Genetic Dissection of a Biological Process
the “Developmental Genetics Paradigm”

To dissect a biological processes one needs to:

a) Identify 'all' of the components (gene products) involved in the process.
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 metazoa (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 the “desired” phenotype.
• 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 made 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 biological process, at any step.
   - Endocytosis, metabolic synthesis and catabolism, cell cycle progression (e.g. DNA synthesis), 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.

<table>
<thead>
<tr>
<th>Sex determination</th>
<th>Cell fate decisions</th>
<th>Control of Cell cycle transitions</th>
</tr>
</thead>
<tbody>
<tr>
<td>♂ vs. ♀</td>
<td></td>
<td>$G_2 \rightarrow M$</td>
</tr>
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</table>
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**

Figure 13-36 Details of the endocytic pathway from the plasma membrane to lysosomes. Maturation of early endosomes to late endosomes occurs through the formation of multivesicular bodies, which contain large amounts of invaginated membrane and internal vesicles (hence their name). Multivesicular bodies move inward along microtubules, continually shedding transport vesicles that recycle components to the plasma membrane. They gradually convert into late endosomes, either by fusing with each other or by fusing with preexisting late endosomes. The late endosomes no longer send vesicles to the plasma membrane.
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

Alternate male or female fate phenotypes
Hedgehog Signaling

Gene 1st identified in Drosophila in Screens for embryonic pattern formation

hh
ptc
ci
PKA
Smo
Cos2
Fu
Su(Fu)

Counter
parts now
identified in
other
animals

Alternate cell fate phenotypes

Forward Genetics - classical developmental genetics

Genetic Screen

Gene mutation
(disrupted process)
Phenotype
Inferred in vivo
Function

Biological Process

Epistasis Analysis

Genetic Pathway

Molecular/Cellular Mechanism of Pathway Execution

Molecular ID of Causal Gene

Genetic Mapping
Whole Genome Sequencing
- Additional Alleles
- Reverse Genetic Phenocopy
- Transgene Rescue
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 odorant 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.

• Programmed 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.
C. elegans as a model for determination of sexual fate

**XX hermaphrodite**

- intestine
- oocytes
- sperm in spermatheca
- vulva
- eggs in uterus
- oocytes
- anus

**XO male**

- intestine
- testis
- sperm
- seminal vesicle
- cloaca
- rays
- fan
- spicule
- vas deferens

>30% of cells sexually specialized
all tissues sexually dimorphic

---

Self-fertile hermaphrodites

- Pharynx
- Intestine
- Embryos
- Distal Tip Cell
- Sperm
- Oocytes

Transparent - visualized using Nomarski microscopy

GFP labeled larval neurons

Androdioecious = hermaphrodite/male
Lineage tree for the *C. elegans* hermaphrodite (somatic cells)

Sulston, Horvitz & Kimble

Blast cells generate larval lineages and cell types

Sulston, Horvitz & Kimble
C. elegans Sex Determination as a model

Phenotypes one can propose to screen for:

<table>
<thead>
<tr>
<th>Transformation of sexual fate with normal differentiation</th>
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<tbody>
<tr>
<td>Soma</td>
</tr>
<tr>
<td>XX</td>
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<td>XX</td>
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<td>XX</td>
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<td>XO</td>
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<tr>
<td>XO</td>
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<tr>
<td>XO</td>
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</tbody>
</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, Mog and others.
Mutant animals are not self-fertile, although male and female animals are cross-fertile.

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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.

\[
\begin{align*}
\text{tra-1} & \quad \text{tra-2} & \quad \text{tra-3} \\
XX & \quad \rightarrow & \quad \text{Masculinized} \ (\sigma \text{ or incomplete } \sigma') \\
\text{fem-1} & \quad \text{fem-2} & \quad \text{fem-3} \\
\text{(XO)} & \quad \rightarrow & \quad \text{Feminized} \ (\varphi) \\
\text{her-1} & \quad \text{XO} & \quad \rightarrow \quad \text{Hermaphrodite} \ ()
\end{align*}
\]
Analysis of new mutations following forward genetic screen

1) Recover the new mutation.

