Chap. 9 - DRUGS TARGETING NUCLEIC ACIDS DNA & RNA

Types of DRUGS ACTING on DNA (in chapter 9)

- Intercalating agents
- Topoisomerase poisons
- Alkylating agents
- Metallating agents
- Chain cutters
- Chain terminators
- Control of gene transcription (and epigenetics)

DRUGS ACTING ON DNA

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
- A preference is often shown for either the minor or major groove
- Intercalation prevents replication and transcription
- Intercalation can inhibit topoisomerases too
Intercalating agents

Proflavine, also called diaminoacridine, is an acriflavine derivative, a disinfectant bacteriostatic against many gram-positive bacteria. It has been used in the form of the dihydrochloride and hemisulfate salts as a topical antiseptic, and was formerly used as a urinary antiseptic. Proflavine is toxic and carcinogenic in mammals and so it is used only as a surface disinfectant or for treating superficial wounds.

Proflavine is also known to have a mutagenic effect on DNA by intercalating between nucleic acid base pairs. It differs from most other mutagenic components by causing base pair-deletions or basepair-insertions and not substitutions.

Planar tricyclic system
The amino substituents are protonated
Used as a topical antibacterial agent in WW II
Targets bacterial DNA
Too toxic for systemic use

as a salt proflavine
is more stable and avoids oxidation

Intercalating agents: Proflavine

Ionic interactions
van der Waals interactions

DNA DOUBLE HELIX
sugar phosphate backbone

also possible along DNA (any cation)
Mg$^{2+}$
Ca$^{2+}$
Na$^+$
K$^+$
R-NH$_3^+$
(others?)
Ethidium bromide is an intercalating agent commonly used as a fluorescent tag (nucleic acid stain) in molecular biology laboratories for techniques such as agarose gel electrophoresis. When exposed to ultraviolet light, it will fluoresce with an orange colour, intensifying almost 20-fold after binding to DNA. It has been commonly used since the 1950s in veterinary medicine to treat trypanosomiasis in cattle, a disease caused by trypanosomes. The high incidence of antibiotic resistance makes this treatment impractical in some areas, where the related isometamidium chloride is used instead. Ethidium bromide may be a mutagen, although this depends on the organism exposed and the circumstances of exposure. Approximately 30,000 people in sub-Saharan Africa get African trypanosomiasis each year and Chagas disease, which causes 21,000 deaths per year mainly in Latin America.

Protozoan trypanosomes spread by the Tsetse Fly, develops over years

Isometamidium is a phenanthidine aromatic amidine with a narrow therapeutic index which has been marketed for over 30 years as both a prophylactic and a therapeutic trypanocidal agent in the field. Isometamidium chloride is used curatively at lower dosage rates, and prophylactically at higher dosage rates.

Indications
Intromidium is indicated for treatment and prevention of trypanosomiasis caused by Trypanosoma spp. in cattle, goats, sheep, camels, horses and dogs. A preventive dosage ensures protection for 2 to 4 months. When clinical cases occur, the whole group should be treated.

Contra indications
Intromidium should not be administered subcutaneously. Avoid concurrent administration of other trypanocidal drugs, particularly diminazene aceturate. Avoid underdosing.
Intercalating agents - anticancer agents

Doxorubicin (also called adriamycin)
1. Intercalates via the major groove of DNA double helix.
2. Blocks the action of topoisomerase II by stabilising the DNA-enzyme complex.
3. Acts as a topoisomerase poison.

Doxorubicin is commonly used to treat some leukemias and Hodgkin's lymphoma, as well as cancers of the bladder, breast, stomach, lung, ovaries, thyroid, soft tissue sarcoma, multiple myeloma, and others. Many combination therapies are used, such as (adriamycin, cyclophosphamide).

Liposomal form
There is a pegylated (polyethylene glycol coated) liposome-encapsulated form of doxorubicin, sold as Doxil. Doxil is used primarily for the treatment of ovarian cancer or AIDS-related Kaposi's sarcoma where the disease has progressed or recurred after platinum-based chemotherapy.

Cyclophosphamide
Common side effects include low white blood cell counts, loss of appetite, vomiting, hair loss, bleeding from the bladder, an increased future risk of cancer, infertility, allergic reactions, and pulmonary fibrosis. Cyclophosphamide is in the alkylating agent and nitrogen mustard family of medications. It works by interfering with the duplication of DNA and the creation of RNA.

The biosynthesis of Doxorubicin uses enzymes found in fat metabolism. This involves using acetyl building blocks (2C) as acetyl CoA. Such molecules are called “kitids” (often multiples of 2C). There are 1000s of compounds that have been discovered from the random synthesis of clusters of proteins working together. Chemists have tried to splice in various combinations of such genes in microorganisms to make novel drugs as potential lead compounds.
Polyethylene glycol (PEG) is a polyether compound with many applications from industrial manufacturing to medicine. A comparison of uricase and PEG-uricase. PEG-uricase includes 40 polymers of 10kDa PEG. PEGylation improves its solubility at physiological pH, increases serum half-life and reduces immunogenicity without compromising activity. Upper images show the whole tetramer, lower images show one of the lysines that is PEGylated.

Dactinomycin
1. Intercalates via minor groove of DNA double helix
2. Prevents unwinding of DNA double helix
3. Blocks transcription by blocking DNA-dependent RNA polymerase

Dactinomycin is a chemotherapy used to treat many cancers, including Wilms tumor, rhabdomyosarcoma, Ewing's sarcoma, trophoblastic neoplasm, testicular cancer, and certain types of ovarian cancer. It is given by injection into a vein. **Common side effects** include bone marrow suppression, vomiting, mouth ulcers, hair loss, liver problems, infections, muscle pains, future cancers, allergic reactions, and tissue death at the site of injection. Use in pregnancy may harm the baby.

