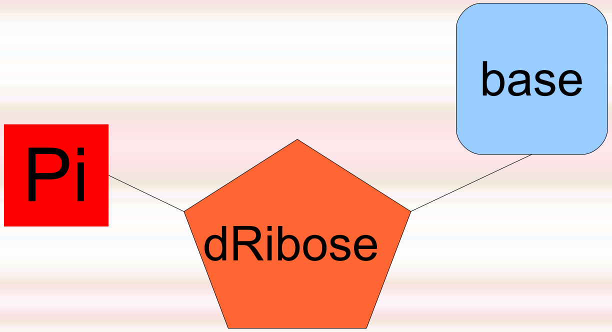
Biomolecules: nucleotides

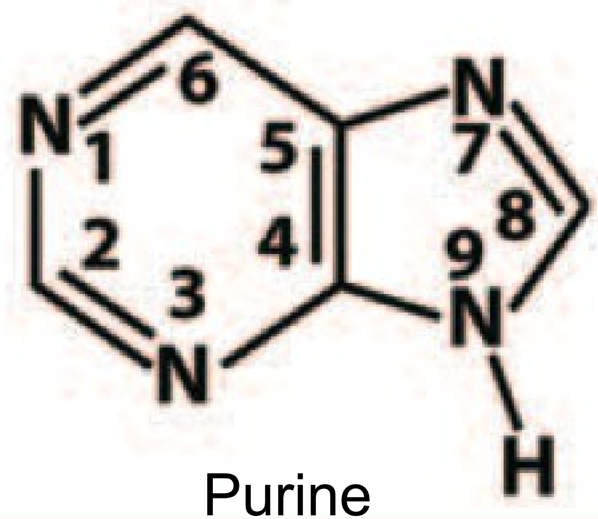
Nucleotides and nucleic acids

- Nucleic acids are linear hetero-polymers adapted to maintain and transmit the genetic information
- Depending on their sugar moiety they can be classified into: ribonucleic acids (RNA) and deoxy-ribonucleic acids (DNA)
- The building blocks of nucleic acids are complex molecules called nucleotides



Nucleotides

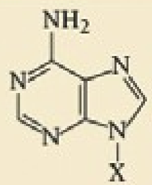
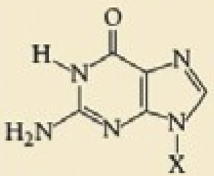
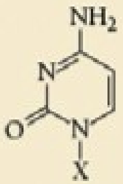
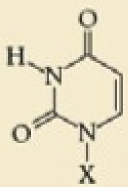
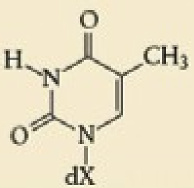
- Each nucleotide is composed by three different chemical entities:
  - 1 to 3 molecules of phosphoric acid
  - 1 cyclic (deoxy)ribose molecule
  - 1 nitrogenous aromatic base (aka nucleobase), chemically derived from (but not synthesized from) either purine or pyrimidine



Purine

Pyrimidine

The structure and nomenclature of the nucleobases:

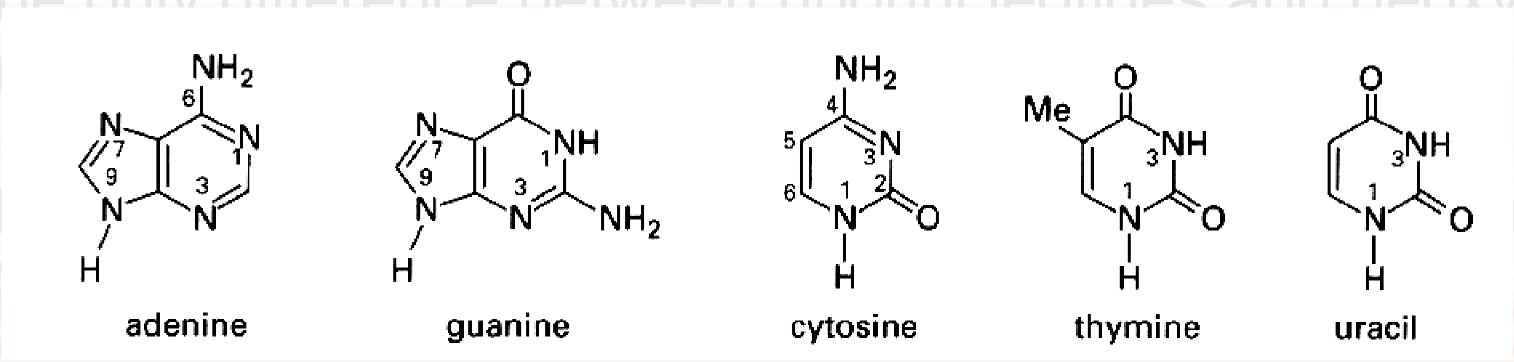
Base Formula	Base (X = H)	Nucleoside (X = ribose[a])	Nucleotide[b] (X = ribose phosphate[a])	
	Adenine Ade A	Adenosine Ado A	Adenylic acid Adenosine monophosphate AMP	
	Guanine Gua G	Guanosine Guo G	Guanylic acid Guanosine monophosphate GMP	
	Cytosine Cyt C	Cytidine Cyd C	Cytidylic acid Cytidine monophosphate CMP	
	Uracil Ura U	Uridine Urd U	Uridylic acid Uridine monophosphate UMP	Only in RNA
	Thymine Thy T	Deoxythymidine dThd dT	Deoxythymidylic acid Deoxythymidine monophosphate dTMP	Only in DNA

