Genetic pathway analysis

Reading: lecture notes

Extragenic suppressors

- Informational suppressors: allele specific, gene nonspecific
- Bypass suppressors (parallel pathways): allele nonspecific, gene specific
- Bypass suppressors (same pathway): allele nonspecific, gene specific
- Interaction suppressors: allele specific, gene specific

Bypass suppressors

- allele nonspecific, gene specific

Suppressors can be mutations in a:

1. Parallel pathway
2. Same pathway

Bypass suppressor in parallel pathway

Gain-of-function mutations in \( CYC1 \) are bypass suppressors of loss-of-function \( CYC7 \) mutants.

\[
\begin{align*}
CYC7 & \quad \rightarrow \quad \text{cytochrome c} \\
CYC1 & \quad \rightarrow \quad \text{cytochrome c} \\
\text{X}CYC7 & \quad \rightarrow \quad \text{cyc7 mutant} \\
CYC1 & \quad \rightarrow \quad \text{cytochrome c}
\end{align*}
\]

Insertion of the transposable element \( Ty1 \) in the \( CYC1 \) gene causes increased expression.

\[
\begin{align*}
\text{XCYC7} & \quad \rightarrow \quad \text{cyc7 mutant} \\
\text{Ty1} & \quad \rightarrow \quad \text{cyc7 mutant}
\end{align*}
\]
Bypass suppressors can be in distinct processes.

Bypass suppressors that are in the same pathway are referred to as epistatic suppressors.

Two types of regulation:
- Positive
- Negative

We can use epistatic suppressors to order genes.  e.g., *ced* apoptosis genes in *C. elegans*

<table>
<thead>
<tr>
<th>Gene 1</th>
<th>Gene 2</th>
<th>1</th>
<th>2</th>
<th>2 is inactive</th>
</tr>
</thead>
<tbody>
<tr>
<td>CED-9</td>
<td>CED-3</td>
<td>OFF</td>
<td>ON</td>
<td>CED-4</td>
</tr>
</tbody>
</table>

*ced*-9(lf) animals die because of widespread apoptosis

*ced*-3(lf) or *ced*-4(lf): all cells than normally die survive

*ced*-9; *ced*-3 or *ced*-9 *ced*-4 double mutants live, and all cells that normally die survive.

Bypass suppression in negative regulatory step.

Gene 1 inhibits gene 2
1 —— 2  2 is inactive

Mutation in 1 causes 2 to be active
\[ \times \] —— 2  2 is active

Suppressor mutation in 2 inactivates 2
\[ \times \] ——\[ \times \] 2 is inactive

Bypass suppression by mutation in a gene downstream in the same pathway (negative regulation).

Cells that normally survive

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

Cells that normally die

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

Bypass suppression in positive regulatory step.

Positive regulatory pathway; signal turns pathway ON

<table>
<thead>
<tr>
<th>Signal</th>
<th>1</th>
<th>2</th>
<th>ON</th>
</tr>
</thead>
<tbody>
<tr>
<td>CED-9</td>
<td>OFF</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mutation in 1 inactivates pathway

| Signal | \[ \times \] | OFF |
|-------|-------------|
| CED-3 | CED-4 |

Suppressor mutation in 2 turns pathway ON

<table>
<thead>
<tr>
<th>Signal</th>
<th>[ \times ]</th>
<th>2'</th>
<th>ON</th>
</tr>
</thead>
</table>

Bypass suppression by mutation in a gene downstream in the same pathway.

The suppressor must be a gain-of-function mutation.
Gain-of-function mutations can be used to order genes in positive regulatory pathway.

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 can artificially create gain-of-function *ced-3* or *ced-4* by overexpressing proteins in specific cells.

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.

Mec-7 is a β tubulin expressed in subset of mechanosensory neurons (e.g., ALM neurons). Use *mec-7* promoter to express *ced* cDNAs.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Promoter</th>
<th>Coding</th>
</tr>
</thead>
<tbody>
<tr>
<td>mec-7</td>
<td>mec-7</td>
<td>coding</td>
</tr>
<tr>
<td>mec-3</td>
<td>mec-7</td>
<td>coding</td>
</tr>
<tr>
<td>mec-4</td>
<td>mec-7</td>
<td>coding</td>
</tr>
</tbody>
</table>

High levels of either CED-3 or CED-4 causes the ALM neurons to die.

The ALMs survive when *ced-4* is overexpressed from the *mec-4* promoter in a *ced-3* background.

The ALMs survive when *ced-4* is overexpressed from the *mec-4* promoter in a *ced-3* background.

<table>
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<tr>
<th>Gene</th>
<th>Promoter</th>
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<tr>
<td>mec-7::ced-3</td>
<td>mec-7</td>
<td>ced-3 survival</td>
</tr>
<tr>
<td>mec-7::ced-4</td>
<td>mec-7</td>
<td>ced-4 (\rightarrow) ced-3 apoptosis</td>
</tr>
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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
CED-9 protein tethers CED-4 to the mitochondrial membrane!

When the apoptotic pathway is activated CED-4 protein moves to the nuclear membrane.

Check out other examples in lecture notes

Note that all of the bypass suppressors, whether in parallel or the same pathway, are predicted to be allele nonspecific.

e.g. ced-3 will suppress any loss-of-function allele of ced-9.

Note also that all of the bypass suppressors, whether in parallel or the same pathway, are predicted to be gene specific.

e.g., ced-3 will suppress ced-9 alleles, but not mutant alleles of other genes; for example, those involved in muscle function.

Genetic analysis can be used to order genes

Genetic pathway

In hermaphrodites

In males

Interaction suppressors

Allele specific, gene specific

mutagen

act1ts/act1ts

Grow at nonpermissve temperature
Isolated sac mutants

act1ts/act1ts; sac6/+ grow

The suppression of a mutant allele and its suppressor is reciprocal

The Model

Protein A Protein B

wild-type phenotype

Protein A* Protein B

mutant phenotype

Protein A* Protein B*

wild-type phenotype (suppressed)

Protein A Protein B*

mutant phenotype
The suppression of the *act1* and *sac-6* alleles is reciprocal: a real example

<table>
<thead>
<tr>
<th>Wild-type phenotype</th>
<th>Mutant phenotype (suppressed)</th>
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<tr>
<td>Act1p  Sac6p</td>
<td>Act1p  Sac6p</td>
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