Cellular & Molecular Biology
SQBS 1143

Chapter 7:
Mutations

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Introduction

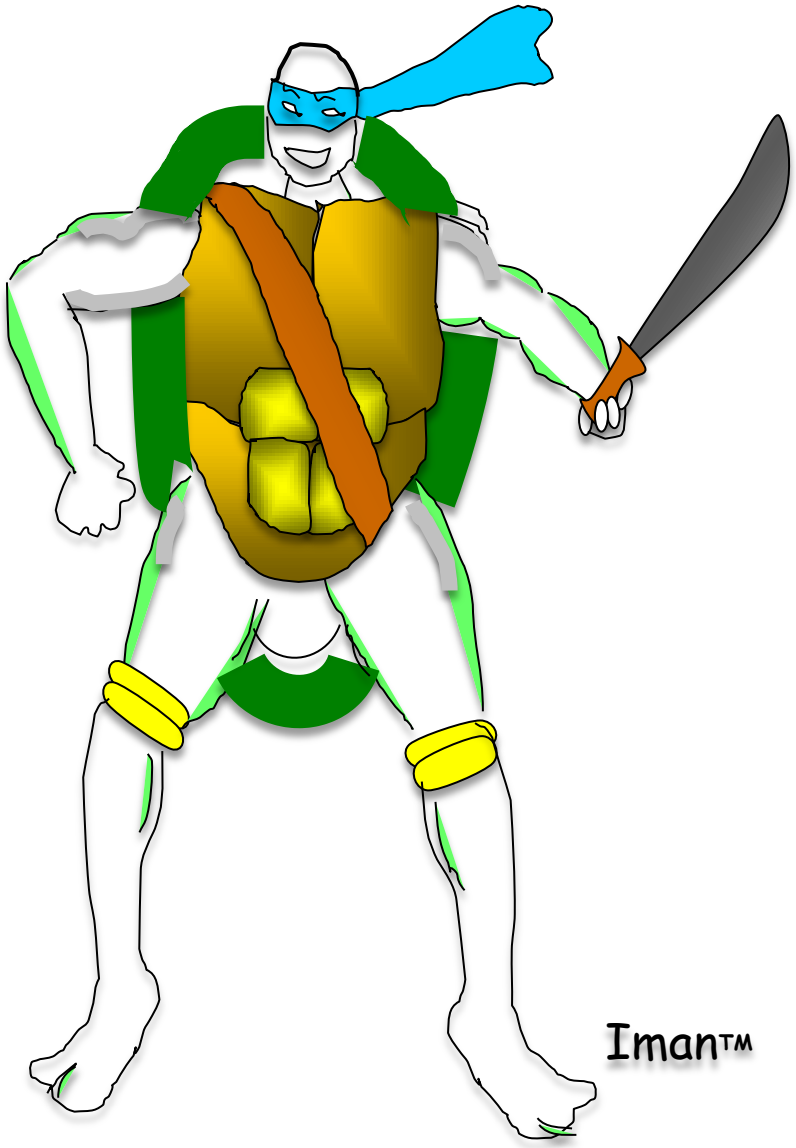
Nature is not perfect and mistakes can happen. Errors may occur in any of the processes of molecular biology. A mistake in a cell's genetic material is known as a **mutation**.

At the molecular level, mutations are **alterations in the DNA molecules** making up the genes. Because of this, mutations will be passed from a parent cell to its descendants; they are **inherited defects**. Then humans carry a mutation in their reproductive cells which leads to observable defect, we talk about inherited disease.

Although we tend to think of inherited condition like diabetes and muscular dystrophy as diseases, we often refer to cleft palates or color blindness as inherited defects. They are all as a result of mutations in our genetic material, **the DNA**.

Not only are some diseases directly caused by mutations but susceptibility to disease is also influenced by genetic constitution. It has been said that disease, except trauma, has a genetic component.

In fact, **all of us are mutants** - many times over – and we all have quite a substantial number of mistakes in our genes. That includes you!



Teenage Mutant Ninja Turtle

There are two main reasons why :

First, there are **many different types of mutations** and most of them have only a very **minor effect**; in fact many appear to cause no noticeable defect at all. Relatively few mutations cause such large changes that they attract our attention.

Secondly, higher organisms have **two copies of each gene**. This means that **if one copy is mutated**, there is a **back-up copy** which can be used.

This is just as well. It has been estimated that a typical human carries enough harmful mutations to total approximately eight lethal equivalents.

This means that if we were haploid, with only a single copy of each gene, we would all be dead.

Mutations Alter the DNA

A mutation is a **change in the base sequence** of the DNA. There are many possible changes we can make. These may be illustrated by considering their effect as shown in the next slide.

WILD TYPE	**THE CAT SAT ON THE MAT**
SUBSTITUTION	**THE RAT SAT ON THE MAT**
INSERTION (SINGLE)	**THE CAT SPAT ON THE MAT**
INSERTION (MULTIPLE)	**THE CATTLE SAT ON THE MAT**
DELETION (SINGLE)	**THE C*T SAT ON THE MAT**
DELETION (MULTIPLE)	**THE CAT *** ON THE MAT**
INVERSION (SMALL)	**THE TAC SAT ON THE MAT**
INVERSION (LARGE)	**TAM EHT NO TAS TAC EHT**

Obviously, such changes alter the meaning of the sentence to varying degrees. Similarly, altering the DNA base sequence has a variety of effect.

To understand these let's recall the **central dogma of molecular biology.**

First, DNA is the genetic material. When DNA molecule replicates, any changes due to mutations of the original DNA base sequence will be duplicated and passed on the next generation. In other words, **mutations are inherited**.

Second, the DNA is used as a template in transcription to make an RNA copy. Therefore the mutation in the DNA sequence will be passed on to the RNA molecule. Finally, the messenger RNA is translated to give protein.

Reason

An altered RNA sequence may be translated into an altered protein. Since cells depend on proteins to carry out all their chemical reactions, the final result of a change in the DNA sequence may be a **defect in the operation of some vital reaction**.

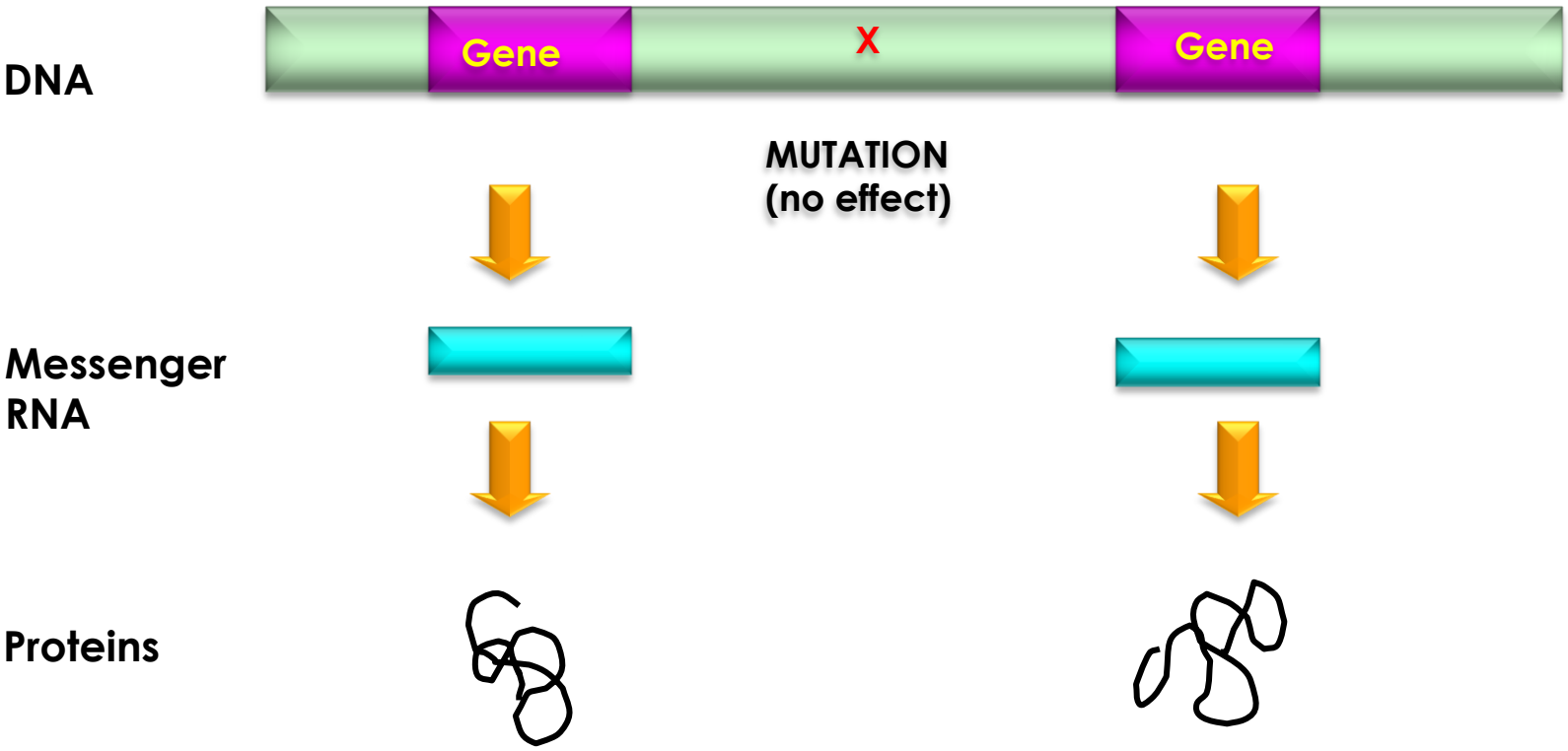
(1) Silent Mutation

A silent mutation is not a mutation that stops the cat on the mat from meowing or a mutation that kills the cat.

It is a mutation in the DNA sequence that **has no effect on the operation of the cell**. In other words, silent mutations do not alter the **phenotype**.

