Lecture 11

Gene Mutations

Let's say that we are investigating the LacZ gene, which encodes the lactose hydrolyzing enzyme ß-galactosidase. There is a special compound known as X-gal that can be hydrolyzed by ß-galactosidase to release a dark blue pigment. When X-gal is added to the growth medium in petri plates, Lac^+ *E. coli* colonies turn blue whereas Lac^- 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 $LacZ^-$ phenotype to be determined. A very large number of different LacZ mutations can be found but they can be categorized into three general types.

Mutation Type	Description
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	A•T → G•C, G•C → A•T
		2-aminopurine	A•T → G•C
Base Modifying Agent	Chemical or photo damage to DNA can be repaired, but repair itself is error prone	Hydroxylamine	G•C → A•T
		EMS	G•C → A•T, C•G or T•A
		UV	All changes
Intercalating Agent	Polycyclic compounds can fit between bases and cause mis-copying by polymerase to add or delete bases	Acridine	Frameshifts (+ or –)
		Proflavine	"
		ICR-191	"

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. Say that you start with a **mi**$^-$ λ phage mutant that makes small plaques. After plating a large number of these mutant phage rare revertants can be isolated by looking for phage that have restored the ability to make large plaques. 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

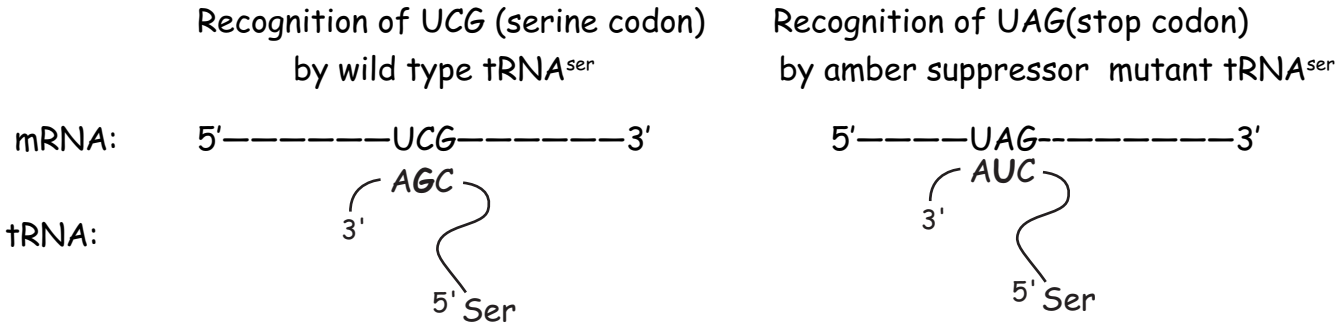
2) **intragenic suppressor** - compensating mutation in same gene

3) **extragenic suppressor** - compensating mutation in different gene

These possibilities can be distinguished in that a revertant that arose by suppression will still carry the starting mutation (now masked by the suppressor mutation), whereas a back mutation will produce a true wild type phage. The general test is to cross the revertant to wild type and to note whether **mi⁻** recombinants are observed. A back mutation crossed to wild type will not produce any **mi⁻** progeny, whereas a revertant that results from an extragenic suppressor will produce many **mi⁻** recombinants. Intragenic suppressors will produce an intermediate result that sometimes can be difficult to distinguish from a back mutation in practice. For example, an intragenic suppressor that lies very close to the original **mi⁻** mutation may be able to produce **mi⁻** recombinants in principle but these recombinants may be too rare to be readily observed.

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 sense. 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 ($tRNA^{ser}$). One of six $tRNA^{ser}$ 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 $tRNA^{ser}$ to recognize a UAG codon and insert tryptophan when a UAG codon appears in a coding sequence.



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

Example: **Pam** designates an amber (nonsense) mutation in the λ phage **P** gene, which is required for λ phage DNA replication. When **λ Pam** phage are grown on *E. coli* with an amber suppressor (Su^+) the phage multiply normally, but when **λ Pam** phage infect a nonsuppressing host (Su^-) the phage DNA cannot replicate.

The combined use of amber mutations and an amber suppressor produces a **conditional mutant**, which is a mutant 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. In a sense, auxotrophic mutations are also conditional because auxotrophic mutants can be grown in the presence of the required nutrient but the mutants will not grow when the nutrient is not provided.