Genetic Dissection of a Biological Process
the "Developmental Genetics Paradigm"

To dissect a biological process one needs to:

a) Identify all of the components (gene products) involved in the process ("parts list")
b) Understand the normal or wild-type function of each component (from phenotype)
c) Understand how the components act together to achieve the process (epistasis analysis).

Alteration of gene activity (mutation, RNAi, etc.) provides the entrée for this understanding.

For model organisms like bacteria and viruses, eukaryotic microbes (e.g., yeasts, Chlamydomonas) and certain metazoans (C. elegans, Drosophila, Arabidopsis, Zebrafish and Mouse),

- A genome wide search for mutants with a desired phenotype that disrupts a specific biological process can be conducted.

- The more one knows about the biological process, the better one can choose a phenotype that probes the process of interest.

- Such mutant hunts can identify genes that function in the process acting at any of a number of levels.

Such a Mutational Genetic approach makes no assumptions about the time, place, or mode of action of the gene product.

Instead, it is dependent on the phenotype of the mutation (get what you look for).

Gene mutations are used to:

1) Identify and study the molecules involved in the biological (developmental) process of interest
2) Study the biology of mutant cells, tissues, animals that have altered properties, defective or inappropriate cell types.
3) Define, in formal terms, the wild type gene function(s).
4) Define the scope and logic of the network of genetic interactions that control the process and to examine in vivo the effects of removing or altering the elements (gene products) of the network.
Two broad classes of gene mutant (or knockdown) phenotypes that affect biological processes.

1) Mutations that block or arrest a process.
   In principle, identifies genes involved in execution of a biological process, at any step.
   - Endocytosis, metabolic synthesis and catabolism, cell cycle progression (e.g. DNA synthesis), tissue (e.g. vulval) development.

2) Mutations in regulators of biological processes – that control two alternative states of a process.
   A “normal” phenotype is observed, but at an inappropriate time, place, position or condition.
   In principle, identifies all genes involved in a signal transduction pathway with a binary "ON" or "OFF" output that controls a process.
   - Most obviously observed in developmental processes involving cell fate decision, but occurs in essentially all biological processes.

Sex determination  |  Cell fate decisions  |  Control of cell cycle transitions
--- | --- | ---
♀ | vulval cell fate | G2 → M-phase

The goal of a genetic dissection is to describe a biological process in formal terms – as a pathway or network of interactions between genes (gene products).

- Such a pathway makes specific predictions about how a biological process works – that can be tested – genetically, molecularly, and developmentally.

Conversely
- Molecular, biochemical, and cell biological experiments are necessary to transform the formal model into concrete mechanisms.

Endocytic pathway, worked out largely from yeast mutants
Pathway for control of G2 to M cell cycle transition worked out largely in the yeasts.

Alternate state phenotypes, G2 arrest or progression through M phase.

Drosophila Sex Determination

Inappropriate male or female fate phenotypes

Hedgehog Signaling

Genes 1st identified in Drosophila in screens for embryonic pattern formation

MH
Gli
CI
Ortholog identified in
Smo
other animals
Su(Fu)
Smo(Pu)

Alternate cell fate phenotypes
Two general approaches are used to identify genes that function in a biological process

1) Forward genetic screens
2) Reverse genetic screens
Examples of some biological processes investigated in *C. elegans* using a forward genetic approach

- Specification of cell fate.
- Guidance of cell and axon migration.
- Programmed neuronal cell death (apoptosis).
- Role of cell lineage and partitioning of maternal information during early development.
- Muscle assembly and function.
- Timing of developmental events (heterochronic mutants).
- Control of nervous system wiring.
- Control of Dauer Larvae formation (sensing food and population density).
- Chemo and adaxial attraction and repulsion.

Examples of Phenotype that is related to biological process of interest

- Guidance of cell and axon migration
  - Screen for mutations that result in misplacement of cell or axon.
- Neuronal cell death
  - Screen for survival of neuronal cell that should undergo apoptosis.

