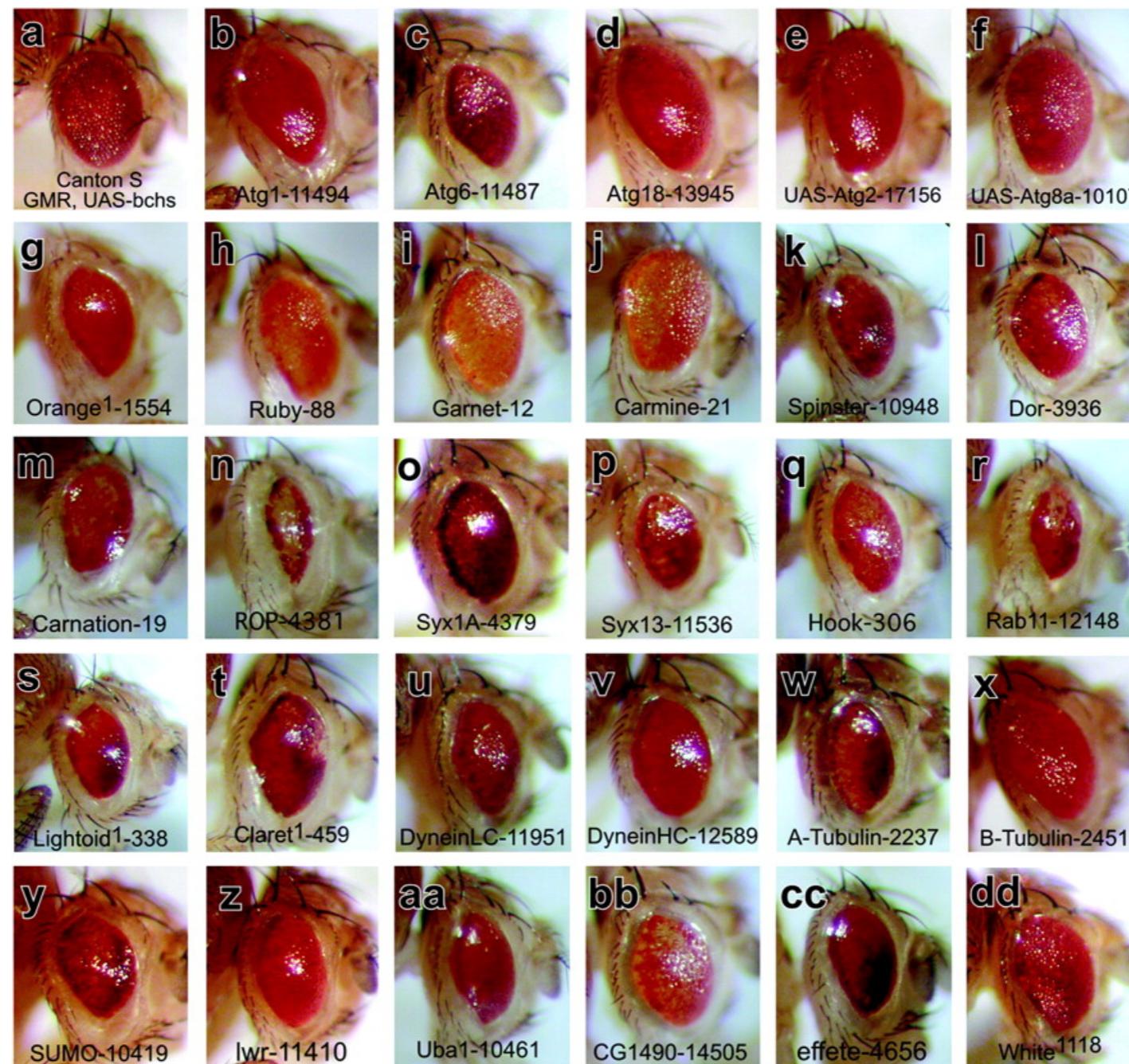
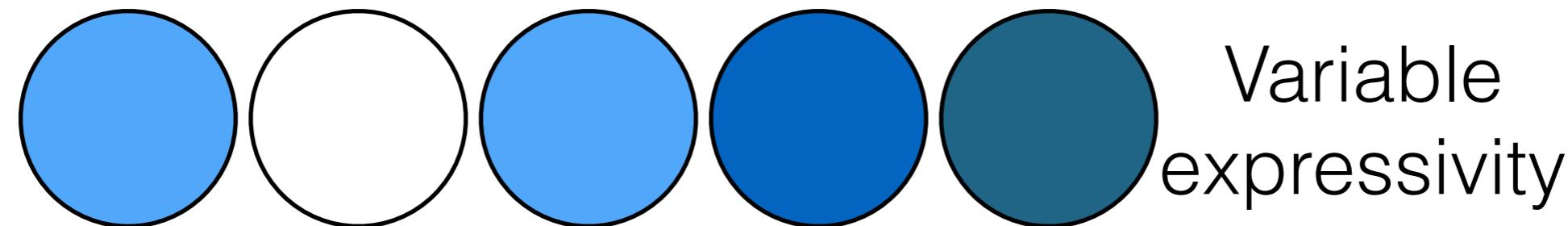
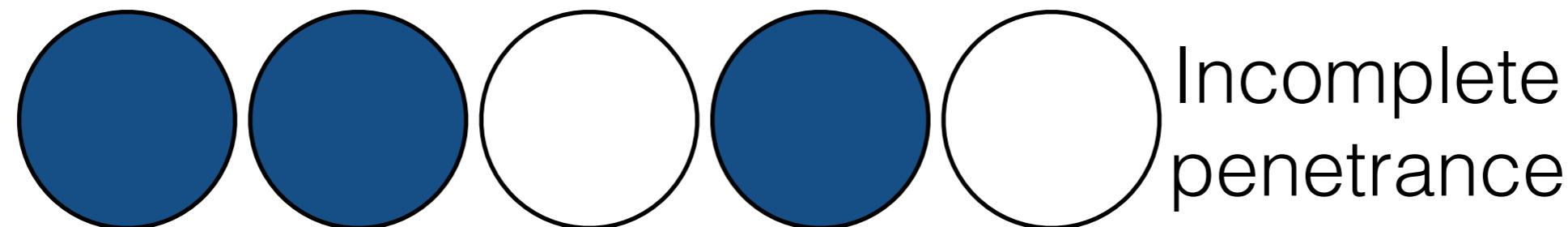
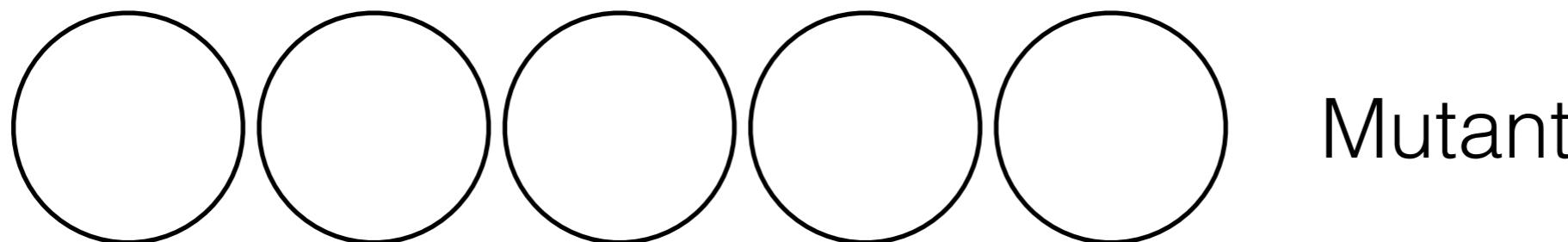
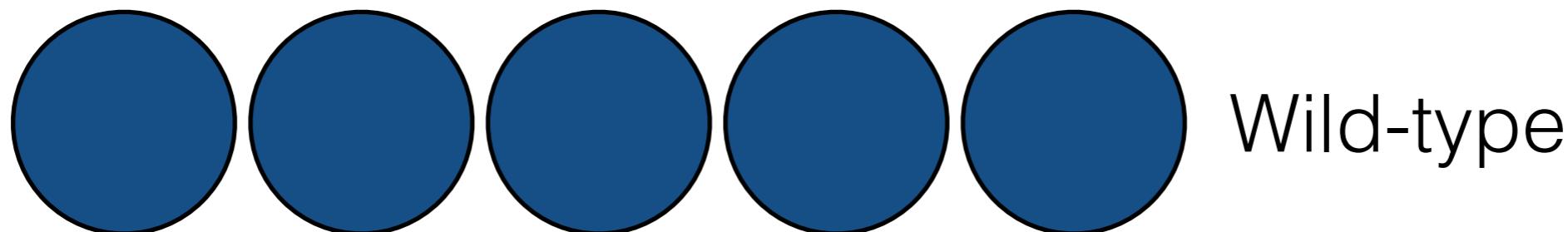


