

# Genetic Adaptation I

Microbial Physiology

Module 3

# Topics

- Topic 1: General Features of the Bacterial Genome
- Topic 2: Plasmids
- Topic 3a: Mutations
- Topic 3b: Repair

# Aims and Objectives

- By the end of this module you should
  - have an understanding of the differences in bacterial and eukaryotic chromosomes and how mutations impact on each
  - know about types of plasmids and what roles they play in microbial genetics
  - have an understanding of mutations and the roles they play in the generation of microbial diversity
  - have an understanding of the microbial defenses against mutations (repair mechanisms)

# Topic 1: Bacterial Genomes

- The genome is the replicating repository of cell's genetic information
- Significant differences between prokaryotes and eukaryotes

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<b>Feature</b>	<b>Bacteria</b>	<b>Eukaryotes</b>
Complement of genes	Monoploid	Diploid
Redundant DNA sequences	No	Yes
Colinearity between genes and proteins	Yes	No
Polycistronic mRNA	Yes	No

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# Complement of Genes

- Prokaryotes are monoploid
  - Eukaryotes are diploid
- Consequences of monoploid genomes
  - Mutations have immediate effect on the organism
    - Effects of mutations (particularly detrimental ones) can be masked by the second allele in a diploid genomes
  - Allow the use of genetic switches in the promoter regions
    - Mechanism of controlling gene function

# Genetic Organisation in Bacteria

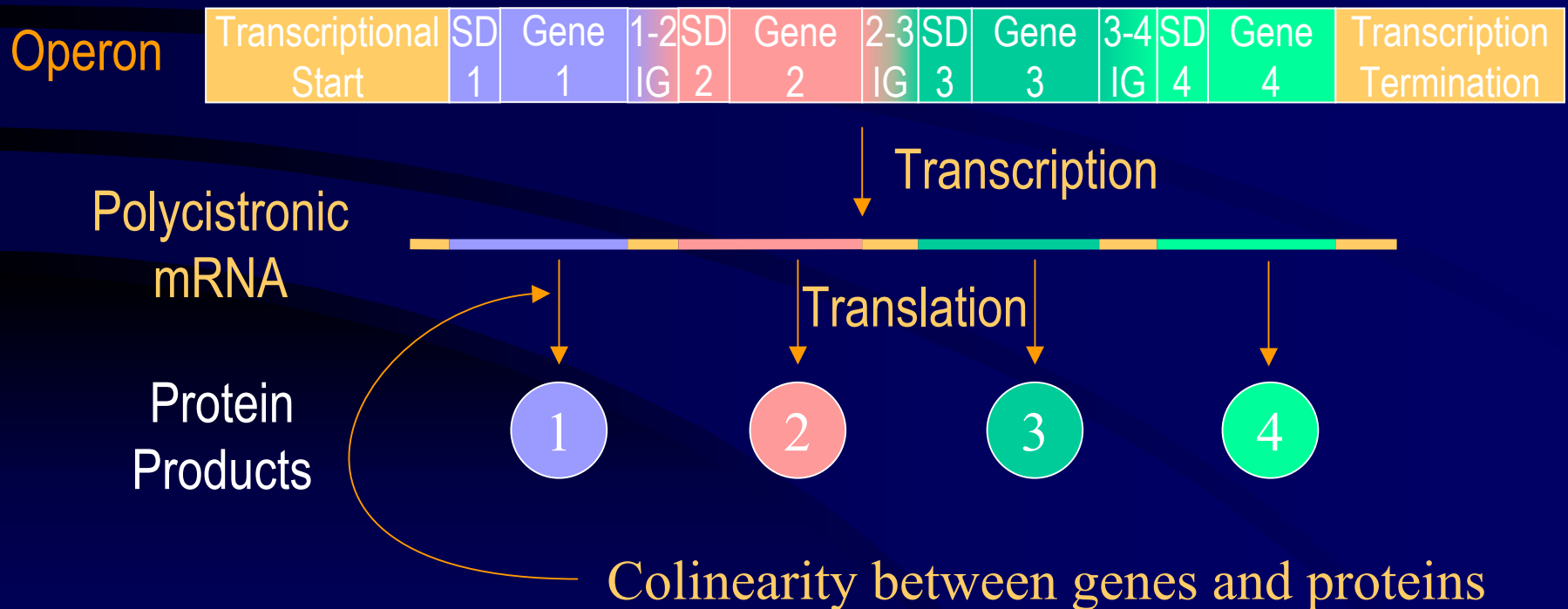
- Single, circular chromosome (generally)
- Compact

Organism	Size (Mb)
<i>Escherichia coli</i>	4.60
<i>Deinococcus radiodurans</i>	3.28
<i>Neisseria meningitidis</i>	2.27
<i>Treponema pallidum</i>	1.14
<i>Saccharomyces cerevisiae</i>	13.0

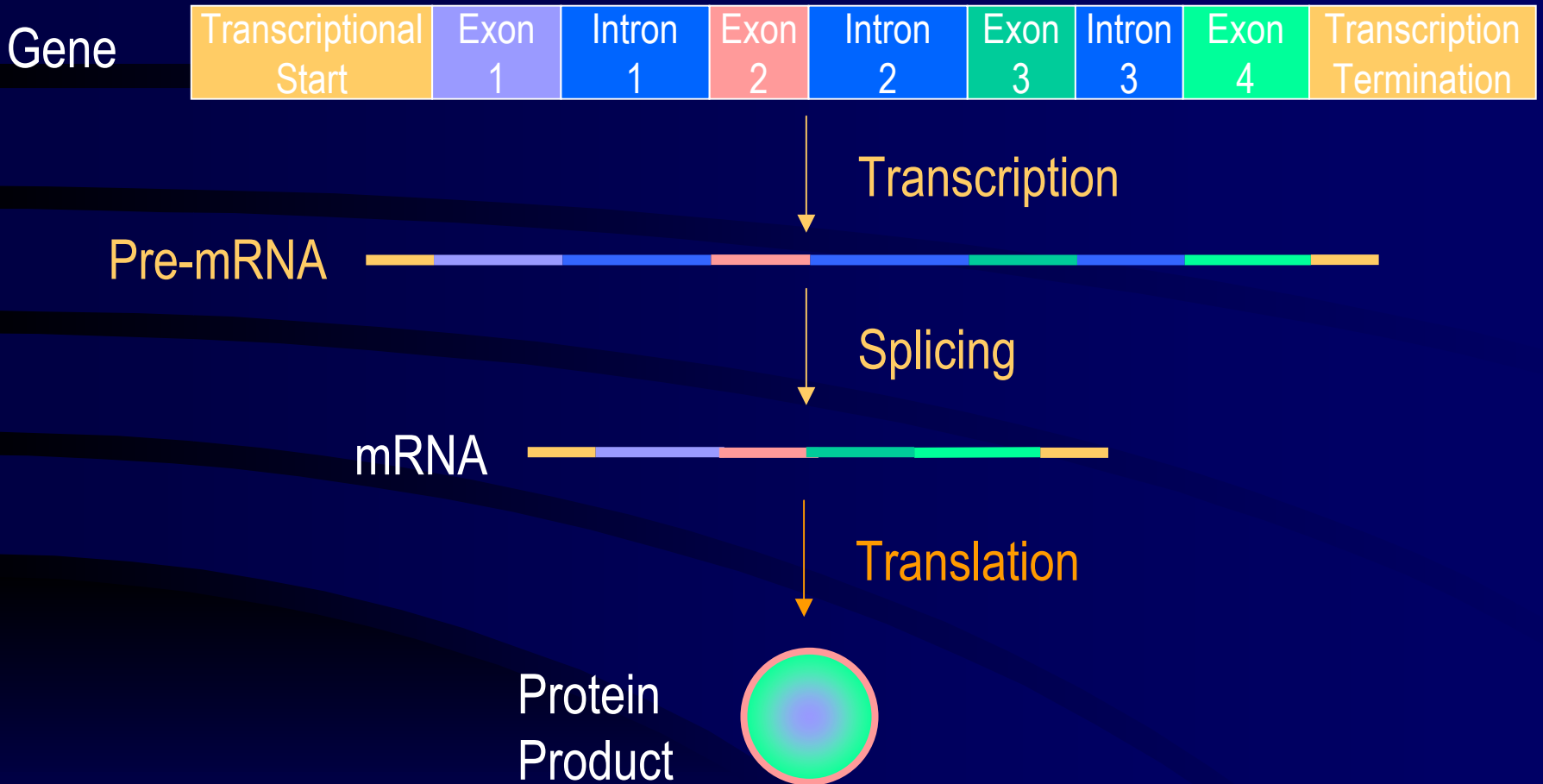
- Leaves little space for ‘useless’ DNA sequences

# Polycistronic mRNA

- Bacterial genes encoding related functions tend to be clustered in such a way that they are transcribed together
  - Polycistronic mRNA



# But in Eukaryotes...





# Arrangement of Genes on the Bacterial Chromosome

- Genes with related functions are arranged in operons and co-transcribed
  - 7 copies of rRNA operons in *E. coli*
- Genes encoding proteins with similar functions map to similar regions of the chromosomes of different organisms
  - Most homologous genes map to the same region of the *E. coli* and *Salmonella enterica* serotype Typhimurium chromosomes
    - Except for a region (~10%) where the order is reversed

# Arrangement of Genes on the Bacterial Chromosome

- Genes with a high frequency of transcription tend to be oriented with respect to the chromosome origin of replication
  - Located near the origin on the chromosome
    - More copies during most of the replication process
  - Oriented so that the genes are transcribed in the same direction as replication occurs
    - Minimises collisions between the transcription and replication machinery

# Topic 2: Plasmids

- Plasmids are
  - Circular, extrachromosomal genetic elements capable of autonomous replication
  - Double-stranded DNA
  - 1 to 200 kb in size
  - Can exist as multiple copies per cell
  - In laboratory strains
    - Rarely encode functions critical for survival
      - Unless under selective conditions
    - Can be lost (or cured) after prolonged propagation