Nucleotides

- The reactions leading to the formation of nucleotides belong to **condensations**:
  - One of the 3 acid groups of phosphoric acid reacts with the alcoholic group in position C5' of (deoxy)ribose → phosphoester bond (sometimes also in C3')
  - The alcoholic group in beta-C1' of (deoxy)ribose reacts with the NH group either in position 9 (Purines) or 1 (Pyrimidines) → N-glycosidic bond
- In the absence of a phosphate group, the ribose-base compound is called **nucleoside**
- At physiological pH, the phosphate groups are ionised, this explains the acidic behaviour of nucleotides (mono-, di-, tri-phosphate) and their preference to form salts with Mg^{2+}

Nucleotides

- In order to avoid confusion in the numbering:
  - all the C and N belonging to the base are numbered follow-ing the purine/pyrimidine convention
  - All the C belonging to the sugar are added a “prime” (ex. C5', C2', etc)
- The only difference between ribonucleotides and deoxyribo-



Nucleotides

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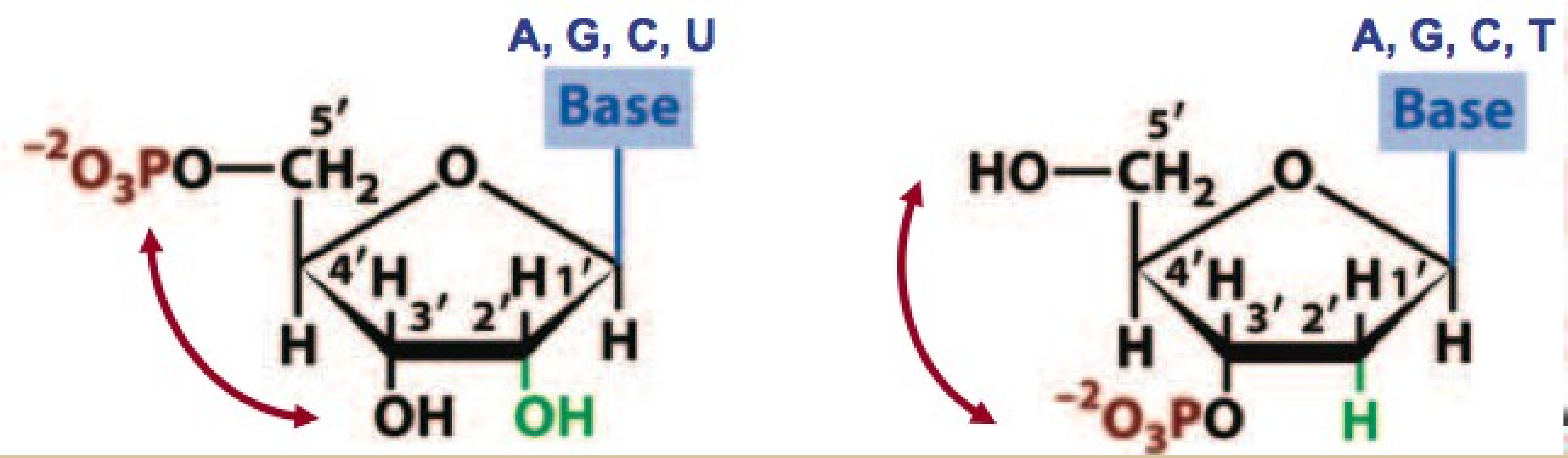
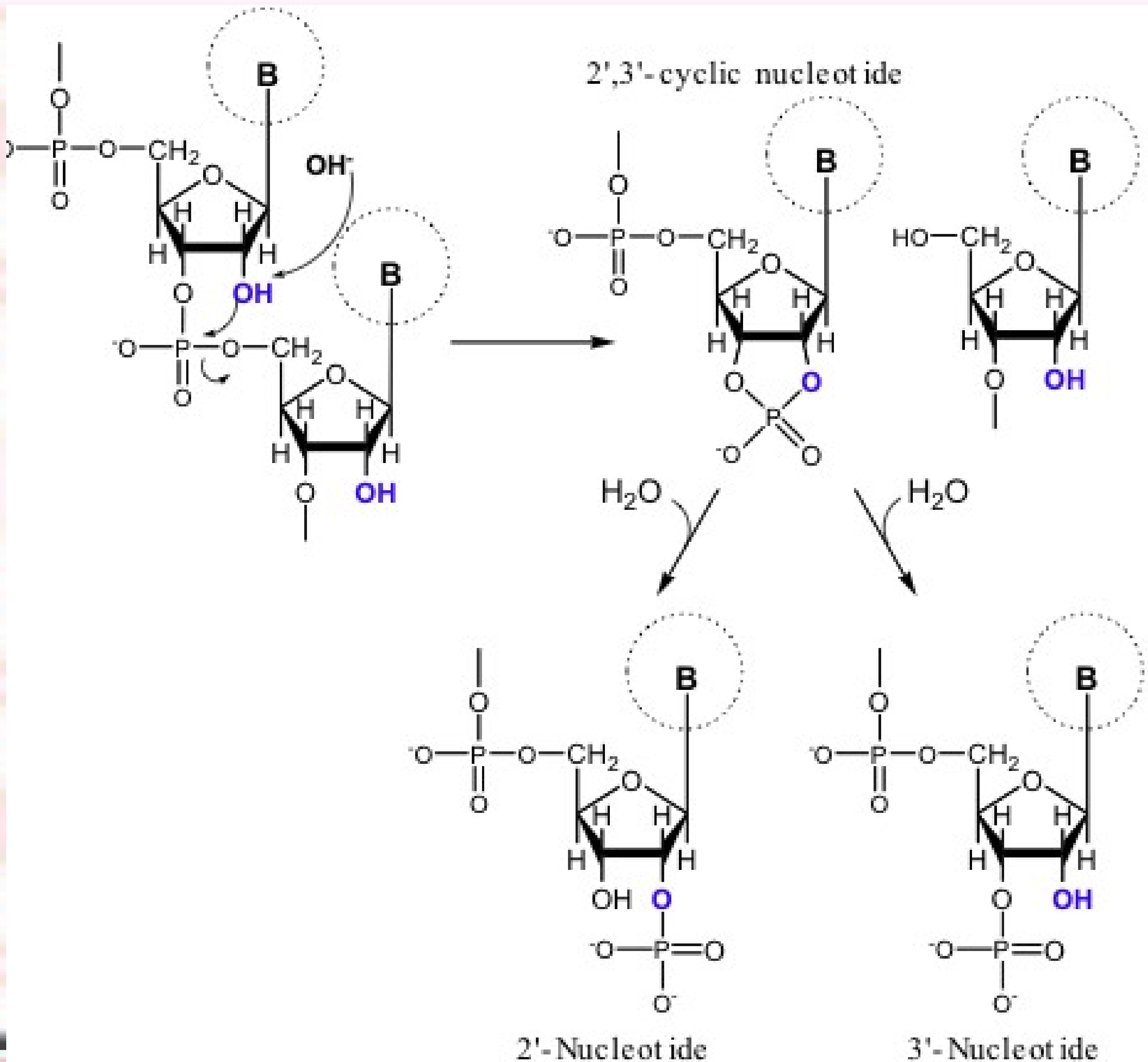
  - all the C and N belonging to the base are numbered following the purine/pyrimidine convention
  - All the C belonging to the sugar are added a “prime” (ex. C5', C2', etc)
- The only difference between ribonucleotides and deoxyribonucleotides lays in the substitution of the alcoholic group on C2' with a Hydrogen
- This will improve the stability towards hydrolysis of about 100-folds of DNA with respect to RNA



