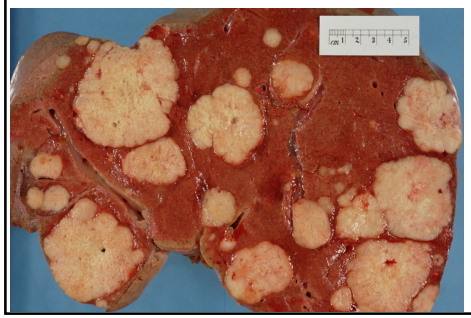
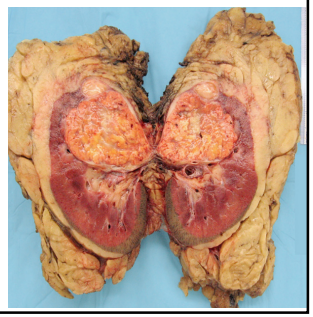
DRUGS ACTING ON DNA

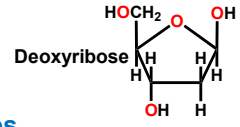




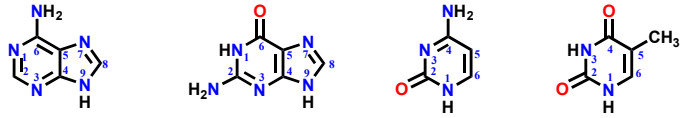
1. DEOXYRIBONUCLEIC ACID (DNA)

1.1 Primary Structure - basic building blocks

Sugar

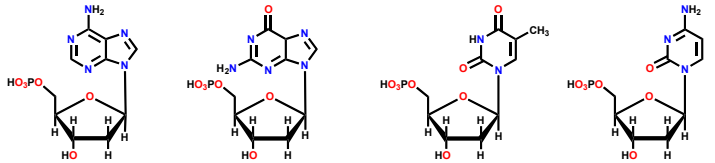


Nucleic acid bases

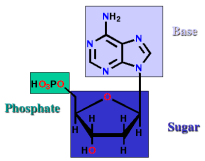


Nucleosides (deoxyribose sugar part + base) do not bear a phosphate group

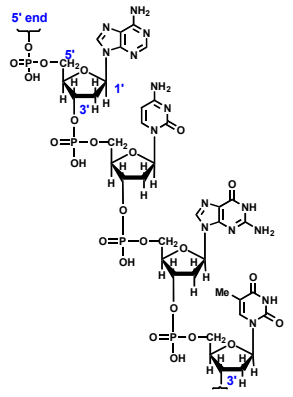
Building blocks – Nucleotides (base+sugar+phosphate)

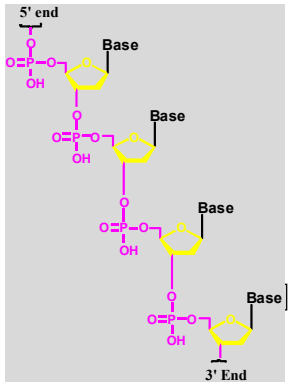


A and **G** are purines **T** and **C** are pyrimidines



Primary Structure (an order of building blocks linking)

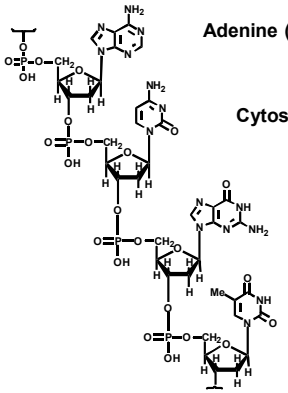




Sugar phosphate backbone

DEOXYRIBONUCLEIC ACID (DNA)

Primary Structure



Adenine (A)

Cytosine (C)

Guanine (G)

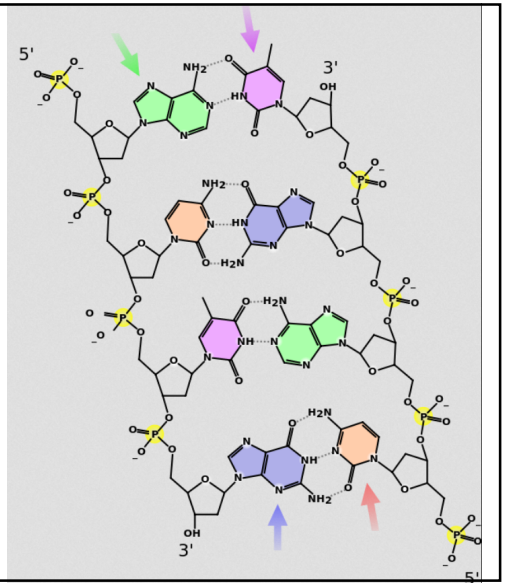
Thymine (T)

Note:

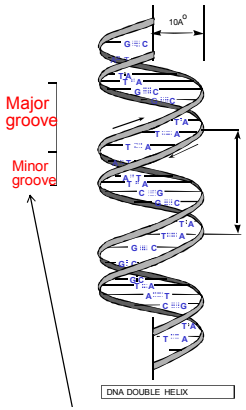
- Sugar phosphate backbone is constant
- Bases attached in apparently random order

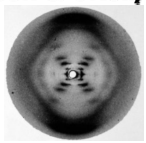
Secondary Structure

A::T, C:::G pairing

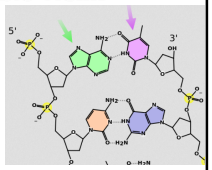


Secondary Structure (Watson & Crick) - Double Helix



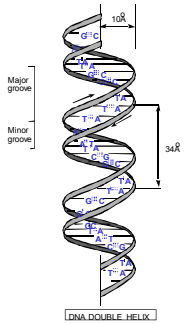


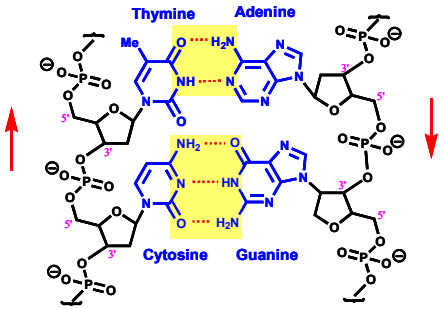
X-ray crystallography of DNA



- Sugar phosphate backbone is ionised and faces outward (favourable interactions with water)
- Nucleic acid bases point inward and pair up A-T or G-C
- Purine pairs with pyrimidine => constant diameter to helix
- Base pairs are stacked (VdW interactions
- Chains drugs complementary because of

the grooves are important for the action of anticancer intercalating





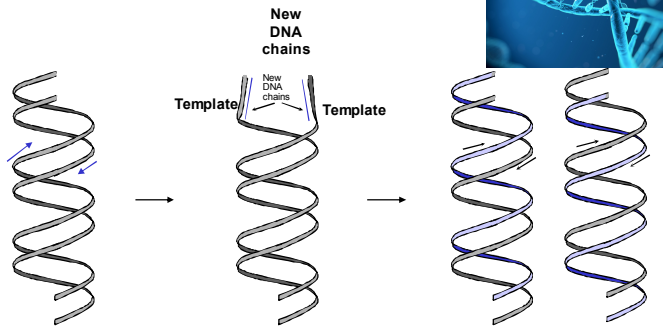
Base Pairing

A-T base pairing involves 2 H-bonds

G-C base pairing involves 3 H-bonds

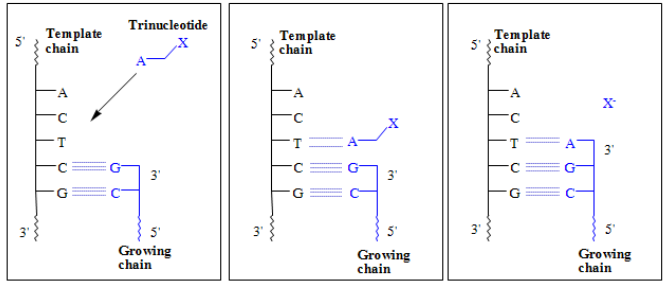
1. DEOXYRIBONUCLEIC ACID (DNA)