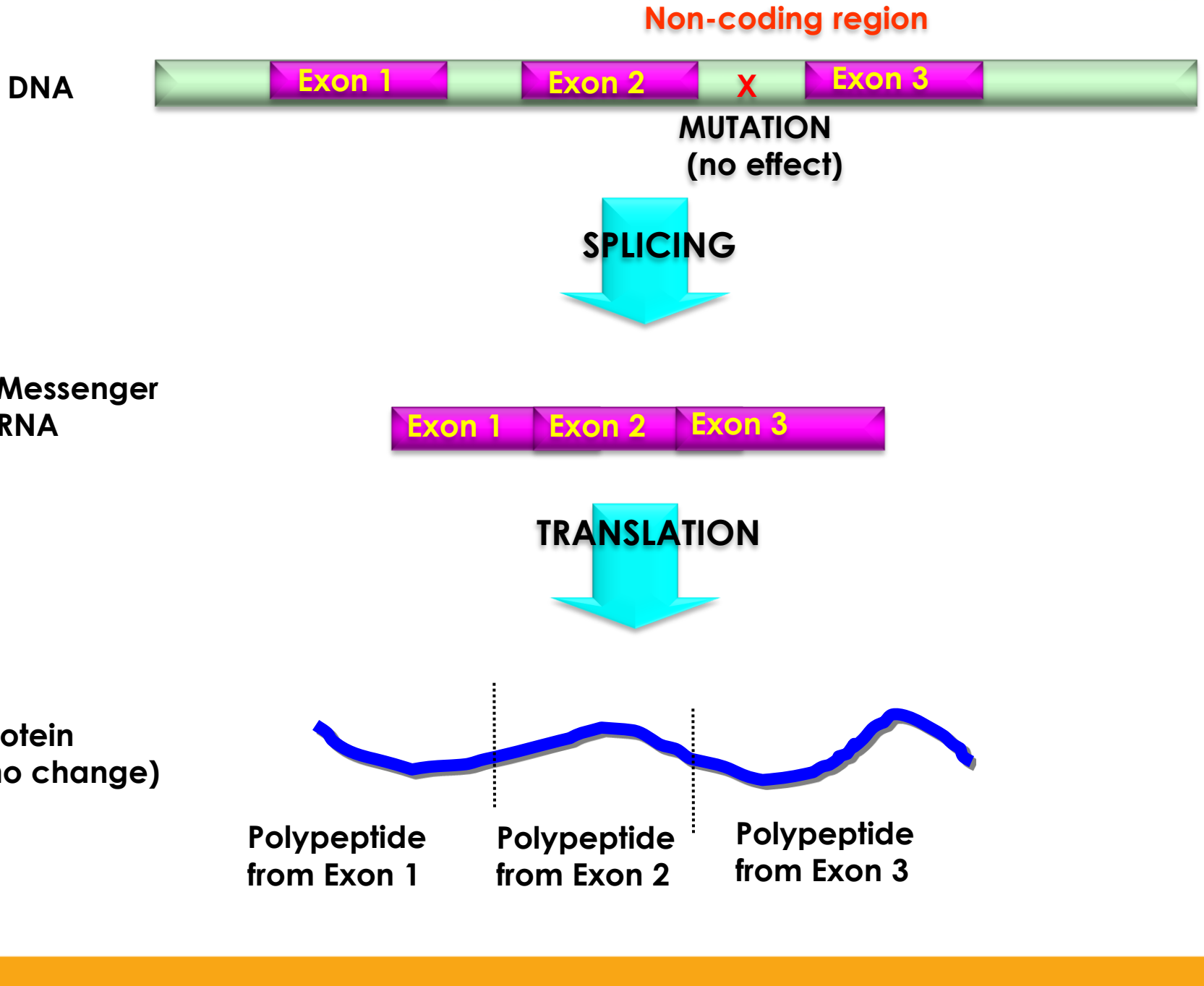
Categories of Silent Mutation

1. One obvious way to get a silent mutation is if the base change **occurs in the non-coding DNA** between genes. Therefore no genes are damaged and no proteins are altered.



2. Higher organisms possess **intervening sequences** within many of their genes. Since the intron is cut out and discarded when the messenger RNA is made, an **alteration in its sequence will not affect the final protein**.

   Note that **not all base changes in an intron are harmless**; we must not alter the few important bases at the splice recognition sites or disaster will result. Nevertheless, most base changes within an intron are also silent mutations.


Non-coding region
DNA
Exon 1
Exon 2
X
Exon 3
MUTATION
(no effect)
SPLICING
Messenger
RNA
Exon 1
Exon 2
Exon 3
TRANSLATION
Protein
(no change)
Polypeptide
from Exon 1
Polypeptide
from Exon 2
Polypeptide
from Exon 3

3. The third main type of silent mutation is within the **coding region** of a gene and **does get passed on to the messenger RNA**. How can this be?

The key is to remember the **genetic code**. Each codon, or group of three bases, is translated into a single amino acid in the final protein product.

However, because there are **64 different codons**, most of the 20 possible amino acids have more than one codon (see **CODON TABLE**) a base change that **converts the original codon** into **another codon that codes for the same amino acid** will have no effect on the final structure of the protein

2nd base (middle)

1st base	U	C	A	G	3rd base
U	UUU Phe UUC Phe UUA Leu UUG Leu	UCU Ser UCC Ser UCA Ser UCG Ser	UAU Tyr UAC Tyr UAA STOP UAG STOP	UGU Cys UGC Cys UGA STOP UGG Trp	U C A G
C	CUU Leu CUC Leu CUA Leu CUG Leu	CCU Pro CCC Pro CCA Pro CCG Pro	CAU His CAC His CAA Gln CAG Gln	CGU Arg CGC Arg CGA Arg CGG Arg	U C A G
A	AUU Ile AUC Ile AUA Ile AUG MET	ACU Thr ACC Thr ACA Thr ACG Thr	AAU Asn AAC Asn AAA Lys AAG Lys	AGU Ser AGC Ser AGA Arg AGG Arg	U C A G
G	GUU Val GUC Val GUA Val GUG Val	GCU Ala GCC Ala GCA Ala GCG Ala	GAU Asp GAC Asp GAA Glu GAG Glu	GGU Gly GGC Gly GGA Gly GGG Gly	U C A G

For example, the amino acid **alanine** has four codons: **GCU**, **GCC**, **GCA** and **GCG**. (Note that we are discussing this in RNA language: these are the codons as found on mRNA.) Since they all have GC as the first two bases, any codon of the form GCX (X = any base) will give **alanine**.

So if we start with **GCC** and mutate the last **C** to an **A**, this changes the codon to **GCA**, we still get **alanine** in the resulting protein. Many other amino acids (such as **valine**, **threonine** and **glycine**) also have sets of four codons in which the last base does not matter.

This is referred to **third base redundancy**. If you examine the codon table, you will see that **altering the third base may have no effect on the protein** that will be made.

In other words, about a third of single base changes will be silent, even if they occur within the protein coding region of a gene.

(2) Missense Mutation

Now for some **bad mutations**. When the change in the base sequence alters a codon, so **one amino acid in a protein is replaced with a different amino acid**, this is called a **missense** mutation.

i. First a **moderately bad mutation**: suppose we change the middle base **C** - of the codon **GCA** that codes for **alanine** to a **G**. We now have **GGA** which will give **glycine**.

Glycine and alanine are not identical but they are both **relatively small and uncharged amino acids**. Replacing alanine with glycine in a protein will probably not radically alter its structure. If we are reasonably lucky, the protein will still work, at least partially.

However, if the exchange is made in a critical region of the protein, such as its **active site**, we may **destroy its activity** completely.

Since the critical regions of most proteins occupy only a small proportion of the total protein sequence, most changes from one amino acid to another amino acid with **similar chemical properties** will be **relatively mild** and usually **non-lethal**.

These are known as **conservative substitutions** (See **CONSERVATIVE SUBSTITUTION**).

CONSERVATIVE SUBSTITUTION

And now for some **truly bad mutations**: suppose we change the middle base - C - of the codon GCA which codes for alanine to an A. We now have GAA which will give **glutamic acid**.

Glutamic acid is **acidic** and carries a strong **negative charge**.

It is most definitely **NOT similar to alanine** and is therefore referred to as a **radical replacement**. Unless we are very lucky, replacing alanine by glutamic acid will **seriously cripple or even totally** incapacitate our protein (see **RADICAL REPLACEMENT**).

RADICAL REPLACEMENT

ii. An interesting and sometimes useful type of missense mutation is the **temperature sensitive mutation**.

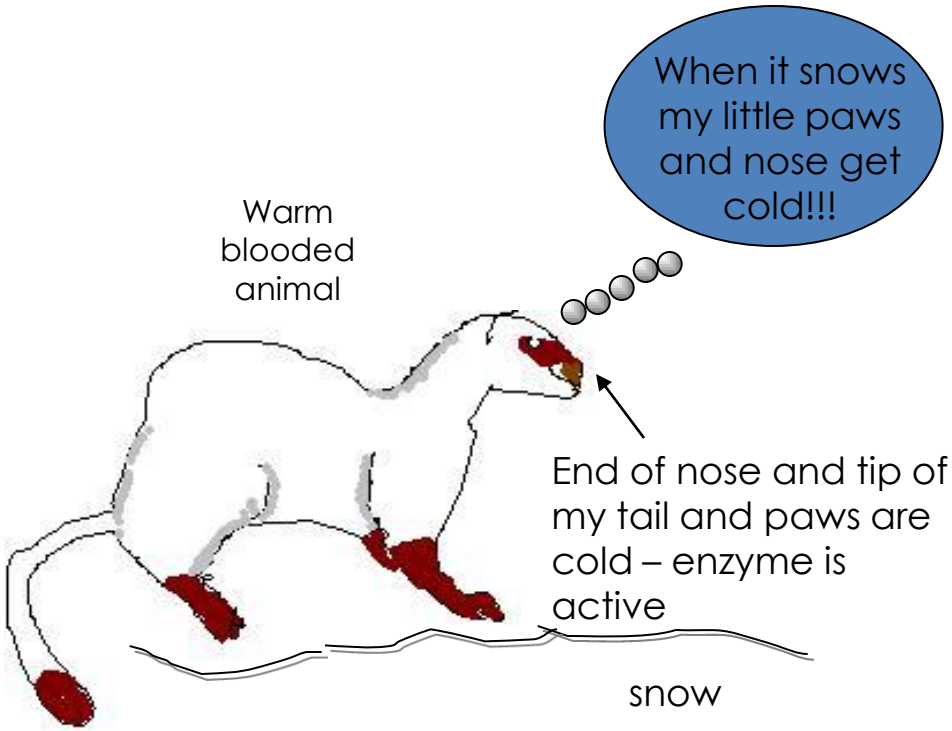
As its name indicates, we get a **protein that folds properly at low temperatures but at high temperatures is unstable and unfolds**.

Consequently, the protein is **inactive at high temperatures**. If a protein is essential, a missense mutation in it will often be **lethal**, and it is difficult to study a non-existent organism.

However, if we have a temperature sensitive mutant, we can grow it and perform experiments at the lower temperature, the **permissive temperature**, where it is alive.

To analyze the damage caused by the mutation, we can shift the temperature up to the temperature which the protein is inactivated and the organism will eventually die.

TEMPERATURE SENSITIVE MUTANT



Quite a few animals have black tips to their paws and tails, even though they are light coloured over the rest of their cute, cuddly, furry bodies. This is due to a temperature sensitive "mutation" in the enzyme responsible for synthesizing melanin, the black pigment in the skin . At the normal temperature of warm-blooded animals, the mutant enzyme is inactive, so melanin is not made over most of the body. However, it is cooler out in the boonies at the tips of the paws, the tail and the nose. Here the enzyme is active, melanin is made, and the tips turn black. What was originally a mutation has become a "normal" form of body colouring for these animals - a useful, or at any rate, a pretty mutation.

(3) Nonsense Mutation

Can things get worse? Why even ask…….
Things can always get worse suppose that we start with the codon **UCG** for **serine**. Let's change the middle base from **C** to **A**. We now have **UAG** which is one of the three **STOP codons** (**UAA**, **UAG**, and **UGA** - **STOP** - remember these!)