Bio393: Genetic Analysis

Genetic interactions: suppression and enhancement





When not every mutant animal has the mutant phenotype... incomplete penetrance

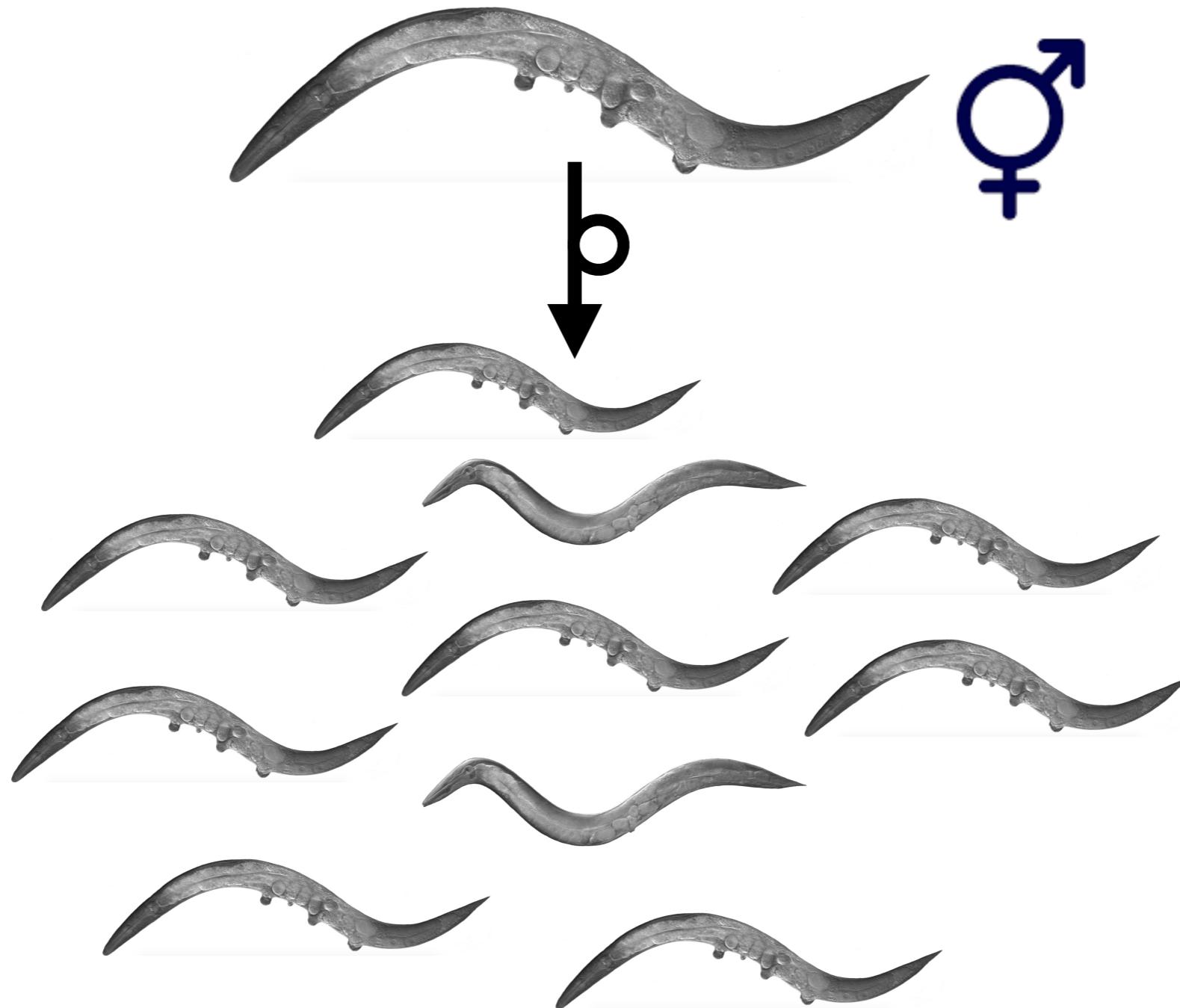


Wild-type worms have one vulva



Multivulva mutant worms have multiple vulvae

When not every mutant animal has the mutant phenotype... incomplete penetrance



8/10 multivulva, mutant allele is 80% penetrant

When a mutant animal is not completely mutant... variable expressivity



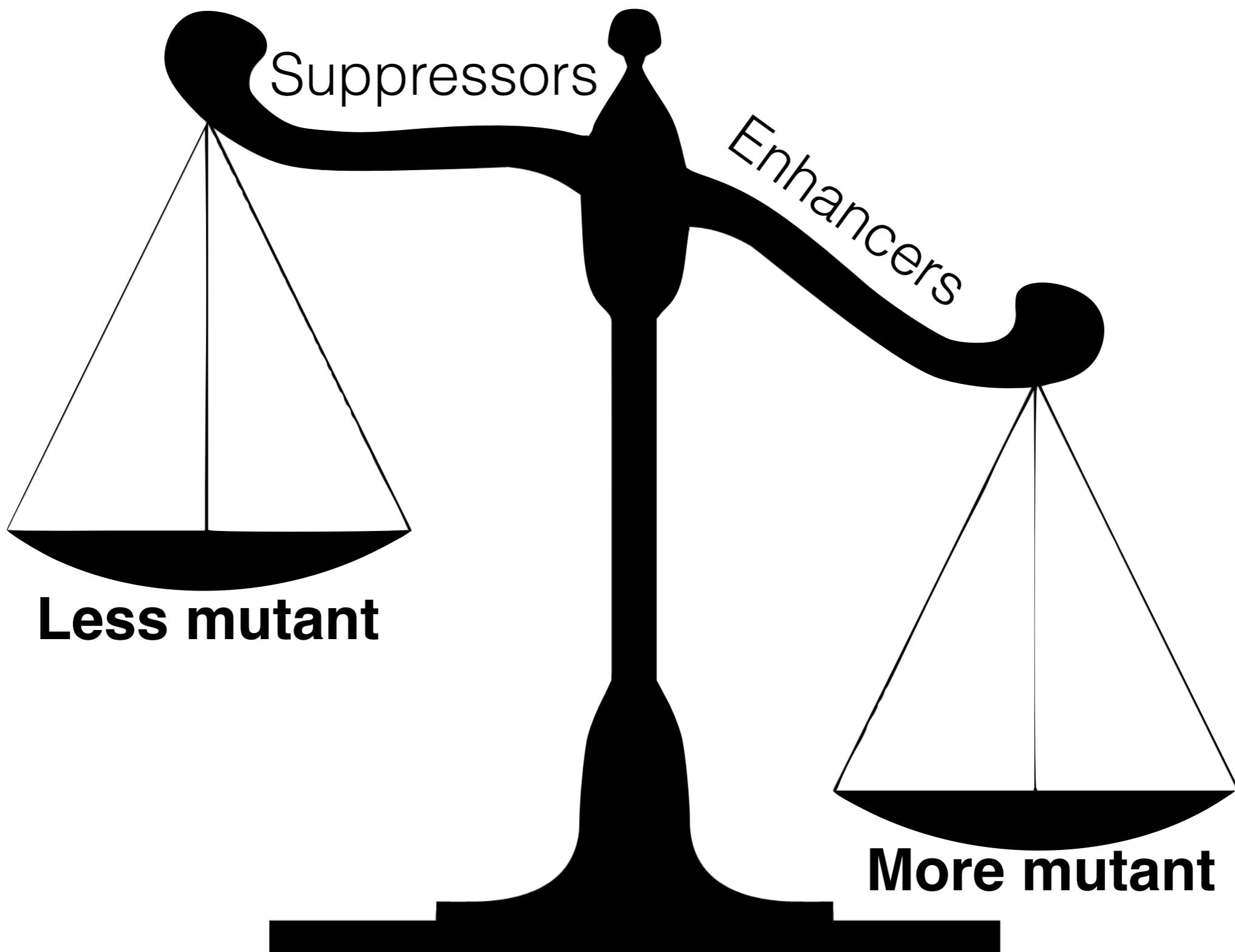
Wild-type worms have one vulva



Multivulva mutant worms have multiple vulvae

An animal only has two extra vulvae instead of three.

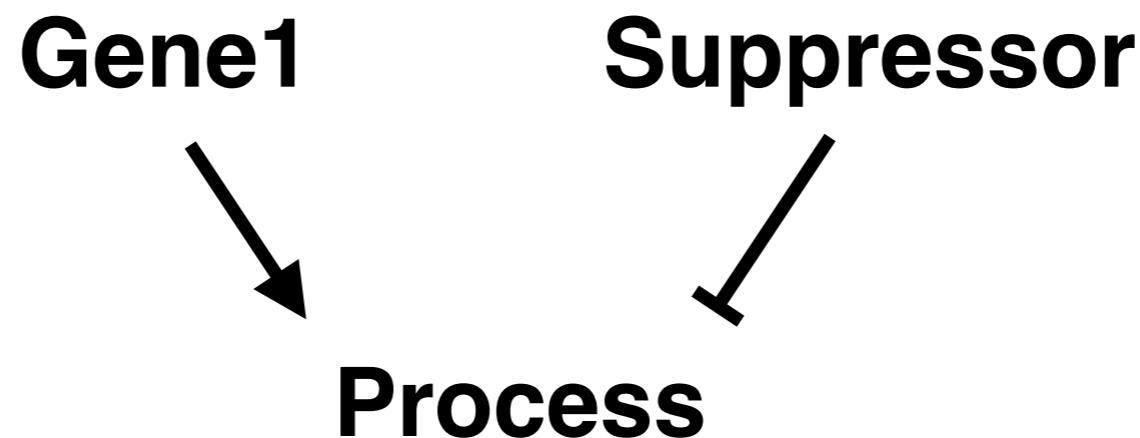
Identification of mutants that modify an existing mutant phenotype



Identification of mutants that modify an existing mutant phenotype

Suppressors make the mutant phenotype less severe.

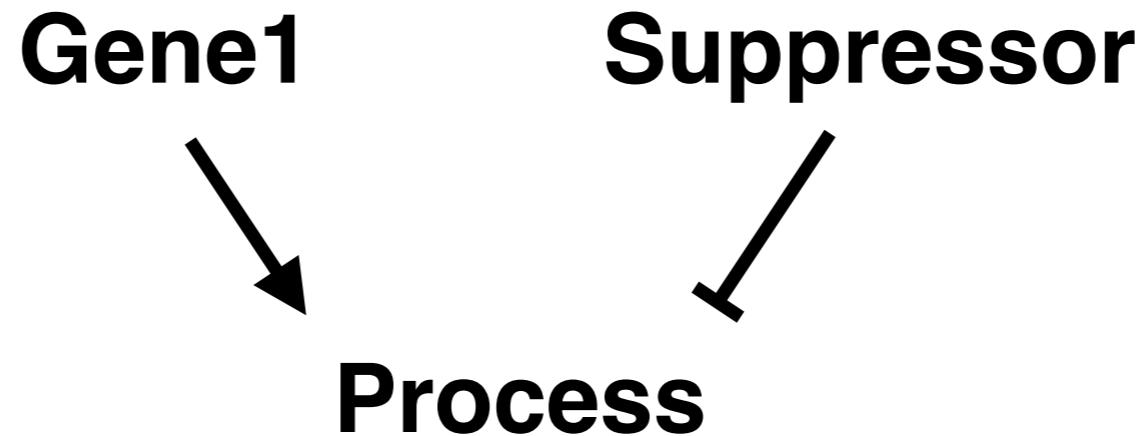
The suppressor gene acts oppositely to the original gene.



Why do we care?

Suppressor genes allow us to build pathways

Gene1 —————→ **Suppressor** —————→ **Process**

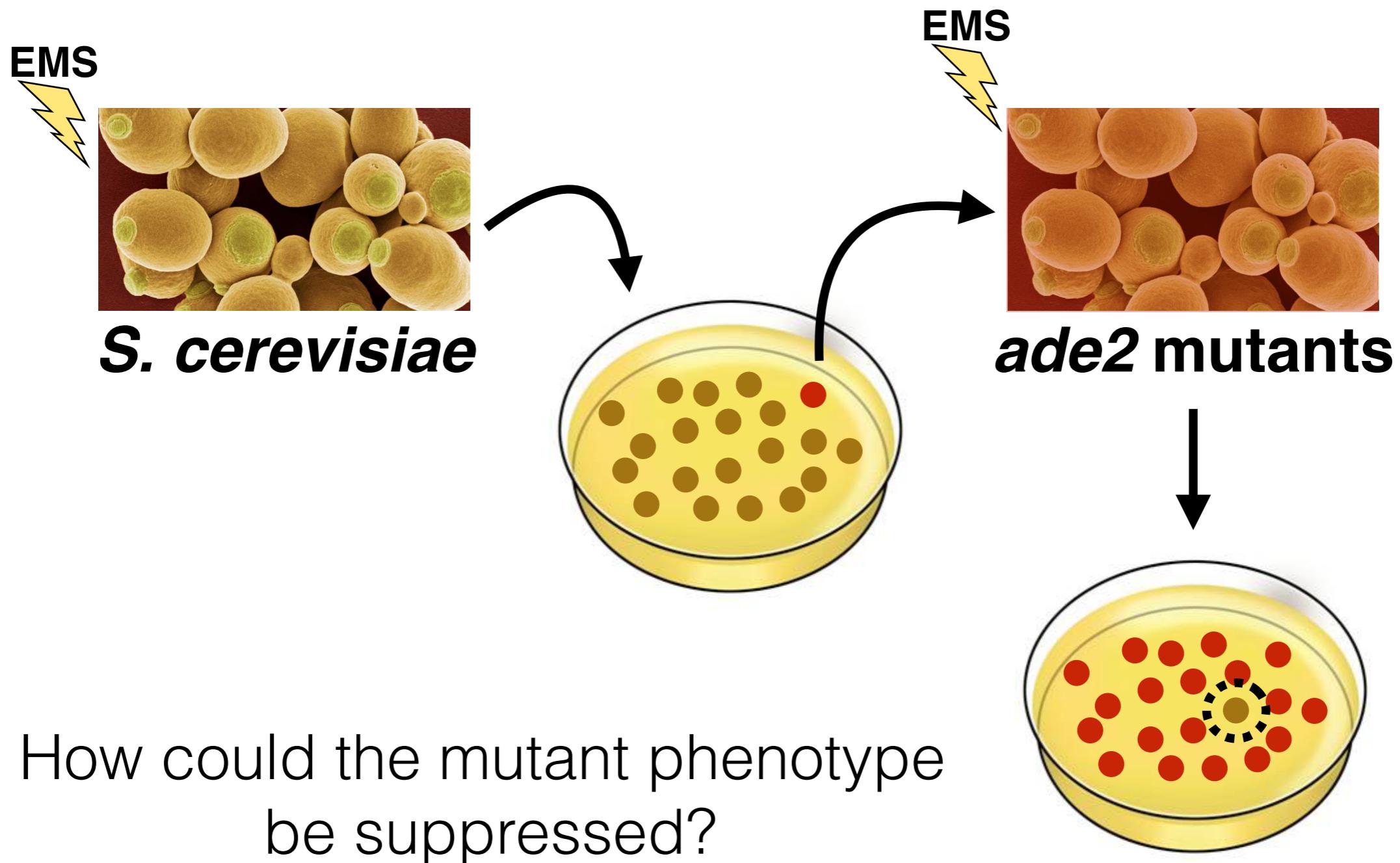


Suppressors come in two general flavors: intragenic and extragenic

Same gene vs. different genes

How to tell?

We want to identify suppressors of the *ade2* mutant phenotype



Three general types of suppressor mutants

1. Intragenic suppressor (revertant or back mutation)

>ADE2 YOR128C SGDID:S000005654
ATGGATTCTAGAACAGTTGGTATATTAGGAGGGGACAATTGGGACGTATGATTGAG
GCAGCAAACAGGCTAACATTAAGACGGAATAACTAGATGCTGAAAATTCTGCCAAA
CAAATAAGCAACTCCAATGACCACGTTAATGGCTCCTTCCAATCCTCTGATATCGAA
AAACTAGCTGAAAAATGTGATGTGCTAACGATTGAGATTGAGCATGTTGATGTCCTACA
CTAAAGAATCTTCAAGTAAAACATCCCATTAAAAATTACCCCTCCAGAAACAATC
AGATTGATACAAGACAAATATATTCAAAAAGAGCATTAAATCAAAATGGTATAGCAGTT
ACCCAAAGTGTCTGTGGAACAAAGCCAGTGAGACGTCCCTATTGAATGTTGGAAGAGAT
TTGGGTTTCCATTCGTCTTGAAGTCGAGGACTTGGCATACGATGGAAGAGGTAACCTC
GTTGTAAGAATAAGGAAATGATTCCGGAAGCTTGGAAAGTACTGAAGGATCGTCTTGT
TACGCCAAAAATGGCACCAATTACTAAAGAATTAGCAGTCATGATTGTGAGATCTGTT
AACGGTTAGTGTCTTACCCATTGTAGAGACTATCCACAAGGACAATATTGTGAC
TTATGTTATGCCCTGCTAGAGTCCGGACTCCGTTCAACTTAAGGCGAAGTTGGCA
GAAAATGCAATCAAATCTTCCGGTTGGTATATTGGTGTGGAAATGTTCTATTAA
GAAACAGGGGAATTGCTTATTAACGAAATTGCCCAAGGCCTCACAACTCTGGACATTAT
ACCATTGATGCTTGCCTCACTTCTCAATTGAAGCTCATTGAGATCAATATTGGATTTG
CCAATGCCAAAGAATTTCACATCTTCTCCACCATTACAACGAACGCCATTATGCTAAAT
GTTCTGGAGACAAACATACAAAAGATAAAGAGCTAGAAACTTGCAGGAAAGAGCATTGGCG
ACTCCAGGTTCTCAGTGTACTTATATGGAAAAGACTCTAGACCTAACAGAAAAGTAGGT
CACATAAATATTATTGCCCTCCAGTATGGCGGAAT A AACAAAGGCTGAACCTACATTACA
GGTAGAACTGATATTCCAATCAAATCTCTGCTGCTCAAAAGTTGGACTTGGAAAGCAATG
GTCAAACCATTGGTTGGAATCATCATGGGATCAGACTCTGACTTGCCTGGTAATGTCTGCC
GCATGTGCGGTTAAAAGATTGGCGTCCATTGAAGTACAATAGTCTTGCTCAT
AGAACTCCACATAGGATGTCAGCATATGCTATTCCGCAAGCAAGCGTGGAAATTAAAACA
ATTATCGCTGGAGCTGGTGGGCTGCTCACTGCCAGGTATGGTGGCTGCAATGACACCA
CTTCCTGTCATCGGTGTGCCGTAAAAGTTCTGTCTAGATGGAGTAGATTCTTACAT
TCAATTGTGCAAATGCCTAGAGGTGTTCCAGTAGCTACCGTCGCTATTAATAAGTACG
AACGCTGCGCTGTTGGCTGTCAGACTGCTTGGCGTTATGATTCAAGTTACAACGAAA
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ACTGTCGGTTACGAAGCTTATCTAGAAAACAAGTAA

