Understanding Mutations

- **Mutation:** A change that occurs in a DNA sequence which can be inherited
    - **cell to cell** or **generation to generation change —>** needs to be **established in a population**
- **Phenotypical changes** are used to track mutations —> change seen has genotypical basis

Example) yeast cultures are grown on plates containing a medium filled with nutrients. A normal plate with the right amount of nutrients will allow for the growth of all yeast cultures.

—> in a plate with medium that is lacking some type of nutrient only some of the yeast cells will grow —> **change or contract that genetic change that causes a particular difference**

> **Wild Type —>** A normal genotype that is the most frequent and came first
>
> **Mutant:** A change in the wild type that may or may not confer a negative impact

- **Mutant:** An organism which experiences a change in DNA sequence
    - most mutations do not have any affect ~ **you cannot tell the difference**
    - can cause neutral changes ~ may have a **phenotypical difference** but is **fully functional**
    - very few are beneficial ~ **improves fitness and highly relative** (only in particular situations can be it better)
    - some cause problems
    - some can be lethal

All dependent on the conditions we use to observe the mutants

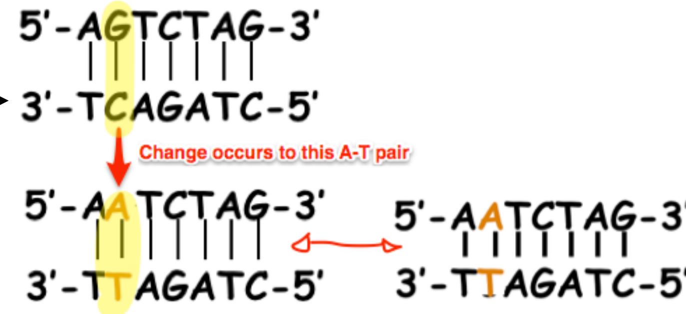
- **Mutations confer some type of phenotype:**
    - Altered appearance
    - Altered growth conditions
    - Altered Behaviour
    - Altered molecules

Different types of mutations can occur:

Small changes ~ **nucleotide base pair substitutions:**

Types of Mutations

Base-Pair Substitution: The nucleotide pair is substituted for another nucleotide pair and this can be:

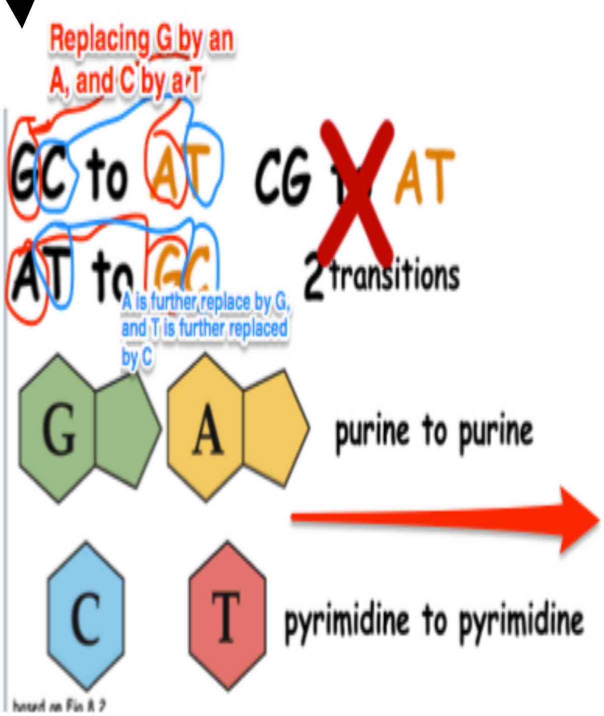


Transitions: same structure substitution in which **purines are replaced by purines** and **pyrimidines and replaces by pyrimidines**

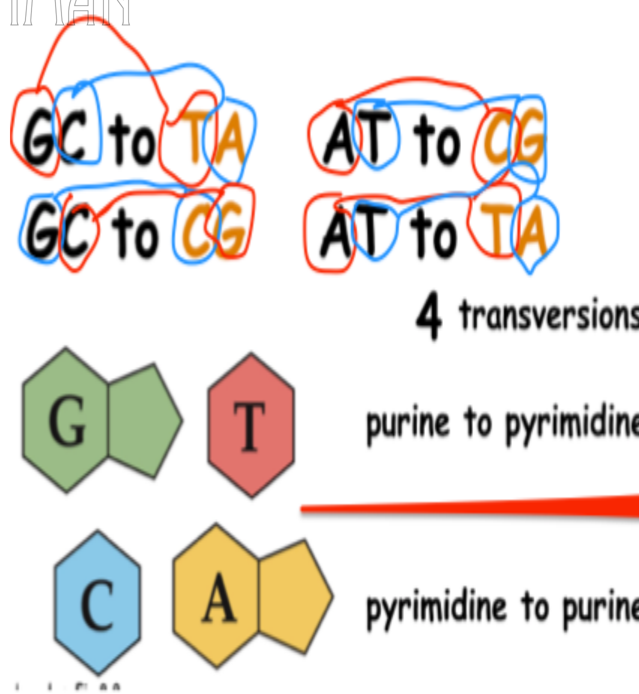
- 2 Transitions can occur

Transversions: Different structure substitutions in which **purines** are replaced by **pyrimidines** and **pyrimidines** are replaces by **purines**

- 4 Transversions can occur

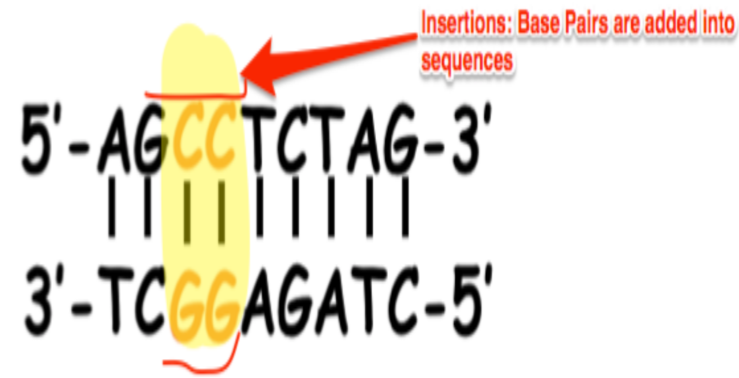


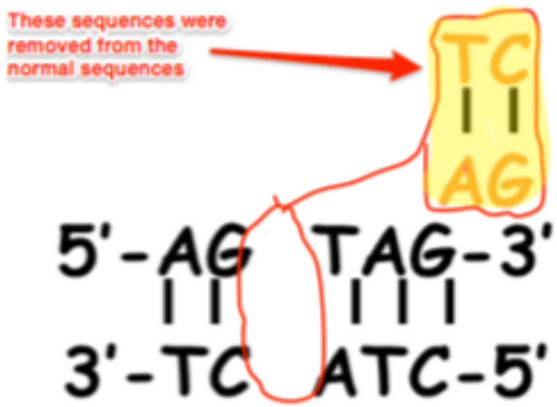
The changes are same structure changes with purines changing to purines and pyrimidines changing to pyrimidines



Transversions are occurring here by which different structural changes can occur
Typically: A-G & T-C
The different structural changes occur when purines are substitutes to pyrimidines and vice-versa so...
-->G-C, G-T
--> C-A, C-G
-->A-T, A-C
--> T-G, T-A

Insertions: The insertion of nucleotides sequences (mono, di, etc….) into the regular sequence

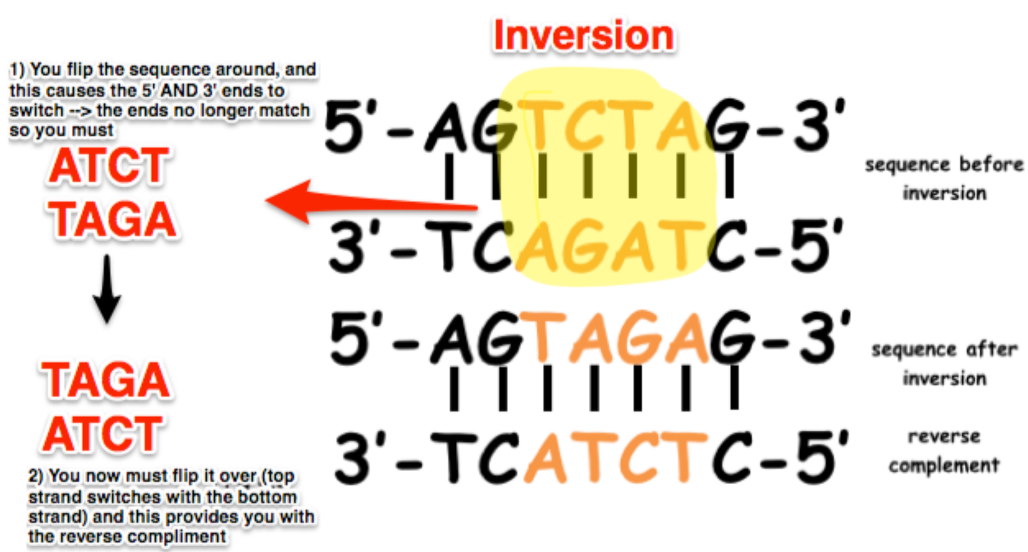


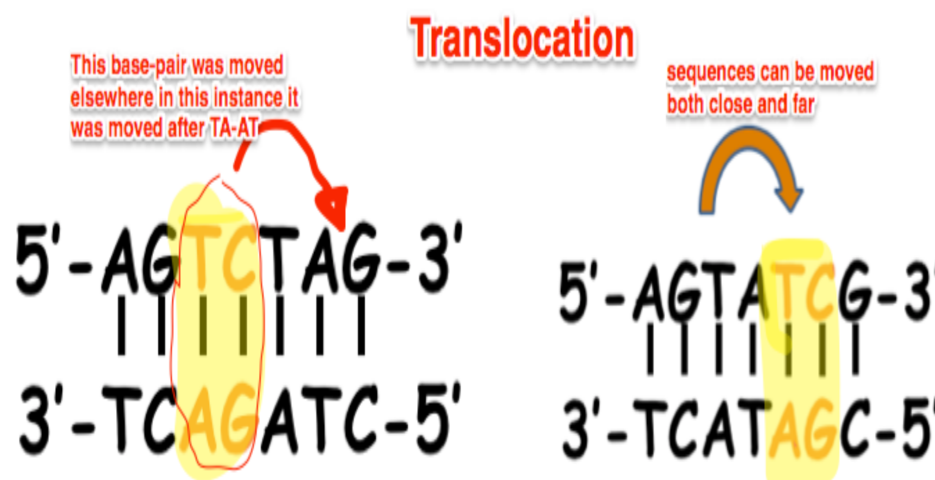


Deletions: Deleting sequences from the original sequence of nucleotides

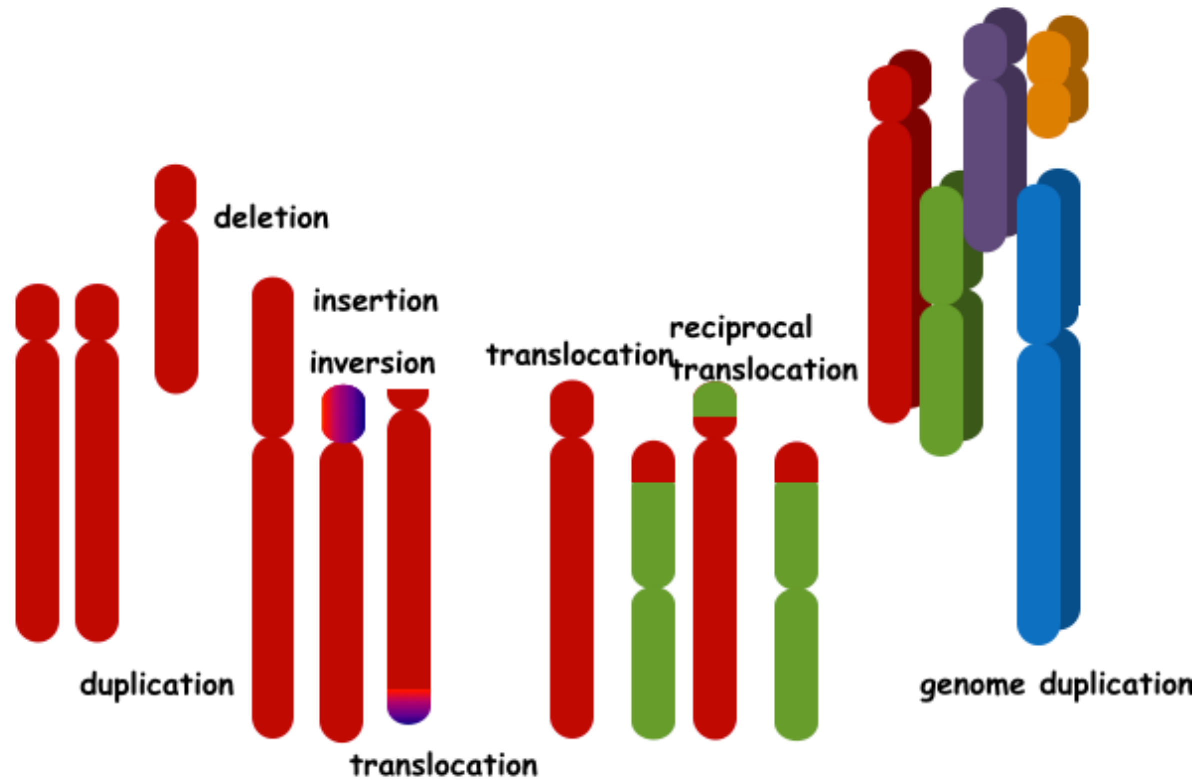
Inversions: Take the specific sequence and turn it around
—> The 5' and 3' ends switch causing the ends to mismatch
—> You must then turn the sequence around and flip it over —> observe that it is the **opposite strand** and in the **other direction ~ reverse compliment**