Replication



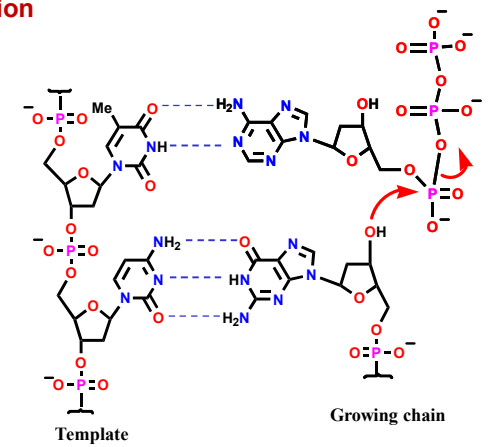
DNA Double Helix | **Replication (two templates)** | **DNA Daughter helices**

Replication *catalysed by enzyme*



This is a way **to pass genetic information** from generation to generation. How can only four bases in nucleotides code 20 amino acids? **Each AA is coded by a combination of three nucleotides** (triplet code. There are **64** = 4Exp3 **ways to combine 4 nucleic bases in sets of three**). Example: Pro has CCU, CCC, CCA, or CCG etc.

Replication

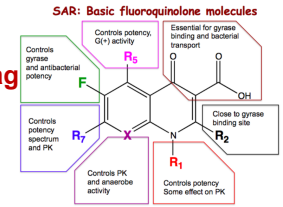


diphosphate leaving group

Tertiary Structure

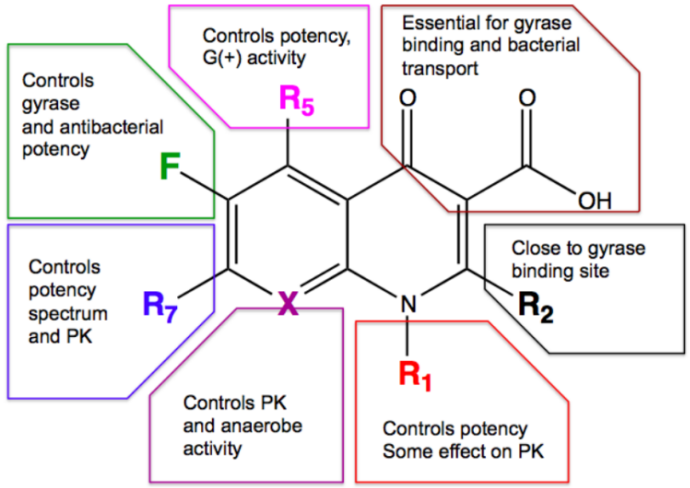
DNA is extremely long (huDNA: ca 2 m), it would not fit into nucleus in its linear form

- **Double helix coils into a 3D shape - supercoiling**
- **Double helix has to unravel during replication**
- **Unravelling leads to strain therefore it needs an enzymes (topoisomerases) that catalysed unraveling cutting and repair of DNA chain**
- **fluoroquinolone is an antibacterial ag that inhibits this enzyme**



***gyrase*, is an enzyme within the class of topoisomerase**

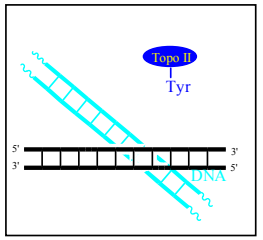
SAR: Basic fluoroquinolone molecules



DEOXYRIBONUCLEIC ACID (DNA)

Action of topoisomerase II

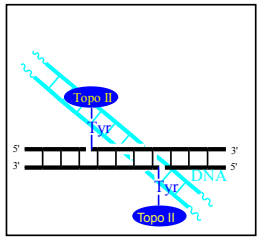
- Relieves the strain in the DNA helix by temporarily cleaving the DNA chain and crossing an intact strand through the broken strand



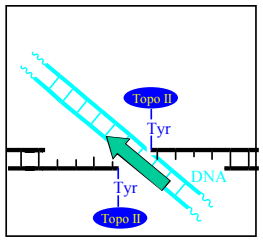
Topoisomerase binds to part of DNA where two regions of double helix are in near proximity.

- Tyrosine residues in the enzyme are involved in the chain breaking process and form temporary covalent bonds to DNA

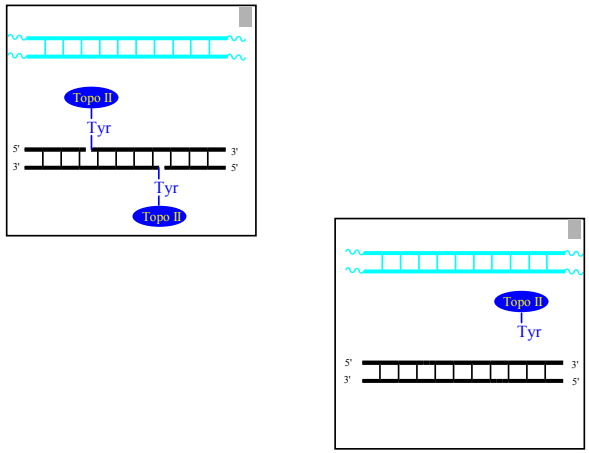
- The enzyme pulls the chains apart to create a gap



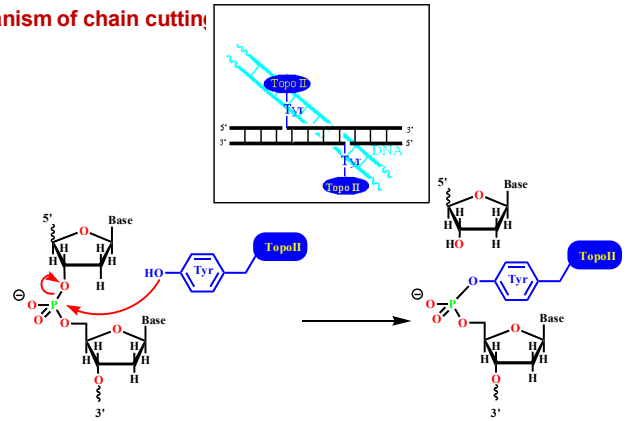
- temporarily cleaving the DNA chain crossing an intact strand through the broken strand and the intact strand of DNA is passed through the gap



• **The break is resealed**



Mechanism of chain cuttin



Topoisomerases are responsible for supercoiling and also uncoiling process, so **inhibition of these enzymes would effectively block transcription and replication**.