Three general types of suppressor mutants

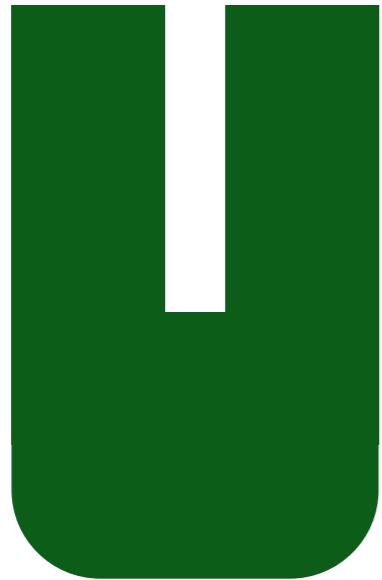
1. Intragenic suppressor (revertant or back mutation)

```
>ADE2 YOR128C SGDID:S000005654
ATGGATTCTAGAACAGTTGGTATATTAGGAGGGGGACAATTGGGACGTATGATTGAG
GCAGCAAACAGGCTAACATTAAGACGGAATACTAGATGCTGAAAATTCTCCTGCCAAA
CAAATAAGCAACTCCAATGACCACGTTAATGGCTCCTTTCCAATCCTCTTGATATCGAA
AAACTAGCTGAAAATGTGATGTGCTAACGATTGAGATTGAGCATGTTGATGTTCCCTACA
CTAAAGAACATCTCAAGTAAACATCCAAATTAAAAATTACCCCTCTCCAGAAACAAATC
AGATTGATACAAGACAAATATTCAGGAGCATTAACTCAAAATGGTATAGCAGTT
ACCCAAAGTGTCCCTGTGGAACAAGCCAGTGAGACGTCCTATTGAATGTTGGAAGAGAT
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GTTGAAAGATAAGGAAATGATTCCGGAAGCTTGGAAAGTACTGAAGGATCGTCCTTG
TACGCCGAAAAATGGCACCATTACTAAAGAATTAGCAGTCATGATTGAGATCTGTT
AACGGTTAGTGTCTTACCAATTGTAGAGACTATCCACAAGGACAATTGAGAC
TTATGTTATGCGCCTGCTAGAGTTCCGGAACCTCAACTTAAGGCGAAGTTGTTGGCA
GAAAATGCAATCAAATCTTCCCGTTGTGGTATATTGGTGTGAAATGTTCTATTAA
GAAACAGGGGAATTGCTTATTAAACGAAATTGCCCAAGGCCTACAACCTCTGGACATTAT
ACCATTGATGCTTGCCTCACTCTCAATTGAAGCTCATTGAGATCAATATTGGATTTG
CCAATGCCAAAGAATTTCACATCTTCTCCACCATTACAACGAACGCCATTATGCTAAAT
GTTCTGGAGACAAACATACAAAGATAAAGAGCTAGAAACTTGCAGGAAAGAGCATTGGCG
ACTCCAGGTTCCCTCAGTGTACTTATATGGAAAAGAGTCTAGACCTAACAGAAAAGTAGGT
CACATAAATATTATTGCCTCCAGTATGGCGGAATGTGAACAAAGGCTGAACCTACATTACA
GGTAGAACTGATATTCAATCAAATCTCTGTGCTCAAAAGTTGGACTTGGAAAGCAATG
GTCAAACCATTGGTTGGAATCATCATGGGATCAGACTCTGACTTGCCGGTAATGTCGCC
GCATGTGCGGTTAAAAGATTGGCGTTCCATTGAAGTGAACATAGTCTCTGCTCAT
AGAACTCCACATAGGATGTCAGCATATGCTATTCCGCAAGCAAGCGTGGAAATTAAAACA
ATTATCGCTGGAGCTGGTGGGCTGCTCACTTGGCAGGTATGGTGGCTGCAATGACACCA
CTTCCTGTATCGGTGTGCCGTAAAAGTTCTGTAGATGGAGTAGATTCTTACAT
TCAATTGTGCAAATGCCCTAGAGGTGTTCCAGTAGCTACCGTCGCTATTAAATAAGTACG
AACGCTGCGCTGTTGGCTGTCAGACTGCTTGGCGTTATGATTCAAGTTACAAACGAAA
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ACTGTCGGTTACGAAGCTTATCTAGAAAACAAGTAA