2) Genotypic characterization. Determine mode of inheritance.
   a) Does it segregate as a single gene?
   b) Is it dominant or recessive?

3) Assign the mutation to a locus by mapping and complementation testing.
   - also need to remove unrelated mutations induced during the mutagenesis by
     outcrossing with wild type, replacing with wild-type chromosomes.

4) Molecular identification of the mutant locus by next generation sequencing
   - usually employing crosses that simultaneously map the phenotype of interest

Next-Generation Sequencing-Based Approaches for Mutation Mapping and
204:451-474

Analysis of new mutations following forward genetic screen (cont.)

5) Phenotypic characterization.
   Which cells have a transformed sexual fate?
   Is the phenotype temperature sensitive or does it show a maternal effect?
   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.

6) 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 (cont.)

7) 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 presented by Hermann Muller
Muller, H. J. 1932. Further studies on the nature and causes of gene mutations.

“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 under EMS mutagenesis conditions in C. elegans).
- Intragenic revertants (true or pseudo) of a lf 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 lf phenotype, and bring to bear other information about the gene, the more informative the explanation of wild type gene function.
unc-104(lf) phenotype | Inferred wild-type function
--- | ---
Uncoordinated (hypomorphic) | Normal movement and viability
Embryonic lethal (null) | 
Microscopy - polarized light and Nomarski: Muscle morphology normal | Probable neurological defect
Histology - loss of neurotransmitter staining | Defect in neurotransmitter synthesis
Electron microscopy
Axon termini lack synaptic vesicles, all remain in cell body | A Kinesin motor subunit necessary for anterograde transport of vesicles to axon termini.
Molecular ID of unc-104 - Defining member of a class of Kinesin Heavy Chain | 

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 1° 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.

- Screen for 2-fold arrested embryos
  Among 15 genes, find *lev-11* tropomyosin, *pat-2* alpha-integrin, *let-2* type IV collagen then
  - Determine 1st deviation from wild-type by
    a) time-lapse video Nomarski Microscopy (800x)
    b) antibody staining

For *lev-11*(*lf*) and *pat-2*(*lf*), elongation is arrested.
\[\therefore\] tropomyosin and \(\alpha\)-integrin are required for embryonic morphogenesis

For *let-2*(*lf*), elongation occurs normally.
\[\therefore\] Type IV basement membrane collagen is not required for embryonic morphogenesis, but is required for maintenance of organ integrity, once formed.
**lev-11 tropomyosin vs. pat-2 α-Integrin phenotypes**

*pat-2(lf) α-integrin mutant embryos fail to assemble thick and thin filaments.*

**: pat-2 α-integrin is necessary for filament 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.

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

- Can reveal affects 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 deficiency *Df*

\[
\begin{array}{c|c|c}
B & B & Df \\
\hline
\text{Less} & \text{More} & \text{mutant} \\
\end{array}
\]
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.*

---

**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>B</th>
<th>B</th>
<th>B</th>
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</thead>
<tbody>
<tr>
<td>A - putative null allele</td>
<td>B</td>
<td>Df</td>
<td>A</td>
</tr>
</tbody>
</table>

Phenotype

<table>
<thead>
<tr>
<th>Less mutant</th>
<th>More mutant</th>
</tr>
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</table>

Additional expectations

\[
\frac{A}{+} = \frac{Df}{+} \\
\frac{A}{A} = \frac{A}{Df}
\]
Types of molecular null mutations

<table>
<thead>
<tr>
<th>Function</th>
<th>Activity null (e.g. missense in catalytic residue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Localization</td>
<td>Metabolic/trafficking defective</td>
</tr>
<tr>
<td>Protein</td>
<td>Protein instability</td>
</tr>
<tr>
<td>Folding modification assembly</td>
<td>Protein null</td>
</tr>
<tr>
<td>Polypeptide</td>
<td>Premature stop</td>
</tr>
<tr>
<td>Translaction</td>
<td>AUG missense</td>
</tr>
<tr>
<td>mRNA</td>
<td>RNA null</td>
</tr>
<tr>
<td>Splicing, nuclear export</td>
<td>Promoter mutant</td>
</tr>
<tr>
<td>Pre-mRNA</td>
<td>Splice site mutant</td>
</tr>
<tr>
<td>DNA</td>
<td>DNA null (deletion)</td>
</tr>
</tbody>
</table>

