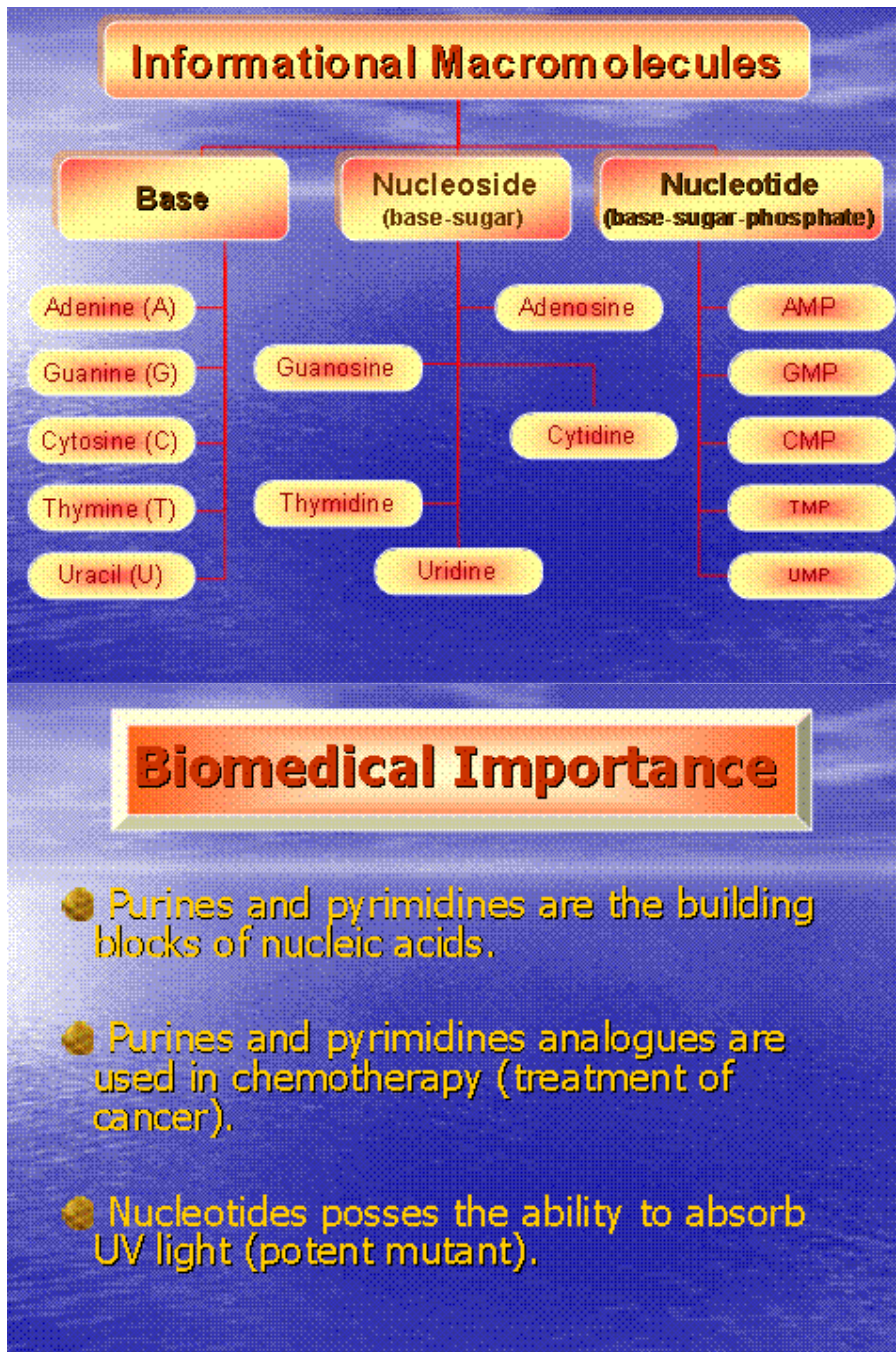




DNA



Biomedical Importance

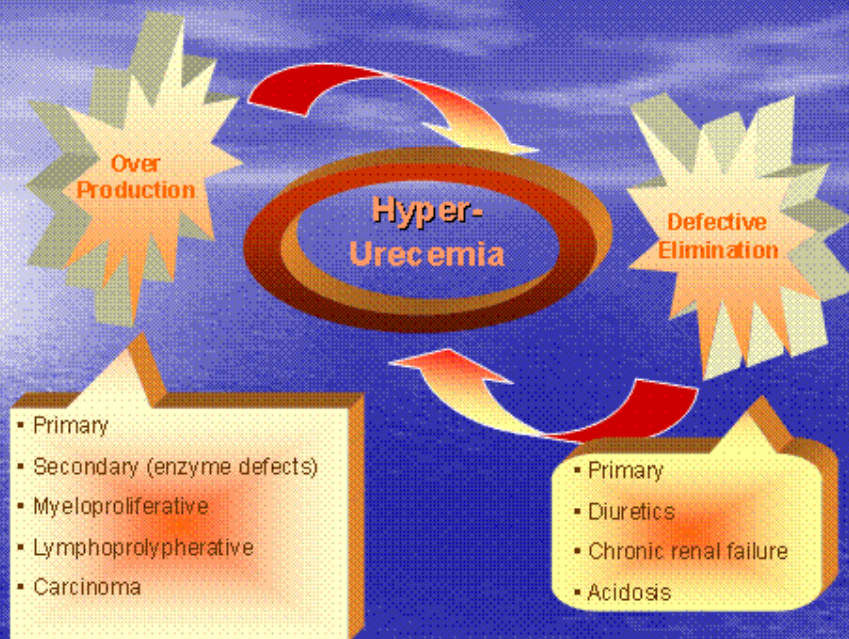
- Nucleotides function in diverse reactions
 - Protein and nucleic acid synthesis.
 - Regulatory functions.
 - Inter- and intracellular signal transduction.
 - Act as second messenger as cAMP and cGMP.
 - Serve as high energy transducer like ATP and GTP.

Purine Catabolism

- Humans convert the adenosine and guanosine to the excreted end product uric acid.
- Normal humans excrete 400-600 mg/24 hours of uric acid.
- Urates are more soluble than uric acid so at pH 5 urine can dissolve one tenth as much urates as urine at pH 7.
- Urinary tract crystals are thus sodium urate proximal to distal and collecting tubules where acidification of urine occurs but uric acid at the distal sites.

Urate Pool

- Normal males pool 1200 mg while females 600 mg.
- Normal serum level of urates in males 2-7 mg/100ml while females 2-6 mg/100ml.
- The pool is affected by
 - Sex (higher in males)
 - Diet (rises with high protein)
 - Obesity (increase)
 - Social class (higher levels in high classes)



Primary Gout

- Metabolic disorder characterized by recurrent attacks of arthritis usually in men.
- Monoarticular (metatarsophalangeal joint of big toe).
- Patients may develop renal stones composed mainly of uric acid.