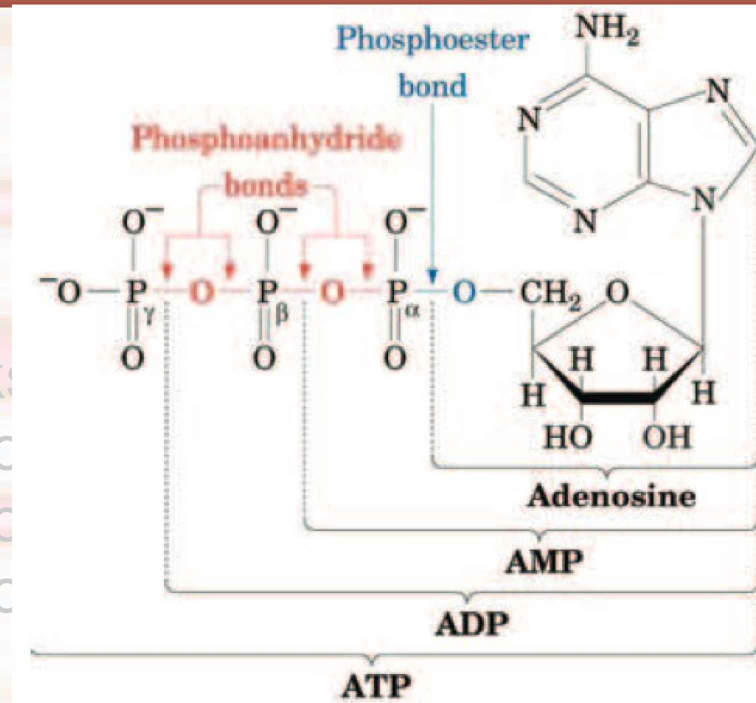
Hydrolysis of RNA



Functions of nucleotides

- Building blocks of RNA and DNA
- Transporters of energy (ATP, GTP)
- Transporters of electrons (FAD, NAD, NADP)
- Transporters of acyl groups (CoenzymeA)
- Signalling molecules (cAMP, c-di-GMP)

- Building blocks
- Transporters c
- Transporters c ... ADP)
- Transporters c ... A)

