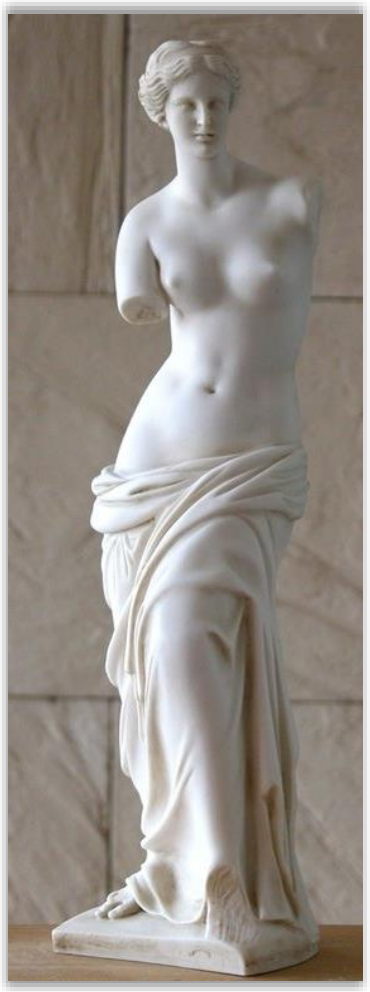
Molecular pathology course for residents in pathology