Dactinomycin, also known as actinomycin D, is the most significant member of actinomycines, which are a class of polypeptide antitumor antibiotics isolated from soil bacteria of the genus Streptomyces. It is one of the older anticancer drugs, and has been used for many years.
Many quinone methides show medicinal properties

Taxodone and its oxidized rearrangement product, taxodione, are diterpenoid quinone methides found in Taxodium distichum (bald cypress), Rosmarinus officinalis (rosemary), several Salvia species and other plants, that display anticancer, antibacterial, antioxidant, antifungal, insecticide, and antifeedant activities.

Intercalating agents and chain cutting - Bleomycins

Notes on 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
- Results in radical formation and chain cutting
- Bleomycin prevents DNA ligase from repairing damage
Bleomycin is a medication used to treat cancer. This includes Hodgkin's lymphoma, non-Hodgkin's lymphoma, testicular cancer, ovarian cancer, and cervical cancer among others. Typically used with other cancer medications, it can be given intravenously, by injection into a muscle or under the skin. It may also be administered inside the chest to help prevent the recurrence of a fluid around the lung due to cancer; however talc is better for this.

Common side effects include fever, weight loss, vomiting, rash, a severe type of anaphylaxis, possible inflammation of the lungs that can result in lung scarring. Chest X-rays every couple of weeks are recommended to check for this. Bleomycin may cause harm to the baby if used during pregnancy. It is believed to primarily work by preventing synthesis of DNA.

Free radical damage to DNA can remove bases and

Iron is believed to be the metal responsible for the creation of free radicals (often hydroxyl) because it exists at the highest concentration of any transition metal in most living organisms.

Free radicals can attack the deoxyribose DNA backbone and bases, potentially causing a number of lesions that can be cytotoxic or mutagenic. Cells have developed complex and efficient repair mechanisms to fix the lesions. In the case of free radical attack on DNA, base-excision repair is the repair mechanism used. Free radical reactions with the deoxyribose sugar backbone (C1', C2', C3', C4' or C5') are initiated by hydrogen abstraction from a deoxyribose carbon, and the predominant consequence is eventual strand breakage and base release.
Topoisomerase medications are split into two main classes: **topoisomerase poisons**, which target the topoisomerase-DNA complex, and **topoisomerase inhibitors**, which disrupt catalytic turnover.

**Topo II poisons**

Examples of topoisomerase poisons include the following:

- **Eukaryotic type II topoisomerase inhibitors** (topo II): amsacrine, etoposide (next slide), etoposide phosphate, teniposide and doxorubicin. These drugs are anti-cancer therapies.

- **Bacterial type II** topoisomerase inhibitors (gyrase and topo IV): fluoroquinolones. These are antibacterials and include such fluoroquinolones as ciprofloxacin.

Some of these poisons encourage the forward cleavage reaction (fluoroquinolones), while other poisons prevent the re-ligation of DNA (etoposide and teniposide).

**Ciprofloxacin** (later slides) targets prokaryotes topo II a thousand times better than it targets eukaryotic topo IIs.

**Topo II inhibitors**

These inhibitors target the N-terminal ATPase domain of topo II and prevent topo II from turning over. Very few of these inhibitors.

An example of topoisomerase inhibitors includes: **ICRF-193**. The structure of this compound bound to the ATPase domain is known. The drug binds in a non-competitive manner and locks down the dimerization of the ATPase domain.

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**Topoisomerase poisons - non-intercalating**

Stabilizes the complex between DNA and topoisomerase enzymes
Used as anticancer agents
Also causes chain cutting

**Etoposide**

Etoposide is used as a form of chemotherapy for cancers such as Kaposi’s sarcoma, Ewing’s sarcoma, lung cancer, testicular cancer, lymphoma, nonlymphocytic leukemia, and glioblastoma multiforme. It is often given in combination with other drugs (such as bleomycin). It is also sometimes used in a conditioning regimen prior to a bone marrow or blood stem cell transplant.

It is given intravenously (IV) or orally in capsule form. If the drug is given IV, it must be done slowly over a 30- to 60-minute period because it can lower blood pressure as it is being administered. Blood pressure is checked often during infusing, with the speed of administration adjusted accordingly.

Etoposide forms a ternary complex (3 way) with DNA and the topoisomerase II enzyme (which aids in DNA unwinding), prevents re-ligation of the DNA strands, and by doing so causes DNA strands to break. Cancer cells rely on this enzyme more than healthy cells, since they divide more rapidly. This causes errors in DNA synthesis and promotes apoptosis of the cancer cell.
Teniposide is a chemotherapeutic medication used in the treatment of childhood acute lymphocytic leukemia, Hodgkin's lymphoma, certain brain tumours, and other types of cancer. It slows the growth of cancer cells in the body (by a ternary complex).

When Teniposide is used with other chemotherapeutic agents, it results in severe bone marrow suppression. Other common side effects include gastrointestinal toxicity, hypersensitivity reactions, and reversible alopecia (hair loss).

Teniposide is a semisynthetic derivative of podophyllotoxin from the rhizome of the wild mandrake (Podophyllum peltatum). More specifically, it is a glycoside of podophyllotoxin with a D-glucose derivative. It is chemically similar to the anti-cancer drug etoposide, being distinguished only by a thienyl where etoposide has a methyl. Both these compounds have been developed with the aim of creating less toxic derivatives of podophyllotoxin.