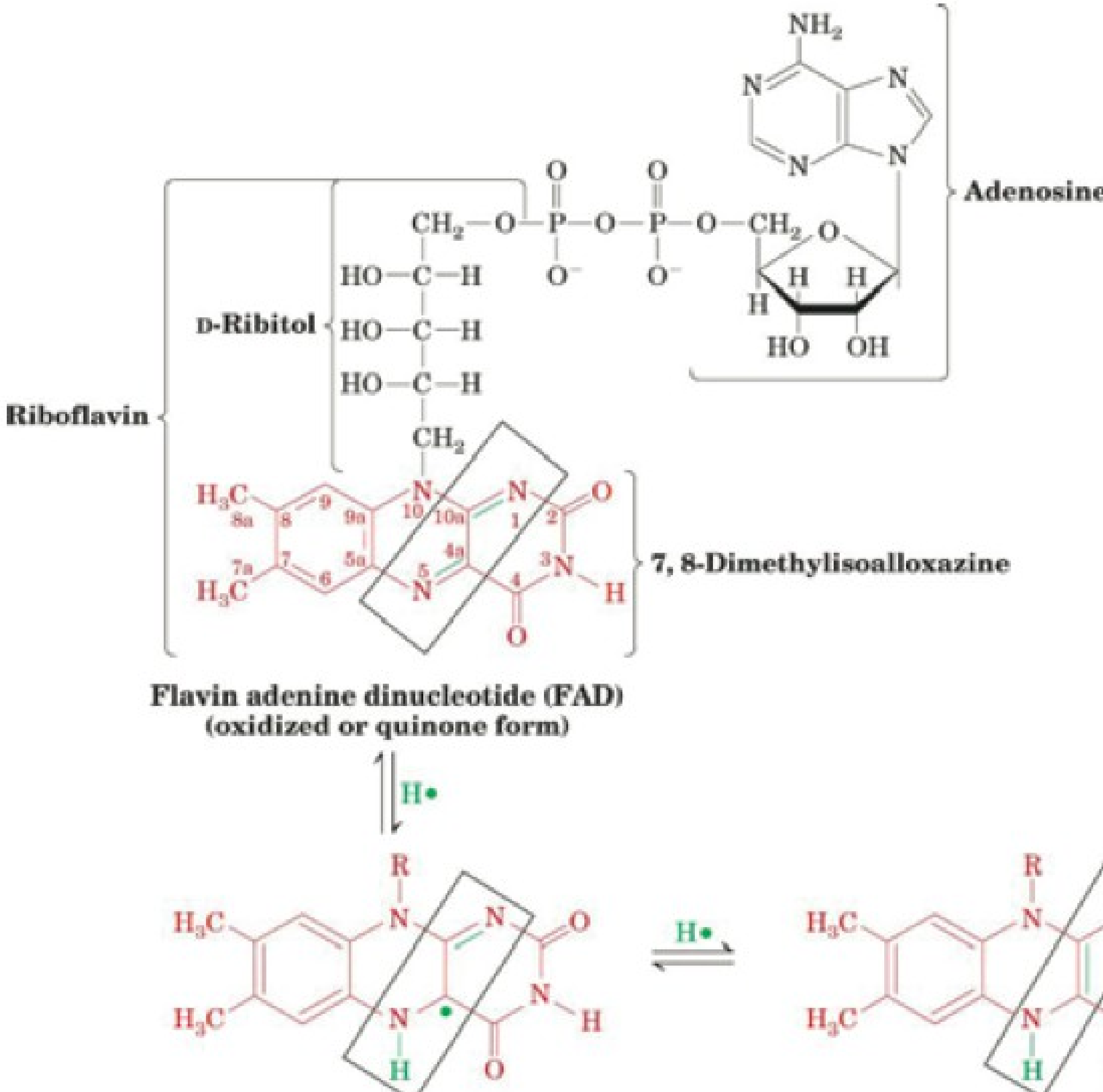


ATP

- It transfers energy in all living organisms
- The energy is stored in the phospho-anhydride bond and released when this group os transferred to another molecule (ΔG= -30.5 kJ/mol)
- Its intracellular concentration is quite low (5mM) but its daily production is about the same as the animal's weight

FAD (flavin adenine dinucleotide)

- In this co-enzyme of redox proteins one ADP is bound to riboflavin (Vitamin B2)
- Many organisms can synthesize riboflavin, men are not capable
- The redox reaction involves the acceptance/release of two electrons in two steps by the dimethyl-isoalloxazine ring
- Oxidised FAD has a bright yellow colour
- Reduced $FADH_2$ has a pale yellow colour

