

## Lecture 12

### Gene Mutations

Let's say that we are investigating the LacZ gene, which encodes the lactose hydrolyzing enzyme  $\beta$ -galactosidase. There is a useful compound known as X-gal that can be hydrolyzed by  $\beta$ -galactosidase to release a dark blue pigment. When X-gal is added to the growth medium in petri plates, Lac<sup>+</sup> E. coli colonies turn blue whereas Lac<sup>-</sup> colonies with mutations in the LacZ gene are white. By screening many colonies on such plates it is possible to isolate a collection of E. coli mutants with alterations in the LacZ gene. PCR amplification of the LacZ gene from each mutant followed by DNA sequencing allows the base changes that cause the LacZ<sup>-</sup> phenotype to be determined. A very large number of different LacZ mutations can be found but they can be categorized into three general types.

<b>Mutation Type</b>	<b>Description</b>
Missense	A base change that converts one codon into another. Many missense mutations are silent because the encoded amino acid remains the same or the amino acid substitution is sufficiently subtle so as not to compromise activity of the enzyme. Missense mutations that have a marked effect often lie in the active site or grossly disrupt protein folding.
Nonsense	A base change that converts a codon within the coding sequence into a stop codon. Note that there is only a limited set of sense codons that can be converted to a stop codon by a single base change. Nonsense mutations lead to a truncated protein product. Nonsense mutations that lie early in the gene sequence will completely inactivate the gene. Sometimes nonsense mutations that lie late in the gene sequence will not disrupt gene function.
Frameshift	The addition or deletion of a base or bases such that the coding sequence is shifted out of register. Note that addition or deletion of a multiple of three bases does not cause a frameshift. After the frameshift mutation is encountered, missense codons will be read up to the first stop codon. Like nonsense mutations, frameshift mutations usually lead to complete inactivation of the gene.

Although many different kinds of mutations occur spontaneously, the frequency with which mutations occur can be increased as much as  $10^3$  fold by treatment of cells with a mutagen. Here are some general categories of mutagens

Type of Mutagen	Mechanism	Examples	Type of Mutations
Base Analog	Analog is incorporated into DNA and can pair with more than one base	5-bromouracil 2-aminopurine	$A \cdot T \rightarrow G \cdot C$ , $G \cdot C \rightarrow A \cdot T$ $A \cdot T \rightarrow G \cdot C$
Base modifying agent	Chemical or photo damage to DNA can be repaired, but repair itself is error prone	Hydroxylamine EMS UV	$G \cdot C \rightarrow A \cdot T$ $G \cdot C \rightarrow A \cdot T$ , $C \cdot G$ , or $T \cdot A$ All changes
Intercalating agent	Polycyclic compounds can fit between bases and cause mis-copying by polymerase to add or delete bases	Acridine Proflavine ICR-191	Frameshifts (+ or -) " "

### Suppressor mutations

A powerful mode of genetic analysis is to investigate the types of mutations that can reverse the phenotypic effects of a starting mutation. Starting with a  $LacZ^-$  mutant of *E. coli* (white on X-gal), we could mutagenize and then plate out a large number of colonies on X-gal plates to screen for rare colonies that were blue on X-gal. These **revertants** could have either been mutated such that the starting mutation was reversed or they could have acquired a new mutation that somehow compensates for the starting mutation.

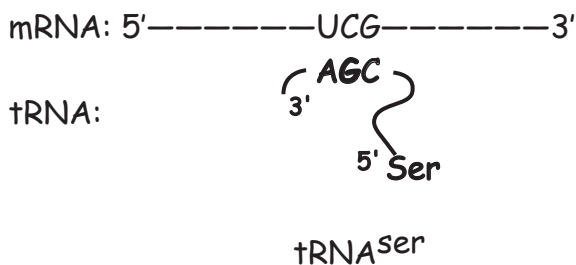
The possibilities are:

- 1) back mutation - true wild type
- 2) intragenic suppressor - compensating mutation in same gene
- 3) extragenic suppressor - compensating mutation in different gene

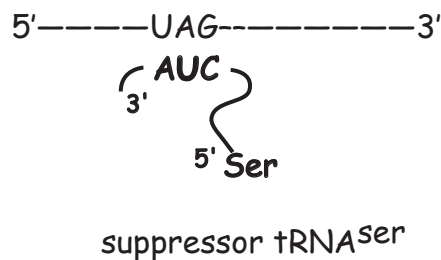
## Nonsense suppressors.

An important class of extragenic suppressor mutations can suppress nonsense mutations by changing the ability of the cells to read a nonsense codon as a sense codon. Such extragenic revertants were originally isolated by selecting for reversion of amber (UAG) mutations in two different genes. Since simultaneous back mutations at two different sites is highly improbable the most frequent mechanism for suppression is a single mutation in the gene for a tRNA that changes the codon recognition portion of the tRNA. For example, one of several possible nonsense suppressors occurs in the gene for a serine tRNA (tRNA<sup>ser</sup>). One of six tRNA<sup>ser</sup> normally contains the anticodon sequence CGA which recognizes the serine codon UCG (by convention sequences are given in the 5' to 3' direction). A mutation that changes the anticodon to CUA allows the mutant tRNA<sup>ser</sup> to recognize a UAG codon and insert tryptophan when a UAG codon appears in a coding sequence.

Recognition of UCG (serine codon)  
by wild type tRNA<sup>ser</sup>



Recognition of UAG(stop codon)  
by amber suppressor mutant tRNA<sup>ser</sup>



The presence of an amber suppressing mutation is usually designated Su<sup>+</sup> whereas a wild-type (nonsuppressing) strain would be designated Su<sup>-</sup>.

Example: LacZ<sup>am</sup> designates an amber (nonsense) mutation in the LacZ gene. Whereas a LacZ<sup>am</sup> E. coli strain is Lac<sup>-</sup> and therefore is white on X-gal plates, a strain with both a LacZ<sup>am</sup> mutation and an amber suppressor (Su<sup>+</sup>) mutation in a tRNA gene might be phenotypically Lac<sup>+</sup> because of extragenic suppression of the LacZ<sup>am</sup> mutation.

The combined use of amber mutations and an amber suppressor produces a conditional mutation, ie a mutation that is expressed under some circumstances but not under others. Conditional mutants are especially useful for studying mutations in essential genes. Another kind of conditional mutation is a temperature sensitive mutation for which the mutant trait is exhibited at high temperature but not at low temperature.