# Plasmids

- In nature...
  - Cells carrying plasmids can have a selective advantage when conditions other than simple nutrient availability determine growth and survival
- Experimental uses...
  - Very important in recombinant DNA technology
  - Method of manipulating and introducing DNA sequences into bacteria
  - Can control expression levels of genes on plasmids by controlling the plasmid copy number and manipulation of the promoter elements of the gene to be overexpressed

# Plasmid Incompatibility Groups

- Plasmids have unique origins or replication
- Cells can carry multiple copies of the same plasmid
  - Eg. pUC119 can exist in up to 700 copies per cell while pBR322 occurs at ~50 copies per cell
- Cells can carry more than one type of plasmid
  - However... each plasmid type must have a different origin of replication
  - Plasmids are classified into incompatibility groups
  - For plasmids to co-exist with a cell, the origin of replication must come from different incompatibility groups

# Conjugative Plasmids

- Plasmids capable of mediating their transfer from one cell to another
  - A process known as conjugation (see Topic 5)
- Transfer occurs from a cell containing the conjugative plasmid (the donor) to a cell that does not have the plasmid (the recipient)
  - Transfer occurs via the formation of a pilus
  - Replication occurs via rolling circle replication
- Some conjugative plasmids can insert into the chromosome
  - Process called integration
  - Plasmids capable of this are known as episomes

# The R Plasmids

- R plasmids carry genes conferring antibiotics resistance
  - Some also carry genes involved with mating
  - Responsible for the transfer of antibiotics resistance genes
- R factors can also be passed across bacterial species
  - Eg. From *E. coli* to pathogenic strains of *Shigella* and *salmonella*
- Bacterial cells are capable of acquiring resistance to multiple antibiotics

# R100

- An R Plasmid
- 89.3 kb
- Confers resistance to sulfonamides, streptomycin, spectinomycin, fusidic acid, chloramphenicol and tetracycline
- Resistance to mercury and cadmium
- Can be transferred between enteric bacteria of the following genera
  - *Escherichia*, *Klebsiella*, *Proteus*, *salmonella* and *Shigella*



# Colicinogenic Plasmids

- Carry genes for proteins that are toxic to closely related bacteria
  - Kills off the opposition
  - These proteins are called colicins (in *E. coli*), subtilisins in (*bacillus subtilis*) etc...
- Colicinogenic plasmids also carry genes responsible for the processing and transporting of the colicin as well as proteins responsible for conferring immunity to the action of the colinic

# The Col Plasmids

- *Escherichia coli* plasmids
- Encode various colicins
- Colicins are released from the producing cell
- Bind to a specific receptor on the surface of a susceptible cell
  - Receptor is usually responsible for transport of an extracellular nutrient into the cell
- On internalised, the colicin disrupts some critical cell function
  - Frequently form ion channels in the cell membrane
  - Leakage of  $K^+$  and  $H^+$  out of the cell
  - Deprives cells of energy

# Cell Functions Coded by Some Plasmids

Group	Function	Example
Fertility plasmids	Transfer of DNA from one cell to another	F plasmids
Resistance plasmids	Resistance to various antibiotics	RP4, R100
	Resistance to mercury and cadmium	R100
	Resistance to UV radiation	ColE1
Col Plasmids	Bacteriocin production	ColE1, ColE2
Virulence factor plasmids	Enterotoxin production	LT2
	Fimbriae production	K88
Metabolic plasmids	Spore formation in streptomycetes	SCP1
Transformation	Formation of crown gall tumours in plants	Ti plasmids ( <i>Agrobacterium</i> )

# Topic 3a: Mutations

- Unlike eukaryotes...
  - Mutations (for better or worse) have an immediate effect on the cell
    - No other allele to mask deleterious effects of mutation
- Important evolutionary process in the generation of microbial diversity
  - Allows the generation of enzymes with new functions when placed under the full pressure of natural selection
  - This, in turn, may result in generation of new microbial species

# Genotype Versus Phenotype

- Genotype
  - The genetic makeup of an organism
  - The complement of genes possessed by an organism
    - eg. *bla*<sup>+</sup> indicates the organism possesses the gene for  $\beta$ -lactamase
- Phenotype
  - The physical (or observed) characteristics displayed by an organism
    - eg. *bla*<sup>+</sup> cells would be resistant to ampicillin
    - These cells would express the Bla protein

# Phenotypic Effects of Mutation

- Lethal mutations
  - Mutations that effect the organisms ability reproduce
- Conditionally-lethal mutations
  - Lethal under certain condition or not lethal under certain conditions
  - Conditions that allow the organism to survive
    - Permissive conditions
  - Conditions that are lethal to the organism
    - Restrictive conditions
  - auxotrophic, temperature-sensitive and supressor-sensitive mutations

# Lethal Mutations

- Lethal mutations usually occur in genes involved with vital cell processes
- For example
  - Lethal mutation in RNA polymerase gene
  - Non-functional RNA polymerase
  - Wide-ranging effect on transcription
  - No mRNA to translate
  - No proteins
  - No cell ☹️

# Nutritional Mutations

- Conditionally-lethal mutations
- Inability to synthesise essential metabolite
  - Wild-type organisms (prototrophic) can synthesise metabolite
  - Metabolites could include purine, pyrimidines or amino acids
- Auxotroph and Prototroph grow in media containing the metabolite (say valine)
  - Permissive condition
- The prototroph will grow in the absence of valine as it can synthesise its own valine whereas the auxotroph will not survive
  - Restrictive conditions



# Temperature-sensitive Mutations

- Conditionally-lethal mutations
- Organisms grow at one temperature (permissive condition) but not at another temperature at which the wild-type organism is usually viable (restrictive condition)
  - Generally heat-sensitive mutants (i.e. Mutant gene product has increased heat lability)
    - Protein may not fold correctly at elevated temperatures but it fine at lower ones
    - Sometimes, protein synthesis may be effected but the protein functions normally irrespective of the temperature

# Types of Mutations

- Macrolesions
  - Deletions
  - Duplications
  - Inversions
  - Insertions
- Microlesions
  - Single-nucleotide pair insertion
  - Single-nucleotide pair deletion
  - Point mutations
    - Silent
    - Missense
    - Nonsense

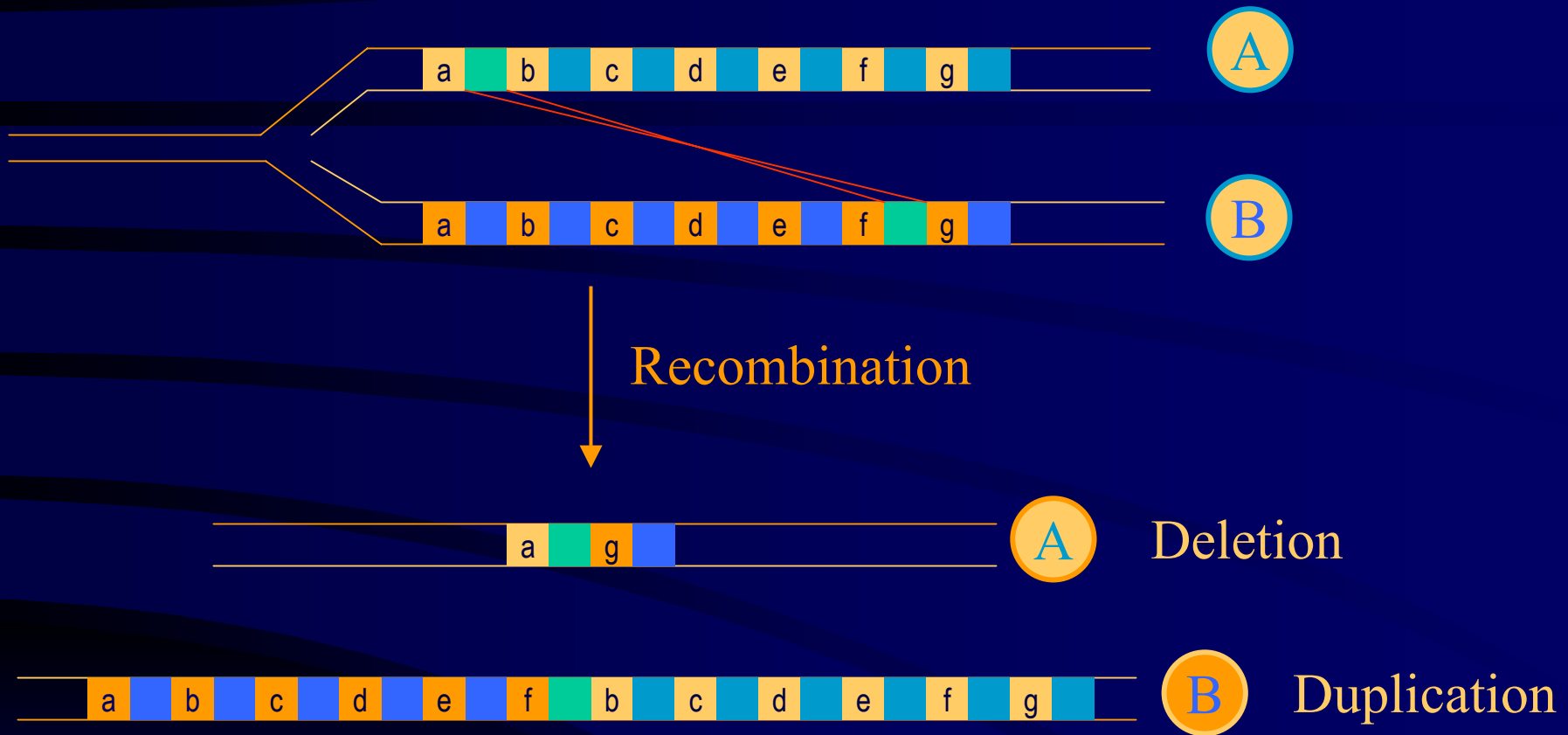
# Deletions

- The loss of a segment of DNA
- ~12% of all spontaneous mutations
- Do not revert
  - Complete loss of DNA is irreversible