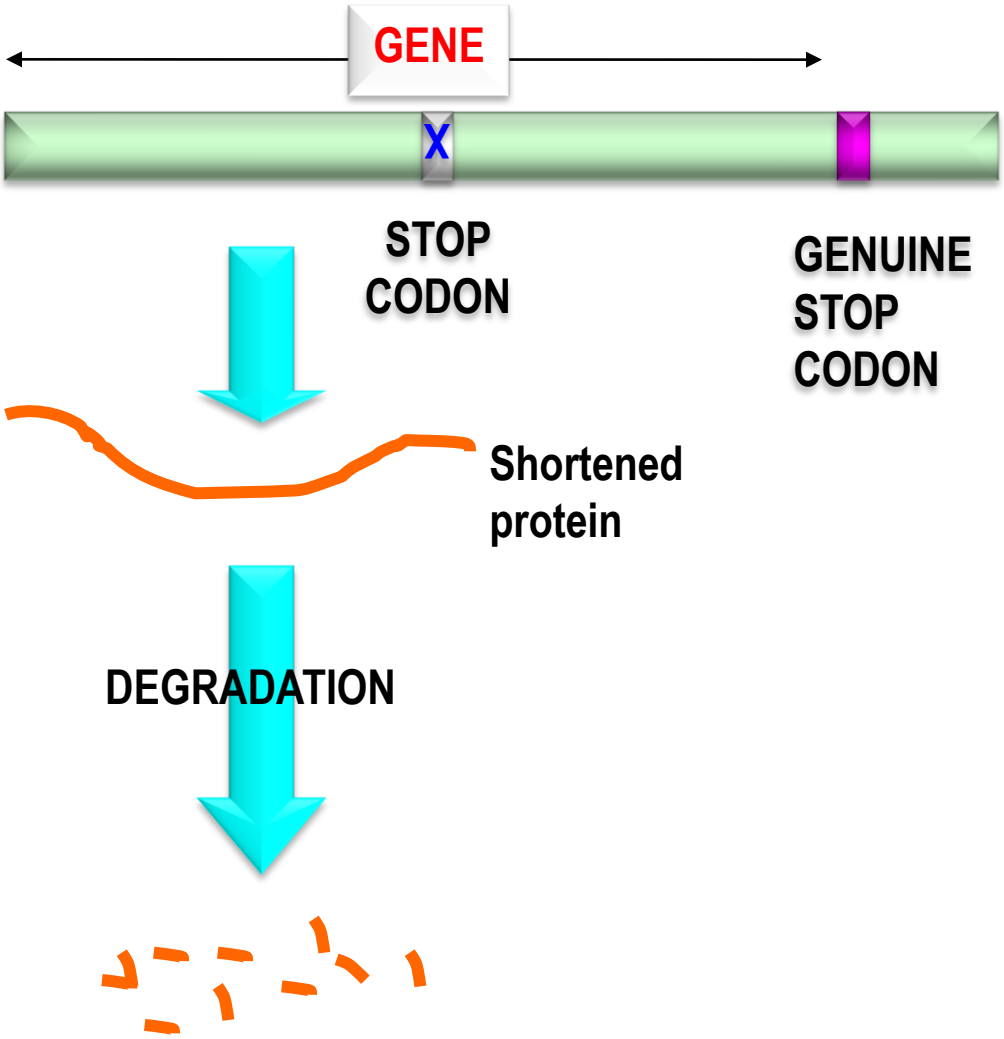
What happens now is that as the ribosome is making our protein it comes to - mutant codon that used to be serine. But this is now a stop codon, so the ribosome, a law abiding citizen, just stops!!! The rest of the protein does not get made.

This makes no sense so it's called a **nonsense mutation** (or sometimes **a chain termination mutation**).

Usually we end up with a shortened polypeptide chain that cannot even fold into a properly folded protein.

Its fate is sealed.
The cell detects and digests unfolded proteins. The result, in practice, is the total **absence of this particular protein**, which well have drastic results. Nonsense mutations are often **lethal**.

Nonsense Mutation



(4) Deletions and Insertions

So far we have been really rather restrained and only changed a single base for another.

We can remove one or more bases of the DNA sequence. Mutations in which bases are removed are known as **deletions**.

Obviously, if we delete the DNA sequence for a whole gene, this is pretty serious. Where is no gene, there will be no messenger RNA. If there is no messenger RNA, there will be no protein.

If there is no protein, there will be no cell - assuming the protein is essential. Large deletions may move part of a gene, an entire or several genes. You might think that the more bases we remove, the worse the mutation. Not necessarily. Consider the following important piece of RNA message and its translation into protein:

RNA Code: **GAG-GCC-GUA-AUC-GAA-UGU -UUG-GCA-AGG -AAA**

Protein: **Glu - Ala - Val - Ile - Glu - Cys - Leu - Ala - Arg - Lys**

Let's delete! First, just one base. Surely, in a DNA molecule with thousands or millions of bases, it will hardly be missed. No way! We'll delete the middle base of the third codon. And here is what happens:

Wild Type: **GAG-GCC-GUA- AUC-GAA-UGU-UUG-GCA-AGG-AAA**

Mutant: **GAG-GCC-G . A - AUC-GAA-UGU-UUG-GCA-AGG-AAA**

But remember that bases are read in **threes**.

This . is not actually there, it represents absence of a base. Therefore, when taken three bases at a time, our mutant sequence will be **grouped differently**.

By removing a single base we have changed the **reading frame**. The RNA will now be translated as follows:

Wild Type

RNA CODE : GAG-GCC-GUA- AUC-GAA-UGU-UUG-GCA-AGG-AAA

Protein : Glu - Ala - Val - Ile - Glu - Cys - Leu- Ala - Arg - Lys.....

Mutant

RNA CODE : GAG-GCC-GAA- UCG-AAU- GUU-UGG-CAA-GGA....

Protein : Glu - Ala - Glu - Leu - Asn - Val - Trp - Gln - Gly.....

We have completely changed all of the amino acids after the deletion point. With just a **single base deletion**, our **protein sequence has been completely disrupted**.

Insertion of a single extra base would have much the same effect. Whenever a mutation changes the reading frame, it is known as **frameshift mutation** and the resulting protein sequence is total drivel.

Deletion or insertion of **two bases would also change the reading frame**, by two spaces in this case, and it would give a similarly disrupted protein.

However, suppose we delete **three** bases:

Wild Type

RNA : **GAG-GCC-GUA-AUC-GAA-UGU-UUG-GCA-AGG-AAA**

Protein : **Glu - Ala - Val - Ile - Glu - Cys - Leu- Ala- Arg- Lys**

Mutant

RNA : **GAG-… GUA – AUC – GAA - UGU – UUG - GCA ….**

Protein : **Glu - … Val - Ile - Glu - Cys – Leu –Ala …..**

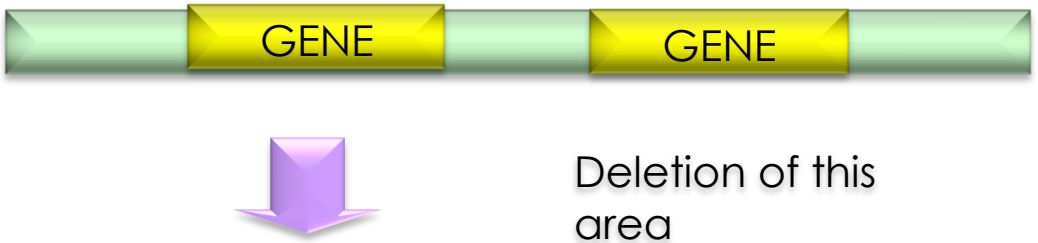
Three bases is a complete codon, so when we translate this sequence to make the protein, we delete an amino acid.

Although we have deleted an amino acid, we did not get out of step during translation; we have **reserved the correct reading frame**.

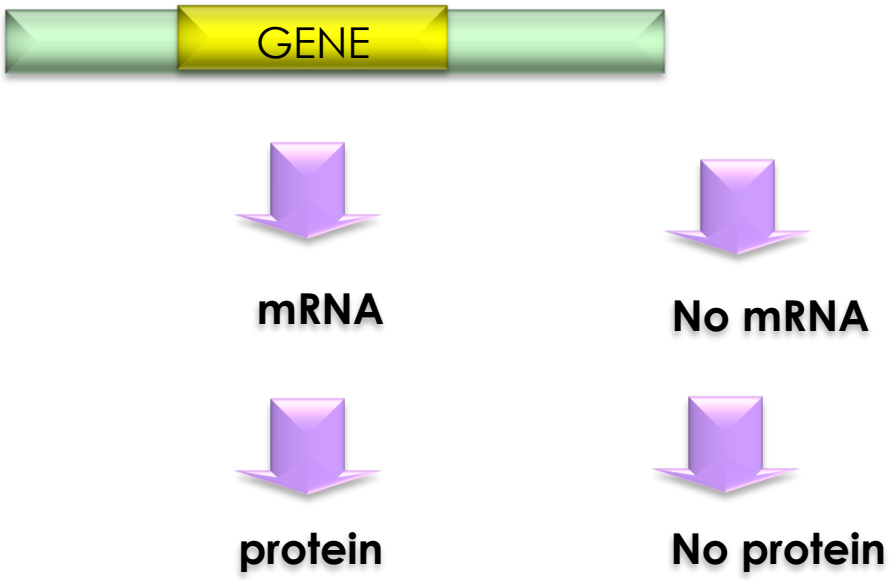
Apart from the single amino acid we lost, the rest of the protein is unchanged.

Large Deletion Mutation

Original DNA



Mutant DNA

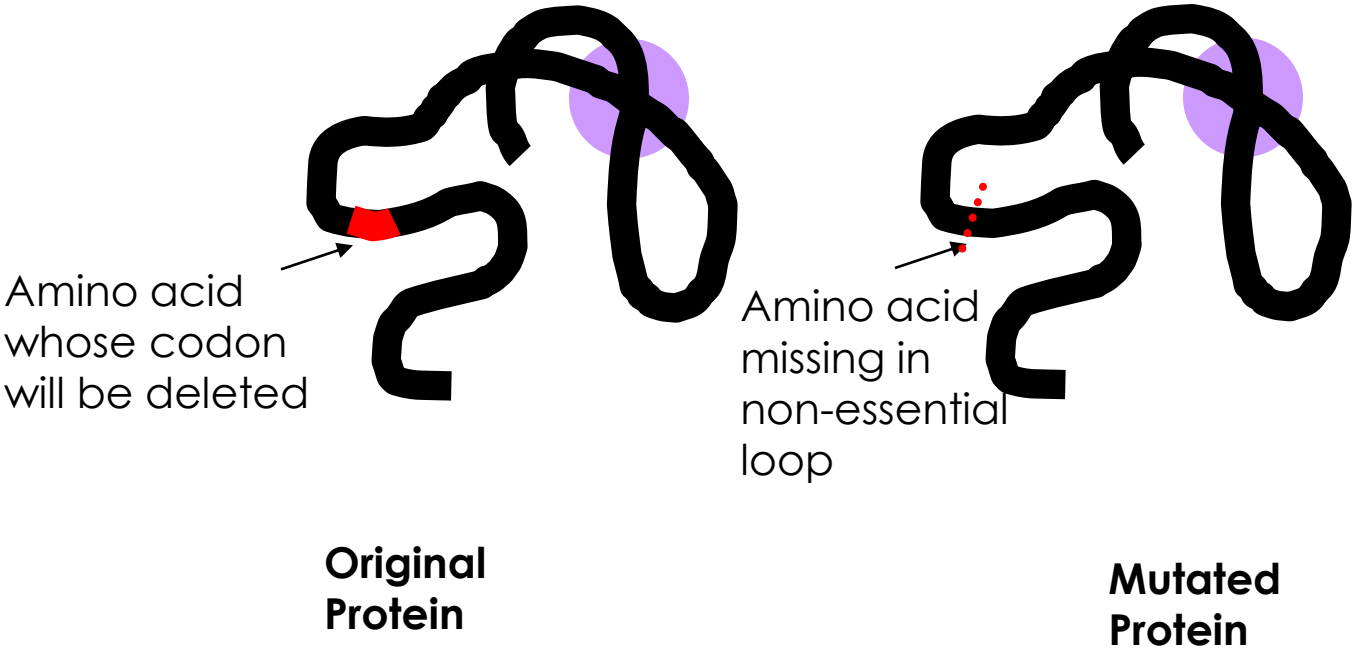


Similarly, a **three base insertion** would **add a single amino acid**, without affecting the rest of the sequence.

If the deleted or inserted amino acid is in a relatively less vital region of the protein, we may actually get away with this and make a functional protein.

We could even get away with adding or deleting more than three bases as long as the number is a multiple of three; in other words, we must add or subtract a whole number of codons in order to avoid the horrible consequences of changing the reading frame.