Camptothecin is a cytotoxic quinoline alkaloid which inhibits the DNA enzyme topoisomerase I (topo I). It was isolated from the bark and stem of Camptotheca acuminata (Happy tree), a tree native to China and used as a cancer treatment in Traditional Chinese Medicine. It showed remarkable anticancer activity in preliminary clinical trials but also low solubility and (high) adverse drug reaction. Because of these disadvantages synthetic and medicinal chemists have developed numerous syntheses of Camptothecin and various derivatives to increase the benefits of the chemical, with good results. Two analogues have been approved and are used in cancer chemotherapy today, topotecan and irinotecan.
Camptothecin binds to the topo I enzyme and DNA complex by hydrogen bonds resulting in a ternary complex, and thereby stabilizing it. This prevents DNA re-ligation and causes DNA damage which results in apoptosis. The most important part of the structure is the E-ring which interacts from three different positions with the enzyme. The hydroxyl group in position 20 forms hydrogen bond to the side chain on aspartic acid number 533 in the enzyme. It is critical that the configuration of the chiral carbon is (S) because (R) is inactive. The lactone is bonded with two hydrogen bonds to the amino groups on arginine 364. Toxicity of CPT is primarily a result of conversion of single-strand breaks into double-strand breaks during the S-phase when the replication fork collides with the cleavage complexes formed by DNA and CPT. The lactone ring in CPT is highly susceptible to hydrolysis. The open ring form is inactive and it must therefore be closed to inhibit topo I. The closed form is favored in acidic condition, as it is in many cancer cells microenvironment. CPT is transported into the cell by passive diffusion.

Topotecan (trade name Hycamtin) is a chemotherapeutic agent that is a topoisomerase I inhibitor. It is a synthetic, water-soluble analog of the natural chemical compound camptothecin. It is used in the form of its hydrochloride salt to treat ovarian cancer, lung cancer and other cancer types. Topotecan was the first topoisomerase I inhibitor for oral use. Why is it more water soluble? Loperamide often taken with topotecan due to severe diarrhea side effect. SN-38 is an antineoplastic drug. It is the active metabolite of irinotecan (an analog of camptothecin - a topoisomerase I inhibitor) but has 1000 times more activity than irinotecan itself. SN-38 and its glucuronide are lost into the bile and feces. It can cause the symptoms of diarrhoea and myelosuppression experienced by ~25% of the patients administered irinotecan.

Loperamide, sold under the brand name Imodium among others, is a medication used to decrease the frequency of diarrhea. It is often used for this purpose in gastroenteritis, inflammatory bowel disease, and short bowel syndrome. It is not recommended for those with blood in the stool. The medication is taken by mouth.
Model of human liver cell showing blood, bile and intestinal compartments, indicating tissue involvement of genes in the irinotecan pathway – eventually leads to inhibition of DNA replication and transcription.

Irinotecan-associated diarrhea sometimes leads to such severe dehydration that it requires hospitalization. This side-effect is treated with an antidiarrheal such as loperamide.

The immune system is adversely impacted by irinotecan, reflected by dramatically lowered neutrophils (white blood cells). The patient may experience a period of neutropenia while the bone marrow increases white cell production to compensate.

Neutrophils make up the majority of circulating white blood cells and serve as the primary defense against infections by destroying bacteria, bacterial fragments and immunoglobulin-bound viruses in the blood. Patients with neutropenia are more susceptible to bacterial infections and, without prompt medical attention, the condition may become life-threatening (neutropenic sepsis). Neutropenia can be acute (temporary) or chronic (long lasting).

The partition-coefficient (P) or distribution-coefficient (D) is the ratio of concentrations of a compound in a mixture of two immiscible phases at equilibrium. This ratio is a measure of the difference in solubility of the compound in these two phases. The partition-coefficient generally refers to the concentration ratio of un-ionized species of compound whereas the distribution-coefficient refers to the concentration ratio of all species of the compound (ionized plus un-ionized) and depends on pH. Usually one phase is water and the other is 1-octanol. The partition coefficient measures how hydrophilic or hydrophobic a chemical substance is. Hydrophobic drugs with high octanol/water partition coefficients are mainly distributed to hydrophobic areas such as lipid bilayers of cells, while low octanol/water partition coefficients are found primarily in aqueous regions such as blood serum.

solubility and partition coefficients of straight chain functional groups in 100 g water

<table>
<thead>
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<th>Solubility</th>
<th>log P_{oct/water}</th>
<th>log P_{oct/100}</th>
<th>log D_{oct/water}</th>
<th>log D_{oct/100}</th>
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<tr>
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</tr>
<tr>
<td>H</td>
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<td>-0.54</td>
<td>+0.33</td>
<td>+0.70</td>
</tr>
<tr>
<td>H</td>
<td>100% miscible</td>
<td>+0.45</td>
<td>+0.59</td>
<td>-0.24</td>
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<tr>
<td>C_{15}OH</td>
<td>100% miscible</td>
<td>+0.79</td>
<td>+0.88</td>
<td>+0.29</td>
</tr>
<tr>
<td>C_{15}OH</td>
<td>100% miscible</td>
<td>+1.31</td>
<td>-0.24</td>
<td>-0.84</td>
</tr>
</tbody>
</table>

There is good correlation of calculated partition coefficients with experimental partition coefficients.

\[
\log P_{\text{oct/H}_2\text{O}} = \log \frac{[\text{compound}]_{\text{octanol}}}{[\text{compound}]_{\text{water}}}
\]

What might a distribution coefficient curve look like for the following compound?