# Duplications

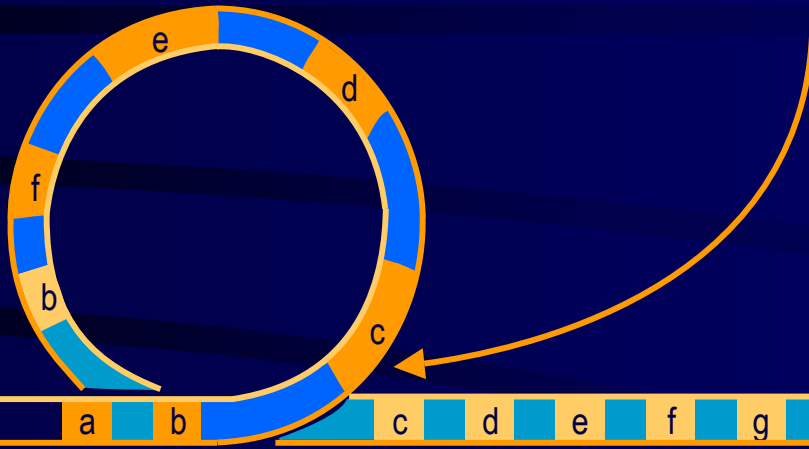
- Duplication of a segment of the chromosome
- Duplication results in large regions of homology present
  - Sites for homologous recombination
  - Can result in further amplification of the duplicated region
- The high degree of homology also results in the instability of the duplications
  - Can be lost through homologous recombination

# Mechanisms of Duplication

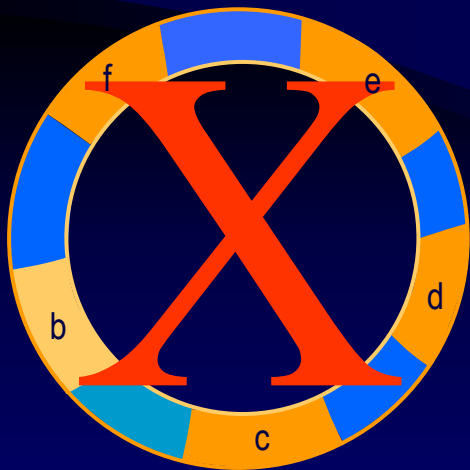


# Loss of Duplications

B

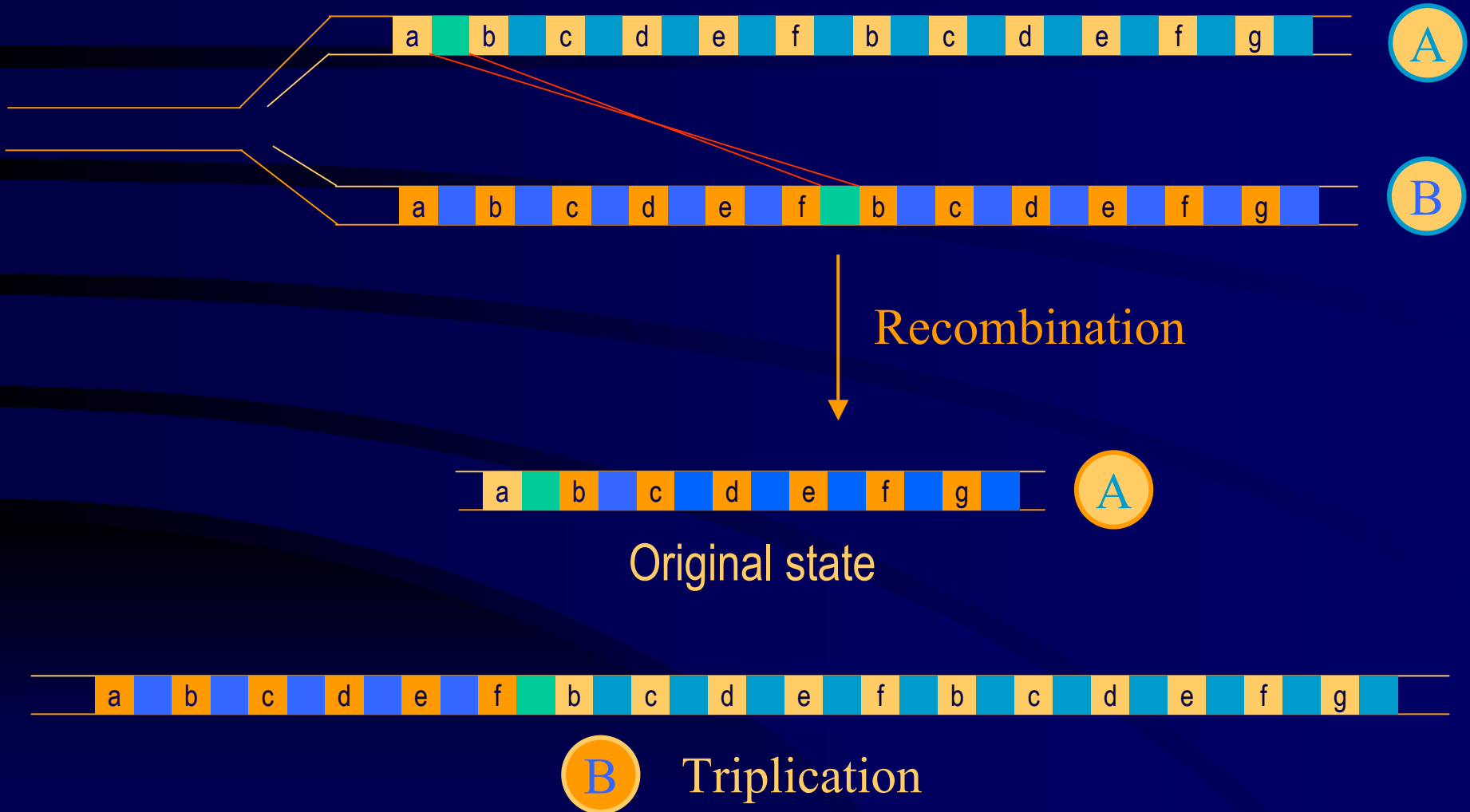


Homologous  
Recombination



Original state

# Amplification of Duplications



# How Are Duplications Maintained?

- Key is natural selection
- If duplicated genes (and their products) provide a selective advantage...
  - Organisms possessing stable duplications will out-compete wild-type organisms or those that have lost duplications
  - Duplications may subsequently be lost when the selective pressure for their retention is gone



# Duplications and Evolution

- Duplicated sequences acquire mutations like all other DNA sequences
- If a duplication is mutated such that it is not transcribed (and the gene product is not crucial for survival), the duplication may be maintained by the cell
  - Less burden as it is not transcribed
  - Acquire mutations more rapidly because the pressures of natural selection are removed

# Duplications and Evolution

- The duplicate will continue to accumulate mutations until...
  1. It accumulates so many mutations that no functional gene products could be produced and it is deleted from the genome;
  2. It reverts, either due to selective pressure or random mutation, to its original function;
  3. Through the accumulation of mutations, it produces a protein with either enhanced activity or a completely new function that provides some selective advantage

# Inversions

- Reversal of the order that the genes are organised on the chromosome
- Rarely has any effect on expression of these genes
- Example:
  - *E. coli* and *S. enterica* serotype Typhimurium have nearly identical physical maps except for a region where the genes have been inverted

# Insertions

- Also called translocations
- Rare events in bacteria
- Movement of a DNA fragment from one region of the chromosome to another without duplication of the genetic material
- Insertions can occur via insertion sequences

# Wild-Type (Normal) DNA Sequence

DNA      5' -ATG AAA ATA GGC GGC CTC ATT CTG ... -3'  
            3' -TAC TTT TAT CCG CCG GAC TAA GAC ... -5'

Transcription

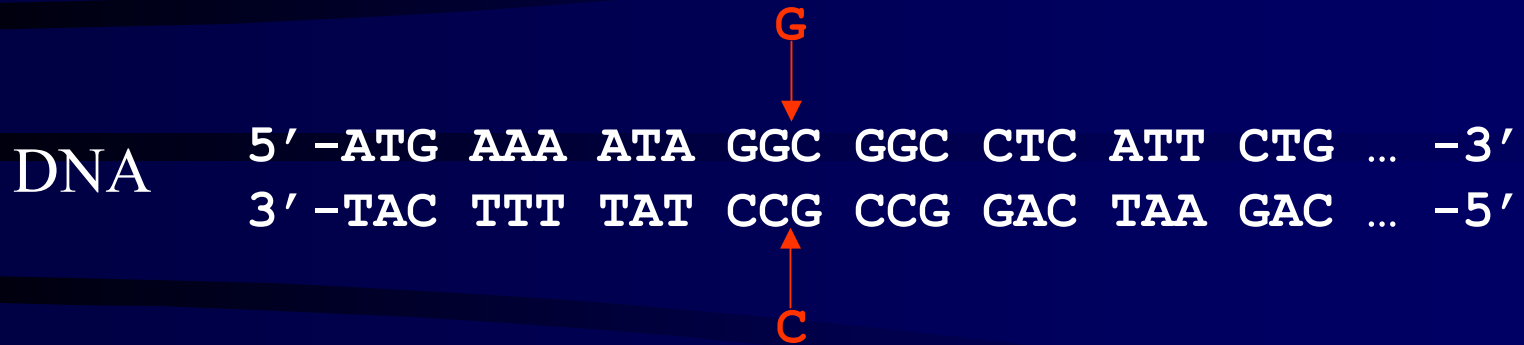
RNA      5' -AUG AAA AUA GGC GGC CUC AUU CUG ... 3'

Wild-type  
Protein



Functional Protein

# Single Nucleotide-pair Insertion



# Single Nucleotide-pair Insertion

Frame Shift



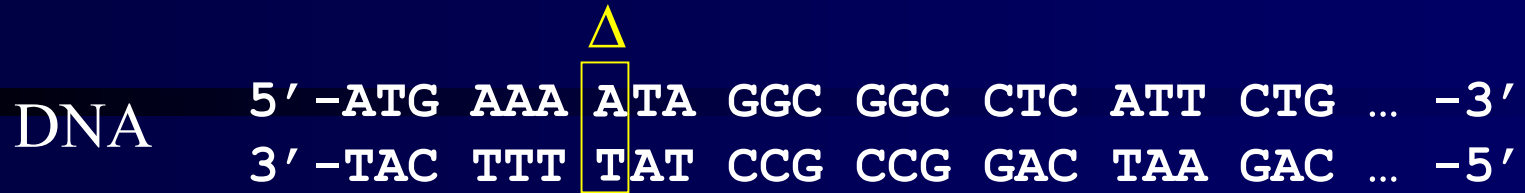
Transcription



Mutant Protein

?