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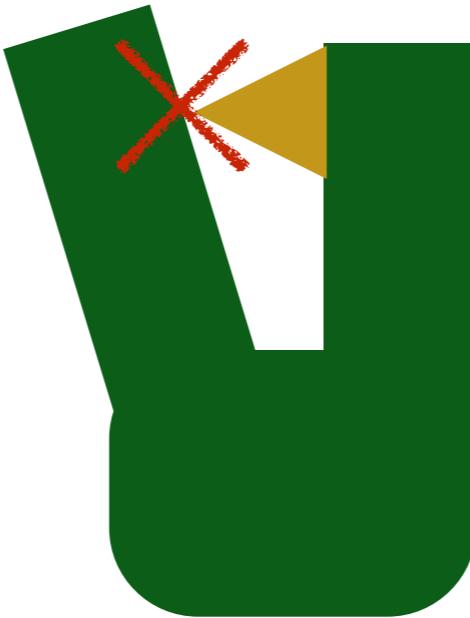
- Absolutely linked to gene
- Same attributes as the wild-type allele
- Extremely rare

Three general types of suppressor mutants

1. Intragenic suppressor (revertant or back mutation)
2. Intragenic suppressor (linked, allele-specific and gene-specific)



Wild-type



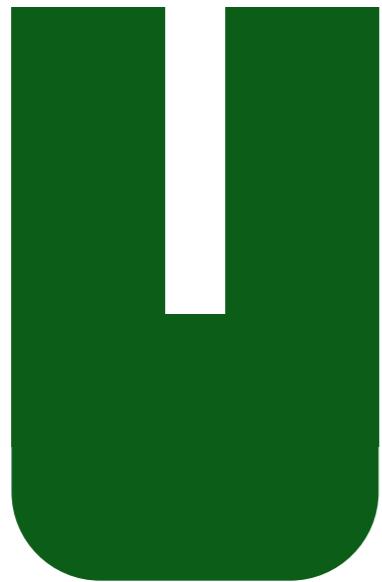
Mutant1



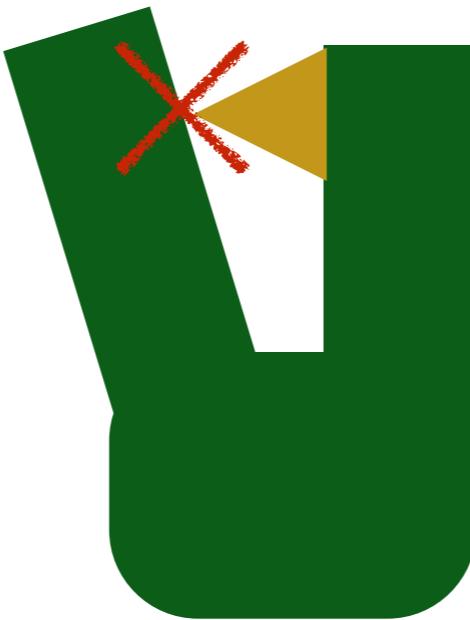
Mutant1 +
Mutant2
(Suppressor)

Three general types of suppressor mutants

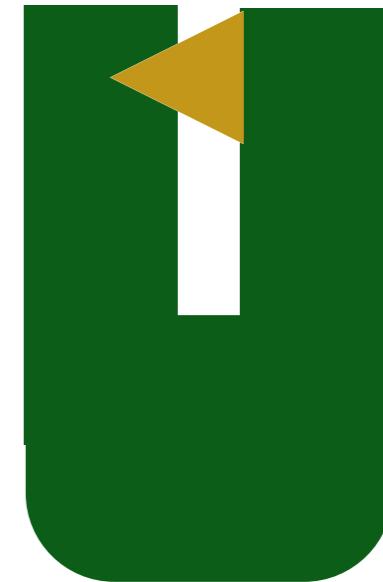
1. Intragenic suppressor (revertant or back mutation)
2. Intragenic suppressor (linked, allele-specific and gene-specific)



Wild-type



Mutant1



Mutant1 +
Mutant2
(Suppressor)

Example of intragenic suppressor: *trpA* in *E. coli*

trpA mutant = needs tryptophan to grow

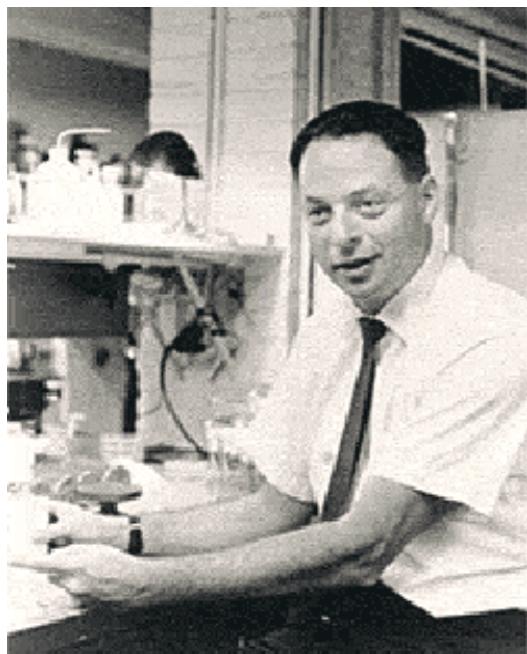
trpA mutant suppressor = grows without tryptophan

trpA WT = G210

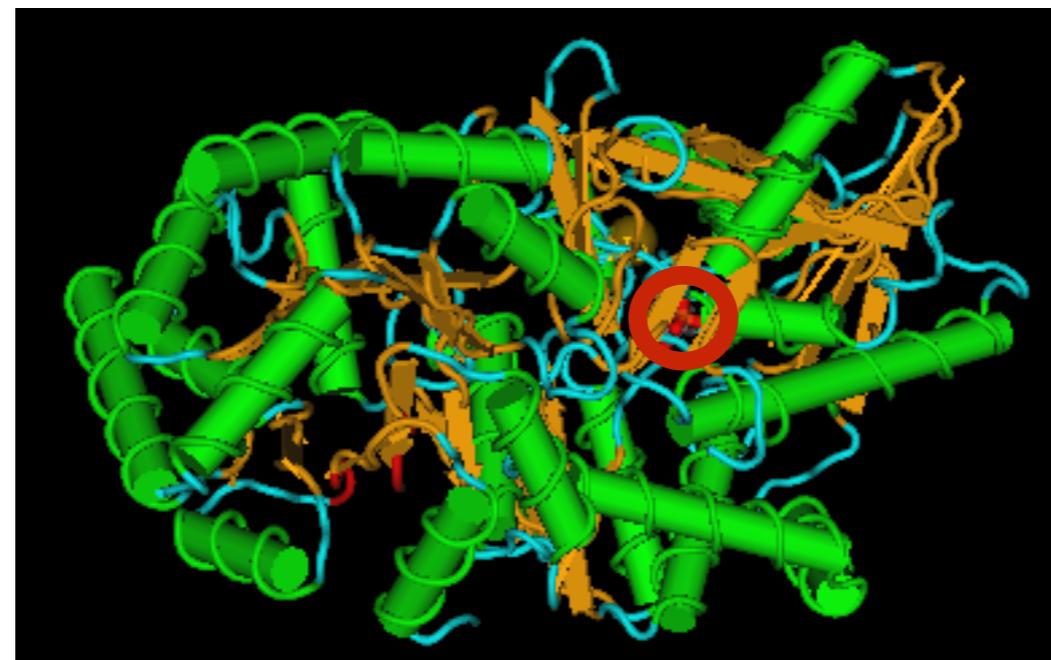
trpA mutant = Q210

trpA mut. sup. = Q210 **C174**

trpA backcross = G210 **C174**

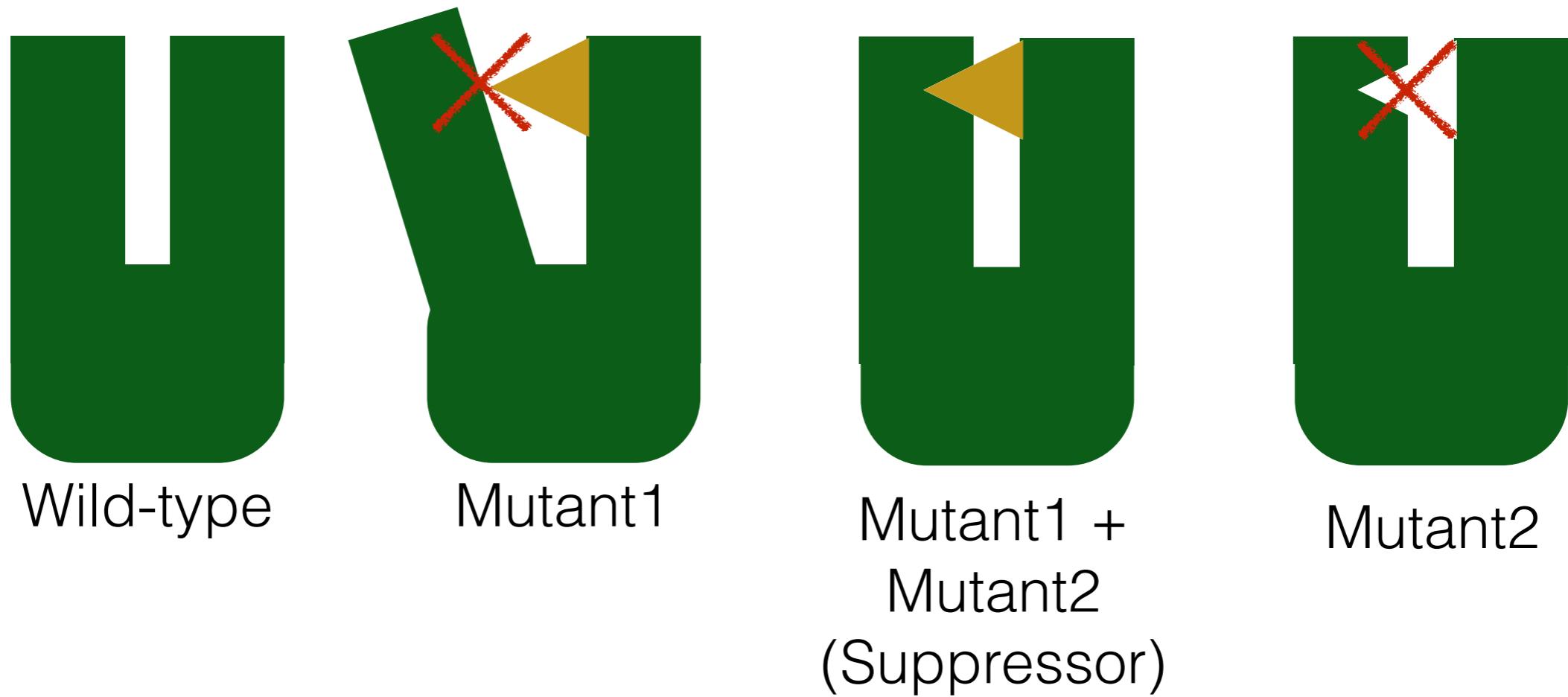


Charles Yanofsky



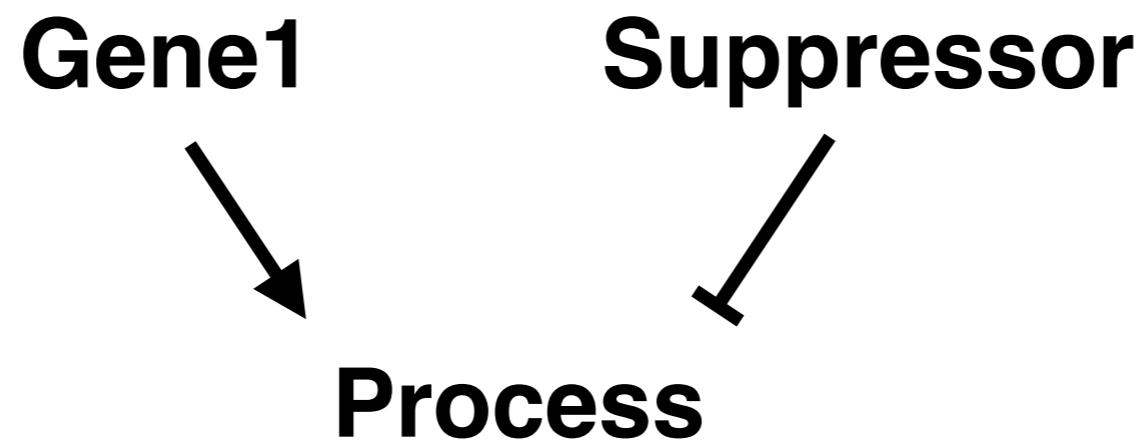
Three general types of suppressor mutants

1. Intragenic suppressor (revertant or back mutation)
2. Intragenic suppressor (linked, allele-specific and gene-specific)



Three general types of suppressor mutants

1. Intragenic suppressor (revertant or back mutation)
2. Intragenic suppressor (linked, allele-specific and gene-specific)
3. Exogenous suppressor
 1. Standard
(allele non-specific, gene or pathway specific)



Gene1 —→ Suppressor —→ Process

Example of extragenic suppressor

gene A inhibits gene B



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



LoF in *B* eliminates its function



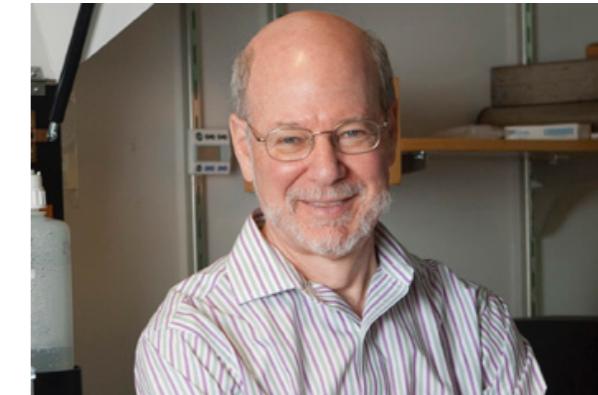
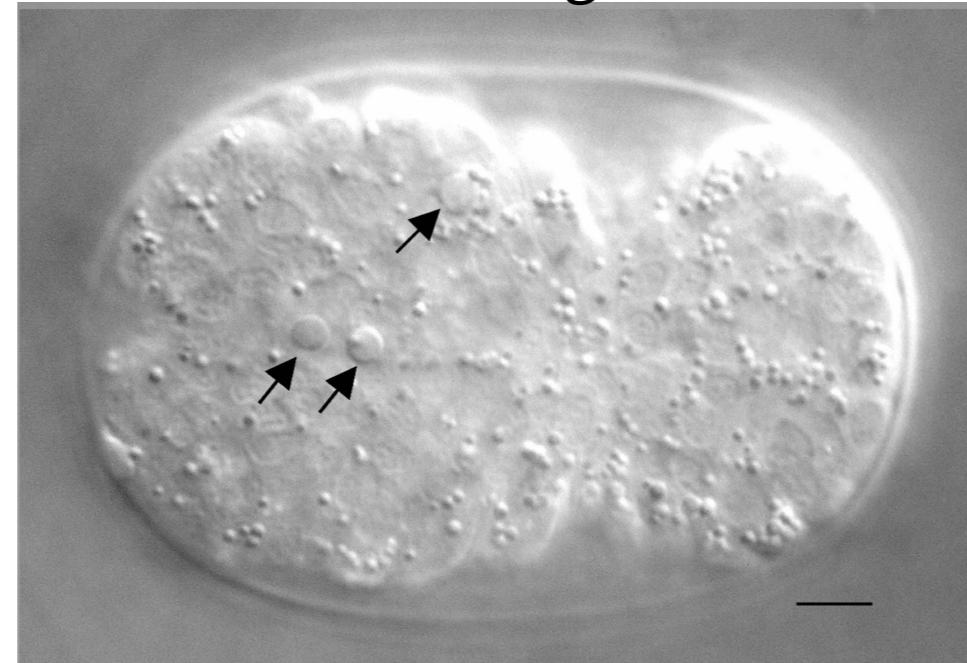