An allelic loss of function series can be generated for some genes.

\[
\begin{align*}
+ & \rightarrow m_1 & \rightarrow m_2 & \rightarrow m_3 & \rightarrow m_4 & \rightarrow m_5 \\
100\% & \rightarrow m_1 & \rightarrow m_2 & \rightarrow m_3 & \rightarrow m_4 & \rightarrow m_5 \\
\text{Increasing expressivity} & \rightarrow \text{Decreasing gene activity} & \rightarrow \text{(possible null)}
\end{align*}
\]

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.
- Normal function is very sensitive to the amount of gene 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)

\[
\frac{+}{+} < \frac{If}{+} \equiv \frac{Df}{+}
\]

Haploinsufficiency is suppressed by addition of a wild type gene copy

\[
\frac{+}{+} \equiv \frac{If}{+} \equiv \frac{Df}{+}
\]

Examples from Drosophila

\textbf{Notch:} \quad \frac{\text{Notch}(lf)}{+} = \text{Notched Wing} \quad \text{versus} \quad \frac{\text{Notch}(lf)}{\text{Notch}(lf)} = \text{Embryonic lethal}

\begin{align*}
\text{actin}(lf) + & \quad \text{Disorganized muscle structure} \quad \text{Flightless} \\
\text{or} \quad \text{myosin}(lf) + & \quad \text{Disorganized muscle structure} \quad \text{Flightless} \\
\frac{+}{+} & \equiv \frac{\text{actin}(lf)}{+} \equiv \frac{\text{myosin}(lf)}{+} \quad \text{Wild type}
\end{align*}

Double mutant \quad \frac{\text{actin}(lf)}{+} ; \frac{\text{myosin}(lf)}{+} \quad \sim \text{Wild type}

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, miRNA, protein, etc. to progeny.

Maternal rescue

<table>
<thead>
<tr>
<th>F1</th>
<th>F2</th>
<th>F3</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="Maternal_rescue.png" alt="Diagram" /></td>
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</tr>
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Mutant zygotic genotype [m(−/−); z(+/−)] but phenotypically wild-type as rescue by + gene product in egg cytoplasm.

Maternal absence

<table>
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<th>F3</th>
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Zygotic genotype wild-type [m(−/−); z(+/−)] (assuming recessive)

Mutant phenotype

Genetics of fem-3(lf)

A) \[ \frac{\text{fem-3(lf)}}{\text{fem-3(lf)}} \] \( \varphi \) \( x \) \( + \) \( + \) \( \sigma \)

\[ m(−/−); z(+/−) \]

\[ \frac{\text{fem-3(lf)}}{+} \]

\[ XX \text{ 15%} \varphi \]

\[ XO \text{ 40% feminized} \sigma \]

B) \[ \frac{\text{fem-3(lf) dpy-20}}{+} \] \( \xrightarrow{+} \) \( \xrightarrow{+} \) \( \varphi \)

\[ \text{fem-3(lf) dpy-20} \]

\[ \frac{\text{unc-24 dpy-20}}{\text{unc-24 dpy-20}} \]

\[ \varphi \]

\[ \text{Look at Dpy non-Unc progeny} \]

\[ \frac{m(+/+); z(+/−)}{unc-24 + dpy-20} \]

\[ XX \text{ 5%} \varphi \]

\[ XO \text{ 100% WT} \]

Df also show this. .:. a haplo-insufficiency for XX function.

XO 100% WT No haploinsufficiency for XO function

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.
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.
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.
• Have been used to isolate EMS loss-of-function alleles of the locus by intragenic reversion (now would likely use CAS9/CRISPR).
• Can be used suppressors and enhancer screens
• Can identify “switch” genes.

