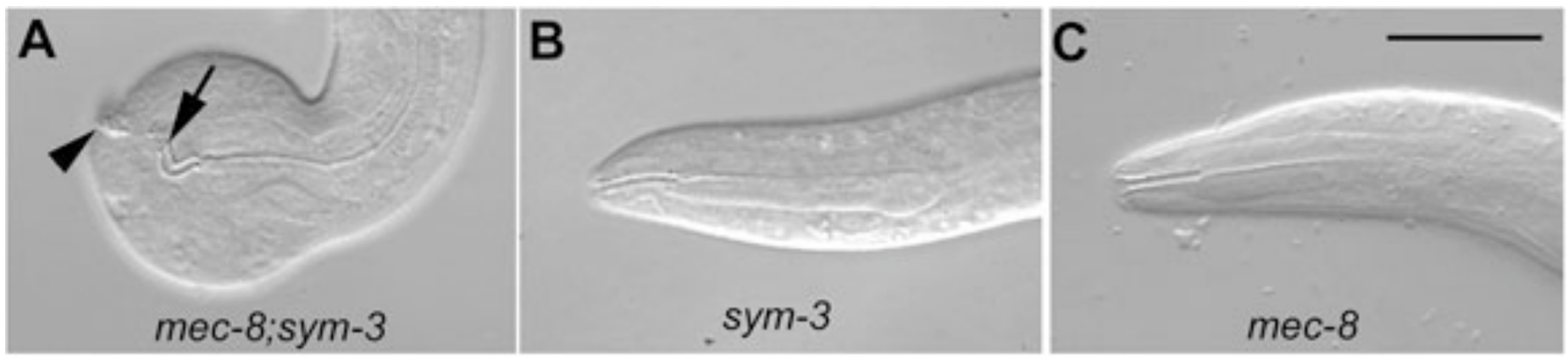
Genetic suppressors and enhancers provide clues to gene regulation and genetic pathways

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) + 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 ⊣ *B* B is inactive

Mutation in *A* causes *B* to be aberrantly active

a ✕⊣ *B* B is active

Mutation in *B* reduces or eliminates its function

a ✕⊣ *b* B is inactive

here, mutation *b* suppresses mutation *a*

we previously looked at an example of such a pathway:

ced-9 ⊣ *ced-4* → *ced-3* → 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)

A → *B* B is active in presence of A

Mutation in *A* results in loss of *B*'s function

a(lf) ✗→ *B* B is inactive

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

a(lf) ✗→ *b(gf)* B is active

here, mutation *b(gf)* suppresses mutation *a(lf)*

Note: this doesn't imply that *b(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.

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 ß 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 <u>either</u> 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 → 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

P$_{mec-7}$*::ced-3*

ced-4 → ***ced-3*** → apoptosis

P$_{mec-7}$*::ced-3*; *ced-4* mutant

~~*ced-4*~~ → ***ced-3*** → apoptosis

...but 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

$P_{mec\text{-}7}$::*ced-4*

ced-4 → *ced-3* → apoptosis

$P_{mec\text{-}7}$::*ced-4*; *ced-3* mutant

ced-4 → ~~*ced-3*~~ survival

Model from epistasis

Cells that normally survive

Cells that normally die

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.

generation	genotype		phenotype
P_0	*unc-54/unc-54*		paralyzed
		self-fertilize	
F_1	*unc-54/unc-54; sup-3/+*		paralyzed
		self-fertilize	
F_2	*unc-54/unc-54; sup3/sup3*		can move!

sup3 was shown to be an <u>unlinked, gene-specific, non-allele-specific</u> suppressor of *unc-54*

This implies that *sup3* is <u>not</u> 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*^{sup3} allele