# Single Nucleotide-pair Deletion





# Single Nucleotide-pair Deletion

Frame Shift

Mutant DNA 5' -ATG AAA TAG GCG GCC TCA TTC TG ... -3'  
3' -TAC TTT ATC CGC CGG ACT AAG AC ... -5'

Transcription

Mutant mRNA 5' -AUG AAA UAG GCG GCC UCA UUC UG ... -3'

Amber Mutant



Wild-type Protein



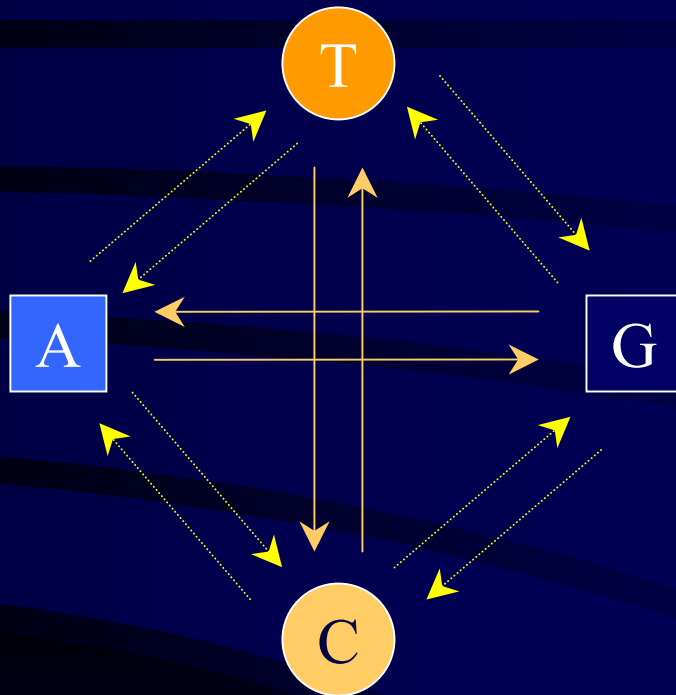
Truncated Protein

? But probably ×

# Base Substitutions in DNA

- Twelve different base substitutions can occur in DNA
  - Transitions
    - 4 possible transitions
    - substitution of one purine for another purine
    - substitution of one pyrimidine for another pyrimidine
  - Transversions
    - 8 possible transversion
    - substitution of a purine for a pyrimidine
    - substitution of a pyrimidine for a purine

# Transitions and Transversions



Purine Transition

Pyrimidine Transition

Transversions

□ Purines

○ Pyrimidines

→ Transitions

→ Transversions

# The Universal Genetic Code

Second Position

		Second Position					
		U	C	A	G		
First (5') Position	U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U	
		UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C	
		UUA Leu	UCA Ser	UAA <i>Ochre</i>	UGA <i>Opal</i>	A	
		UUG Leu	UCG Ser	UAG <i>Amber</i>	UGG Trp	G	
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U		
	CUC Leu	CCC Pro	CAC His	CGC Arg	C		
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A		
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G		
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U		
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C		
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A		
	AUG MET	ACG Thr	AAG Lys	AGG Arg	G		
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U		
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C		
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A		
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G		
		Third (3') Position					

# Silent Mutations

- Mutations that arise from the degenerate nature of the genetic code
  - Do not result in a change in the amino acid sequence
  - Rarely have any effects on expression



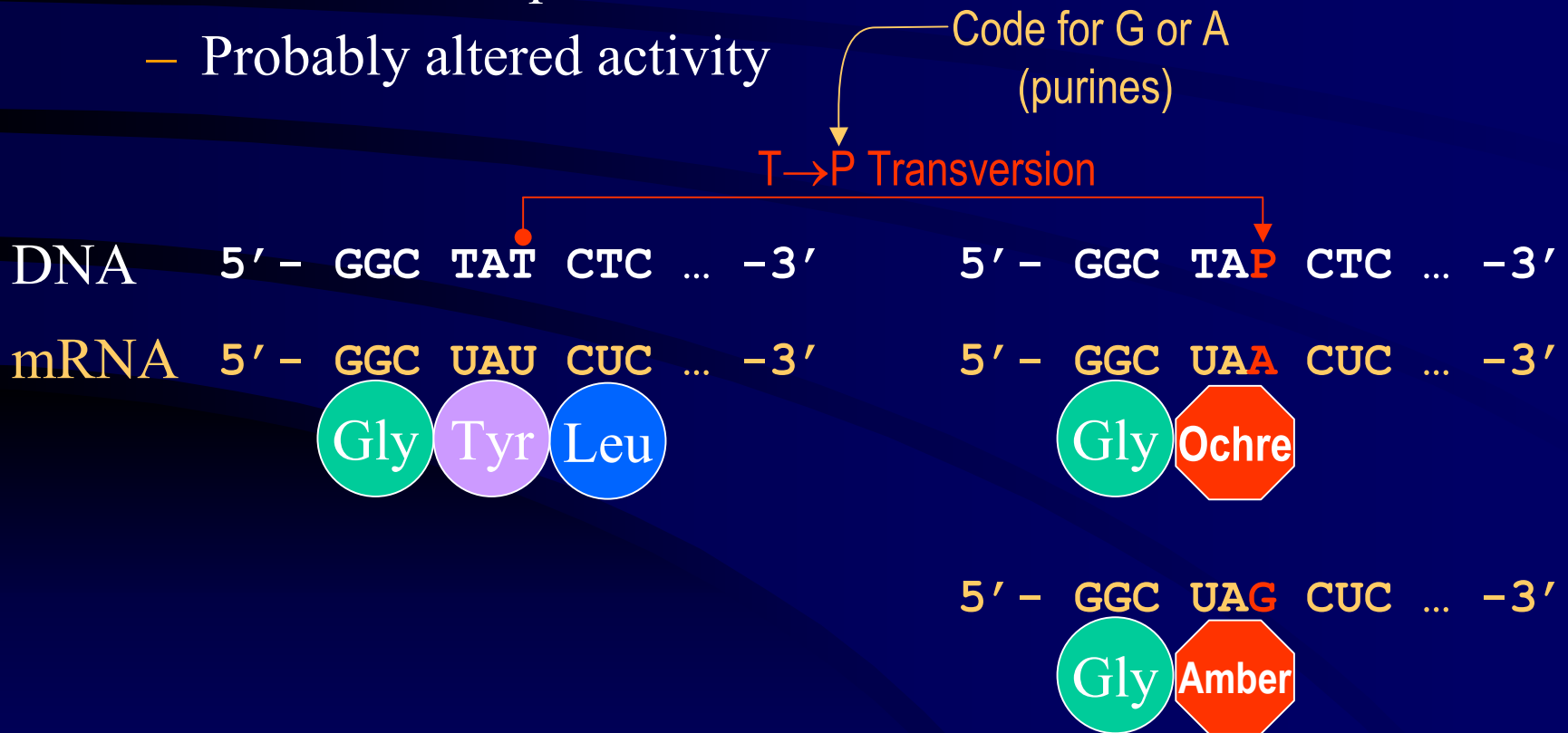
# Missense Mutations

- A mutation that results in the incorporation of a different amino acid as a result of the changed triplet codon
  - May have some effect on protein activity depending on the importance of the residue mutated
    - i.e. active site residue



# Nonsense Mutations

- Mutation in a codon that results in the incorporation of a stop codon
  - Truncates the protein
  - Probably altered activity



# Inducing Mutations: Radiation

- Ionizing radiation
  - Sufficient exposure to ionizing radiation induces lethal mutations
    - Used as a method of sterilisation (gamma radiation)
- UV light
  - Induces the formation of covalent links between adjacent thymidine nucleotides
    - Thymidine dimers
    - Cannot act as template for DNA polymerase



# Inducing Mutations: Chemical

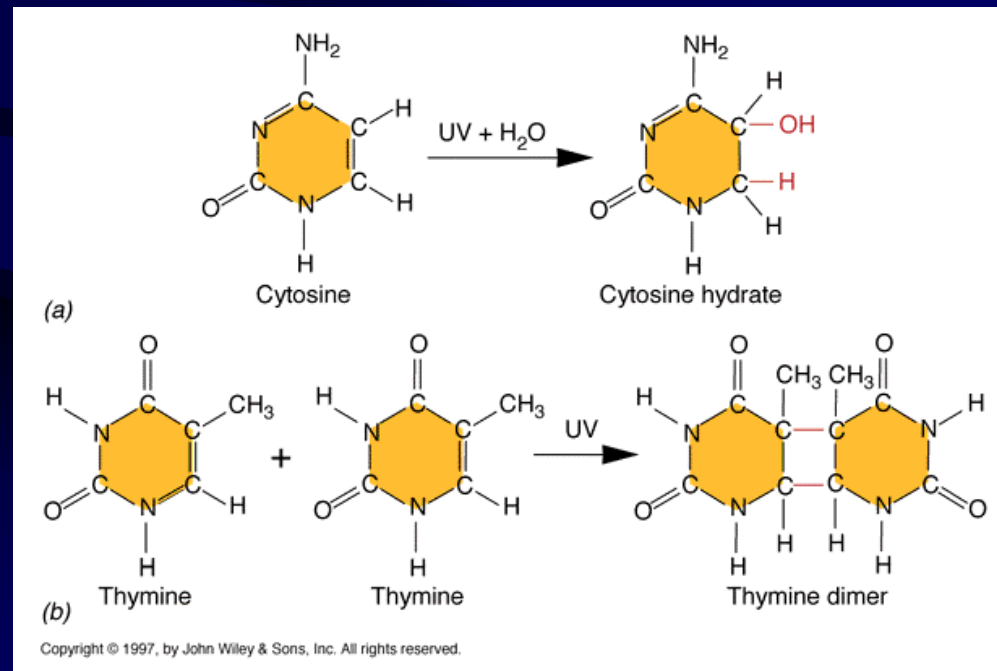
- Some chemicals increase the frequency with which mutations occur
  - Mutagens
- Hydroxyalamine
  - Chemically modifies cytosine to uracil
    - Base pairs with A instead of G
      - Results in a GC to AT transition
- Nitrosoguanidine
  - Alkylates nitrogenous bases
  - Results in AT to GC transitions