Action of topoisomerase I

Topoisomerase I is similar to II. It **relieves the torsional stress of supercoiled DNA during replication, transcription and repair.** It **cleaves only one strand of DNA**. Tyrosine residue is linked to the 3´phosphate end of the DNA strand (**rather than both 5´ends by Topoisomerase-II**). This forms cleavable complex with a **single-strand break**. Relaxation of torsional strain takes place either by:

a/ allowing the **intact strand to pass through the nick** or

b/ by **free rotation of the DNA about the uncleaved strand**

Once the torsional strain of the DNA has been relieved, the enzyme rejoins the cleaved strand of DNA and departs.

Topoisomerase IV is a bacterial enzyme, important target for the fluoroquinolone agents.

Chromatin is the **combination of DNA and proteins** that make up the contents of the nucleus of a cell. The primary functions of chromatin are: **to package DNA into a smaller volume to fit in the cell, to strengthen the DNA to prevent DNA damage**, and to **control gene expression** and **DNA replication**. The primary protein components of chromatin are **histones** that compact the DNA. Chromatin is only found in eukaryotic cells.

DNA is not an isolated molecule **within nucleus**, it **is associated with histones** (proteins) that form with associated DNA **NUCLEOSOME** which occurs regularly along the length of the chromatin and **plays a crucial role in the regulation of DNA**

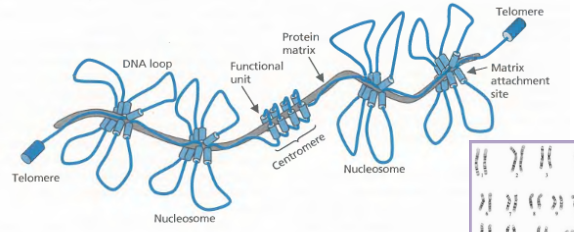
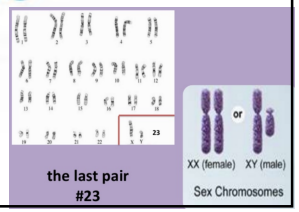


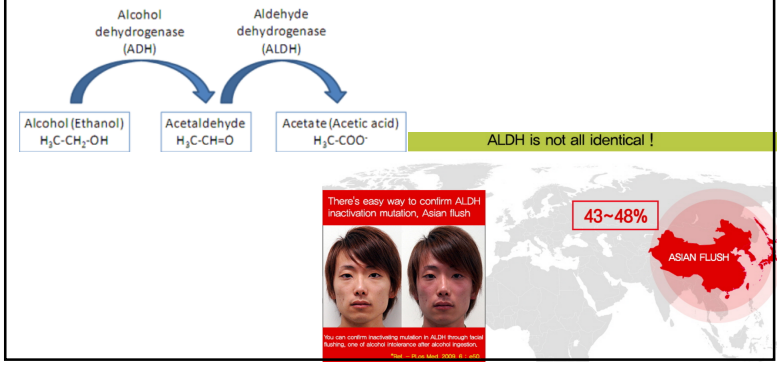
FIGURE 21.5 Chromatin, telomeres and DNA.



Did you know? The length of all joined DNA from an adult body has 111 mld km that is more as the distance between Earth and Pluto (7.5 mld km)!

Adult human body consists from ca 3.72 x 10E13 cells.
Current lenght of human DNA is ca 3 m.
Length of all joined human DNA from one adult body is:
3.72 x 3 x 10E13 m = 11.16 x 10E10 km = 111 mld km !!!
Earth Neptun distance is 4.4 mld km.
Earth Pluto distance is 7.5 mld km.

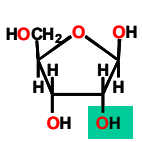
GENETIC POLYMORPHISM: replication process is not perfect, occasionally a **mutation** can occur. If it is not fatal, it is **carried out through generations**. This leads to **different individuals** having slightly **different gene sequences** (in average 1 on 1 000 base pairs between individuals). Nucleic acid acts as the code for amino acids in proteins. **Mutation in proteins** causes **individual susceptibility to diseases and also to drugs**. Patient´s genome is a way to predict and prevent disease and also to choose the ideal drug therapy = **PERSONALISED MEDICINE**

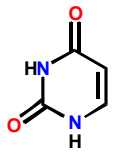


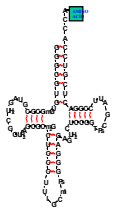
RIBONUCLEIC ACID (RNA)

Primary structure

- Similar to DNA with the following exceptions:
  - Ribose is used instead of deoxyribose
  - Uracil (demetylated T) is used rather than thymine



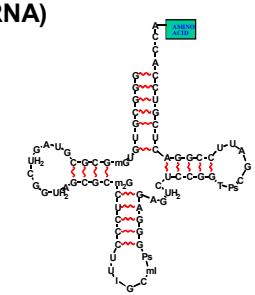




A/U and C/G pairing within one chain!

Secondary RNA structure

- single stranded
- some regions of helical secondary structure exist due to base pairing within the same strand (see t-RNA)
- adenine pairs to uracil (A / U)
- guanine pairs to cytosine (C / G)



Tertiary RNA structure

- Three types of RNA are involved in protein synthesis

- **Messenger RNA (mRNA)**
  relays the code for a protein from DNA to the protein production site

- **Transfer RNA (tRNA)**
  the adapter unit linking the triplet code on mRNA to specific amino acids

- **Ribosomal RNA (rRNA)**
  present in ribosomes (the production site for protein synthesis).
  Important both structurally and catalytically

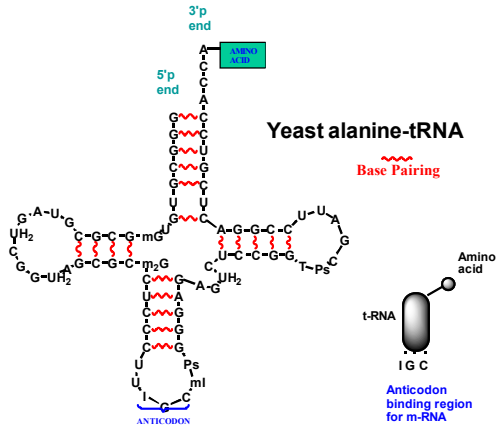
RIBONUCLEIC ACID

(RNA-crucial „middle man" between DNA and proteins)

Tertiary structure

less common bases in RNA
ml Methylinosine
I Inosine
UH2 Dihydrouridine
T Ribothymidine
Ps Pseudouridine
mG Methylguanosine
m2G Dimethylguanosine