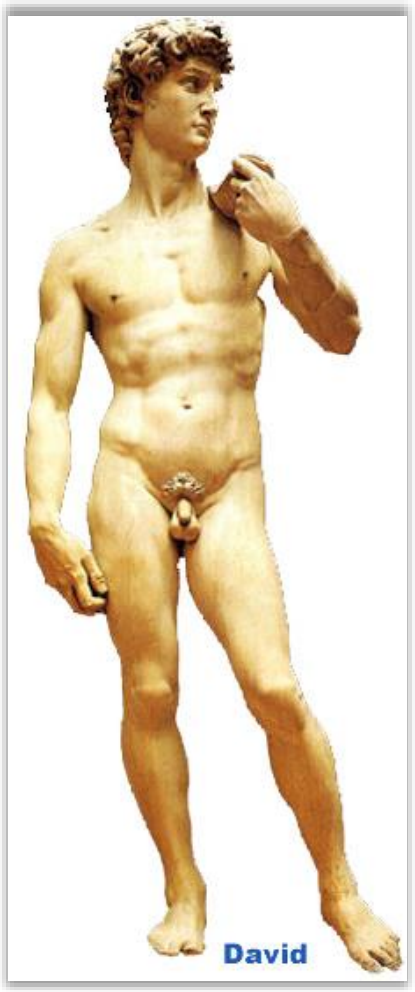
An introduction to cell biology

N. Van Roy (University of Ghent)

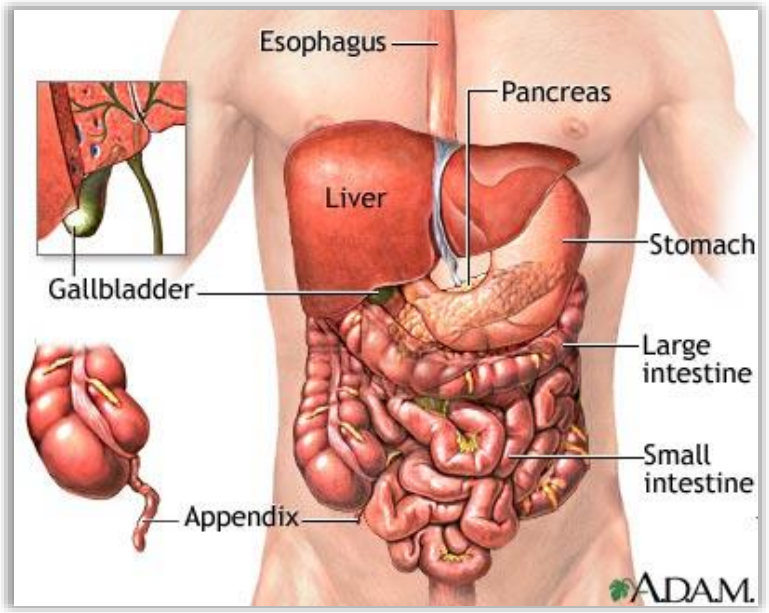
GHENT UNIVERSITY

Constituents of the human body



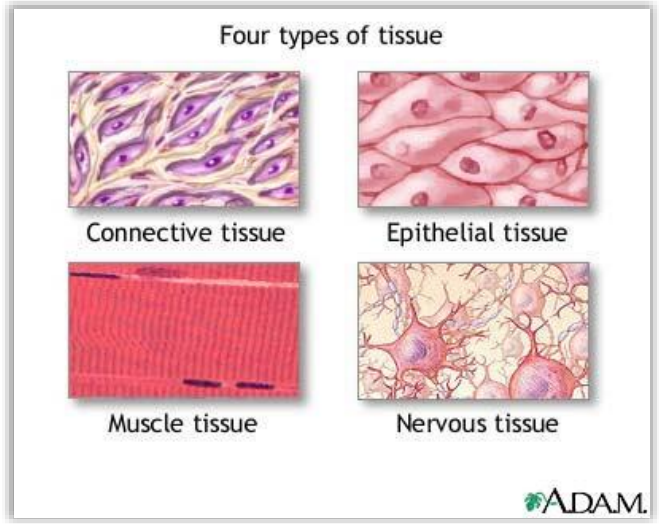


body=10^{14} cells

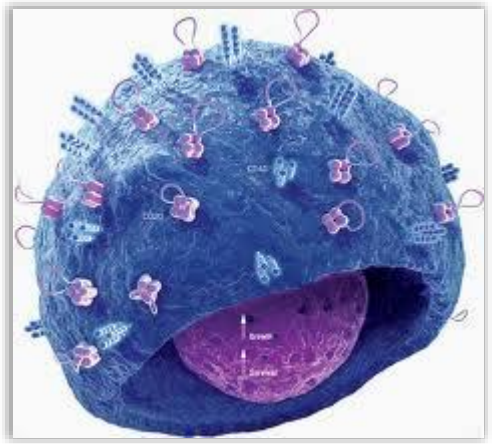


organs

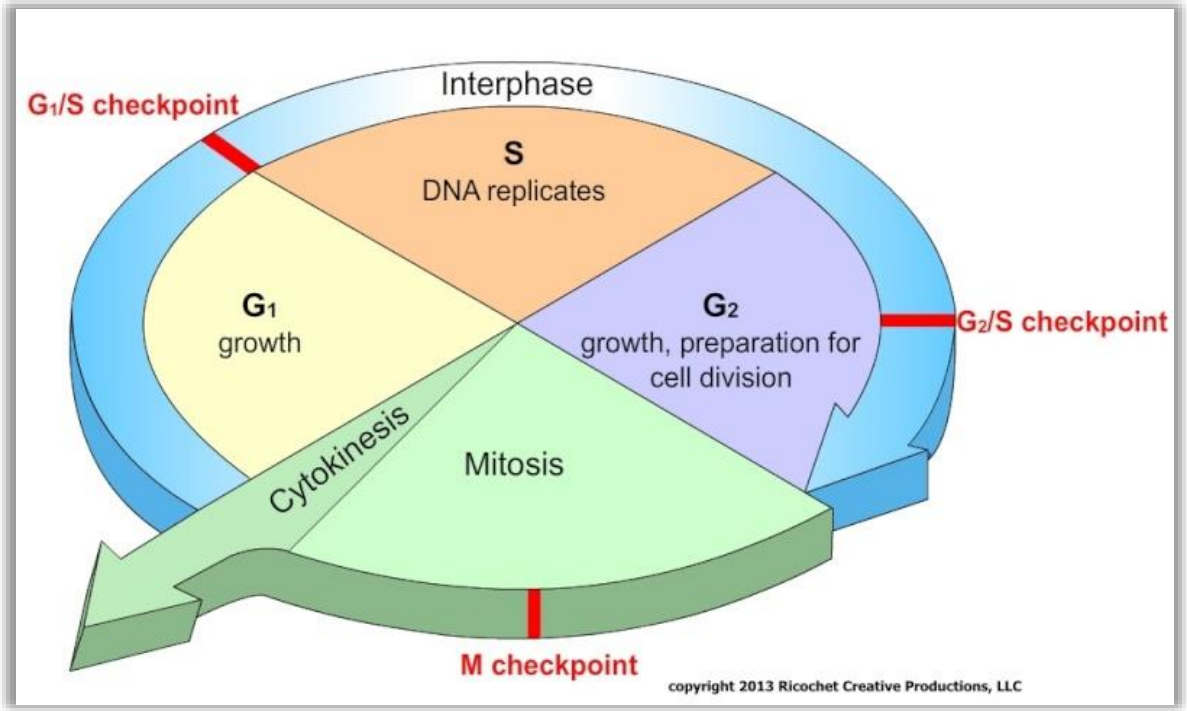
tissues



cell



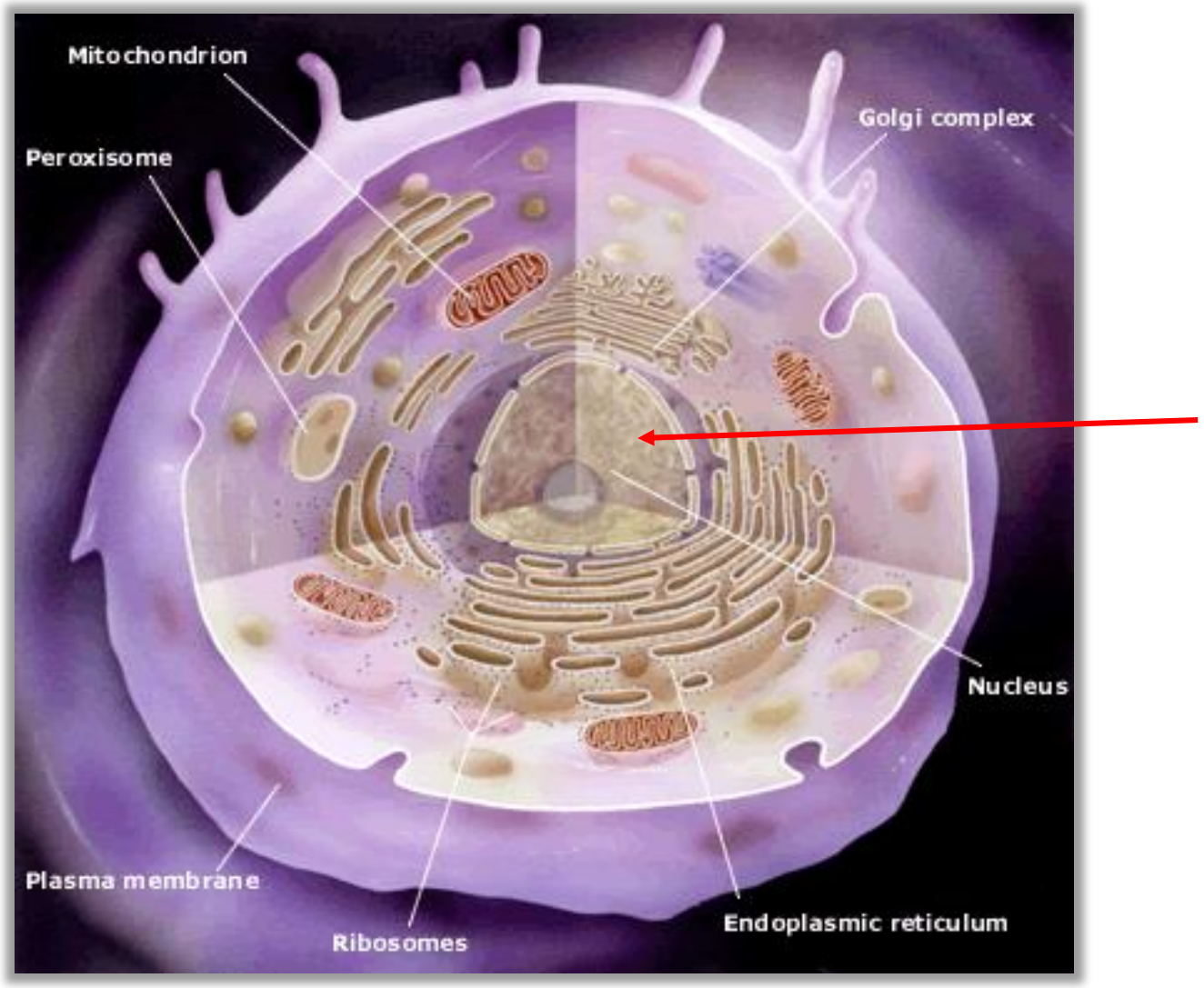
How do we obtain so many cells ?



