Genetic suppressors and enhancers provide clues to
gene regulation and genetic pathways

**Suppressor mutation**: a second mutation results in a less
severe phenotype than the original mutation

Suppressor mutations can be *intragenic* or *extragenic*

**Enhancer mutation**: a mutation in another gene results in a
more severe phenotype than the original mutation

Phenotype \((m_1 + m_2)\) > Phenotype \((m_1) + \text{Phenotype } (m_2)\)
Extragenic suppressors

On Monday we discussed:

1. Interaction suppressors: allele specific, gene specific

2. Informational suppressors: allele specific, gene nonspecific

Today:

3. Bypass suppressors: allele-nonspecific, gene-specific
   a. Bypass suppressors in the same pathway
   b. Bypass suppressors in parallel pathways

Bypass suppressors can reveal a great deal about the molecules and pathway(s) that contribute to specific cell functions.
In pathways involving negative regulation, L.O.F. mutations in downstream genes can suppress mutations in upstream genes.

Gene A inhibits gene B
(could be either via the gene’s expression or the protein’s function)

\[ A \rightarrow B \rightarrow \text{B is inactive} \]

Mutation in A causes B to be aberrantly active

\[ a \rightarrow \times \rightarrow B \rightarrow \text{B is active} \]

Mutation in B reduces or eliminates its function

\[ a \rightarrow \times \rightarrow b \rightarrow \text{B is inactive} \]

Here, mutation \( b \) suppresses mutation \( a \)

We previously looked at an example of such a pathway:

\[ \text{ced-9} \rightarrow \text{ced-4} \rightarrow \text{ced-3} \rightarrow \text{apoptosis} \]

L.O.F. mutations in ced-9 (which are lethal) can be suppressed by mutations in ced-3 or ced-4.
In pathways involving positive regulation (e.g., signaling pathways), usually only G.O.F. mutations in downstream genes can suppress mutations in upstream genes.

Gene A activates gene B
(could be either via the gene’s expression or the protein’s function)

\[
\text{A} \rightarrow \text{B} \quad \text{B is active in presence of A}
\]

Mutation in A results in loss of B’s function

\[
a(\text{lf}) \xrightarrow{\times} B \quad \text{B is inactive}
\]

gain-of-function mutation in B eliminates dependence on A

\[
a(\text{lf}) \xrightarrow{\times} b(\text{gf}) \quad \text{B is active}
\]

Here, mutation \(b(\text{gf})\) suppresses mutation \(a(\text{lf})\)

Note: this doesn’t imply that \(b(\text{gf})\) looks just like wild-type - usually there is a good reason that a protein depends on a signal or interaction with another protein for its function.
Gain-of-function mutations can be used to order genes in positive regulatory pathway.

$ced-9 \Rightarrow ced-4 \Rightarrow ced-3 \Rightarrow$ apoptosis

Since loss-of-function mutations in $ced-3$ and $ced-4$ result in a loss of apoptosis, can’t order genes with these mutations.

But... you can artificially create gain-of-function $ced-3$ or $ced-4$ by overexpressing the genes in specific cells.
MEC-7 is specialized β tubulin expressed in subset of mechanosensory neurons (e.g., ALM neurons).

Tubulin is a highly expressed gene (strong promoter)

Test: construct and inject artificial genes that express either ced-3 or ced-4 from the mec-7 promoter

High levels of either CED-3 or CED-4 cause the ALM neurons to die
We can now ask whether CED-3 activates CED-4 or CED-4 activates CED-3.

CED-3 $\rightarrow$ CED-4  

or

CED-4 $\rightarrow$ CED-3
The ALMs die when *ced-3* is overexpressed from the *mec-4* promoter in a *ced-4* background.

