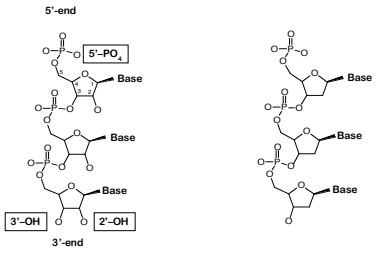
RNA and DNA Consist of Polymerized Nucleotides



Ribonucleic acid (RNA)

Deoxyribonucleic acid (DNA)

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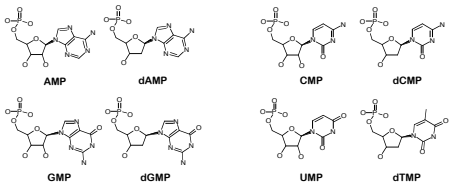
Components of RNA and DNA



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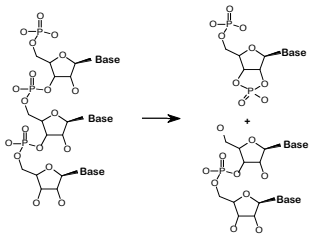
RNA Is More Labile Than DNA

RNA is labile in a water solution because the phosphate groups can form phosphodiester bonds with both the 2' and 3' –OH groups of ribose.



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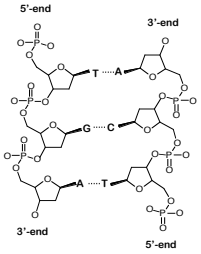
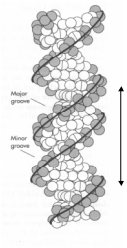
The Strands of DNA are Anti-Parallel



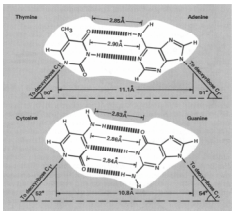
Structure of Double-Stranded DNA

B-form of DNA



10 base pairs
3.4 nm

Base-pairing of B DNA



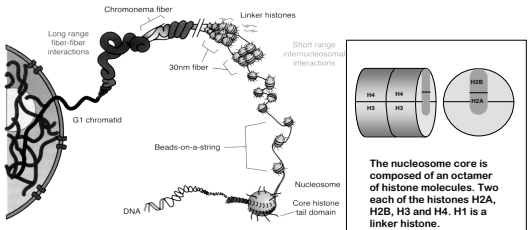
The Size of Cellular DNA

Escherichia coli (a bacterium) has one DNA molecule (chromosome) with a size of ca. 10^6 nucleotides

Homo sapiens cells have 46 chromosomes, each consisting of one DNA molecule, with a total size of ca. 10^9 nucleotides

Nuclear DNA Forms Chromatin

Histones are among the most conserved proteins in eukaryotes



The nucleosome core is composed of an octamer of histone molecules. Two each of the histones H2A, H2B, H3 and H4. H1 is a linker histone.

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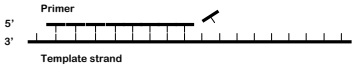
Synthesis of DNA

Enzymes catalysing the synthesis of DNA: DNA polymerases.

DNA polymerases have some general properties

- **DNA molecules are extended at the 3'-end. DNA is synthesised in the 5' to 3' direction**
- **Deoxynucleoside triphosphates (dATP, dCTP, dGTP, dTTP) are used as building blocks**
- **The polymerisation reaction is directed by A–T, G–C base pairing to a template strand**



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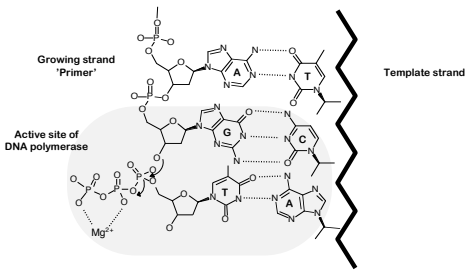
Polymerisation of deoxynucleotides