- Serum urates exceeds solubility levels.
- Sodium urate crystals deposit in soft tissues and joints (tophi).
- Macrophages phagocytose crystals and cause damage to membranes within leucocytes.
- Lysosomal contents are released causing gout manifestations.

Diagnosis Of Gout

- **Probable Diagnosis**
 - High plasma urate level (some patients have normal urate levels).
- **Definitive Diagnosis**
 - Aspiration of the joint fluid during attack, deposited crystals are seen microscopically .
 - Needle shaped birefringent, appear yellow when their long axis is parallel to the plane of polarized light & blue when perpendicular .

Treatment of Gout

- Anti-inflammatory drugs in acute attack.
- Decrease plasma urate levels (by weight reduction, drugs, diet).
- Allopuranol inhibits xanthine oxidase \longrightarrow ↓ urate level and ↑ urinary excretion of xanthine and hypoxanthine.

Hyperurecemia

● Secondary Gout

- Starvation
- Diuretic therapy
- Psoriasis
- Cytotoxic therapy
- Chronic renal disease
- Myeloproliferative and Inherited metabolic disorder

● Lesh – Nyhan Syndrome

- Defect in hypoxanthine guanine phosphoribosyl transferase (HGPRT).
- Purines could not be converted to nucleotides (converted to urates).
- Urinary urates increased and uric acid calculi may form in urinary tract.