Translocations: move a base-pair elsewhere —> can move close or far and can be variable amount of base-pairs that are moved





- **Large changes** usually occur by **mechanism of mutations to chromosomes**
    - **Deletions:** regions of chromosomes are largely deleted
    - **Insertions:** the chromosome length is extended by some type of chromosomal insertion
    - **Duplication:** duplication of the whole chromosome occurs
    - **Inversion:** regions of the chromosome are turned around and flipped over —> creates the **reverse compliment (**opposite strand in other direction)
    - **Translocation:** movement of chromosomal fragments either **between** or **within** chromosomes.
    - **Genome Duplication:** duplication by means of a **whole-genome** fashion
        - Remember we are dealing with all the genetic info that an organism is comprised of
        - Can have consequential implications and duplications are species dependent



Overall bigger chunks of the genetic info can be altered at the chromosomal level(large changes) **vs changes in the nucleotide sequences** conferring smaller mutational changes.

- Ultimately, mutations tend to be **rare events** with **mutations occurring** on a **2-12x10^-6 mutational basis per gene** / **per each gamete……** the question remains then

When do such mutations happen?

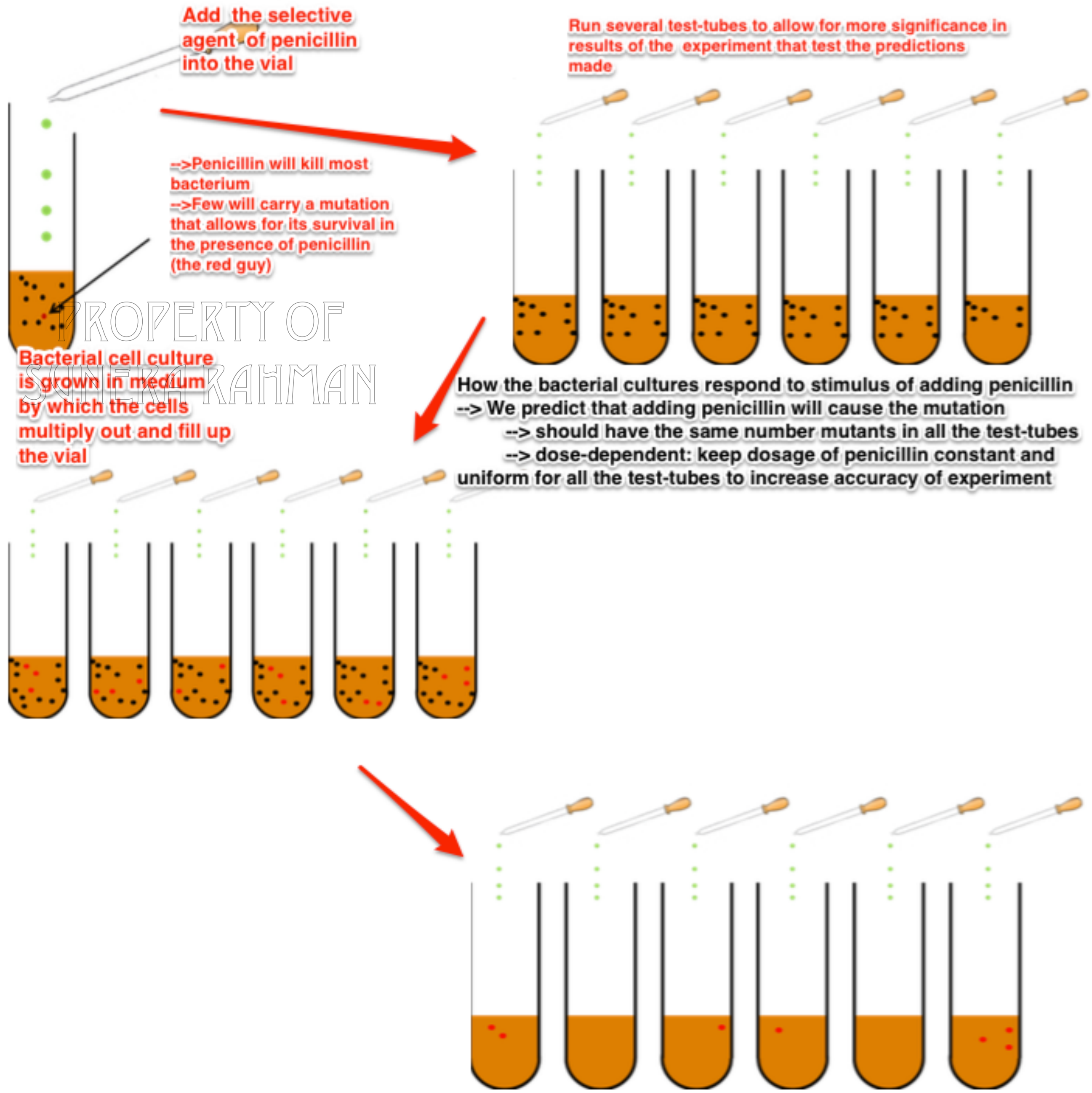
Do they occur as a response to environmental stimulus?

Is it just based on random chance?

Experimental Research: Does change occur in regards to external stimulus or are the changes **gradual** and occur on a basis of some type of **selective agent** (characteristic or trait that gives individuals in the population and advantage for survival)

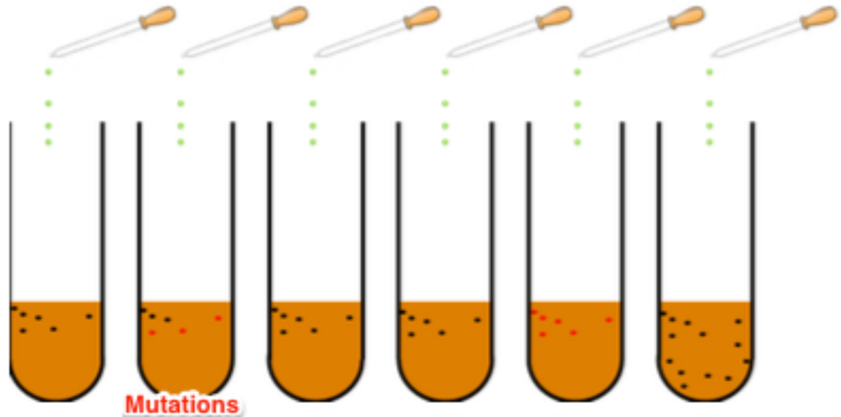
- We add penicillin to impose the mutational change in the bacterium
- Same number of bacteria affected ~ same number of changes per vial

Do mutations occur in response to stimulus



Testing whether the mutation occurs randomly overtime

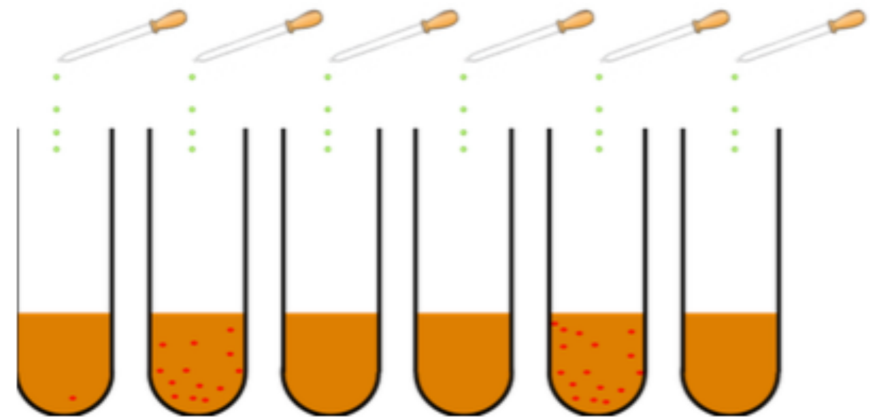
- **Changes happen over-time randomly and we can only see them if we change environmental conditions**



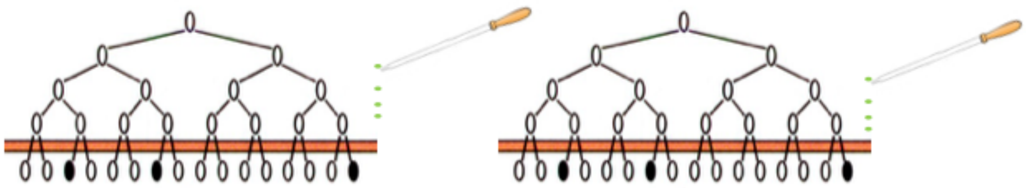
Mutations occurred at a different point in the vial

The mutation occurred early in some of the vials and continues to multiply

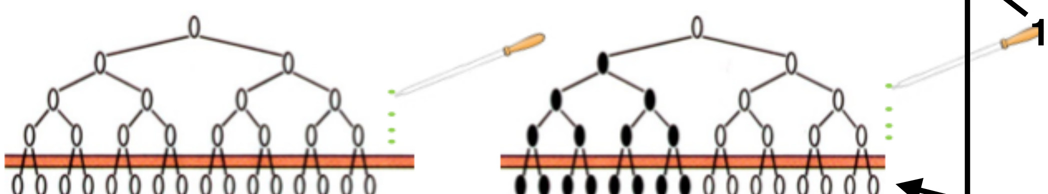
If we add selective agent of penicillin to the cultures it brings about this result:
-->All mutant forms survive
-->All the wild-type bacterium end up dying



bacterial resistance is response to an environmental stimulus (penicillin)



resistance happens spontaneously at some time

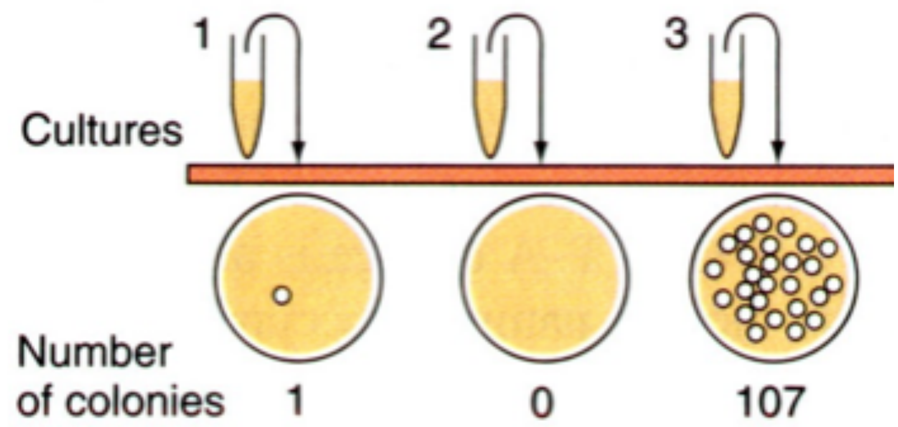


Bacterial Culture Tree of Life Analysis

1. **Top picture:** The changes occur as the stimulus is added…. penicillin creates an environment that causes most bacteria to die early on in the TOL but some survive into the next generation
2. **Bottom Picture:** Resistance to penicillin spontaneously came about at some point and in some lineage of the bacterium
   - **Divergence:** The lineage of bacterium split into 2 linages one that had the allele for resistance and one that lacked such an allele

Fluctuation Test

- Take the contents of the vial and plate it on agar with the **antibiotic:** ampicillin
    - **offspring of the mutants (**the bacterium that survive penicillin exposure) should be able to survive
    - **lots of mutants will bring about lots of colonies**
    - **Conclusion:** Response does not occur in regards to a selective agent as the outcome was seen for the first time on the agar plate —> **So did the response occur in regards to adding a change in the environmental conditions where the bacterial cultures will grow(**adding AMP to plates)**?**



