Lecture 2-3: Using Mutants to study Biological processes

Objectives:

1. Why use mutants?

2. How are mutants isolated?

3. What important genetic analyses must be done immediately after a genetic screen for mutants?

4. What information can be learned from double mutant analysis?
Reading

• References:


Why use mutants?

A. Mutant phenotypes provide information about the biological role of the gene product in an organismal or cellular context (in vivo) by providing information concerning what goes wrong when the product does not function properly.

B. A cloned gene can provide biochemical information about its product (enzyme, transcription factor, structural protein).

Researchers need both phenotypic and biochemical information about their gene to understand its role.

The identification of a gene by mutant phenotype = forward genetics.

Using a cloned gene to find a mutant phenotype = reverse genetics.
Scope of the problem

- All genes in Arabidopsis have been identified through genome sequencing so cloned genes are readily accessible.

But of 27,000 genes:

<table>
<thead>
<tr>
<th># genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Known biochemical/ cellular/biological function</td>
</tr>
<tr>
<td>Predicted biochemical function from annotation</td>
</tr>
<tr>
<td>Unknown</td>
</tr>
</tbody>
</table>
Goal: Identify genes with a role in a specific biological process by finding mutants defective in that process.

Mutants provide

-- information concerning the role of the gene in vivo.
-- method by which to clone the gene that is mutated.
Mutagenesis

1. Mutants are generated by exposing a population of organisms to a mutagen and allowing the individuals in the population to reproduce.

   mutagens = irradiation (UV, Xray, fast neutron, etc.), chemicals (ethyl methane sulfonate, nitrosoguanidine etc.), insertional elements (transposons, TDNA)

2. The mutagen induces multiple mutations in the genome of the cells exposed (M1 generation; mutations are heterozygous in diploids).
   --Those mutations in germline cells are passed on to the next generation (M2 generation).
   --In plant species that self fertilize (eg. Arabidopsis) the M2 population will include some plants homozygous for mutations.

Therefore: The M2 generation is typically screened for mutant phenotypes
Mutagenesis in Arabidopsis

Mutagenized cells are heterozygous in diploids

Westhoff  Fig. 3.1
Mutagenesis in Arabidopsis

Westhoff  Fig. 3.1
Genetic Nomenclature (Arabidopsis, yeast)

A gene is typically named after the mutant phenotype or the biological function with which it was identified.

Mutant phenotype = George

Gene name (abbreviation) = GEORGE (uppercase, italics) = GEO

mutant alleles = geo-1 (lowercase italics)
geo-2
geo-3

dominance/recessiveness is not indicated

protein = GEO (uppercase, no italics)
Basic Genetic Analysis of Mutants
(what you should know genetically about any mutant you find)

You screened a large mutagenized (M2) population and found three plants with curled leaves, sepals and petals.

Hypothesis: Each plant is homozygous for a recessive allele of a single nuclear gene that is needed for the leafy organs of the plant to develop normally.

Phenotype = Curly Leaf (Crl) Gene = CRL alleles = crl-1, crl-2, crl-3

How do you test your hypothesis?
What are the competing hypotheses?
Basic Genetic Analysis of Mutants

1. Is the mutant phenotype heritable?

Allow the plant to self fertilize. Does the phenotype show up in the next generation?

Yes → heritable
No → phenotype is probably not due to mutation.
Basic Genetic Analysis of Mutants

2. Is the mutant phenotype due to a recessive, codominant or dominant mutant allele?

Cross the mutant plant to wild type. If the F1 progeny phenotype is:
   i) wild type then the mutant allele is recessive (most common)
   ii) mutant then the mutant allele is dominant
   iii) between wild type and mutant then the mutant allele is co-dominant
Basic Genetic Analysis of Mutants

3. Is the phenotype of a each mutant due to mutation of one or more than one nuclear genes?

Self fertilize the F1 plants and determine the number and type of mutant phenotypes among the F2 progeny.

\( \frac{3}{4} \) wild type, \( \frac{1}{4} \) curly leaves, petals, sepals (mutant allele recessive) or \( \frac{3}{4} \) curly leaves, petals, sepals, \( \frac{1}{4} \) wild type (mutant allele dominant)

= single nuclear mutation

9/16 wild type, 3/16 curly leaves (normal petals and sepals), 3/16 curly sepals and petals (normal leaves), 1/16 curly leaves, petals, sepals

= two nuclear genes, one required for normal leaf development and another required for normal floral organ development.
Basic Genetic Analysis of Mutants

(Co)Segregation analysis:

If two aspects of a phenotype segregate together in an F2 population then the mutation(s) causing the phenotypes are closely linked and may be caused by a single mutation.

If two aspects of a phenotype can be observed separately in an F2 population then they are not caused by the same mutation and are due to mutations in at least two different genes.
Basic Genetic Analysis of Mutants

Three Crl mutants were found. Do they represent mutations in three different genes or three alleles of the same gene?

If the mutants are recessive to wild type and the phenotype segregate as a single nuclear gene then the question can be answered by a:

Complementation test
## Complementation test

<table>
<thead>
<tr>
<th>Mutant 1</th>
<th>Mutant 2</th>
<th>Mutant 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crl</td>
<td>Crl</td>
<td>Crl</td>
</tr>
</tbody>
</table>

All mutants are recessive to wild type. All segregate as single nuclear genes.