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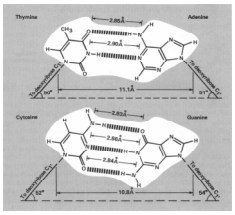
Fidelity of DNA synthesis

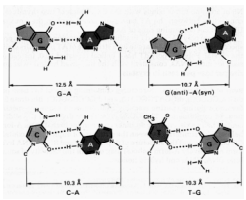
In mammals, the error frequency of DNA synthesis must be $<10^{-9}$

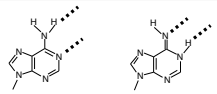
The base pairing mechanism leads to one error in ~10^4 polymerised nucleotides

Reasons for Incorrect Base-pairing



Correct base-pairing

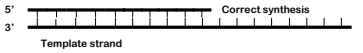




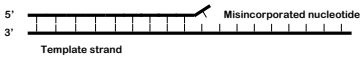
Tautomer of A can base pair with C instead of T

Correction of Replication Errors

Proofreading activity of DNA polymerases

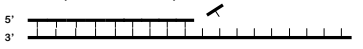


Template strand



Template strand

3' to 5' exonuclease activity of DNA polymerase catalyses the removal by hydrolysis of 3'-terminal nucleotides that are not base-paired to the template strand.

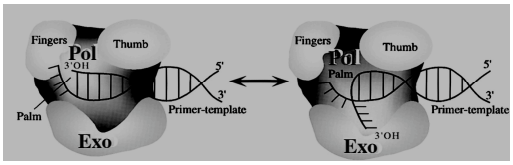


Template strand

With this mechanism the error frequency of DNA synthesis is decreased to ~10^{-6}.

Correction of Replication Errors

DNA polymerase I from *E. coli* has two catalytic activities, although the enzyme consists of a single polypeptide chain.

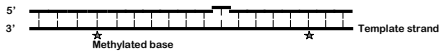


View of a DNA polymerase bound to a primer-template junction in its synthesis (left) and proofreading (right) modes Pol denotes the polymerase active site; Exo, the 3' to 5'exonuclease active site.

From Baker & Bell (1998) Cell, **92**:295

Correction of Replication Errors

Postreplication mismatch repair



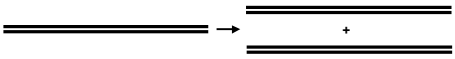
In DNA, some of the C (and A) bases are methylated. Methylation is a slow process. Newly replicated DNA is undermethylated.

Mismatch repair enzymes recognise mismatched nucleotides and remove the nucleotide in the undermethylated strand.

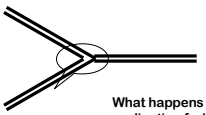
The mismatch repair system increases the fidelity of DNA replication to ~10^{-9}.

Replication of Chromosomal DNA

Semiconservative DNA replication

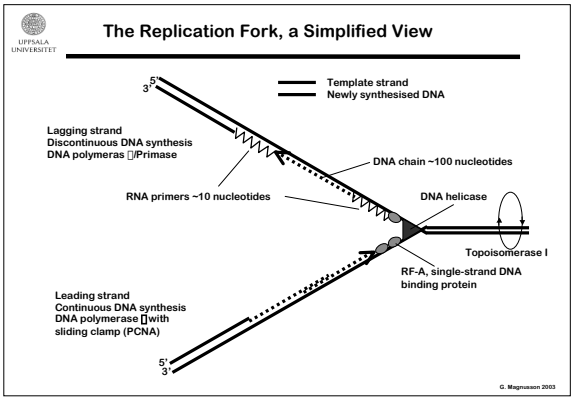


A half-replicated DNA molecule



What happens at the replication fork?