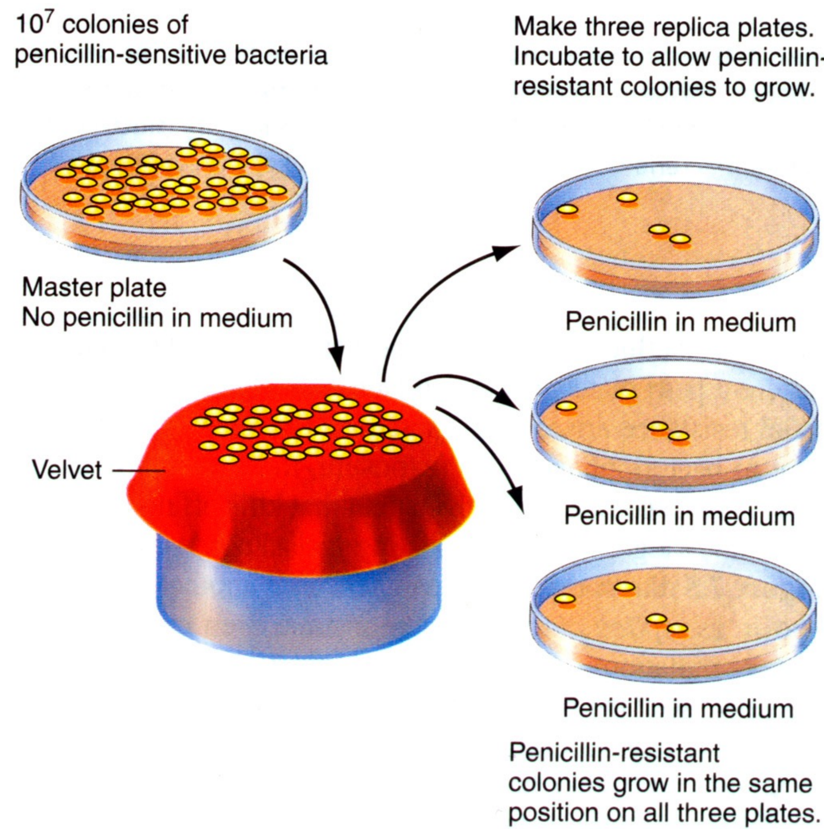
Replica Plating

1. Take master plate containing penicillin-sensitive bacterial colonies and **press onto velvet** to allow **transfer of cells**
- **Same pattern of cells** is observed on the velvet

2. Take fresh plates with penicillin containing medium and **transfer the cells onto the velvet**

3. Mutants from the penicillin sensitive plate will be able to survive, while **wild-types** will die
- certain colonies will be of the **mutant offspring** while others will not and those that are will survive
- Coincidently each plate consists of a replica of the same cells and this can be seen by how the pattern of resistant colonies is retained on all 3 plates.

Conclusion: Mutant bacterial population already existed upon spontaneous arrival in the population and upon exposure to penicillin those colonies were the only ones that survived thereby demonstrating how the environmental change likely did not induce the change (**the spots on the plates would be at different locations in this case)**

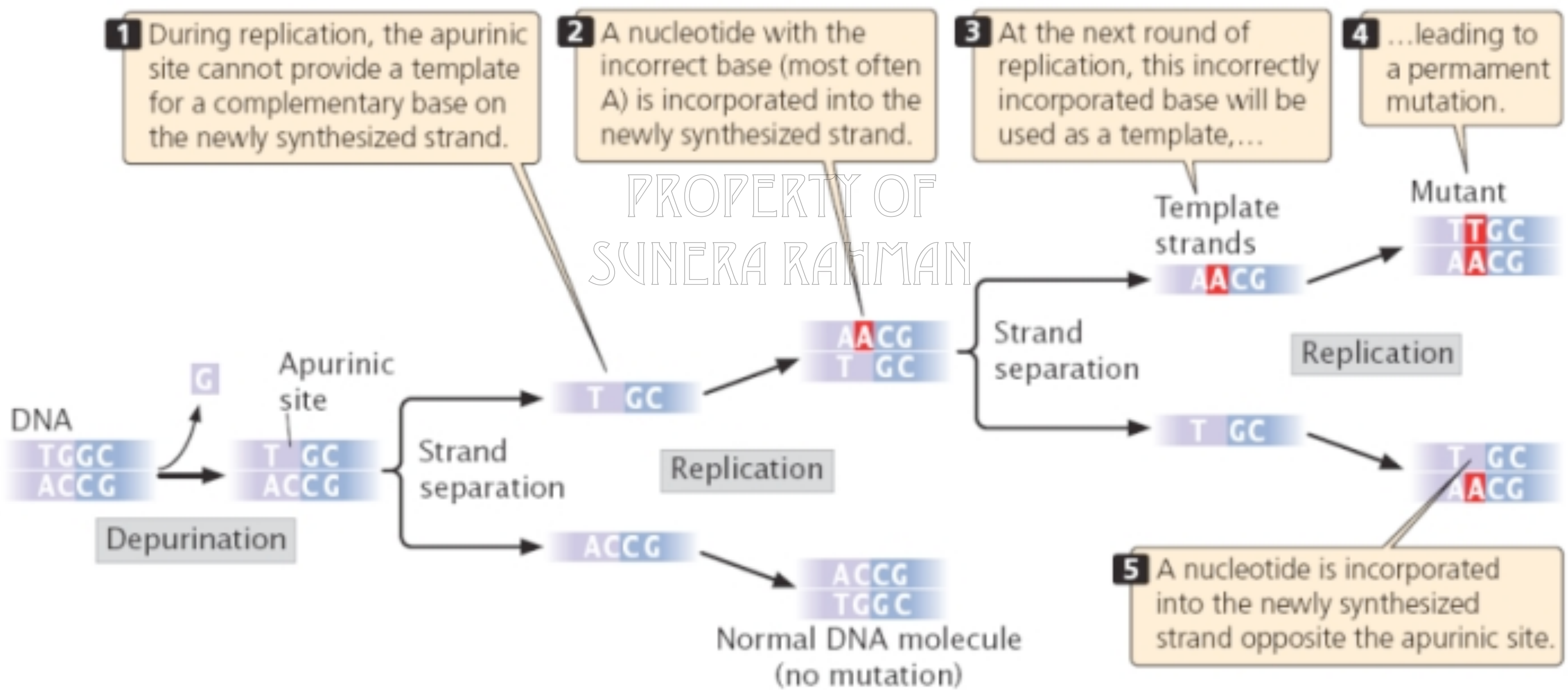


Mutations can occur for many different reasons!

- Things like **errors during replication, free radicals, and UV damage** can all cause mutations
- Mutations can be **classified** into **different sub-groups**

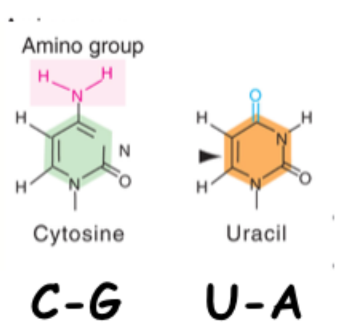
1. Spontaneous Mutations

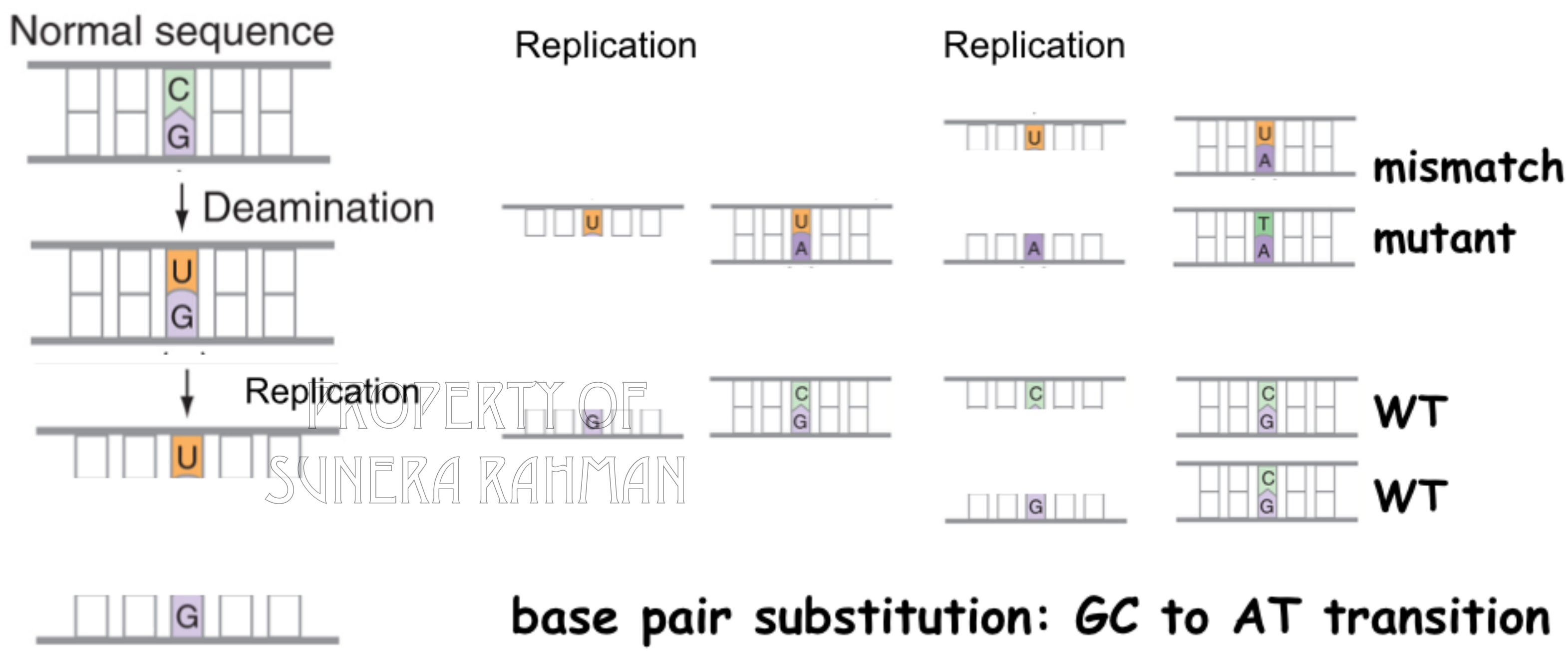
- Changes happening overtime and occurring by varying means including:
  - **Depurination:** Disturbance of the chemical bond connecting a **purine (adenine/guanine)** to the **deoxyribose-phosphate backbone** by some kind of spontaneous break



- It takes **2 rounds of replication** for this mutation to become permanent
  - As we know A/G are purines and therefore this change of incorporating A after the first around of replication causes that mutant to produce a strand that has pyrimidine in replacement of the purine
  - **Result: G/C** is replaced by **T/A = G—>T transversion** mutation.

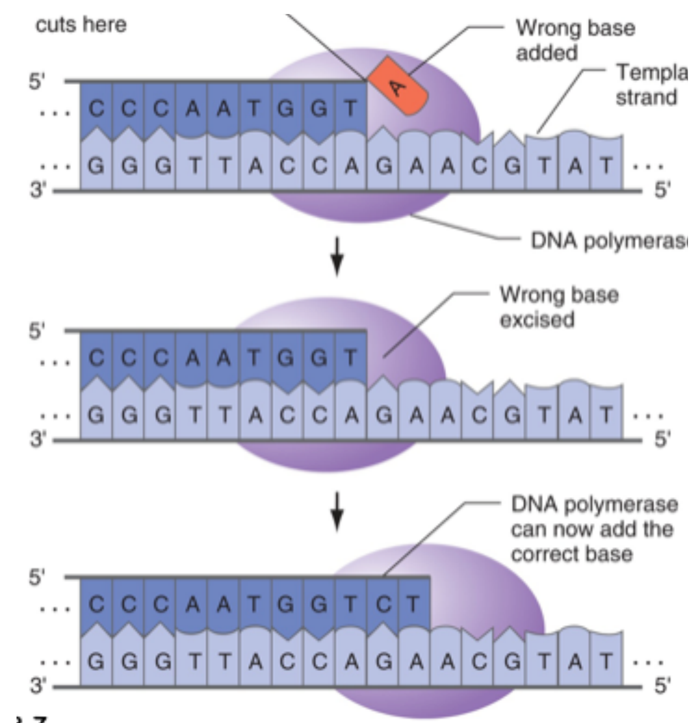
Deamination: The **loss of the amino group** from **cysteine** in its **C-G** base-pair within the DNA sequence —> causes it to **become uracil** (RNA) —> **base-pairing properties change** and **U** pairs with **A**





- **Result:** Upon another round of replication will **A** pair with **T** —> **A/T mutant sequence is established into the population —> C—> T point mutation generated = Transition**