**ced-4 mutant**

\[
\begin{align*}
\text{ced-4} & \quad \rightarrow \quad \text{ced-3} \\
\times & \\
\text{survival}
\end{align*}
\]

\[P_{\text{mec-7::ced-3}}\]

\[
\begin{align*}
\text{ced-4} & \quad \rightarrow \quad \text{ced-3} \quad \rightarrow \quad \text{apoptosis}
\end{align*}
\]

\[P_{\text{mec-7::ced-3}; \text{ced-4 mutant}}\]

\[
\begin{align*}
\times & \quad \rightarrow \quad \text{ced-3} \quad \rightarrow \quad \text{apoptosis}
\end{align*}
\]
...but the ALMs **survive** when *ced-4* is overexpressed from the *mec-4* promoter in a *ced-3* background.

**ced-3** mutant

\[ \text{ced-4} \rightarrow \text{ced-3} \quad \text{survival} \]

\[ P_{\text{mec-7::ced-4}} \]

\[ \text{ced-4} \rightarrow \text{ced-3} \rightarrow \text{apoptosis} \]

\[ P_{\text{mec-7::ced-4}; \text{ced-3} \ \text{mutant}} \]

\[ \text{ced-4} \rightarrow \text{ced-3} \quad \text{survival} \]
Model from epistasis

Cells that normally survive

CED-9
ON
CED-4
OFF
CED-3
OFF

Cells that normally die

CED-9
OFF
CED-4
ON
CED-3
ON
Changes in gene dosage can result in extragenic suppressors.

Homozygous unc-54 (myosin heavy chain) mutants are paralyzed. As we discussed on Monday, this makes it easy to find suppressors.

**sup3** was shown to be an unlinked, gene-specific, non-allele-specific suppressor of unc-54. This implies that **sup3** is not an intragenic revertant or an informational suppressor (e.g., a nonsense suppressor).

**sup3** is actually an allele of myo-3, which encodes a normally minor myosin heavy chain, but its expression is increased ~3-fold in the myo-3<sup>sup3</sup> allele.

<table>
<thead>
<tr>
<th>Generation</th>
<th>Genotype</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>P₀</td>
<td>unc-54/unc-54</td>
<td>paralyzed</td>
</tr>
<tr>
<td>F₁</td>
<td>unc-54/unc-54; sup-3/+</td>
<td>paralyzed</td>
</tr>
<tr>
<td>F₂</td>
<td>unc-54/unc-54; sup3/sup3</td>
<td>can move!</td>
</tr>
</tbody>
</table>
In budding yeast, it is common to screen systematically for “high-copy suppressors”

*Overexpression* of one gene can sometimes compensate for loss or reduction of another (related?) gene.

- **library of wild-type genes**
  - **2-micron plasmid** (50-100 copies per cell)
  - selectable marker e.g. *URA3*
- culture of mutant yeast
- plate on uracil- medium
- replica plate to conditions where original mutant can’t grow
- sequence plasmids from suppressed colonies to identify high-copy suppressor genes

1. **culture of mutant yeast**
2. **library of wild-type genes**
3. **2-micron plasmid** (50-100 copies per cell)
4. selectable marker e.g. *URA3*
5. plate on uracil- medium
6. replica plate to conditions where original mutant can’t grow
7. sequence plasmids from suppressed colonies to identify high-copy suppressor genes
The properties of the starting mutation you use for a suppressor screen will determine what kind(s) of suppressors you can expect to isolate.

If \( m \) is...
- a deletion of the gene
- a premature stop codon
- a missense mutation that destabilizes the protein

Then \( s \) can be:
- a bypass (extragenic) suppressor
- bypass suppressor, nonsense suppressor, RNA editing mutant...
- an interacting protein, a heat-shock protein, a compensatory mutation in the same gene or an interacting gene, etc.
Genetic suppressors and enhancers provide clues to gene regulation and genetic pathways

**Suppressor mutation**: a second mutation results in a less severe phenotype than the original mutation

Suppressor mutations can be *intragenic* or *extragenic*

**Enhancer mutation**: a mutation in another gene results in a more severe phenotype than the original mutation

Phenotype \((m_1 + m_2) > \text{Phenotype } (m_1) + \text{Phenotype } (m_2)\)
The properties of the starting mutation you use for a suppressor screen will determine what kind(s) of enhancers you can expect to isolate.

