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

\[
\text{Phenotype (} m_1 + m_2 \text{)} > \text{Phenotype (} m_1 \text{)} + \text{Phenotype (} m_2 \text{)}
\]
Screens for suppressors and enhancers are powerful tools to identify other genes that act in the same pathway/process as a known gene of interest.

Suppressor screening in *C. elegans* is identical to a search for a revertant, or reverse mutation:

```
“forward”

| wild type | mutagen | mutant |

```

```
“reverse”

| mutant | mutagen | revertant (wild type or more wild type) |
```

Note: don’t confuse reverse mutations with reverse genetics
A “revertant” isolated in this manner may result from a new mutation in the same gene (in which case it’s called a revertant or an intragenic suppressor), or a mutation in a different gene, in which case it’s an extragenic, or second-site, suppressor.

P₀  m/m

F₁  m/m; s/+  self-fertilize

F₂  m/m; s/s

A dominant suppressor may have no phenotype on its own, or it may have a recessive phenotype.
One class of intragenic suppressors: true revertants

Simplest case of reversion: restoration of the original protein sequence
Another simple type of intragenic suppressor: partial revertants

DNA ..ATTCTTCAT.. → ..ATTTTTCAT..
RNA ..AUUCUUCUAU.. → ..AUUUUUCAU..
Protein ..Ile Leu His.. → ..Ile Phe His..

Ile (isoleucine) is chemically more similar to leucine (Leu) than phenylalanine (Phe) and may be less disruptive of the protein’s function.
Slightly more complicated intragenic suppressors: compensatory mutations

A second mutation offsets the damage created by the first mutation

Here, an interaction in the 3D structure of the protein between leucine and tyrosine was disrupted by mutation of the leucine to phenylalanine, but the structure was restored by mutation of the tyrosine to isoleucine.
Extragenic suppressors

1. Interaction suppressors: allele specific, gene specific
   These are compensatory mutations in a different gene

2. Informational suppressors: allele specific, gene nonspecific
   (we’ll also cover these today)

On Wednesday:

3. Bypass suppressors (parallel pathways): allele nonspecific, gene specific

4. Bypass suppressors (same pathway): allele nonspecific, gene specific (for example: ced-3 or ced-4 mutations suppress ced-9 mutations)

\[ \text{ced-9} \rightarrow \text{ced-4} \rightarrow \text{ced-3} \rightarrow \text{Apoptosis} \]
Interaction suppressors restore protein-protein interactions

protein B  protein A
missense mutation in gene A disrupts interaction
missense mutation in gene B restores interaction

Interaction suppressors can identify other important genes in a pathway of interest, and can tell you a lot about how proteins interact.

They are both allele-specific and gene specific
“Informational” suppressors are extragenic mutations that enable a mutated gene to function (usually only partially)

Two types that we will discuss:

nonsense suppressors
amber (UAG), ochre (UAA), opal (UGA)

smg suppressors
“Amber” mutations are nonsense mutations from any coding codon (often Tyr) to a UAG stop codon.

To understand how nonsense suppressors work, we have to talk a little bit more about translation...

Translation is the process by which the ribosome matches each codon to the “anticodon” of a tRNA molecule, which is directly attached to the encoded amino acid. This amino acid is then transferred from the tRNA to the peptide chain.
An “amber suppressor” is a mutation in a tRNA gene that enables the ribosome to put an amino acid at a UAG codon.

NOTE: amber suppressor will suppress any amber mutation, but not other nonsense mutations (opal or ochre), missense mutations, frameshift mutations or deletions. Nonsense suppressors are therefore allele specific but gene nonspecific in their suppression.
An amber mutation in tRNA$^{\text{Tyr}}$ is in the anticodon loop.

**Note:** It would obviously be bad if all UAG stop codons were replaced with Tyrosine.

Would you expect a nonsense suppressor to be dominant or recessive?

Read about this on pp. 290-91 (Ch. 8)
smg suppression
(Suppressors with morphological defects in the genitalia)

This class of suppressors was discovered in *C. elegans* and *S. cerevisiae* but they exist in all eukaryotes.

Mutations in seven different genes (*smg-1*-7) were isolated as allele-specific suppressors of *tra-2*, *lin-29*, and *unc-54*.

Like nonsense suppressors, these are informational suppressors as they are allele specific and gene nonspecific.
The *unc-54* gene encodes muscle myosin

Homozygous *unc-54* mutants are paralyzed

Some mutations in *unc-54* mapped to the 3’ UTR??

Some mutations in *unc-54* mapped to the 3’ UTR??

5’UTR

3’UTR

transcription, mRNA processing

translation

Myosin heavy chain

Head

Neck

ELC

RLC

Tail
Suppressors screens for *unc-54* suppressors identified a number of *smg* genes. Mutations in these genes stabilize the *unc-54* mRNA.