Interfase=G1+S+G2

Cell cycle ~24 hr

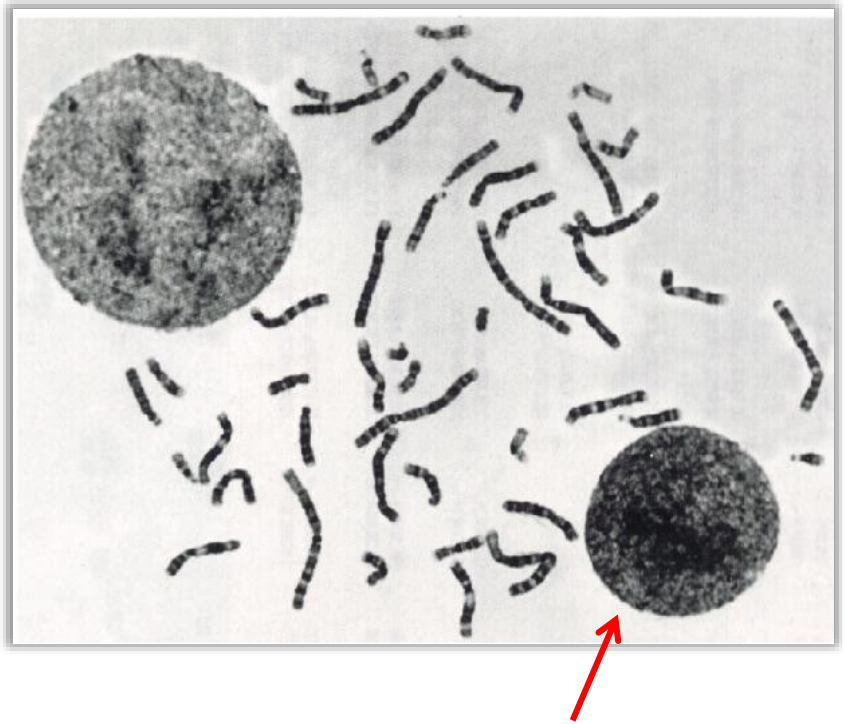
The cell's interior



CHROMOSOMES

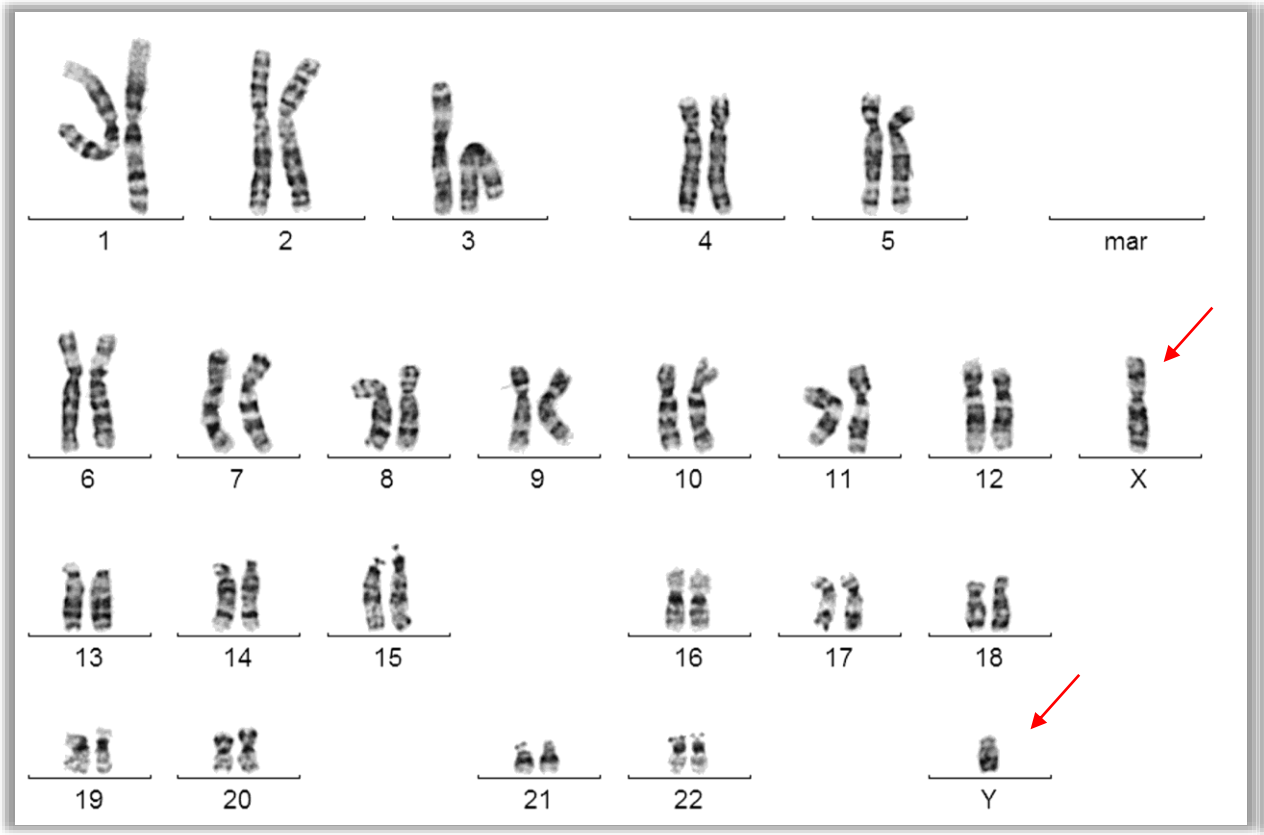
- “coloured bodies”
  chroma: colour *soma*: body
- only visible during cell <u>division</u>