The Replication Fork, a Simplified View



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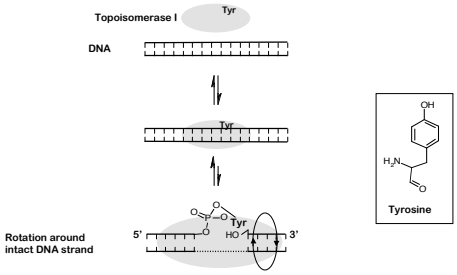
Reaction Catalysed by DNA Topoisomerase I



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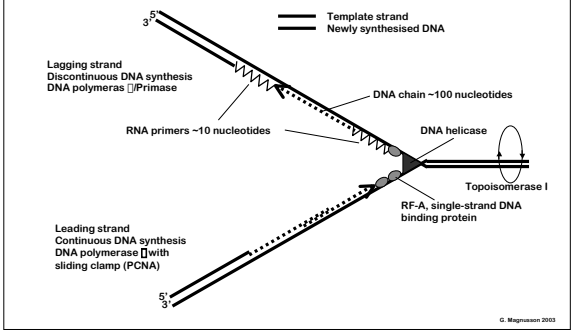
The Replication Fork, a Simplified View



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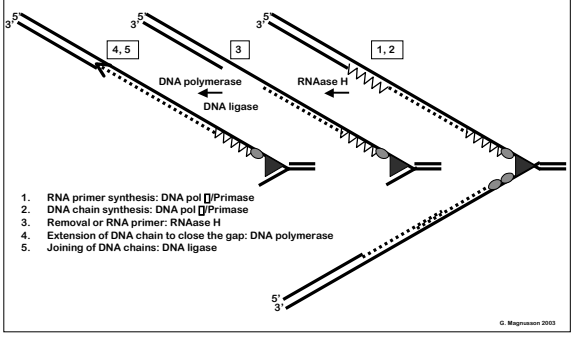
Lagging Strand Synthesis



1. RNA primer synthesis: DNA pol α/Primase
2. DNA chain synthesis: DNA pol δ/Primase
3. Removal or RNA primer: RNAase H
4. Extension of DNA chain to close the gap: DNA polymerase
5. Joining of DNA chains: DNA ligase

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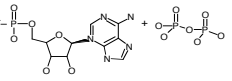
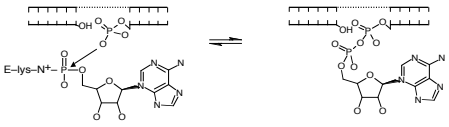
Reactions Catalysed by DNA Ligase

1 $E\text{–lys–}NH_2 + ATP \rightleftharpoons E\text{–lys–}N^+\text{–}$ 

2 

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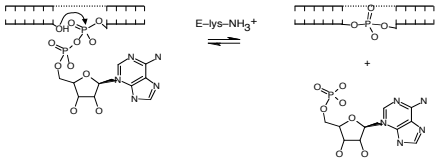
Reactions Catalysed by DNA Ligase

3 

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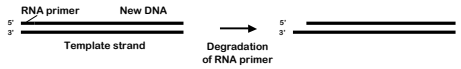
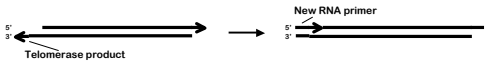
Replication of Chromosomal Ends (Telomers)



Loss of genetic information at chromosomal ends is prevented by the addition of nontemplated nucleotides by telomerase



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Mechanism of Telomerase Activity

Telomerase is a DNA polymerase that uses an RNA template. This template is a subunit of the enzyme and consists of an RNA molecule with a AAUCCC sequence repeat.

In the new-born, the telomeres have a length ~10^{-4} nucleotides with ca. 10^2 unpaired nucleotides at the 3'-end.

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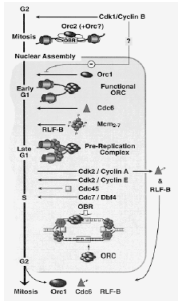
Initiation of DNA-Synthesis at an Origin



From Bogan et al. (2000) J. Cell. Physiol. Vol. 184, 139

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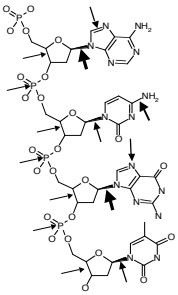
Frequent Spontaneous DNA Damage

Frequency of individual lesions of nucleotides is suggested by the size of the arrows.