  Genes where lf and gf have opposite phenotypes.

  fem(lf) ♀ “OFF” (no product)
  fem(gf) ♂ abnormally “ON”

Gene activity of the fem locus, as defined by the lf and gf mutations, acts in a regulatory or binary switch to determine ♀ or ♂ fate (both necessary and sufficient).
Deducing wild type gene function from only a gain of function mutation can be misleading

- **Example A**
  \[
  \frac{\text{unc-93(gf)}}{\text{unc-93(gf)}} \text{ Unc Vs. } \frac{\text{unc-93(null)}}{\text{unc-93(null)}} \text{ Wild type}
  \]

- **Example B**
  \[
  \frac{\text{unc-54(gf)}}{\text{unc-54(gf)}} + \text{ Unc} \quad \frac{\text{unc-54(null)}}{\text{unc-54(null)}} + \text{ Wild type}
  \]
  \[
  \frac{\text{unc-54(gf)}}{\text{unc-54(gf)}} \text{ lethal Vs. } \frac{\text{unc-54(null)}}{\text{unc-54(null)}} \text{ Unc}
  \]

**Example C**

\[
\frac{\text{mec-4(lf)}}{\text{mec-4(lf)}} \text{ Wild type}
\]
\[
\frac{\text{mec-4(lf)}}{\text{mec-4(lf)}} + \text{ Wild type}
\]

\[
\frac{\text{mec-4(gf)}}{\text{mec-4(gf)}} \text{ Worm movement is touch insensitive (neuronal morphology normal)}
\]
\[
\frac{\text{mec-4(gf)}}{\text{mec-4(gf)}} \text{ Necrotic cell death of touch cell neurons. (worm movement is touch insensitive)}
\]

*mec-4* encodes a ENaC sodium channel subunit

*mec-4(gf)* - channel is open all the time.
Genetic behavior of gain of function alleles

Hypermorph - increased wild-type activity

\[
\frac{m}{Df} < \frac{m}{+} < \left( \frac{m}{+}; \frac{m}{m} \right)
\]

<table>
<thead>
<tr>
<th>phenotype</th>
<th>WT</th>
<th>More mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Removing (+) gene activity makes the phenotype more wild type.</td>
<td>Adding (+) gene activity makes the phenotype more mutant.</td>
<td></td>
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</tbody>
</table>

Antimorph - (a subset of which are called dominant negative)

Poisonous product that can interfere with wild type. Acts in the same phenotypic direction as \( l_f \).

\[
\frac{m}{+} < \frac{m}{+} < \left( \frac{m}{Df}; \frac{m}{m} \right)
\]

<table>
<thead>
<tr>
<th>phenotype</th>
<th>WT</th>
<th>More mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adding (+) gene activity makes the phenotype more wild-type. (+) competes with antimorphic product.</td>
<td></td>
<td></td>
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There are two types of antimorphic poisonous gene products
(1) Dominant negative

Poisoning gene product is part of a homo-multimer (dimer, tertamer)

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)

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: \( \frac{null}{null} \) or \( \frac{m}{Df} \) < \( \frac{m}{m} \)

Less mutant More mutant

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

\[
\begin{align*}
\frac{m}{Df} & \quad \frac{m}{m} & \frac{m}{+} & \frac{m}{+} \\
\end{align*}
\]

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

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

\textit{tra-1}(lf) \enspace XX \sigma \quad \text{OX} \sigma \quad \therefore \textit{tra-1}(+) is necessary for \varphi development.

Strong \textit{tra-1}(gf')

\begin{align*}
\varphi & \equiv \frac{\textit{tra-1}(gf')}{Df} \equiv \frac{\textit{tra-1}(gf')}{+} \\
\end{align*}

No change with increasing dose: constitutive. Unclear if hypermorph or neomorph.

Weaker \textit{tra-1}(gf'')

\begin{align*}
\text{For XX:} & \quad \frac{\textit{tra-1}(gf'')}{Df} \equiv \frac{\textit{tra-1}(gf'')}{+} \equiv \varphi \\
\text{For XO:} & \quad \frac{\textit{tra-1}(gf'')}{Df} < \frac{\textit{tra-1}(gf'')}{+} < \frac{\textit{tra-1}(gf'')}{+} \equiv \varphi \\
\text{phenotype} & \quad \text{Increasing feminization with increase in dose} \quad \therefore \text{Hypermorphic allele}
\end{align*}
Complex *tra-1* gain of function mutations.

