymes

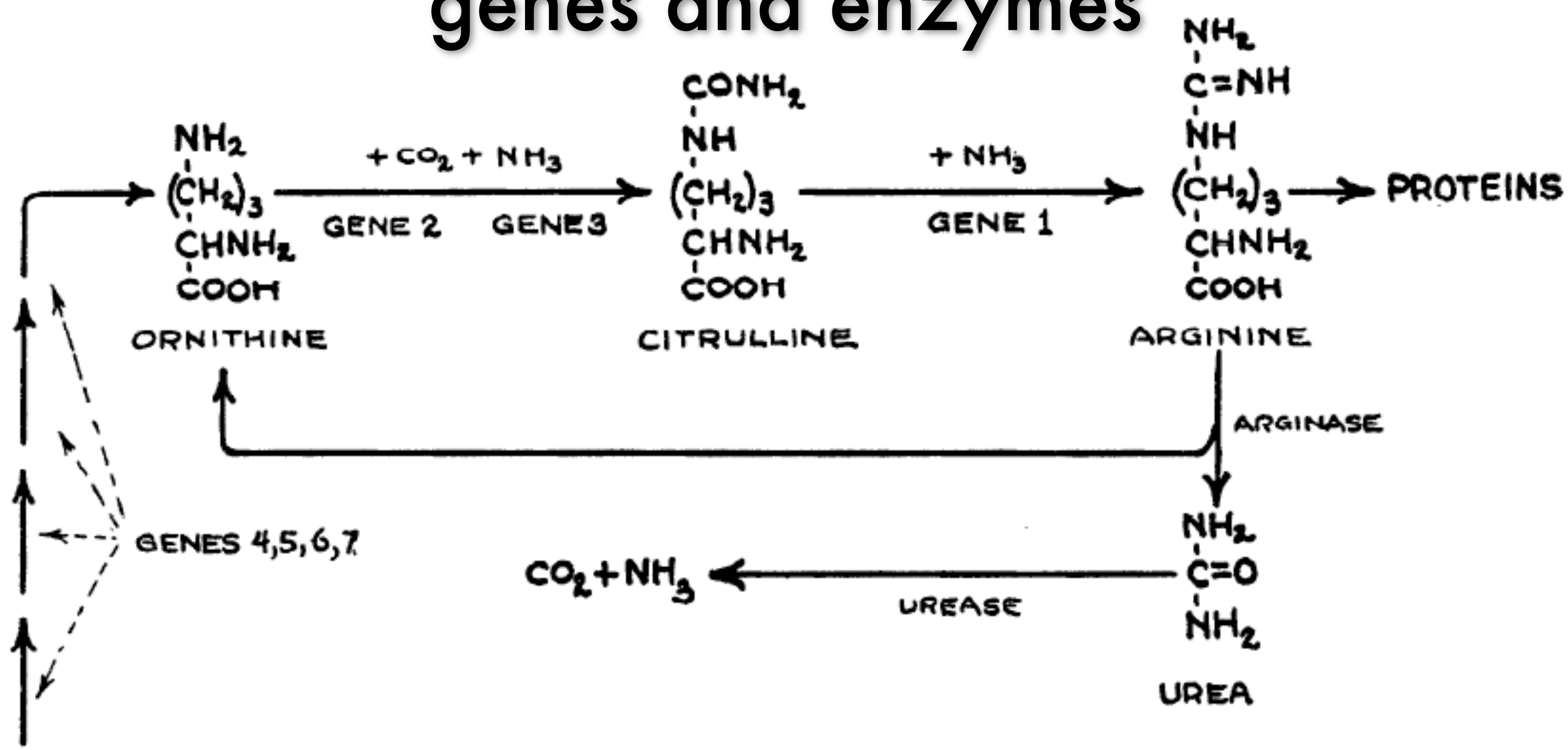


Fig. 3. Ornithine cycle in Neurospora.