In Frame Deletion Mutations

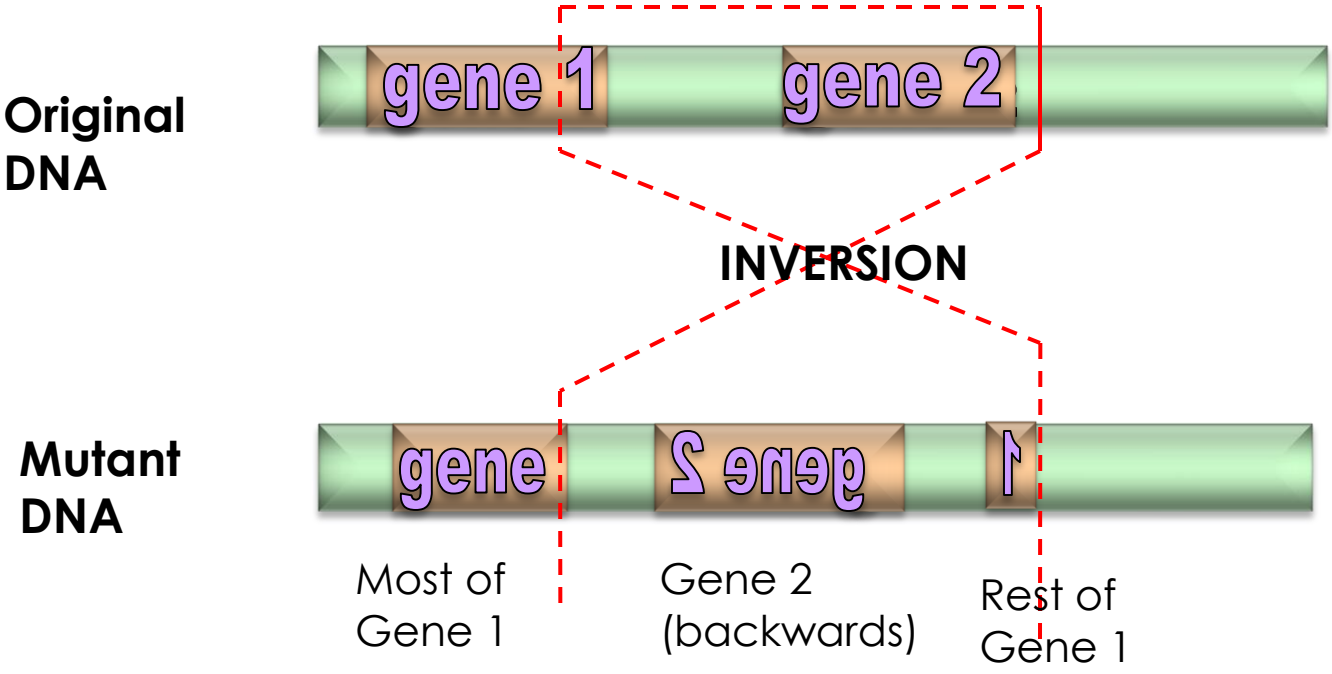


In much the same way we could add or remove a finger of your hand without killing you; you would just find it hard to get gloves that fit!

Compare that to the effects of changing the reading frame, which would be replacing an arm with a totally different body part.

(5) Rearranging DNA: Inversions and Translocations

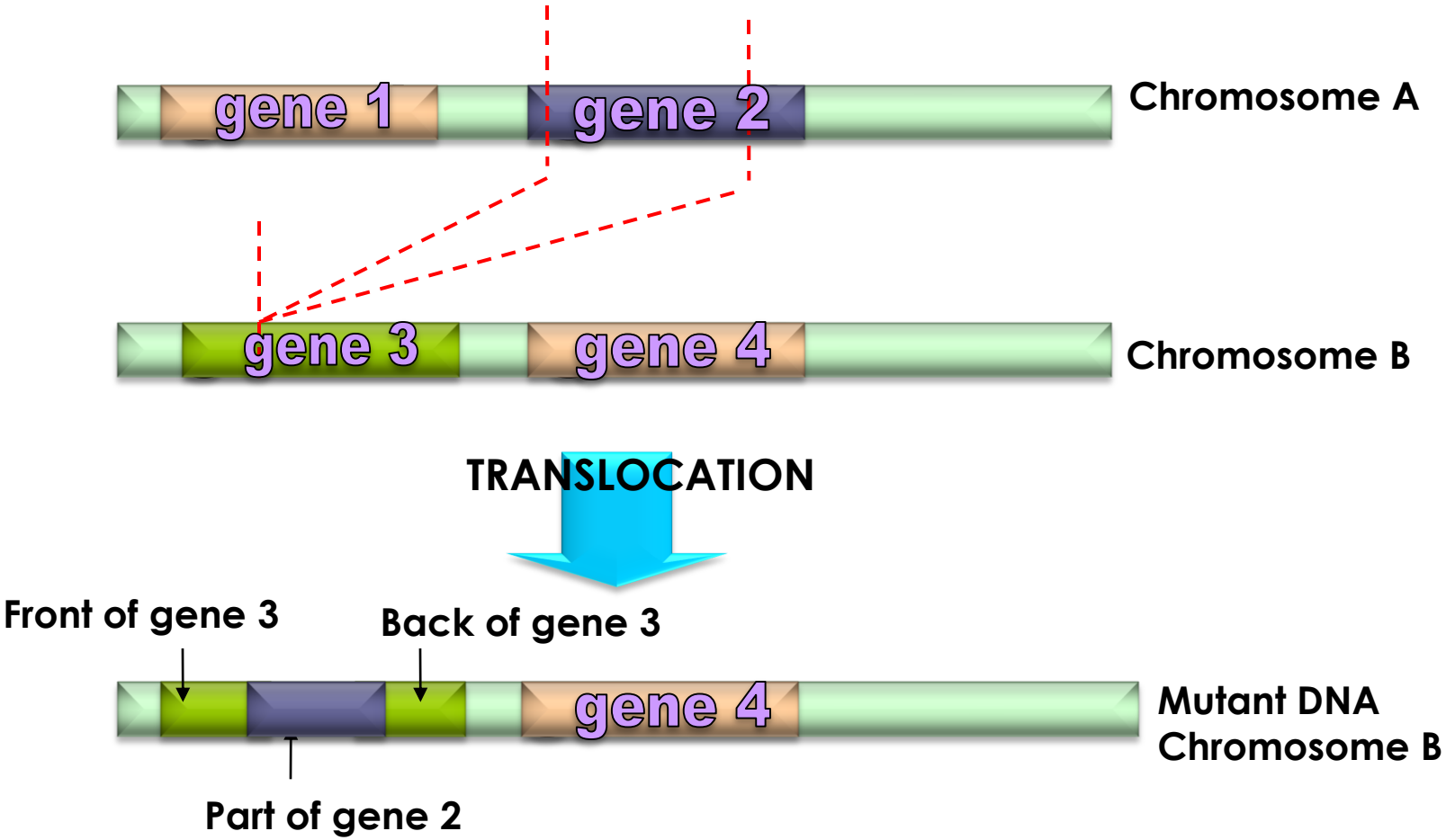
An **inversion** is just what its name implies, an inverted segment of the DNA. As you might imagine, reading a stretch of DNA backwards gives a ghastly mess. Inversions are definitely bad news.


Original DNA
gene 1
gene 2
INVERSION
Mutant DNA
gene
gene 2
1
Most of Gene 1
Gene 2 (backwards)
Rest of Gene 1

A **translocation** is when a section of DNA is removed from its original position and moved to another location, either on the same chromosome, or on a completely different chromosome.

If an intact gene is merely moved from one place to another, it may still work and little damage result. On the other hand, if, say, **half of a gene is moved** and **stuck somewhere else** in the **middle of another gene**, the result is chaotic and severely harmful.

Translocation mutation



What Causes Mutations?

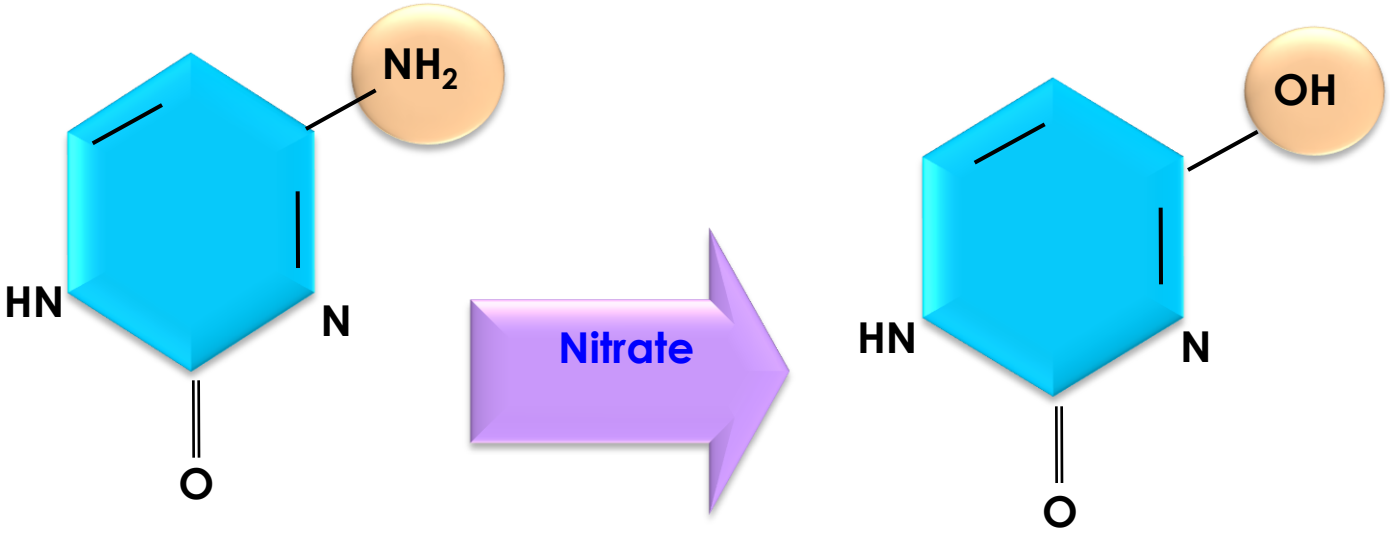
Mutations may be caused by agents that damage the DNA, and these are often known as **induced mutations**. Agents that mutate DNA are called **mutagens** are of two main types: chemical mutagens **(toxic chemicals)** and physical mutagen **(radiation).**

Even if there are no dangerous chemicals or radiation around, mutations will occur, though less frequently. These are known as **spontaneous mutations** and they are due to errors in DNA replication. The enzymes of DNA replication are not perfect and sometimes make honest mistakes.

The most common types of toxic chemicals react with DNA and alter the chemical structure of the bases. For example, **EMS** (**ethyl methane sulfonate**) is a mutagenic chemical widely used by molecular biologists. It adds a methyl group to bases in DNA and so changes their shape.

Nitrate is a chemical at replaces amino groups with hydroxyl groups and so converts the base cytosine to uracil.

Nitrate Converts Cytosine To Uracil



CYTOSINE

URACIL

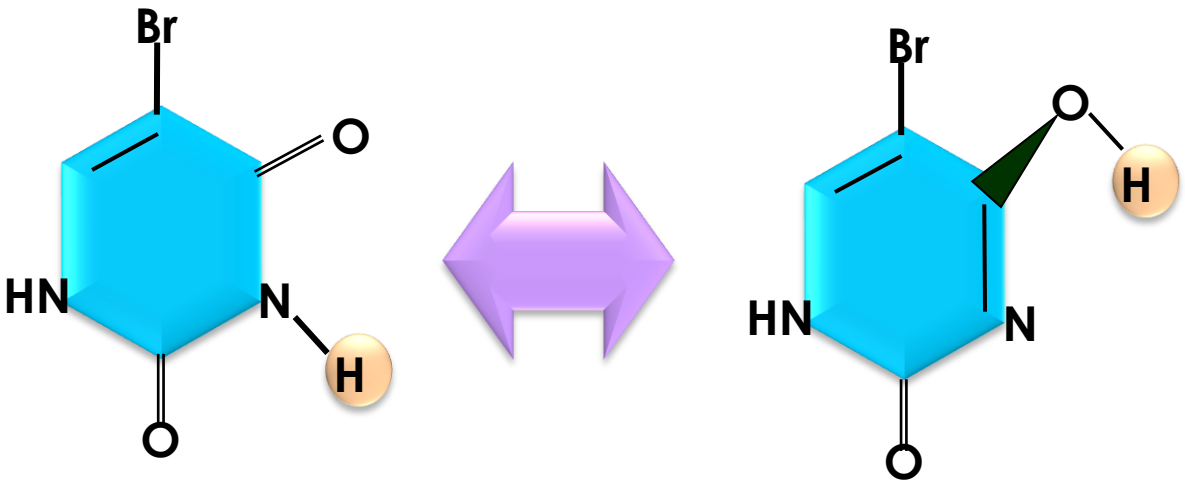
When the time comes for DNA replication, the DNA polymerase is confused by - altered bases and puts in wrong bases in the new strand of DNA it is making.