How many genes have been identified? Possibilities: 1, 2, 3

<table>
<thead>
<tr>
<th>Test</th>
<th>Deduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutant1 x Mutant 2</td>
<td>Mutant1 x Mutant 2</td>
</tr>
<tr>
<td>Crl x Crl</td>
<td>crl1-1/ crl1-1</td>
</tr>
<tr>
<td></td>
<td>crl1-2/ crl1-2</td>
</tr>
</tbody>
</table>

### Result

<table>
<thead>
<tr>
<th>F1</th>
<th>F1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crl</td>
<td>crl1-1/ crl1-2</td>
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### Conclusion:

Mutants 1 and 2 fail to complement and must be homozygous for mutations in the same gene.
## Complementation test

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</tr>
<tr>
<td></td>
<td>CRL 1/CRL 1</td>
</tr>
<tr>
<td></td>
<td>CRL 2/CRL 2</td>
</tr>
<tr>
<td></td>
<td>crl 2-1/crl 2-1</td>
</tr>
</tbody>
</table>

### Result

- **F1**
  - Normal leaves, sepals, petals

### Conclusion:

- Mutants 1 and 3 are in different complementation groups. Mutants are homozygous for mutant alleles of different genes.

- To have a wild type phenotype the F1 must be heterozygous for mutant alleles of two different genes.

### Conclusion:

- The three Crl mutants identify two different genes required for normal leafy organs. CRL1 and CRL2
Double Mutant Analysis

Purpose:

To examine the functional relationship between two genes required for the same biological process by examining the phenotype of an organism that is homozygous for mutations in both genes.
Double Mutant Analysis

Process:

1. Construct double mutants by crossing two mutants homozygous for different mutations (if not possible because of lethality or sterility heterozygotes can be used).

2. Identify the double mutant class in the F2 population by phenotype if possible. The identity of double mutants (genotype) can be verified by crossing to homozygous parents (test cross). Otherwise one may have to rely on the frequency in the F2 as proof of identity.

3. Generally double mutant phenotypes fall into three classes based on a comparison with parental phenotypes: additive, epistatic, synergistic.
# Double Mutant Analysis

<table>
<thead>
<tr>
<th>Genes</th>
<th>GEO and HAU</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Class</strong></td>
<td><strong>genotype</strong></td>
</tr>
<tr>
<td>Parental</td>
<td>geo/geo, HAU/HAU</td>
</tr>
<tr>
<td>Parental</td>
<td>GEO/GEO, hau/hau</td>
</tr>
<tr>
<td>Double mutant</td>
<td>geo/geo, hau/hau</td>
</tr>
</tbody>
</table>
Double Mutant Analysis

**Additive phenotype:** Geo Hau = Geo + Hau

Implies that GEO and HAU function independently and on different processes.


Conclusion: GEO and HAU function independently on different aspects of root development (GEO is required for normal root growth and HAU is required for root hair development.

\[ \text{GEO} \rightarrow \text{straight roots} \text{ = positively regulate, promote,} \]

\[ \text{HAU} \rightarrow \text{root hairs} \text{ = normal root} \]
b) **Epistatic phenotype**: Geo Hau = either Geo or Hau

i) If Geo = Hau = Geo Hau then epistasis implies that both genes function in the same pathway and that each gene is essential to the process.


\[
\begin{align*}
\text{GEO} &\rightarrow \text{HAU} &\rightarrow& \text{Normal root growth} \\
\text{or} \\
\text{HAU} &\rightarrow \text{GEO} &\rightarrow& \text{Normal root growth} \\
\text{or} \\
\text{HAU} &+ \text{GEO} &\rightarrow& \text{Normal root growth}
\end{align*}
\]
b) **Epistatic phenotype**: Geo Hau = either Geo or Hau

   ii) If Geo is opposite in phenotype to Hau. There are two possibilities:

A. If Geo Hau = Hau, the result implies that Geo and Hau are in the same pathway and Geo is a negative regulator of Hau.

   Eg. Geo = long roots, Hau = short roots Geo Hau = short roots. **HAU** is required to promote root growth and **GEO** is a negative regulator of **HAU**.

   = negatively regulate, inhibit, suppress

   GEO  HAU  More root growth
Double Mutant Analysis

B. If Geo Hau = Hau, the result implies that Geo and Hau are in the same pathway and Hau is a negative regulator of Geo.

Eg. Geo = long roots, Hau = short roots Geo Hau = long roots. GEO is required to suppress root growth and HAU is a negative regulator of GEO.

HAU ---| GEO ---|-- More root growth
c) **Synergistic phenotype**: Geo Hau >>> Geo or Hau.

Implies that GEO and HAU contribute independently to the same process.

Eg. Geo = long roots (wt = 2 cm, Geo = 4 cm)  Hau = long roots (Hau = 4 cm)  Geo Hau = very long roots (20 cm). GEO and HAU negatively control root growth independently of one another and are performing partially redundant functions.