- mixture of *gf* and *lf* characteristics (mx for "mixomorph")

For **XO**:

\[
\frac{\text{tra-1(mx)}}{Df} < \frac{\text{tra-1(mx)}}{+} < \frac{\text{tra-1(mx)}}{\text{tra-1(mx)}}
\]

<table>
<thead>
<tr>
<th>genotype</th>
<th>phenotype</th>
<th>increased dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{tra-1(mx)}) Df</td>
<td>Increased feminization</td>
<td>&quot;Hypermorph, increased activity&quot; (More mutant)</td>
</tr>
</tbody>
</table>

For **XX**:

\[
\frac{\text{Df}}{+} \equiv \frac{\text{tra-1(mx)}}{+} ; \frac{\text{tra-1(mx)}}{Df} \equiv \text{Masculinized}
\]

*tra-1(mx)* is less able than *tra-1(+) in promoting ♀ development in **XX** animals – this is like *tra-1(lf)*.

∴ "Hypomorphic - partial If"

---

**gf mutations can reveal regulatory domains**

<table>
<thead>
<tr>
<th>fem-3(lf)</th>
<th>Soma</th>
<th>germline</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>XX</strong></td>
<td>-</td>
<td>Feminized</td>
</tr>
<tr>
<td><strong>XO</strong></td>
<td>Feminized</td>
<td>Feminized</td>
</tr>
</tbody>
</table>

∴ *fem-3(+) is necessary for the ♀ fate in the germline and soma.*

<table>
<thead>
<tr>
<th>fem-3(gf)</th>
<th>Soma</th>
<th>germline</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>XX</strong></td>
<td>WT</td>
<td>Masculinized</td>
</tr>
<tr>
<td><strong>XO</strong></td>
<td>WT</td>
<td>WT</td>
</tr>
</tbody>
</table>

Tissue specific gain-of-function- excessive specification of male germline fate.
- *fem-3(gf)* point mutations are in the 3' untranslated region of the RNA.
- Do not affect *fem-3* RNA levels or protein sequence.

• Region defined by mutations is a negative regulatory site for translational repression.
• Repression allows specification of ♀ fate.
• *gf* mutations block binding/activation of repressor.
Necessary:
Loss of function mutations provide evidence that a gene is necessary for a biological process.

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, a negative result (no phenotype, or a loss of function phenotype) from overexpression cannot be interpreted.

---

**Human genetic variation - missense changes**

- Vast majority of coding sequence variation in human genetics (from forward genetic screens) are missense mutations.
- Often unclear whether/how a missense mutation affects gene activity.
- In human genetics, missense changes where it is unclear if it contributes to phenotype are called “variants of unknown significance” (VUS).

In human genetics, correlative information provides support for a VUS having a functional consequence (pathogenic).

1) Is VUS1 found in control populations?
2) Does VUS1 recur in patients with the same phenotype?
3) Use bioinformatics prediction tools to determine if VUS is deleterious (PolyPhen, Sift, etc.)
Using model organisms to investigate human genetic variation

- How does one experimentally determine if a VUS contribute to phenotype and assess its genetic mechanism?
- If there is an orthologous in a model organism, use genome engineering (CRISP/Cas9) to generate the VUS1 mutation.

In model organisms one can ask
a) Does \( \frac{\text{VUS1}}{\text{VUS1}} \) have a phenotype?

b) Does \( \frac{\text{VUS1}}{\text{null}} \) have a phenotype?

Note that the phenotype in the model organism may be different than in humans (called phenologs).
If the null/null for the gene containing VUS1 does not have a phenotype, then unlikely that VUS1/VUS1 show a phenotype*.

~90% of genetically based disease has a dominant mode of presentation.

c) Does \( \frac{\text{VUS1}}{+} \) or \( \frac{\text{gene (0)}}{+} \) have a phenotype?