Phenotype is assessed by microscopy

*C. elegans* is transparent, allowing assessment of cell identity with visible light (Nomarski microscopy) and fluorescent microscopy
- GFP or other fluorescent proteins that report on cell identity, function, gene/gene product expression, protein localization & function, small molecule reporter (Ca++), dyes that report activity or concentration (e.g. fat level).

**Mutant Screens in *C. elegans***

1) Screen for desired phenotype in the F2 generation
   - Usually identify recessive loss of function mutations.
2) Screen for desired phenotype in the F1 generation
   - Usually identify dominant gain of function mutations
3) Screen for desired phenotype in the F3 generation
   - Can identify maternal effect, usually loss of function mutations.

*If the gene mutation results in sterility or lethality, usually have to conduct a clonal screen (single hermaphrodite per plate), where the mutation can be recovered/propagated from heterozygous sibs.*
C. elegans as a model for determination of sexual fate

**Graphical Abstract**

- **XX hermaphrodite**
- **XO male**

> 30% of cells sexually specialized, all tissues sexually dimorphic

**Self-fertile hermaphrodites**

1. Transparent - visualized using Nomarski microscopy
2. GFP labeled larval neurons

**Lineage tree for the C. elegans hermaphrodite (somatic cells)**

Sulston, Horvitz & Kimble
Blast cells generate larval lineages and cell types

Sex specific cell lineage generates the sexually dimorphic structures.

C. elegans Sex Determination as a model

Phenotypes one can propose to screen for:

Transformation of sexual fate with normal differentiation

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Sex</th>
<th>Sex fate</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>XX</td>
<td>♀</td>
<td>♀</td>
<td>Wild type</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>♂</td>
<td>Wild type</td>
</tr>
<tr>
<td>XX</td>
<td>Partial ♂ or ♂/intersexual</td>
<td>Fem/Fog</td>
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<tr>
<td>XX</td>
<td>♂</td>
<td>♂</td>
<td>Male</td>
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<tr>
<td>XX</td>
<td>♀</td>
<td>♀</td>
<td>Wild type</td>
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<tr>
<td>XX</td>
<td>♀</td>
<td>♂</td>
<td>Her</td>
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<tr>
<td>XX</td>
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<td></td>
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</table>
Screen for recessive Sex Determination mutations in XX animals

1) Screen with dissecting microscope (25-50X mag)
2) Screen using Nomarski DIC microscope (>500X mag)

Find XX male/intersex, female, male and others.
Mutant animals are not self-fertile, although male and female animals are cross-fertile.

Screen for mutations affecting XO animals

a) Use a him mutation to generate XO animals
b) Use dpy-21 to distinguish between XX (Dpy) and XO (non-Dpy) animals.

Find XO hermaphrodites (fertile) and females.

Screen for masculinized XX animals using X-linked markers

- dpy-7 and unc-18 are X-linked morphological markers that are less than 1 cM (map unit) apart.
- Non-Dpy non-Unc males are XX
- Mutation in a him gene will give Dpy males (dpy-7/0) and Unc males (unc-18/0)
Genes identified from the screens.

- **tra-1** XX Masculinized (♂ or incomplete ♀)
- **tra-2** XX
- **tra-3**

- **fem-1** XX Feminized (♀)
- **fem-2** XX
- **fem-3** (XO)

- **her-1** XO Hermaphrodite (♀)

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Analysis of new mutations following forward genetic screen (genotype)

1. Recover/propagate the new mutation in subsequent generations.
   a) Does it segregate as a single gene?
   b) Is it dominant or recessive?
3. Assign the mutation to a locus by genetic mapping and complementation testing.
4. Standard worm EMS mutagenesis results in ~2500 de novo variants, with ~500 coding changes, the vast majority are unrelated to the gene of interest.
   - need to remove unrelated mutations induced during the mutagenesis by autocrossing with wild type, replacing with wild-type chromosomes.
5. Molecular identification of the mutant locus by whole genome sequencing
   - usually employing crosses that simultaneously map the phenotype of interest

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Analysis of new mutations following forward genetic screen (phenotype)