Another type of chemical mutagen mimics the bases found in natural DNA. For example, the chemical **bromouracil** resembles thymine in shape. It is converted by the cell to the DNA precursor.

Bromouridine triphosphate and DNA polymerase will then **insert this by mistake** where thymine should have gone. Mimics acting like this are called **base analogs**.

Fortunately, bromouracil can **change between two alternative shapes** like Dr. Jekyll and Mr. Hyde. In its evil Mr. Hyde form it **resembles cytosine** and **pairs with guanine**.

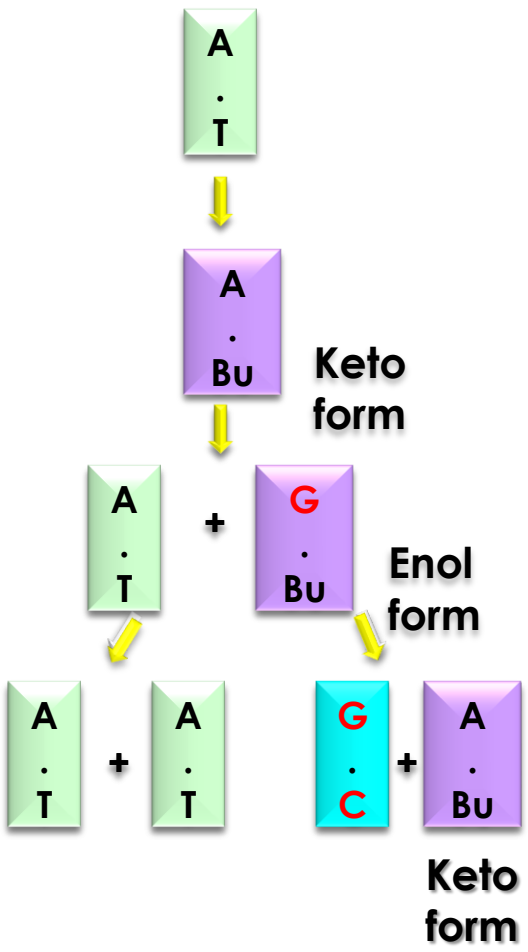
Bromouracil induced Mutations

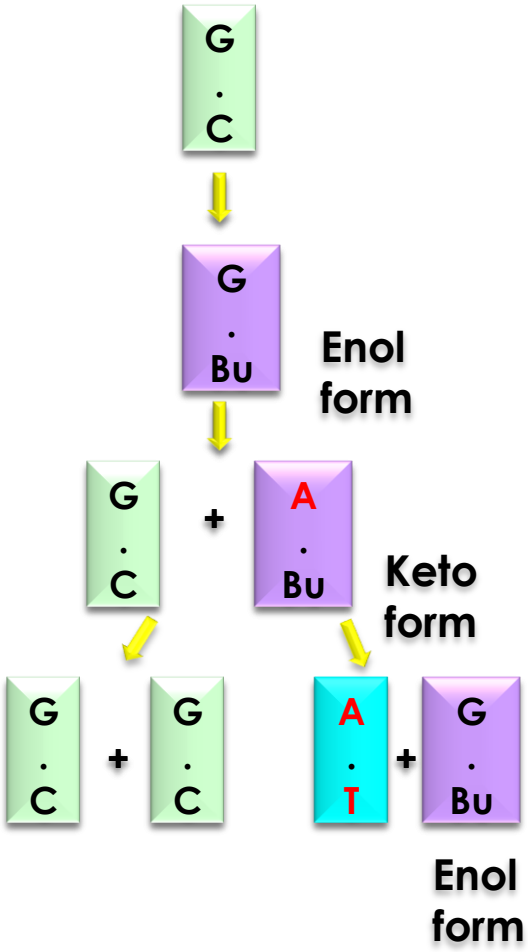


Looks like T (keto form) Pairs with A

Looks like C (enol form) Pairs with G

If **bromouracil is in its misleading form** when DNA polymerase arrives, a **G will be put into new strand opposite the bromouracil instead of A**.





Error in replication: A.T→G.C

Error in incorporation: G.C→A.T

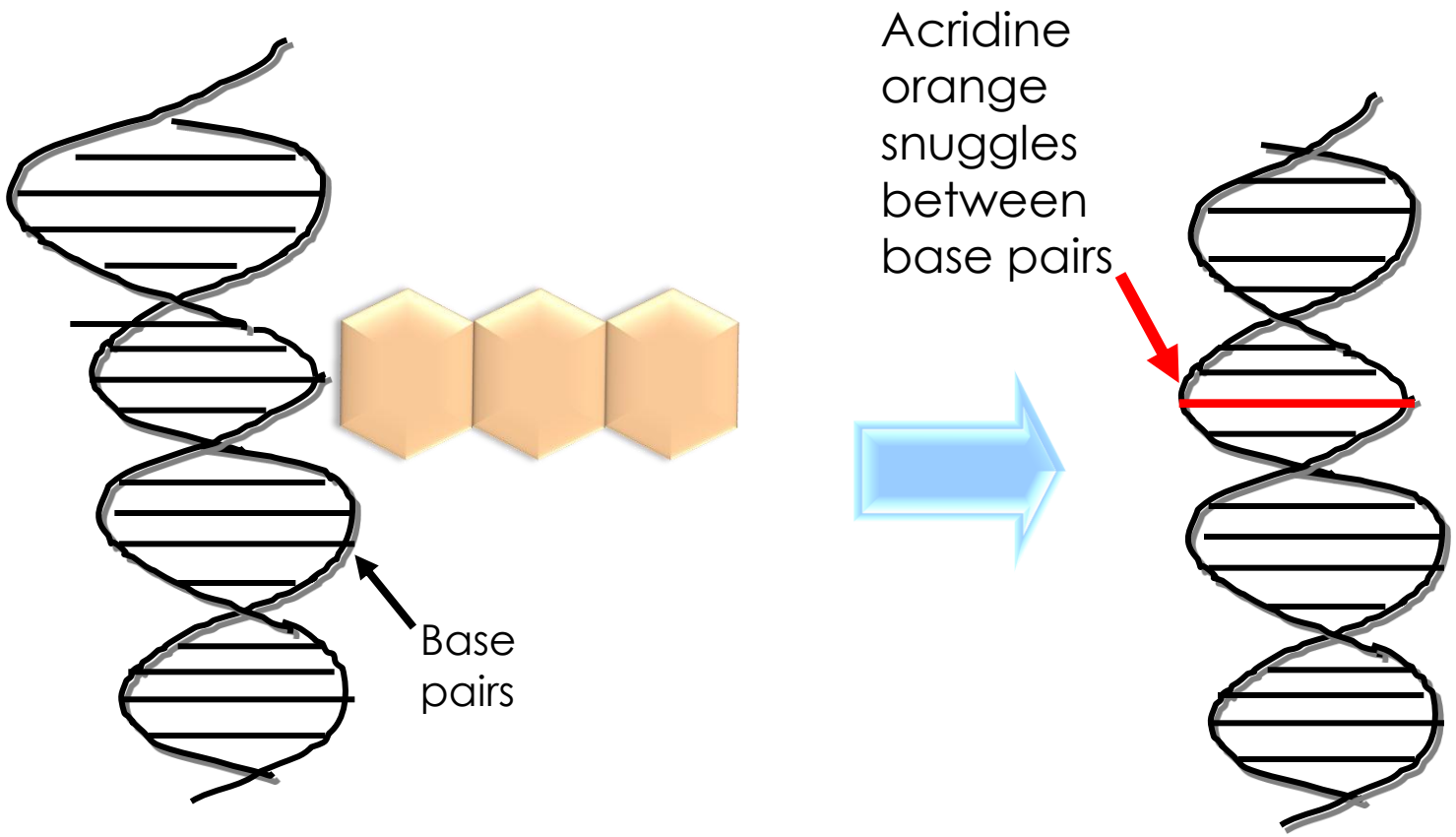
A. During replication, BU, in its **usual keto** form, substitutes for T and the replica of an initial A.T pair becomes an A.Bu pair. In the first mutagenic round of replication the BU, in its **rare enol** form, pairs with G. In the next round of replication the G pairs with a C, completing the transition from an A.T to a G.C pair.

B. During the replication of G.C, pair a BU in its **rare enol** form pairs with a G. In the next round of replication the BU is again in the **common keto** form and it pairs with A, so that the initial G.C pair becomes an A.T. The replica of the A.Bu pair produced in the next round of replication is another A.T pair.

A more subtle form of **chemical mimicry** consists of **imitating the structure** of a **base pair** rather than a single base. For example, **acridine orange** has three rings and is about the size and shape of a base pair.

Acridine orange is not actually incorporated into the DNA. Instead it **squeezes in between the base pairs** in DNA that already exists (see **ACRIDINE ORANGE IS AN INTERCALATING AGENT**). This is referred to as **intercalation**.

Acridine Orange is an Intercalating Agent



When it is time for DNA replication, the DNA polymerase thinks the intercalating agent is a base pair and it **puts an extra base** when making a new strand.

As discussed above **insertion of an extra base** will **change the reading frame** of the protein coded by a gene. Since this will **completely destroy the function** of the protein, **intercalating agents** are **definitely very bad**.

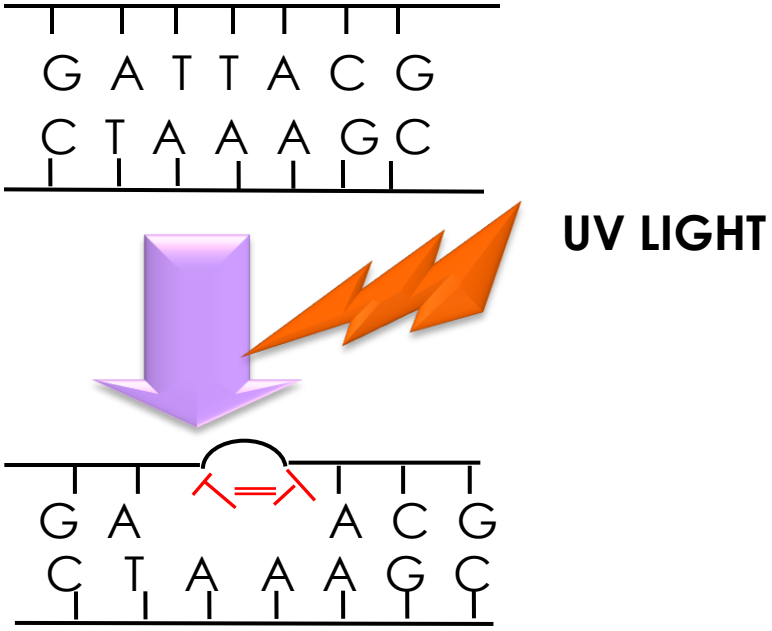
A **teratogen** is an agent that causes **abnormal development of the embryo** leading to "monstrosities," that is to say, **gross structural defects** (*teras* means monster in Greek).