Mistakes during replication: Spontaneous mutations can arise during replication but luckily **repair mechanisms** like the **proofreading enzyme DNA Polymerase** exist to correct such errors —> every time a base is inserted will this enzyme check to see if it was done right, **if not it goes backwards and takes it out ~ 3' to 5'exonuclease ability**



Slippage: during replication to **DNA sequences** that **contain long runs or repeats (tandem repeats)**, can some of those repeated sequences **slip-out** under **clumsy watch** of **DNA Polymerase**

- **Tandem repeats** are unstable areas of the genome that are more susceptible to **insertions/ deletions** resulting in **genome rearrangements**

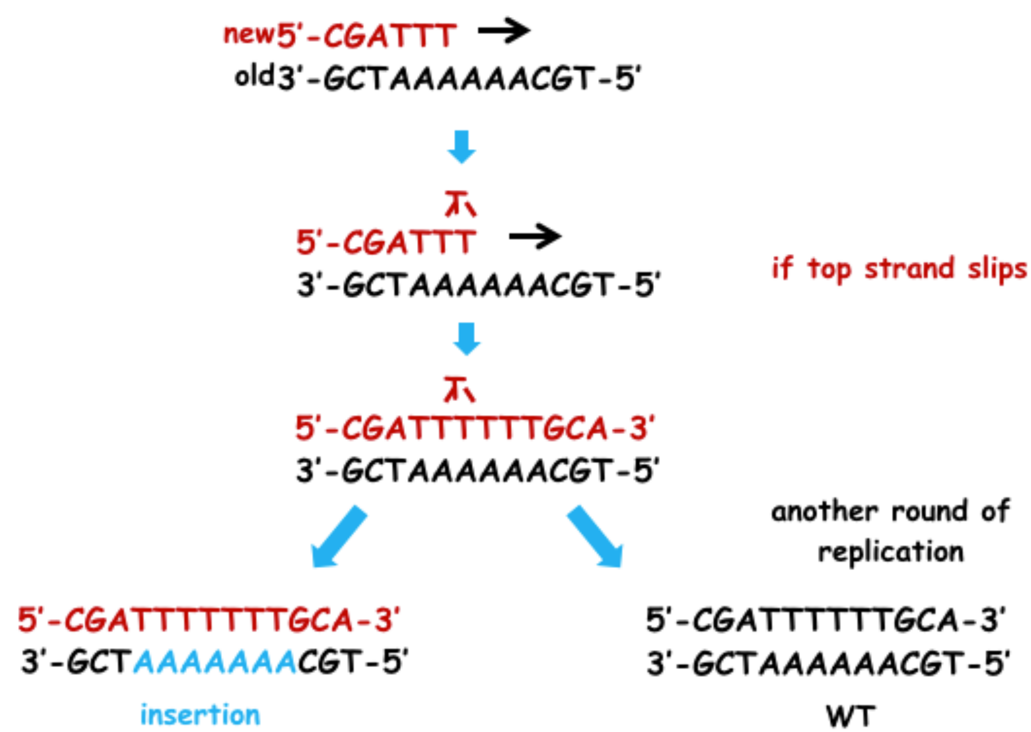
Top-Strand Slippage

- DNA Polymerase is probably high as fuck and isn't paying attention to actual amount of repeated nucleotides in template strand—> stoner problems = **replication stopped &** polymerase temporarily released (**tryna grab some munchies**)

- The **newly synthesized strand** strand **slips out** & **pairs with another direct repeat upstream**

- DNA polymerase reattaches itself onto the template strand at the same position but **mistakingly backtracks** and **repeats insertion** of the previously added nucleotide sequences (burnt out and being lazy so mans fuck up again**)**—> **repeats on template strand now replicated twice** in daughter strand

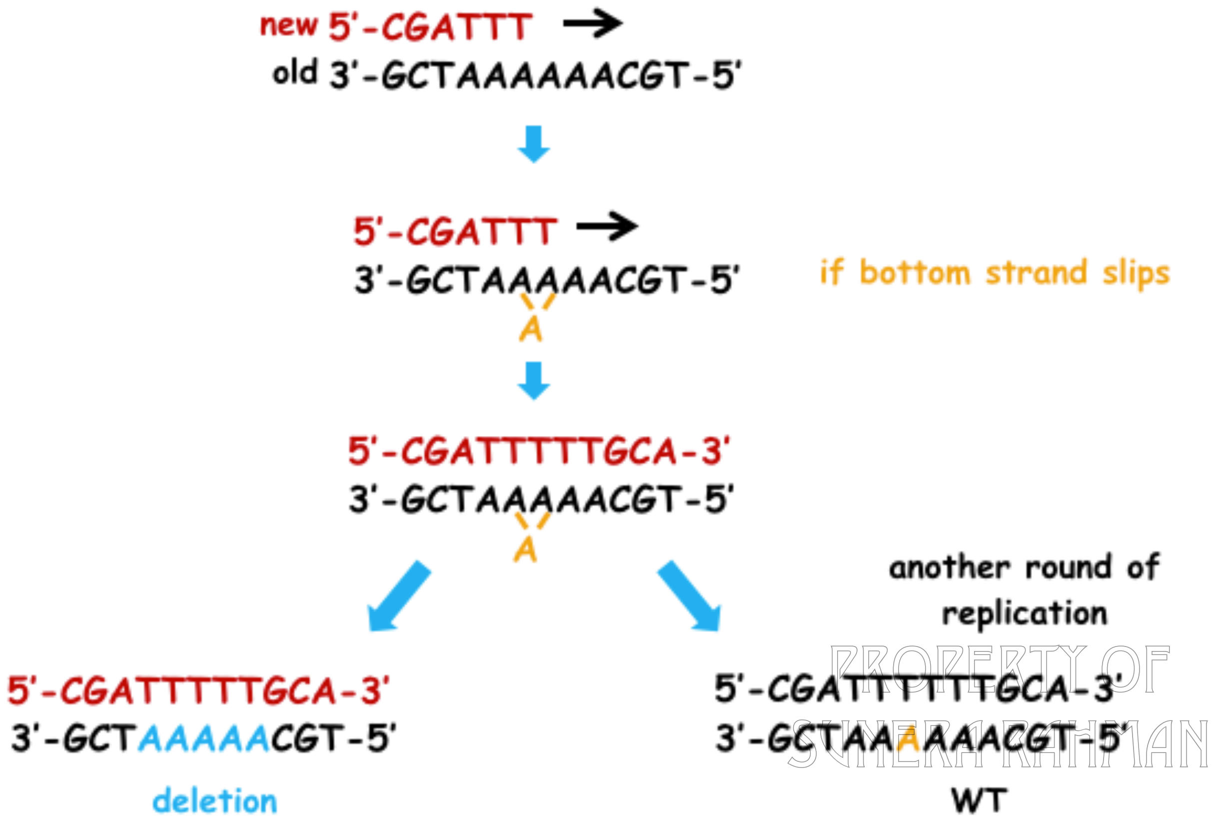
- **Expansion** of replication region occurs by insertion of new nucleotides —> **template** and **daughter strands no longer pair properly**



Upon another round of replication

- To compensate for mismatch do **nucleotide excision repair proteins** come in and **expand the top strand sequence —>** replication of this strand produces daughter helices with extra nucleotide—> **INSERTION**

- **Bottom strand** generates a **wild-type** strand with the **necessary amount of nucleotides (**in this case, **6t's)**



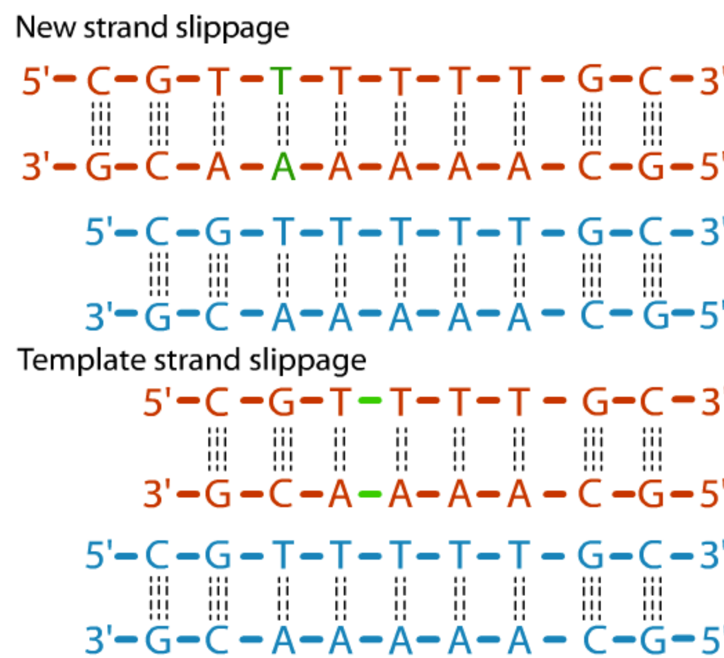
Bottom Strand-Slippage: DNA polymerase is trippin mad ballz and fucks up during synthesis of daughter strand in tandem repeat areas of the genome —> **replication stopped &** polymerase temporary released

•Bottom strand slips out a bit

•DNA Polymerase reattaches where it left off and **proceeds** to **synthesize** the top strand without notice of replication error (the slippage occurred to template strand and this is the base by which the daughter strand is synthesized from …**so how would polymerase recognize an error**)

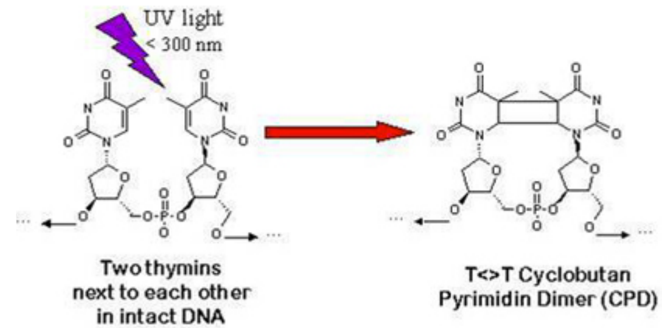
- **Result:** Upon another round of replication will…
  - **5'—3'** lagging strand as template: One of the daughter helices will now have one less nucleotide —> **deletion**
  - **3'—5'** leading strand as template: Expansion of replication region occurs in which new nucleotides are added —> **template strand has correct amount of nucleotides again —>** newly synthesized daughter helices are WILD-TYPE

OVERALL IDEA OF SLIPPAGE



NOTE: Slippage in areas of Tandem repeats with trinucleotides (**agaagaaga)** can cause severe genomic alterations leading to diseases such as **Huntington's Disease & Fragile X Syndrome**

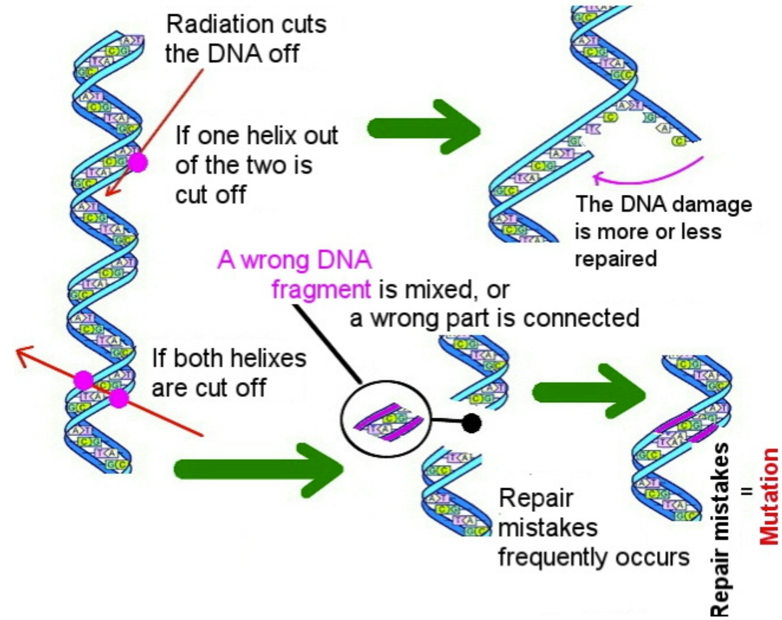
2. <u>Induced Mutations</u> **Chemical/Physics mutagens** attacking DNA ~ **DNA DAMAGE** increases rate of mutation



Physical Mutagens: Radiation sources inflicting damage to DNA

Examples:

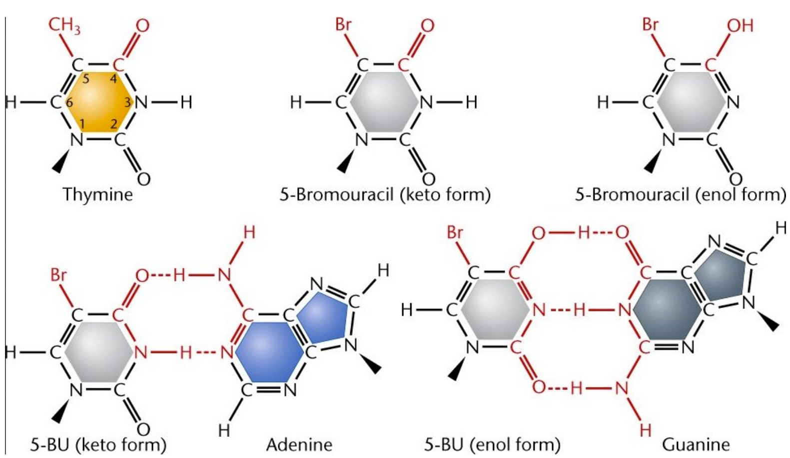
- Damage from **UV Rays (200-300nm)** occurs as it **produces pyrimidine dimers (**covalent bonds cross link adjacent pyrimidine bases in DNA) ~ **molecular lesions (cyclobutane) —> result:** alters DNA structure and inhibits proper functioning of DNA polymerase (**arrests DNA replication**)



- **Ionizing Damage (**X-rays, gamma rays, beta particles**): generates free radicals** within the cell —> produces **ROS~reactive oxygen species —>** causes **single/double stranded breaks** in the double-helix with the latter being the more harmful of the 2

Chemical Mutagens: replacement, alteration, or structural changes to a base and its properties

Examples:



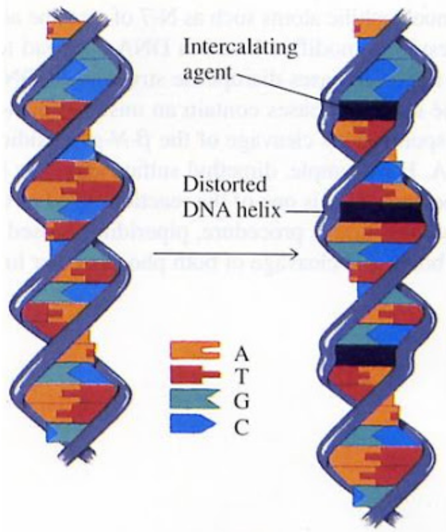
•**Base Analogs:**
almost identical chemicals that can substitute into the normal nucleotide base

•An example of such would be **5-bromouracil** which is structurally similar to thymine and therefore can be mistakingly substituted in during replication by Polymerase —> **produces unnoticeable change at first**

—> **5BU alternates** in its keto/enol form

•During **next round** of replication it may switch into **enol form** and **instead pair with guanine** vs adenine —> **mispairing**

Base Alterations: attachment of **alkyl, methyl, ethyl** to the side chains of nucleotide bases o**r removal of side chain components** in such bases.



Intercalating Agents: molecules that can insert itself between the bases of DNA and produce a **frameshift mutation** during replication
•**polycyclic, aromatic, and planar** ligands of appropriate size can open space between DNA base pairs and **unwinding/rising** of the helix —> **local structural changes** leading to modifications that can cause **changes in functionality**

Biological Agents

Viruses: Insertion of virus DNA into the genome can cause **disruption** of normal **genetic function**

Transposons ~ Jumping Genes: fragments of DNA that constantly relocate themselves within the genome and that can potentially cause a mutational changes if it jumps into a gene

Bacteria: the presence of certain types of bacteria in an organism can produce negative effects such as inflammation which induces the formation of **ROS (reactive oxygen species) —>** DNA Damage = reduced efficiency of repair mechanisms —> **increases mutation rate**

Endogenous vs Exogenous

3. <u>Endogenous Agents:</u> **internal metabolic processes** that lead to can genetic changes thereby increasing likelihood for mutations

- Majority of mutations in human tissue comprise of endogenous origin
    - Errors in DNA repair mechanisms (Slippage
    - Toxic by-products of cellular metabolism (**ROS)**
    - Nucleotide Imbalance

4. <u>Exogenous Agents:</u> **external factors** (from the outside) that can be of chemical constituency in which upon application to the organism cells can it cause changes to the DNA —> **carcinogenic agents (**pollutants, human-made chemicals, pharmacological compounds, radiation, etc)

Other shit we should probably consider..

Toxicity~Genotoxicity: Chemical agents that damage the genetic info within a cell and can potentially induce mutations —> **can lead to cancer**

•can have direct/indirect effects on DNA

•May just kill the organism instead of just changing the DNA

Mutagenicity: physical, biological, or chemical agents (**mutagens)** that can induce a genetic mutation

•frequency of mutations increases

•does not necessarily cause cancer

All mutagens are genotoxic, however, not all genotoxic substances are mutagenic

To wrap this section up

When deciding what mechanism of action causes which mutation, many considerations to be taken

Spontaneous vs Induced: The example is radiation from the sun (UV rays)

- exposure to sunlight on a normal day would bring about **spontaneous mutations** as we are in a normal environment and not doing anything unusual —> **pyrimidine dimers**
- intentional over exposure to sunlight (bitches trying to get orange and shit) can be considered a means to **induce mutation**

  —> Same agent but depending on the circumstances will the mechanism that brings about such a mutation(**pyrimidine dimers**) be different

Repair Systems for Mutations

For each type of DNA damage, the cell has evolved a specific method of repairing the damage or eliminating the damaging compound

Proof Reading: DNA polymerases contain proofreading activities that are primarily involved in replication error repair

- **error** detected —> DNA replication **halted** —> polymerases **move backward** and **remove nucleotides** from daughter DNA chain until wrong nucleotide is removed —> continue the **forward replication** process

Base excision repair: DNA repair mechanism by which a mismatched or modified base is **excised** and **replaced**

- Both pathways are initiated by the **recognition of mismatch/modification —> glycosylase removes** the **base** from the **sugar-phosphate backbone**

2 pathways of pair can be taken:

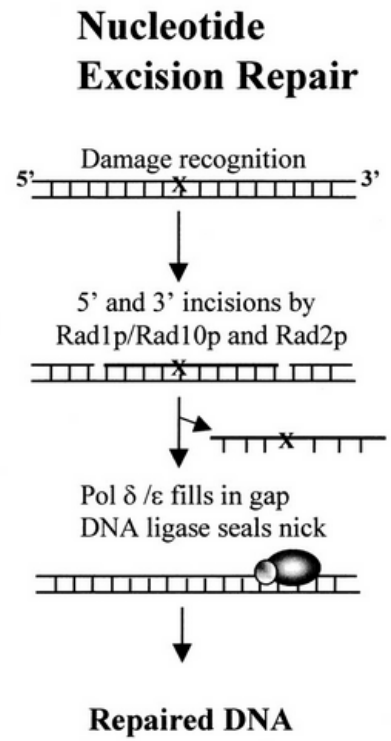
1. An **endonuclease** comes in and **cleaves** the sugar-phosphate backbone at the **3' end** of the **abasic site** (where no purine/pyrimide exists) —> A **second endonuclease** comes in and cleaves at the 5' end of the abasic site —> **result:** nucleotide falls out with nothing left to hold it in place = 1-nucleotide wide s**ingle-stranded gap**

2. An endonuclease comes in and cuts the sugar-phosphate backbone at the **5' end** of the **abasic site —> deoxyribophosphodiesterase** cuts out the sugar-phosphate section of the abasic site —> **result:** 1-nucleotide wide s**ingle-stranded gap**

- **DNA Polymerase** re-enters and starts synthesizing DNA —> **DNA Ligase** comes in to seal the sugar-phosphate backbone

Nucleotide Excision Repair: removes damage from **pyrimidine dimers** and **chemical adducts** which are **easily recognized** since they both cause significant **distortion** in the **DNA helix.**

- Targets actively transcribed regions of the genome
- **Separation** of **double helix** at lesion site —> **single-strand incision** at 5' and 3' ends —> **excision(cutting out)** of the lesion containing **single-stranded DNA fragment** —> **DNA polyermase** continues replication and **ligase fills** in the **gap**



- **Note:** Only one strand is damaged and this allows the other **undamaged single-strand** to be used as a **template** for **DNA polymerase** to **synthesize** the needed **complimentary strand**

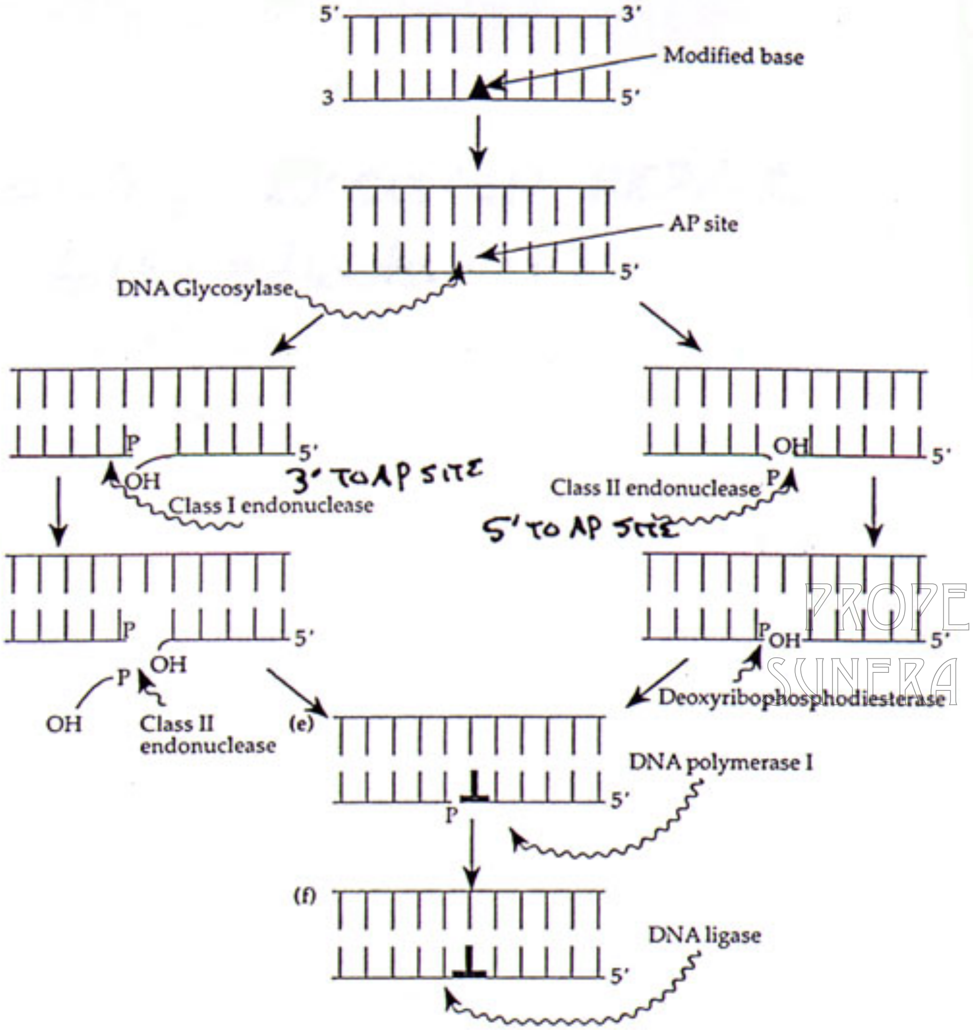
Methyl-directed Mismatch Repair: mechanism that recognizes and replaces insertions, deletions, and mismatches that arise during DNA replication/recombination as well as repairing some forms of DNA damage