Implies more variety in RNA secondary and tertiary Structure – no double helix

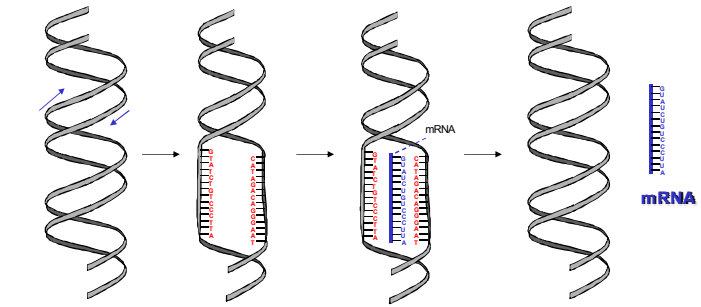


Anticodon: - contains 3 bases that are specific for the attached amino acid complementary triplet code on m-RNA (the codon)

RIBONUCLEIC ACID (RNA)

Transcription - code redrawing to mRNA

The copying of a segment of DNA which codes for a specific

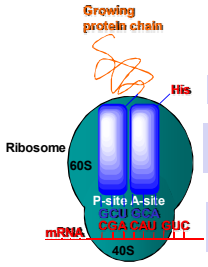


Translation - protein synthesis

Endoplasmatic reticulum: cellular organelle for proteosynthesis on ribosomes

rRNA is a major component of each ribosomal subunit (ca 2/3 of mas of ribosome).

rRNA has a major catalytic role in proteosynthesis.



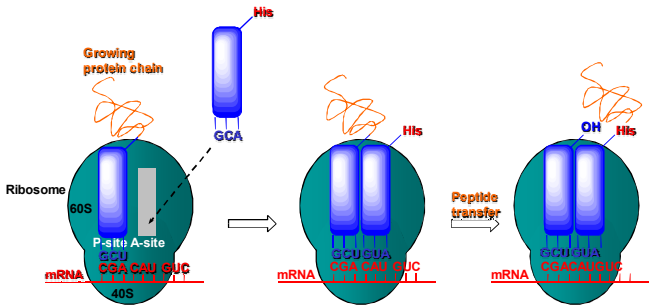
HIS codes: CAU, CAC, CAA, CAG

each AA residue needs different mRNA

anticodon on tRNA
codon on mRNA

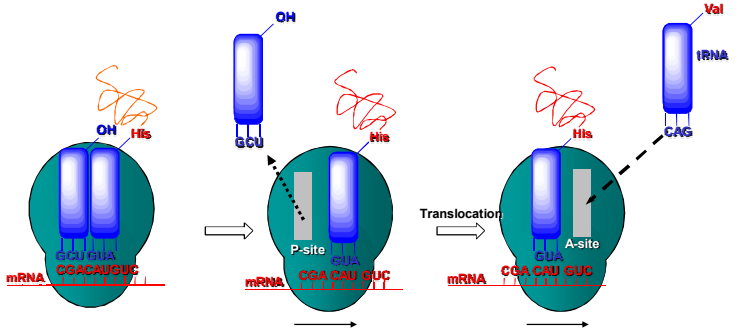
2. RIBONUCLEIC ACID (RNA)

2.5 Translation - protein synthesis



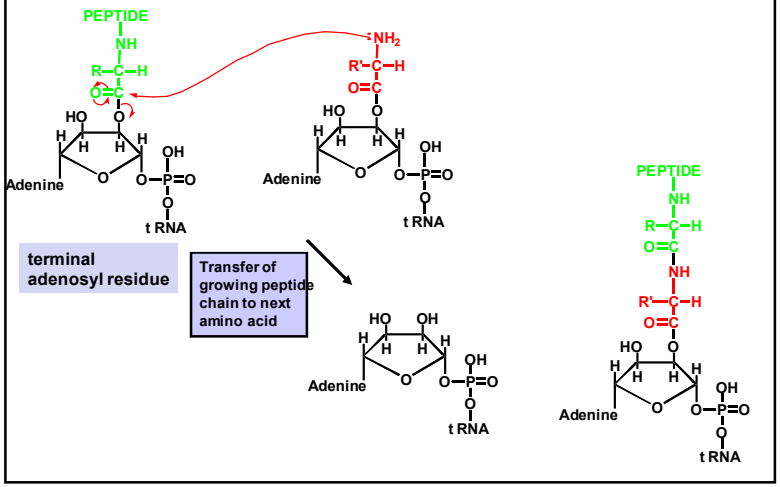
2. RIBONUCLEIC ACID (RNA)

2.5 Translation - protein synthesis



2. RIBONUCLEIC ACID (RNA)

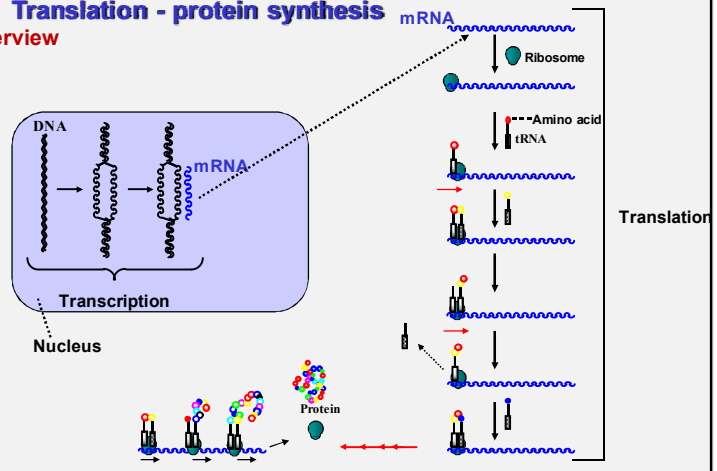
2.5 Translation - protein synthesis



2. RIBONUCLEIC ACID (RNA)

2.5 Translation - protein synthesis

Overview



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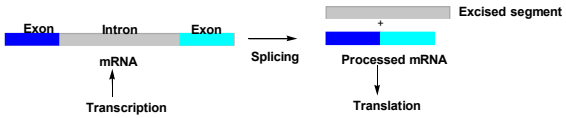
Modifications of mRNA prior to translation



- a middle section (intron) is excised
- end regions (exons) are then spliced together
- splicing requires an RNA / protein complex called a spliceosome
- the RNA in spliceosome is called small nuclear RNA (snRNAs < 300 nucleotides)
- snRNAs align the target mRNA by base pairing to it
- DNA mutation => new splice site => different mRNA => defective protein: 15% of genetic diseases

GENETIC ILLNESSES: results in **defective or non-expression of** particular **proteins**

ALBINISM skin, hairs, eyes lack pigment (**defective enzyme tyrosinase**: copper containing enzyme that catalyses first stages of pigment **melanin**) ca 90 mutations identified resulting one or more AA being altered in tyrosinase. Mutations which alter AA in the active site are most likely to result in loss its activity.