Three general types of suppressor mutants

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2. Intragenic suppressor (linked, allele-specific and gene-specific)
3. Exogenous suppressor
 1. Standard
(allele non-specific, gene or pathway specific)
 2. Bypass suppressor (suppresses null allele,
allele non-specific, gene or pathway specific)



Example of bypass extragenic suppressor

Programmed cell death
in *C. elegans*



Bob Horvitz

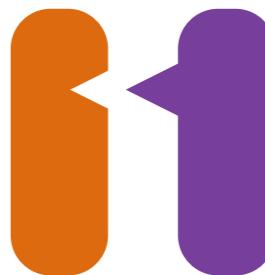
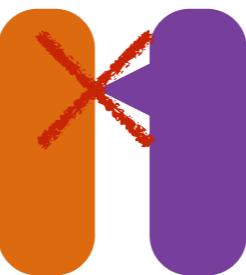
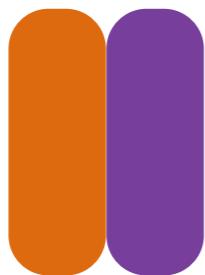


ced-9(0) = too much cell death = dead animal

ced-9(0); ced-3(0) = no cell death, suppression, live animal

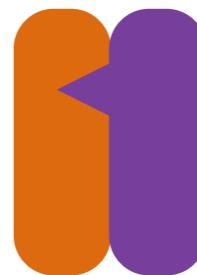
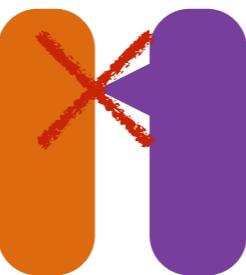
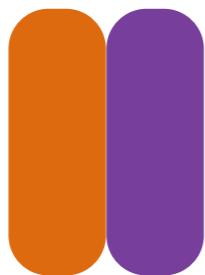
Three general types of suppressor mutants

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3. Exogenous suppressor
 1. Standard
 2. Bypass suppressor (suppresses null allele, allele non-specific, gene or pathway specific)
 3. Interactional suppressor (gene-specific, allele-specific)



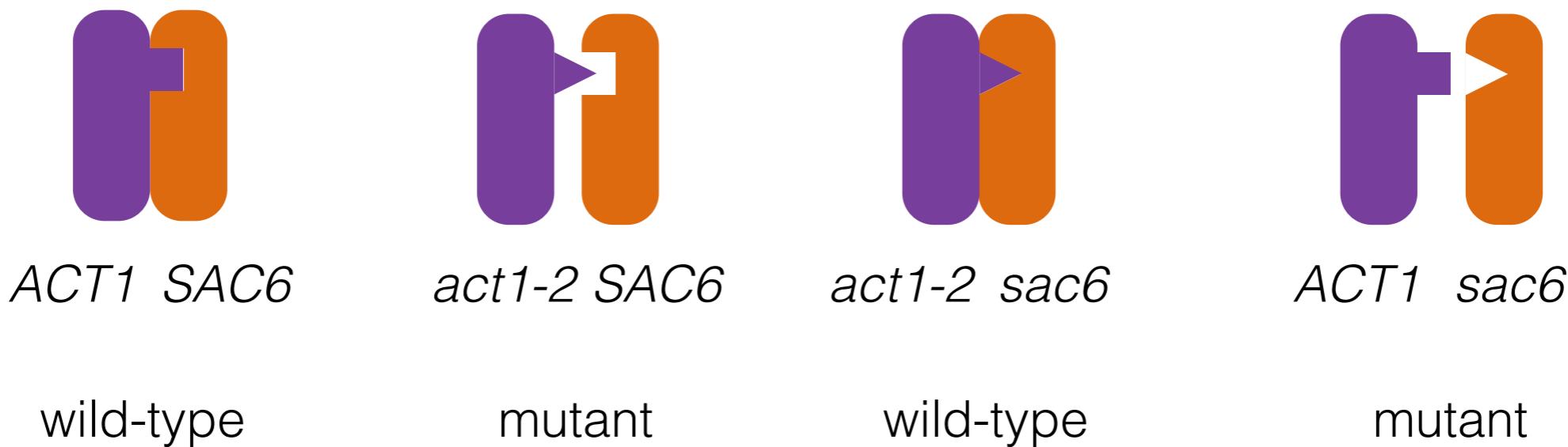
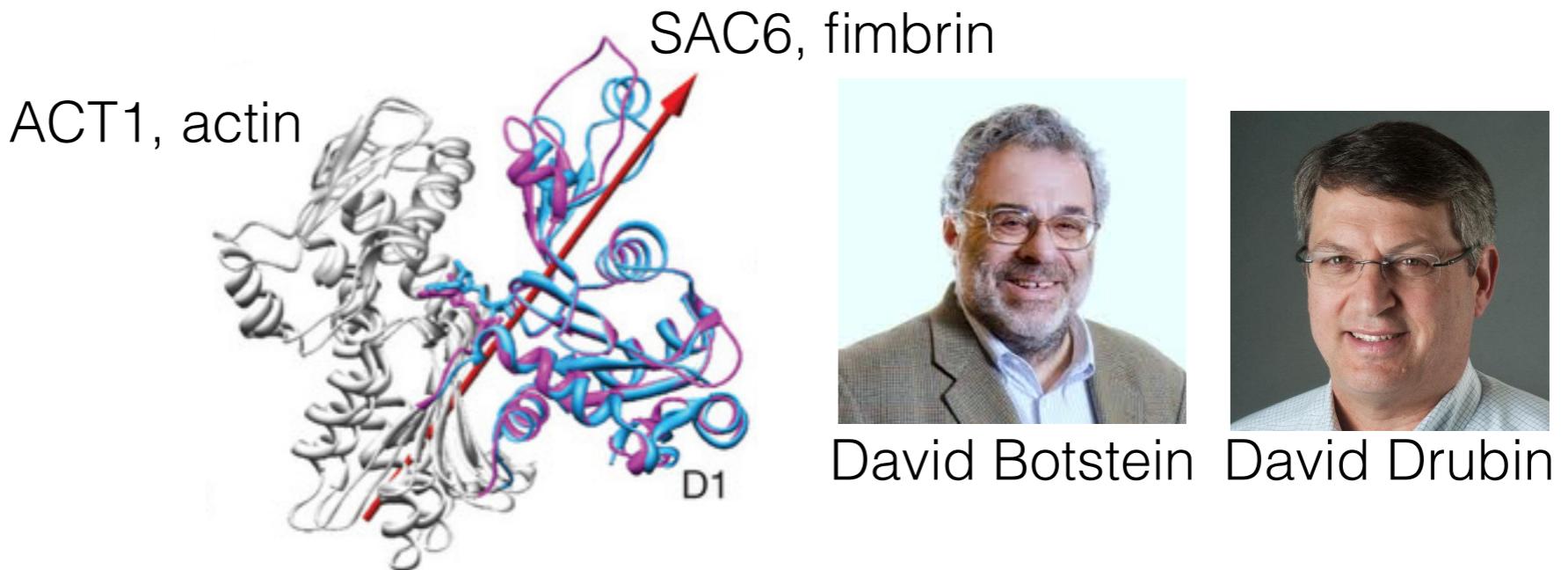
Three general types of suppressor mutants

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 1. Standard
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 3. Interactional suppressor (gene-specific, allele-specific)



Example of extragenic interaction suppressor

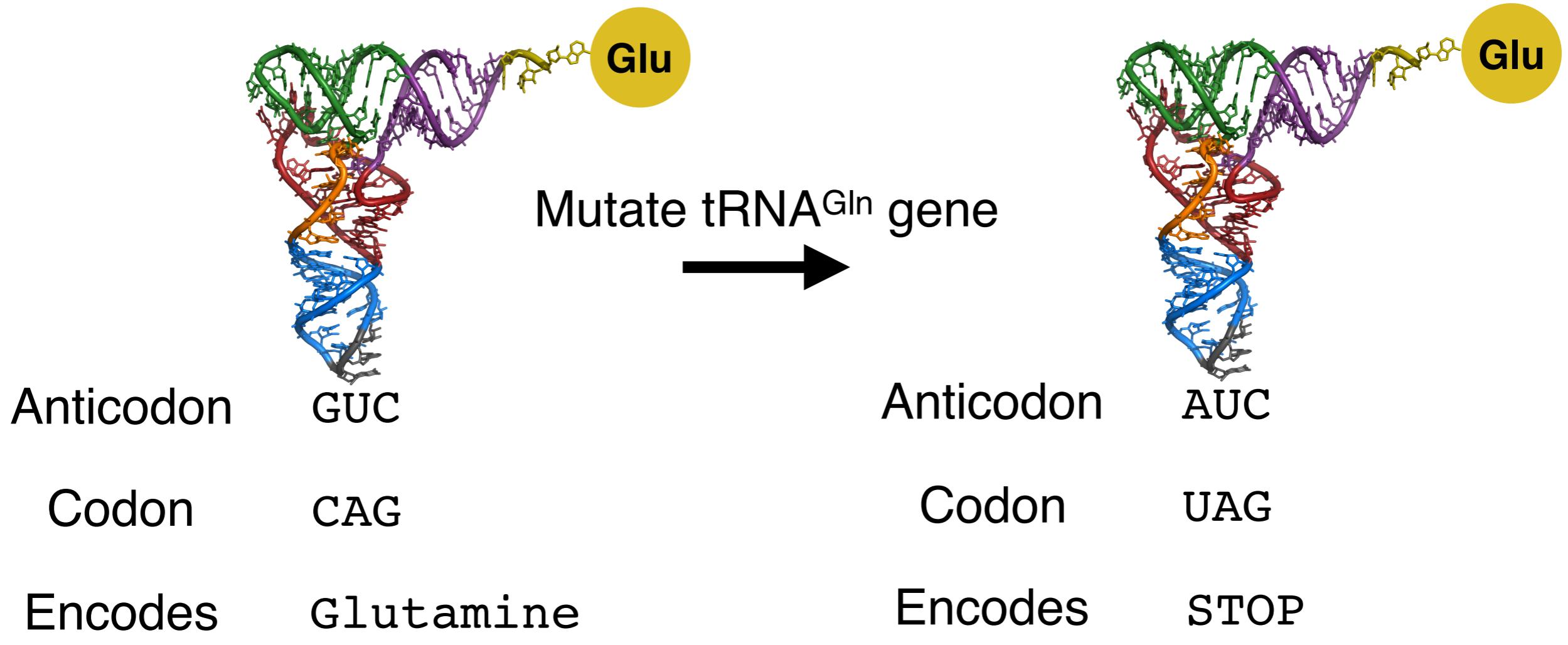
Yeast suppressors of *ACT1* mutants



Three general types of suppressor mutants

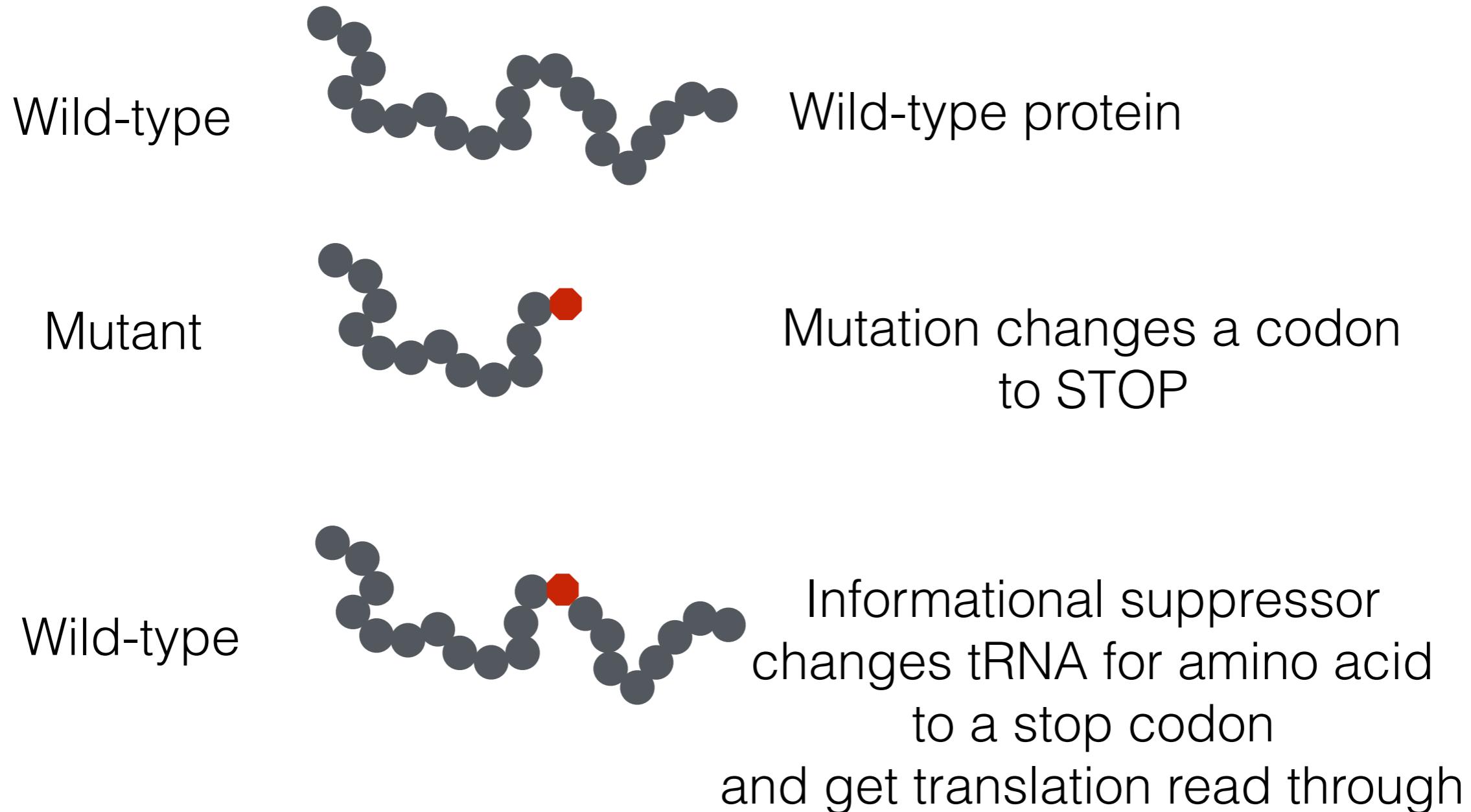
1. Intragenic suppressor (revertant or back mutation)
2. Intragenic suppressor (linked, allele-specific and gene-specific)
3. Exogenous suppressor
 1. Standard
 2. Bypass suppressor
(allele non-specific, gene or pathway specific)
 3. Interactional suppressor