6. Phenotypic characterization.
   Which cells have a transformed sexual fate?
   a) penetrance: % of individuals of a given genotype that display a phenotype.
   b) expressivity: the degree to which the phenotype is displayed among individuals of a given genotype.
7. Determine if the mutation is pleiotropic.
   Does the mutant display other phenotypes not obviously related to sex determination?
   - Pleiotropy suggests that the gene is involved in other processes.
   - Pleiotropy can make it difficult to isolate and characterize null mutations, as the phenotype of interest can be masked by the other phenotypes.
Analysis of new mutations following forward genetic screen (phenotype)

**8)** The final morphological phenotype/ the phenotype used in the screen is usually far removed from the initial defect caused by the mutation.

- Determine earliest time when phenotype deviates from wild-type. The more markers for the biological process of interest, the greater the depth of understanding.

Isolation of multiple alleles of a gene of interest

- More fully deduce the biological functions of the gene.
- Useful for further genetic manipulations (epistasis, isolation of suppressors or enhancers).
- Provides a more rapid route of gene ID from whole genome sequencing.
- Generates important reagents for structure/ function analysis.

Two broad classes of mutations.

Extending the nomenclature first described by Hermann Muller:


"loss of function" (lf) alleles - reduce (hypomorph) or eliminate (null, amorph) activity of gene or gene product. Usually recessive.

"gain of function" (gf) alleles: increase activity (hypermorph), poisonous activity (antimorph) or novel activity (neomorph) of a gene or gene product. Usually dominant.
Characteristics of loss of function (lf) alleles

- if alleles act in the same phenotypic direction as a deletion of the locus, classically defined from genetic deficiencies, Df, which are multi-locus deletions.
- if alleles act in the same phenotypic direction as RNAi of the gene.
- if alleles are isolated at high frequency (~5x10^-4 for an "average" gene under EMS mutagenesis conditions in C. elegans).
- Intragenic revertants (true or pseudo, from phage genetics) of a if allele are isolated at low frequency.
- Usually recessive.

Loss-of-function (lf) alleles define what processes fail to occur or occur incorrectly in the partial or complete absence of the gene product.

- Wild-type gene function is therefore necessary (directly or indirectly) to promote the normal process.

In a formal sense - wild-type gene function is defined as what is necessary for the converse of the loss of function mutant phenotype or for what processes are missing in the mutant.

The greater the extent that one can describe if phenotype, and bring to bear other information about the gene, the more informative the explanation of wild type gene function.
**unc-104(lf) phenotype**

<table>
<thead>
<tr>
<th>Inferred wild-type function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal movement and viability</td>
</tr>
<tr>
<td>Probable neurological defect</td>
</tr>
<tr>
<td>Defect in neurotransmitter synthesis</td>
</tr>
<tr>
<td>Axon terminals lack synaptic vesicles, all remain in cell body</td>
</tr>
<tr>
<td>Molecular ID of unc-104</td>
</tr>
<tr>
<td>Defining member of a class of Kinesin Heavy Chain</td>
</tr>
</tbody>
</table>

**The terminal phenotype of the mutant cell/animal is often far removed from the initial defect that results from the gene mutation.**

To determine what is the 1st defect:
- Search for the first phenotypic deviation from wild-type
- Use to determine if gene affects biological process of interest
- Use in the definition of wild-type gene function

**Example of 3 classes of mutants that have the same terminal phenotype but arise by different mechanisms.**

**What genes function in Embryonic Morphogenesis (elongation) in C. elegans?**

Block in Morphogenesis gives a 2-fold arrested embryo/worm as the terminal phenotype.
- **Primary screen**: 2-fold arrested embryos
  - Among 15 genes, find 4e-22 tropomyosin, pat-2 alpha-integrin, iso-2 Type IV collagen
- **Secondary screen**: Determine 1st deviation from wild-type by
  a) time-lapse video Nomarski Microscopy
  b) antibody staining
For let-2(lf), elongation occurs normally. 
.: Type IV basement membrane collagen is not required for embryonic morphogenesis, but is required for maintenance of organ integrity, once formed.