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

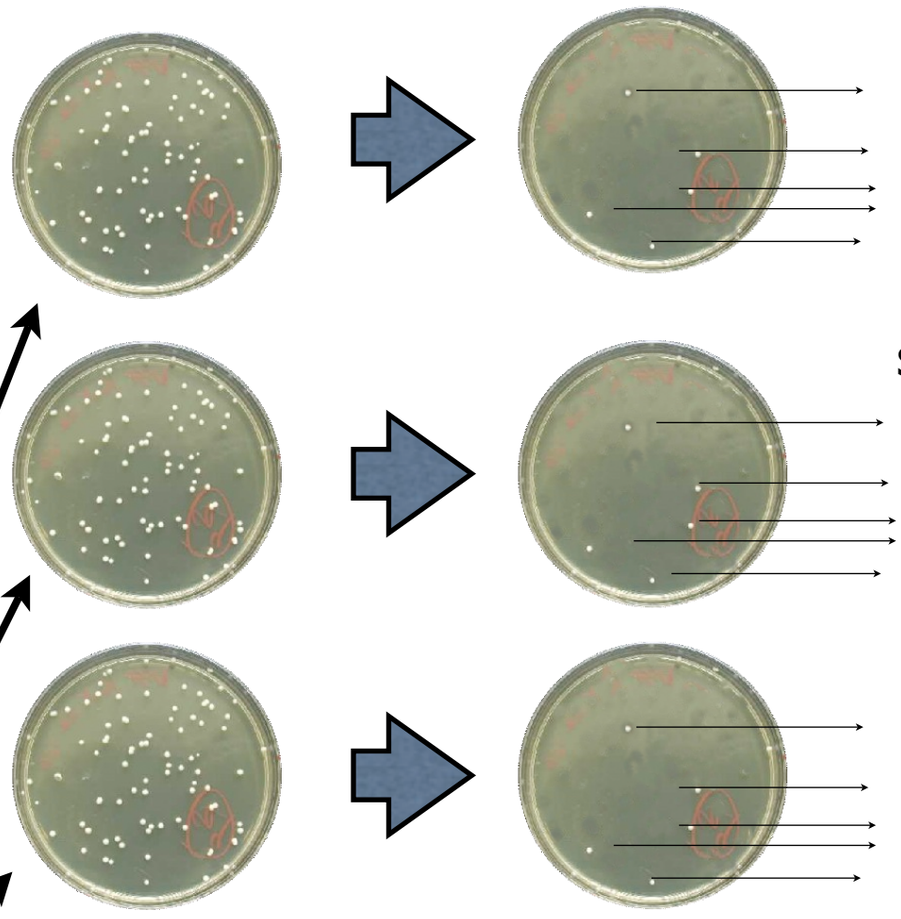
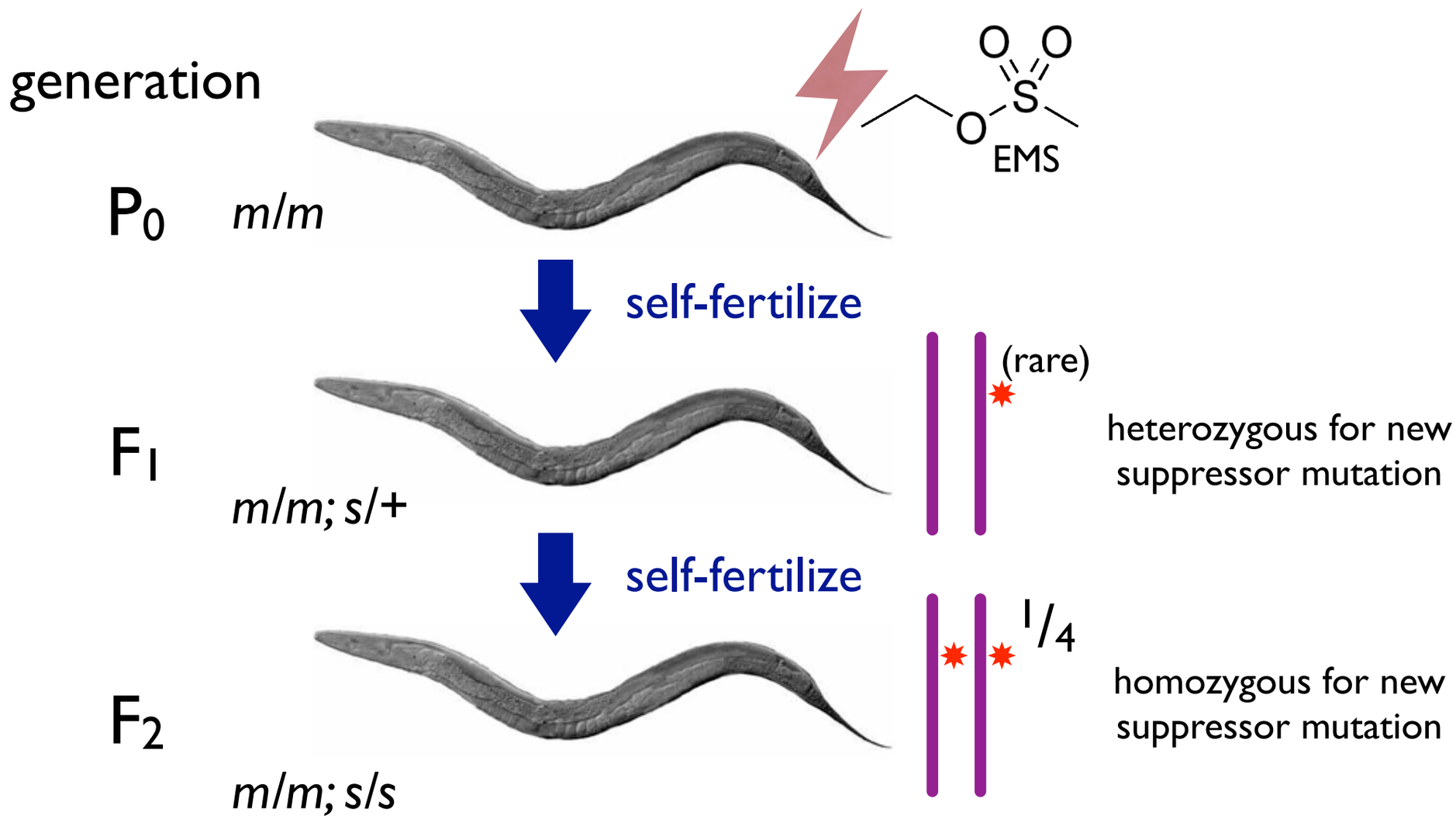


