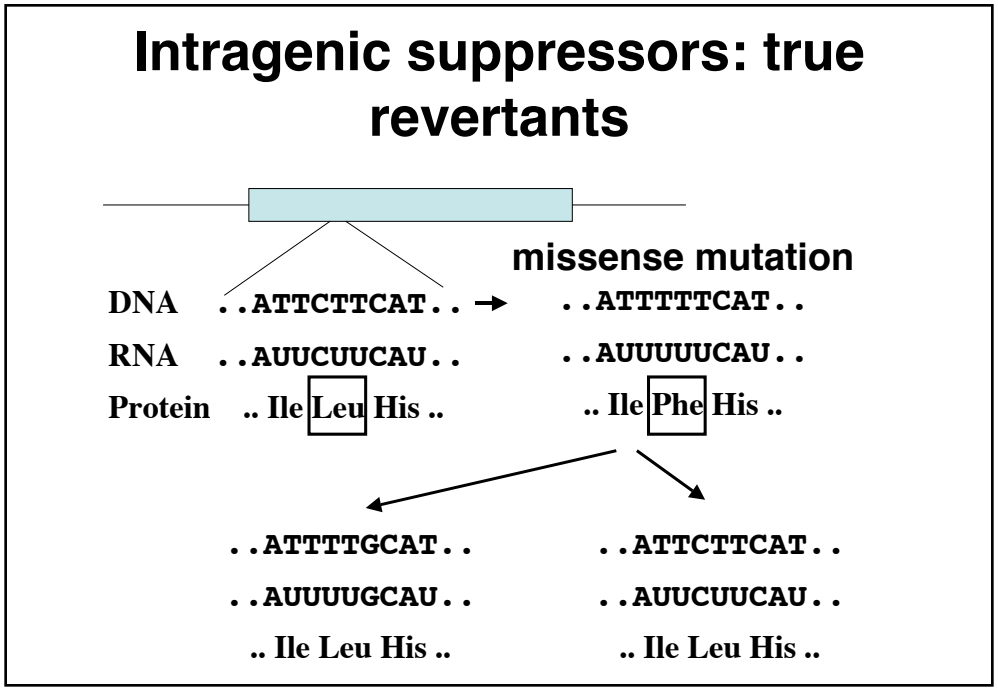
Suppressor Genetics: intragenic and informational suppressors

We have covered one approach used in genetics to study gene function: classical forward genetics where an investigator selects or screens for mutants defective in a cellular process.

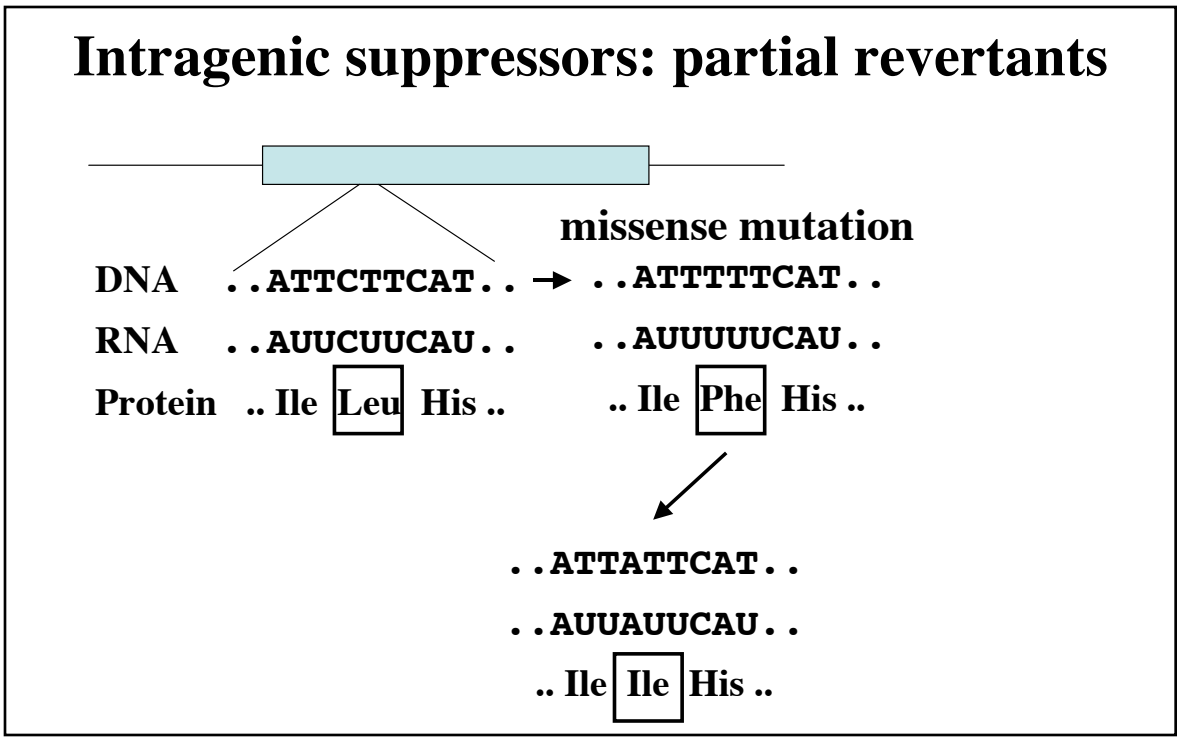
Another approach to studying a gene's function is to look for interacting genes. Mutations in interacting genes are often isolated as suppressors of mutations in the gene that is being studied. In other words, you mutagenize a mutant, and look for mutations that suppress the mutant phenotype; i.e., you select or screen for animals that appear wild type. These strains are referred to as revertants, since the mutant phenotype has been reverted to wild type or closer to the wild-type phenotype. Revertants can either be intragenic or extragenic.