For lev-11(lf) and pat-2(lf), elongation is arrested. 
.: tropomyosin and α-integrin are required for embryonic morphogenesis. 

Basement membrane failure causes collapse of epidermis and muscle cell detachment.

lev-11 tropomyosin vs. pat-2 α-Integrin phenotypes

Deductions/ conclusions
part-2(0) α-integrin mutant embryos fail to assemble thick and thin filaments. 
.: pat-2 α-integrin is necessary for myofilament assembly.
lev-11(lf) tropomyosin mutant embryos have normal thick and thin filament organization, but fail to contract. 
.: Tropomyosin is not necessary for assembly of the myofilament lattice but is necessary for contraction.

Muscle structure assessed with antibody staining.

Hypomorphic allele: partial reduction in function or activity - usually less severe than a null mutation. Often with variable penetrance & expressivity.

- Can reveal effects of partial activity - such as tissue specific functions.
- Can define domains of the product necessary for specific functions.
- Important for analysis if the null phenotype of the gene is pleiotropic (e.g. lethal)
- Temperature sensitive conditional alleles (ts - heat sensitive; cs - cold sensitive)
  a) Determine time during process (cell cycle, development etc) when product acts.
  b) Ideal for use in isolation of extragenic suppressors and enhancers.

Genetic definition of a hypomorphic allele (B)
Mutant phenotype of a hypomorphic allele is enhanced in trans to a null allele, for example a gene deletion or multi-locus deletion or deficiency (abbreviated as Df). 

<table>
<thead>
<tr>
<th>B</th>
<th>Df</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less</td>
<td>More</td>
</tr>
<tr>
<td>mutant</td>
<td>mutant</td>
</tr>
</tbody>
</table>
Null (or amorphic) alleles: the complete absence of gene function.

- Used to deduce the range of wild-type gene function.
- Used in epistasis analysis.
- Used in functional (molecular, phenotypic) studies of cells/organisms that completely lack the gene product.

fem-1(null) (aka fem-1(0))

XX are ♀ (germline transformation)
XO are ♀ (germline and somatic transformation)

In the absence of the fem-1 product, female development occurs in both germline and soma of XX and XO.

∴ fem-1(+) is necessary for ♂ development in both the germline and soma of XX and XO.

fem-1 (hypomorph)  

XX are ♀ (germline transformation)
XO are normal ♂

Full range of processes that require fem-1(+) are not revealed in the hypomorph.

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Genetic definition of Null (amorph) alleles

- Often has complete penetrance and expressivity.
- Should not be enhanced by RNAi of the same locus (unlike hypomorphic alleles).
- Should behave in genetic tests like a deletion of the gene or a multi-locus deletion (Df).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phenotype</th>
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<tbody>
<tr>
<td>♀</td>
<td>♀</td>
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<td>♀</td>
<td>♀</td>
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A - putative null allele
B - hypomorphic allele

Genotype $A < B = Df$

Phenotype Less mutant
More mutant

Additional expectations
$A^+$ = Df

Types of molecular null mutations

- Activity null: In a decrease in catalytic activity
- Molecular assays for determining 'nullness' depend on sensitivity of the assay.
- Proteolytic: Proteolytic enzyme
- Nonsense: Nonsense mediated RNA decay
- Drugs resistant
- DNA null: DNA null

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<table>
<thead>
<tr>
<th>Function</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>Folding/translation</td>
</tr>
<tr>
<td>Pre-edit</td>
<td>splicing/nuclear export</td>
</tr>
<tr>
<td>DNA</td>
<td>transcription</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Localize</th>
<th>Trafficking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypomorphic alleles: the complete lack of any of these molecular steps.</td>
<td></td>
</tr>
</tbody>
</table>
An allelic loss of function series can be generated for some genes.