plate on uracil- medium

replica plate to conditions where original mutant can't grow

sequence plasmids from suppressed colonies to identify high-copy suppressor genes

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.

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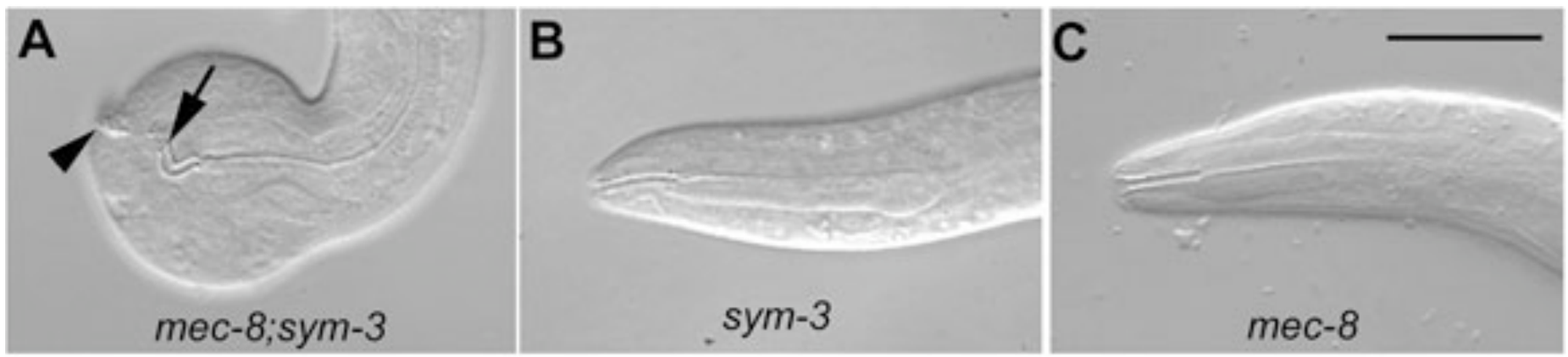
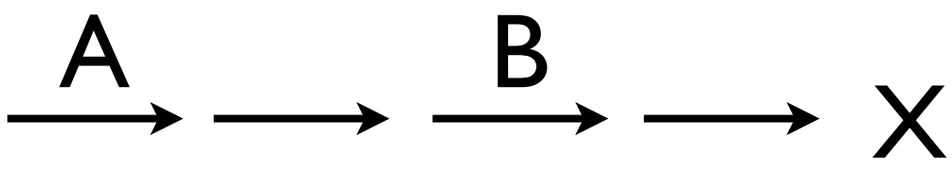


The properties of the starting mutation you use for a suppressor screen will determine what kind(s) of enhancers you can expect to isolate