Intragenic suppressors

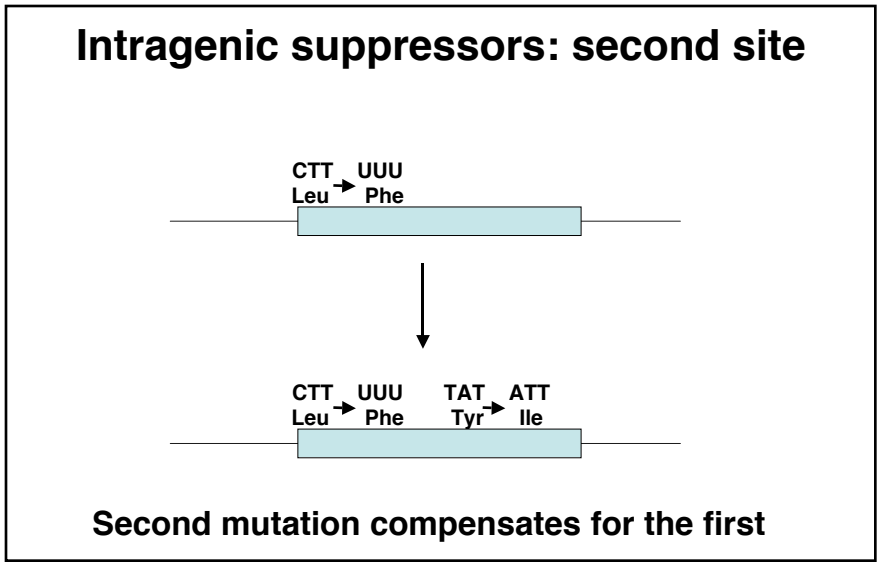
There are several types of intragenic suppressors. Sometimes a mutant protein can be restored to wild type. Let's consider a gene that has a CUU codon, which encodes a leucine that is mutated to a UUU codon to generate a mutant protein where phenylalanine replaces Leu. This is the forward mutation. There are several types of revertants that could be isolated, all of which restore normal or nearly normal protein function. A revertant that changes the UUU codon back to CUU or to UUG, both of which encode Leu, is called a true revertant because they restore the protein back to wild type.



UUU could also be changed to AUU, which encodes Ile. Because Ile is structurally more similar to Leu than Phe, this could restore protein function. In cases were the revertant results in a different, but more conservative change than the original mutation, protein function is often partially restored, and this type of revertant is referred to a partial revertant.