<table>
<thead>
<tr>
<th>Allele</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>m1</td>
<td>Normal</td>
</tr>
<tr>
<td>m2</td>
<td>Reduced</td>
</tr>
<tr>
<td>m3</td>
<td>Further reduced</td>
</tr>
<tr>
<td>m4</td>
<td>Markedly reduced</td>
</tr>
<tr>
<td>m5</td>
<td>Null</td>
</tr>
</tbody>
</table>

100% WT

Increasing expression

Decreasing gene activity (possible null)

Ranking of a series of alleles that differ “quantitatively” in the amount of residual gene activity.

Dominant loss of function mutations: Haploinsufficiency

Two doses of gene product expression are required.

- Gene where normal function is very sensitive to the amount of product - reducing by a factor of 2 is not sufficient for normal function.
- Most genes do not show obvious haploinsufficiencies (is clearly selected against in nature).

- Titration experiments in yeast suggest that at least some gene products are in vast excess (>10 fold).

Haploinsufficiency is suppressed by addition of a wild type gene copy

<table>
<thead>
<tr>
<th>Condition</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Notch(lf)</td>
<td>Notched Wing</td>
</tr>
<tr>
<td>Notch(lf)</td>
<td>Embryonic lethal</td>
</tr>
<tr>
<td>ac:n(lf)</td>
<td>Disorganized muscle structure</td>
</tr>
<tr>
<td>Notch(lf)</td>
<td>Flightless</td>
</tr>
<tr>
<td>myosin(lf)</td>
<td>Flightless</td>
</tr>
<tr>
<td>ac:n(lf)</td>
<td>Wild type</td>
</tr>
</tbody>
</table>

Examples from Drosophila

- Compensation for reduced amount of gene product - suggests that haploinsufficiency is caused by an imbalance of thick and thin filament components.
Parental effects - genotype of the parent determines phenotype (penetrance/expressivity) of progeny

Cytoplasmic maternal effects - species that produce large eggs that donate mRNA, mRNAs, protein, etc. to progeny.

Maternal rescue

F1

Mutant zygotic genotype [m(-/-); z(-/-) but phenotypically wild-type as rescue by + gene product in egg cytoplasm]

F2

Mutant phenotype

Maternal absence

F1

Zygotic genotype wild-type [m(-/-); z(-/-)] (assuming recessive)

But phenotypically mutant as gene product (from mother) required prior to activation of zygotic gene expression.

Genetics of fem-3(lf)

A) fem-3(lf) fem-3(lf) ♀ x + ♂
m(-/-); z(-/-) fem-3(lf) + XX
15% ♀ 45% feminized ♀

B) fem-3(lf) unc-24 ♀
XO
unc-24 ♀
du-20 + +
♂
fem-3(lf) + unc-24 ♀
Dpy + unc-24 +
♀
5% ♀

Results from A) and B) also shows that there is a maternal absence effect for fem-3 function in XO, and to a lesser extent XX animals.

C1) fem-3(lf) unc-7 unc-7 [X] ♀
fem-3(lf) + ♀
unc-7 + +
♂
m(-/-); z(-/-) unc-7 fem-3(lf) unc-7 +
XX 100% ♀

♀. No maternal rescue of XX animals (Dpy non Unc)

C2) unc-7/+ [X] feminized XO Partial feminization XO (Unc)
♂
cunc-7 + unc-7 fem-3(lf) unc-7 +
XX dpy-20 25% ♀

♀. Partial maternal rescue of XO animals (Dpy, Unc)

D) fem-3(lf) unc-7 unc-7 [X] ♀
fem-3(lf) + ♀
unc-7 + +
♂
unc-7 + +
♀
Complete feminization XO (Unc) ♀
Characteristics of gain-of-function alleles

- Usually dominant
- gf alleles isolated at low frequency, as they disrupt gene function in specific ways (loss of regulation, poisoning, etc.)
- Intragenic EMS revertants of gf alleles are isolated at high frequency - because they are loss of function mutations in cis to the gain-of-function mutation.

<table>
<thead>
<tr>
<th>of (mutation)</th>
<th>mutagenesis</th>
<th>of (WT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dominant phenotype</td>
<td>Wild type phenotype</td>
<td></td>
</tr>
</tbody>
</table>

Characteristics of gain-of-function alleles (cont.)