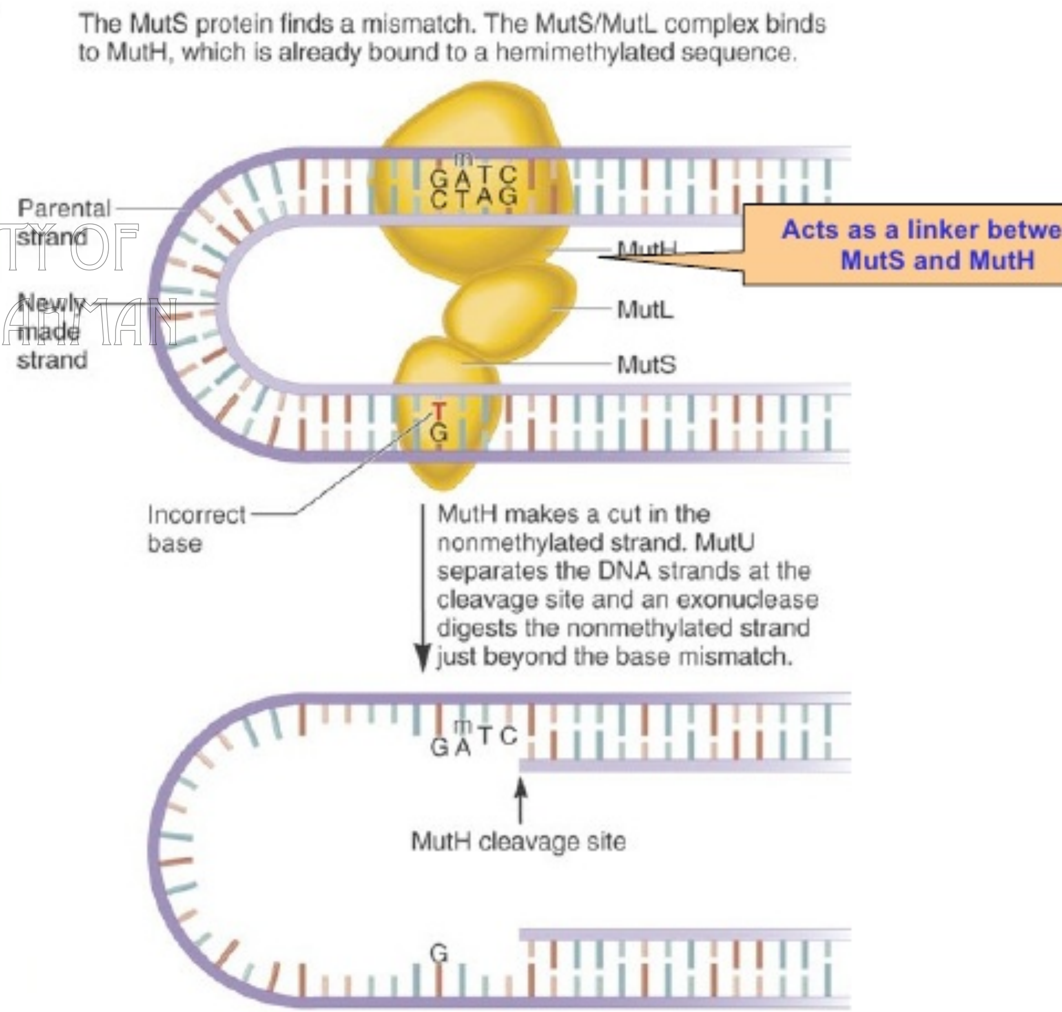
- specifically the methyl-directed mechanism is only seen in **e.coli** bacterium while eukaryotes and other bacterium use a different means of mismatch repair
  1. mismatch can be detected shortly after DNA replication since the **parental strand** is already **methylated** but the **newly synthesized daughter strand** is not methylated until a few minutes after replication thus allowing a mode of distinguishing between the 2 strands
  2. **Enzymes** will bind to the mismatch site and form a complex that binds to other proteins and use a looping mechanism to interact with the **non-methylated strand**
  3. The **non-methylated strand** is cut and then further **digested** by an exonuclease from the non-**methylated site** all the way to the **mismatched area**
  4. **Result:** Gap is filled by **Polymerase III** and sealed by **ligase**

Overview of How Repair Mechanisms Work

1. **Initial recognition** by an enzyme which recognizes specific types of damage
2. **Damage** is either directly **repaired** or **removed** [Often done by protein-complex interactions]
3. Removal leaves behind **small** (**abasic site)** or **large gaps** (**many nucleotides removed**)
4. The **gap** is **filled** in by DNA Polymerase
5. DNA **ligase seals** the **backbone** of the DNA

Base Excision Repair

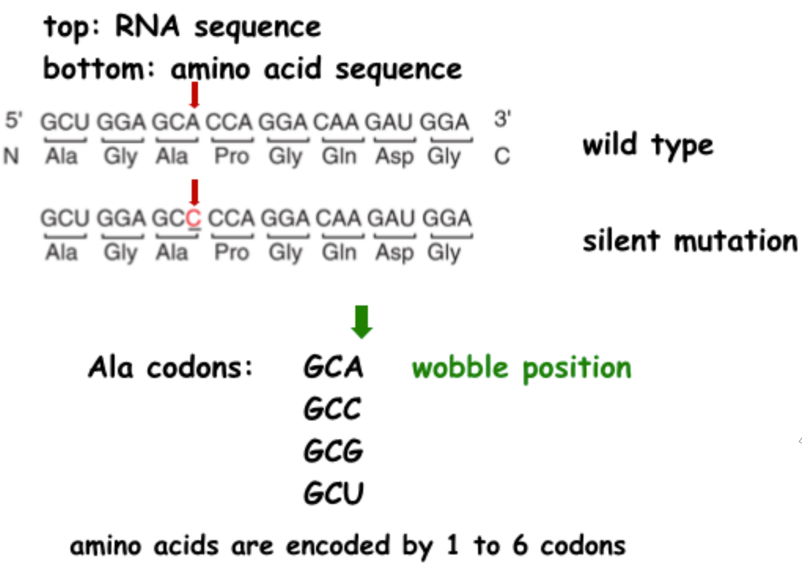




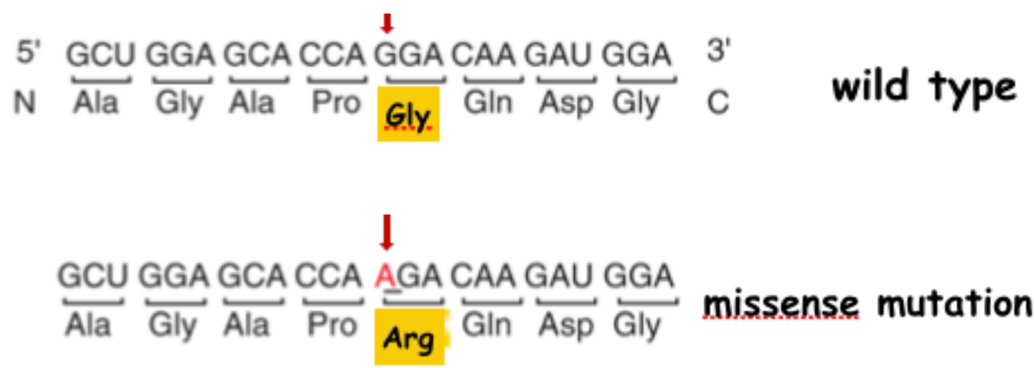
Methyl-directed mismatch repair in *E. coli*

Allele Classification

- Changes to the genome occur by alterations to DNA but only **after translation** are the effects of such changes observed



- **Methionine:** This is the amino acid that is coded for by **AUG** and it represents the start of an amino acid sequence. It is the start codon and is only coded for by that one sequence of AUG
- **Alanine:** An example of an amino acid that is coded for by multiple sequences of codons (**GCA, GCC, GCG, GCU)**
- **Silent Mutation:** A **change that occurs** at the **level of DNA (transcription)** but at the **protein** level (translation) nothing really changes —> image on left demonstrates effect of change the 3rd base on the codon sequence by which that change still calls for the same amino acid and hence the effect of the change at the DNA level is not conveyed in the final product
- **Wobble Position:** 3rd base of a codon sequence in which changes to that nucleotide have little to no effect on the product of the gene since it tends to still code for that same amino acid

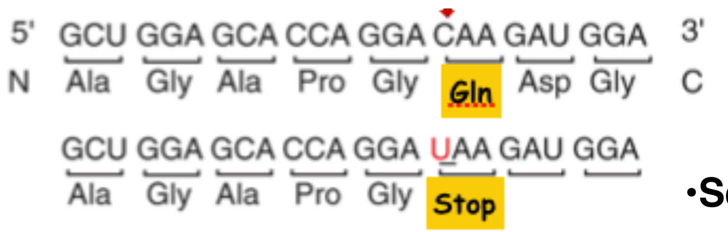


- **First position:** If changing the first position in a codon sequence, some kind of effect is expected since this position **is critical**. A change in the first position calls for a different amino acid altogether
- **Missense Mutation:** A different amino acid coding for what should have actually been there —> image on right shows how changing the **G** in **glycine** with **A** incorporates **arginine** instead

- **Result of this change:** Contextual to extent of change itself —> **where in the sequence is the base change happening, whether the change occurs on catalytic portion of a protein thereby affect its catalytic ability**

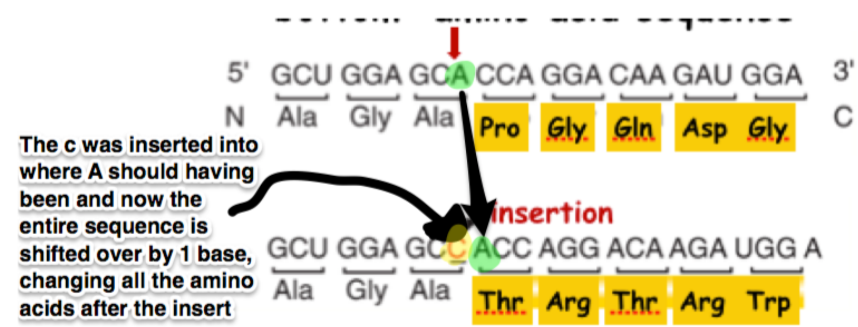
Stop Codon: termination sequence of a polypeptide change in which **no tRNA's** are **available** to attach an amino acid to the translating chain —> **protein truncated** —> **termination signal**

- coded for by **UAA, UAG, UGA**



- **Non-sense mutation:** A point mutation in the DNA sequence that not only changes the amino acid itself but also changes the entire polypeptide chain by calling for a **premature stop codon** and producing a **truncated , incomplete,** and **non-functional** protein
- **Severity** of its effect is dependent on **where the premature stop codon is located**
- If **mutation** occurs towards **end of DNA sequence**, few amino acids are lost and **effect** can be **minimal** (only slightly shorter)
- If **mutation** occurs towards beginning of the DNA sequence then a lot of amino acids will be excluded from the polypeptide chain—> **produces protein** that is very much **destroyed** and will likely confer a **severe negative impact**

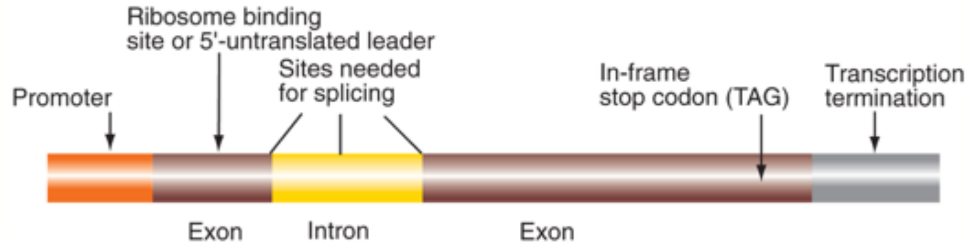
- **Frameshift Mutation:** Shifting the reading frame that will be interpreted by the ribosomal complex for attachment of amino acids by tRNA's
- The effect of a frameshift mutation is really dependent on the amount of nucleotides that are shifted (**3** inserts shift a whole fucking codon)



Note: The insert of the nucleotides themselves aren't what poses the severity of this change but instead the shifting caused by the insertion is what poses the largest problem

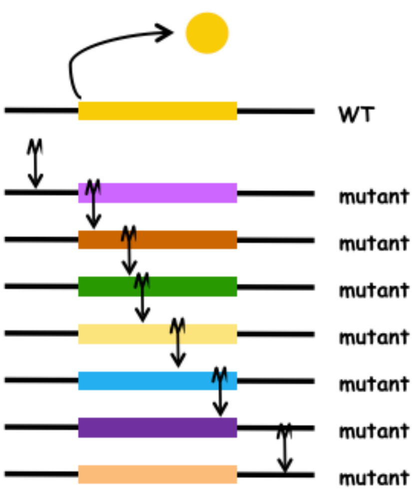
- **Insertion:** as seen on the image in the right another **C** was added next to the C already present in the sequence coding for alanine —> **everything** is **shifted over by 1 nucleotide** thereby causing all other triplets to also shift by 1 nucleotide and **call** for **very different amino acids** than what should have been there

Not all mutations affect sequences that are going to be translated into proteins….