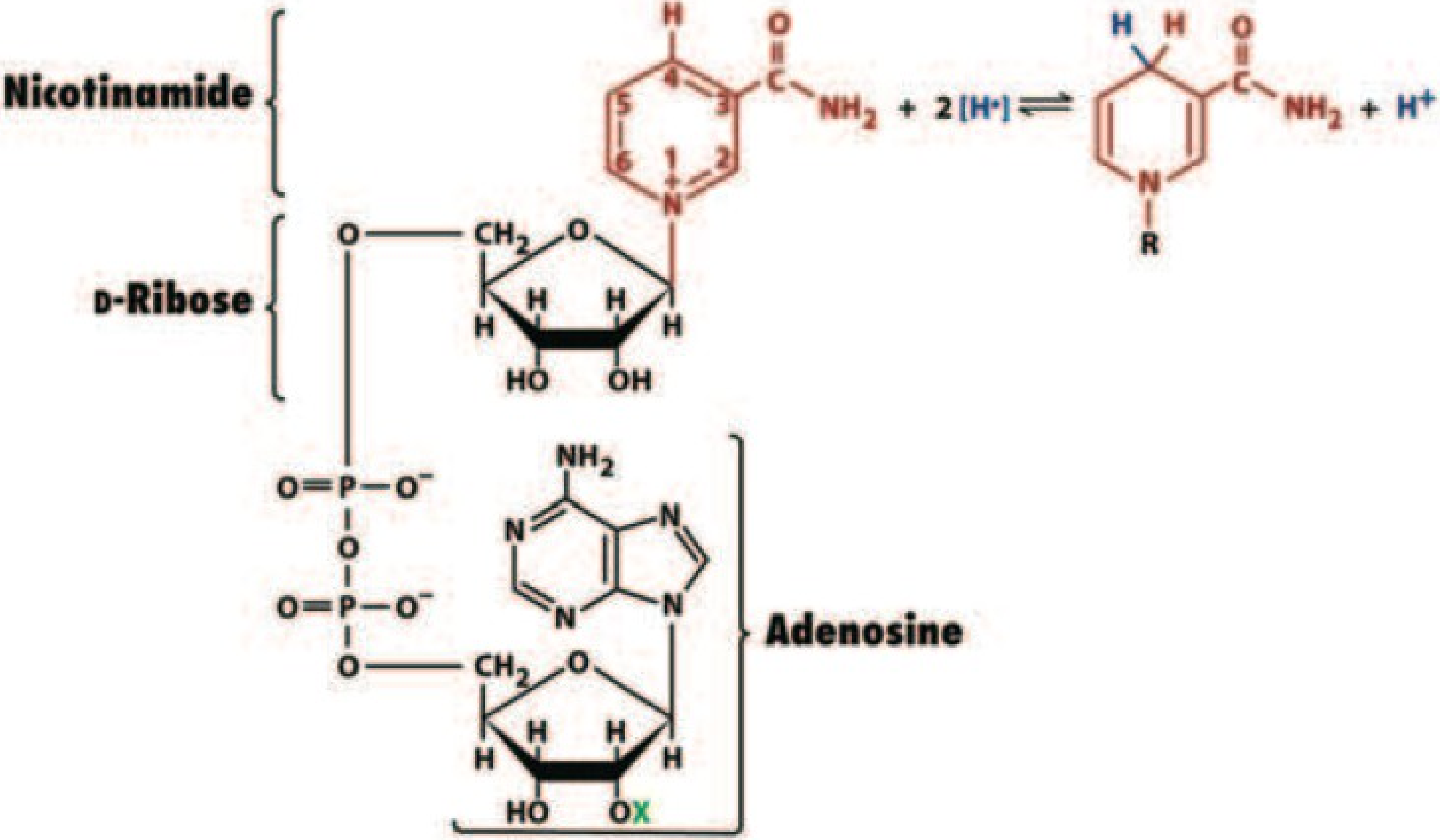


Flavin adenine dinucleotide (FAD)
(oxidized or quinone form)

FADH₂ (reduced or hydroquinone form)

NAD – NADP (nicotinamide adenine dinucleotide - phosphate)

- In this molecule ADP is bound to another ribose which is N-glycosilated to nicotinamide (a derivative of niacin, vitamine B3)
- Niacin can either be synthesized from Trp or taken from the diet, its deficiency causes Pellagra
- The redox centre is indeed on the nicotinamide ring, where a quaternary N acts as an electrons trap
- Both NAD^+ and $NADP^+$ transfer 2 electrons (one ion H^-)

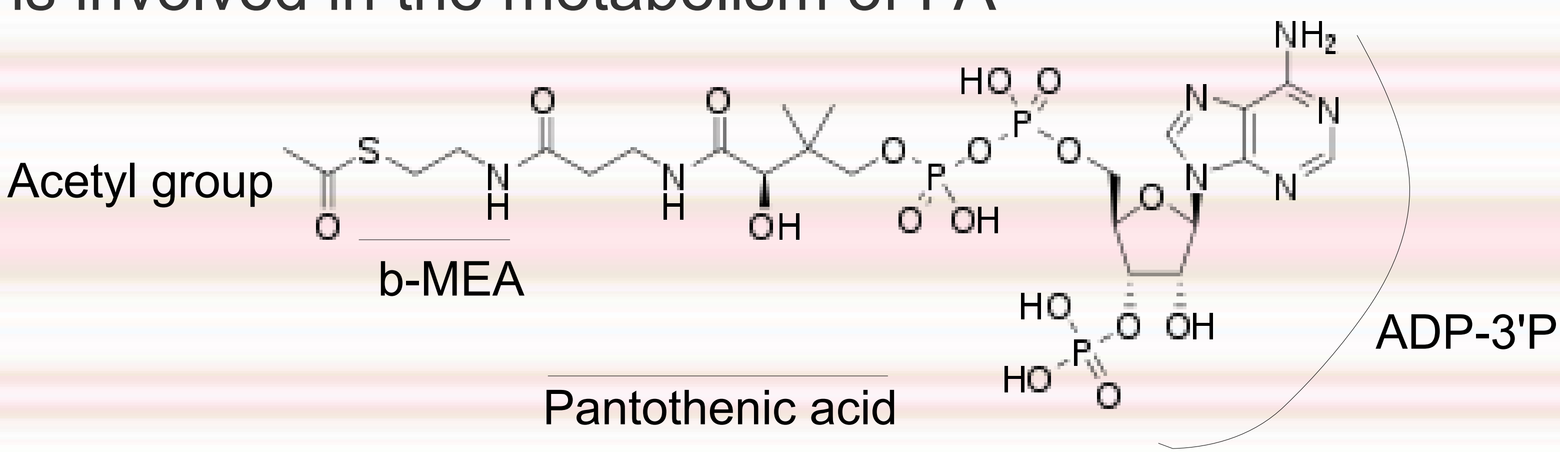


X = H   Nicotinamide adenine dinucleotide (NAD^+)

X = PO_3^{2-} Nicotinamide adenine dinucleotide phosphate ($NADP^+$)

CoA (coenzyme A)

- This transporter has a central role in the metabolism since it transfers acyl groups (2 C atoms) to acceptors
- It is made by adeosine-3'-phosphate-5'-diphosphate linked to pantothenic acid and beta-mercaptoethylamine (b-MEA)
- This thiol derivative is the carrier of acyl groups
- The thiol-ester bond has a stored energy greater than that of ATP (ΔG= 31.5 kJ/mol)
- It is involved in the metabolism of FA

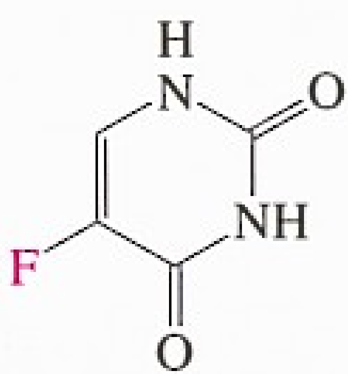


Synthetic analogues of nucleotides

- Heterocycles synthesized to interfere with DNA replication used in chemotherapy