Chromosomes after banding

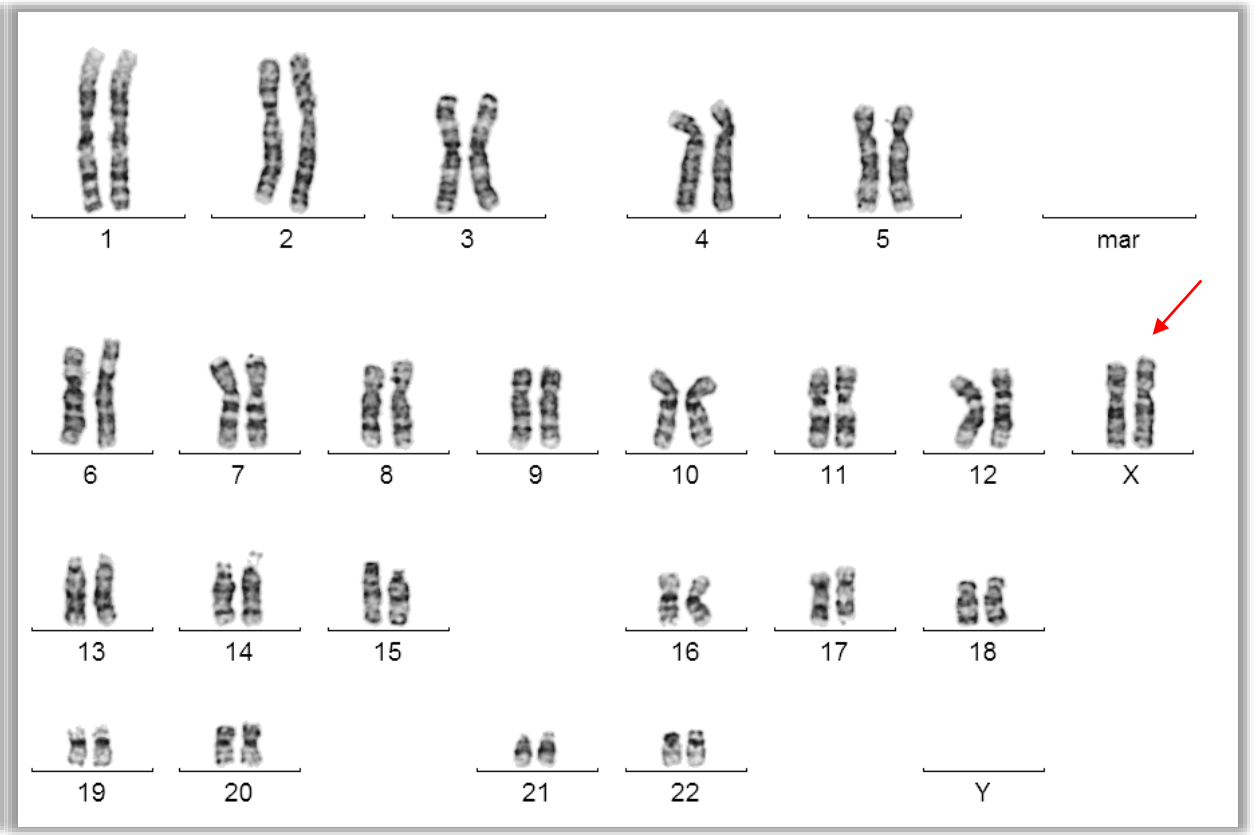


Interphase nucleus

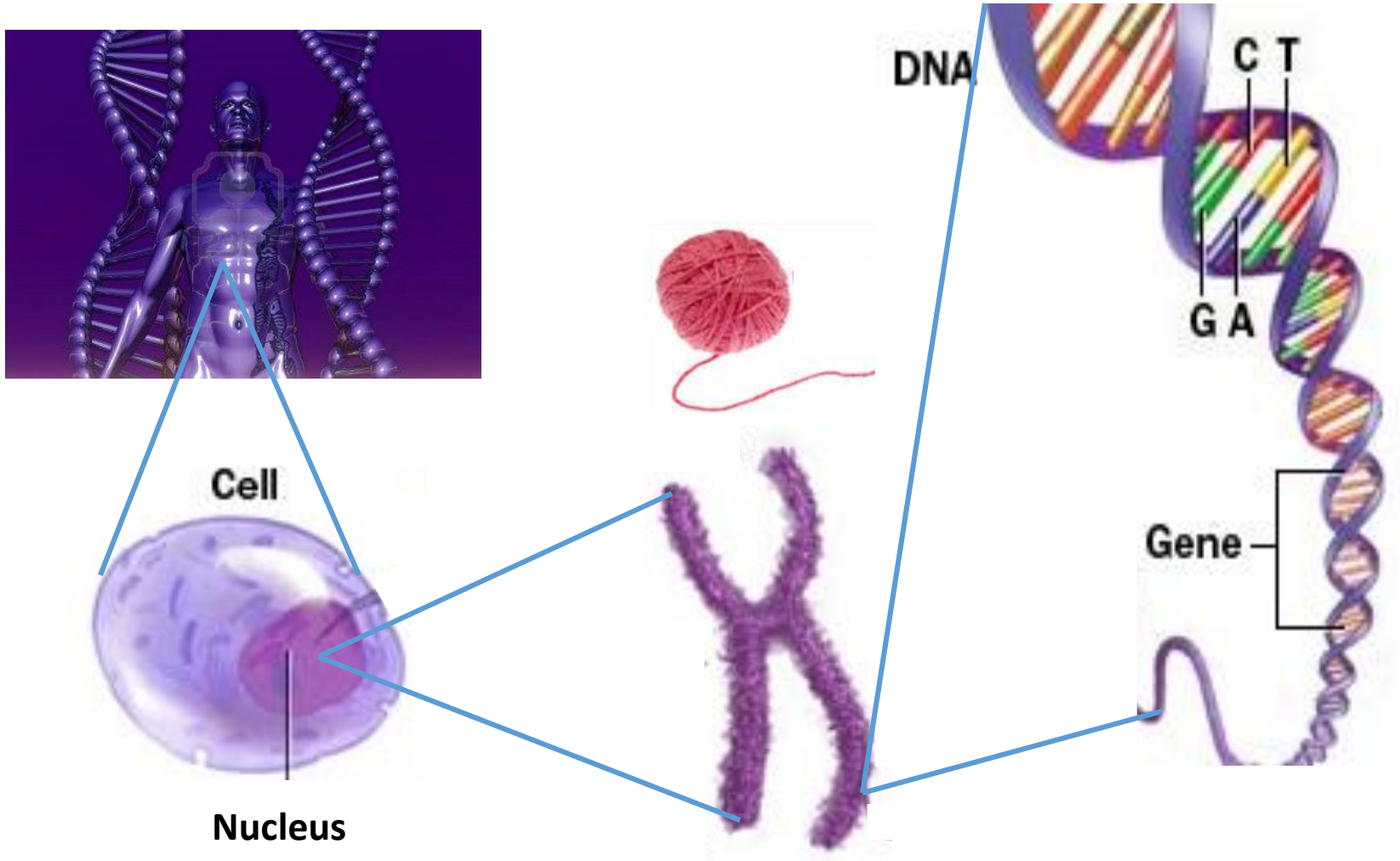
Normal male karyotype: 46, XY



Normal female karyotype: 46, XX



DNA : molecule of life



10 trillion cells in a human body

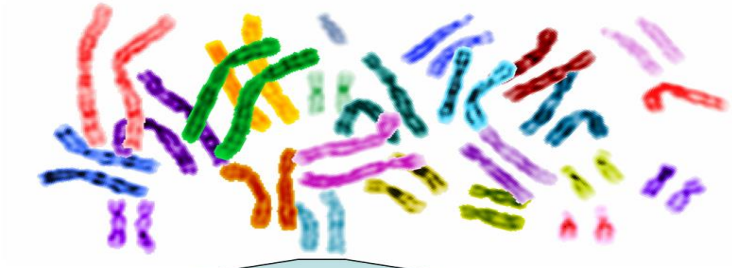
The human genome

- DNA in mitochondria (prokaryotic):
  mtDNA: localized in mitochondria
  (= structures/organells in eukaryotic cells that convert food to energy).

- DNA in nucleus (nuclear genome):
  - 23 pair chromosomes:
    - 22 pair autosomes (1-22)
    - 2 sex chromosomes (X and Y)
  - ~ 22.000 genes
  - 3 billion bp or 6 billion nucleotides