The most famous example is **thalidomide** which resulted in the birth of malformed children often missing arms or legs, etc. Teratogens are simply mutagens which have **spectacular effects on animals**.

Some forms of radiation cause mutations. High frequency electromagnetic radiation - **ultraviolet radiation** (UV light), **X-rays** and **gamma rays** (g-rays) - directly damage DNA.

Ultraviolet radiation makes two neighboring thymine bases react with each other to give thymine **dimers** (see **ULTRAVIOLET LIGHT AND THYMINE DIMERS**).

Ultraviolet Light and Thymine Dimers



These confuse DNA polymerase which will make mistakes when synthesizing a new strand of DNA.

Ultraviolet radiation is emitted by the sun. Most of it is absorbed by the ozone layer in upper atmosphere, so it does not reach the surface of the earth.

If the ozone layer is destroyed by the chlorinated hydrocarbons used in aerosol sprays and refrigerants, the amount of UV reaching us increase drastically.

But don't worry! Long before this, the increased UV radiations, the increased UV would kill all the plants.

There would be nothing your single mouth to eat and would starve in dignity.

In the early days of molecular biology, **X-rays** were used to generate mutation in the laboratory, X-ray to produce multiple mutations and often yield arrangements of the DNA such as **deletions**, **inversions, translocations**.

And that is why, when you are given a chest X-ray, your procreative organs are shielded with a lead apron, the geneticist's equivalent of a bullet proof vest.

In addition to **electromagnetic radiation**, there are other forms of radiation such as α-particles and β-particles emitted by radioactive materials along with γ-rays.

There are also cosmic rays which, as you might guess, some from outer space. Most α-particles are too weak to penetrate skin and it is the β-particles which you need to worry about.

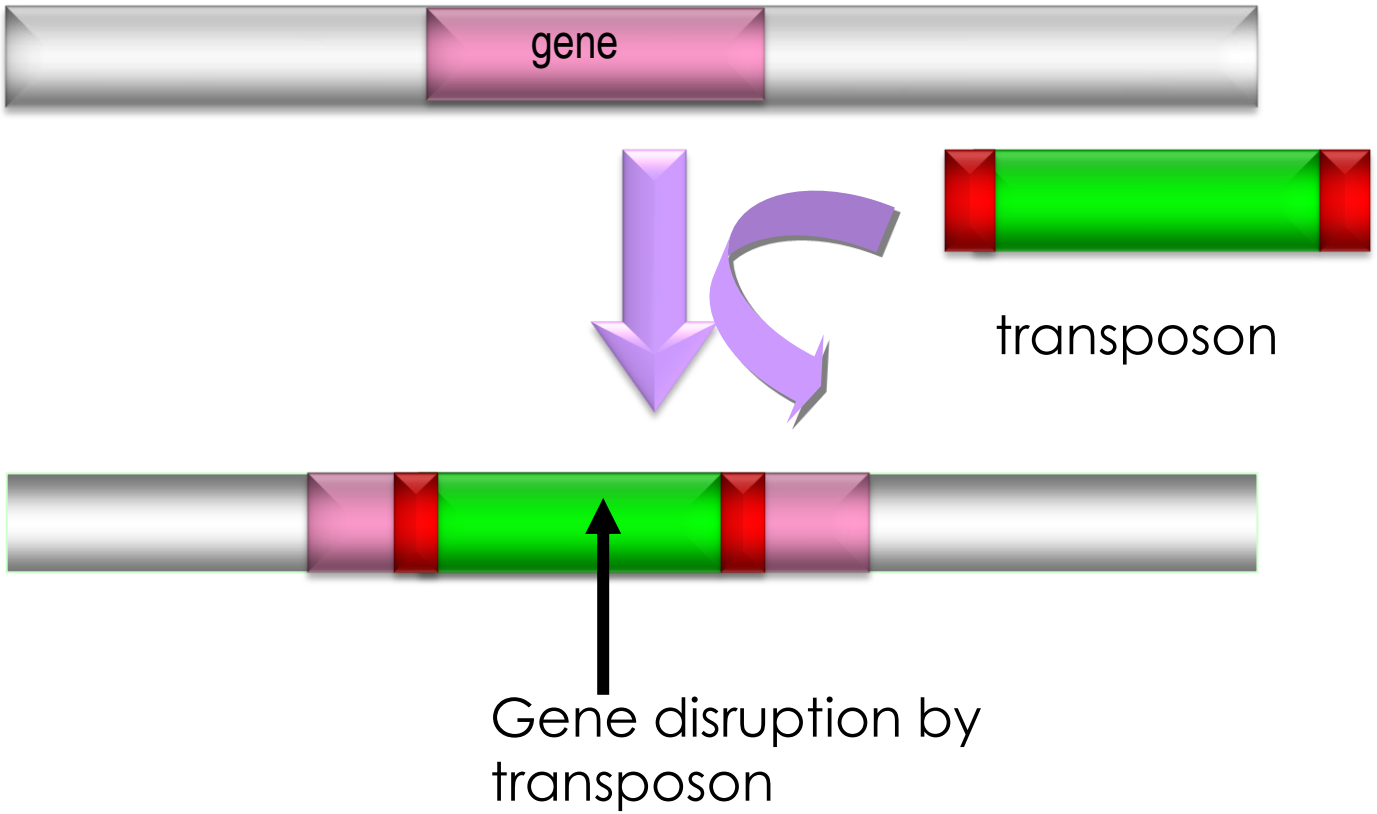
Mutation Caused by Insertion of Transposon

Insertion of an unrelated stretch of DNA into the middle of a gene we have drastic effects. A variety of DNA sequences are known that can move around from place to place on the chromosome. These are referred to as **transposon** or **jumping genes**.

Sometimes, when relocating, they **spontaneously insert themselves** into the **middle of another gene** (see **MUTATION BY TRANSPOSON INSERTION**). This **disrupts the target gene** and completely **abolishes its proper function**.

Although these mutations are insertions, they are really quite distinct in their origin from the smaller insertions described above which are due to chemicals or to mistakes made by DNA polymerase.

Mutation by Transposon Insertion



Genetically-engineered Gene Disruption

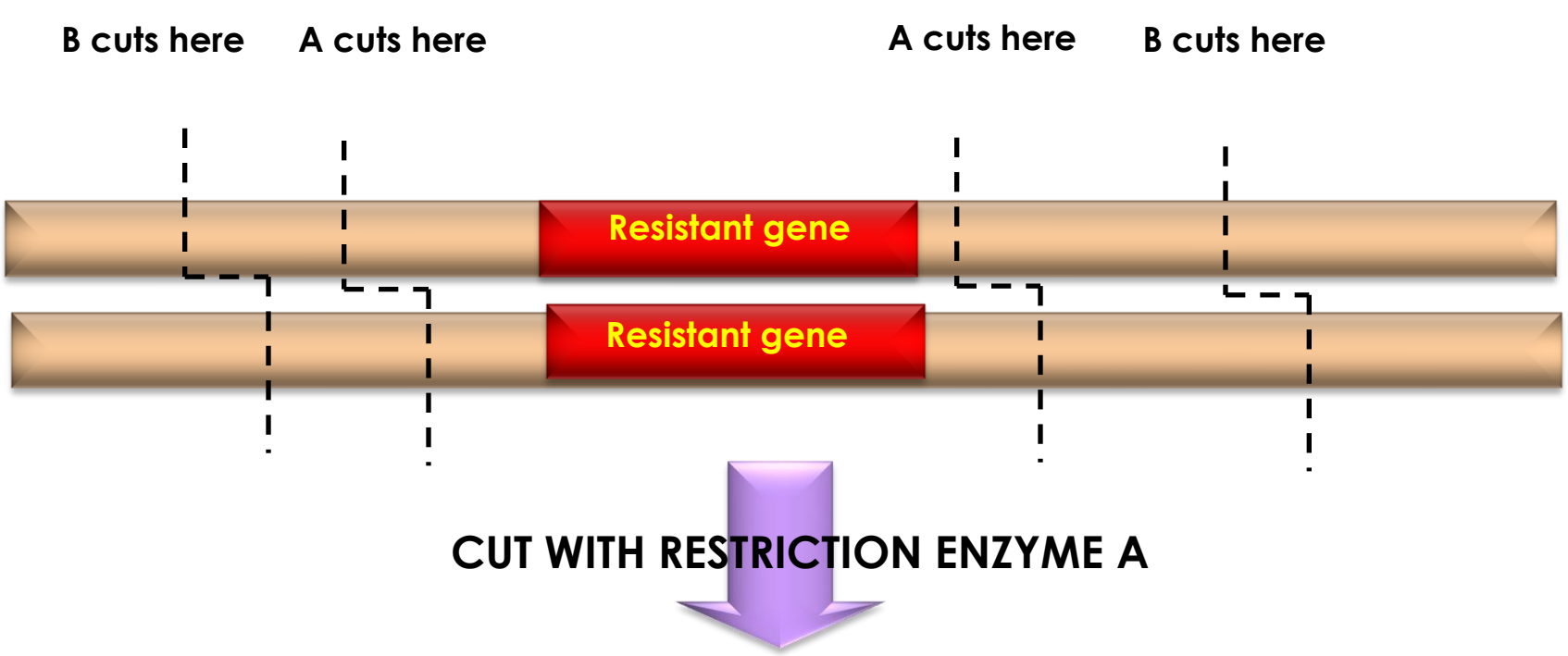
Mutations that serve to completely inactivate a gene are useful in genetic analysis. So scientists sometimes deliberately insert foreign DNA into genes to disrupt them and then study the results.

To do this it is necessary to clone the gene and carry it on some convenient vector such as bacterial plasmid.

For disruption, a deliberately designed segment of DNA is used. Known as **gene cassette (see GENE CASETTE)**, it carries a gene for resistance to some antibiotic such as **chloramphenical and kanamycin**.

This way, like insert DNA can easily be detected because cells carrying it will carry some resistance to the antibiotic. At each end, the cassette has several convenient restriction enzyme sites.