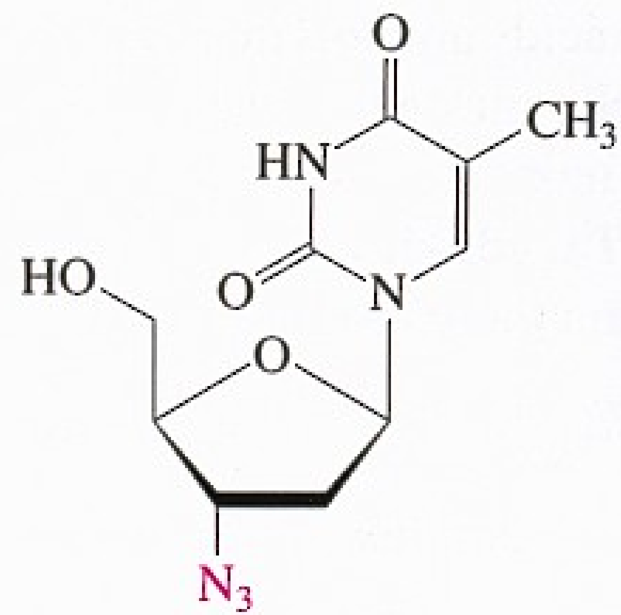
Examples:

- 5-F-uracil used in anti-cancer therapy
- Acyclovir used against herpes viruses
- AZT used against HIV



5-Fluorouracil
(Fluracil)

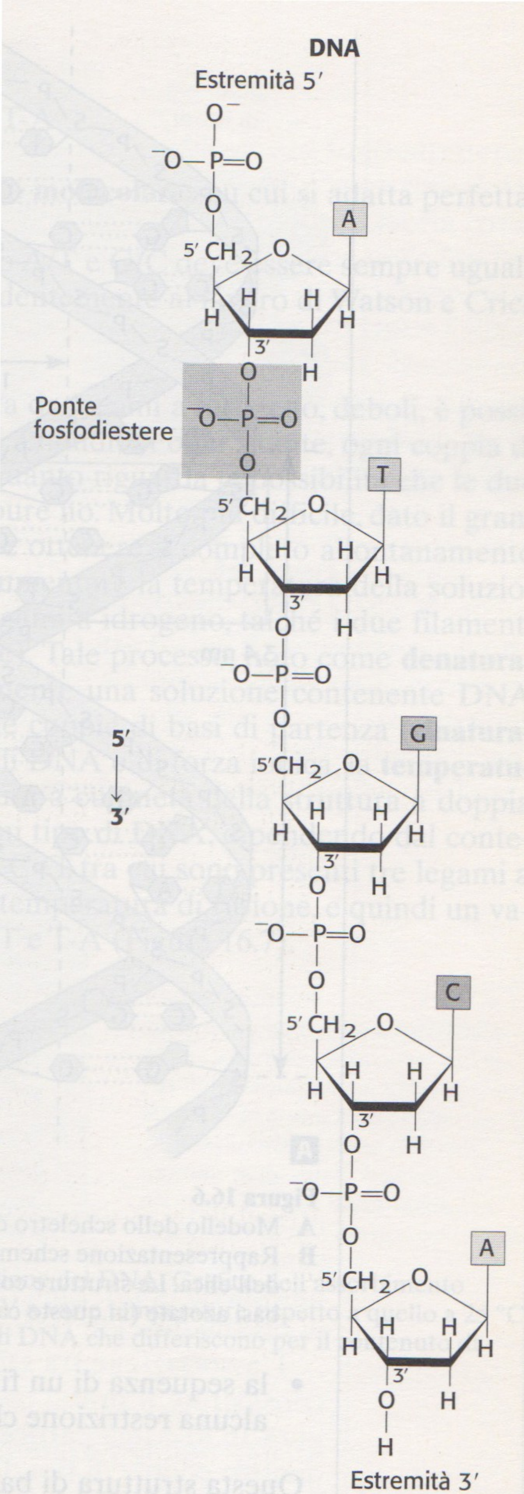
9-[(2-Hydroxyethoxy)methyl]guanosine
(Acyclovir)



3′-Azido-3′-deoxythymidine
(Zidovudine, or AZT)

Nucleic acids: DNA and RNA

- The genetic information must be stably stored in small volumes for long times
- The same information must be available to be read and used:
  - Transcription is the process in which DNA is copied into RNA
  - Translation is the process in which mRNA is converted into proteins
- The information in DNA and RNA must be accessible to proteins and/or other nucleic acids
- The offspring must have the same set of instructions of the parents:
  - Replication of DNA in a semiconservative way