# Repair Mechanisms

- Mutations happen!!
- Part of the function of dsDNA is to maintain the integrity of the genetic code
- Repair mechanisms exist to fix mutations as they arise
- Four basic mechanisms
  - Photoreactivation
  - Mismatch Repair
  - Excision Repair
  - SOS Repair

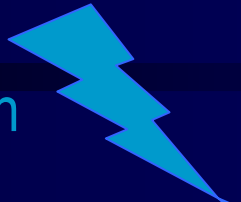
# Photoreactivation

- Removes thymidine dimers
  - Uses the photoreactivation enzyme PRE (a photolyase) to break the covalent linkage between thymidine bases
  - PRE only functions in the presence of light
  - Requires no digestion or gap-filling enzymes

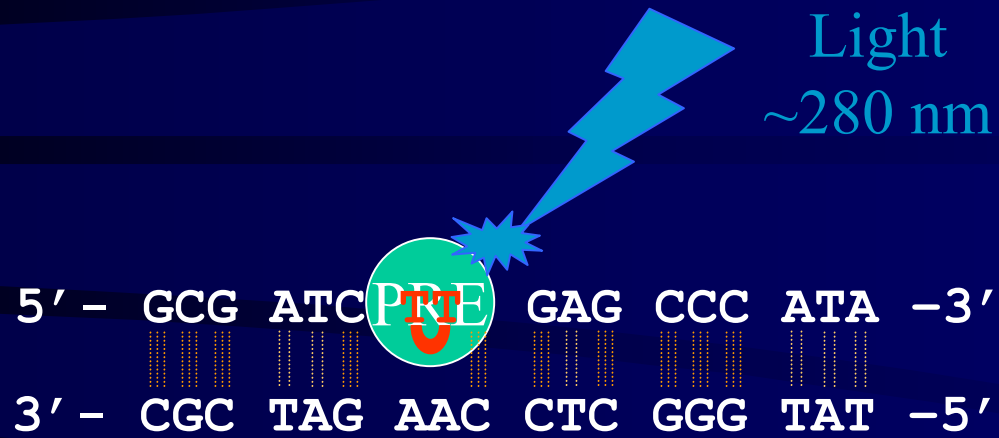


# Photoreactivation

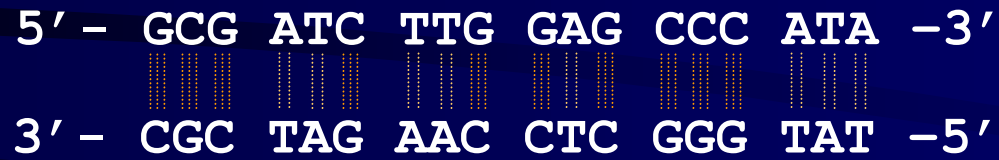
UV  
Irradiation



# Photoreactivation



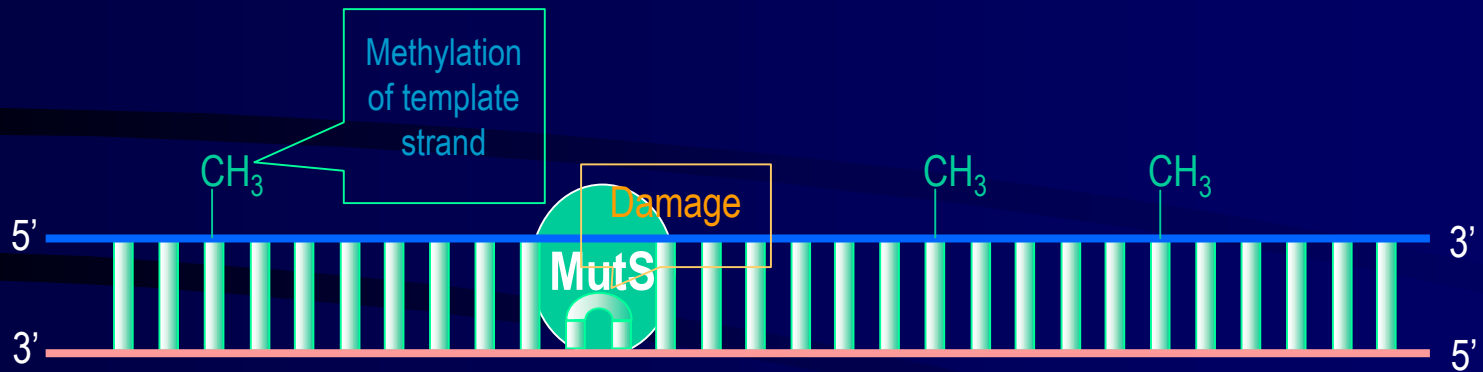
# Photoreactivation



# Mismatch Repair

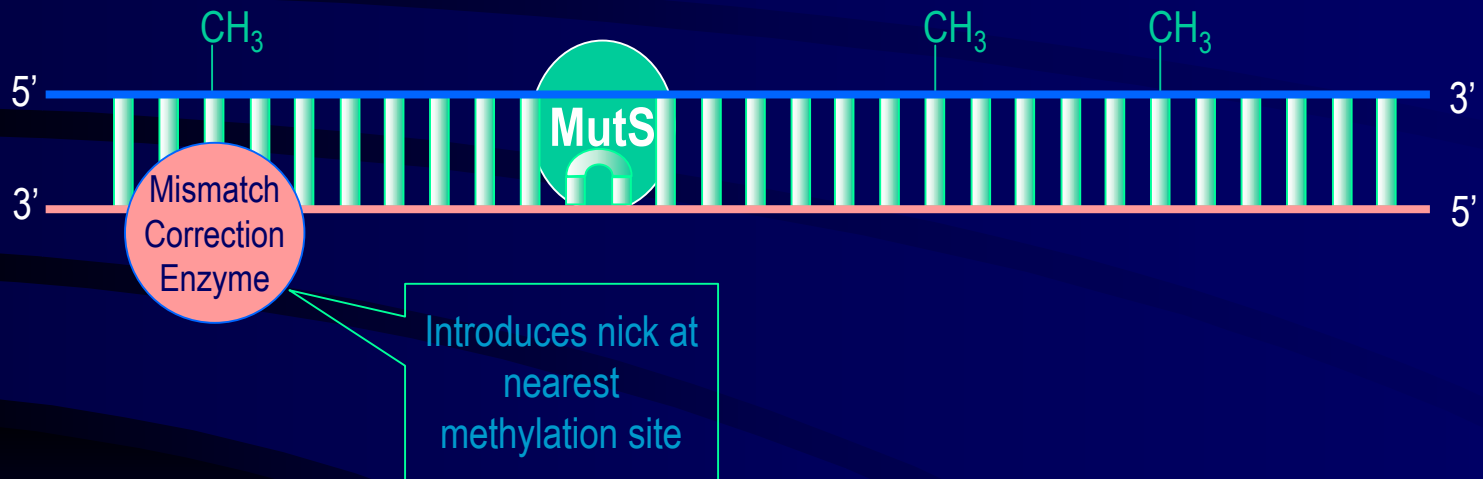
- Recognizes and corrects residual errors left uncorrected by DNA polymerase
  - Errors occur in the newly synthesised strand leaving the original template strand with the correct sequence
- Corrections are made by the multimeric mismatch correction enzyme
  - Mismatch repair also requires numerous other enzymes such as DNA helicase, DNA pol III, exonuclease I and DNA ligase