Phenylketonuria absence or deficiency phenylalanin hydroxylase, this enzyme converts Phe to Tyr. If the enzyme is not working **Phe concentration in blood rises** together with some metabolic products like phenylpyruvate **=> severe mental retardation**

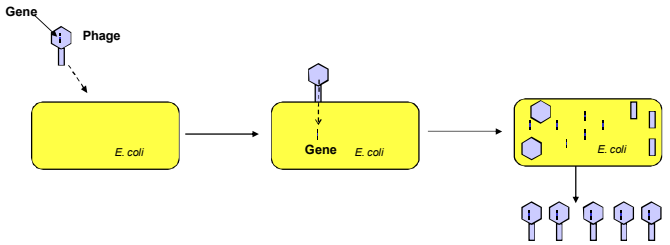
Haemophilias: one of the **coagulation factor is deficient => uncontroling bleeding** after an injury (before they died young, today intravenous infusion of missing coagulation factor (purified from blood plasma) after injury, problem UK 1979-1985 observed 1 200 HIV infections as a result of infected blood products, also HepB, C therefore nowadays **recombinant DNA technology produced blood coagulation factors** to exclude infections, unfortunately some patients produce immune response to the infused c. factor, solution could be a gene therapy => introduction of genes that would produce the right c. factor in the body).

Muscular dystrophy: incidence (1 / 3 500 males), **absence of protein dystrophin** that has important structural role in cells. Its absence causes in muscle deterioration. (gen therapy)

Cancer is associated with genetic defects which result in molecular signalling defects in the cells.

3. GENETIC ENGINEERING

Amplification of a gene



- Gene can be inserted into a bacteriophage
- Bacteriophages are viruses which infect bacterial cells
- Multiple copies of the bacteriophages are produced along with the gene

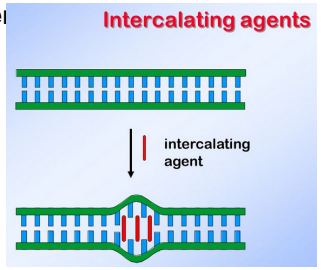
DRUGS ACTING ON DNA

- Intercalating agents
- Topoisomerase poisons
- Alkylating agents
- Metallating agents
- Chain cutters
- Chain terminators
- Control of gene transcription

Intercalating agents

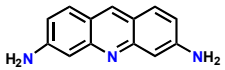
Mechanism of action

- contain planar aromatic or heteroaromatic ring systems
- planar systems slip between the layers of nucleic acid pairs and disrupt the shape of the helix
- preference is often shown for the minor or major groove
- **intercalation prevents replication and transcription**
- intercalation **can inhibit topoisome**



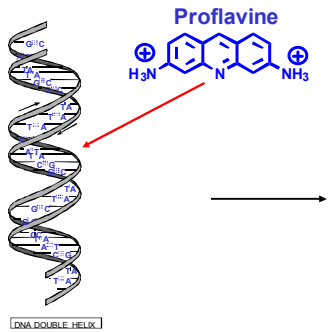
Intercalating agents

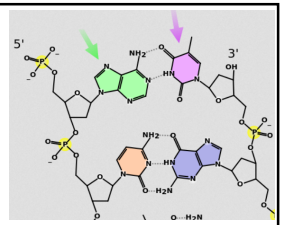
Proflavine

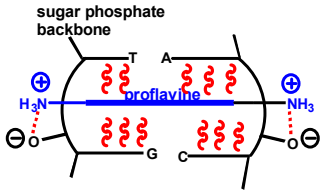


- **planar** tricyclic system
- the amino substituents are **protonated and charged**
- targets bacterial DNA
- used as a **topical antibacterial agent** in the second world war
- **too toxic for systemic use**

Intercalating agents





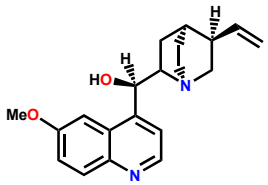


van der Waals interactions

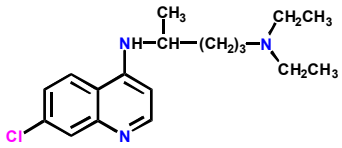
Ionic interactions

Intercalating agents

antimalarial agents



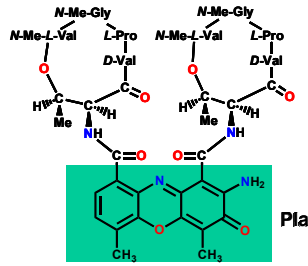
Quinine



Chloroquine

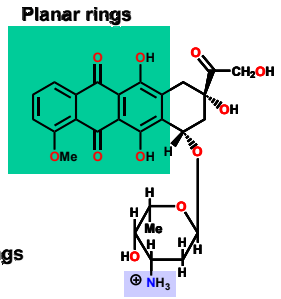
Intercalating agents

anticancer agents



Planar rings

Planar rings



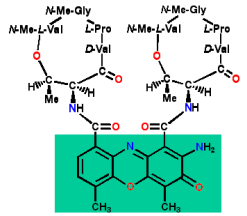
Dactinomycin

Extra binding to sugar phosphate backbone by cyclic peptide

Doxorubicin (Adriamycin)

Extra binding to sugar phosphate backbone by NH_3

Dactinomycin

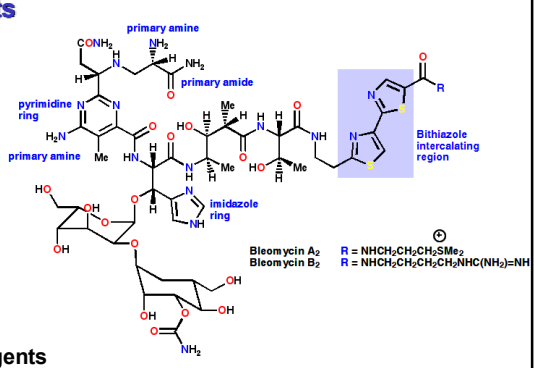


Dactinomycin

- **intercalates via minor groove** of DNA double helix
- **prevents unwinding** of DNA double helix
- **blocks transcription** by blocking DNA-dependent RNA polymerase
- **intercalates** via the **major groove** of DNA double helix
- **blocks** the action of **topoisomerase II** by stabilising the DNA-enzyme complex

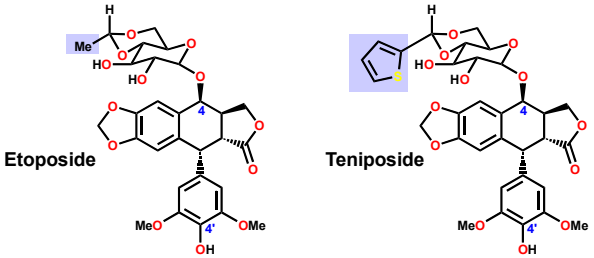
Intercalating agents

Bleomycins



- used as **anticancer agents**
- **intercalated** by means of bithiazole ring system
- **ferrous ion** then **chelated by nitrogens of the primary amines, amide and pyrimidine ring**
- reaction with oxygen results in a ferric ion and **reactive oxygen species (ROS)**
- results in **radical formation and DNA chain cutting**
- bleomycin **prevents DNA ligase from repairing damage**

Topoisomerase poisons - non-intercalating

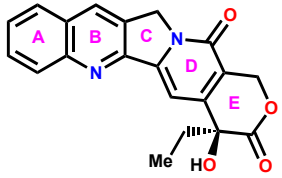


etoposide and teniposide

- used as **anticancer agents**
- **stabilise the complex** between **DNA** and **topoisomerase** enzymes
- also cause **chain cutting**