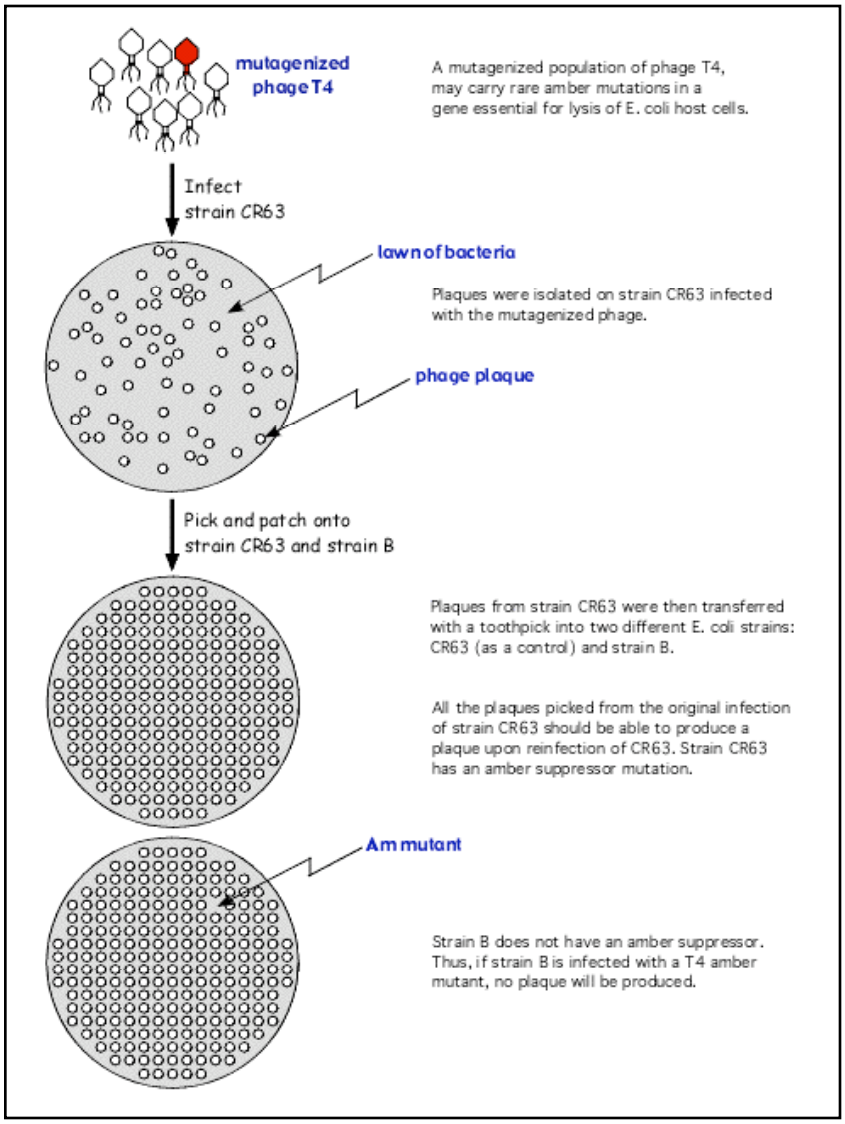


Often times, a revertant is caused by a change in another amino acid in the protein. This type of intragenic suppressor is referred to as second site because they define a different position in the protein.



Extragenic suppressors
Another type of second site suppressor is a mutation in another gene that compensates for the original lesion, also known as an extragenic suppressor. This type of suppressor will be the focus of the remainder of this lecture and the next lecture. Extragenic suppressors can be of various types.

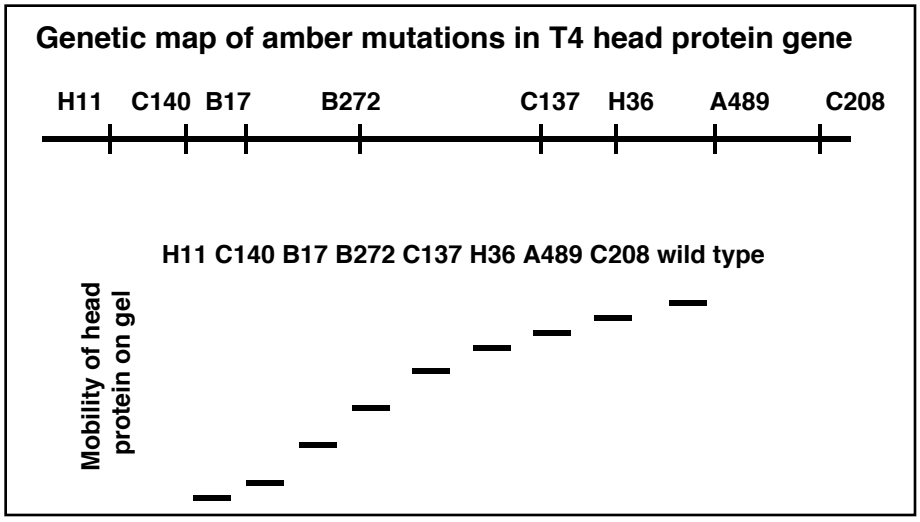
Informational suppressors
A mutant gene can sometimes be restored to near normal function by modifying the machinery in which genetic material is transcribed and translated. Suppressors that restore gene function by alteration of this machinery are known as informational. Information suppressors can be identified by their genetic behavior: they are allele specific, gene nonspecific suppressors. We will consider two types of informational suppressors here: nonsense suppressors and *smg* suppressors.