● Von Gierk's Disease

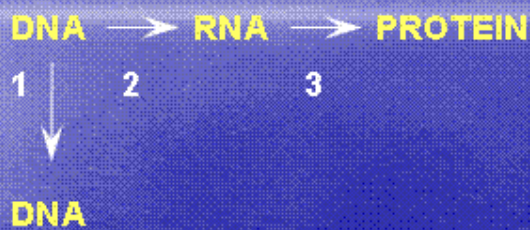
- Defect in glucose 6 phosphatase.
- Purine over production and hyper urecemia.
- Lactic acidosis elevates renal threshold for urates.

Nucleic Acid Function & Structure

Biochemical Importance

- Chemical basis of heredity and genetic diseases resides in deoxy ribonucleic acid (DNA) structure.
- DNA directs synthesis of ribonucleic acid (RNA) which directs the protein synthesis.

THE FLOW OF GENETIC INFORMATION



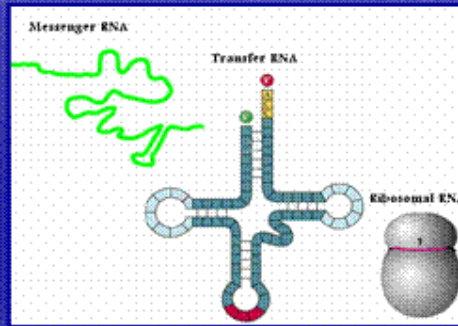
- | | |
|------------------|---------------------|
| 1. REPLICATION | (DNA SYNTHESIS) |
| 2. TRANSCRIPTION | (RNA SYNTHESIS) |
| 3. TRANSLATION | (PROTEIN SYNTHESIS) |

Nucleic Acids

DNA



RNA



Nucleic Acids

DNA

- Sugar: 2' deoxyribose
- Double stranded right helix
- Deoxyribonucleotides
 - Adenine - Guanine
 - Cytosine - Thymine
- Guanine = Cytosine
- Adenine = Thymine
- Cannot be hydrolyzed by alkali

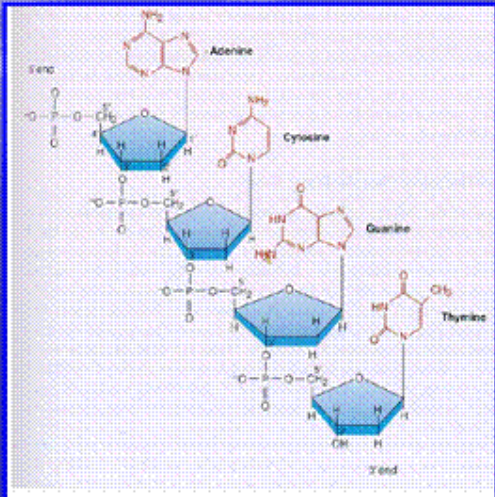
RNA

- Sugar: ribose
- Single stranded
- Ribonucleotides
 - Adenine - Guanine
 - Cytosine - Uracil
- Guanine ≠ Cytosine
- Adenine ≠ Uracil
- Can be hydrolyzed by alkali

DNA Primary Structure

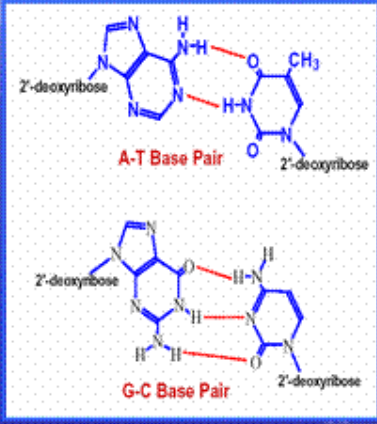
Polynucleotides

- Singly esterified 5' phosphate of a nucleotide can esterify another OH group forming a diester.
- 3' 5' Phosphodiester bonds form the backbone of the polynucleotides