(gene-specific, allele-specific)
 4. Informational suppressor
(gene-nonspecific, allele-specific)

Informational suppressors are allele-specific but gene-nonspecific



Many amber (UAG) stop codons will be read through

Informational suppressors are allele-specific but gene-nonspecific



Nonsense suppression as therapy

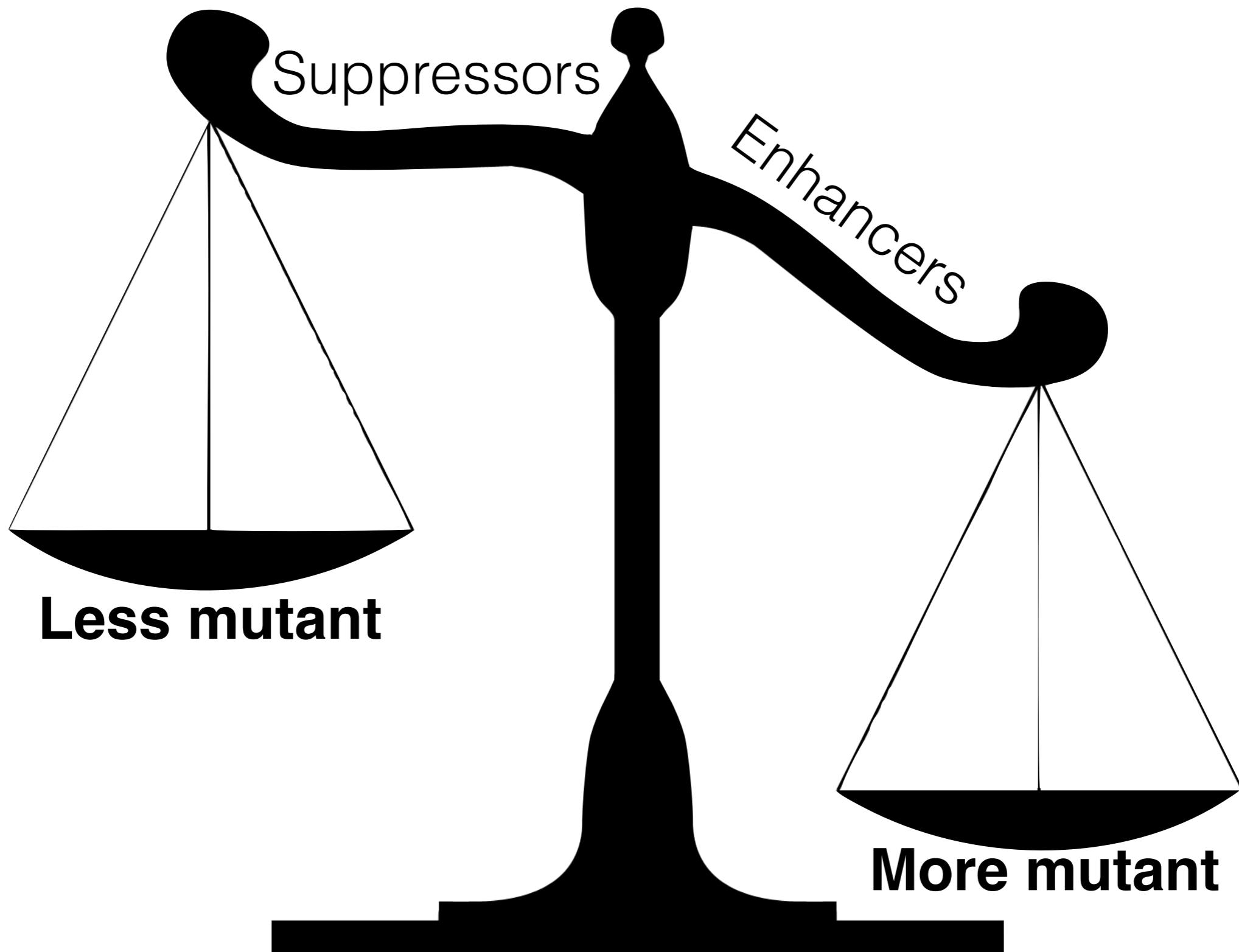


- Cystic Fibrosis (CFTR)
- Duchenne muscular dystrophy (dystrophin)
- Beta thalassaemia (beta-globin)
- Hurler syndrome (alpha-L iduronidase)
- Ullrich disease (collagen type VI)

Three general types of suppressor mutants

1. Intragenic suppressor (revertant or back mutation)
2. Intragenic suppressor (linked, allele-specific and gene-specific)
3. Exogenous suppressor
 1. Standard
 2. Bypass suppressor
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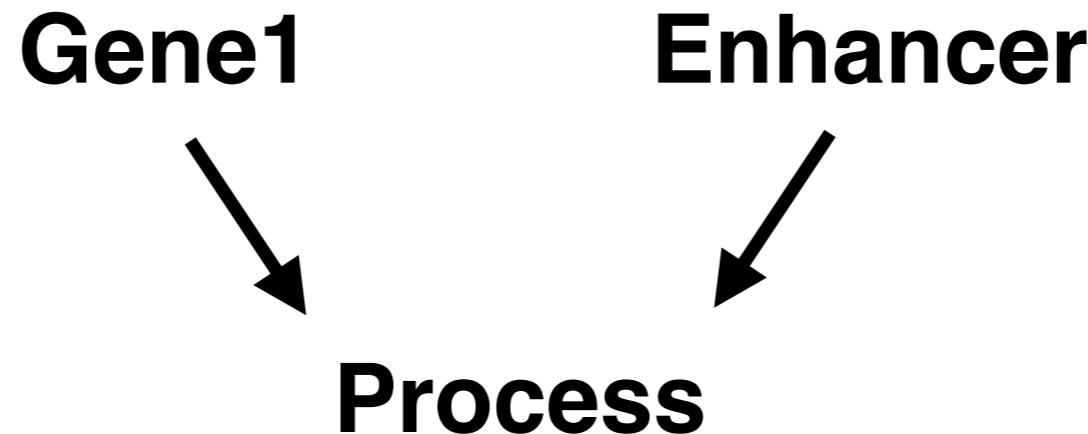
Identification of mutants that modify an existing mutant phenotype



Identification of mutants that modify an existing mutant phenotype

Enhancers make the mutant phenotype worse.

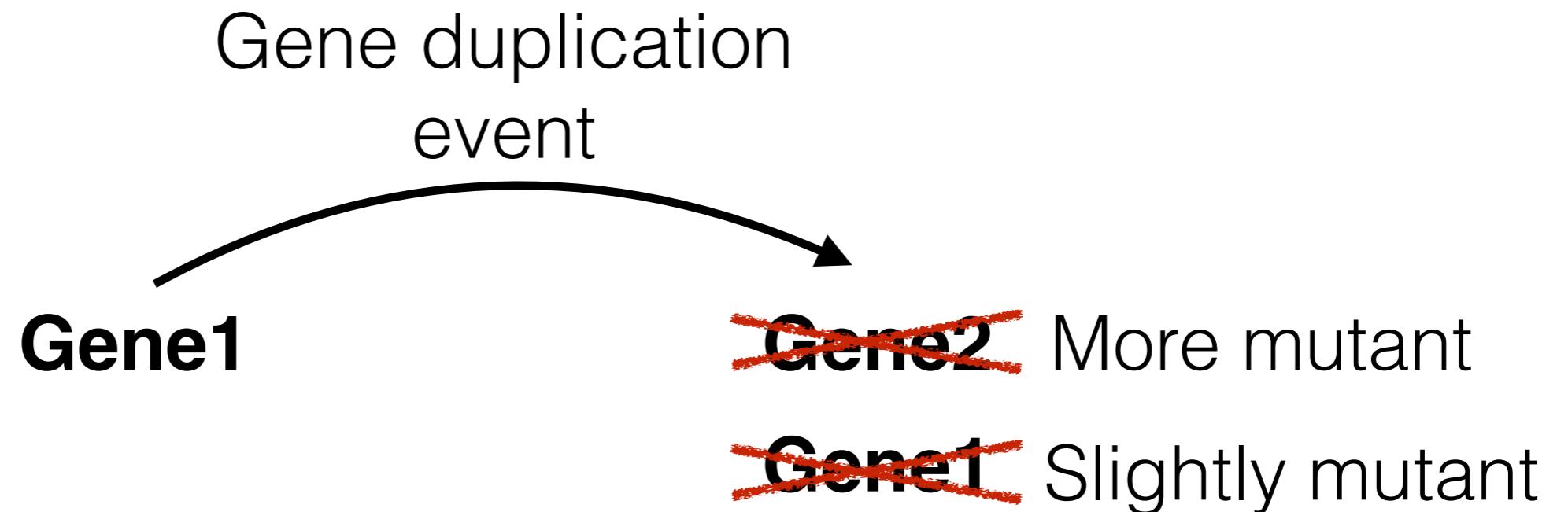
The normal function of the enhancer gene acts with the same effect as the original gene.



Why do we care?

Enhancers act in redundant processes

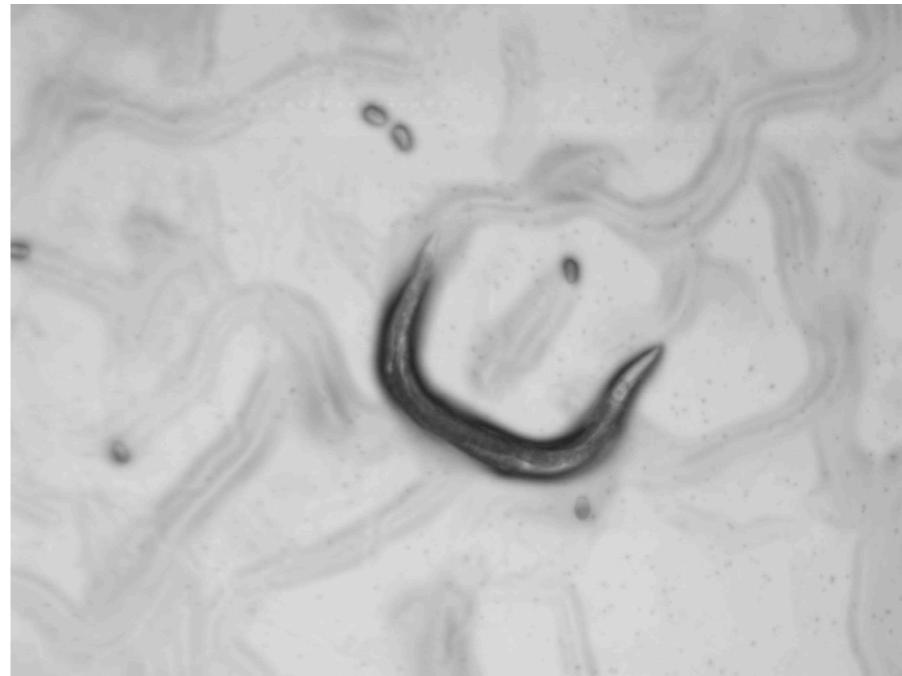
1. Gene families (redundancy by similarity of function)



Example of gene family redundancy: cuticle collagens



wild-type



mutant

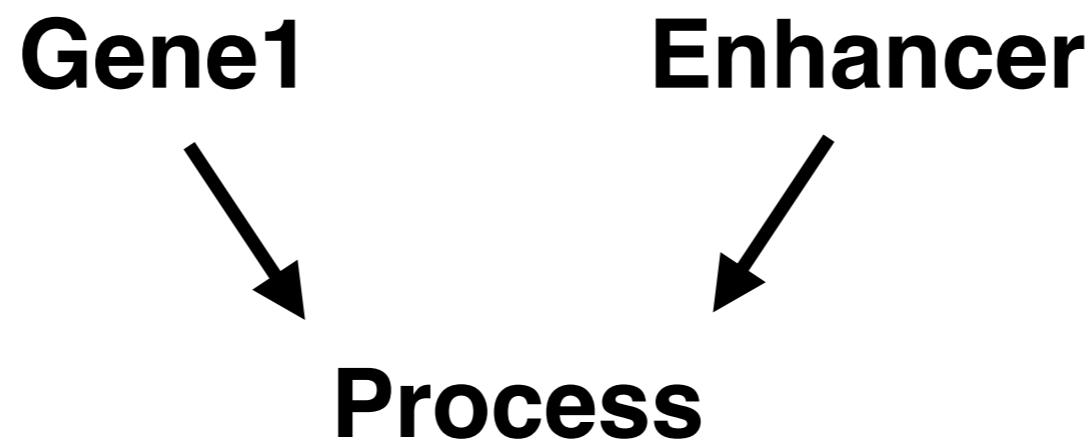
rol-8(0) = partial Roller

rol-8(0); sqt-1(0) = more Roller

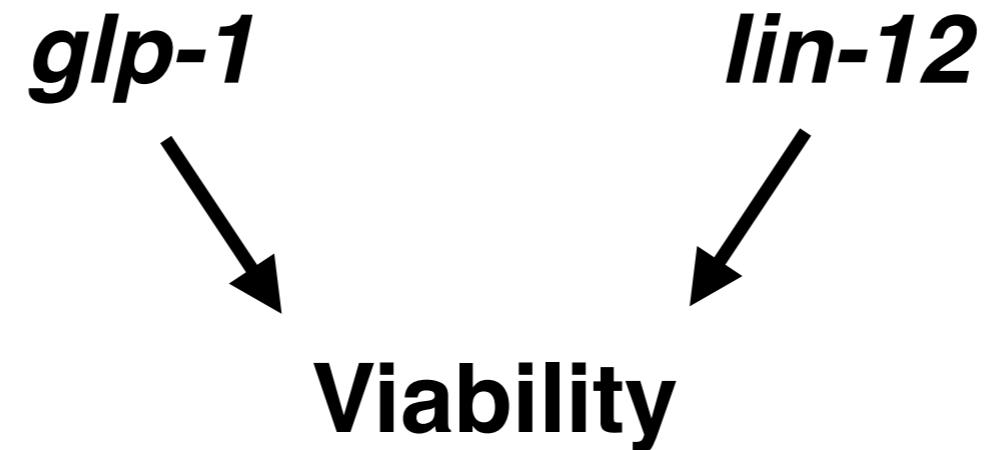
rol-8(0); sqt-1(0); sqt-2(0) = even more Roller

Enhancers act in redundant processes

1. Gene families (redundancy by similarity of function)
2. Parallel pathways (redundancy by similarity of process)



Example of pathway redundancy



glp-1(0) = germ line under proliferation
lin-12(0) = cell-fate specification defect