From Beadle (1945) *Physiology Reviews:*

In the analysis of "arginineless" mutants, Srb and Horowitz made use of these types of genetic tests. Characterization of the seven genetically distinct mutants in terms of their requirements for normal growth showed that one responded only to arginine, two grew normally in the presence of either arginine or citrulline, while the remaining four responded to arginine, citrulline, or ornithine. It is significant that for the conversion of citrulline to arginine which is most probably a single reaction, only one gene is known. Two genes are known for the conversion of ornithine to citrulline and there are good chemical reasons for supposing that this transformation involves two reactions with carbamino ornithine as an intermediate. The reactions by which plants synthesize ornithine from simpler constituents are not known. Four genes have been shown to be concerned with reactions leading to ornithine and on the basis of the one-gene-one-reaction concept this would imply at least four reactions in its synthesis, an implication that is certainly not unreasonable from a chemical standpoint.

Interpretation of epistasis experiments requires knowledge about the biochemical function of the genes in question

Are you looking at a (linear) biosynthetic or breakdown pathway?

A → B → C → D

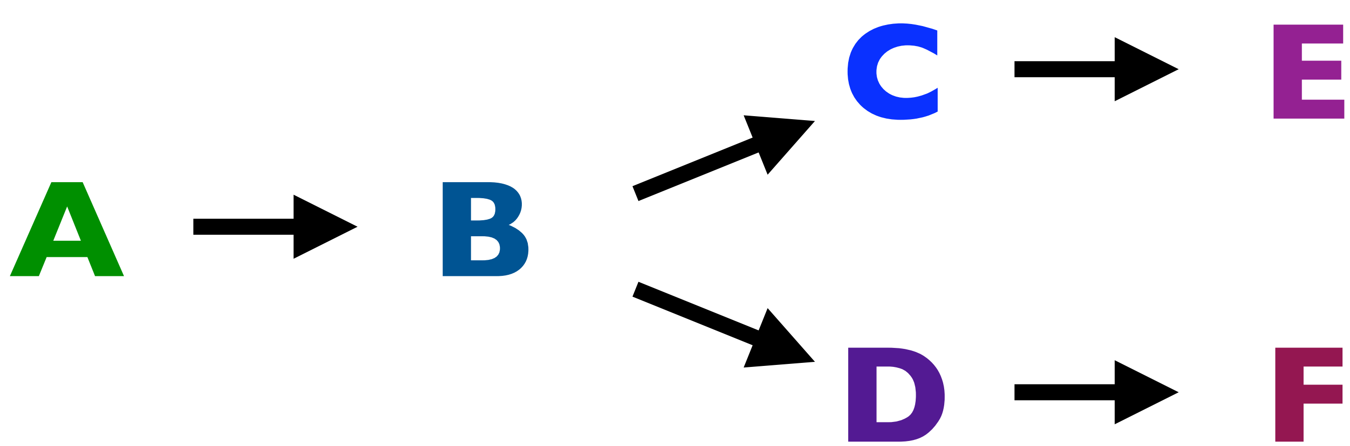
A’ → B’ → C’ → D’ (toxic!)

Examples:

Alcohol Dehydrogenase (in liver) can convert allyl alcohol, to acrolein, which is toxic