Topoisomerase poisons - non-intercalating

Examples - Quinolones and fluoroquinolones

- Synthetic agents used as antibacterial agents
- Stabilise complex between bacterial DNA and topoisomerases
- Binding site for agents revealed once DNA strands are ‘nicked’

The quinolones are a family of synthetic broad-spectrum antibiotic drugs. They can be isolated from natural sources (such as plants, animals and bacteria) and act as natural antimicrobials.

Quinolones exert their antibacterial effect by preventing bacterial DNA from unwinding and duplicating. The majority of quinolones in clinical use are fluoroquinolones, which have a fluorine atom attached to the central ring system, typically at the 6-position or C-7 position.

Fluoroquinolones are especially important for hospital-acquired infections in which resistance to older antibacterial classes is suspected. Treatment guidelines recommend minimizing the use of fluoroquinolones and other broad-spectrum antibiotics in less severe infections and in those in which risk factors for multidrug resistance are not present. Side effects are severe so quinolones are contraindicated unless no other safe alternative antibiotic exists.
Tertiary Structure

1. Double helix coils into a 3D shape – supercoiling
2. Double helix has to unravel during replication
3. Unravelling leads to strain
4. Relieved by enzyme-catalyzed cutting and repair of DNA chain
5. Quinolone and fluoroquinolone antibacterial agents inhibit this enzyme

Nalidixic acid (1962) was the first of the quinolone family of antibiotics. The quinolones are a family of synthetic broad-spectrum antibiotic drugs. Quinolones, and derivatives, have also been isolated from natural sources (such as plants, animals and bacteria) and can act as natural antimicrobials and/or signalling molecules. Quinolones prevent bacterial DNA from unwinding and duplicating.

Ciprofloxacin (1980) is used to treat a number of bacterial infections including bone and joint infections, intra abdominal infections, certain type of infectious diarrhea, respiratory tract infections, skin infections, typhoid fever, and urinary tract infections, among others. A single F at position 6 greatly increased both activity and cellular uptake. The piperizine ring (ionized) at position 7 improved pharmacokinetics. Replacement of the N8 nitrogen reduced adverse reactions and increased activity against S. aureus.

Topoisomerase poisons - non-intercalating

Examples - Quinolones and fluoroquinoxilones

- Drug molecules are stacked in the bound complex
- Bound to DNA and enzyme by hydrogen and ionic bonds
Topoisomerases I form a covalent intermediate in which the active site tyrosine becomes attached to the 3’ phosphate end of the cleaved strand rather than the 5’ phosphate end.

The TOP1 protein of humans has been subdivided into four regions. The N-terminal 214 amino acids are dispensable for relaxation of supercoiling activity in vitro. The N-terminal domain is followed by a highly conserved, 421 amino acid core domain containing all of the catalytic residues except the active site tyrosine. This is followed by a poorly conserved linker domain of 77 amino acids. Finally there is a 53 amino acid C-terminal domain. The active site Tyr723 is found within the C-terminal domain.

After the TOP1 covalently attaches to the 3’ end of the broken strand, supercoiling of the DNA is relaxed by controlled rotation of DNA about the intact strand. Then the 5’ hydroxyl end of the broken DNA strand can reverse the phosphotyrosyl bond, enabling the release of TOP1 and religation of the DNA. The nicking and closing reactions are fast, and about 100 cycles can occur per second.

One of the first inhibitors shown to target TOP1 is irinotecan. Irinotecan is an analogue of the cytotoxic natural alkaloid camptothecin, obtained from the Chinese tree Camptotheca acuminata. Irinotecan is especially effective through its metabolic product SN-38. Irinotecan and SN-38 act by trapping a subset of TOP1-DNA cleavage complexes, those with a guanine +1 in the DNA sequence. One irinotecan or SN-38 molecule stacks against the base pairs flanking the topoisomerase-induced cleavage site and poisons (inactivates) the TOP1 enzyme.

DRUGS ACTING ON DNA  Alkylating agents

Nucleophilic atoms on nucleic acid bases

https://en.wikipedia.org/wiki/Alkylating_antineoplastic_agent
Alkylation agents

- Contain highly electrophilic groups
- Form covalent bonds to nucleophilic groups in DNA (e.g. 7-N of guanine)
- Prevent replication and transcription
- Useful anticancer agents
- Toxic side effects (e.g. alkylation of proteins)
- Can cause interstrand and intrastrand crosslinking if two electrophilic groups present
- Alkylation of nucleic acid bases can result in miscoding

Crosslinking is possible for doubly electrophilic compounds

Intrastrand crosslinking

Interstrand crosslinking

Normal base pairing

Cytosine

Guanine

Guanine prefers keto tautomer

Miscoding resulting from alkylated nucleic acid bases

Thymine

Alkylated guanine

Abnormal base pairing. Alkylated guanine prefers enol tautomer

How many possible tautomers are there for DNA bases?

Cytosine

Thymine

Adenine

Guanine

Normally, has 2 H-bonds with adenine

Changes charge and changes shape.
Alkylating agents

Chlormethine (nitrogen mustard)

\[
\text{MeN} \quad \text{Cl} \quad \text{Cl}
\]

Bendamustine is a nitrogen mustard used in the treatment of chronic lymphocytic leukemia and lymphomas. It belongs to the family of drugs called alkylating agents. It is also being studied for the treatment of sarcoma. It is also being investigated for the non-cancer diseases.

- Secretly used medicinally in 1942 (WW II), reduced white blood count, many side effects, including increased cancer risk later
- Causes intrastrand and interstrand cross-linking
- Prevents replication
- Mono-alkylation of guanine also possible
- Analogues with better properties have been prepared

Chlormethine is also used to synthesize pethidine. How might this be done? In 1975 pethidine was opioid of choice of 60% of doctors for acute pain and 22% for chronic severe pain.

