Genetic Analysis: the Terminology

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Genetic Terminology

ATG

UAA

* = mutation

Recessive

*/ = mutant phenotype;
*/+ = wild-type phenotype
*/+ = mutant phenotype

Dominant

Allelism - Complementation

Amorphic/Null or Hypomorphic--
Hypomorph: */ has weaker mutant phenotype than */null.
*/ has less activity of gene product than */null.
Null—no functional gene product

How define a Null allele?

-- */Deletion = */
Beware of stop codons!

Haploinsufficient--
*/+, mutant phenotype; lof mutation
1/2 the normal dose is not sufficient for normal development

loss-of-function (lof)--
* causes reduction in activity of gene product

Hypermorphic/ Gain of function--
gof*/+, often opposite phenotype to lof/lof*. Can be overexpression, overactivity, or unregulated activity of gene product.
Double heterozygote--
An individual that is heterozygous for two different mutations in two DIFFERENT genes
\[ \text{bmp2b}^{c305a+/+}, \text{smad5}^{c270a+/+} \]

Transheterozygote--
An individual that is heterozygous for two different mutations in the SAME gene
\[ \text{bmp2b}^{c305a/}, \text{bmp2b}^{c24} \]

Genetic Interaction--
One example: a mutant phenotype observed in double heterozygous embryos, which is not observed in either single heterozygote

<table>
<thead>
<tr>
<th>Parent 1</th>
<th>Parent 2</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{bmp2b}^{c305a/+} )</td>
<td>( \text{smad5}^{y40a/+} )</td>
<td>(weaker than either +/- phenotype)</td>
</tr>
<tr>
<td>WT</td>
<td>WT</td>
<td></td>
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What does it mean? What does it mean if the double heterozygotes do not show a phenotype?

Double mutant of \( \text{bmp2b}; \) \( \text{smad5} \): Linear pathway v parallel pathway

What about “genetic interactions” with morpholino knockdown phenotypes? No and Yes—can more effectively manipulate doses, BUT must do in controlled manner, e.g. double injections

<table>
<thead>
<tr>
<th>Temperature-sensitive---</th>
<th>less activity at higher temp., more gene activity at lower temp.</th>
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<tbody>
<tr>
<td>Why useful?</td>
<td></td>
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Maternally expressed gene-- a gene expressed in the oocyte and persisting in the egg, which may function in embryogenesis

Maternal effect mutation--- Mutant mother is cause of embryonic phenotype; genotype of father/progeny irrelevant.

<table>
<thead>
<tr>
<th>Female</th>
<th>Male</th>
</tr>
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<tbody>
<tr>
<td>*/+</td>
<td>*/+</td>
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Maternal-zygotic (MZ) mutation---
Loss of maternal AND zygotic gene product cause of embryonic phenotype; a maternally and zygotically expressed gene functioning in embryonic development

Homozygotes show phenotype. Reciprocal cross—no phenotype
A Few more genetic terms

**Antimorph (genetic) / Dominant Negative (misexpression)**
--- the mutant gene product is antagonistic to wild type activity; often dominant, but can be recessive.

* = antimorphic mutation

*/+ versus null /+ ?? Strength of phenotypes

*/+ Stronger mutant phenotype than null /+

*/+ Less activity of gene product than null /+

**Penetration** --- for a given genotype, the proportion of individuals that exhibit a phenotype. Incomplete v. Complete

**Expressivity** --- the range of phenotypes observed for a given genotype

--- Primary v Secondary Defect
--- Primary defects are those caused directly by the mutant gene.
--- Secondary defects are those caused subsequent to the primary defect and are caused by the primary defect. Examples: 1° -- no heart beat  2° -- 2 dpf small head, eyes

Why important?

--- Specific v Non-specific phenotypes
--- Specific: loss of dopaminergic neurons at 5 dpf with no other defects
--- Non-specific: loss of dopaminergic neurons with loss of gut, liver, jaw differentiation; if it were examined, all late organ development arrests. Type of gene mutant: e.g. DNA polymerase subunit etc.

Why such a late phenotype? DNA polymerase protein and transcript are maternally loaded in egg and very stable. Beware! Don't take myopic view of phenotypes.
Genetic Epistasis

How do the 3 recessive limbless genes, \textit{nls (no limbs)}, \textit{ton (trunk only)}, \textit{lls (lost limbs)} relate to each other and \textit{*/+ (leggy)}? Which acts upstream and downstream of the other? How test?

Epistasis Test--Make double mutants

---in a double mutant animal, mutation of one gene (the epistatic one) masks the mutant phenotype of a second gene

\begin{itemize}
  \item \textit{nls/nls; */+}
  \item \textit{ton/ton; */+}
\end{itemize}

---mutants must have opposite phenotypes to each other to evaluate genetic epistasis

\textit{Null alleles IMPORTANT for lof alleles}

If \textit{nls/nls; */+} has no limbs, then \textit{leggy} phenotype \textit{depends} on \textit{nls}; \textit{nls} is downstream of \textit{leggy}, \textit{leggy} \textit{--------> nls}

If \textit{ton/ton; */+} has ectopic limbs, then \textit{leggy} phenotype does \textit{NOT depend} on \textit{ton} and thus \textit{ton} is either \textit{upstream} of \textit{leggy}, \textit{ton} \textit{--------> leggy} \textit{OR} in a \textit{parallel} pathway to \textit{leggy}.

These epistasis results are \textit{consistent} with the pathway: \textit{ton} \textit{--------> leggy} \textit{--------> nls}

The epistatic gene is \textit{DOWNSTREAM}. 

\textit{*/+ = ectopic limbs, we’ll call it \textit{leggy} (constitutive activity)}
Genetic Epistasis (another example)

The epistatic gene is DOWNSTREAM

Recessive mutant gene extra limbs, xlb, with ectopic limbs (similar to leggy). Where does it act relative to other genes, nls, ton, lls, and leggy?

Epistasis Test--Make double mutants
Which ones?
- nls / nls ; xlb / xlb  ?limbless or ectopic limbs?
- ton / ton ; xlb / xlb  Genes acting antagonistically to each other
- lls / lls ; xlb / xlb

Gene Order?
If limbless phenotype, then nls phenotype does NOT depend on wild type gene product of xlb, so xlb is NOT downstream of nls. The epistasis result is consistent with xlb -------- l nls
xlb could also function in parallel to nls.

If ectopic limbs, then xlb phenotype does NOT depend on nls, thus nls is NOT downstream of xlb. Epistasis consistent with nls ------ xlb

Molecular Epistasis

Does the expression of llb (limbless) depend on ton or als?
Can decipher gene order for genes with same phenotypes.
Can we infer Biochemical Interactions from this Gene Order?  

NO

It provides a molecular FRAMEWORK,
Generally a pathway of genes acting in a process

Genetics does NOT replace Biochemistry
AND
Biochemistry does NOT replace Genetics

Combining Genetics & Biochemistry is very powerful