Topoisomerase poisons

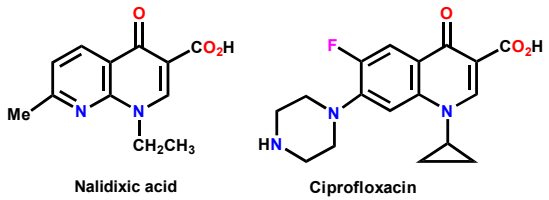
Camptothecin



- **semi-synthetic** analogues used as anticancer agents
- **stabilises complex** between **DNA** and **topoisomerase I**
- **single-strand breaks** accumulate in the DNA chain
- **irreversible double-strand breaks** occur during transcription

Topoisomerase poisons

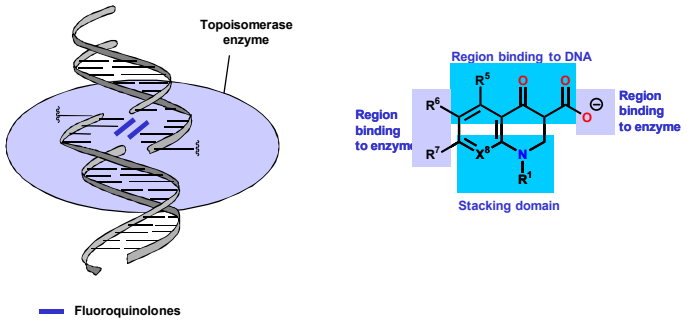
Quinolones and fluoroquinilones



- synthetic agents used as **antibacterial agents**
- **stabilise complex** between **bacterial DNA and topoisomerases**
- **binding site for agents** revealed **once DNA strands are 'nicked'**

Topoisomerase poisons

Quinolones and fluoroquinolones



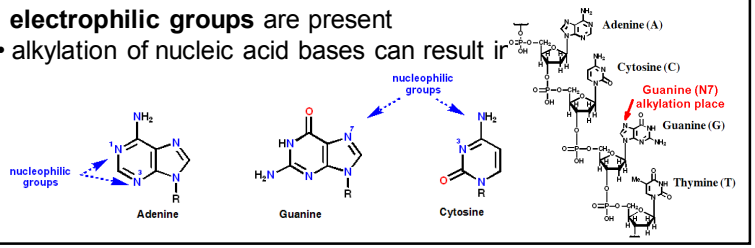
- **four molecules are stacked in the bound complex**
- they **bound to DNA and enzyme** by hydrogen and ionic bonds

Alkylating agents

- useful **anticancer agents**
- **contain** highly **electrophilic group**
- **form covalent bonds** to nucleophilic groups **in DNA**

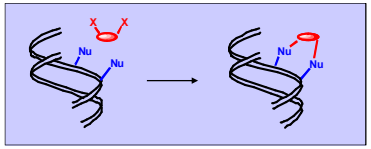
(e.g. 7-N of guanine)

- **prevent replication and transcription**
- **toxic** side effects (e.g. alkylation of proteins)
- can cause **interstrand and intrastrand cross-linking if two electrophilic groups** are present
- alkylation of nucleic acid bases can result in

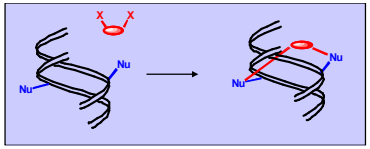


Alkylating agents

Cross linking



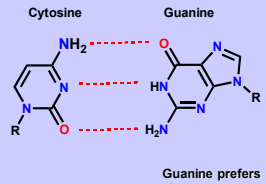
Intrastrand cross linking



Interstrand cross linking

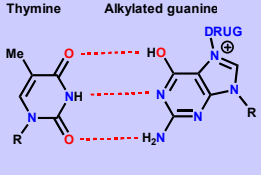
Alkylating agents

Normal base pairing



normal C / G base pairing

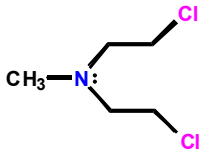
Miscoding resulting from alkylated nucleic acid bases



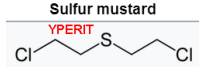
abnormal T / G base pairing, alkylated guanine prefers enol tautomer

Alkylating agents

Chlormethine (nitrogen mustard)



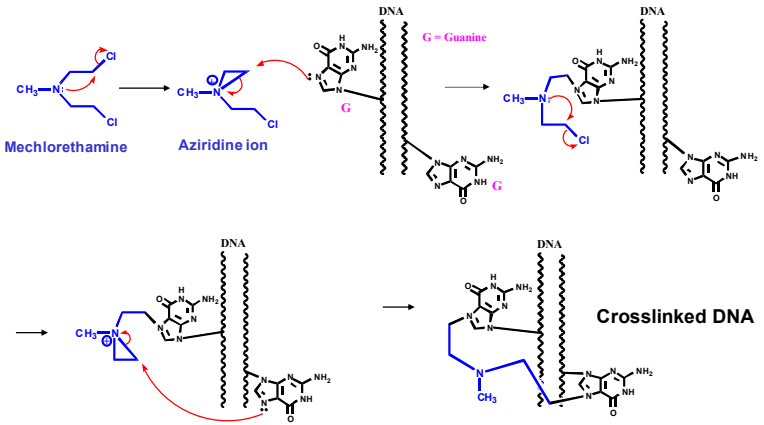
Sulfur mustard



in 1917 Yprés Belgium, oil no odour ,but side compounds smelling as mustard, garlic, onion, burned gum

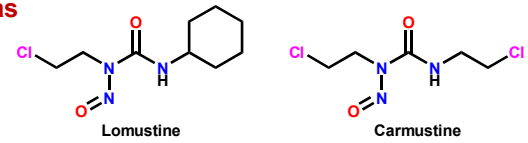
- used medicinally in 1942
- causes **intrastrand and interstrand DNA cross-linking**
- **prevents DNA replication**
- mono-alkylation of guanine also possible
- analogues with better properties have been prepared

Mechanism of action

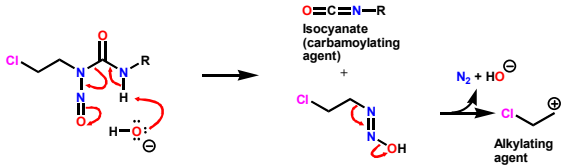


Alkylating agents

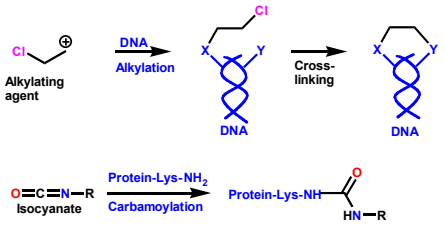
Nitrosoureas



decompose in a body to form an **alkylating agent** and a **carbamoylating agent**

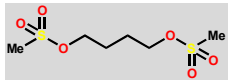


Nitrosoureas



- alkylating agent causes **interstrand cross-linking**
- **cross linking** between G-G or G-C
- **carbamoylating agent reacts with lysine residues** on proteins
- may **inactivate DNA repair enzymes**