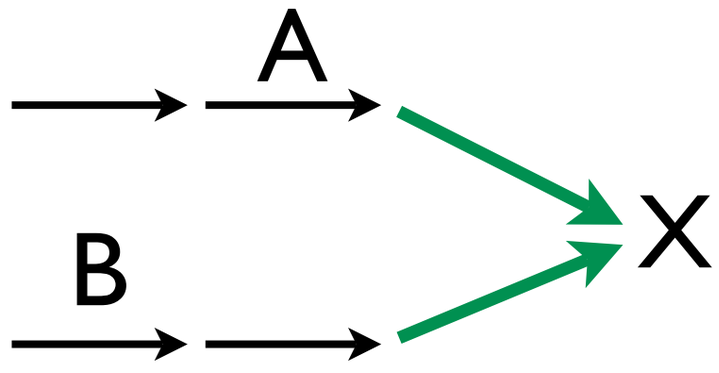
It may be easier to find an enhancer of a mutation that is a hypomorph (reduction-of-function) than a null (complete L.O.F.)



a and *b* are hypomorphic mutations in two genes in the same essential pathway, and together they fatally cripple the pathway.

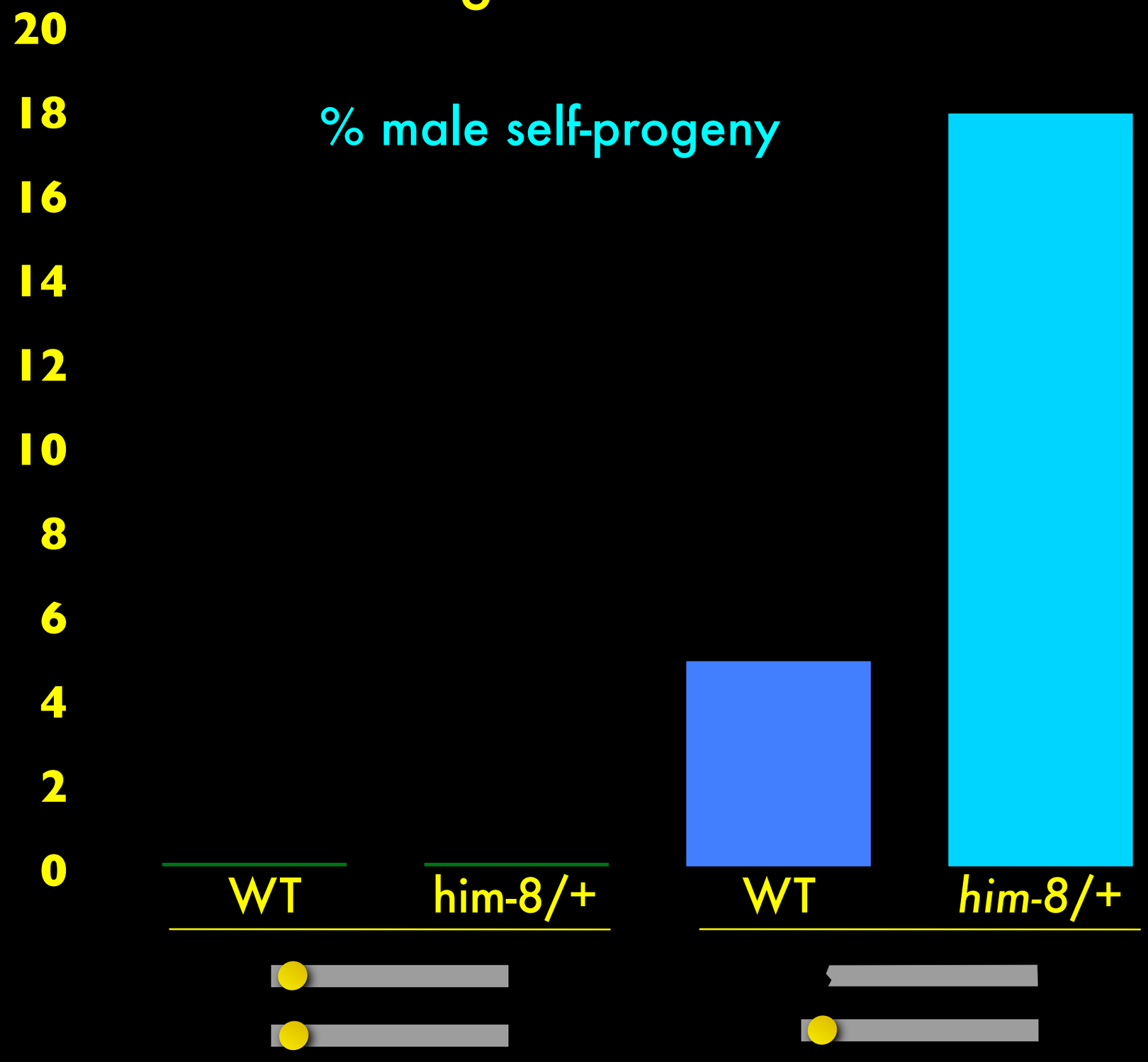
Note: sometimes in a situation like this you will see "nonallelic noncomplementation": *aABB* is normal, *AAbB* is normal, but *aAbB* shows a mutant phenotype.