Alkylating agents

Chlormethine

Mechanism of action

How can DNA heal itself? Are there other possible leaving groups? How do they compare to chloride? Will they survive a journey through the body?

Mechlorethamine

Aziridine ion

DNA

G = Guanine

Crosslinked DNA
Alkylating agents
Nitrosoureas

Mechanism
Decompose in body to form an alkylating agent and a carbamoylating agent

Notes
• Alkylating agent causes interstrand crosslinking
• Crosslinking 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 (dimesylate). Causes interstrand crosslinking

Mechanism
Alkylating agents

Dacarbazine

- Prodrug activated by demethylation in liver
- Decomposes to form a methyldiazonium ion
- Alkylates guanine groups (methylates here)

Mechanism

- Cyt P-450 oxidation with cytochrom P-450
- Diazomethane (very toxic and explosive gas), usually made as basic ether solution using Diazald.
- Diazomethane

DRUGS ACTING ON DNA - Alkylating agents

Example - Mitomycin C

- Prodrug activated in the body to form an alkylating agent
- One of the most toxic anticancer drugs in clinical use

Mitomycin C is a mitomycin that is used as a chemotherapeutic agent by virtue of its antitumour activity. It is given intravenously to treat upper gastro-intestinal cancers (e.g. esophageal carcinoma), anal cancers, and breast cancers, as well as by bladder instillation for superficial bladder tumours. It causes delayed bone marrow toxicity and therefore it is usually administered at 6-weekly intervals. Prolonged use may result in permanent bone-marrow damage. It may also cause lung fibrosis and renal damage.
Coenzyme Q10, also known as ubiquinone, is a coenzyme that is ubiquitous in the bodies of most animals. It is a 1,4-benzoquinone, where Q refers to the quinone chemical group and 10 refers to the number of isoprenyl chemical subunits in its tail. This fat-soluble substance, which resembles a vitamin, is present in most eukaryotic cells, primarily in the mitochondria. It is a component of the electron transport chain and participates in aerobic cellular respiration, which generates energy in the form of ATP. Ninety-five percent of the human body's energy is generated this way. There are three redox states of CoQ10: fully oxidized (ubiquinone), semiquinone, and fully reduced (ubiquinol). The capacity of this molecule to act as a two-electron carrier (moving between the quinone and quinol form) and a one-electron carrier (moving between the semiquinone and one of these other forms) is central to its role in the electron transport chain due to the iron–sulfur clusters that can only accept one electron at a time, and as a free radical-scavenging antioxidant.
In the bacterium Legionella pneumophila, mitomycin C induces competence for transformation. Natural transformation is a process of DNA transfer between cells, and is regarded as a form of bacterial sexual interaction. In microbiology, genetics, cell biology, and molecular biology, competence is the ability of a cell to take up extracellular ("naked") DNA from its environment in the process called transformation. Competence may be differentiated between natural competence, a genetically specified ability of bacteria which is thought to occur under natural conditions as well as in the laboratory, and induced or artificial competence, which arises when cells in laboratory cultures are treated to make them transiently permeable to DNA. In the natural world DNA usually becomes available by death and lysis of other cells, but in the laboratory it is provided by the researcher, often as a genetically engineered fragment or plasmid. During uptake, DNA is transported across the cell membrane(s), and the cell wall if one is present. Once the DNA is inside the cell it may be degraded to nucleotides, which are reused for DNA replication and other metabolic functions. Alternatively it may be recombined into the cell’s genome by its DNA repair enzymes. If this recombination changes the cell’s genotype the cell is said to have been transformed.

**DRUGS ACTING ON DNA**

**Metallating agents**

- Neutral inactive molecule acting as a prodrug
- Platinum covalently linked to chloro substituents
- Ammonia molecules act as ligands (RNH₂)
- Activated in cells with low chloride ion concentration
- Chloro substituents replaced with neutral water ligands
- Produces positively charged species
- Binds to DNA in regions rich in guanine units
- Intrastrand links rather than interstrand
- Localised unwinding of DNA double helix
- Inhibits transcription

**Cisplatin**, cisplatinum, platamin, neoplatin, cismaplat or cis-diamminedichloridoplatinum(II) (CDDP) is a chemotherapy drug. It was the first member of a class of platinum-containing anti-cancer drugs, which now also includes carboplatin and oxaliplatin. These platinum complexes react in the body, binding to DNA and causing the DNA strands to crosslink, which ultimately triggers cells to die in a programmed way.

**Carboplatin**, is a chemotherapy drug used against some forms of cancer (mainly ovarian carcinoma, lung, head and neck cancers as well as endometrial, esophageal, bladder, breast and cervical; central nervous system or germ cell tumors; osteogenic sarcoma, and as preparation for a stem cell or bone marrow transplant). It has vastly reduced side effects compared to cisplatin. Cisplatin and carboplatin belong to the group of platinum-based antineoplastic agents, and interact with DNA to interfere with DNA repair.
Bleomycin is mostly used to treat cancer. This includes Hodgkin's disease, non-Hodgkin's disease, testicular cancer, ovarian cancer, and cervical cancer. It can be given intravenously, by injection into a muscle or under the skin. It may also be administered inside the chest to help prevent the recurrence of a fluid around the lung due to cancer.