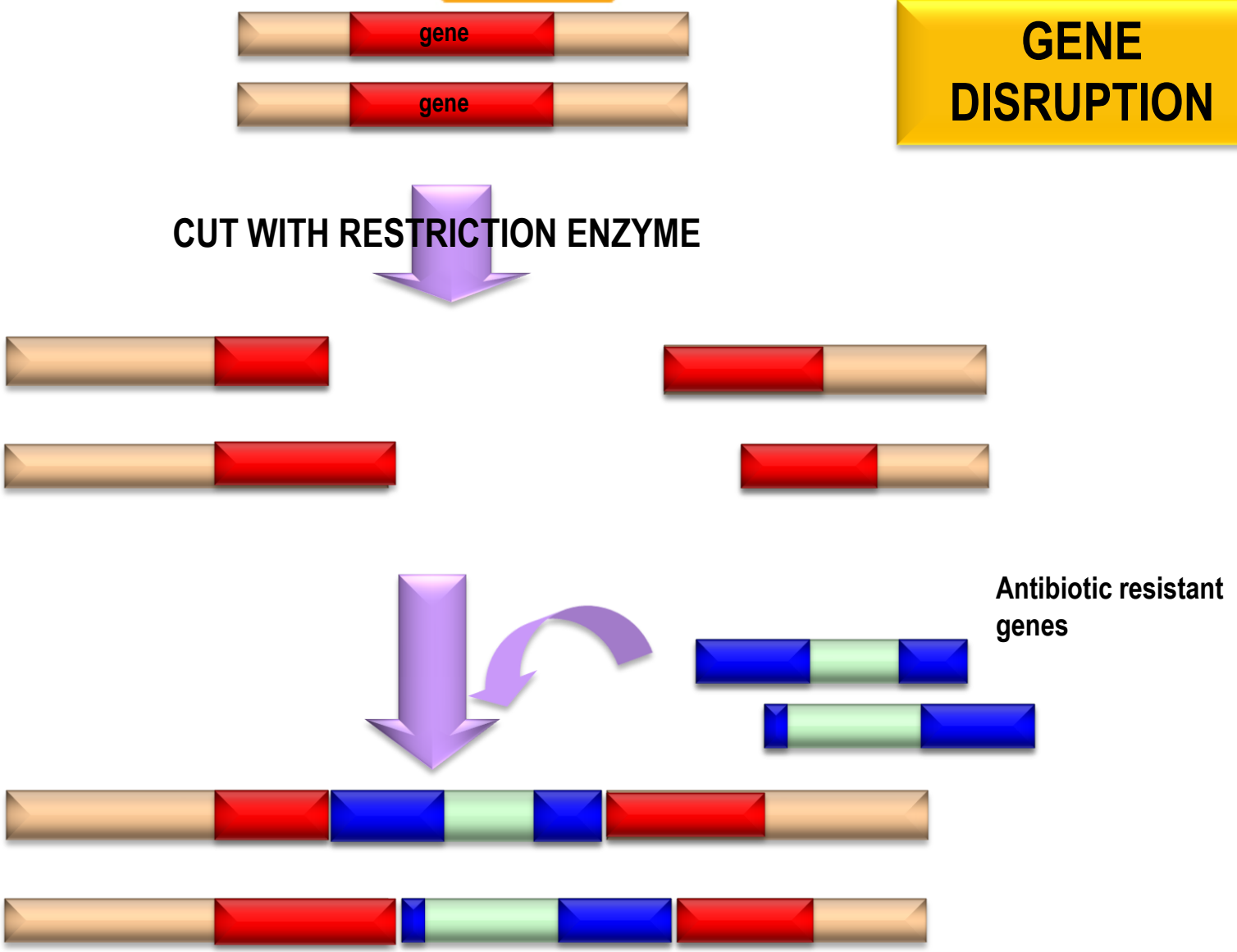
Gene Casette



The target gene is cut open with one of these restriction enzymes and the casette is cut from its original location with the same enzyme.

The cassette is then ligated into the middle of the target gene (see **GENE DISRUPTION**). The plasmid carrying the disrupted gene can now be put back into the organism from which it came.

GENE DISRUPTION



Mutational Hot Spots

If the same gene is mutated thousands of times, are the mutations all different and are they distributed at random throughout the DNA sequence of that gene?

Well, many of them are. However, here and there in the DNA sequence, you will find a location where mutations happen many times more often than average.

All the mutations occur at such a site will usually be identical. These are called **hot spot**.

Most hot spots are due to the presence of occasional **methylcytosine bases** in the DNA. These are made from cytosine after DNA synthesis and they pair correctly with guanine just like normal cytosine.

However, every now and then one of these **methylcytosine** bases **spontaneously disintegrates to give methyluracil. This pairs with adenine**, not with guanine and so when the DNA is replicated next, an error will happen.

Reversion and Suppression

Suppose we have a mutant and its DNA gets zapped again. There is a small chance that the second mutation will reverse the effect of the first. The process is called **reversion**. Reversion refers to the **observable outward characteristics** of our organism: it is a **phenotypic** term.

The likelihood that exactly the one base out of millions that was **previously mutated**, will be the very one to **mutate again** is extremely **low**. Those rarities where the original base sequence is exactly restored are **true revertants**.

More often our **revertants actually contain a second base change** that cancels out the effect of the first one. These are **second-site revertants**. Let's consider two examples.

The simplest to understand is if the original mutation was a frameshift mutation due to **deletion or insertion of a single base**. This alters the reading frame and disrupt the protein sequence:

Wild Type

WtDNA: GAG - GCC - ATC - GAA - TGT- TTG -GCA - AGG-AAA

Protein : Glu – Ala – Ile – Glu – Cys – Leu – Ala – Arg - Lys

Original Deletion Mutant

DNA : GAG -G.C - ATC -GAA - TGT- TTG- GCA - GTG - TTG - GCA -AGG

Grouped as : GAG – GCA – TCG – AAT –GTT – TGG…..

Protein : Glu - Ala – Ser - Asn – Val – Trp ……..

But suppose we now insert an extra base a little way further along the sequence. This second site insertion will restore original reading frame.

Revertant

DNA: GAG – G.C – AATC – GAA –TGT – GCA – GTG – TTG – GCA ….

Grouped as : GAG – GCA – ATC – GAA – TGT – GCA …..

Protein : Glu - Ala – Ile- Glu – Cys – Leu ……..

Although the DNA sequence is not identical to its original state, the protein has been exactly restored. Similarly, **an insertion mutation can be corrected by a second-site deletion**.

The **key to success when reverting** is to **restore activity** to the protein, not to get obsessive-compulsive about the DNA sequence.

A less obvious but more frequent case is where the original mutation as a **base change**. Consider a protein with 100 amino acids whose correct 3-D structure depends on the interaction between a positively charged amino acid at position **25** to a negatively charged one at position **50**.

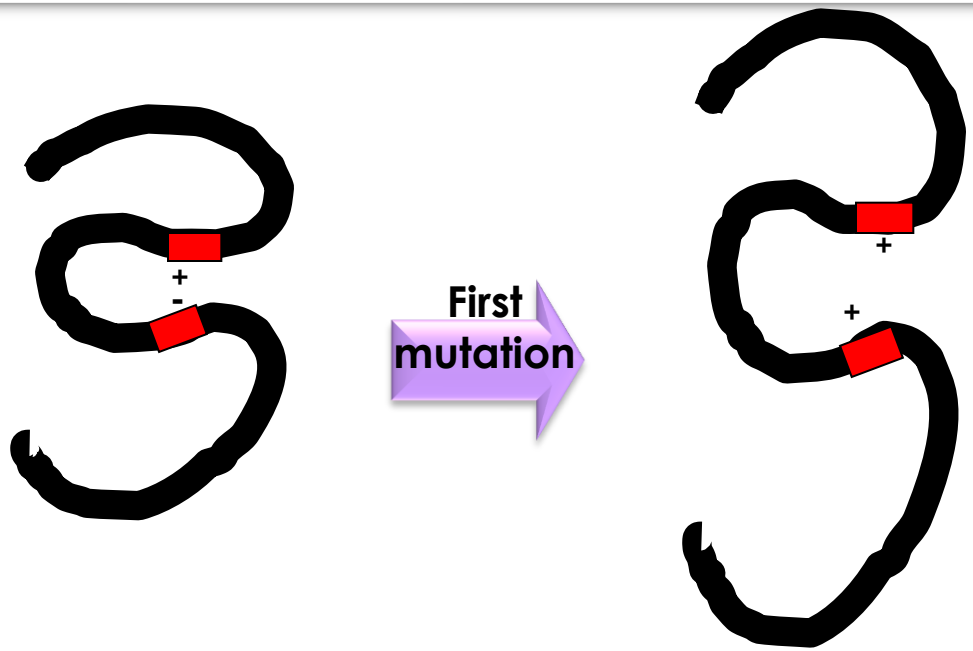
Suppose the original mutation changes codon no. **50** from **GAA** for **glutamic acid** (**negatively charged**) to **AAA** which encodes **lysine**, a **positively charged amino** (see **MISFOLDED MUTANT PROTEIN**). The protein's folding is now disrupted.

We could make a true revertant by replacing **AAA** with **GAA**. However, suppose instead we mutate codon No. **25** to give a negatively charged amino acid. We now have a negative charge at position No. **25** and a positive charge at No. **50**.

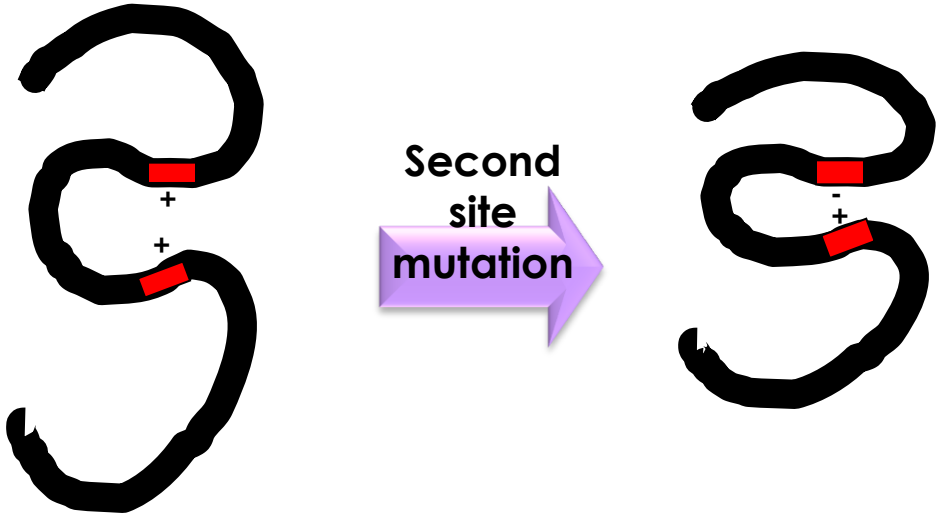
We have now restored the reaction between these two regions and the protein will fold O.K. again (see **SECOND SITE REVERSION CURES DEFECT**).

Will the revertant protein work correctly? Sometimes, sometimes not - it depends on other factors, such as whether these alterations change the active site.

Misfolded Mutant Protein



SECOND SITE REVERSION CURES DEFECT



Detection Mutagenic Chemicals by Reversion

Chemical mutagens can be detected by the **Ames test**, used routinely industry and government agencies to screen chemicals for possible dyes, and many other chemicals are now checked by the test that examines its effect on bacteria in culture.

Mutants of the bacterium *Salmonella typhimurium* carrying mutations in the **genes for histidine synthesis** are used in the Ames test. Since they **can no longer make the amino acid histidine**, these mutants **cannot grow unless given histidine**.

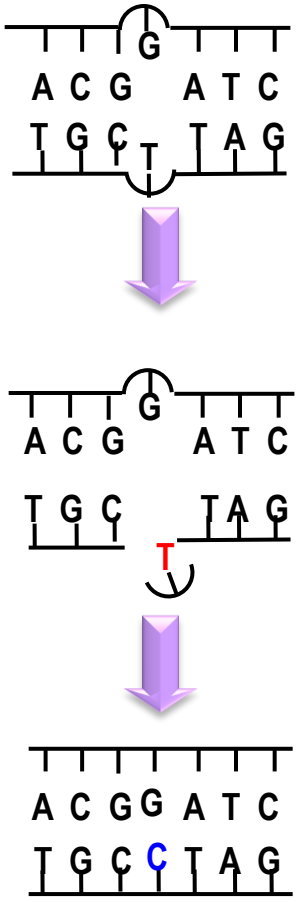
When large numbers of these mutants, bacteria are placed on growth medium lacking histidine, just a **few colonies** appear. These are **revertants** since **reversions are actually mutations back to the original state**; the frequency of reversion is also increased by mutagenic agents.

Different types of original mutant, for example, base changes or mutations, are used to screen for different classes of mutagenic agent. Clever, huh!

DNA Repair

Even if your genes are damaged, all is not lost. Most cells contain a variety of damage control systems and some of these can repair damaged DNA. There are several DNA repair systems, designed to deal with different problems and they are often rather complicated.