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



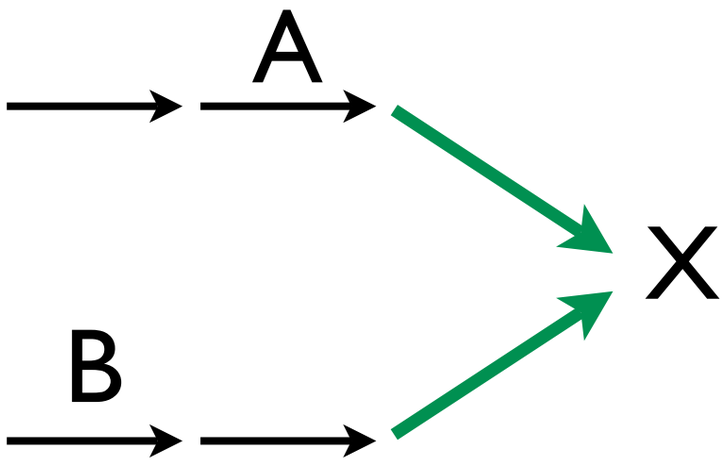
If two pathways contribute to outcome X, then mutations in B will enhance the effect on X of mutations in A (and vice versa)

An example of a genetic enhancer from the last lecture: *him-8* mutations show dominant genetic enhancement of Pairing Center mutations

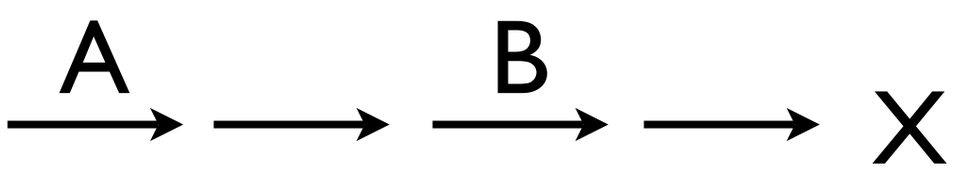


One type of synthetic interaction: *synthetic lethality*

aB (haploid) or *aaBB* (diploid)	viable (maybe sick)
Ab (haploid) or *AAbb* (diploid)	viable (maybe sick)
ab (haploid) or *aabb* (diploid)	dead



"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.