A dominant mode of action of hypermorphic and antimorphic mutations, to the extent to which it has been analyzed, shows conservation between orthologs in different species (e.g., activated Ras, antimorphic myosin).

Haploinsufficiency for orthologous genes is significantly less conserved. Different species titrate gene activity differently.
**Screens vs. Selections**

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

**Selection:** Only viable and fertile animals or cells are obtained.
- a) Drug resistance
- b) Suppression (extragenic) of lethality
  -- less labor intensive than a screen
  ➞ $10^6 \sim 10^8$ 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)*)

unc-5 is tightly linked to *fem-1*

\[\begin{array}{c}
P^0 \ x \ unc-5 \ then \ X \ fem-1(ts) \ XO \ \varnothing \ (permissive \ temp) \\
F1 \ \ x \ unc-5 \ + \ fem-1(ts) \ ; \ unc-5 \ + \ fem-1(ts) \ ; \ unc-5 \ (restrictive \ temp) \\
Non-Unc \ \varnothing \ \ x \ WT \ \varnothing \\
\end{array}\]

Recover new *fem-1* allele in 2\textsuperscript{nd} generation as Unc \varnothing \ \frac{unc-5 \ fem-1(*)}{unc-5 \ fem-1(*)}
**Complementation Tests: informative exceptions**

**Intragenic complementation**

\[
\begin{align*}
\text{gene-a(x1)} & \quad \text{Mutant phenotype} \\
\text{gene-a(x1)} & \quad \text{Mutant phenotype} \\
\text{gene-a(x2)} & \quad \text{Mutant phenotype} \\
\text{gene-a(x2)} & \quad \text{Mutant phenotype}
\end{align*}
\]

Trans-heterozygote: \( \frac{\text{gene-a(x1)}}{\text{gene-a(x2)}} \) Wild-type (or more wild-type than either allele alone)

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

**Intergenic non-complementation**

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

Trans-heterozygote: \( \frac{\text{gene-a(x10)}}{\text{gene-a(x20)}} \) Wild-type

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

For example

\[
\begin{align*}
A^- & \quad A^- & \quad A^+ & \quad A^+ \\
B^- & \quad B^+ & \quad B^- & \quad B^+ \\
\quad \text{Each mutant} & \\
\quad \text{Only functional complex}
\end{align*}
\]

Complementation tests with dominant mutations are hard to interpret.
Dissection of a process that does not obviously effect viability (e.g. sex determination) is nonetheless likely to employ essential genes.

Lethality can make it difficult to identify a genes 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 \[ \rightarrow \] that is a recessive Let
  - intragenic revertants that are recessive Let
- Non-complementation screen
  \[ \text{F1 if } \frac{m}{Df} \equiv M \] phenotype, viable & fertile
  
Can get new alleles (m') that are null

\[ \frac{m'}{m} \equiv M, \text{ where } \frac{m'}{m'} \text{ is Let} \]

Alternatively, suppressor and enhancer mutant screens

---

**Genetic interaction screens**

**Suppression:**\[ a^B + \quad \rightarrow \quad a^b^* \]

<table>
<thead>
<tr>
<th>Mutant phenotype</th>
<th>Wild-type or Near wild-type phenotype</th>
</tr>
</thead>
</table>

**Enhancement:**\[ a^B^+ \quad \rightarrow \quad a^b^* \]

| Wild type phenotype \text{ or} Partial phenotype | Mutant phenotype \text{ or Qualitative or quantitative increase in phenotype} |

What one obtains depends on:
1) Nature of starting allele \( a^- \) (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

\[ A^+B^+ \quad a^\text{-null} \text{ mutant, } B^+ \quad a^-,B^+ \]

Epistatic suppression

\[ A^+ \quad B^+ \quad a^- \quad B^+ \quad a^- \quad B^- \]

ON \quad OFF \quad "OFF" \quad ON \quad "OFF" \quad "OFF"

Wild-type \quad Mutant \quad "Wild-type"

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 protein.
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