- Mutations can occur to non-coding sequences in the promoter regions of the DNA sequence ~ **regulatory sequences** —> can affect how well a particular gene is transcribed
    - can increase/decrease gene expression
    - affects the point at where transcription/translation will occur
    - can stop gene production all together
    - changes in the ribosome binding site can affect translation levels
    - changes in splicing sites can influence what is being translated

How mutations affect protein function



•the wild-type sequence is making proteins as normal

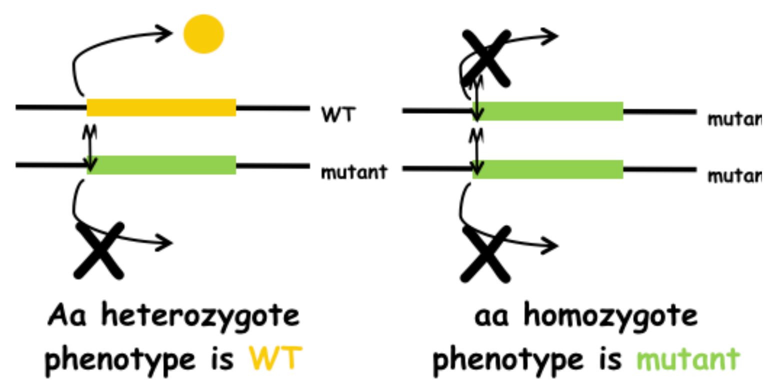
•mutations to the wild-type gene can occur **sporadically, anywhere** along the gene ——> If **mutations occur** at **different locations** on the **same gene** this will create different **mutant alleles**

•Many mutations to a particular gene produce mutant alleles of that particular gene that can be classified into 2 categories: **Loss of function alleles and Gain of function alleles**

Things to keep in mind in this section

- **Wild-type genes** are yellow
- **Functional wild-type** proteins are round and yellow
- **Mutant alleles** will be of different

We Begin this Epic Journey with Loss of Function Alleles



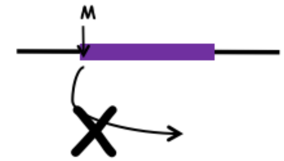
- Most loss of function alleles are **recessive** and display a **homozygous** phenotype
    - If you have a **heterozygote** containing a **recessive mutant allele** with a **dominant** wild-type allele the product will display the **wild-type phenotype**
    - a recessive homozygote for the mutant allele will display a mutant phenotype

- If the mutant allele is dominant, a heterozygote for the wild-type and mutant will have a **mutant phenotype**

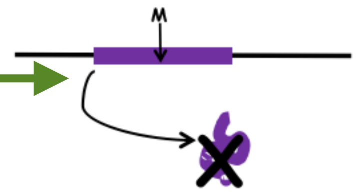
Types of Mutant Loss of Function Alleles

•**Null Allele:** No protein is made at all, **stop codon** is introduced early

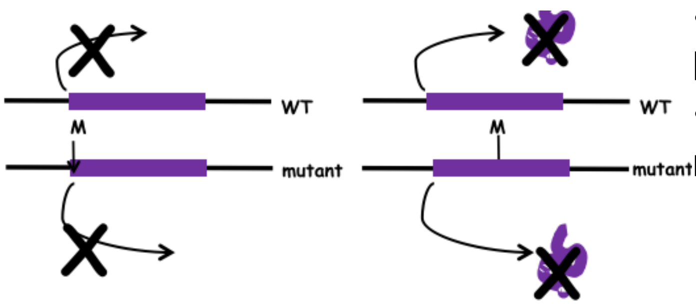
•As you can see, the mutation occurs early on in the DNA sequence and therefore the amino acid sequence is halted early on in translation stopping protein synthesis altogether



•**Amorphic allele: subclass** of **null allele** in which a **deformed protein** is made and this **deformity** is **severe** enough for the **protein to not function at all**



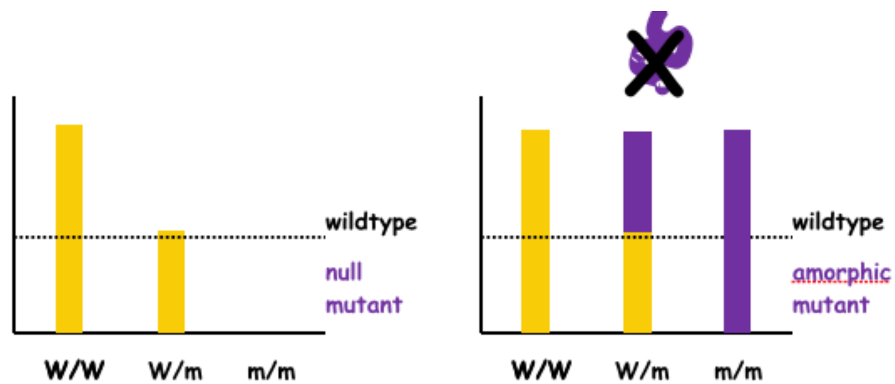
•this mutation seems to occur in the middle of the gene implicating that some amino acids sequences are incorporated into the chain before it is prematurely truncated.



•These **type of mutations** are typically **recessive** and therefore **heterozygotes** tend be of **wild-type phenotype**

•Homozygotes for null or amorphic alleles will of course have a mutant phenotype

How allele type affects protein concentration and how much protein is actually made



- **First Graph:** As we know the null allele stops protein synthesis altogether and no protein is made
  - •**Homozygote for WT(**dominant)**:** High concentration of functional WT protein
  - •**Heterozygote** for **null mutation**: protein concentration is at what is necessary for function but less than what would have been for **W/W**
  - •**Homozygote** for null mutation: No protein is produced at all….

Amorphic Graph

•**Homozygote for WT:** High concentration of WT protein

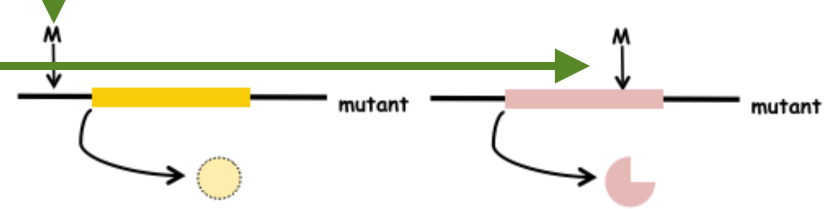
•**Heterozygote for amorphic mutant: Half** the protein produced is **normal WT** while the other half is of the **deformed non-functional type**

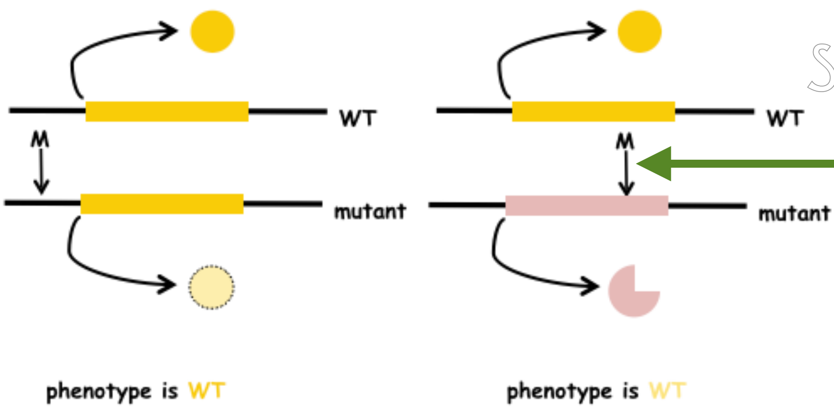
•**Homozygote for amorphic mutant:** High concentration of the deformed, non-functional mutant proteins —> WHAT A FKN WASTE.

• the lack of wild-type protein and the existence of the **null mutants** in its place produces wild-types that change into mutants as seen in **Arabidpsis Thaliana —>** Flower now has missing reproductive organs

•**Hypomorphic Alleles:** Mutations that reduce a genes functionality without completely eliminating its function

- •If you have a Wild-Type gene and the **mutation** is in the **promoter region** you may no longer have a **high rate of transcription**
- •If you have a Wild-Type gene with the mutation within the coding sequence this can affect how well the protein functions = **loses some of ability —> changed protein form —> result: weaker function/lower activity**





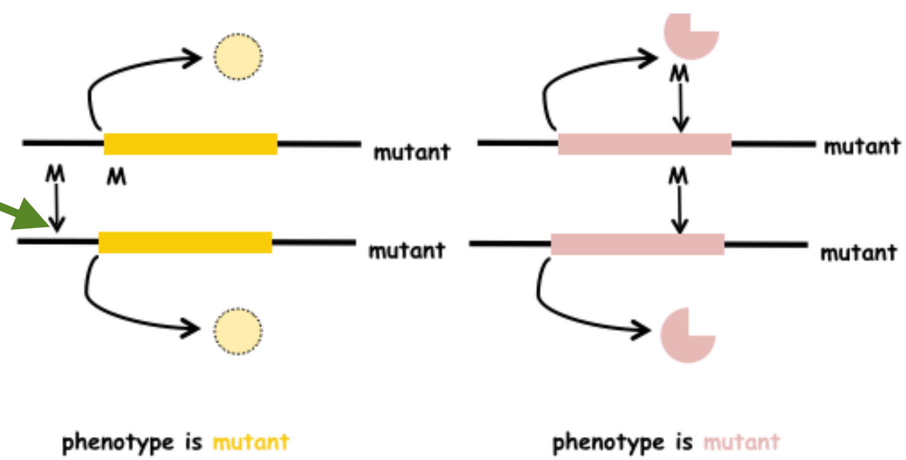
•If **the hypomorphic mutant allele** is in the promoter region causing a **lowered rate** of **transcription** and **heterozygous** with the **Wild-Type** —> phenotype will still be WT

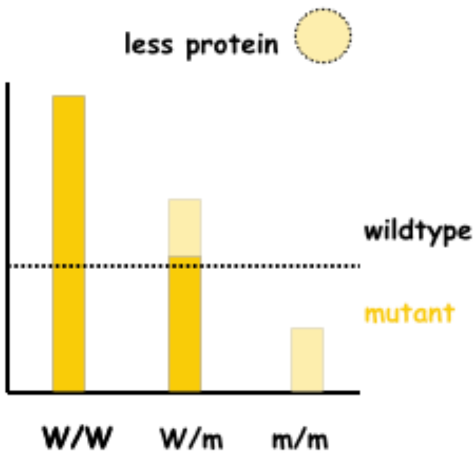
•If the **hypomorphic mutant allele** is **within** the **coding region** and **heterozygous** with the **WT** —> Phenotype will still be WT

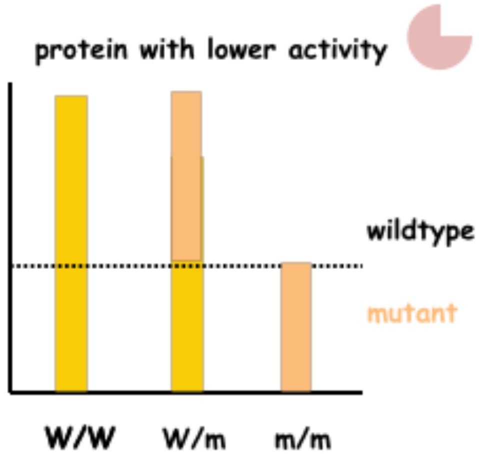
•Both still allow for a **sufficient amount** of **normally working protein**

• If the **hypomorphic mutant allele** is in the **promoter region** and is **homozygous** the phenotype will be mutant and cause an overall low rate of transcription for this protein ~ **low yield**

•If the **hypomorphic mutant allele** is **within** the **coding region of the gene** and **homozygous** the **protein** will **not functional correctly** altogether





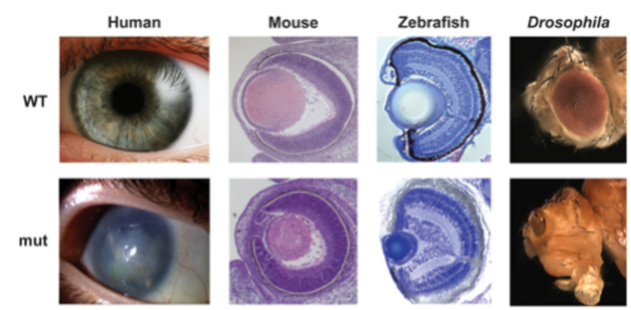


•**Heterozygote** for **hypomorphic mutation in promoter sequence**: sufficient amount of WT protein is still being made but definitely a lot less than what would have been for **W/W**
•**Homozygote** for **hypomorphic mutation in the promoter region**: Since the rate of transcription is lower altogether a very minimal amount of normal protein is made

•**Heterozygote** for **hypomorphic mutation** within **coding region**: protein concentration is high but you have large amounts of the **WT protein** and **mutant improperly functioning protein —>** probably competes with each other or mutant disturbs WT function —> overall **lower protein activity**
•**Homozygote** for **hypomorphic mutation** within **coding region**: **optimal** amount of **deformed protein** is made—> p**rotein activity** is significantly lower

Loss of Function Example: A mutation exists in drosophila that can potentially cause this organism to be eyeless

- •This mutation effects **eye development** and **different alleles** to this gene have **different affects**
    - No eyes = **null**
    - Small eyes = **hypomorphic**
    - Reduced eye sizes = **hypomorphorphic**
    - •**Pax6** in mice and **aniridia** in humans
- •Impact of such a mutation can vary depending on where this mutation occurs



Loss of function alleles can affect genes where the protein product is required in 2 places

Example: Consider a gene whose protein product is required for wing function and leg development