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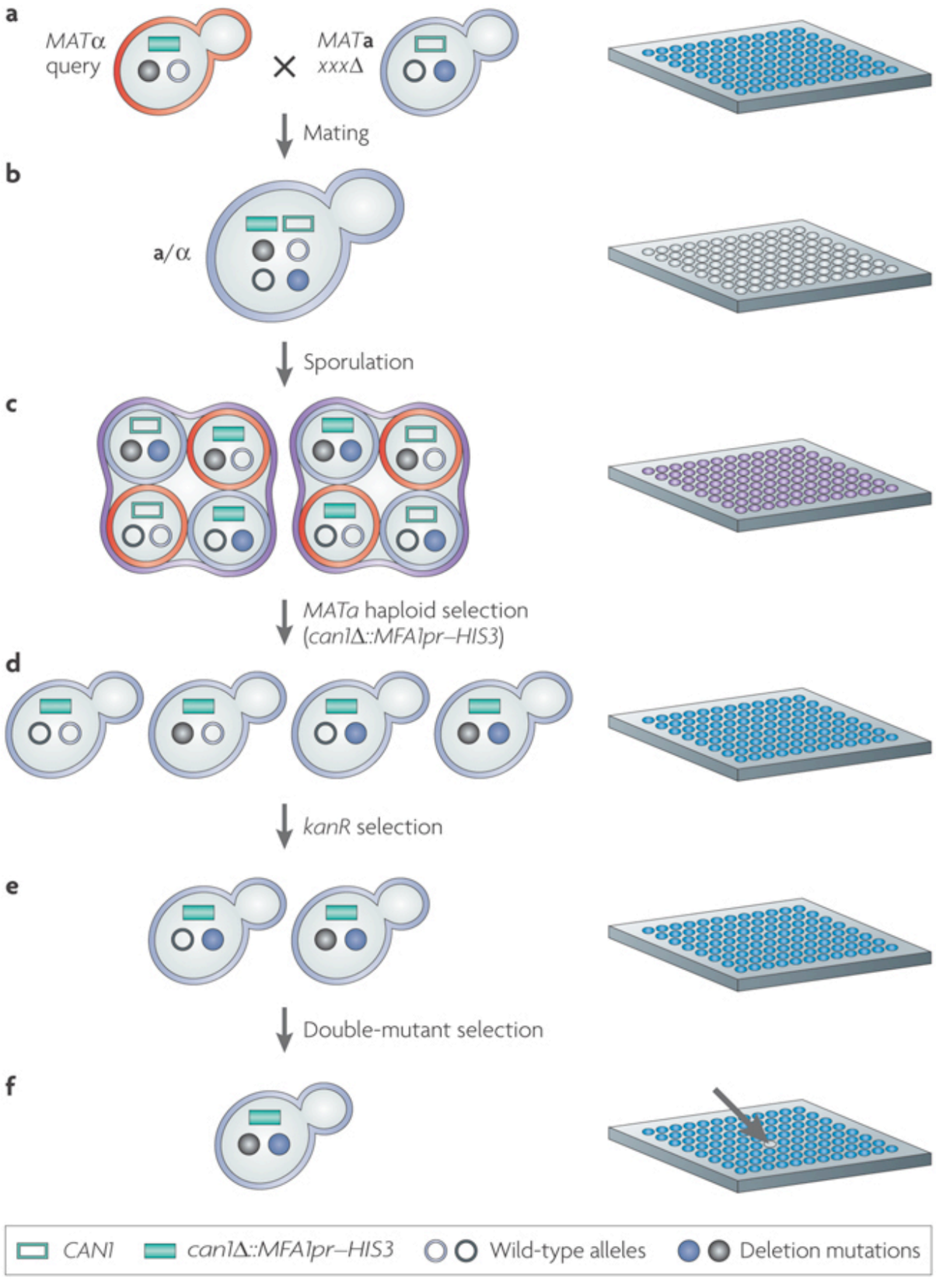
High-throughput synthetic lethality analysis in yeast



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)

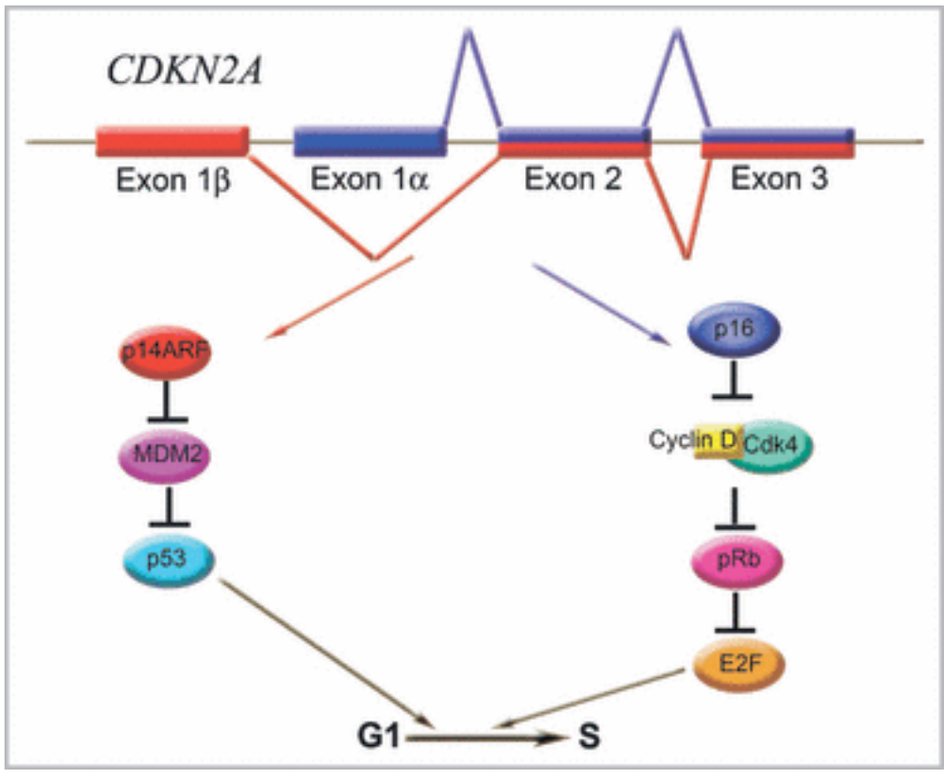
PTC THERAPEUTICS ® is developing drugs that they hope will suppress the reduced transcription of mutant genes