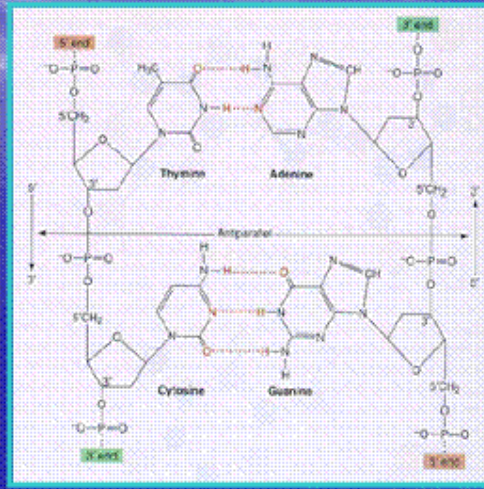
DNA Primary Structure

- The two strands of DNA are held together by hydrogen bonds.
- Pairing between nucleotides are specific
 - Adenine = Thymine (2 H bonds)
 - Guanine = Cytosine (3 H bonds)



DNA Primary Structure

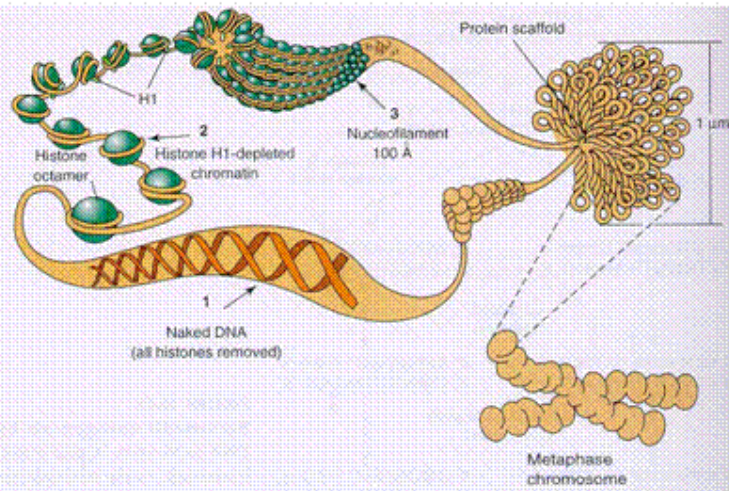
- The two strands of DNA are antiparallel.
- Genetic information resides in one strand (template).
- The other strand is the coding strand matching the RNA transcript that encodes the protein.



DNA Organization

- Double stranded DNA helix has a length (1 meter) thousands of times the diameter of the nucleus.
- DNA in eukaryotes is associated with a variety of proteins → chromatin structure.
- Most of the DNA is associated with histones to form nucleosome.
- DNA is organized into chromosomes (23 pairs).
- 90% of DNA is constitutive transcriptionally inactive generally associated with nucleosomes and not sensitive to digestion by nucleases.
- Inactive chromatin is densely packed (heterochromatin).

Packing into chromosomes



Distinctive Types of DNA

- **Telomeres:** ends of chromosomes
 - e.g. (G₄T₂)
- **Single copy sequences:** the genes
 - single nucleotide polymorphisms, **SNPs** ("snips")
- **Moderately repetitive sequences:**
 - short (100-400bp) interspersed elements (**SINEs**)
eg Alu repeats 400,000 per haploid
 - long (5000-7000bp) interspersed elements (**LINES**, L1 elements) e.g. Kpn1 repeats 100,000 per haploid

Distinctive Types of DNA

- **Highly repetitive sequences:**
 - highly repetitive tandem repeats (“satellite” DNA) near the centromeres, 50% of repeat DNA
 - microsatellites (1-5bp) eg dinucleotide repeats, usually in blocks less than 150bp in length
 - hypervariable minisatellites (10-60bp); these are regions identified as **variable number tandem repeats, VNTRs**
 - **simple sequence repeats, SSRs** typically 4 bp elements

Basic Elements of Each Chromosome

- **Must contain a centromere**
 - The protein complex called the kinetochore forms at the centromere and attaches to the mitotic spindle
 - allows 1 copy of each duplicated chromosome to be pulled into each daughter cell
- **Must contain 2 telomeres**
 - Element of repeated sequences at the ends of chromosomes that allow efficient replication
- **Must contain origins of replication**
 - Regions where duplication of the DNA begins