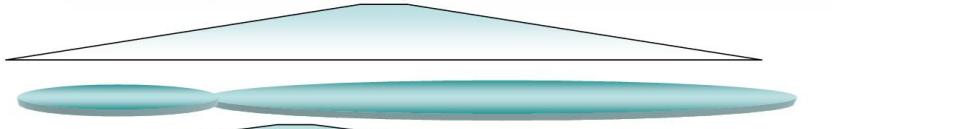
The human genome reads like a book

Human genome

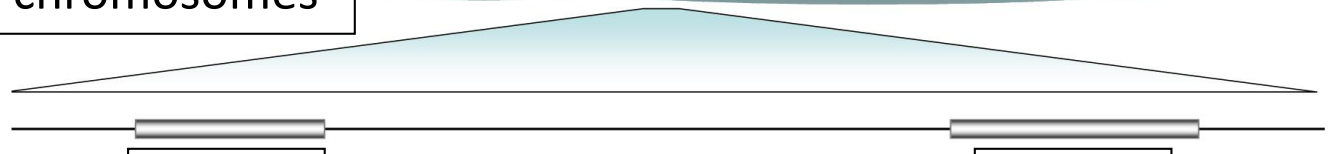


Manual of the cell

23 pair chromosomes



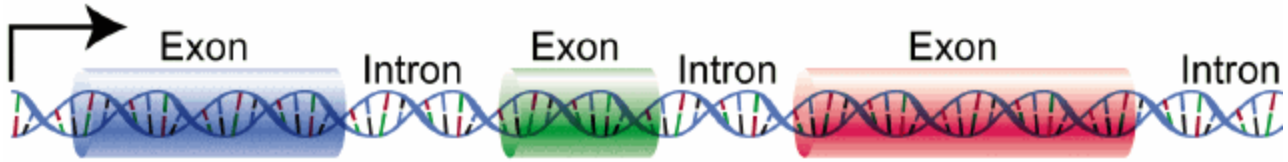
23 chapters



gene

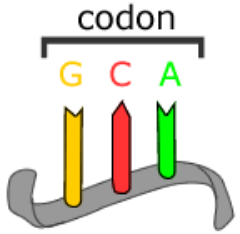
gene

~19.000 stories/recipes



Paragraphs

advertisements

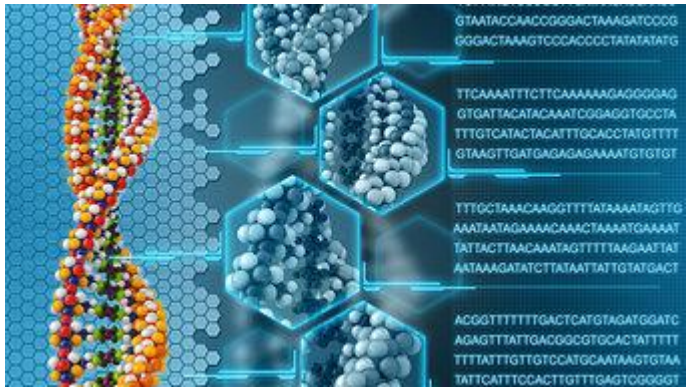


Words

codon = 3 nucleotides (letters)

The human genome

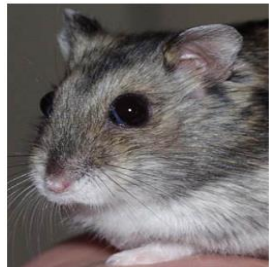
- total: 3 billion bp, ~19.000 genes
- gene sequences: ~5%
  - exons: 1.5%
  - introns: 3.5%
- chromosome 1: 250Mb
- chromosome 22: 50Mb
- gene: about 3.000 bp
- function of ~50% of the genes is unknown
- ‘junk’ DNA: ~95%
- ‘repeats’ : >50%
- low variability: 99,8% identical between individuals
- variation: per 10 million (SNPs)
- mean: 3 million differences between 2 individuals



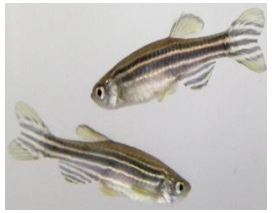
Variations in genomes


99,8%: 0,2% = 6 miljoen verschillen


98%

70%



Organisation of genomes: number of genes/chromosomes says nothing about complexity of an organism

organism	genome	number genes	agreement
bacterie	4,6 million bp	~4.500	-
fruitfly	165 million bp	~13.600	50%
human	**3 billion bp**	**~19.000**	**100%**
mouse	3 billion bp	~30.000	~90%
plant	125 million bp	~25.000	-
worm	97 million bp	19.099	40%
yeast	12 million bp	~6.000	31%

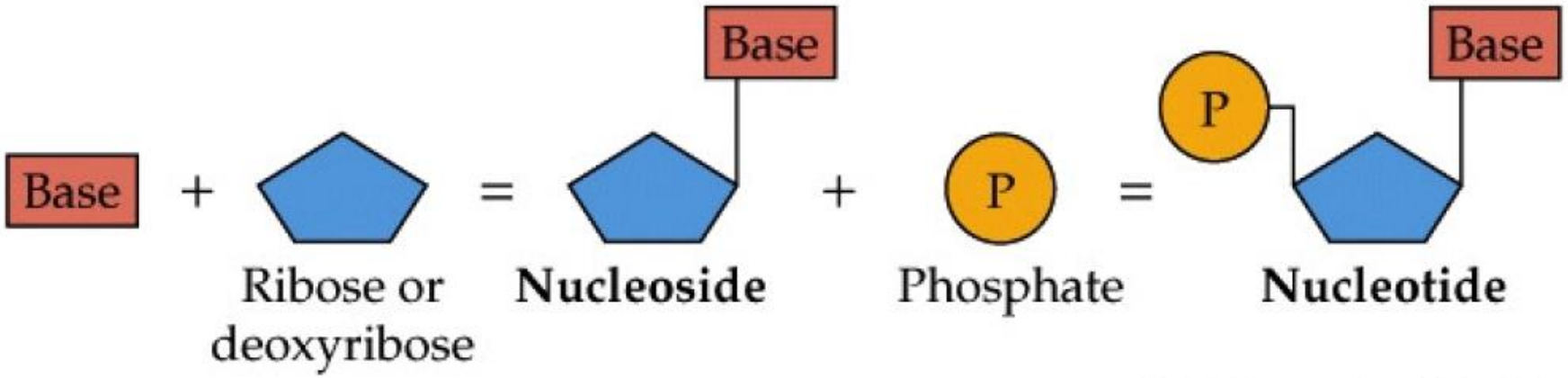
Human:

- Small number of genes !
- Genome with complex structure

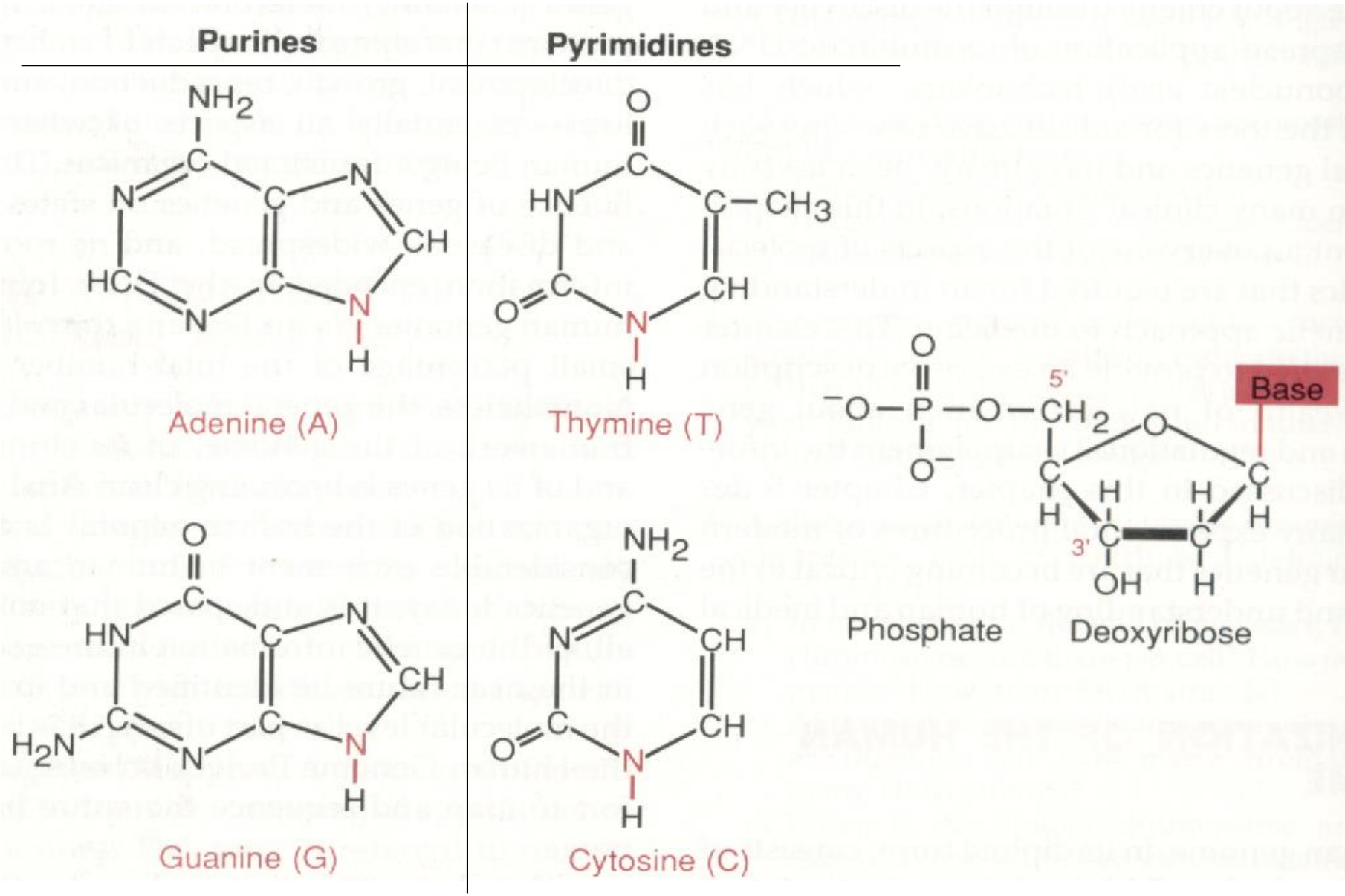
DNA, carrier of genetic information

- DNA = deoxyribonucleic acid
- DNA = polymer of nucleotides
- nucleotide:
  - Pentose sugar (5C) (deoxyribose)
  - N-containing bases:
    - Cytosine (C) and thymine (T) (pyrimidines)
    - Adenine (A) and guanine (G) (purines)
    - phosphorgroup