Nucleic acids structure

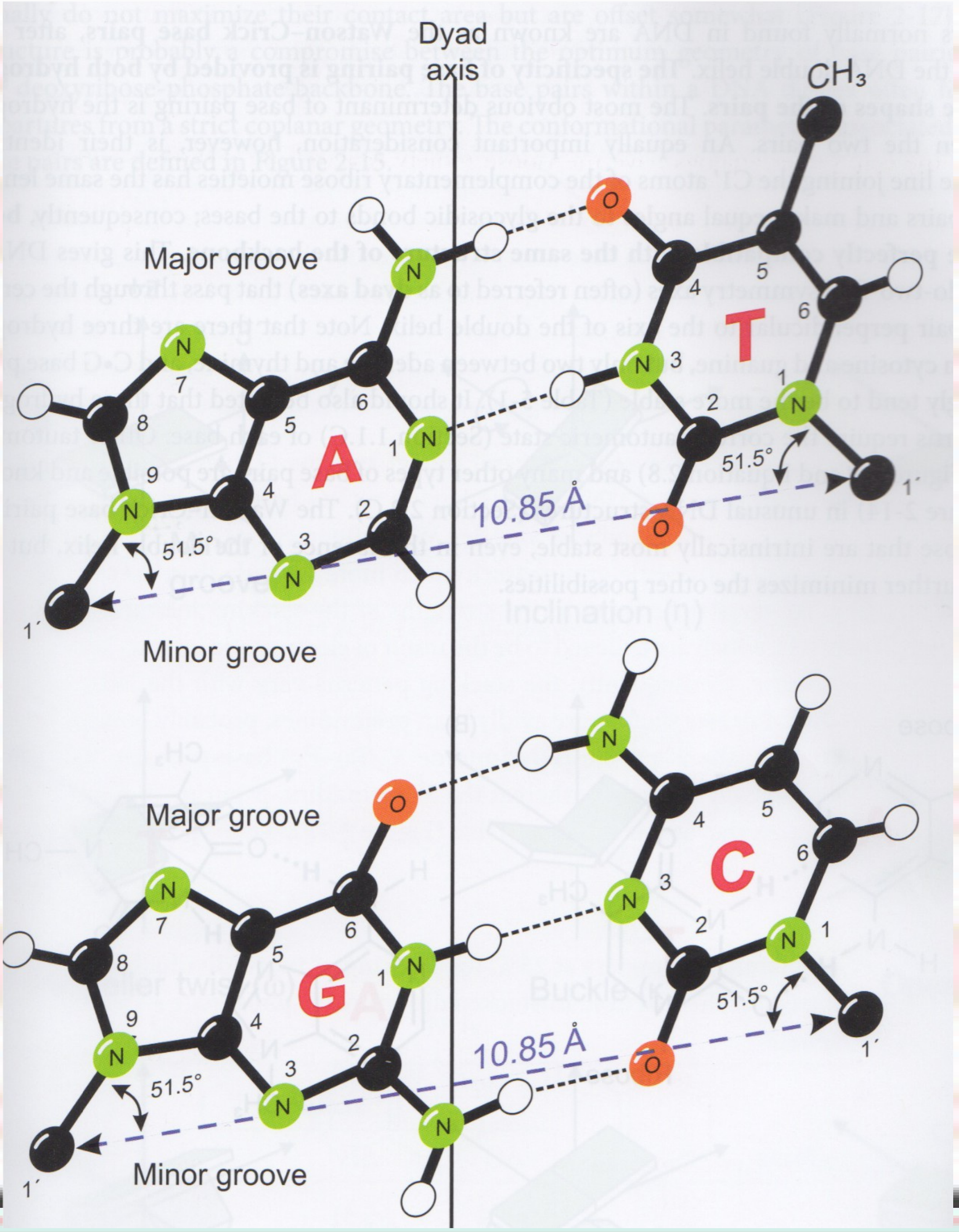
- Nucleic acids are polymers of nucleotides in which the phosphate group bridges the 5'OH of the n position to the 3'OH of the (n-1) position → phosphodiester bond
- The scaffold is the linear sugar-phosphate polyanion
- The sequence of the bases is the information → free heteropolymer
- The heteropolymer is directional:
  - 5' terminus: first residue with a 5'-phosphate group not involved in any other bonds
  - 3' terminus: last residue carrying a free 3'OH group
- The sequence is always written 5' → 3' since this is the way it is polymerized

Base pairing

- The bases can form directional hydrogen bonds in pairs:
  - A with T (and T with A) in DNA only
  - A with U (and U with A) in RNA only
  - C with G (and G with C) in both DNA and RNA

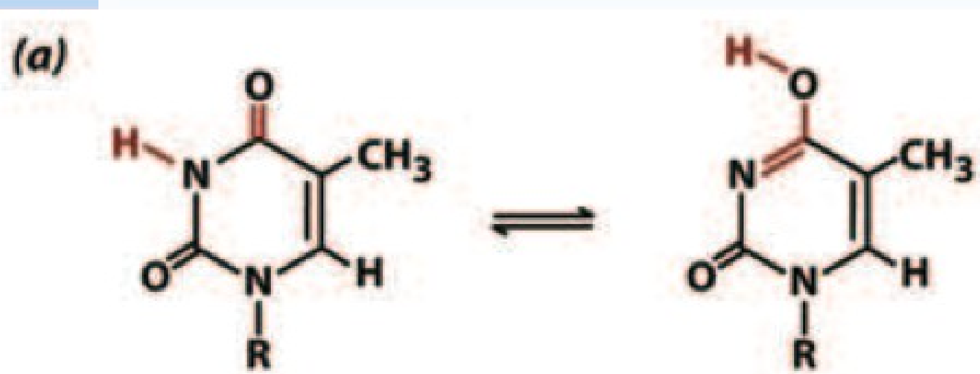
- Several factors limits all the possible pairing to the above mentioned:
  - the size of the rings (Pu-Pu is too large, while Py-Py is too narrow when two filaments are coupled)
  - A:T can make 2 H-bond; G:C can make three
  - The polarity of H-bond in corresponding positions of these couples is matching (ex. T has a C=O facing a NH_2 of A → acceptor facing donor, while G has a C=O in that position)
  - A:U is possible since the only difference between the two bases (extra CH_3 in C3 of U) is off the side involved in H-bonds