Chromatin Remodeling

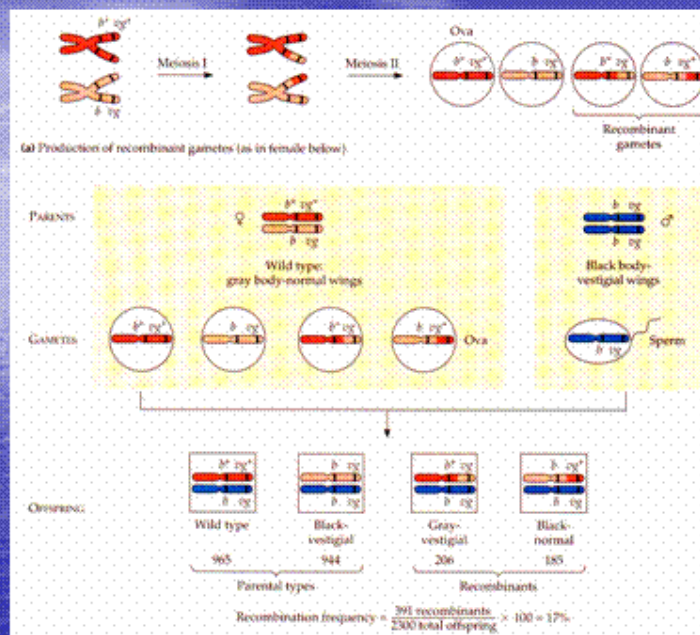
- Histone modifications affect chromatin structure and function
- The N-term of the core histones highly conserved
- Acetylation tends to destabilize chromatin structure, associated with transcriptional activation
- Other modifications include methylation and phosphorylation

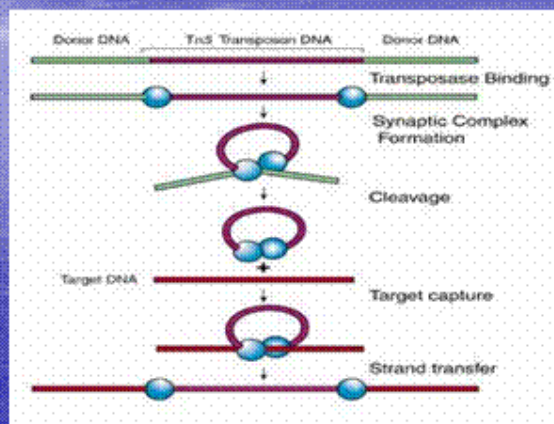
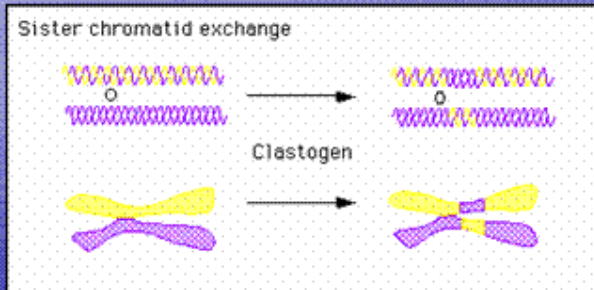
DNA Organization

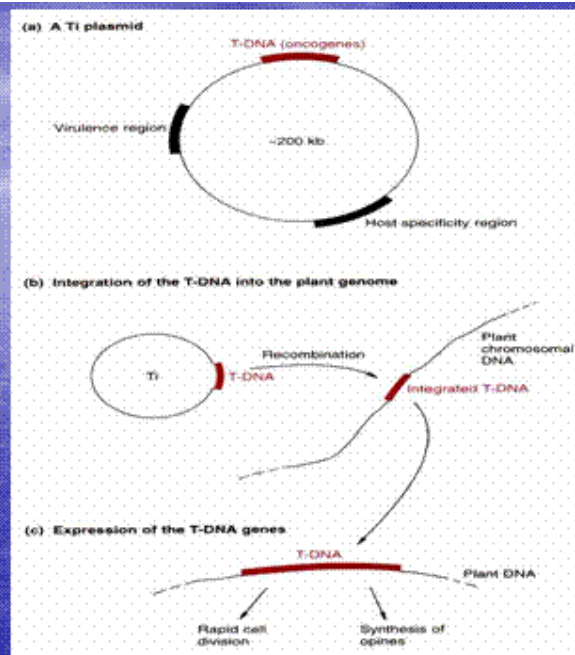
- Transcriptionally active DNA (genes) (euchromatin) is often clustered in regions and are sensitive to nuclease attack.
- Some regions are hypersensitive to nucleases and mostly contain transcription control sites.
- Genes may be separated by inactive chromatin.
- Transcription unit (portion of the gene that is copied by RNA polymerase) consists of coding regions of DNA (exons) interrupted by intervening sequences of non coding DNA (introns).