Nonsense mutations and their suppressors
Nonsense mutations were initially defined by a class of bacteriophage T4 mutations called *amber*. These mutations are conditionally lethal, growing on certain *E. coli* strains but not others.

These mutations defined many different complementation groups, but the basis of their phenotype was not understood. Work by Sydney Brenner in the

1960s showed that these *amber* mutations were nonsense mutations and that the variant *E. coli* strains that allowed growth of these mutant T4 phage contained nonsense suppressors. Brenner had isolated 10 T4 *amber* mutations in the gene 23, which encodes the major T4 head protein. Genetic crosses between these mutations and the analysis of intragenic recombination defined a fine structure map of the gene. The primary sequence of the head protein had been determined from protein sequencing and digestion of the head protein with trypsin and chymotrypsin generated eight distinct protein fragments that could be separated electrophoretically. Because the sequence was known, the position of each digestion fragment in the protein was known. If total protein of infected cells is labeled with radioactive amino acids and then digested with trypsin and chymotrypsin the head protein fragments can be easily seen since more that 50% of the total protein is head protein. When each of the *amber* gene 23 mutants were used in these experiments they gave different numbers of head protein fragments. For example one mutation positioned at one end of the map generated only one fragment from the amino terminus of the protein, whereas a mutation positioned at the other end of the gene 23 map produced seven fragments and was missing the carboxy terminal fragment. The mutations in the middle gave more or fewer fragments depending on whether the mutations mapped closer to one end or the other. Brenner and his coworkers proposed that nonsense codons generated interrupted polypeptide chain assembly. These results also provided evidence that the nucleotide sequence defined by the genetic map and the protein sequence were colinear.

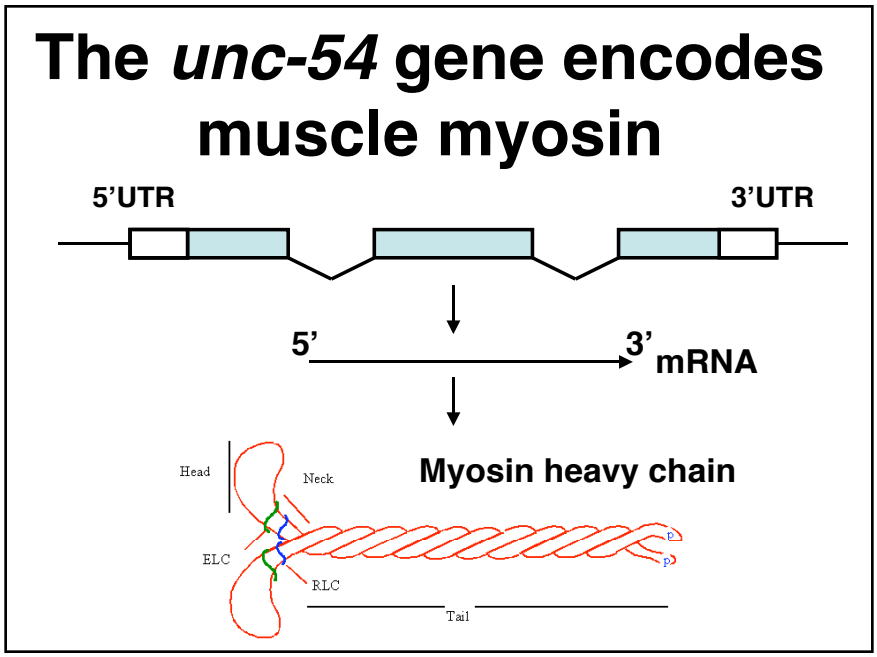


When the same experiments with the mutants were conducted in a permissive *E. coli* strain, each mutant phage made full length head protein. Brenner proposed that the variant strain contained a nonsense suppressor. This result was consistent with other genetic observations. Work on other genes showed that only some mutations in those genes were suppressed in this *E. coli* strain. The suppressor was eventually