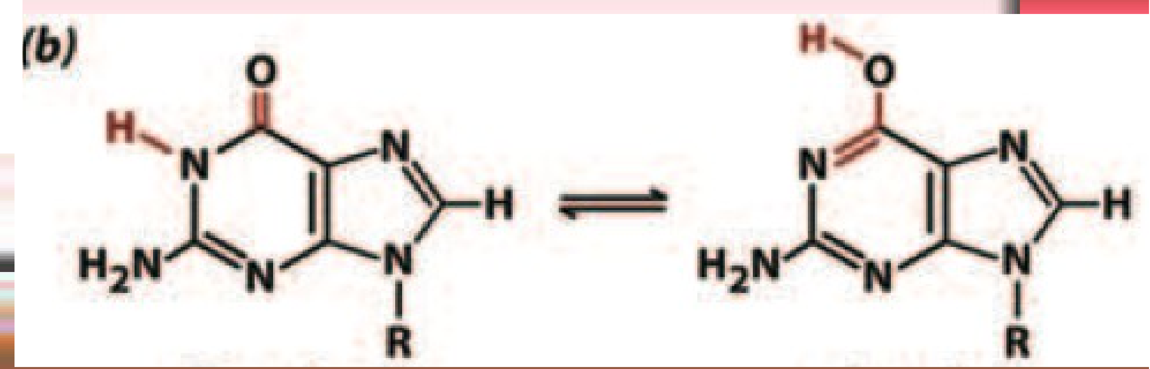
Base pairing



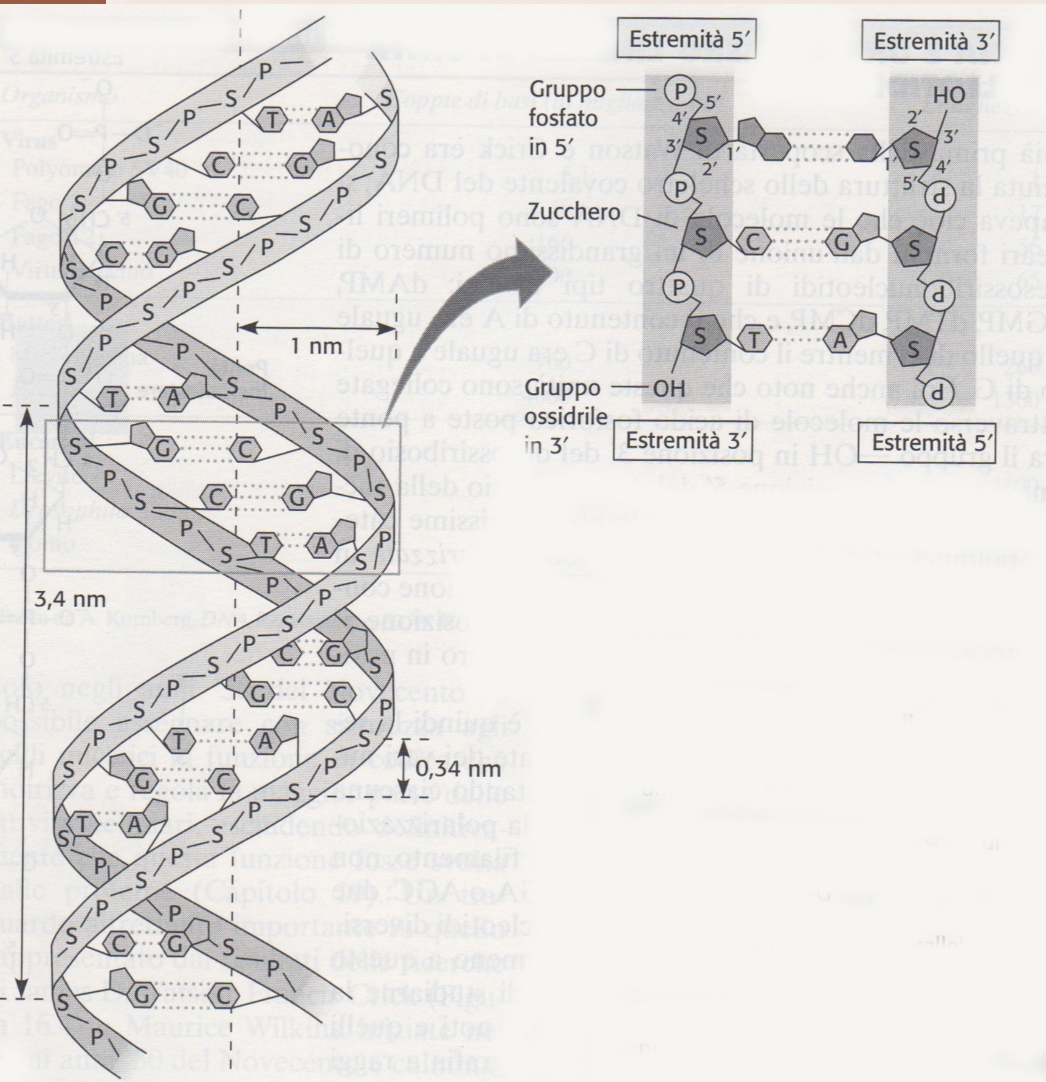
Keto-enol tautomerism

- The bases can undergo spontaneously tautomerism of C=O into C-OH, this is specially true for T (panel a) and G (panel b), but can also happen to C and U
- The interconversion of the two forms involves the movement of a proton and the shifting of bonding electron
- DNA double helix is only compatible with all the bases in their keto-form
- Within this frame, the most important tautomerism is the conversion of T, since its enol-form can only pair with G (3 H-bonds) and not with A → induced mutation





DNA structure



- DNA is the nucleic acid of chromosomes
- It is always made by two paired filaments running in opposite direction
- This results into a double helix in which each filament is the template of the next generation's replicative event
- The complementarity is given by the reciprocal base pairing
- A small repertoir of base pairing is fundamental to keep errors to a minimum in both replication and transcription /translation

RNA structure

- RNA is always present as a single filament, but folds into it-self in a larger variety of shapes, which includes short helical regions in which base-pairing can occur (stem-loop regions)
- RNA can be classified based on its function:
  - mRNA (the string directly relating DNA to protein) → no fixed tertiary structure
  - tRNA (adaptor molecule carrying aa to the ribosome) → specific L-shape almost independent of the sequence
  - rRNA (structural and functional constituent of the ribosome) → complex structure to host accessory scaffolding pro-teins and regulatory factors