Genetic analysis - II: Pathways

Genetics 371B Lecture 26 16 Nov. 1999

Determining the order of action of genes

- One approach: provide the intermediate that the mutant can't make…

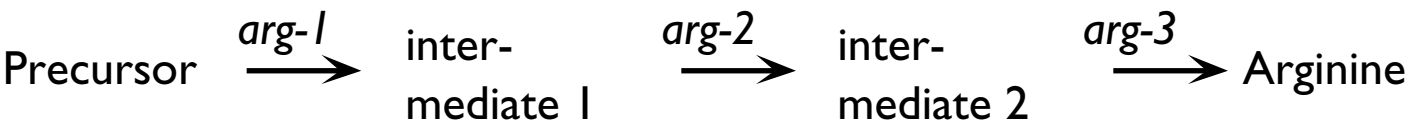
  [Analogy: restoring an assembly line]

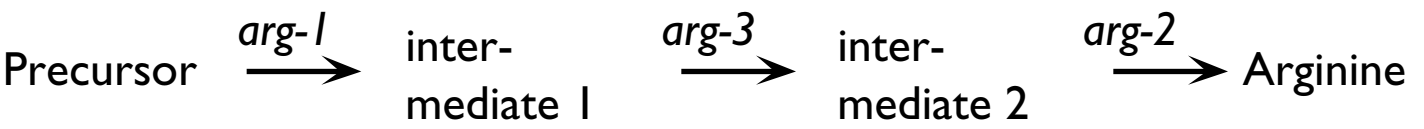
- Disadvantage: need to know the intermediates in the pathway

  Example: arginine synthesis defects in Neurospora

arg-1, *arg-2*, *arg-3*: wildtype alleles of all 3 needed for Arg synthesis

6 possible linear pathways:





etc.

Predictions:

If the first pathway is correct,

◇ arg-1 mutants –

◇ arg-2, arg-3 mutants –

Intermediates: ornithine, citrulline

Experiment:

Add one supplement at a time to the growth medium; ask: does the mutant show growth? ("+" = growth, "-" = no growth)

	Supplement:			
	None	Ornithine	Citrulline	Arginine
Wildtype	+	+	+	+
arg-1	-	+	+	+
arg-2	-	-	+	+
arg-3	-	-	-	+

Interpretation:

arg-3 is not rescued by of the intermediates—

arg-2 is helped by citrulline but not by ornithine—

arg-1 can grow on any of the intermediates—

The correct pathway:

Precursor → Ornithine → Citrulline → Arginine

A genetic way of ordering the pathway:

Epistasis analysis

Compare double mutant phenotype with single mutants

Advantage: don't need to know intermediates, just need distinct phenotypes for the various mutations

e.g., coat color in mammals

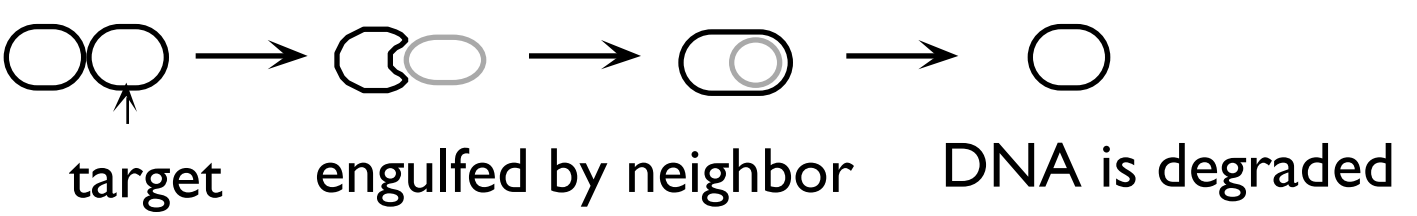
Consider two genes: **C** and **E**

ccE_ : albino (no pigment)
C_ee : no color in coat

cc ee double mutant: albino

Interpretation:

Another example: programmed cell death (apoptosis) in *C. elegans*



Mutant gene	**phenotype**
ced-3	cells live
ced-2	cells die, not engulfed
nuc-1	cells die and engulfed, DNA not degraded
Double mutants	
ced-3, ced-2	cells live
ced-2, nuc-1	cells die, but are not engulfed
ced-3, nuc-1	cells live

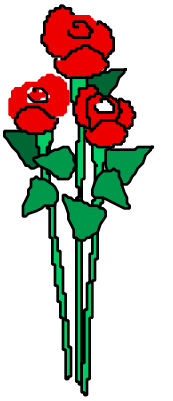
An example of a **negative interaction**:
Rb and E2F

rb^- : cells enters S phase

$E2F^-$: cell does not enter S phase

double mutant: cell does not enter S phase

An exercise:



Mutational analysis of flower color was undertaken in a plant species that normally makes red flowers. The mutations fell in three complementation groups: **A**, **B**, and **D**. The phenotypes of single and double null mutants are listed:

Mutant	**Phenotype**
a$^-$	purple flowers
b$^-$	red flowers
d$^-$	white flowers (no color)
a$^-$ b$^-$	red flowers
a$^-$ d$^-$	white flowers
b$^-$ d$^-$	white flowers

Deduce the pathway of flower color production.

Extra challenge: How might the **b$^-$** mutant have been detected?

To be discussed on Monday, Nov. 22