Genetic material can be rearranged

- Chromosomal recombination
 - Exchanged genetic information between similar chromosomes.
- Sister chromatid exchange.
- Transposition
 - small DNA elements transpose themselves in and out affecting the function of the neighboring DNA.
- Chromosomal integration occurs with some viruses.

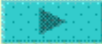






Genetic material can be rearranged

• Gene Conversion

• Similar sequences of homologous or non homologous chromosomes pair up and eliminate mismatched sequences which may lead to accidental fixation. 

• Immunoglobulin rearrange


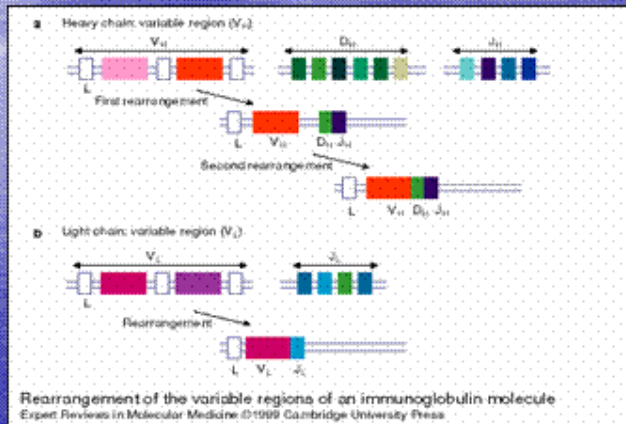
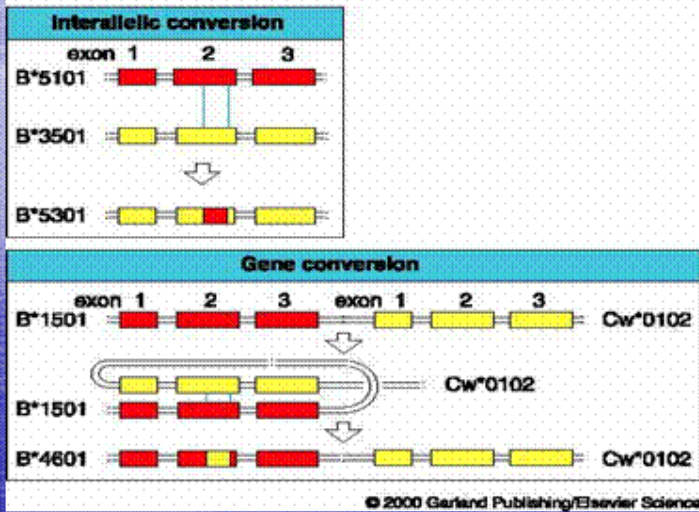
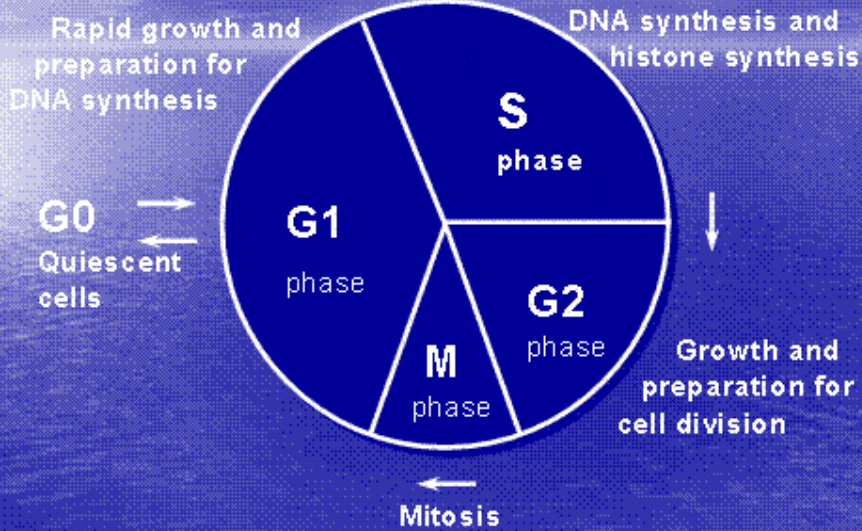
• V_L and C_L genes for a single immunoglobulin are widely separated in germ line DNA. In DNA of differentiated immunoglobulin producing cell the same V_L and C_L genes are physically closer and in the same transcription unit. 