It may be easier to find an enhancer of a mutation that is a hypomorph (reduction-of-function) than a null (complete L.O.F.)

If two pathways contribute to outcome \( X \), then mutations in \( B \) will enhance the effect on \( X \) of mutations in \( A \) (and vice versa).

\[ A \rightarrow B \rightarrow X \]

\( a \) and \( b \) are hypomorph mutations in two genes in the same essential pathway, and together they fatally cripple the pathway.

Note: sometimes in a situation like this you will see “nonallelic noncomplementation”:

\( aABB \) is normal, \( AAbB \) is normal, but \( aAbB \) shows a mutant phenotype.

It is possible to enhance a null mutation if there is a parallel pathway that partially compensates for the function of the gene.

\[ A \rightarrow B \rightarrow X \]

If two pathways contribute to outcome \( X \), then mutations in \( B \) will enhance the effect on \( X \) of mutations in \( A \) (and vice versa).
An example of a genetic enhancer from the last lecture: *him*-8 mutations show dominant genetic enhancement of Pairing Center mutations.
One type of synthetic interaction: *synthetic lethality*

\[ aB \text{ (haploid) or } aaBB \text{ (diploid)} \quad \text{viable (maybe sick)} \]

\[ Ab \text{ (haploid) or } AAbb \text{ (diploid)} \quad \text{viable (maybe sick)} \]

\[ ab \text{ (haploid) or } aabb \text{ (diploid)} \quad \text{dead} \]

\[ \text{“X” is something essential that can be accomplished by either the pathway involving A or the pathway involving B. The two pathways are (partially) redundant.} \]

\[ a \text{ and } b \text{ are hypomorphc mutations in two genes in the same essential pathway, and together they fatally cripple the pathway.} \]
High-throughput synthetic lethality analysis in yeast

Robots pick yeast strains and replica plate them.
Enhancers, suppressors, and human disease

Nonsense suppression has been proposed as a therapy for diseases arising from premature termination codons (PTCs), which include:

* Cystic fibrosis (CFTR)
* Duchenne muscular dystrophy (dystrophin)
* Beta thalassaemia (β-globin)
* Hurler syndrome (alpha-L iduronidase)
* Ullrich disease (collagen type VI)

is developing drugs that they hope will suppress the reduced transcription of mutant genes

Most drugs, in fact, aim to act as chemical suppressors of aberrant processes that lead to disease.
Enhancers, suppressors, and human disease

In many cases, whether or not a mutation causes a disease in an individual reflects the complex genetic background of that person. We are not highly inbred (like worms or mice) - there is a huge amount of genetic variation in people, which can collectively suppress or enhance the effects of specific mutations that promote disease.

Enhancer Example I: Melanoma

Mutations in CDKN2A and other basic cell cycle control genes are associated with increased risk of melanoma.

The risk is much higher in fair-skinned people (esp. red-haired, freckly people), who often carry specific alleles of the melanocortin-1-receptor (MC1R)

In other words, CDKN2A and MC1R alleles enhance each other with respect to the phenotype of melanoma.
Enhancers, suppressors, and human disease

In many cases, whether or not a mutation causes a disease in an individual reflects the complex genetic background of that person. We are not highly inbred (like worms or mice) - there is a huge amount of genetic variation in people, which can collectively suppress or enhance the effects of specific mutations that promote disease.

Enhancer Example II: Triplet expansion diseases

Triplet expansion diseases are inherently unstable and therefore especially sensitive to suppressors and enhancers.

Heterozygous mutations in repair genes can strongly exacerbate (enhance) triplet expansion syndromes.