- Intercalating agent (shown earlier)
- Abstracts H from DNA to generate radicals using oxidized iron complex
- Radicals react with oxygen resulting in chain cutting
- Bleomycin also inhibits repair enzymes

The calicheamicins are a class of enediyne antitumor antibiotics derived from the bacterium Micromonospora echinospora, with calicheamicin g1 being the most notable. It was isolated originally in the mid-1980s from the chalky soil, or “calichi pits”, located in Kerrville, Texas. It is extremely toxic to all cells. N-acetyl dimethyl hydrazide calicheamicin was developed and marketed as targeted therapy against the non-solid tumor cancer acute myeloid leukemia (AML). Calicheamicin γ1 and the related enediyne esperamicin are the two of the most potent antitumor agents known.

Bergman reaction (1972)
Chain cutters

Mechanism

Cycloaromatization

Michael addition

Oxidative cleavage
Neocarzinostatin is a macromolecular chromoprotein enediyne antitumor antibiotic secreted by Streptomyces macromomyceticus. It consists of two parts, a labile chromophore (shown) and a 113 amino acid protein to which the chromophore is tightly and non-covalently bound with high affinity ($K_d \approx 10^{-10} M$). The non-protein component is a very potent DNA-damaging agent; However it is extremely unstable and the role of the protein is to protect it and release it to the target DNA. Opening of the epoxide under reductive conditions present in cells creates favorable conditions for a Bergman cyclization, that leads to formation of benzyne, followed by DNA strand cleavage. As a medicine it is among the most potent known, and in Japan it is the only analog used clinically (against liver cancer).

Neocarzinostatin likely evolved to kill bacteria that compete with the producing organism. Because it achieves this by causing DNA damage, however, it is capable of harming tumor cells, as well and was developed for its anticancer properties.

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 added to growing DNA chain and acts as chain terminator

Azidothymidine (AZT) reduces the replication of the virus and leads to improvements in both symptoms and blood tests. It can also be used to prevent HIV transmission, such as from mother to child during the period of birth or after a needle stick injury. Used by itself in HIV-infected patients, AZT slows HIV replication in patients, but does not stop it entirely. HIV may become AZT-resistant over time, and therefore AZT is now usually used in conjunction with other anti-HIV drugs in the combination therapy called highly active antiretroviral therapy (HAART = highly active antiretroviral therapy).
Azides are very reactive and lose N₂ easily (best leaving group in chemistry)

**Chain terminators**

**Acyclovir (Zovirax)**

**Famciclovir (Famvir)**

Acyclovir, is an antiviral medication. It is primarily used for the treatment of herpes simplex virus infections, chickenpox, and shingles.

Famciclovir is a guanosine analogue antiviral drug used for the treatment of various herpesvirus infections, most commonly for herpes zoster (shingles).

**Notes:**
- Prodrugs used as antiviral agents
- Same mechanisms of action as AZT
- Used vs herpes simplex and shingles
Chain terminators

a) Normal replication

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DNA template
Growing chain
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b) Chain termination

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DNA template
Growing chain
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Mechanism of reverse transcription in class VI virus ssRNA-RT, human immunodeficiency virus (HIV). Colors mark complementary sequences (not drawn to scale). Reverse transcription occurs in the cytoplasm of host cell. In this process, viral ssRNA is transcribed by the viral reverse transcriptase protein (RT) into double stranded DNA. Reverse transcription takes place in 5' → 3' direction. tRNA ("cloverleaf") hybridizes to PBS and provides -OH group for initiation of reverse transcription. Key: (U3 - promoter region), (U5 - recognition site for viral integrase); (PBS - primer binding site); (PP - polypurine section).

1) Strong stop complementary DNA (cDNA) is formed.
2) Template in RNA:DNA hybrid is degraded by RNase H domain of reverse transcriptase
3) DNA:tRNA is transferred to the 3'-end of the template (synthesis "jumps").
4) First strand synthesis takes place.
5) The rest of viral ssRNA is degraded by RNase H, except for PP site.
6) Synthesis of second strand of ssDNA is initiated from the 3'-end of the template. tRNA is necessary to synthesis of complementary PBS
7) tRNA is degraded
8) After another "jump", PBS from the second strand hybridizes with the complementary PBS on the first strand.
9) Synthesis of both strands is completed by the DNAP function of reverse transcriptase.

Both dsDNA ends have U3-R-U5 sequences, so called long terminal repeat sequences (3'LTR and 5'LTR, respectively). LTRs mediate integration of the retroviral DNA into another region of the host genome. Very error prone because no proof reading.
DRUGS ACTING ON DNA | Control of gene transcription

Notes:
- Design of synthetic molecules capable of controlling gene transcription
- Molecules capable of recognizing and binding to specific base pairs
- Hairpin polyamides containing heterocyclic rings are capable of binding to the minor groove
- Binding 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 rRNA | Antibiotics – attack bacteria

Chloramphenicol (vs typhoid)

Rifamycins

Chlortetracycline (Aureomycin)

Streptomycin

Erythromycin

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Chloramphenicol is an antibiotic useful for the treatment of a number of bacterial infections. This includes as an eye ointment to treat conjunctivitis. By mouth or by injection into a vein it is used to treat meningitis, plague, cholera, and typhoid fever (largely drug resistant now). Monitoring of blood levels of the medication and blood cell levels every two days is recommended during treatment. Common side effects include bone marrow suppression, nausea, and diarrhea. The bone marrow suppression may result in death. To reduce the risk of side effects treatment duration should be as short as possible. Chloramphenicol is extremely lipid-soluble; it remains relatively unbound to protein and is a small molecule. It penetrates effectively into all tissues of the body, including the brain. Chloramphenicol increases the absorption of iron.