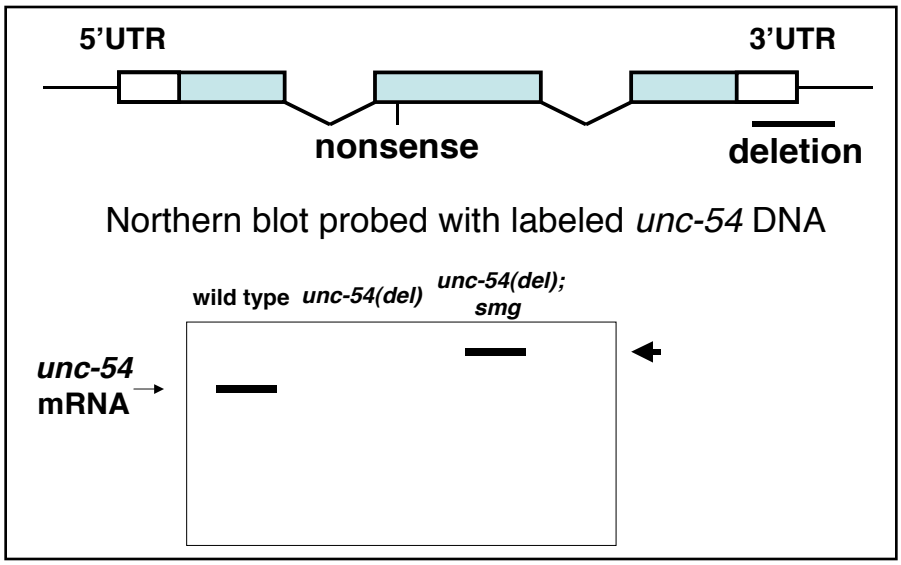
isolated and shown to be a $tRNA^{Tyr}$ with a mutation in the anticodon loop that now recognized the UAG amber stop and put a Trp in the growing peptide chain. Thus the suppressor in the strain is an allele specific, gene nonspecific suppressor. Information suppressors of any type are always allele specific, gene nonspecific.

smg suppression

Mutations in six *C. elegans* genes act as recessive suppressors that are allele specific and gene nonspecific. These genes are referred to as *smg* (suppressor with morphological defects in genitalia - I don't make up these names) because they are suppressors and cause defects in morphogenesis of the hermaphrodite vulva and male tail. These *smg* mutations were all isolated as suppressors of specific mutations in three genes, *tra-2*, *lin-29* and *unc-54*. We will only consider the *unc-54* suppression screen here. The *unc-54* gene encodes the major heavy chain myosin in *C. elegans*, and mutations in this gene cause animals to be paralyzed because their muscles don't contract. The *unc-54* mutants, however, are viable and fertile. A specific deletion mutation of *unc-54* that is missing 3' untranslated sequences but contains all *unc-54* coding sequences leads to an unstable RNA and greatly reduced levels of myosin in homozygous mutant animals. Hermaphrodites homozygous for this mutation were mutagenized and their F2 progeny were screened for suppressed animals that move. All of the suppressors that were isolated in these screens also suppress specific mutations in other genes but don't suppress other *unc-54* mutations. *smg* suppressible mutations are usually weak mutations that don't completely eliminate gene activity, and where genes containing these *smg* suppressible alleles have been cloned, it has been found that the mutations cause the RNAs to be unstable, similar to the situation for the *unc-54* allele. Analysis of *unc-54*



mRNA in *smg; unc-54* animals shows that the *smg* mutation restores normal levels of *unc-54* RNA, but increased levels of wild-type *unc-54* mRNA is not seen in *smg* mutants carrying the wild-type *unc-54* gene. It has been proposed that the *smg* genes are part of a surveillance system that detects and degrades aberrant RNAs resulting from errors of transcription, mRNA processing or mRNA transport.



One interesting feature of this work comes from the analysis of nonsense mutations in the *unc-54* gene. Nonsense mutations normally produce mRNAs that are unstable, and the mRNA of *unc-54* nonsense mutations are rapidly degraded. However, in a homozygous *smg* background, the *unc-54* mRNAs containing the nonsense mutations are stable and accumulate. This is interesting for two reasons. First, it suggests that the *smg* system may also function to remove mRNAs that have been mistranscribed and contain premature stop codons. Second, some of these *unc-54* nonsense mutations are dominant in a *smg* background. It has been proposed that in a *smg* background these truncated proteins accumulate because the RNAs are not degraded and dimerize with wild-type myosin to form inactive dimer, and hence, a dominant Unc-54 phenotype.