Figure 3.23

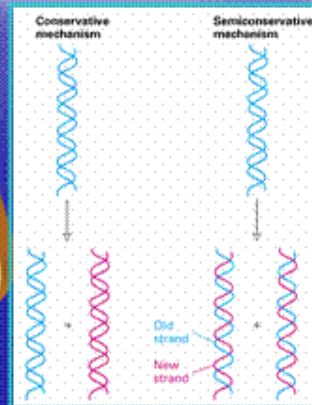


DNA Replication



DNA Replication

- Replication occurs in a semi-conservative manner.
- The two strands separate and each act as template for a new complementary strand synthesis.
- The two daughter molecules each contains only one strand of the parent molecule.

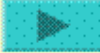




DNA Replication

- Identification of the origins of replication.
- Unwinding of the double strands
- Separation of the strands and formation of replication fork (DNA helicase, primase, DNA polymerase, SSBs).
- Initiation of DNA synthesis and elongation.
- Ligation of the newly synthesized DNA segment.
- Reconstitution of chromatin structure.



DNA Replication

- DNA polymerase only synthesizes DNA in the 5', 3' direction.
- DNA strands are anti parallel.
- Leading strand (forward) the DNA is synthesized continuously.  
- Lagging strand (Retrograde) DNA is synthesized in fragments (Okazaki fragments). 
- The entire process takes about 9 hours.

Types of DNA Damage

□ Single base alteration

- Depurination
- Deamination of cytosine to uracil
- Deamination of adenine to hypoxanthine
- Alkylation of a base
- Insertion or deletion of a nucleotide
- Base analogue incorporation

□ Two base alteration

- UV light induced thymine-thymine dimer
- Bifunctional alkylating agent crosslinkage

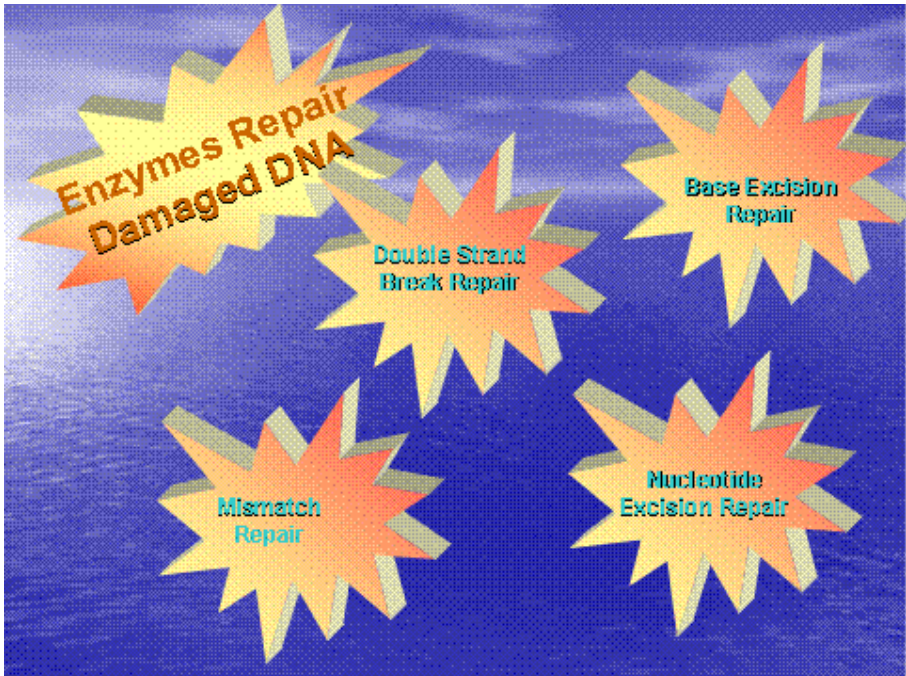
Types of DNA Damage

□ Chain breaks

- Ionizing radiation
- Radioactive disintegration of the backbone
- Oxidative free radical formation

□ Cross linkage

- Between base of the same or opposite strands
- Between DNA and protein molecules



Repair Mechanisms

- Mutations that occur during DNA replication are repaired when possible by **proof-reading** by the DNA polymerases.
- Mutations that are not repaired by proof-reading are repaired by mismatched (post-replication) **repair** followed by **excision repair**.
- Mutations that occur spontaneously at any time are repaired by excision repair (**base excision or nucleotide excision**).