Depurination is the most frequent lesion ~10^4/day/cell.

Deamination of C to form U occurs ~10^3 times per day in a cell.



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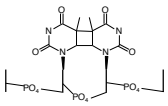
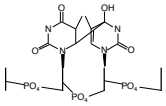
UV-Induced Damage to DNA

Absorption of UV-radiation (200–320 nm) by DNA induces two major types of potentially mutagenic lesions. Skin cells not protected by pigmentation accumulate ~10^4 lesions per hour when exposed to strong sun-light.



DNA backbone



Cyclobutane thymine dimer kinks DNA 7°–9°

Thymine (6–4) pyrimidone photoproduct kinks DNA 40°

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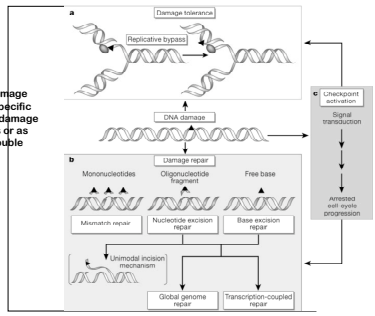
Mechanisms of DNA Repair

In the cell, DNA damage is recognised by specific proteins either as damage to individual bases or as distortion of the double helix.



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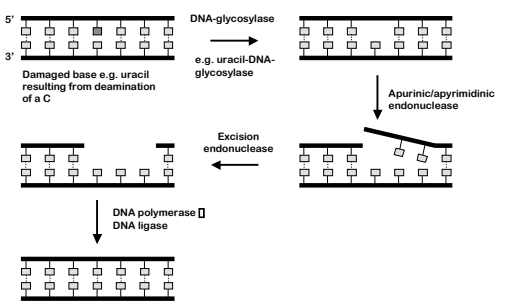
Base Excision Repair of DNA



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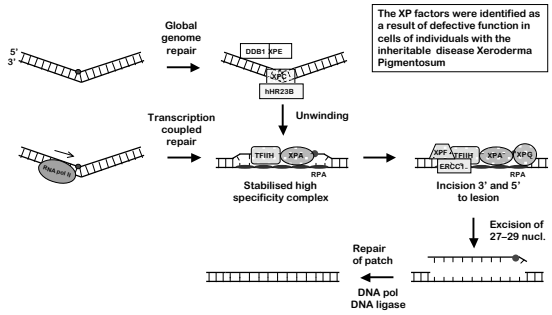
Repair of Lesions Distorting the DNA Helix



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Ames' Mutagen Test

- Salmonella bacteria requiring histidine for growth
- Use strains with several different types of mutations in the *his* genes
- Treat bacteria with test chemical, directly or after metabolic activation in liver cell extract
- Plate bacteria on agar in histidine deficient medium
- Count colonies and calculate mutation frequency

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Mouse Carcinogen Test

- Treat animals with test substance. For substances with low potency, use high doses or many animals
- TD_{50} (mg/kg bodyweight/day during lifetime) calculated as the dose of chemical that would decrease the number of tumour-free animals by 50%
- Tumours have to be analysed by a pathologist
- TD_{50} for various substances has a range of $>10^7$

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Risk Assessment

- All environmental risks cannot be avoided
- There is no inherent difference in the toxicity of natural and synthetic substances
- Human exposure has to be considered in the assessment of risk
- HERP (human exposure rodent potency). Percentage of the rodent potency (TD_{50}) received by a human during lifetime exposure

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HERP

HERP (%)[a]

- **3.6** Beer, 500 ml (etyl alcohol, 23 ml/day)
- **0.4** Typical indoor air (formaldehyde, 600 µg/day)
- **$1x10^{-6}$** Lindane[b] (32 ng/day)

[a]A HERP value of 100 means that the exposure is identical to the TD_{50} in rodent tests.
[b]Lindane is a mutagenic insecticide that is banned from use in Swedish agriculture.

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