

BCMB2X01

Biochemistry and Molecular Biology (I)

SI 2018

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Lecture 1: Introduction

DNA gets converted into RNA (transcription) which gets converted into protein (translation).

The process of transcription is generally much simpler than translation because of the fact that DNA and RNA are both made of nucleotides – the order of bases codes the information – there this process is the simple matter of copying.

4 nucleotides → 20 amino acids – side chains can be hydrophobic, hydrophilic, positive or negative.

- The order of these amino acids gives it its characteristic functions.

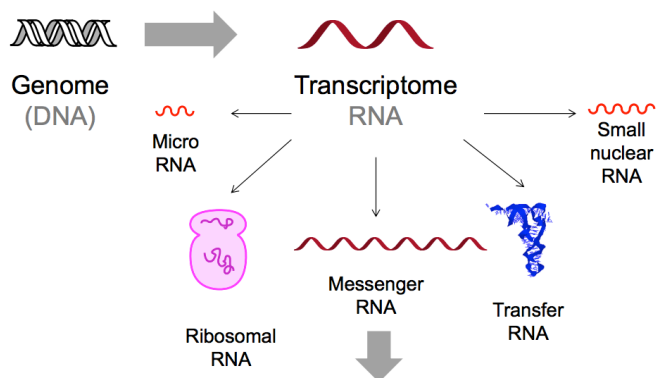
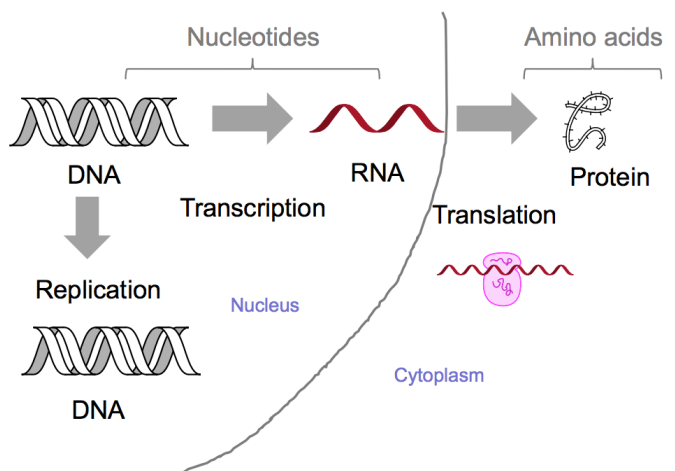
Replication – DNA is copied.

Eukaryotic cell – all the nucleic copying happens within the nuclear membrane, and all protein synthesis happens in cytoplasm.

- Therefore, there are heaps of options because the cell will only send out the RNA that it wants.

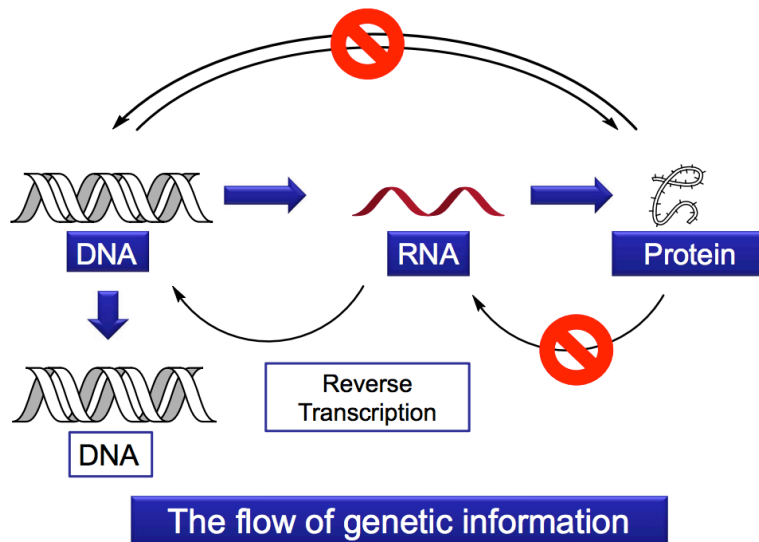
Terminology:

- Genome: DNA
- Transcriptome: RNA
- Proteome: protein

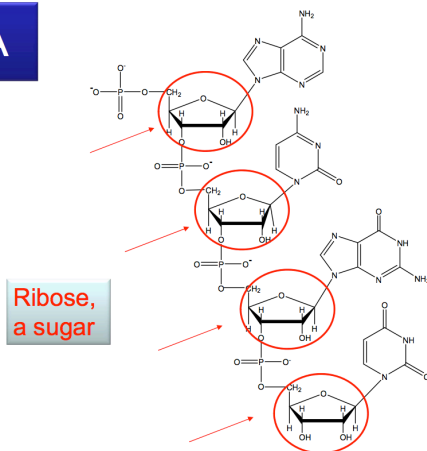


Micro RNA – involved in regulating the rate of translating and what is translated.

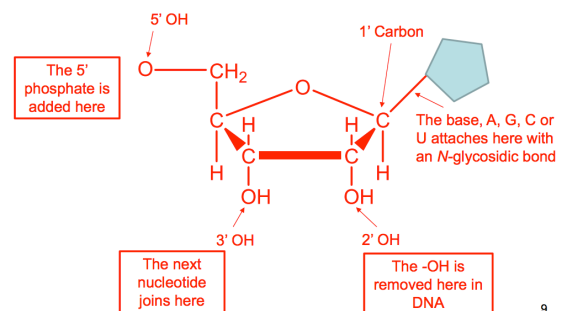
Eukaryotic cells have introns which are non-coding regions – 98% of genome does not code for protein.



RNA



RNA ribose



It doesn't matter which nucleotide it is, the backbone is the same – chain of ribose attracted by a phosphate di-ester bond.

- This backbone has chemical properties which then get exploited in the name.

RNA

The sugar phosphate backbone

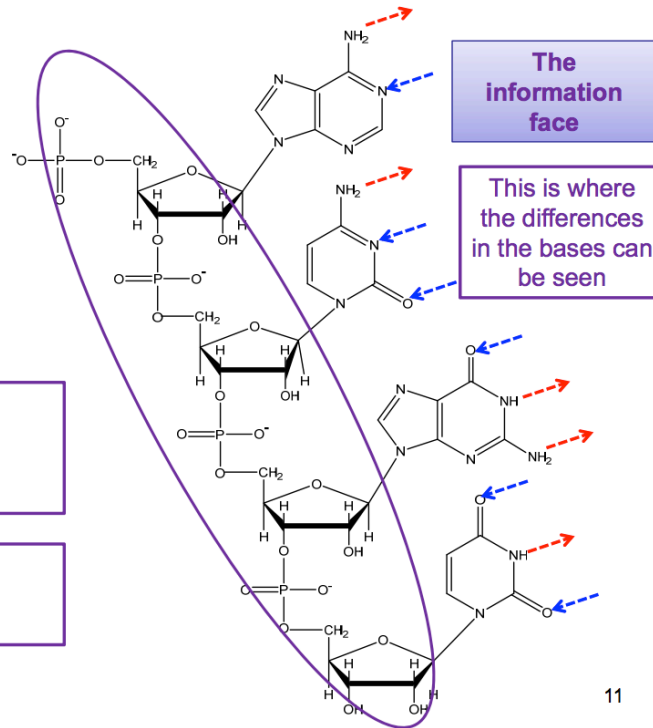
Common, no matter what base is attached

Properties of the sugar phosphate backbone:

- Negative charge
- hydrophilic

Applications:

- Electrophoresis
- Ethanol precipitation



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DNA and RNA behave like charged polymer of carbohydrates. Information is buried in the information face – donors have hydrogen, acceptors don't have hydrogen.