# Mismatch Repair

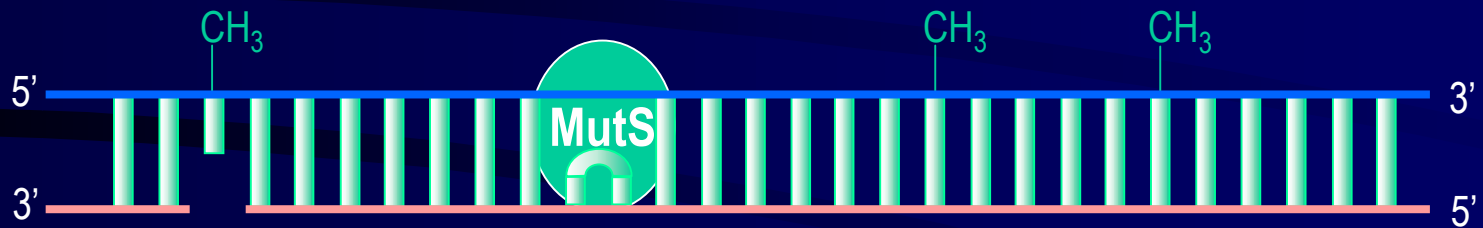




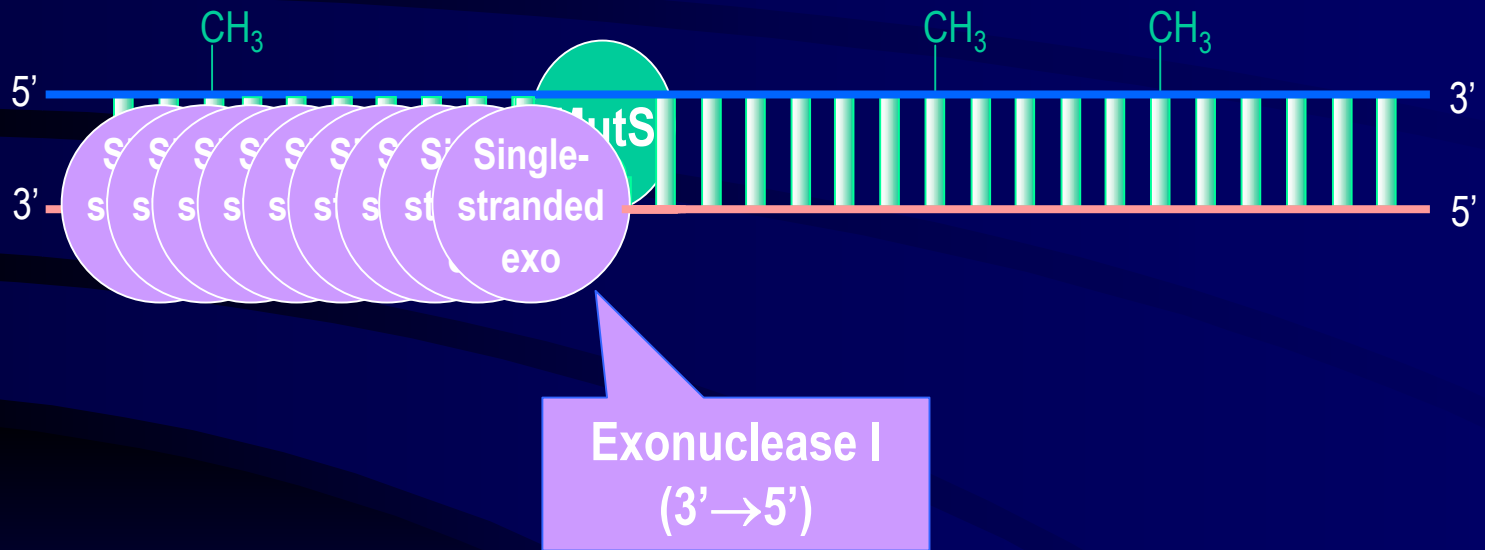
# Mismatch Repair



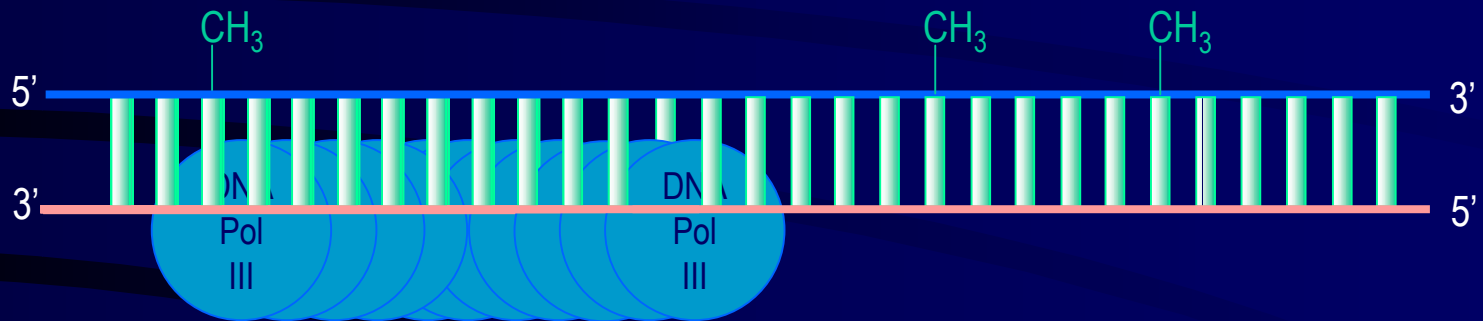
# Mismatch Repair



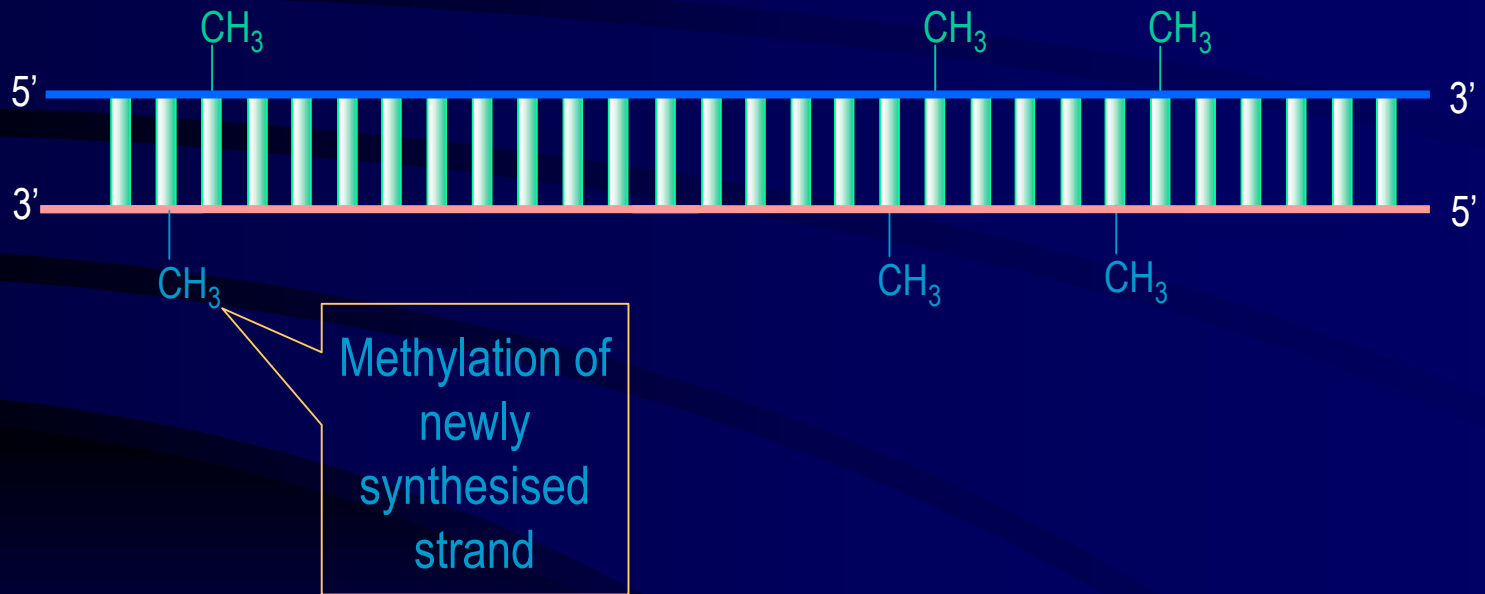
# Mismatch Repair



# Mismatch Repair



# Mismatch Repair



# Excision Repair

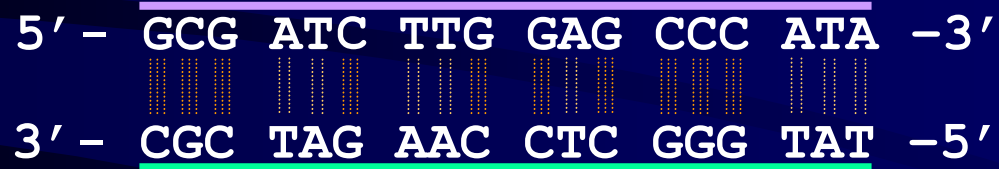
- Corrects damaged DNA by removing nucleotides and resynthesising the region
- Two main types of excision repair
  - Base excision repair
  - Nucleotide excision repair

# Base Excision Repair

- Performed by DNA glycosylases
  - Cleave the N-glycosyl bonds that connects the purine or pyrimidine base to the deoxyribose-phosphate backbone
- Example:
  - Uracil is sometimes inserted into DNA instead of thymidine
    - May arise through the deamination of cytosine
    - Uracil is excised by uracil *N*-glycosylase