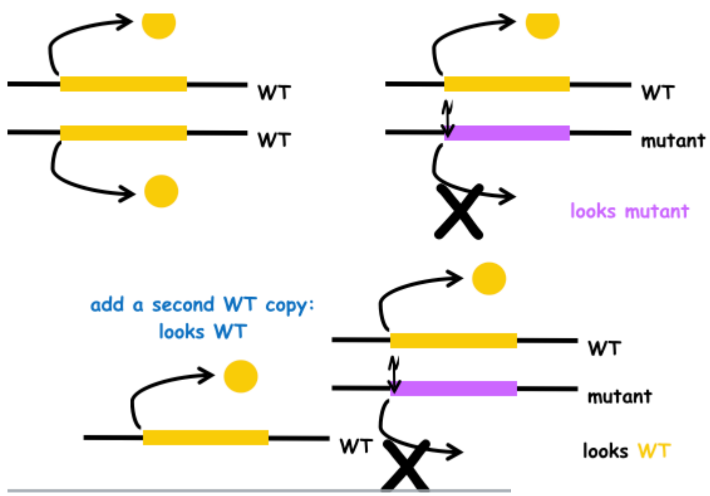
•**Hypomorphic allele:** normal legs are formed but reduced wing function
•**Null allele:** affects both legs and wings and causes them to have reduced function —> does not always mean something isn't formed

Incomplete Dominance: Many different alleles can work together to produce intermediate gene products which convey intermediate phenotypes (a phenotype conferred by the combination of these genes)
Example: Pigment of flowers is coded for by many several pigment coding genes

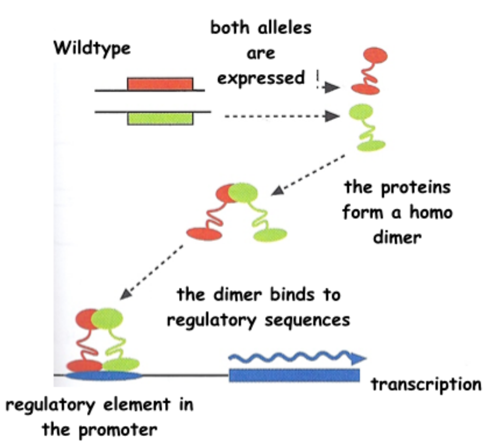
- r^+ **—>** Wild-Type that produce **RED** colour
- r^0 **—> Null allele** that is the default and produces no pigment at all = **WHITE**
- r^{50} **—> Loss of function allele:** always making **half the pigment ~ loss of dominance = 1/2 RED**

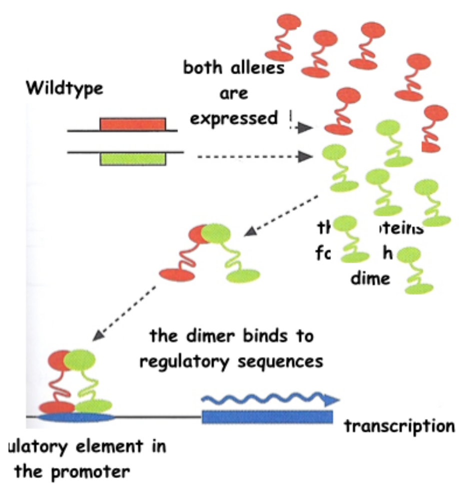
Haplo-insufficiency: One WT allele is not enough for a WT phenotype ~ Organism only has 1 copy of of the functional allele coding for the WT phenotype but both are needed to bring about the proper genetic condition

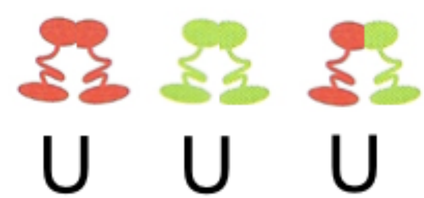
- You would need both Wild-type alleles to manifest the WT phenotype, and lack of one can lead to **autosomal dominant disorders**

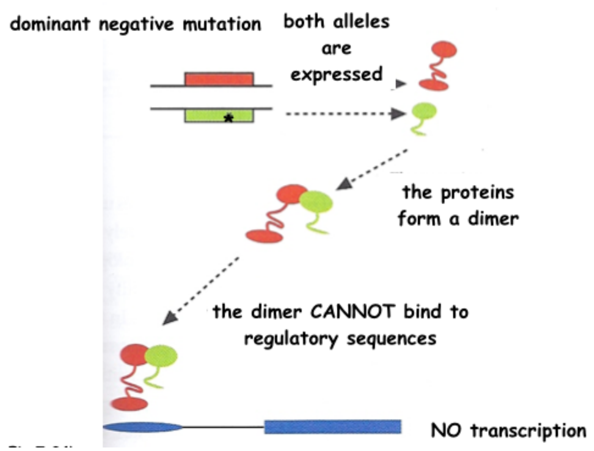


- **Testing for haploinsufficiency:** The observed phenotype may look mutant due to some observable change in normal characteristic of the gene product —> add a second WT Copy and if this causes the phenotype to be of normal regular function, you will know it was haploinsufficient.
- **Dominant negative ~ antimorphic:** mutations that fuck with Wild Type alleles thereby fucking with WT phenotype —> interferes or blocks wild type function ~ **poisons novel function**
- In the images below, you can see that there are 2 wild type alleles that will go on to make very specific proteins
- **Protein interaction domain**: allows the proteins to interact with one another to form a dimer —> binds to promoter sequence ~ **transcription factors —>** binds to DNA binding site
- All the proteins can interact with one another even if they are distinct versions of the gene (different alleles)









•The allele with the start is a **mutant version of the allele —>** makes **truncated protein** that can still form a dimer with the other protein
•**Only** the **red protein** contains the **DNA binding domain**
——> The protein interaction still occurs but the dimer can no longer bind to the promoter sequence since the mutant green protein has a structural malfunction —> **no transcription** as 2/3 of all the dimers lack the proper DNA binding site (only the red-red dimer can bind) —> **dominant negative loss of function mutation**

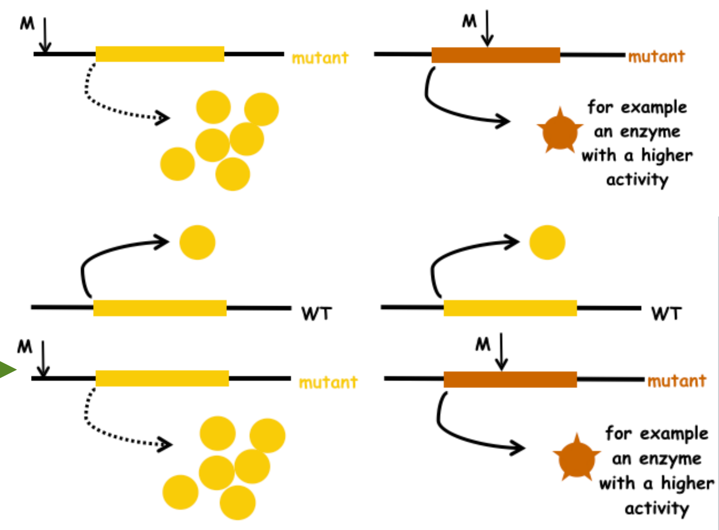
Gain of Function Alleles

Gain of Function: Gaining or **further expressing** something, or attaining expression at a different location

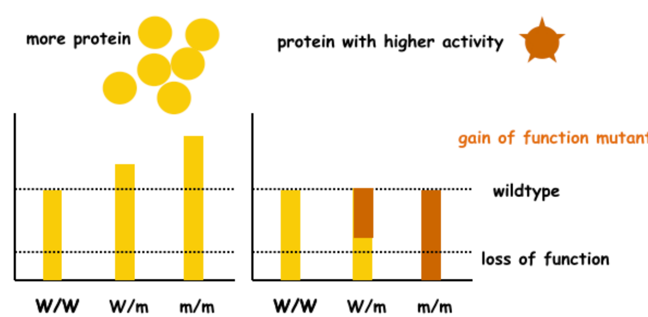
- •Gaining something new doesn't necessarily relate to improvement

•**Hypermorphic:** A mutation that **increases** the amount or **normal protein** made or **produces** a **protein** that **functions better (**enzyme with higher activity level)

- •**Heterozygotes:** Tends to be **dominant** and conveys a **mutant phenotype ~** so if you have a WT and a Mutant version will the mutant version cause a greater increase in the amount of normal protein made and therefore it takes precedence over the WT which makes a **baseline** amount.



- • **Homozygous genotypes for hypermorphic mutants:** An even more active version of the protein in comparison to heterozygotes
- •**Example:** Mice contain **voltage gated sodium channels** coded for by a **wt** allele
  - •**possum:** the mutant allele for the sodium channel that allows for greater passage of salt through pores —> **benefits** itself as it has better mechanism but **non-beneficial** for the mouse who responds to salt increase with **behavioural changes**



•**Neomorphic Allele:** the very **rare** mutation of introducing a **new function**

- •**Example) enzyme** is mutated causing it to **function** at a **different location—>** at this new location it **recognizes a different substrate** —> **produced a new product**
- • **slight changes** induced by neomorphic mutations can **modify protein function** in which **if it selected for it will stay in the population**

•**Ecoptic expression of neomorphic allele:** gene expressed in location where it is usually unexpressed

•**Example —> homeotic mutations:** mutations causing the replacement of one organ for another

•So lets look at this ugly ass **drosophila fly:** The **antennapedia gene** is responsible for the development of legs and is expressed in certain segments of the fly **(thorax ~** specifically **2nd thoracic segment)**

•ecotopic expression of this gene in the head causes the legs to come out of the head .. mans look mangled

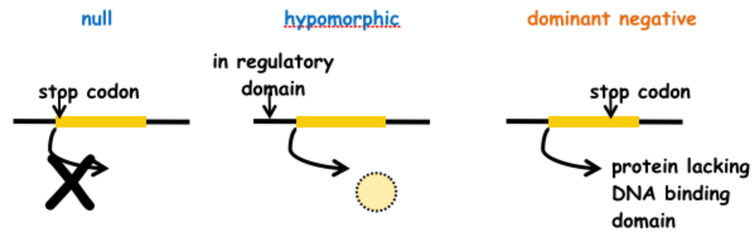
SUMMARY/FINAL NOTES OF ALLELE CLASSIFICATION!

• Mutations affecting function are **allele dependent not gene dependent .. AKA.** different versions of alleles (alleles carrying mutations) in the same gene can be **loss of function** or **gain of function**

•Mutations that occur in regulatory/promoter sequences ultimately **affect transcription levels**

• It is **very difficult** to **predict** how a **particular mutation** will effect its functions and thus it is best to **experimentally analyze the mutants**

•We often times like to artificially synthesize mutants and allocated them to specific places within the genome so we can test function!



Mutations at the level of DNA	Loss of Function Mutations	Gain of Function Mutations
Silent mutation: change occurs at wobble position (3rd) causing a base change but calling for the same amino acid during translation —> typically no phenotypic effect •sometimes induces change in biochemical pathway of protein created (change in base effects protein folding/developmental pathways)	**Null Mutations:** no protein is made at all, stop codon is introduced early —> protein synthesis is stopped altogether —> **Amorphic allele:** subclass of null in which deformed protein is made and such a deformity is severe enough for protein to be non-functional	**Hypermorphic:** A mutation that **increases** the amount or **normal protein** made or **produces** a **protein** that **functions better (**enzyme with higher activity level)
Missense mutation: change that causes polypeptide chain to be replace with a different amino acid	**Hypomorphic Alleles:** Mutations that reduce a genes functionality without completely eliminating its function —> when in promoter region it can cause decrease in levels of expression for the protein	**Neomorphic Allele:** the very **rare** mutation of introducing a **new function** —>**Ecoptic expression of neomorphic allele:** gene expressed in location where it is usually unexpressed
Nonsense Mutation: A point mutation in the DNA sequence that not only changes the amino acid itself but also changes the entire polypeptide chain by calling for a premature stop codon and producing a truncated , incomplete, and non-functional protein	**Incomplete Dominance:** The production of intermediate gene products which convey phenotypes made by a combination of genes —> **phenotype** will be one that can express null alleles with wt, or hypomorphic and (any combo of such) —> each allele will generate some kind of effect and the outcome will be a mixture of such effects	
Frameshift Mutation: Some type of insertion into the DNA sequence that ultimately causes the reading frame for its translation to be shifted —> **codons now call for different aa's** altogether	**Haplo-insufficiency:** One WT allele is not enough for a WT phenotype ~ Organism only has 1 copy of of the functional allele coding for the WT phenotype but both are needed to bring about the proper genetic condition **Antimorphic:** Mutations that fuck with Wild Type alleles thereby fucking with WT phenotype —> interferes or blocks wild type function ~ **poisons novel function**	