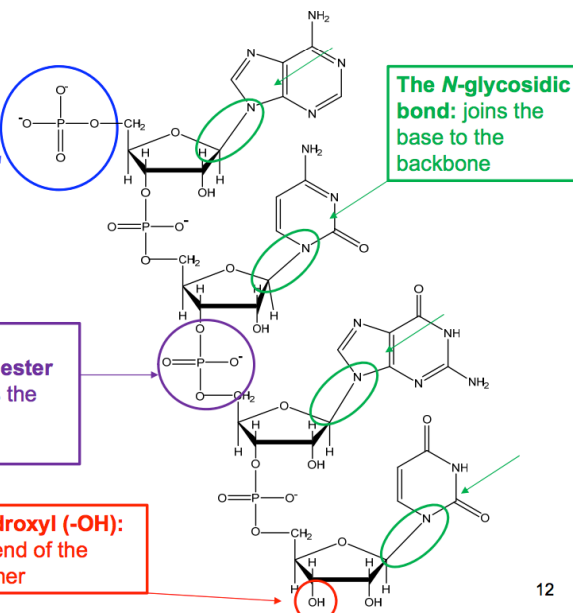
Bases attached to sugars by a N-glycosidic bond

RNA

5' phosphate:
The start of the polymer

The phosphodiester bond:
joins the monomers together

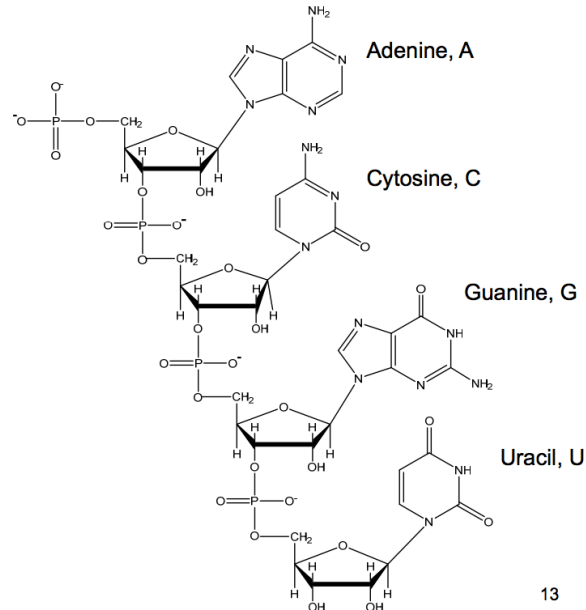
3'hydroxyl (-OH):
The end of the polymer



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RNA

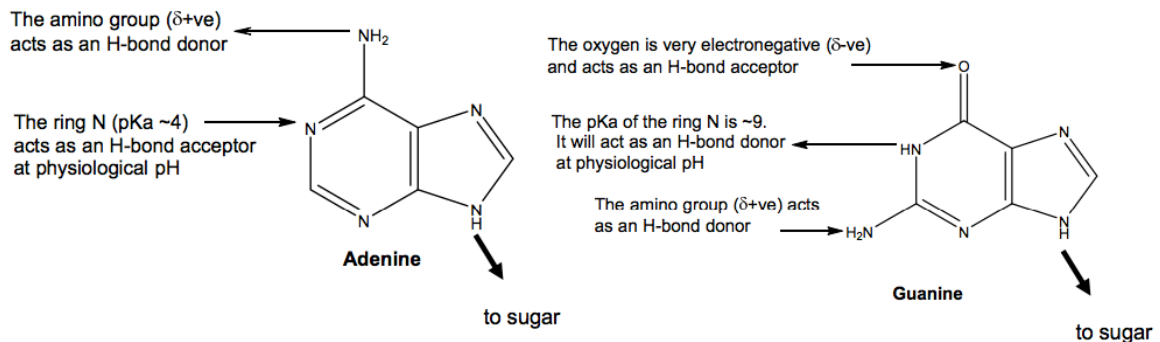
bases



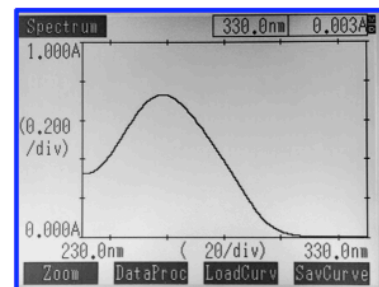
pKa – pH where 50% of functional group protonated – other 50% lost protons. (50% disassociation).