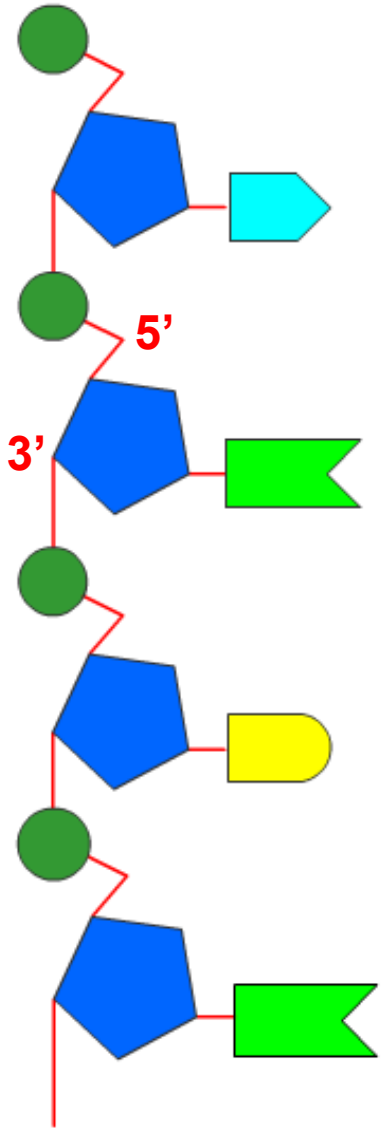
nucleoside

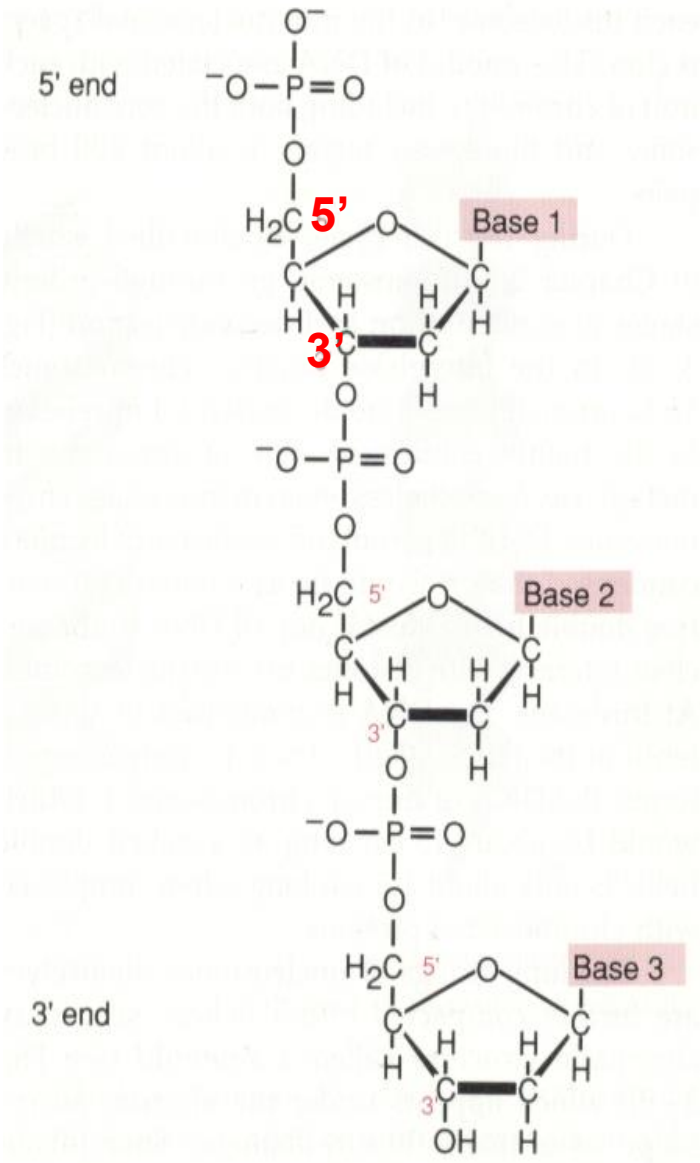


The organic bases

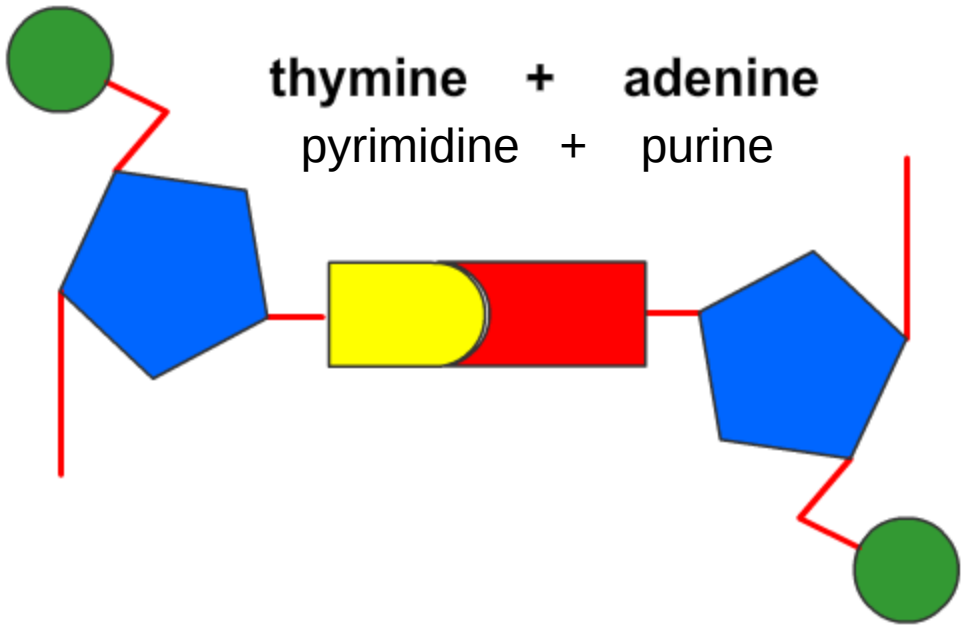


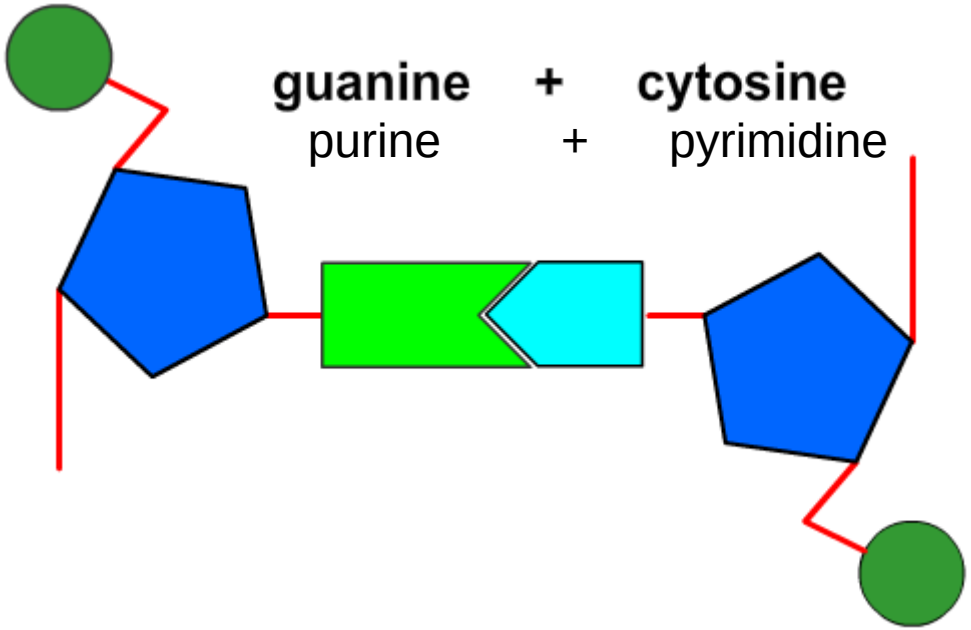
nucleotides polymerise via 3’-5’ phosphodiësterbounds in polynucleotide chains

