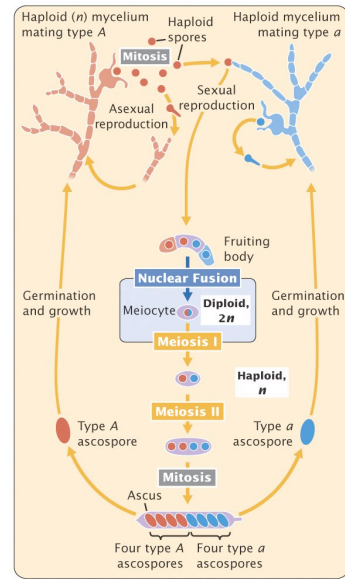


Gene function

The idea that genes control visible traits was always clear. What was not clear was how they did it. Why should carrying one allele produce red flowers, yet carrying another produce white? Why were some alleles dominant and others recessive? What about co-dominance?

Although early work pointed to a link between genes and metabolism, it wasn't until the work of Beadle & Tatum that this was made explicit.

The bread mold
Neurospora had
many useful traits



Searching for auxotrophic mutants

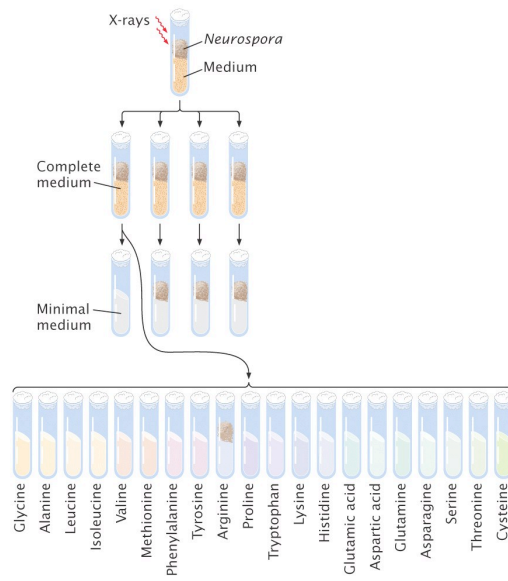


Table 15.1

Growth of arginine auxotrophic mutants on minimal medium with various supplements

Mutant Strain Number	Ornithine	Citrulline	Arginine
Group I	+	+	+
Group II	-	+	+
Group III	-	-	+

Note: + indicates growth; - indicates no growth.

One gene-one enzyme

- Because the original experiment dealt with biochemical pathways, it was not surprising that all the defects were in enzymes. The concept was later modified to one gene-one polypeptide

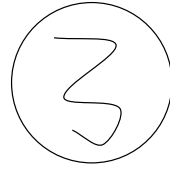
Dominant/recessive

- The idea that a gene was responsible for the production of a specific protein provided an understanding of much of classical genetics.
- In most cases a 'dominant' gene is simply an allele that provides the functional version of the enzyme. As long as at least one copy is present then the enzyme functions normally. For the 'recessive' allele to be visible, both copies of the gene must code for the non-functional form of the enzyme

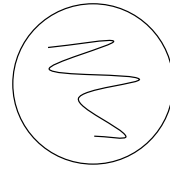
Crossfeeding

- Another series of observations also could be explained. If two mutants, both unable to perform a specific function (i.e. make histidine) are placed close to each other on the same minimal plate, they often will show some growth.
- This phenomenon is termed crossfeeding:

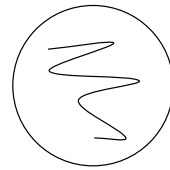
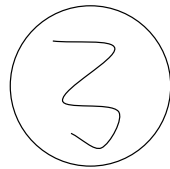
Mutant A streaked on minimal plate



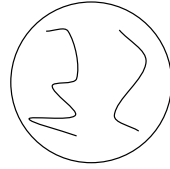
Mutant B streaked on minimal plate



Leave overnight



Both mutants streaked on same
minimal plate (not touching)



Leave overnight

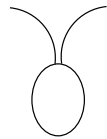


Both show growth

This is usually
because each mutant
makes something
that the other
cannot. If this can
diffuse through the
agar, then the other
mutant can grow.

Complementation

- Crossfeeding is an example of a wider phenomenon, termed complementation. The previous experiment can be done within a single cell.
- If the two mutants are mated then both sets of genetic information can coexist in the same cell.



Wild-type *Chlamydomonas*:
motile flagella

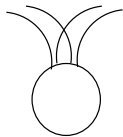


pf-1 mutant: paralyzed



pf-14 mutant: paralyzed

Mate two mutants: cells
fuse to form dikaryon



All four flagella
start to beat

This process can occur
because each of the
mutant flagella is
missing a different
component, which can
be supplied by the
other cell

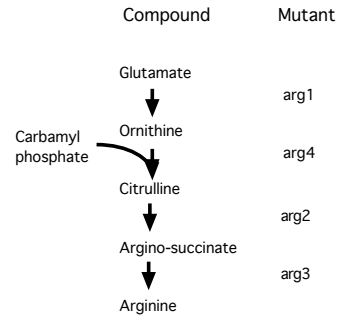
The cistron

- In general, if two genes can complement each other it means they are coding for different proteins. This functional definition of a gene gave rise to the term 'cistron'
- For the most part gene and cistron refer to the same thing, but the use of cistron implies that it has been identified based on functionality

Pathway mapping

- A second use of the Beadle and Tatum approach was to use mutants to map biochemical pathways.
- Just as the various classes of mutants they studied always could be assigned to a specific place in a given pathway, the same could be done with any sequential process

Arginine biosynthesis



Initially the pathway shown was not known. All that was available were the four different mutants, each of which prevented the biosynthesis of arginine. The first step is to find out if the four mutations are in the same cistron, or different cistrons.

First: test all of the mutants in pairs

	arg1	arg2	arg3	arg4
arg1	-	+	+	+
arg2		-	+	+
arg3			-	+
arg4				-

Since each complements all the others, we have four different cistrons

Look for growth when given intermediates

	Min	glu	orn	cit	a/suc	arg
arg1	0	0	+	+	+	+
arg2	0	0	0	0	+	+
arg3	0	0	0	0	0	+
arg4	0	0	0	+	+	+

Since none grown when only supplemented with glutamate, all must be blocked later in the pathway.

The mutant that will only grow when given arginine must be the last step in the pathway, so arg3 is defective in the last step. The next to last must be arg2 and so on.

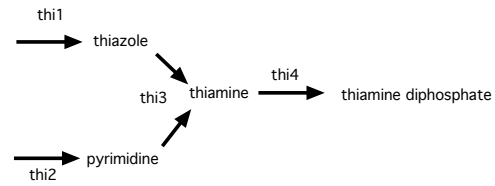
The final sequence of enzymes used must be:

Start arg1 arg4 arg2 arg3 arginine

In many cases each mutant will also accumulate the precursor: so arg2 will tend to have excess citrulline

The same process can also be used with branched pathways: i.e in thiamine biosynthesis

	Min	thiazole	pyrimidine	thiamin	thiPP
thi1	0	+	0	+	+
thi2	0	0	+	+	+
thi3	0	0	0	+	0
thi4	0	0	0	0	+



Note thi1 accumulates pyrimidine, thi2 accumulates thiazole and thi3 will accumulate both

- This approach can even be used for assembly of structures. A mutant early in the process leaves a partially completed structure. The later in the process the mutant occurs, the more complete the structure is.