The rifamycins are a group of antibiotics that are synthesized either naturally by the bacterium Amycolatopsis rifamycinica or artificially. They are a subclass of the larger family of ansamycins. Rifamycins are particularly effective against mycobacteria, and are therefore used to treat tuberculosis, leprosy, and mycobacterium avium complex (MAC) infections.

Isoniazid is a prodrug and must be activated by a bacterial catalase-peroxidase enzyme in Mycobacterium tuberculosis called KatG, which catalyzes the formation of the isonicotinic acyl radical, which spontaneously couples with NADH to form the nicotinoyl-NAD adduct. This complex binds tightly to the enoyl-acyl carrier protein reductase known as InhA, thereby blocking the natural enoyl-AcpM substrate and the action of fatty acid synthase. This process inhibits the synthesis of mycolic acids, which are required components of the mycobacterial cell wall. A range of radicals are produced by KatG activation of isoniazid, including nitric oxide.

Rifamycins have been used for the treatment of many diseases, the most important one being HIV-related tuberculosis.
Streptomycin is an antibiotic used to treat a number of bacterial infections. This includes tuberculosis, Mycobacterium avium complex, endocarditis, brucellosis, Burkholderia infection, plague, tularemia, and rat bite fever. For active tuberculosis it is often given together with isoniazid, rifampicin, and pyrazinamide.

Common side effects include feeling like the world is spinning, vomiting, numbness of the face, fever, and rash.

Streptomycin is a protein synthesis inhibitor. It binds to the small 16S rRNA of the 30S subunit of the bacterial ribosome, interfering with the binding of formyl-methionyl-tRNA to the 30S subunit. This leads to codon misreading, eventual inhibition of protein synthesis and ultimately death of microbial cells through mechanisms that are still not understood. Speculation on this mechanism indicates that the binding of the molecule to the 30S subunit interferes with 50S subunit association with the mRNA strand. This results in an unstable ribosomal-mRNA complex, leading to a frameshift mutation and defective protein synthesis; leading to cell death. Humans have ribosomes which are structurally different from those in bacteria, so the drug does not have this effect in human cells. Streptomycin is an antibiotic that inhibits both Gram-positive and Gram-negative bacteria, and is therefore a useful broad-spectrum antibiotic.

Chlortetracycline (trade name Aureomycin, Lederle) is a tetracycline antibiotic, the first tetracycline to be identified. It was discovered in 1945. Duggar identified the antibiotic as the product of an actinomycete. In veterinary medicine, chlortetracycline is commonly used to treat conjunctivitis in cats and other animals. Chlortetracycline may increase the anticoagulant activities. The risk or severity of adverse effects can be increased when chlortetracycline is combined with acitretin, adapalene, or alitretinoin.
**Erythromycin** is an antibiotic useful for the treatment of a number of bacterial infections, including respiratory tract infections, skin infections, chlamydia infections, pelvic inflammatory disease, and syphilis. It may also be used during pregnancy to prevent Group B streptococcal infection in the newborn. An **eye ointment is routinely recommended after delivery** to prevent eye infections in the newborn. **Common side effects** include abdominal cramps, vomiting, and diarrhea. More serious side effects may include Clostridium difficile colitis, liver problems, prolonged QT, and allergic reactions. **It is generally safe in those who are allergic to penicillin**.

Erythromycin displays **bacteriostatic** activity or inhibits growth of bacteria. By binding to the 50s subunit of the bacterial rRNA complex, Erythromycin **interferes with aminoacyl translocation**, preventing the transfer of the tRNA bound at the A site of the rRNA complex to the P site of the rRNA complex. Without this translocation, the A site remains occupied, thus the addition of an incoming tRNA and its attached amino acid to the nascent polypeptide chain is inhibited. This interferes with the production of functionally useful proteins, which is the basis of this antimicrobial action.

**Antisense Therapy** is a form of treatment for genetic disorders or infections. When the genetic sequence of a particular gene is known to be causative of a particular disease, it is possible to synthesize a strand of nucleic acid (DNA, RNA or a chemical analogue) that will bind to the messenger RNA (mRNA) produced by that gene and inactivate it, effectively turning that gene "off". This is because mRNA has to be single stranded for it to be translated. Alternatively, the strand might be targeted to bind a splicing site on pre-mRNA and modify the exon content of an mRNA. This synthesized nucleic acid is termed an "anti-sense" oligonucleotide (ASO) because its base sequence is complementary to the gene's messenger RNA (mRNA), which is called the "sense" sequence (so that a sense segment of mRNA " 5'-AAGGUC-3'
" would be blocked by the anti-sense mRNA segment " 3'-UUCCAG-5'").
Advantages

• Same effect as an enzyme inhibitor or receptor antagonist
• Highly specific where the oligonucleotide is 17 nucleotides or more
• Smaller dose levels required compared to inhibitors or antagonists
• Potentially less side effects

Disadvantages

• ‘Exposed’ sections of mRNA must be targeted
• Instability and polarity of oligonucleotides (pharmacokinetics)
• Short lifetime of oligonucleotides and poor absorption across cell membranes

DRUGS ACTING ON mRNA

Antisense Therapy

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Micro-RNA (miRNA)

• Short segments of double stranded RNA
• Recognised by enzyme complex RISC to produce single stranded RNA – small interfering or small inhibitory RNA (siRNA)
• Binds to complementary region of mRNA
• mRNA is cleaved by enzyme complex

The RNA-induced silencing complex, or RISC, is a multiprotein complex, specifically a ribonucleoprotein, which incorporates one strand of a single-stranded RNA (ssRNA) fragment, such as microRNA (miRNA), or double stranded small interfering RNA (siRNA). The single strand acts as a template for RISC to recognize complementary messenger RNA (mRNA) transcript. Once found, one of the proteins in RISC, called Argonaute, activates and cleaves the mRNA. This process is called RNA interference (RNAi) and it is found in many eukaryotes; it is a key process in gene silencing and defense against viral infections.
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 mRNA molecules

Problems
- siRNAs need to be metabolically stable
- Need to reach target cells
- Need to enter target cells

Epigenetics – a new frontier: DNA methylation, methylcytosine, histone acylation
Five major families of histones exist: H1/H5, H2A, H2B, H3 and H4. Histones H2A, H2B, H3 and H4 are known as the core histones, while Histones H1 and H5 are known as the linker histones.