- Can act as acid or conjugate base e.g. carboxylic acids or amino group.
- Can be COOH or COO-

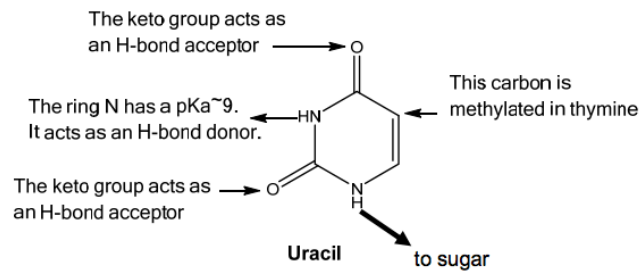
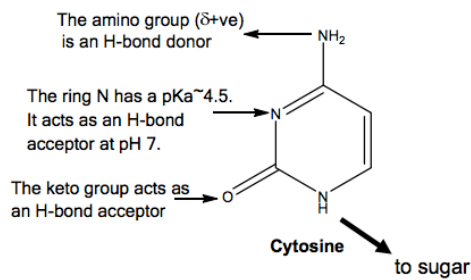
Lower pKa – less affinity for the protons therefore can take a pretty weak base to pull it off.



The aromatic character of the 4 bases gives the DNA and RNA its UV absorbance (pi electron clouds above and below the flat rings)



260nm.



Is this UV absorbance going to change with the source or sequence of the nucleic acid?

- Bases are giving the absorbance but most bases have relatively same absorbance.
- If all 4 bases absorb roughly the same, the sequences do not matter.
- If double stranded – absorbs less

RNA and DNA difficult to distinguish because same bases.

Experimental Applications

To promote base pairing:

- Lower the temperature
- Increase the ionic strength
- Keep the pH ~ 7

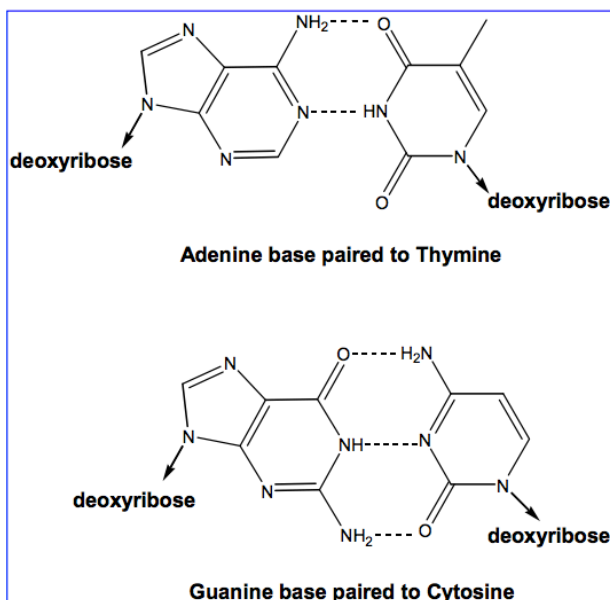
To disrupt base pairing:

- Increase the temperature
- Reduce ionic strength
- Increase the pH > 9

Melting temperature, T_m :

The temperature when the 2 strands come apart

- Increases with increasing $\%(G+C)$
- Increases with increasing ionic strength
- Increases with length



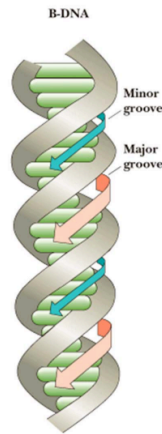
The aromatic character of the 4 bases gives the DNA and RNA its UV absorbance, π electron clouds above and below the flat rings

- The grooves on the surface gives proteins access to the base pairs

- essential if they are to read the sequence
- proteins mainly access the major groove.

Why?

The major grooves are the window into the base sequence and the information...."the soul of the DNA"



- Major groove the protein can get in to read the sequence.

DNA and RNA are copied by a set of enzymes called polymerases

There are RNA and DNA polymerases; the naming is based on the product made, not the template used – RNA makes RNA, DNA makes DNA

There are DNA polymerases which make DNA from an RNA template; reverse transcriptase.