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

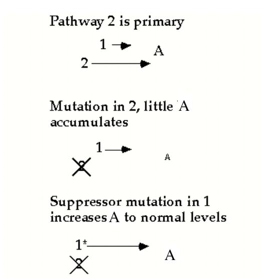
Bypass suppressors
allele nonspecific, gene specific

Suppressors can be mutations in a:

1. Parallel pathway

2. Same pathway

Bypass suppressor in parallel pathway



Bypass suppression by mutation in a gene in a parallel pathway.

Gain-of-function mutations in *CYC1* are bypass suppressors of loss-of-function *CYC7* mutants.

CYC7 → **cytochrome c**

CYC1 → cytochrome c

~~*CYC7*~~ X

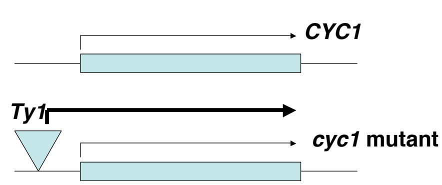
cyc7 **mutant**

CYC1 → cytochrome c

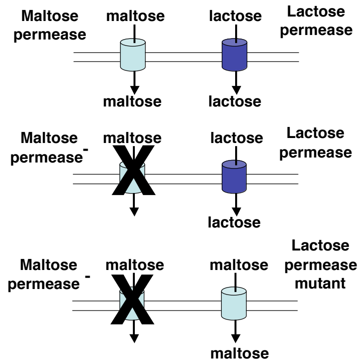
Insertion of the transposable element *Ty1* in the *CYC1* gene causes increased expression.

~~*CYC7*~~ X

CYC1 → **cytochrome c**



Bypass suppressors can be in distinct process.



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

Two types of regulation:

Positive

Negative

Bypass suppression in negative regulatory step.

Gene 1 inhibits gene 2

1 ——┤ 2   2 is inactive

Mutation in 1 causes 2 to be active

~~1~~ ——┤ 2   2 is active

Suppressor mutation in 2 inactivates 2

~~1~~ ——┤ ~~2~~   2 is inactive

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

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

CED-9 ——┤ CED-3 CED-4

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.**

Cells that normally survive

CED-9 ON ——┤ CED-3 CED-4 OFF

Cells that normally die

CED-9 OFF   CED-3 CED-4 ON

Bypass suppression in positive regulatory step.

Positive regulatory pathway; signal turns pathway ON

Signal ——→ 1 ——→ 2   ON

Mutation in 1 inactivates pathway

Signal ——→ ~~1~~   OFF

Suppressor mutation in 2 turns pathway ON

Signal ——→ ~~1~~ ——→ 2* ON

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

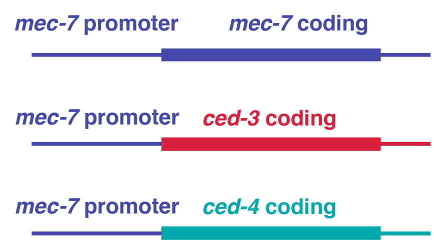
The suppressor must be a gain-of-function mutation.

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.

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



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

Can now ask whether CED-3 activates CED-4 or CED-4 activates CED-3.

CED-3 → CED-4

OR

CED-4 → CED-3

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

ced-4 mutant

~~*ced-4*~~ *ced-3* survival

mec-7::ced-3

ced-4 → *ced-3* → apoptosis

mec-7::ced-3; ced-4 mutant

~~*ced-4*~~ *ced-3* → apoptosis

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

ced-3 mutant

ced-4 ~~*ced-3*~~ survival

mec-7::ced-4

ced-4 → *ced-3* → apoptosis

mec-7::ced-4; ced-3 mutant

ced-4 → ~~*ced-3*~~ survival

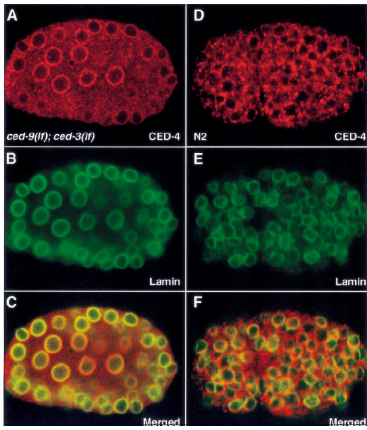
Model from epistasis

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



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

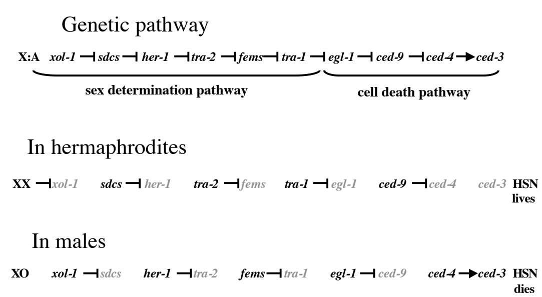
Note that all of the bypass suppressors, whether in parallel or the same pathway, are predicted to be allele nonspecific.

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



Interaction suppressors

Allele specific, gene specific

mutagen

act1^{ts}/act1^{ts}

Grow at nonpermissive temperature
Isolated ***sac*** **mutants**

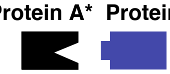
act1^{ts}/act1^{ts}; sac6/+ **grow**

The suppression of a mutant allele and its suppressor is reciprocal
The Model

Protein A Protein B

wild-type phenotype

Protein A* Protein B

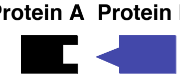


mutant phenotype

Protein A* Protein B*

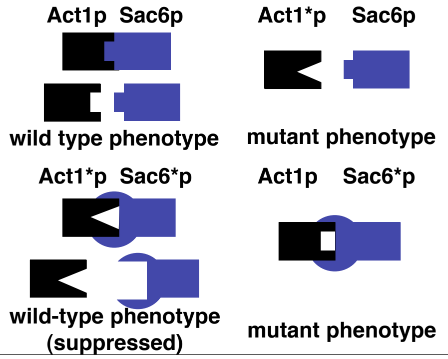
wild-type phenotype (suppressed)

Protein A Protein B*



mutant phenotype

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



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