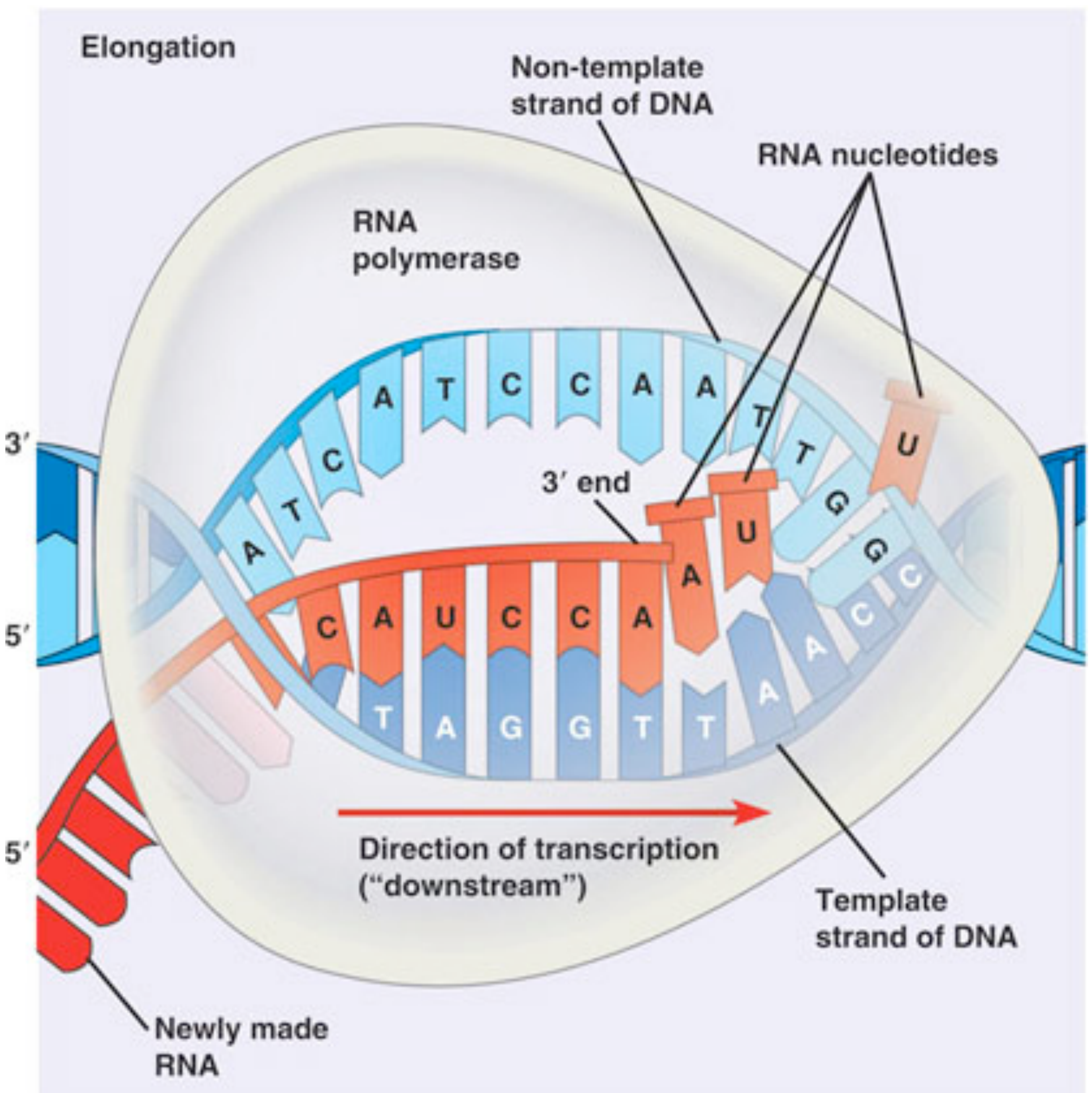
the URA3 gene in budding yeast converts 5-Fluoroorotic Acid (FOA) to 5’fluorodeoxyuridine (FldU)

Does the pathway branch?



So how do genes encode proteins?