- Hypermorphic gf alleles of regulatory-binary switch genes often act in the opposite phenotypic direction as loss of function (or Df) of a gene. Examples: sex determination genes, vulval cell fate genes.

- Antimorphic gf alleles usually act in the same phenotypic direction as loss of function (Df) in the gene. Example: unc-54 myosin

Uses of gain-of-function alleles

- Can provide information about gene function or regulation not available from lf alleles.
- Can identify “switch” genes.
  - Genes where if and gf have opposite phenotypes.
  - fem(II) ♀ "OFF" (no product)
  - fem(II) ♂ "ON"
  - fem(IV) ♀ "OFF" (no product)
  - fem(IV) ♂ "ON"
Gene activity of the fem locus, as defined by the if and gf mutations, acts in a regulatory or binary switch to determine ♀ or ♂ fate (both necessary and sufficient).
- Can be used in suppressor and enhancer screens.
Deducing wild type gene function from only a gain of function mutation can be misleading

- Example A
  - \( \text{unc-93}(\text{gf}) \) Vs. \( \text{unc-93}(\text{null}) \)
  - Wild type

- Example B
  - \( \text{unc-54}(\text{gf}) \) Vs. \( \text{unc-54}(\text{null}) \)
  - \( \text{lethal} \)

Example C

- \( \text{mec-4}(\text{lf}) \) + \( \text{Wild type} \)
- \( \text{mec-4}(\text{gf}) \) – Worm movement is touch insensitive (neuronal morphology normal)
- \( \text{mec-4}(\text{gf}) \) – Necrotic cell death of touch cell neurons. (worm movement is touch insensitive)

\text{mec-4} encodes a ENaC sodium channel subunit

Genetic behavior of gain of function alleles

Hypermorph – increased wild-type activity

\[
\frac{m}{m} \ x \ \frac{m}{m} \ < \ \frac{m}{m} \text{ or } \frac{m}{m}
\]

Phenotype

- WT
- More mutant

Removing (+) gene activity makes the phenotype more wild type.

Adding (+) gene activity makes the phenotype more mutant.
Antimorph – (a subset of which are called dominant negative)
Poisonous product that can interfere with wild type. Acts in the same phenotypic direction as \( \text{lf} \).

\[
\begin{array}{c|c}
\text{phenotype} & \text{WT} & \text{More mutant} \\
\hline
\text{m} & \frac{m}{m} & \frac{m}{m} \\
\hline
\end{array}
\]

Adding (+) gene activity makes the phenotype more wild-type. (+) competes with antimorphic product.

There are two types of antimorphic poisonous gene products

(1) Dominant negative
Poisoning gene product is part of a homo-multimer (dimer, tertamer, etc.)

\[
\begin{array}{c}
\begin{matrix}
\text{A} & \text{A} & \text{A} & \text{A} & \text{A} & \text{A}^* \\
\end{matrix}
\end{array}
\]
Heterozygote

Multimer is not fully functional as mutant * subunit(s) poisons the function of the wild type subunit(s).
Phenotype is usually partial loss of function, with penetrance/expressivity depending on dose. Can not be stronger than null, by definition.

(2) Poisoning another gene product
(usually acting in the same process)

\[
\begin{array}{c}
\begin{matrix}
\text{A} & \text{B} \\
\end{matrix}
\end{array}
\]

If the antimorphic phenotype is more severe than the null phenotype of the locus, then the antimorphic product must be poisoning another gene product.

This is often observed as \( \frac{\text{null}}{\text{null}} \) or \( \frac{\text{m}}{\text{m}} < \frac{\text{m}}{\text{m}} \)

Less mutant More mutant unc-54(gf) is an example
Neomorph – novel activity, increased non-wild-type or inappropriate activity

\[
\frac{m}{Df} \quad \frac{m}{m} \quad \frac{m}{s} \quad \frac{s}{s}
\]

Uncertain what changing dose of (+) will do. Depends on mode of gf mutant activity.