Histones make five types of interactions with DNA:

1. Helix-dipoles form alpha-helixes in H2B, H3, and H4 cause a net positive charge to accumulate at the point of interaction with negatively charged phosphate groups on DNA
2. Hydrogen bonds between the DNA backbone and the amide group on the main chain of histone proteins
3. Nonpolar interactions between the histone and deoxyribose sugars on DNA
4. Salt bridges and hydrogen bonds between side chains of basic amino acids (especially lysine and arginine) and phosphate oxygens on DNA
5. Non-specific minor groove insertions of the H3 and H2B N-terminal tails into two minor grooves each on the DNA molecule

Histones are highly basic facilitating DNA-histone interactions and contribute to their water solubility.

Histones are subject to post translational modification by enzymes primarily on their N-terminal tails, but also in their globular domains. Such modifications include methylation, citrullination, acetylation, phosphorylation, SUMOylation, ubiquitination, and ADP-ribosylation. This affects their function of gene regulation.

In general, genes that are active have less bound histone, while inactive genes are highly associated with histones during interphase. Histones are evolutionarily conserved, and any deleterious mutations appear to be severely maladaptive. All histones have a highly positively charged N-terminus with many lysine and arginine residues.

Methylations in biochemistry
Citrullination or deimination is the conversion of the amino acid arginine in a protein into the amino acid citrulline. Enzymes called peptidylarginine deiminases (PADs) replace the primary ketimine group (=NH) by a ketone group (=O).

Citrullination controls the expression of genes, particularly in the developing embryo. The immune system often attacks citrullinated proteins, leading to autoimmune diseases such as rheumatoid arthritis and multiple sclerosis.

Citrulline is not one of the 20 standard amino acids encoded by DNA in the genetic code. Instead, it is the result of a post-translational modification.

Citrullination is distinct from the formation of the free amino acid citrulline as part of the urea cycle.

Arginine is positively charged at a neutral pH, whereas citrulline is uncharged. This increases the hydrophobicity of the protein, leading to changes in protein folding. Therefore, citrullination can change the structure and function of proteins.

Proteins that normally contain citrulline residues include myelin basic protein (MBP), filaggrin, and several histone proteins, while other proteins, like fibrin and vimentin, can get citrullinated during cell-death and tissue inflammation.

Fibrin and fibrinogen may be favored sites for arginine deimination within rheumatoid joints. Test for presence of anti-citrullinated protein (ACP) antibodies are highly specific (88-96%) for rheumatoid arthritis (RA), about as sensitive as rheumatoid factor (70-78%) for diagnosis of RA, and are detectable from even before the onset of clinical disease.

Acylations in biochemistry

Histone proteins are acetylated and deacetylated on lysine residues in the N-terminal tail as part of gene regulation. The regulation of transcription factors, effector proteins, molecular chaperones, and cytoskeletal proteins by acetylation and deacetylation is a significant post-translational regulatory mechanism. These regulatory mechanisms are analogous to phosphorylation and dephosphorylation by the action of kinases and phosphatases. Not only can the acetylation state of a protein modify its activity but there has been recent suggestion that this post-translational modification may also crosstalk with phosphorylation, methylation, ubiquitination, sumoylation, and others for dynamic control of cellular signaling.
Small Ubiquitin-like Modifier (or SUMO) proteins are a family of small proteins that are covalently attached to and detached from other proteins in cells to modify their function. SUMOylation is a post-translational modification involved in various cellular processes, such as nuclear-cytosolic transport, transcriptional regulation, apoptosis, protein stability, response to stress, and progression through the cell cycle. SUMO proteins are similar to ubiquitin, and SUMOylation is directed by an enzymatic cascade analogous to that involved in ubiquitination. In contrast to ubiquitin, SUMO is not used to tag proteins for degradation. Mature SUMO is produced when the last four amino acids of the C-terminus have been cleaved off to allow formation of an isopeptide bond between the C-terminal glycine residue of SUMO and an acceptor lysine on the target protein.

Ubiquitin is a small (8.5 kDa) regulatory protein that has been found in almost all tissues (ubiquitously) of eukaryotic organisms. There are four genes in the human genome that produce ubiquitin: UBB, UBC, UBA52 and RPS27A. Ubiquitination can affect proteins in many ways; it can signal for their degradation via the proteasome, alter their cellular location, affect their activity, and promote or prevent protein interactions. Ubiquitination is carried out in three main steps: activation, conjugation, and ligation, performed by ubiquitin.

ADP-ribosylation is the addition of one or more ADP-ribose moieties to a protein. It is a reversible post-translational modification that is involved in many cellular processes, including cell signaling, DNA repair, gene regulation and apoptosis. Improper ADP-ribosylation has been implicated in some forms of cancer. It is also the basis for the toxicity of bacterial compounds such as cholera toxin, diphtheria toxin, and others.