Found to be homologous

glp-1(0); lin-12(0) = dead

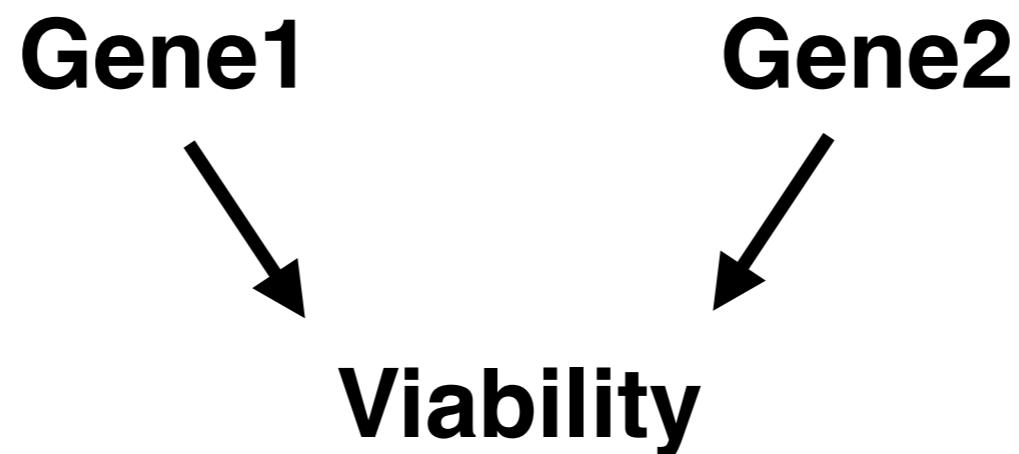
Enhancers act in redundant processes

1. Gene families (redundancy by similarity of function)
2. Parallel pathways (redundancy by similarity of process)
3. Intergenic noncomplementation

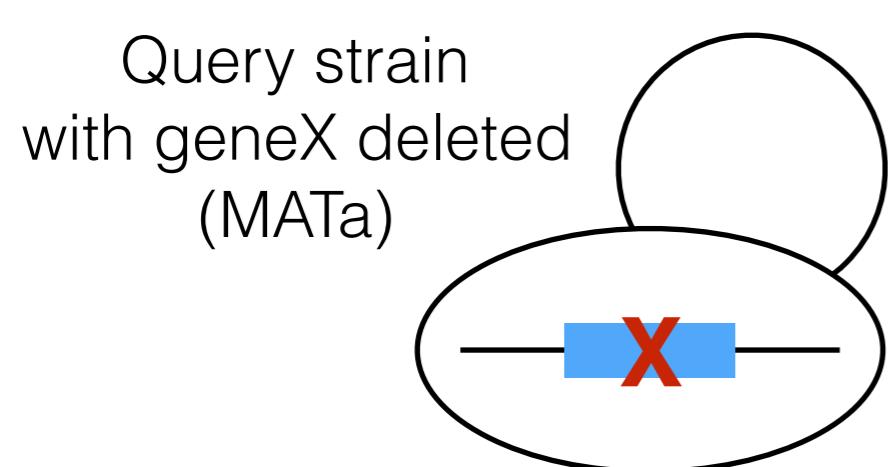
$$\begin{array}{c} \text{Gene1} \quad + \\ \hline + \quad \text{Gene2} \end{array}$$

Enhancers act in redundant processes

1. Gene families (redundancy by similarity of function)
2. Parallel pathways (redundancy by similarity of process)
3. Intergenic noncomplementation
4. Synthetic lethality



Synthetic lethal screens in yeast

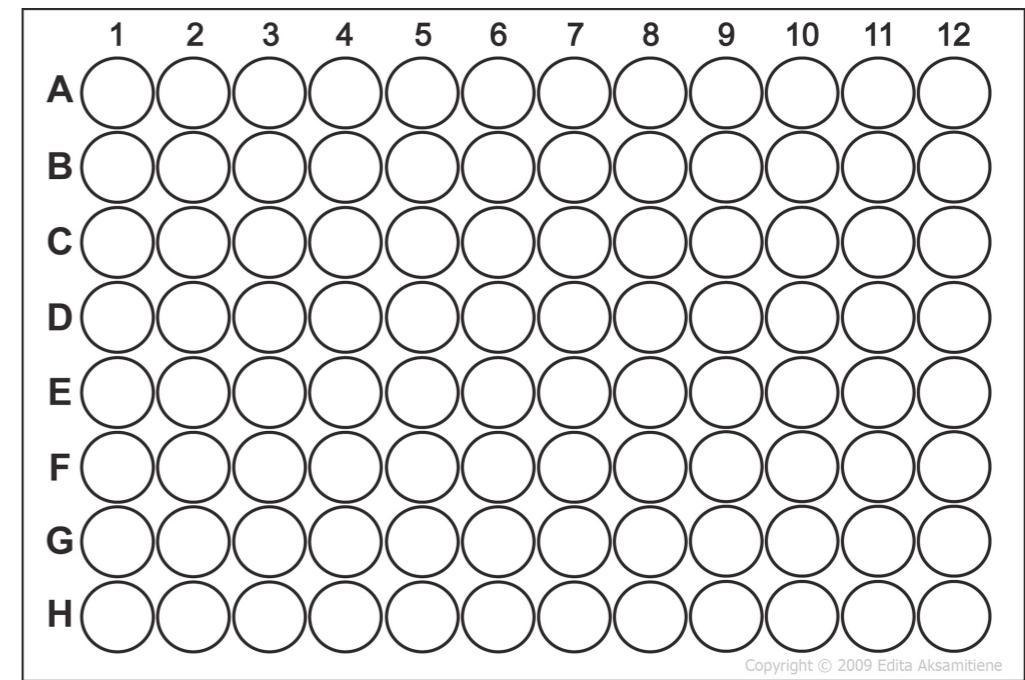
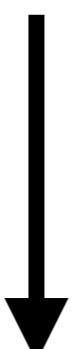


X

Mating and
sporulation of diploids

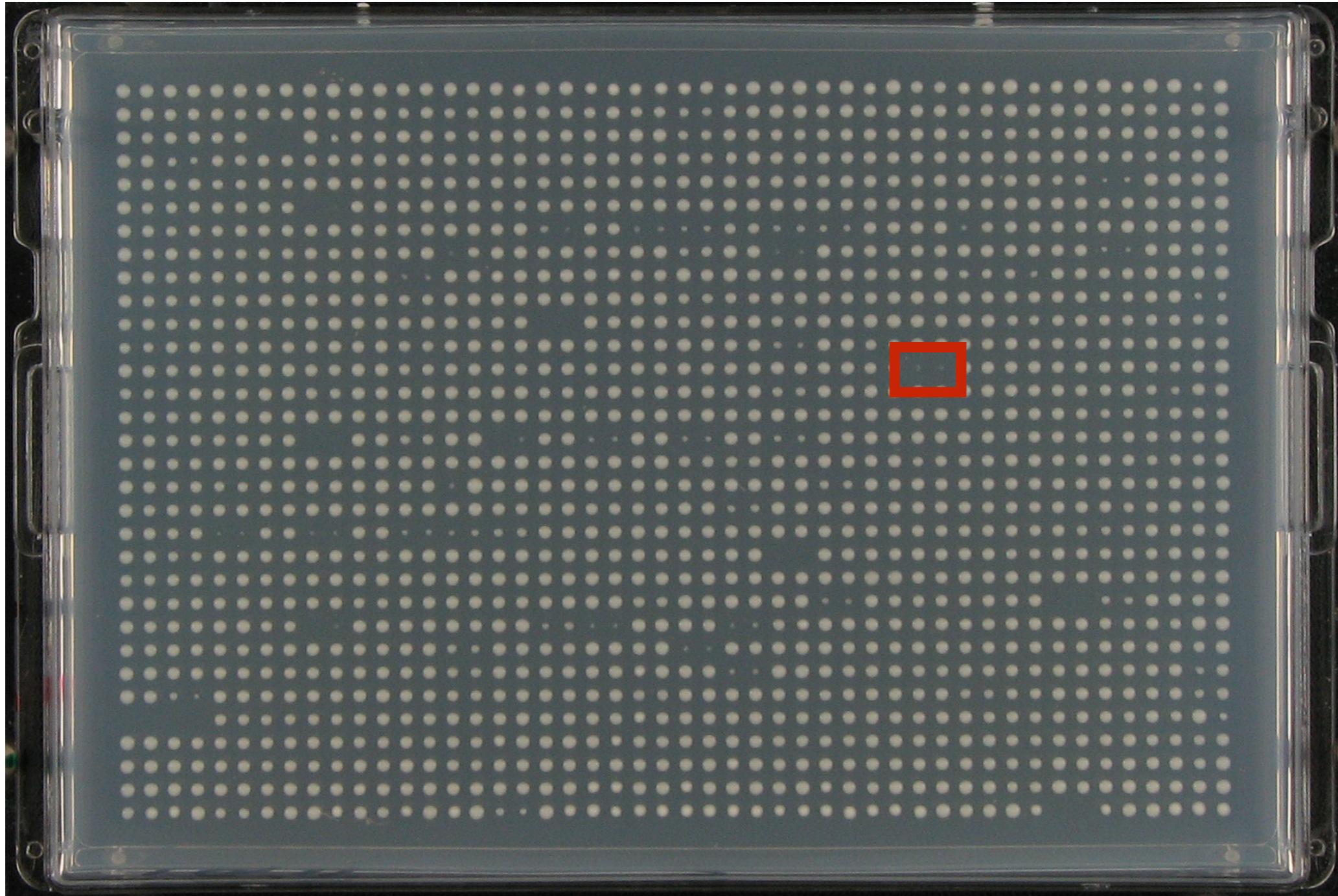


Selection of double mutants



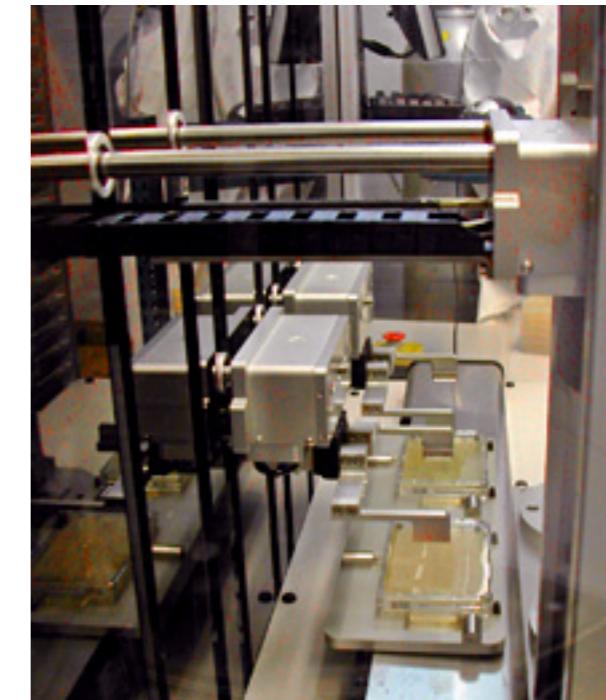
96 deletion collection strains
(MAT α)

Synthetic lethal screens in yeast



Two 96-well plates arrayed in duplicate
384 total wells

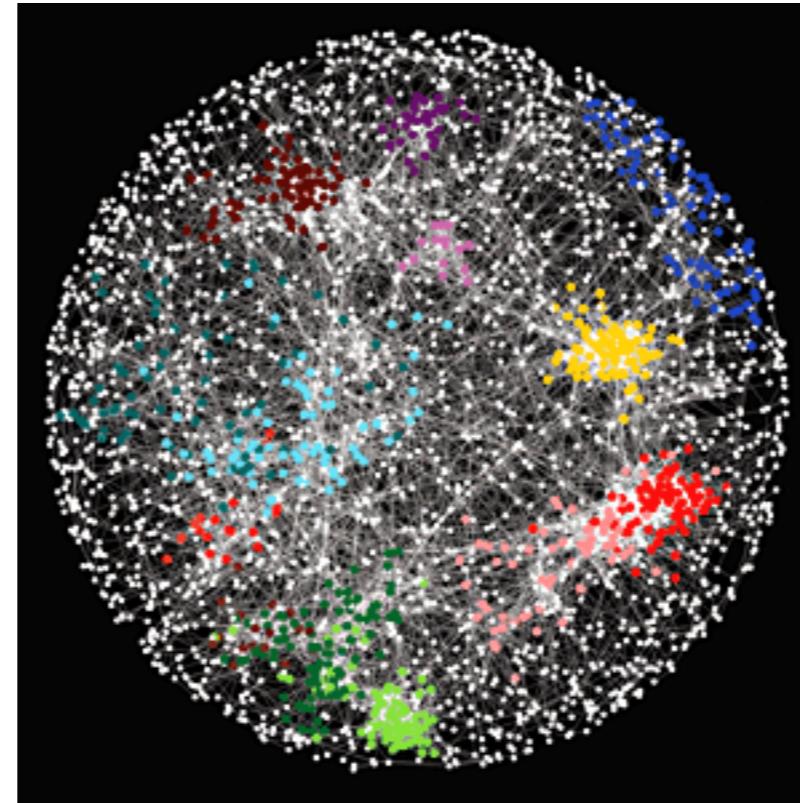
Synthetic lethal screens in yeast



Genetics at a massive scale

Mutation in every gene
crossed with a mutation in every other gene

Synthetic lethal screens in yeast

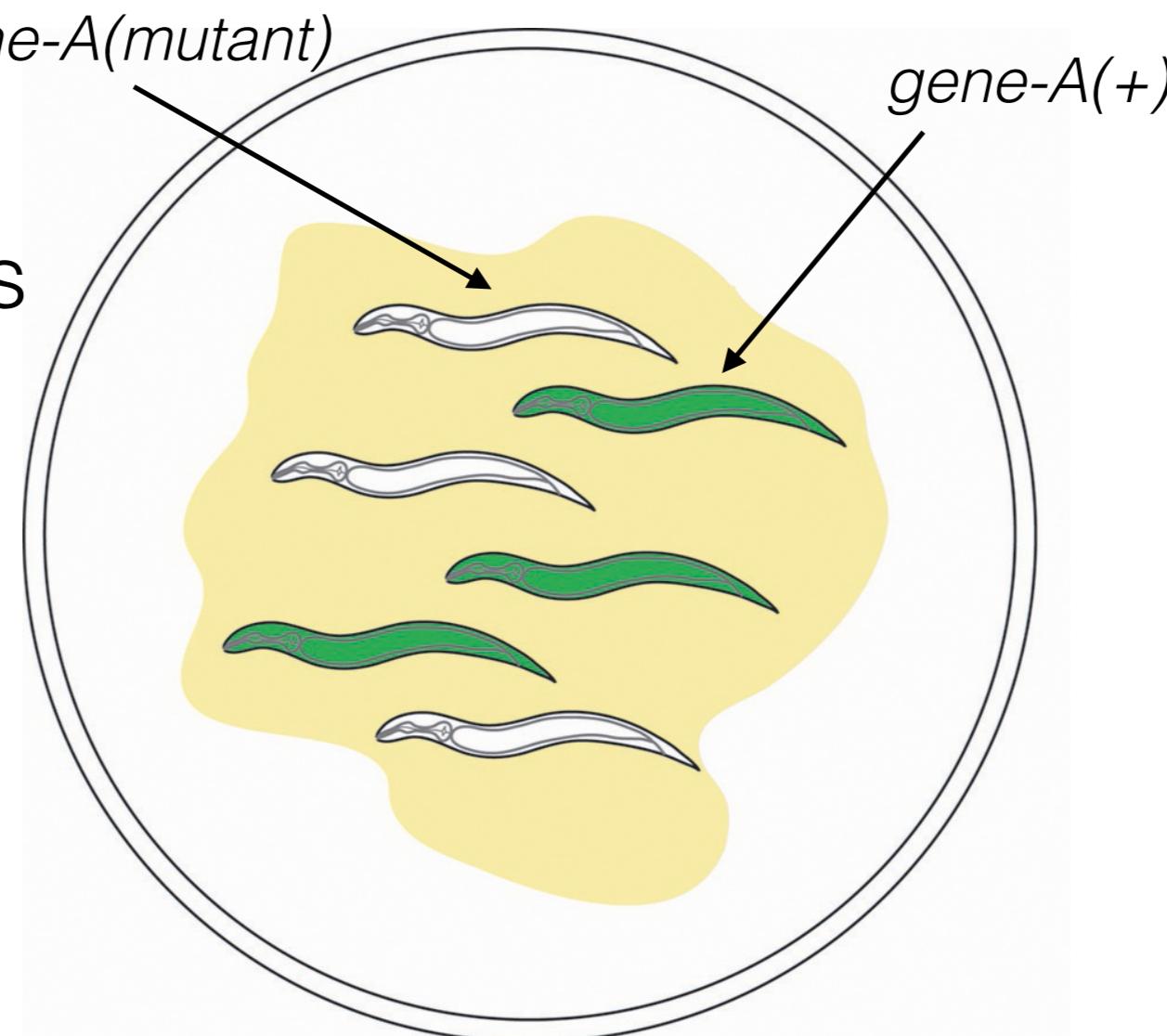


hairballs!

Mutation in every gene
crossed with a mutation in every other gene

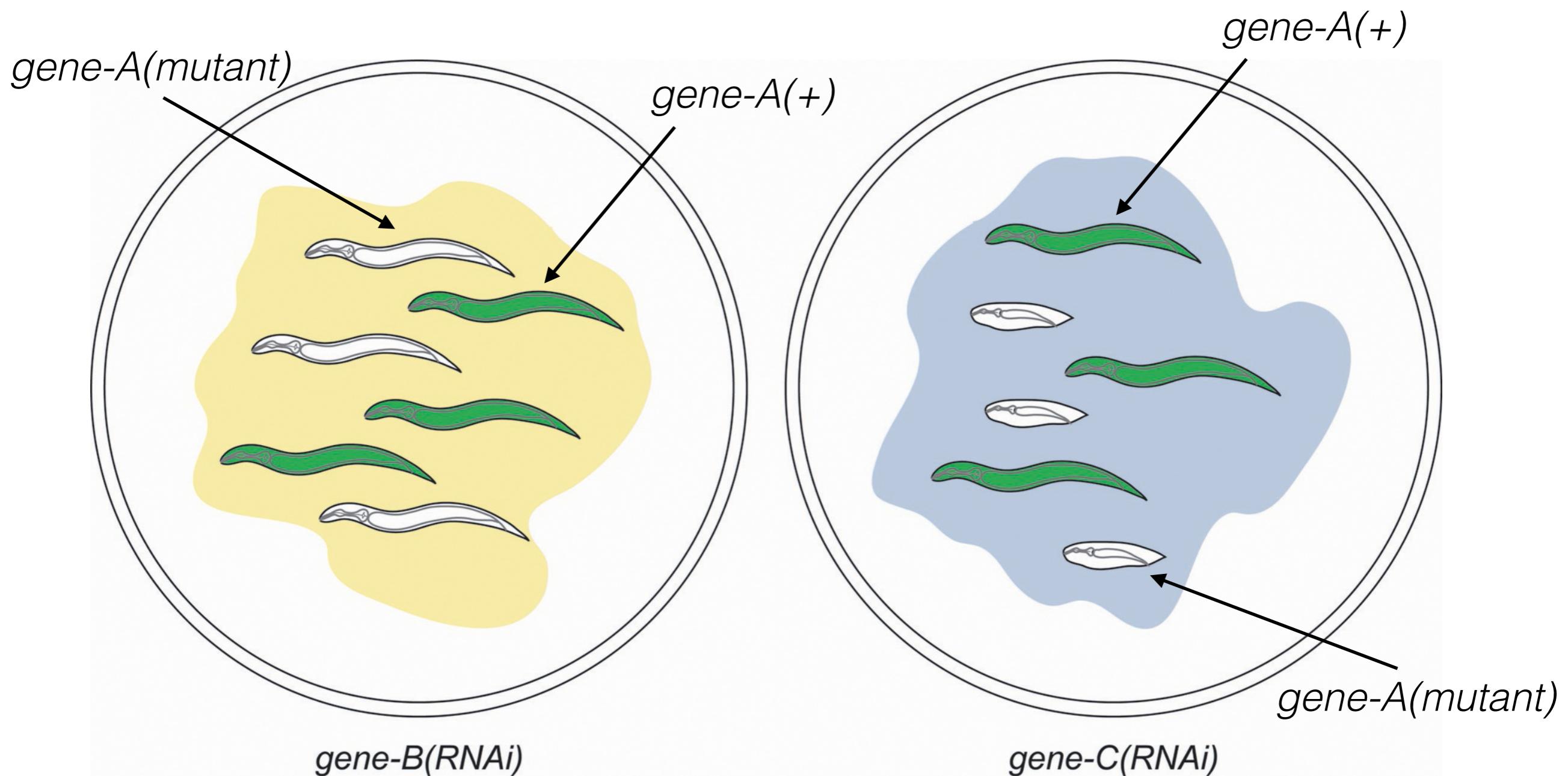
Synthetic lethal screens in *C. elegans*

Non-green worms
are mutant



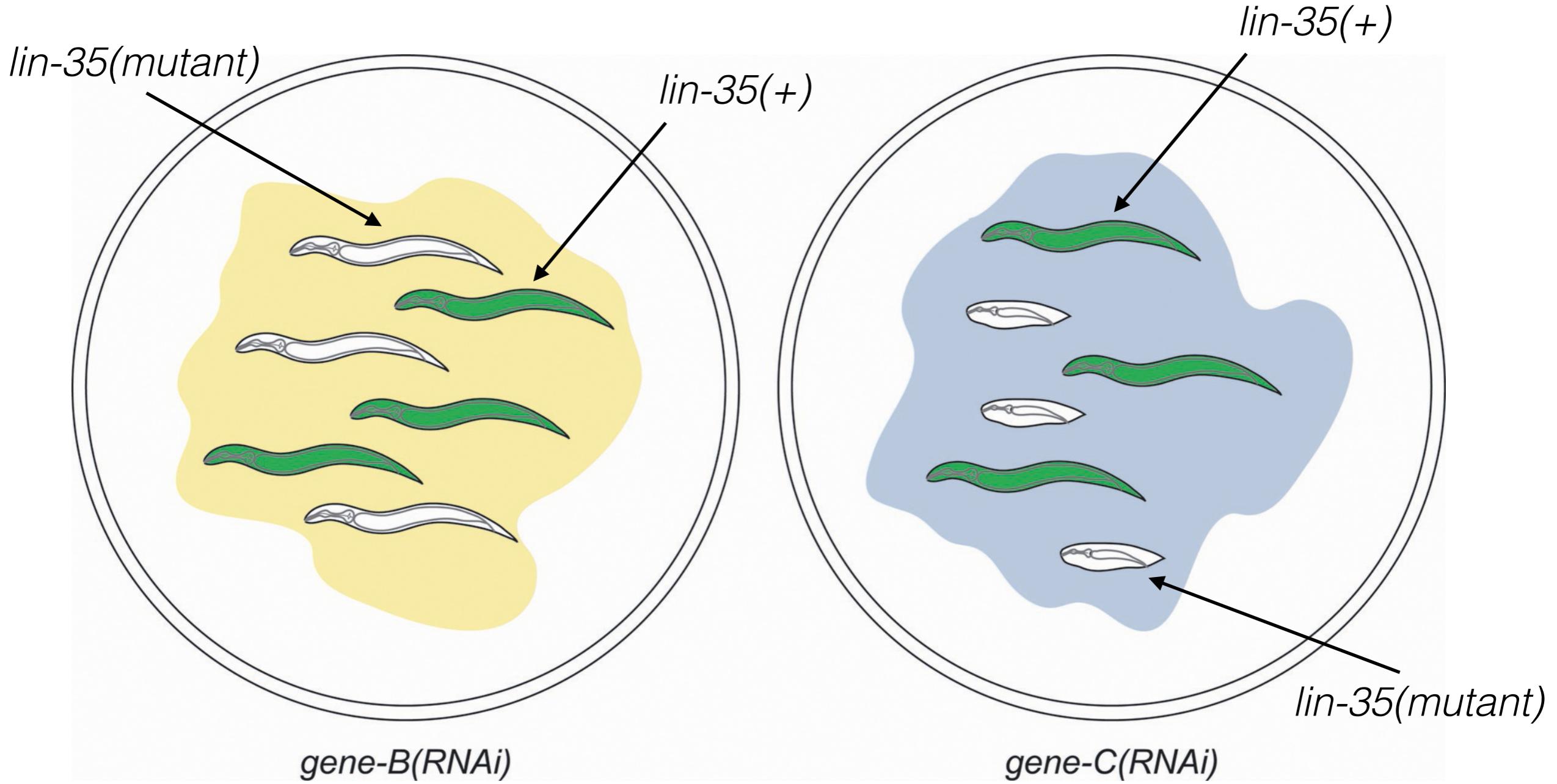
Nearly every gene in the worm can be inactivated
by RNA interference

Synthetic lethal screens in *C. elegans*



Loss of *gene-A* and *gene-C* is synthetic lethal

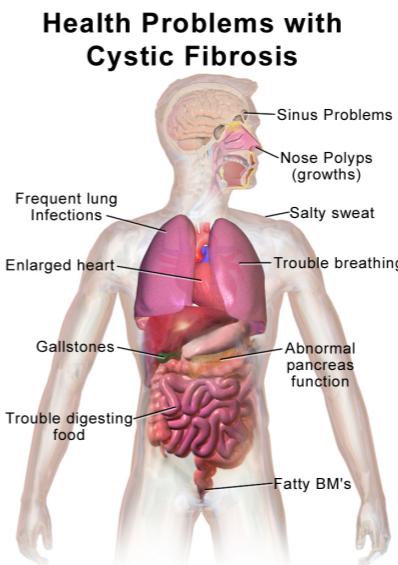
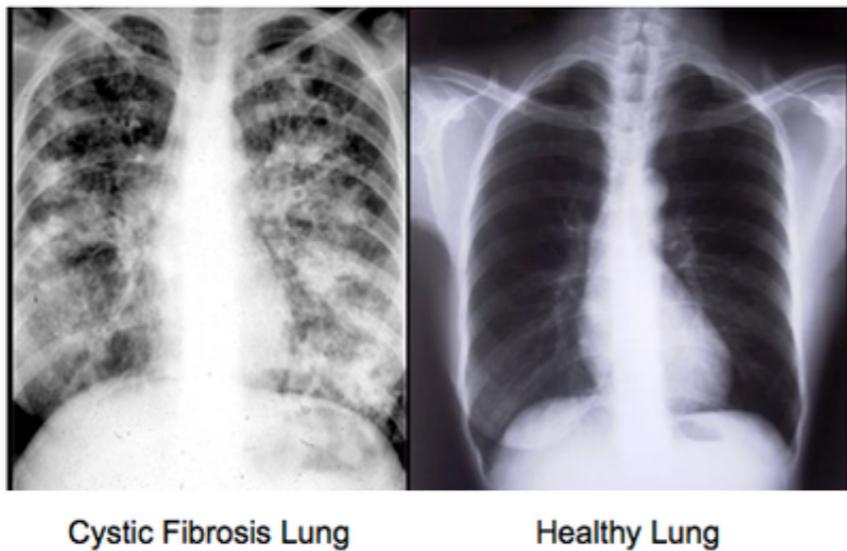
What do we learn?



lin-35 = Retinoblastoma (Rb) homolog in *C. elegans*