\text{tra-1(gf)} Mutations:

- \text{tra-1(lf)} XX ♀
  \quad \therefore \text{tra-1(lf)} \ is \ necessary \ for \ ♀ \ development.

\begin{align*}
\text{Strong tra-1(gf') for both XX and XO:} \\
\frac{♀}{Df} \equiv \frac{tra-1(gf')}{♀} & = \frac{tra-1(gf')}{♀} \\
\text{No change with increasing dose: constitutive. Unclear if hypermorph or neomorph.}
\end{align*}

- \text{tra-1(gf'') for XX:} \\
\frac{♀}{Df} \equiv \frac{tra-1(gf'')}{♀} & = \frac{tra-1(gf'')}{♀} \\
\frac{♀}{♀} \equiv \frac{tra-1(gf'')}{♀} & = \frac{♀}{♀} \\
\text{More mutant: Increasing feminization with increase in dose: \therefore \ Hypermorphic allele.}

\text{tra-1(mx) is less able than tra-1(lf) in promoting ♀ development in XX animals - this is like tra-1(lf)}

\text{♂, Hypermorphic - partial lf}
gf mutations can reveal regulatory domains

\[ \text{fem-3(}f\text{)} \]

<table>
<thead>
<tr>
<th>Soma</th>
<th>Germine</th>
</tr>
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<tbody>
<tr>
<td>XX</td>
<td>Femurized</td>
</tr>
<tr>
<td>XO</td>
<td>Femurized Femurized</td>
</tr>
</tbody>
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\[ \text{fem-3(}f\text{)} \]

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<thead>
<tr>
<th>Soma</th>
<th>Germine</th>
</tr>
</thead>
<tbody>
<tr>
<td>XX</td>
<td>WT Masculinized</td>
</tr>
</tbody>
</table>

- Tissue specific gain-of-function: excessive specification of male germine fate.
- \(\text{fem-3(}gf\text{)}\) point mutations are in the 3' untranslated region of the RNA.
- Do not affect \(\text{fem-3}\) RNA levels or protein sequence.
- B region defined by mutations is a negative regulatory site for transcriptional repression.
- \(\text{gf}\) mutations block binding/activation of repressor.

Sufficient:
Hypermorphic gain of function mutations or overexpression of the wild type gene product can provide evidence for sufficiency.
For example, overexpression of TRA-1 in XO animals converts them into females.

However, no phenotype, a loss of function or a novel phenotype from overexpression can not be interpreted.

Necessary:
Loss of function mutations provide evidence that a gene is necessary for a biological process.

Screens vs. Selections

**Screen:** Inspection of progeny from a mating scheme for a phenotype.
- Whole animal level - microscopy
- Single cell level - microscopy, sorting
- Molecular/biochemical assays
  - Labor intensive, time consuming
  - \(10^4\) - \(10^6\) mutagenized haploid genomes

**Selection:** Only viable and fertile animals or cells are obtained.
- Drug resistance
- Suppression (extragenic) of lethality
  - Less labor intensive than a screen
  - \(10^6 - 10^8\) mutagenized haploid genomes can isolate rare alleles of a locus.
Isolation of additional if alleles of a locus by non-complementation screen of F1 animals

A new (fem-1) allele should fail to complement an existing allele (fem-1[ts])

\[ \text{unc-5} \times \text{fem-1[ts]} \] [permissive temp]

\[ \text{F1} \]

\[ \begin{align*}
\text{unc-5} & \quad \text{fem-1[ts]} \\
+ & \quad + \\
\text{Non-Unc} & \quad \text{Non-Unc}
\end{align*} \]

\text{X WT} \phi

Recover new fem-1 allele in 2nd generation as Unc

Complementation Tests: informative exceptions

Intragenic complementation

\[ \begin{align*}
\text{gene-a(x1)} & \quad \text{Mutant phenotype} \\
\text{gene-a(x2)} & \quad \text{Mutant phenotype} \\
\text{gene-a(x1)} & \quad \text{Wild-type (or more wild-type than either allele alone)}
\end{align*} \]

Trans-heterozygote: \[ \text{gene-a(x1)} \quad \text{gene-a(x2)} \]

Suggests that gene-a has two separate functions/domains, one disrupted by mutation x1, the other by mutation x2.