Alkylating agents

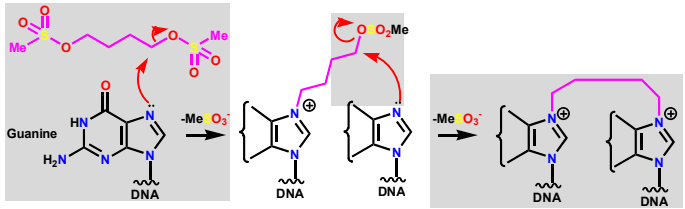


Busulfan

synthetic agent used as **anticancer agent**

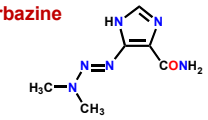
causes **interstrand cross-linking**

Mechanism

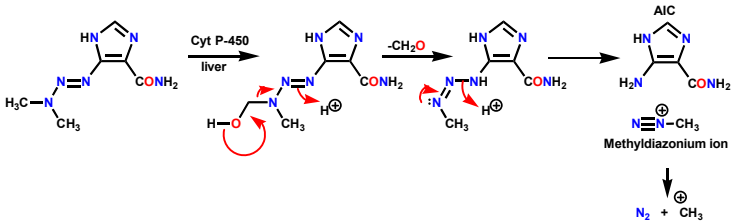


Alkylating agents

Dacarbazine

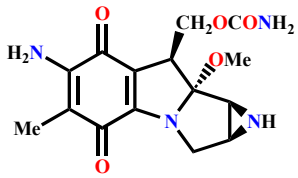


- **prodrug activated** by demethylation **in liver**
- decomposes to **form a methyldiazonium** ion
- **alkylates guanine groups**



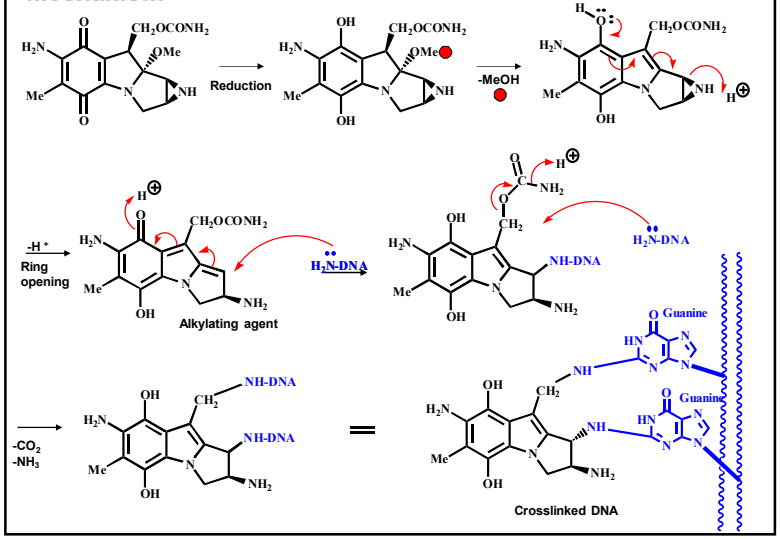
Alkylating agents

Mitomycin C



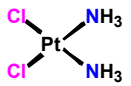
- **prodrug** activates in the body to form an alkylating agent
- **one of the most toxic anticancer drugs in clinical use**

Mechanism



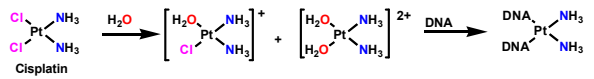
DRUGS ACTING ON DNA

Metallating agents



Cisplatin

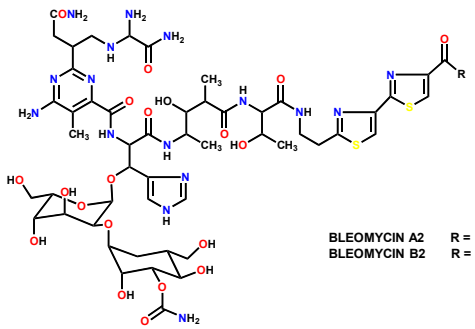
- neutral inactive molecule acting as a **prodrug**
- **activated in cells with low chloride ion concentration**
- **chloro** substituents **replaced with water** ligands
- **produces positively charged species**



- **binds to DNA** in regions rich in **guanine units**
- **intrastrand DNA** links rather than interstrand
- causes localised **unwinding** *(rozpletanie)* **of DNA double helix**
- **inhibits transcription**

DRUGS ACTING ON DNA

Chain cutters



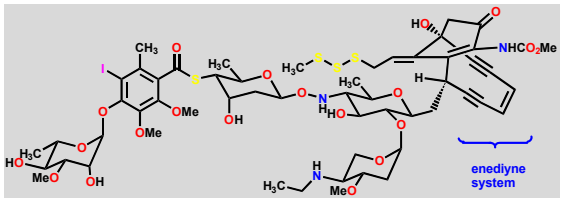
Bleomycin
used in skin cancer

BLEOMYCIN A2 R = $NHCH_2CH_2CH_2{}^+SMe_2$
BLEOMYCIN B2 R = $NHCH_2CH_2CH_2CH_2NHC(NH_2)=NH$

- **intercalating agent**
- abstracts H from DNA to **generate radicals** that with oxygen resulting in ROS causing in **DNA chain cutting**
- **also inhibits repair enzymes**

Chain cutters

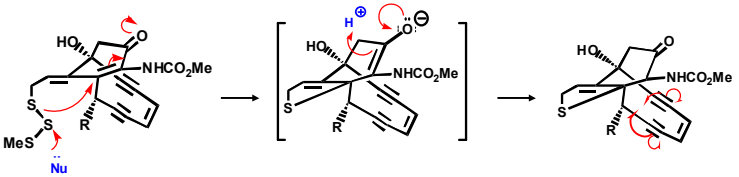
Calicheamicin γ_1^I
Antitumour agent



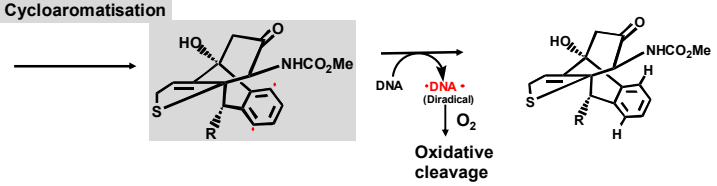
- **generates DNA diradical** that reacts with oxygen results in **chain cutting**