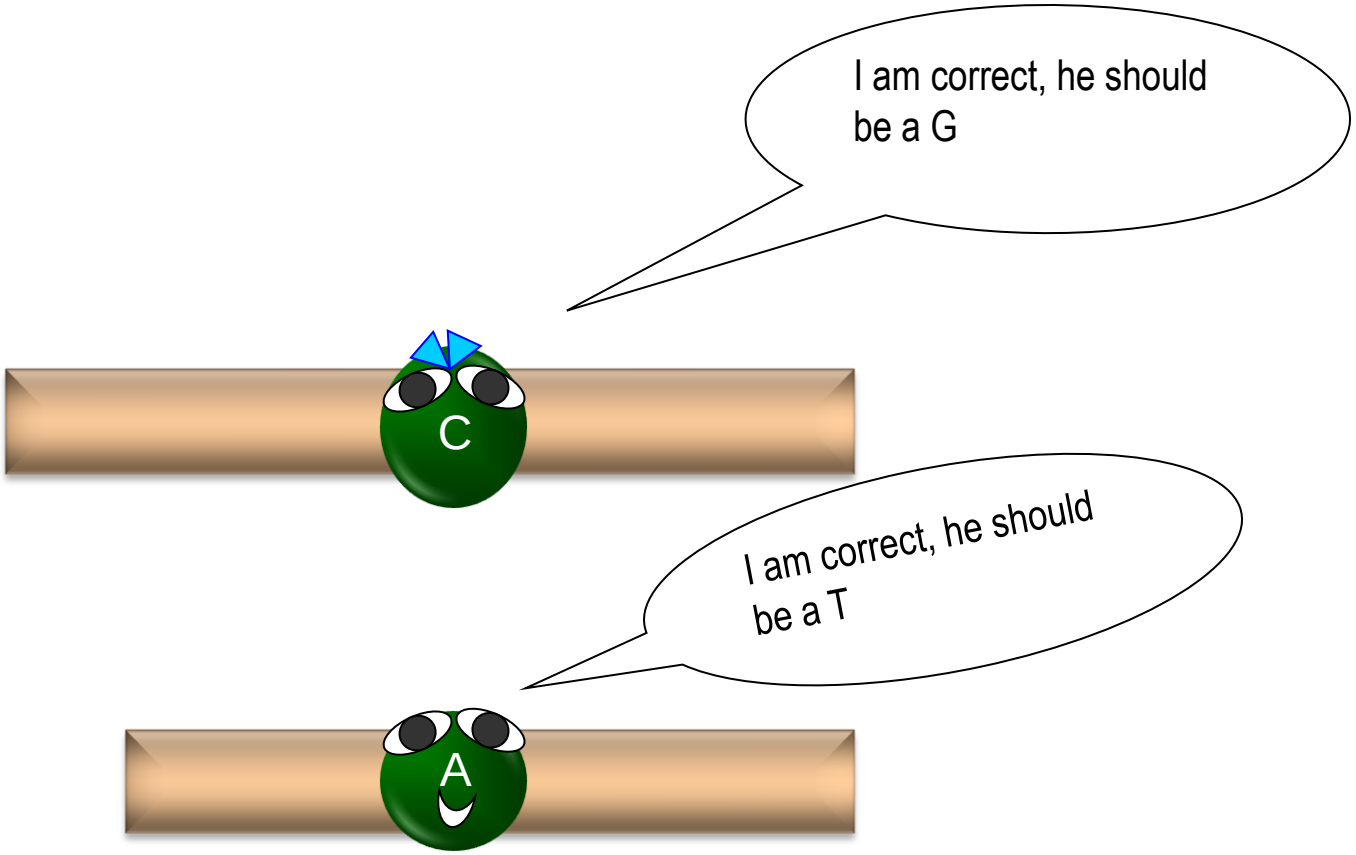
A variety of mutations may result in a base pair that doesn't actually pair properly. In other words, **two bases opposite each hydrogen bond correctly**. This will cause a slight bulge in the DNA double helix that alerts the proteins of the system (see **MISMATCH REPAIR SYSTEM**). This repair system cuts out the wrong base and fills in the gap with the right base to make a correctly bonded base pair.



MISMATCH REPAIR SYSTEM

But wait a moment! Which of the two mispaired bases was the wrong one? We need to know which strand came from the mother cell and which was the recently synthesized (and error carrying) daughter strand (see **BASE ACCUSATION**).

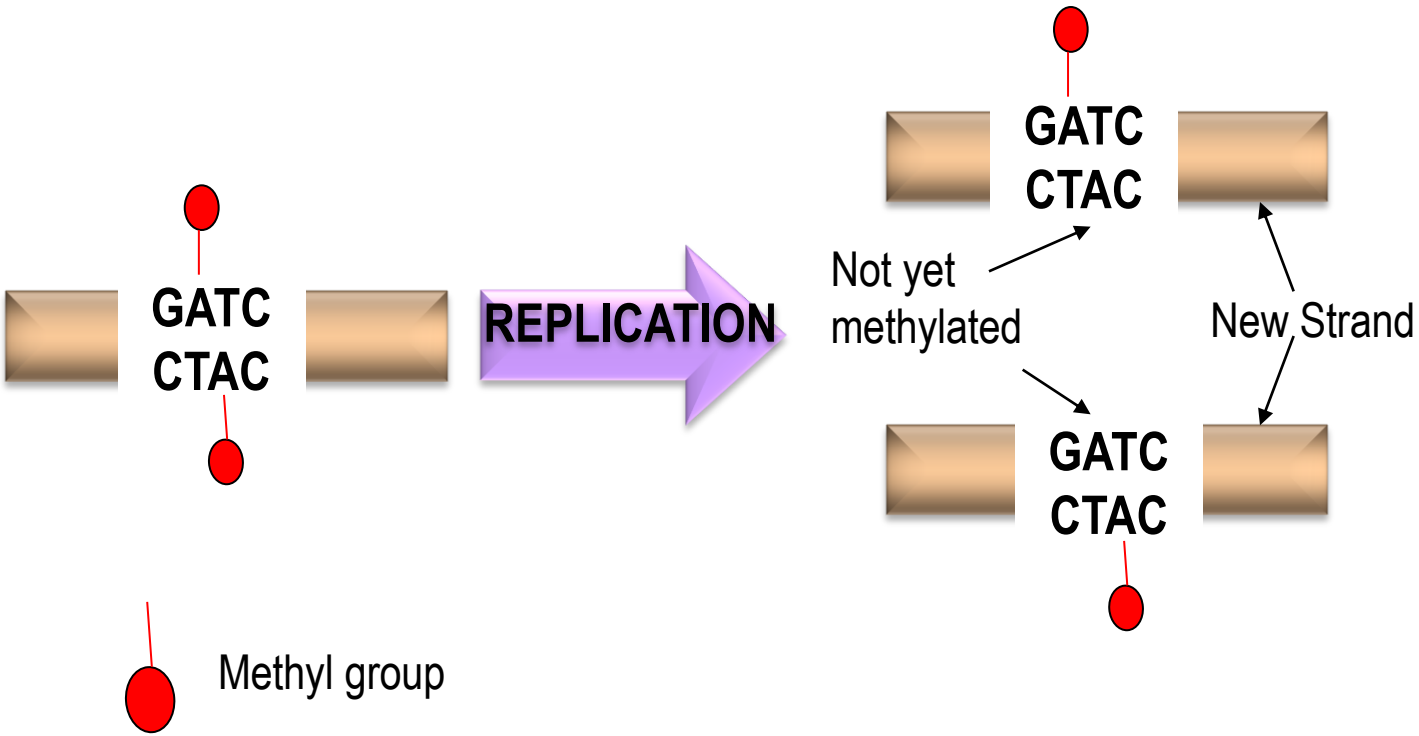
Base Accusations



Bacteria such as *Escherichia coli*, the DNA is tagged (labeled) to indicate this. Wherever the sequence GATC occurs, it has the **methyl group** stuck on to the adenine.

This modification occurs sometime after the DNA has duplicated. So the new strand in a recently replicated DNA molecules not yet have its GATC sequences methylated (see **GATC TAGGING ALLOWS STRAND IDENTIFICATION**).

This allows the mismatch pair system to tell which is the newest strand. Different organisms have different tagging systems, but in principle remains the same.



GATC TAGGING ALLOWS STRAND IDENTIFICATION

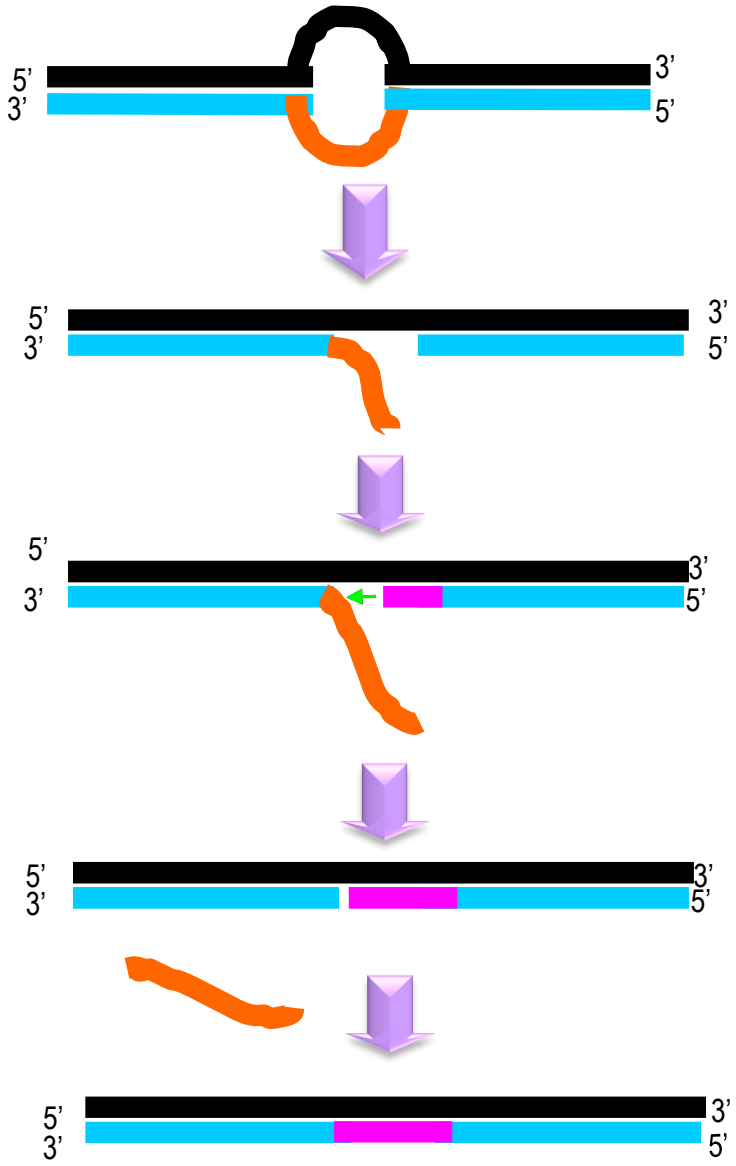
The most widely distributed system for dealing with mutated DNA is excision repair in which a stretch of damaged DNA is cut out and the resulting gap filled in with new DNA. This is often referred to as **'cut and patch repair'** system.

For example a thymine dimer caused by radiation will make the DNA bulge. First, a cut is made on one side of the bulge. Then DNA polymerase I makes a short replacement strand for the damaged region. As DNA polymerase I moves along, it also eat away the old strand.

Finally the old strand is cut off and the new segment is ligated into place (see **CUT AND PATCH REPAIR**). Pol I is better at needlework than your mother; when it has finished patching your genes, there are no visible stitches to show where the new piece is!

Mutations are of vital importance to us all for two main reasons. First, if a mutation occurs in the reproductive cells and can be inherited, it may have major effects on the lives of those who receive it. Second, mutations that are not inherited arise better in cells of the body, may cause cancer.

CUT AND PATCH REPAIR



References:

- Madigan, M.T., Martinko, J.M., Dunlap, P.V. and Clark, D.P. (2009). Brock Biology of Microorganisms: Pearson Education, USA.

- Clark, D.P. and Russel, L.D. (2000). Molecular Biology Made Simple and Fun: Cache River Press, USA.