Intergenic non-complementation

\[ \begin{align*}
\text{gene-a(x10)} & \quad \text{Wild-type} \\
\text{gene-a(x10)} & \quad \text{Wild-type} \\
\text{gene-a(x1)} & \quad \text{Mutant phenotype} \\
\text{gene-a(x20)} & \quad \text{Mutant phenotype} \\
\text{gene-a(x10)} & \quad \text{gene-a(x20)}
\end{align*} \]

Trans-heterozygote: \[ \text{gene-a(x10)} \quad \text{gene-a(x20)} \]

Indicates that gene-a and gene-b participate in the same process and may physically associate (directly or indirectly in a complex).

For example

\[ \begin{align*}
A & \quad B \\
A & \quad B
\end{align*} \]

Complementation tests with dominant mutations are hard to interpret.
Dissection of a process that does not obviously affect viability (e.g. sex determination) is nonetheless likely to employ essential genes. Lethality can make it difficult to identify a gene's involvement in a process as animals/cells die prior to the time of scoring phenotype.

How to identify such genes genetically?
- Hypomorphic alleles
  - gf alleles
    - Intergenic revertants that are recessive
  - Non-complementation screen
    - F1 if \( \frac{m}{M} \) phenotype, viable & fertile
    - Can get new alleles \( m' \) that are null
      - \( \frac{m}{m} \equiv M \), where \( \frac{m'}{M} \) is Let

Alternatively, enhancer and suppressor mutant screens

---

**Genetic interaction screens**

**Enhancer screens**: identify genes that act in the same pathway or process, particularly when starting with a hypomorphic mutation, or genes acting in parallel when starting with a null allele.

**Suppressor screens**: can identify negative regulators of the pathway or process under investigation, particularly when starting with a hypomorphic mutation as loss of a negative regulator will increase activity of the pathway.

Negative regulators of a pathway are excellent drug targets for a disease where a pathway is partially crippled by a hypomorphic mutation, as drugs typically inactivate a gene product and inactivation of a negative regulator will increase pathway activity.

---

**Genetic interaction screens**

**Enhancement**: 
\[
\begin{align*}
\sigma^B & \quad \sigma^b' \\
\text{Wild type phenotype} & \quad \text{Mutant phenotype} \\
\text{Partial phenotype} & \quad \text{Qualitative or quantitative increase in phenotype}
\end{align*}
\]

**Suppression**: 
\[
\begin{align*}
\sigma^B & \quad \sigma^b' \\
\text{Mutant phenotype} & \quad \text{Wild-type or near wild-type phenotype}
\end{align*}
\]

What one obtains depends on:
1) Nature of starting allele \( \sigma \) (null, hypomorph, gf etc.)
2) New mutant as a recessive (F2) or dominant (F1)
3) Nature of mutagen
### Classes of suppressors

a) Gene specific; Allele nonspecific

#### Bypass suppression

<table>
<thead>
<tr>
<th>Wild-type</th>
<th>Mutant</th>
<th>&quot;Wild-type&quot;</th>
</tr>
</thead>
</table>

#### Epistatic suppression

<table>
<thead>
<tr>
<th>Wild-type</th>
<th>Mutant</th>
<th>&quot;Wild-type&quot;</th>
</tr>
</thead>
</table>

### Classes of suppressors (cont.)

a) Gene specific; Allele specific (non Null)

#### Suppression by restoration of physical interaction

Allele specific suppression suggests, but does not prove that A and B gene products physically interact.

Yeast two-hybrid system an alternative in vivo method for detecting physical interactions between proteins.

### Classes of suppressors (cont.)

a) Gene nonspecific; Allele specific

#### Suppression of Nonsense mutations (which can be in many genes)

- tRNA amber (UAG stop) suppressors
- Loss of nonsense mediated mRNA Decay (NMD) pathway

#### Suppression of unstable proteins

- Reduction of function of proteolysis machinery