Chain cutters

Mechanism

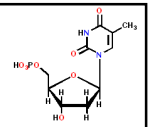


Cycloaromatisation



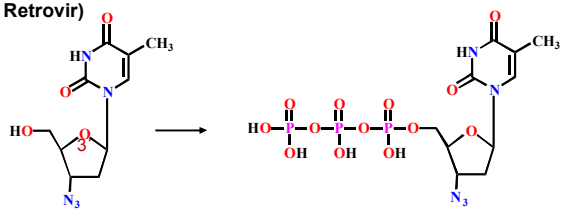
DRUGS ACTING ON DNA

Chain terminators



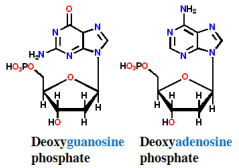
Deoxythymidine phosphate

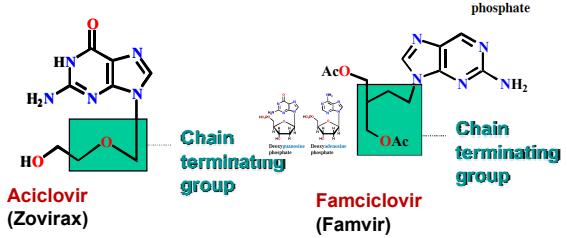
Azidothymidine (AZT)
(Zidovudine; Retrovir)



- azidothymidine is a **prodrug** used in the **treatment of HIV**
- AZT is **phosphorylated to a triphosphate in the body**
- Triphosphate has **two mechanisms of action**:
  - **inhibits** a **viral** enzyme (**reverse transcriptase**)
  - is incorporated into growing DNA chain and **acts as chain terminato**

Chain terminators



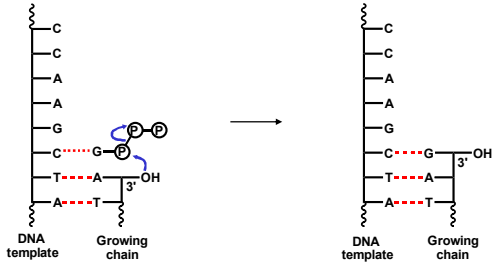


- **prodrugs** used as **antiviral agents**, the same mechanisms as
- used **against herpes simplex** and **shingles**

DRUGS ACTING ON DNA

Chain terminators

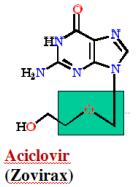
a) Normal replication

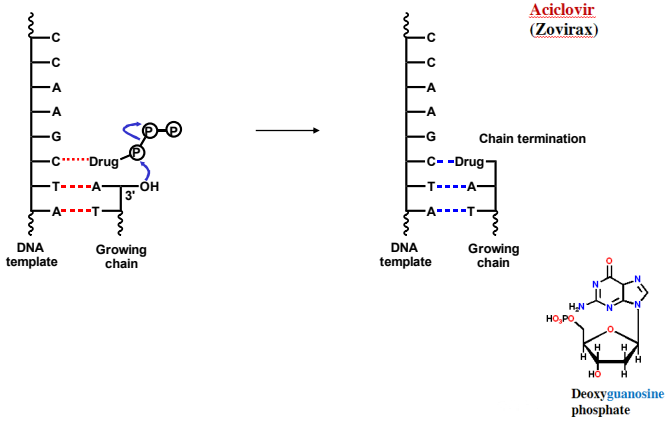


DRUGS ACTING ON DNA

Chain terminators

b) Chain termination





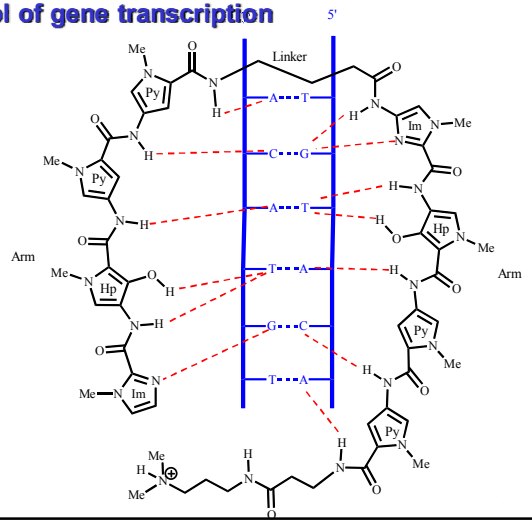
DRUGS ACTING ON DNA

Control of gene transcription

- design of synthetic **molecules** capable of **controlling gene transcription**
- molecules capable of recognising and **binding to specific base pairs**
- **hairpin** polyamides containing heterocyclic rings are **capable of binding**
  to the **minor groove** (involves amide groups and heterocycles)
- particular patterns of heterocyclic rings allow **recognition of particular**
  base pairs
- capable of **inhibiting transcription**
- designed to bind to regulatory element of a gene

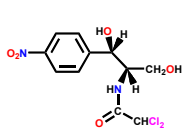
DRUGS ACTING ON DNA

Control of gene transcription

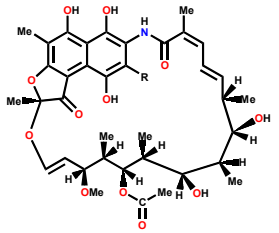


DRUGS ACTING ON rRNA

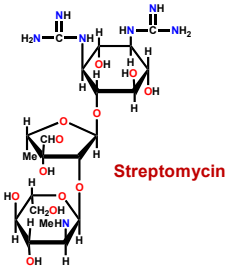
Antibiotics



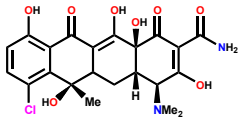
Chloramphenicol
(vs typhoid)



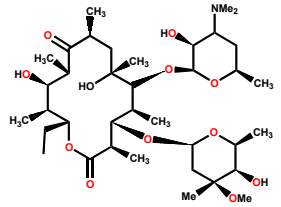
Rifamycins



Streptomycin



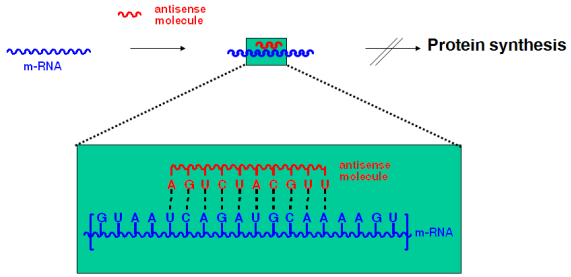
Chlortetracycline
(Aureomycin)



Erythromycin

DRUGS ACTING ON mRNA

Antisense Therapy



Advantages

- **highly specific when** the oligonucleotide conteins **17 nucleotides or more**
- **smaller dose** levels **required compared to inhibitors**
- potentially **less side effects**

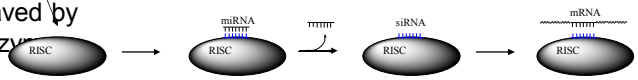
Disadvantages

- **instability and polarity of oligonucleotides** *(pharmacokinetics)*

DRUGS ACTING ON mRNA

Micro-RNA (miRNA)

- **short segments** of double **stranded RNA**
- **recognised by enzyme** complex RISC (***RNA-induced silencing complex***) **to produce** single stranded RNA - small interfering or small inhibitory RNA (**siRNA**)
- **binds to complementary region of mRNA** that is then cleaved by enzyme



DRUGS ACTING ON mRNA

Micro-RNA (miRNA)

Advantages

- **siRNAs** have **potential to be used in gene therapy**
- **greater efficiency** in silencing mRNA **than conventional antisense therapy**
- one siRNA could lead to **cleavage of several mRNAs**

Problems

- **siRNAs need to be metabolically stable**
- **need to reach and enter target cells**