Mismatched (post-replication) repair

- Repair replication error up to 2 – 5 b & loops

- The parental DNA strands are methylated on certain adenine bases

5'
3'

CH₃

- Mutations on the newly replicated strand are identified by scanning for mismatches prior to methylation of the newly replicated DNA

- The mutations are repaired by excision (exonuclease) repair mechanisms

- After repair, the newly replicated strand is methylated

CH₃

Base Excision Repair

- Repair spontaneous chemical or radiation damage to a single base.

- Base removal by various N-glycosylases

- uracil N glycosylase (targets spontaneously deaminated C)

- A basic sugar removal, replacement.

- In eukaryotes pol β repairs this lesion.

ATGUGCATTGA

TACGGCGTAACT

↓ uracil DNA glycosylase

ATGC GCATTGA

TACGGCGTAACT

↓ repair nucleases

AT GCATTGA

TACGGCGTAACT

↓ DNA polymerase β

ATGCCGCATTGA

TACGGCGTAACT

↓ DNA ligase

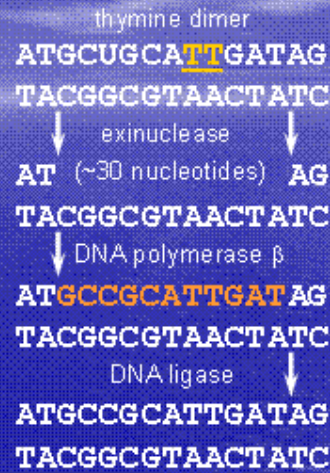
ATGCCGCATTGA

TACGGCGTAACT

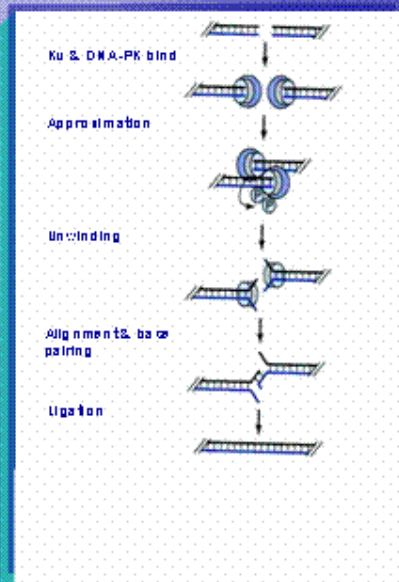
Nucleotide Excision Repair

❖ Repair spontaneous chemical or radiation damage to a DNA segment.

❖ Exinuclease remove ~ 30 base oligomer, replacement.



Double Strand Break Repair



- **Purpose**
 - For immunoglobulin gene rearrangement in B and T cells.
 - Repair of ionizing radiation and oxidative damage.
- Ku heterotetramer binds ends and recruits DNA-PK.
- Phosphorylation of DNA-PK on opposite ends.
- Ku helicase is activated
- Base pairing between single strand ends of fragments; repair

Xeroderma Pigmentosum

- Autosomal recessive genetic disease.
- Characterized by marked sensitivity to sunlight (ultra violet).
- Associated with a 2000-fold increase of sunlight-induced skin cancer and with other types of cancer such as melanoma and premature death.
- The defect involves the repair of the damaged DNA (Thymine dimers).
- Low activity of the nucleotide excision repair caused by mutations in the genes.

Ataxia telangiectasia

- Caused by mutations in gene that **detects DNA damage**
- Increased risk of X-ray
- Increased breast cancer in carriers

Fanconi anemia

- Increased risk of X-ray
- Sensitivity to sunlight

Bloom syndrome

- Caused by mutations in **DNA helicase gene**
- Increased risk of X-ray & sensitivity to sunlight.

By Prof. Dr Hala El-Said

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