Northern blot probed with labeled *unc-54* DNA.

**Diagram:**

- 5’UTR
- **STOP**
- 3’UTR

otted type | *unc-54* (del) | *unc-54*(del); *smg*
Smg mutations can also suppress mRNA instability caused by nonsense mutations.

Northern blot probed with labeled *unc-54* DNA.

*unc-54* mRNA stabilization of the RNA allows more time for readthrough.
The smg genes encode components of a mechanism that degrades mRNAs with premature stop codons - “nonsense-mediated decay” (NMD)
Meiosis in C. elegans

- Meiosis
- Premeiotic
- Pachytene
- Diplotene
- Early meiosis
- Transition zone
- Embryos
- Sperm
- Diakinesis
- Oocytes

adult hermaphrodite
All stages of meiosis can be seen in the microscope.

Dissected gonad from an adult *C. elegans*
Lack of crossover recombination can be detected genetically or cytologically.

Wild-type

- 6 bivalents

rec-

- 12 univalents

Oocytes at diakinesis
Chromosome segregation during meiosis is accomplished through homolog pairing, synapsis, and recombination.
pairing/synapsis

recombination

segregation
spo-11 is required for crossing-over in *C. elegans*

**Wild-type**

- 6 bivalents

**spo-11**

- 12 univalents

**oocytes at diakinesis**
\[ \gamma \text{-irradiation partially bypasses the requirement for SPO-11} \]

**Graph:***

- **X-axis:** Brood 1, Brood 2, Brood 3, Brood 4
- **Y-axis:** Progeny per 100 spo-11 parents
- **Legend:**
  - Green diamond: Irradiated
  - Pink square: Control

**Observations:**
- Brood 1: Irradiated has a higher progeny count than control.
- Brood 2: Progeny count decreases for irradiated but remains low for control.
- Brood 3: Progeny count for irradiated increases compared to control.
- Brood 4: Progeny count for irradiated stabilizes but remains higher than control.
Irradiation generates crossovers in the *spo-11* mutant.

\[
\begin{align*}
\frac{\text{spo-11}}{\text{spo-11}} & \quad \text{IV} ; \\ 
\text{dpy-3 unc-3} & \quad + \\ 
+ & \quad + \\ 
X & 
\end{align*}
\]

Look for Unc non-Dpy and Dpy non-Unc recombinants among the adult progeny:

- 19/93 recombinant
- 5/29 recombinant
**spo-11** is required for crossing-over in *C. elegans*

<table>
<thead>
<tr>
<th>genotype</th>
<th>recombinant chromosomes*</th>
<th>total chromosomes</th>
<th>map distance (cM)</th>
<th>% of control map distance</th>
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<tbody>
<tr>
<td>+/- or spo-11/+</td>
<td>503</td>
<td>1332</td>
<td>37.8</td>
<td>100</td>
</tr>
<tr>
<td>spo-11/spo-11</td>
<td>0</td>
<td>240</td>
<td>&lt;0.5</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

* *dpy-3 – unc-3* interval on *X* chromosome
Homolog pairing occurs normally in *C. elegans* lacking double-strand breaks.

Dernburg et al., 1998
Each of the 6 chromosomes in *C. elegans* has a special region called a "**Pairing Center**" that plays an important role in homologous recombination.
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Villeneuve (1994) *Genetics* 136, 887-902
Each of the 6 chromosomes in *C. elegans* has a special region called a “Pairing Center” that plays an important role in homologous recombination.

...but the determinants of homologous recognition are not restricted to this region of the chromosome.
The Synaptonemal Complex (SC) is built in discrete steps.

The CENTRAL ELEMENT normally polymerizes only after homolog pairing.

AXIAL ELEMENTS form between sisters prior to and independently of homolog pairing.

C. elegans SC.
Central elements usually fail to polymerize between X chromosomes lacking Pairing Centers.

Wild type

\(\alpha\)-HTP-3 Axial Element

\(\alpha\)-SYP-1 Central Element

meDf2 (X PC Δ)
*him*-8 mutations show dominant genetic enhancement of Pairing Center mutations

% male self-progeny

WT  him-8/+  WT  him-8/+
Synapsis of the X chromosomes requires the X Pairing Center and the him-8 gene.
him-8 encodes a C2H2 zinc finger protein

C2H2 Zn fingers

Amino acid changes in mutant him-8 alleles
HIM-8 localizes to chromosome foci

transition zone → pachytene
HIM-8 localizes to the Pairing Center region of the X chromosome and associates with the nuclear envelope.
Each chromosome binds a specific HIM-8/ZIM protein to accomplish homologous pairing and synapsis.

Transition zone

Pachytene