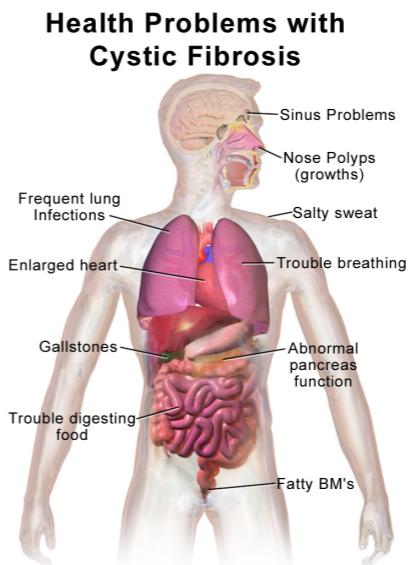
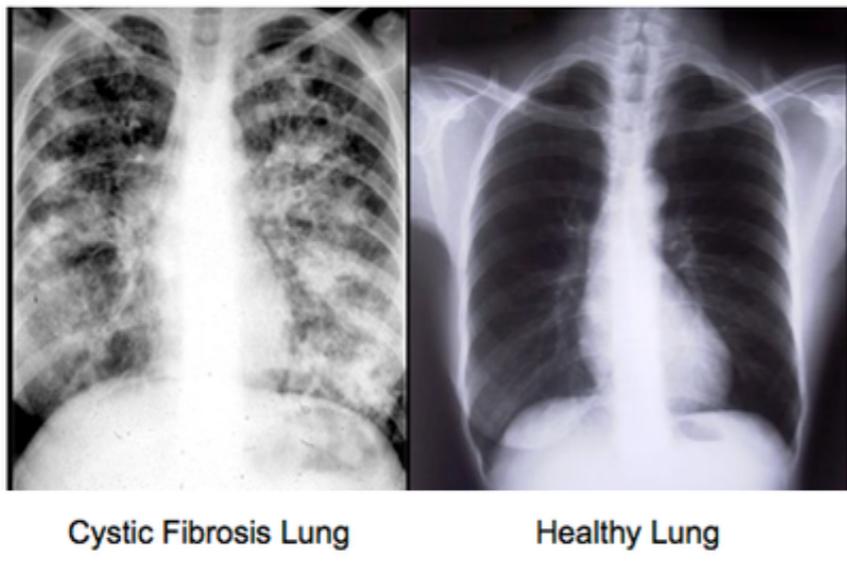
Identify genes that when lost cause *Rb* mutants to die
=drug targets!

What about cystic fibrosis and today's topic?

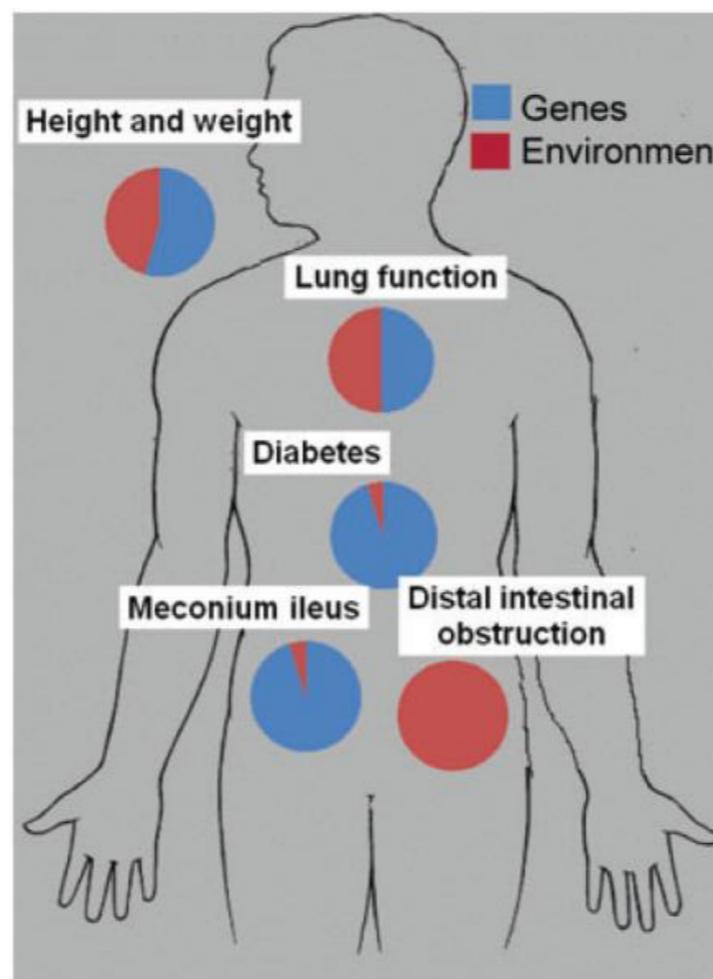


1. Autosomal recessive disorder
2. Not caused by chromosomal aberrations or meiotic NDJ
3. Mapped to chromosome 7
4. Mutations in CF gene are null or hypomorphs
5. Compound heterozygosity (failure to complement) is common

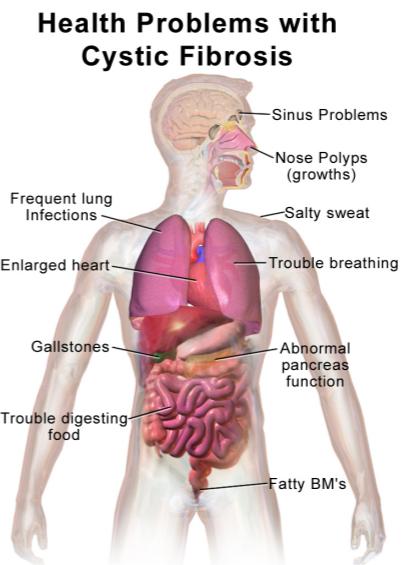
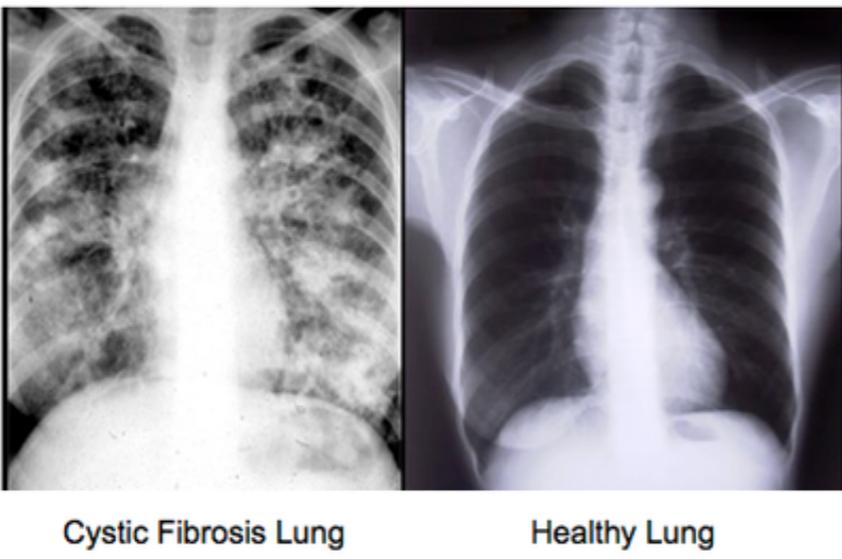
What about cystic fibrosis and today's topic?



Genetic modifiers are enhancers of CF



What about cystic fibrosis and today's topic?



Gene	Pulmonary Function (FEV1)	<i>P. aeruginosa</i>	Intestinal Obstruction	Diabetes	Liver Disease
		Acquisition/Colonization			
<i>ADIPOR2</i>			Possible effect ⁸⁷		
<i>EDNRA</i>	Probable effect ⁷⁹				
<i>IFRDI</i>	Possible effect ⁷⁴				
<i>IL8</i>	Possible effect ⁷⁵				
<i>MBL2</i>	Probable effect ^{55, 62, 64, 70, 94}	Probable effect ^{60, 70, 94}			
<i>MSRA</i>			Probable effect ⁸⁶		
<i>SERPINA1</i>	No effect ^{57, 95, 98}	Likely no effect ^{97, 99, 100}		Possible effect ⁴⁹	
<i>TCF7L2</i>				Probable effect ³⁷	
<i>TGFB1</i>	Probable effect ^{57, 59, 69, 70, 73, 78}	No effect ^{70, 72, 73, 78, 101}			Likely no effect ^{49, 101}

Probable effect: Association observed in ≥ 3 independent populations with ≥ 1000 participants in aggregate.

Possible effect: Association observed in ≥ 2 independent populations with ≥ 500 participants in aggregate.

Likely no effect: No association observed in ≥ 2 independent populations with ≥ 500 participants in aggregate.

No effect: No association observed in ≥ 3 more independent populations with ≥ 1000 participants in aggregate.

N.B.: Some studies include replication populations, which are treated as separate independent populations.