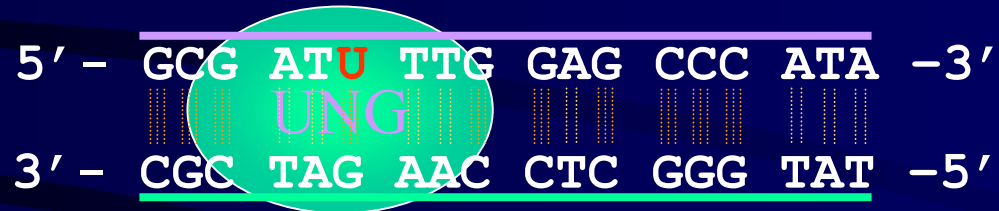
# Base Excision Repair

Deamination  
of cytosine

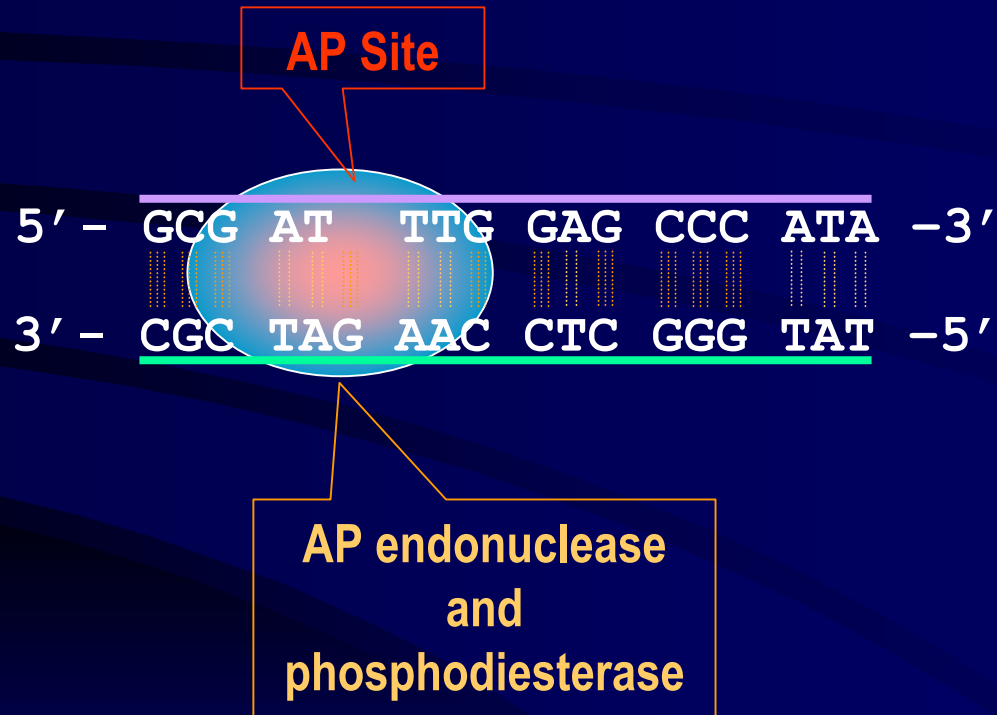




# Base Excision Repair

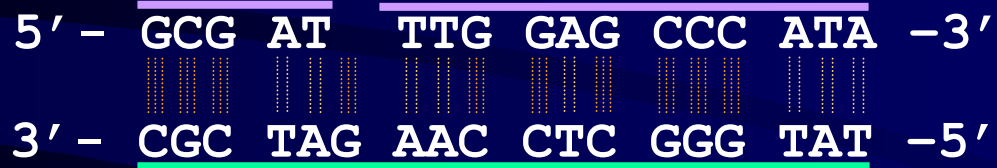


# Base Excision Repair



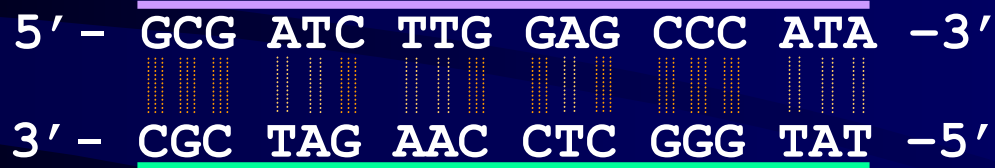
# Base Excision Repair

Single-stranded  
break



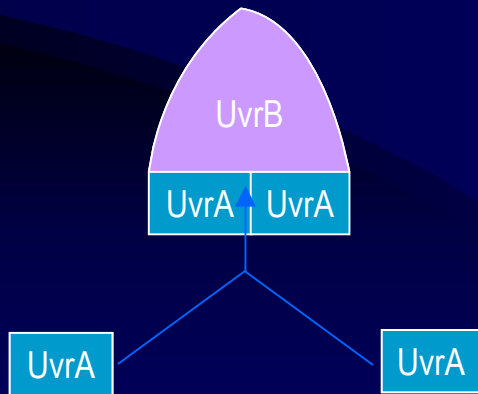
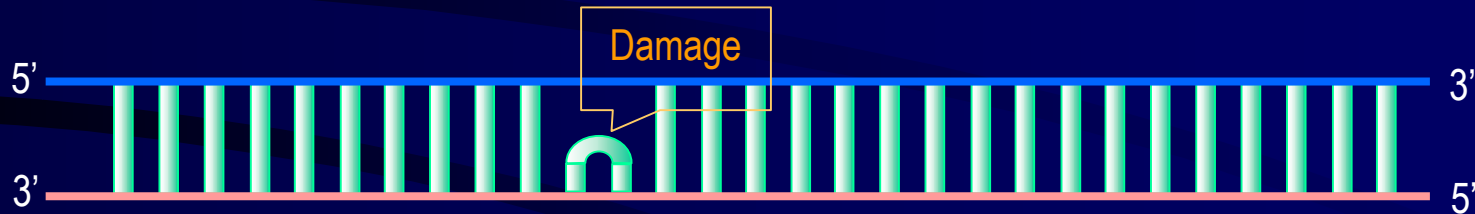
# Base Excision Repair

Gap filled by DNA polymerase  
and nick sealed with ligase

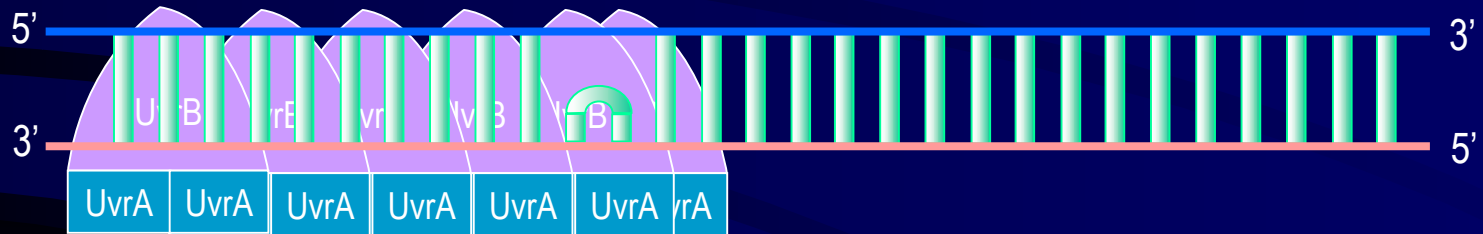


# Nucleotide Excision Repair

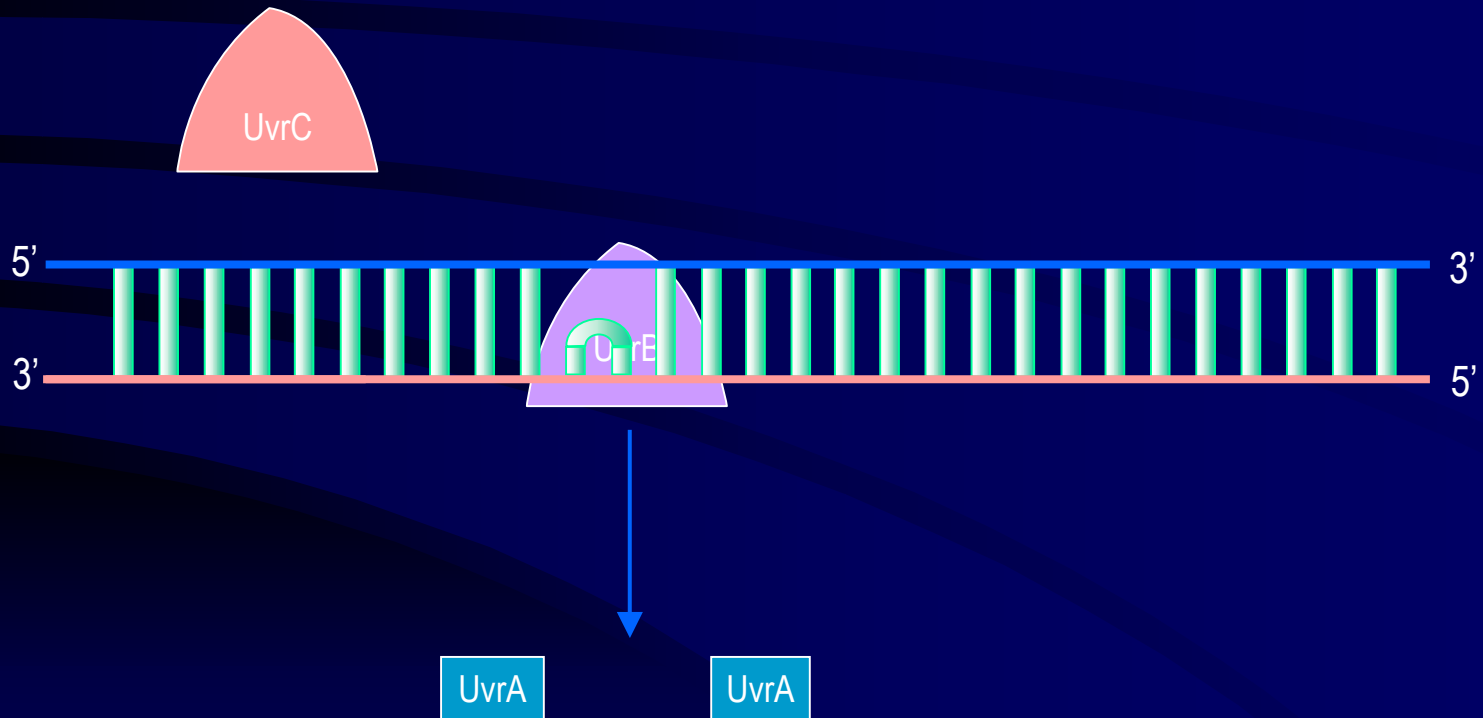
- Repairs pyrimidine (thymidine) dimers
- Excised by UvrABC