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.

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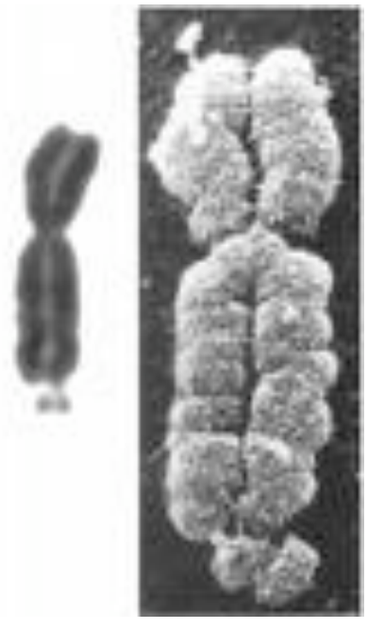
Disease	Symptoms	Gene	Locus	Protein
Non-coding repeats				
Friedreich ataxia	Ataxia, weakness, sensory loss	*FXN*	9q13–q21.1	Frataxin
Fragile X syndrome A	Mental retardation	*FMR1*	Xq27.3	Fragile X mental retardation 1 protein
Fragile X syndrome E	Mental retardation	*FMR2*	Xq28	Fragile X mental retardation 2 protein
Dystrophia myotonica 1	Weakness, myotonia	*DMPK*	19q13	Dystrophia myotonica protein kinase
Spinocerebellar ataxia 8	Ataxia	Antisense to *KLHL1*	13q21	Undetermined
Spinocerebellar ataxia 12	Ataxia	*PPP2R2B*	5q31–q33	Regulatory subunit of the protein phosphatase PP2A
Huntington disease-like 2	Chorea, dementia	*JPH3*	16q24.3	Junctophilin 3
Polyglutamine disorders				
Spinal and bulbar muscular atrophy	Weakness	*AR*	Xq13–q21	Androgen receptor
Huntington disease	Chorea, dementia	*IT15*	*4p16.3*	Huntingtin
Dentatorubral-pallidoluysian atrophy	Ataxia, myoclonic epilepsy, dementia	*DRPLA*	12p13.31	Atrophin 1
Spinocerebellar ataxia 1	Ataxia	*SCA1*	6p23	Ataxin 1
Spinocerebellar ataxia 2	Ataxia	*SCA2*	12q24.1	Ataxin 2
Spinocerebellar ataxia 3 (Machado–Joseph disease)	Ataxia	*SCA3/MJD*	14q32.1	Ataxin 3
Spinocerebellar ataxia 6	Ataxia	*CACNA1A*	19p13	α_{1A}-voltage-dependent calcium channel subunit
Spinocerebellar ataxia 7	Ataxia	*SCA7*	3p12–p13	Ataxin 7
Spinocerebellar ataxia 17	Ataxia	*TBP*	6q27	TATA box binding protein
Polyalanine disorders*				
Oculopharyngeal dystrophy	Weakness	*PABPN1*	14q11.2–q13	Poly(A)-binding protein 2
Congenital central hypoventilation syndrome	Respiratory difficulties	*PHOX2B*	4p12	Paired-like homeobox 2B
Infantile spasms	Mental retardation, epilepsy	*ARX*	Xp22.13	Aristaless-related homeobox, X-linked

Enhancer Example II: Triplet expansion diseases



Fragile X syndrome

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.