Complementation tests
“Complementation group” equals “Gene”

If two mutations failed to complement,
   they are alleles of the same gene
   they are allelic to each other
   they belong to the same complementation group

If two mutations complements each other,
   they are alleles of different genes
   they are not allelic to each other
   they belong to different complementation groups
Lecture 5: Genetic interactions and epistasis

A. Epistasis in a biochemical pathway
B. Epistasis in a regulatory pathway
C. Additive interactions
D. Synergistic interactions
E. Suppressions

Read 14.7 (p632-634); p434-435; 428-429
Fig. 14.36; 10.32; 10. 27; 10.28
epistasis analyses (genetic interactions among different mutations)

A. Flavonoid biosynthetic pathway in maize

\[ \text{COO}^- \quad \text{CH}_2-\text{CO}-\text{SCoA} \]

\[ \text{C2} \]

\[ \text{CHI} \]

\[ \text{F3H} \]

\[ \text{A1} \]

\[ \text{A2} \]

\[ \text{BZ1} \]

\[ \text{BZ2} \]

\[ \text{Mt1, Mt2} \]

\[ \text{Peonidin-3-(p-coumaroyl)-rutinoside-5-gluciside} \]

\[ \text{bronze} \]

\[ \text{red} \]
WT: Red
Mutations in c2, a1, a2: Colorless
Mutations in bz1, bz2: bronze

Double mutants

C2/a1: colourless—but uninformative
bz1/a1: colorless—a1 comes before bz1
bz2/a1: colorless—a1 comes before bz2

For biosynthetic pathways, the phenotype of the earlier gene in the pathway shows in the double mutant. ie. the earlier-step mutant is epistatic to the late-step mutant

Determine relationship between a1 and c2 by feeding experiment:
add flavanone (naringenin): c2+naringenin = red
   a1+naringenin = colorless
Fig. 7.20  Biochemical Pathways

(a) Isolation of arginine auxotrophs
1. Wild-type
   X rays
   Mutagenized conidia
   Fruiting bodies
   Ascii
   Ascospores dissected and transferred; one to each culture tube
2. Tubes of complete medium inoculated with single ascospores.
   Germination, production of conidia
3. Conidia from each culture tested on minimal medium.
   No growth = nutritional mutant
4. Conidia from cultures that fail to grow on minimal medium are tested on minimal medium supplemented with individual amino acids.
   Addition of arginine restores growth, reveals arginine auxotroph.

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

<table>
<thead>
<tr>
<th>Mutant strain</th>
<th>Supplementation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nothing</td>
</tr>
<tr>
<td>Wildtype: Arg⁺</td>
<td>+</td>
</tr>
<tr>
<td>Arg-E⁻</td>
<td>-</td>
</tr>
<tr>
<td>Arg-F⁻</td>
<td>-</td>
</tr>
<tr>
<td>Arg-G⁻</td>
<td>-</td>
</tr>
<tr>
<td>Arg-H⁻</td>
<td>-</td>
</tr>
</tbody>
</table>

(c) Inferred biochemical pathway

Gene:
- ARG-E
- ARG-F
- ARG-G
- ARG-H

Enzymes:
- Acetylornithinase
- Ornithine transcarbamylase
- Argininosuccinate synthetase
- Argininosuccinate lyase

Reactions:
- N-acetylornithine → Ornithine → Citrulline → Argininosuccinate → Arginine

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**B. Regulatory pathways**

Signal → A → B → C → D → gene expression

→ Positive action-stimulate next step.
   Null mutation makes insensitive to signal

—→ Negative action-represses next step.
   Null mutation makes the gene turned on at all time (constitutively)

| b⁻: gene expression never turned on even in the presence of the signal |
| d⁻: gene expression constitutively on even in the absence of signal |

b⁻d⁻ = d⁻: constitutively on

For regulatory pathways, the phenotype of the later-acting genes shows in the double mutant.

ie. the later-acting mutant is epistatic to the earlier-acting mutant
For regulatory pathways, the phenotype of the later-acting genes shows in the double mutant. i.e., the later-acting mutant is *epistatic* to the earlier-acting mutant.
C. Additive pathways

Double mutants of dissimilar phenotypes produce a combination of both phenotypes.

Indicate that the two mutations are in genes acting in separate pathways.

\[ ap2-2 \text{ (flower abnormal)} \times gl \text{ (no trichome)} \]

\[ \text{ap2-2} \text{ gl double mutant} \]

abnormal flower and no trichome
D. Synergistic interactions (enhancement)

Two genes may act at the same step of a pathway
Or in parallel or (redundant) pathways
E. Suppression

Intragenic suppressors

Extragenic suppressors

Allele-specific suppression

Suppressors are defined classically as mutations that correct the phenotypic defects of another mutation without restoring its wild-type sequence. Suppressors may be intragenic (affecting the same gene) or they may be extragenic (affecting a different gene).
Intragenic suppressors

WT

E. coli tryptophan synthase

mut1

Fig. 14.36

WT

Tyr
Gly

mut1

Tyr
Glu

mut1 mut2

Cys
Glu
Intragenic suppressor

Frameshift mutation caused by a single base insertion can be suppressed by a second mutation that cause a single base deletion downstream from the first mutation. See Fig. 10.27-10.28 and p 428-429
Extragenic suppressors

Mutation in one gene could correct the effect of a mutation in another gene

Nonsense (information) suppressor

Mutations in genes whose protein products interact
Nonsense (information) suppressors

In c. elegans, eight suppressors encode identical tRNAs in which a single C→T substitution changes the anticodon of a tRNATrp gene from 5′–CCA–3′ to 5′–CUA–3′. The anticodon change thus allows mutant tRNAs to read the amber codon UAG.
Extragenic suppressors

Particularly useful during genetic analyses, because they often identify additional components of a biological system or process.