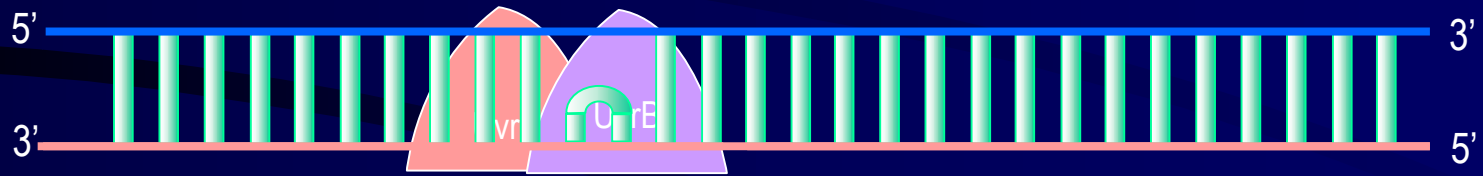
# Nucleotide Excision Repair



# Nucleotide Excision Repair

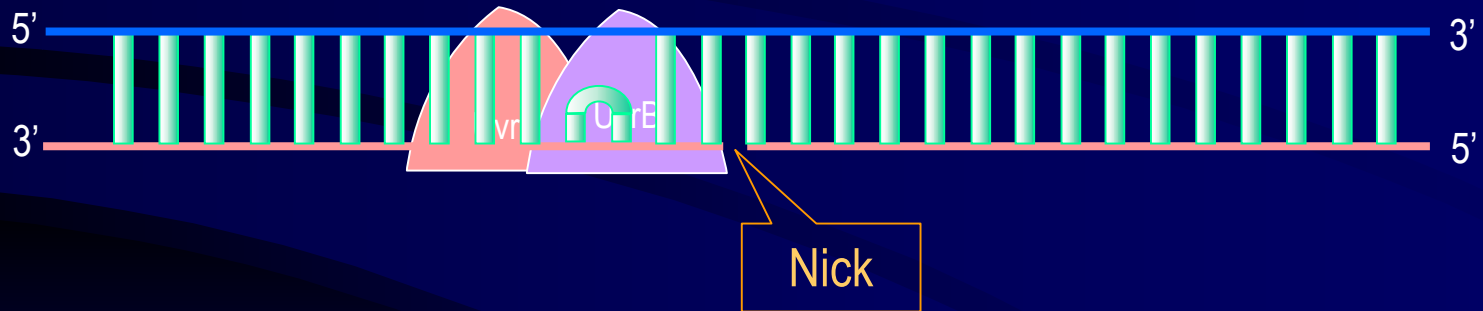


# Nucleotide Excision Repair

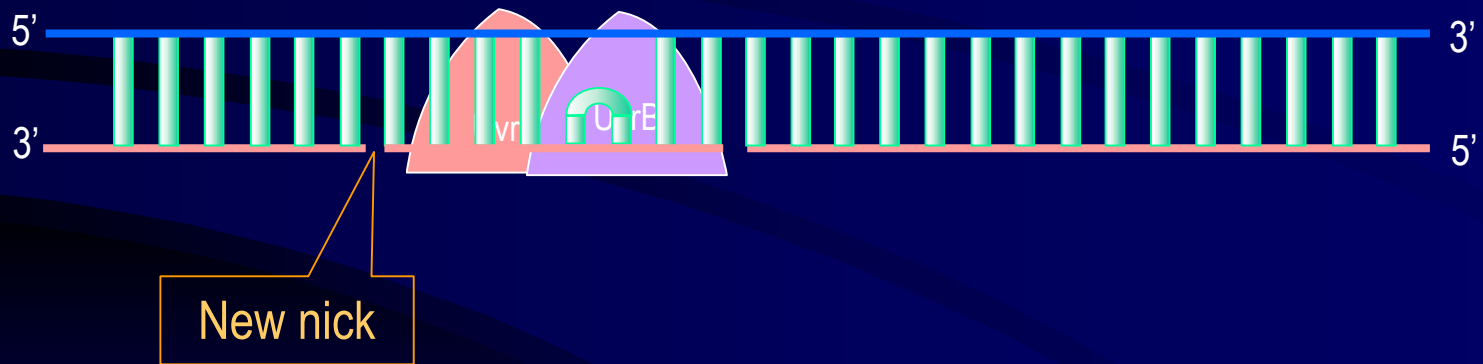




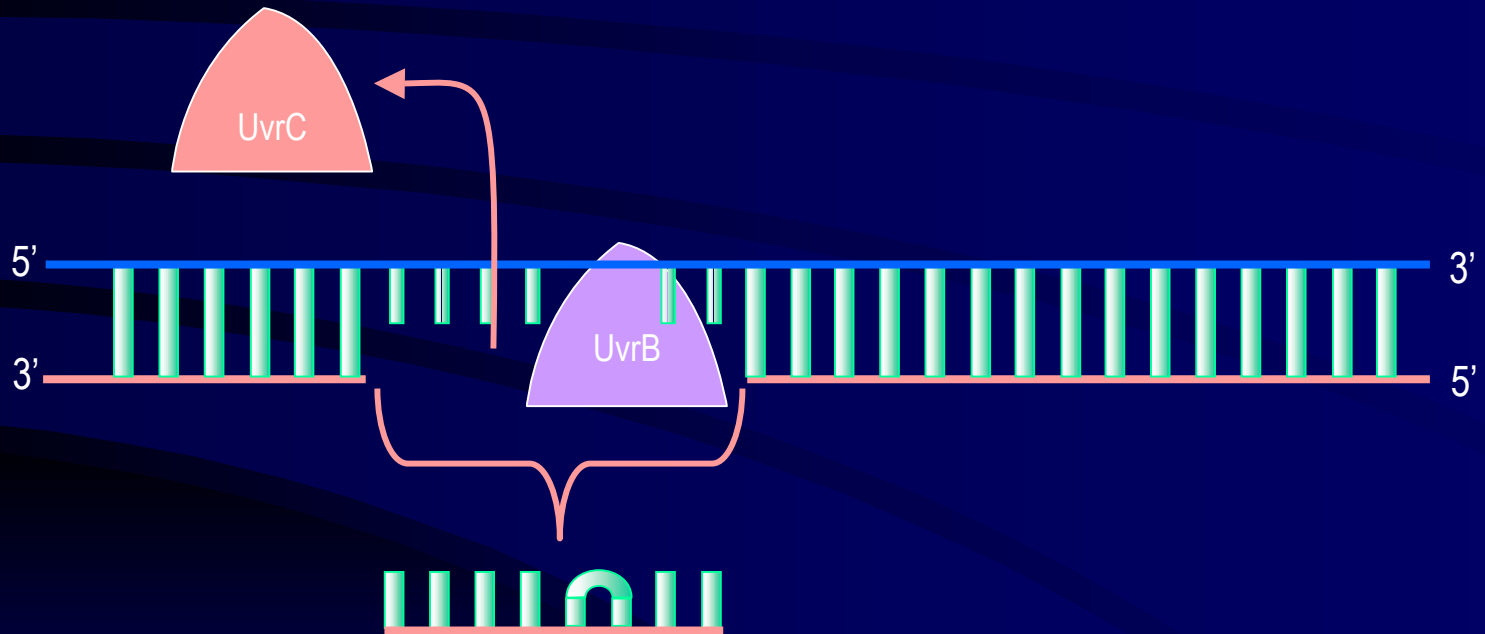
# Nucleotide Excision Repair



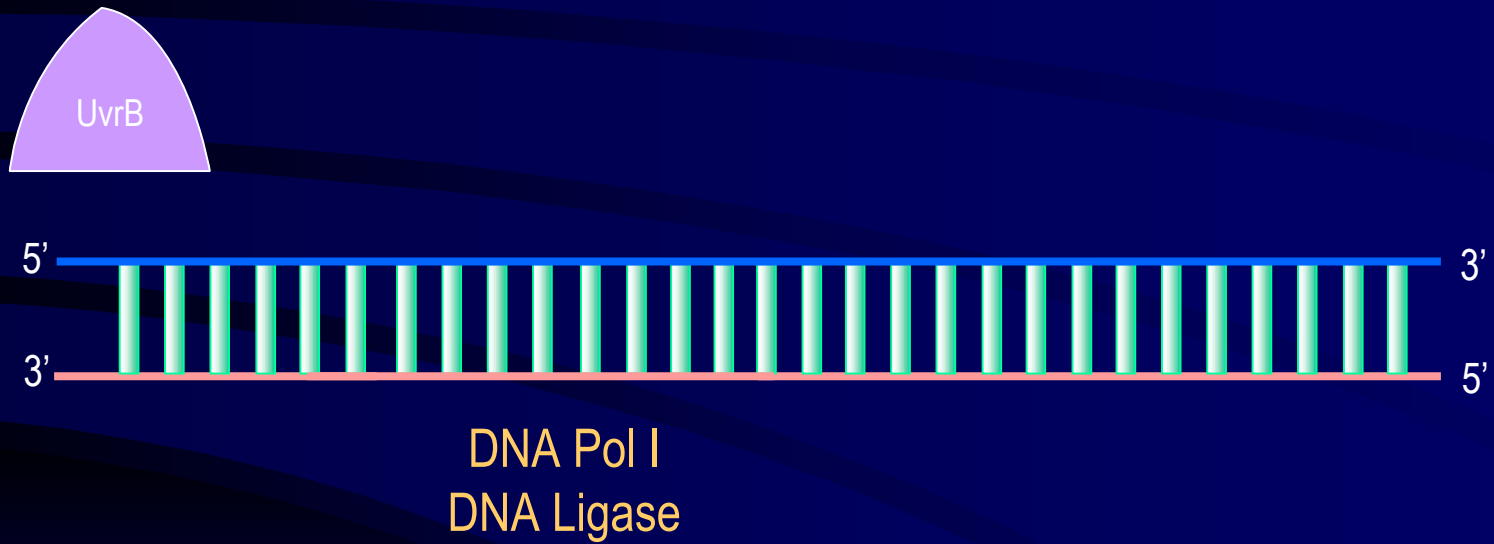
# Nucleotide Excision Repair



# Nucleotide Excision Repair



# Nucleotide Excision Repair



# SOS Repair

- When all else fails...
- Radical, multi-functional repair system designed to save the cell under conditions of persistent DNA damage
- Cell division ceases
- Functioning of the SOS system is controlled by the *lexA-recA* regulon
  - Regulon = set of operons that are coordinately regulated
  - Represses over 20 genes
    - Includes *lexA* and *recA*

# Activation of SOS Repair

- System is activated by a temporary stalling of DNA replication
- RecA is activated by interaction with oligonucleotides produced as a result of DNA damage
- RecA proteolytically cleaves several DNA-binding proteins that usually repress transcription
  - Including LexA
    - LexA usually represses the SOS repair system
  - With LexA inactivated, the levels of RecA increase, which in turn increases that rate of DNA repair

# SOS Repair

- DNA gaps are sometimes filled in without template
  - i.e. the repair mechanisms fill in the gaps without copying the template
  - Creates lots of errors (mutations)

# Learning Exercises and Suggested Reading

- Reading
  - Atlas: Principles of Microbiology
    - Chapter 7, Section 7.1
- Learn about the AMES test