The Genetic Code enables the ribosome to translate each "codon" (3 bases) into one amino acid

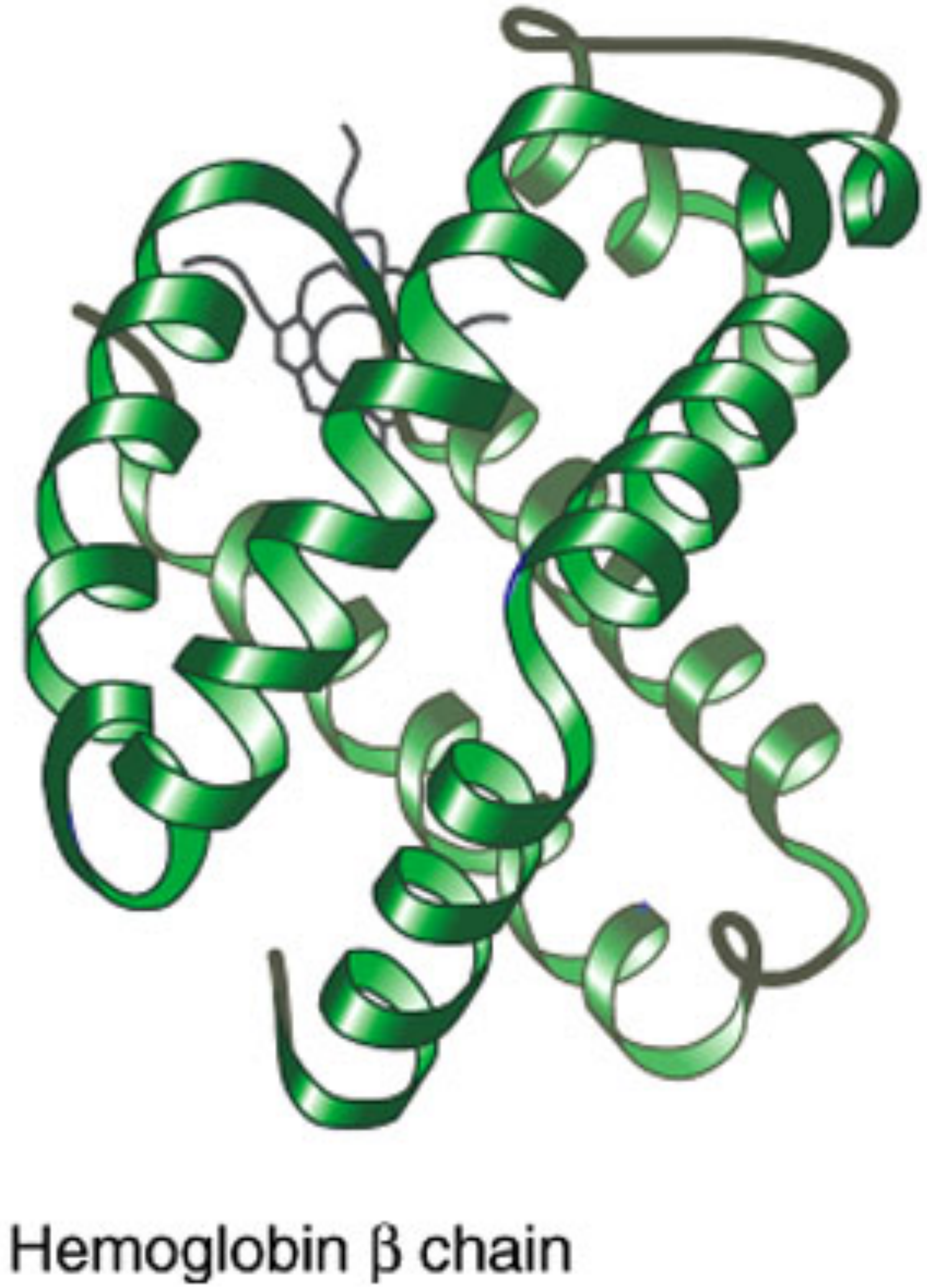


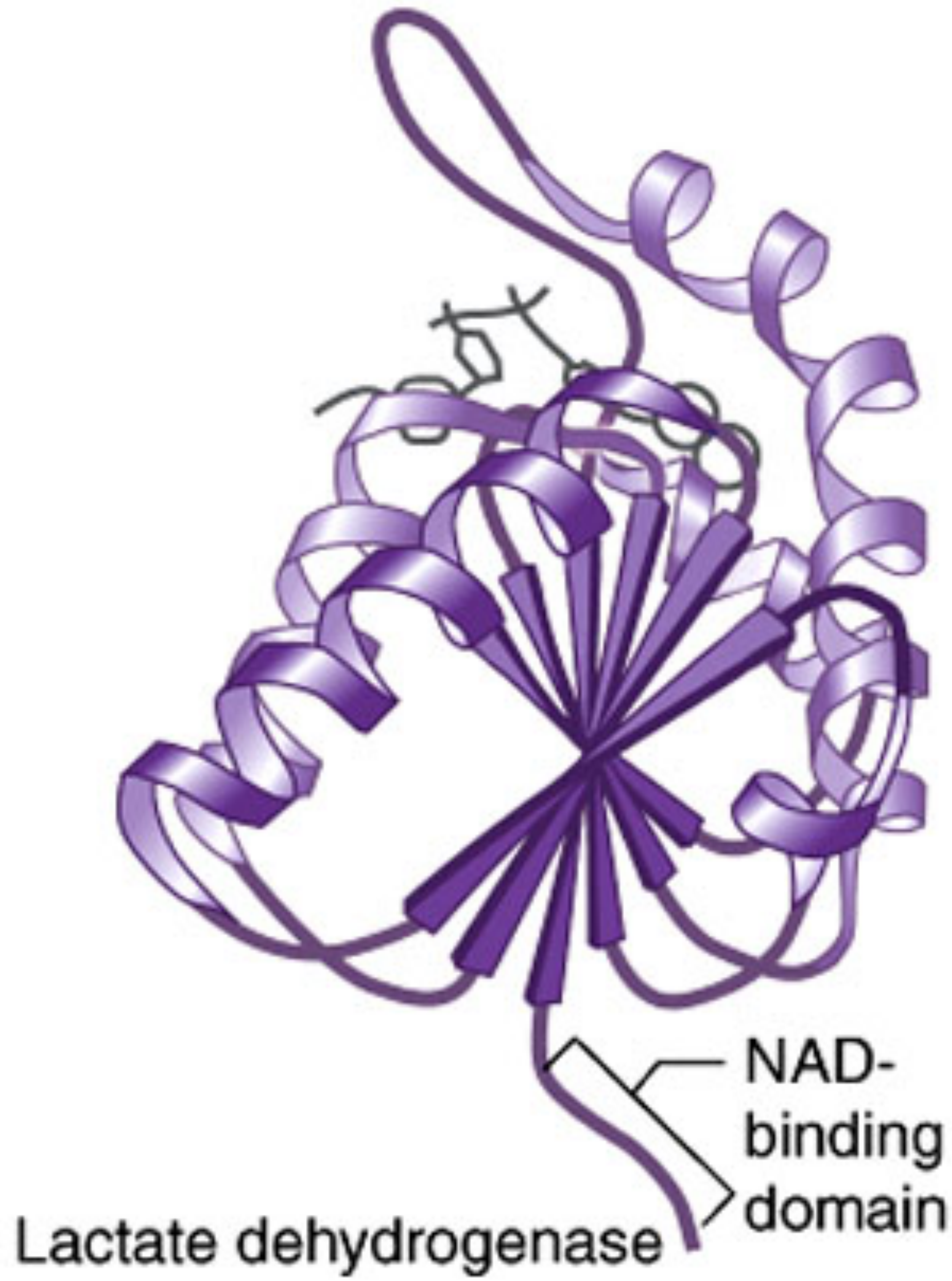
Second letter

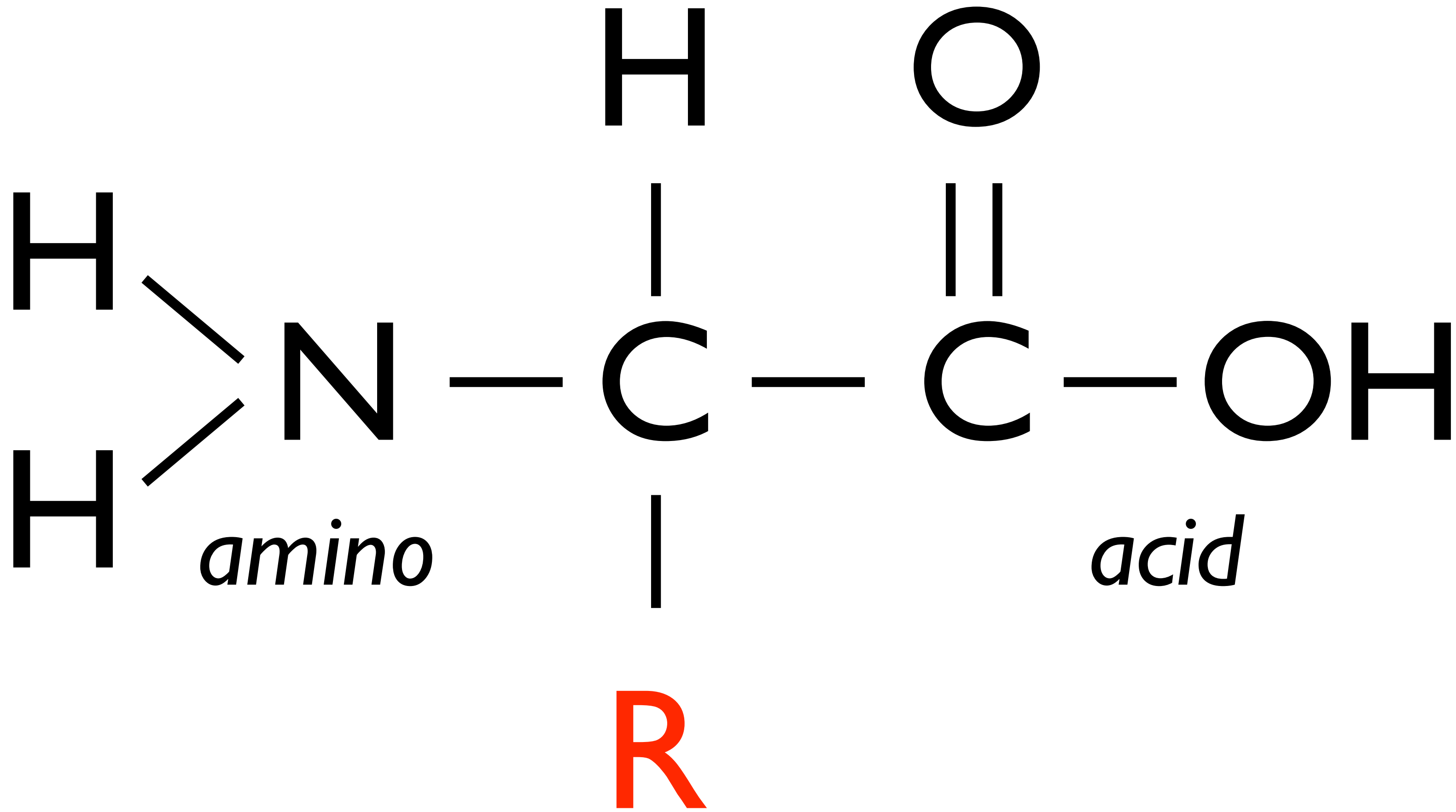
First letter	U	C	A	G	Third letter
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
U	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
U	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
U	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
C	CUC Leu	CCC Pro	CAC His	CGC Arg	C
C	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
C	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
A	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
A	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
A	AUG Met	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
G	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
G	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
G	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

Universal genetic code

Amino acid sequences determine the 3D structures and functions of proteins







R = a “side chain.” It can be really simple (glycine=H) or more exotic...

tyrosine: