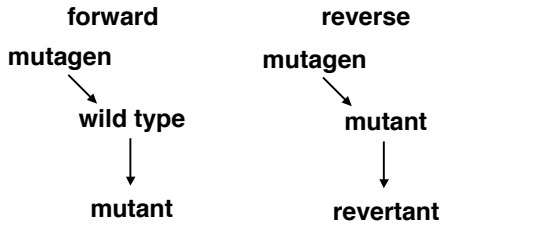
Suppressor genetics

Reading: lecture notes

Screens

1. Direct screen: phagocytosis and apoptotic genes in *C. elegans*
2. Mosaic screen: tumor suppressor genes
3. Revertant screens

Forward and reverse mutations



Don't confuse reverse mutations with reverse genetics

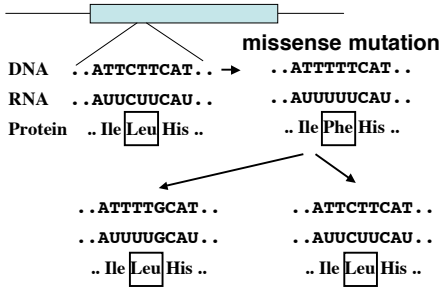
A revertant contains the original mutation and a suppressor mutation.

The suppressor can be:

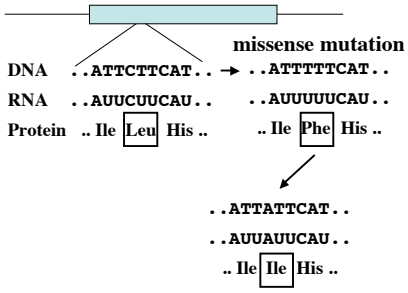
Intragenic: in the same gene

Extragenic: in another gene

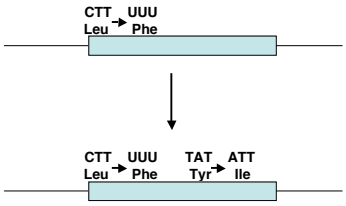
Intragenic suppressors: true revertants



Intragenic suppressors: partial revertants



Intragenic suppressors: second site



Second mutation compensates for the first

Extragenic suppressors

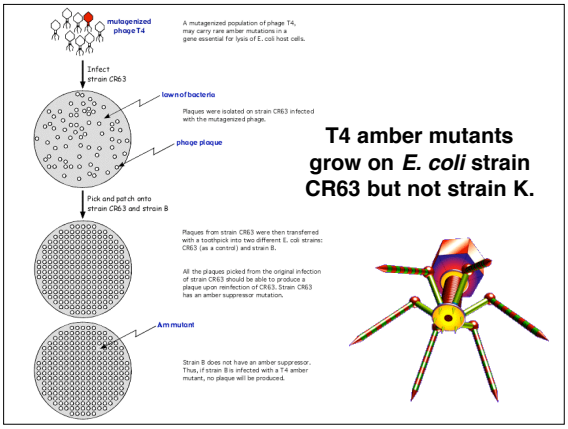
1. Informational suppressors: allele specific, gene nonspecific
2. Bypass suppressors (parallel pathways): allele nonspecific, gene specific
3. Bypass suppressors (same pathway): allele nonspecific, gene specific
4. Interaction suppressors: allele specific, gene specific

Informational suppressors

Two types that we will discuss:

Nonsense suppressors

smg suppressors



T4 amber mutants grow on *E. coli* strain CR63 but not strain K.

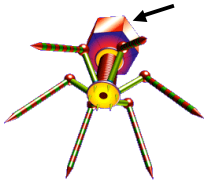
How amber mutants got their name.

Charley agreed to help look for the mutants, picking 2000 plaques in the first try. We also managed to convince Harris Bernstein (then a graduate student working on *Neurospora* genetics) to help and offered him the dubious reward of naming the mutants after him. Harris had the nickname Immer Wieder Bernstein ("Forever Amber" in German). That night we isolated several of the desired mutants and named them "amber mutants."

Dick Epstein letter to Frank Stahl

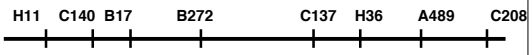
Amber mutations can be in many different T4 genes. (and *E. coli* genes)

The T4 head protein gene.



Amber mutations are nonsense mutations

Genetic map of amber mutations in T4 head protein gene

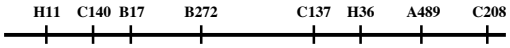


Protein size

wild type
C208
A489
H36
C137
B272
B17
C140
H11

So if amber mutations are nonsense mutations, why do nonsense mutants grow on strain CR63?

Genetic map of mutations in T4 head protein gene



on strain B
wild type
C137

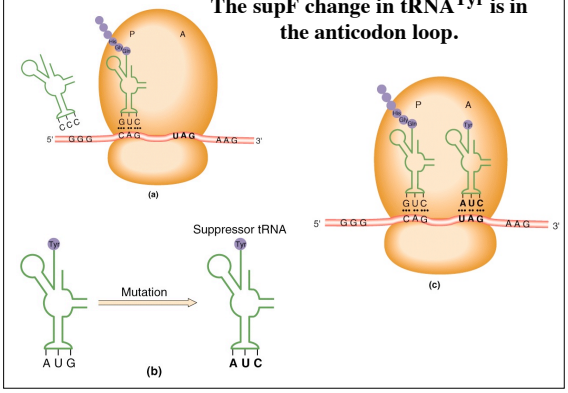
on strain CR63
wild type
C137

Because strain CR63 makes full length T4 head protein!

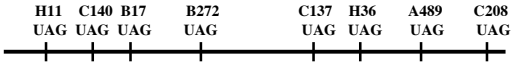
The difference between the two *E. coli* strains can be mapped.

The difference, known as *supF*, mapped to a $tRNA^{Tyr}$ gene.

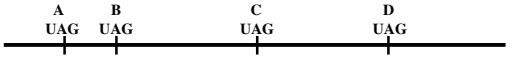
The supF change in $tRNA^{Tyr}$ is in the anticodon loop.



Genetic map of mutations in T4 head protein gene

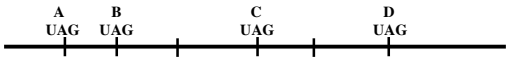


Genetic map of the *E. coli phoA* gene



The *supF* suppressor will suppress UAG amber mutations in the T4 head protein gene and in the *phoA* gene and in many other genes. Therefore the suppressor is gene nonspecific.

Genetic map of the *E. coli phoA* gene



UAA stop

F to G missense

But *supF* will not suppress other nonsense mutations (opal and ochre), missense mutations, frameshift mutations or deletions. Therefore *supF* is allele specific in its suppression.

A couple of final questions.

Will *supF* suppress all UAG nonsense mutations?

Why do *supF E. coli* live?

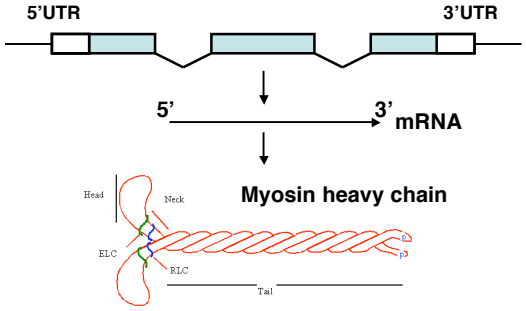
smg suppression

(Suppressors with morphological defects in the genitalia)

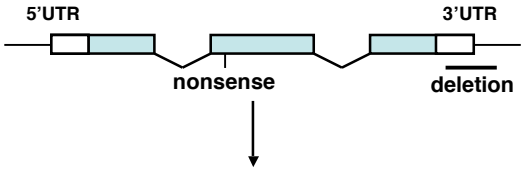
Informational suppressors
Allele specific, gene nonspecific

Mutations in seven genes isolated as allele-specific suppressors of *tra-2, lin-29,* and *unc-54.*

The *unc-54* gene encodes muscle myosin



unc-54 mutants are paralyzed



No myosin--animals paralyzed

Suppression of the Unc-54 locomotion defect identified recessive *smg* suppressors

EMS

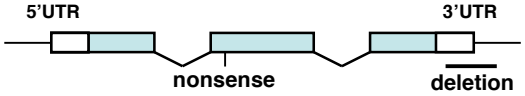
***unc-54(del)/unc-54(del)* hermaphrodites**

F1

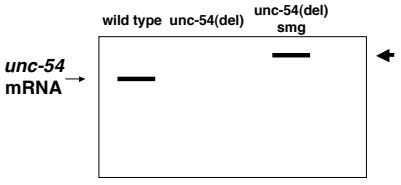
F2 suppressed animals that move

(unc-54/unc-54; smg/smg)

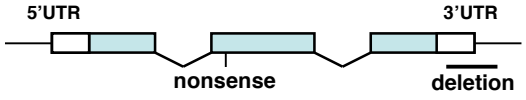
Suppression caused by stabilizing abnormal mRNA



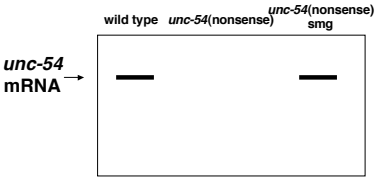
Northern blot probed with labeled *unc-54* DNA



***Smg* mutations can also suppress mRNA instability caused by nonsense mutations (but not Unc phenotype).**



Northern blot probed with labeled *unc-54* DNA



Model

***smg* genes encode components of a mRNA surveillance system that degraded abnormal.**

nonsense mediated mRNA decay