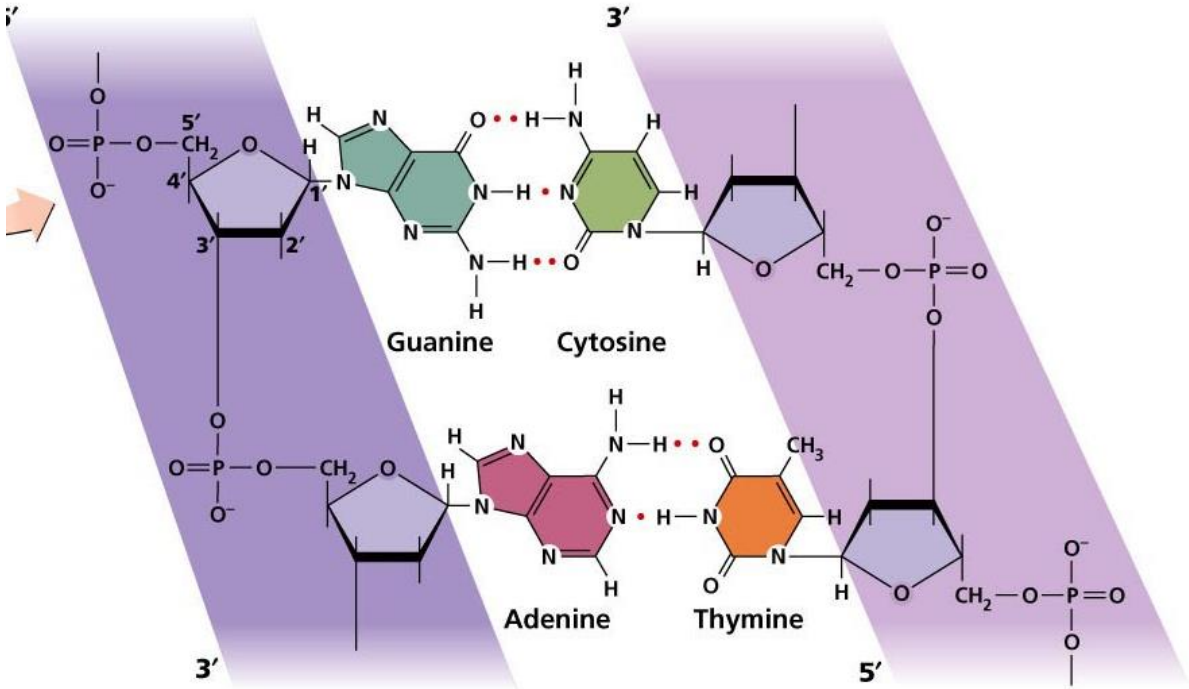




Nucleotides can pair via their bases

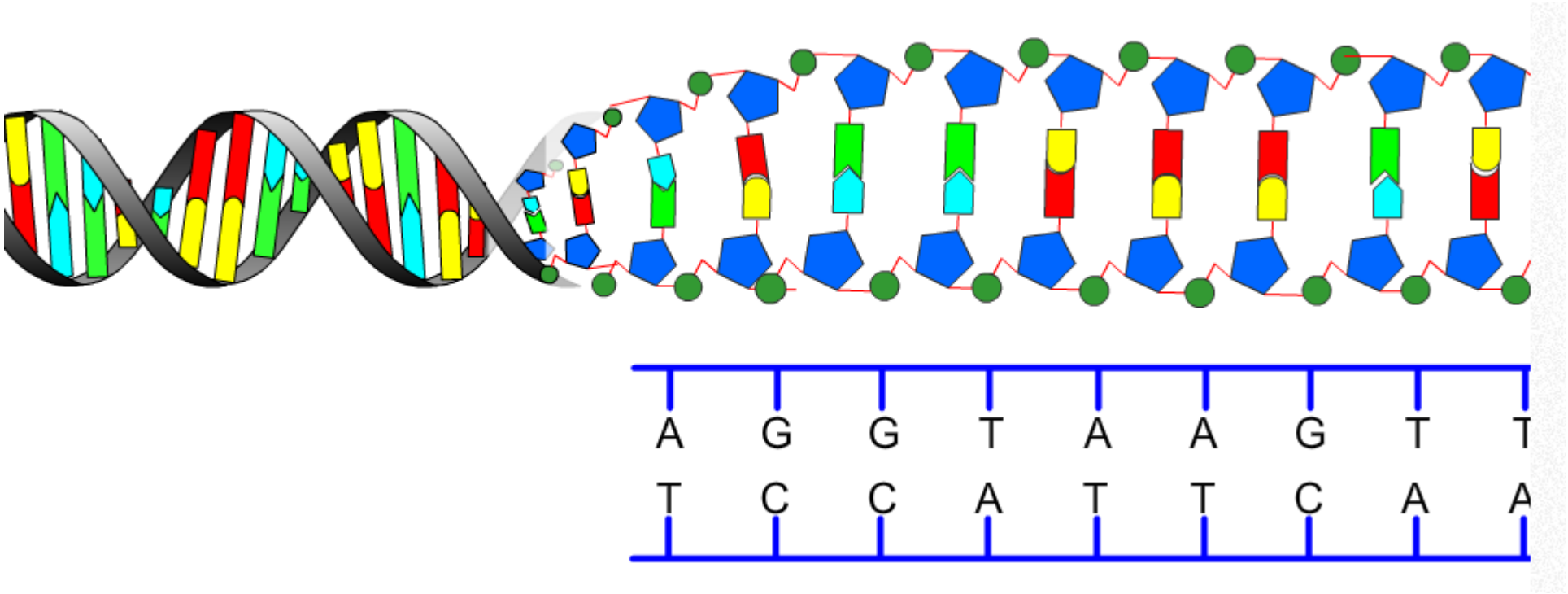






DNA consists of 2 chains of nucleotides

Double helix structure



DNA is basically a very long linear structure

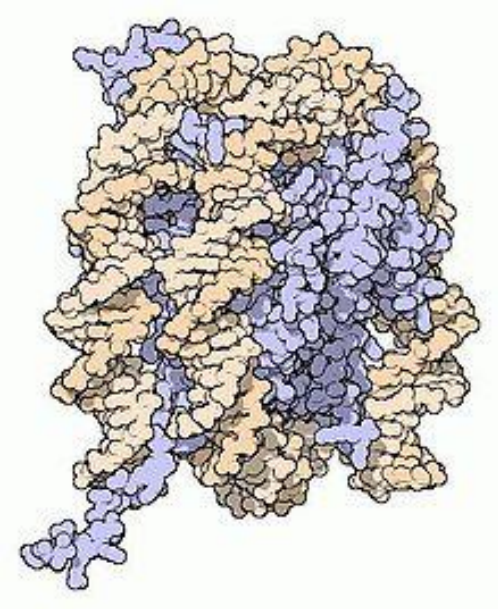
Altogether, unwind chromosomes correspond to 2 meter of “linear” DNA housed into ~4 x 10^{-18} m^3 nucleus

DNA has to be packed

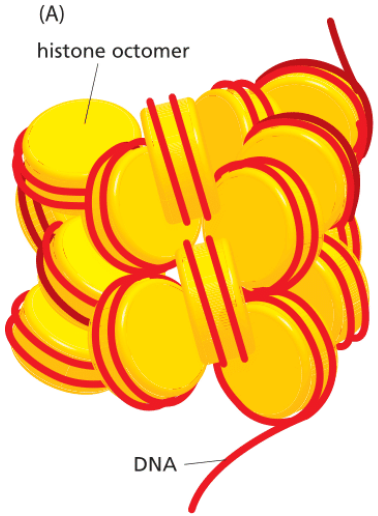
Chromosome: extremely compacted DNA

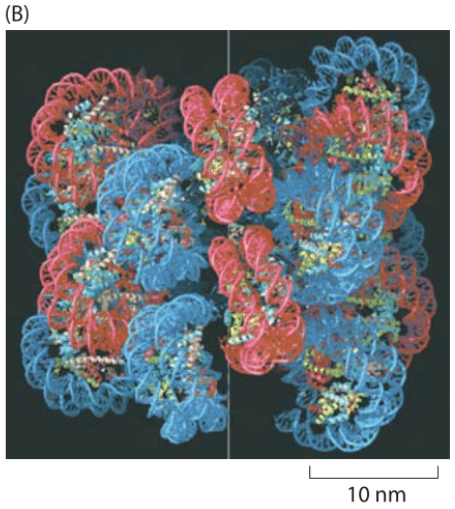
- DNA strand wound around protein complex

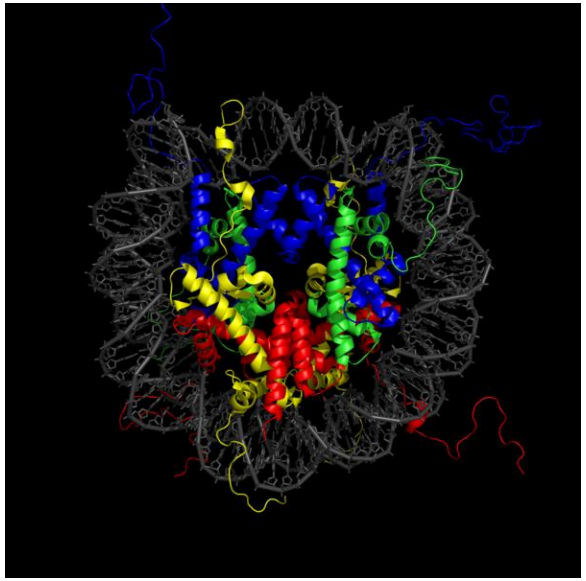
  = **nucleosome** (turn of 1 DNA chain; ± 200 bp)
  comparable to beads on a string



Nucleosome (DNA in yellow histones in blue)



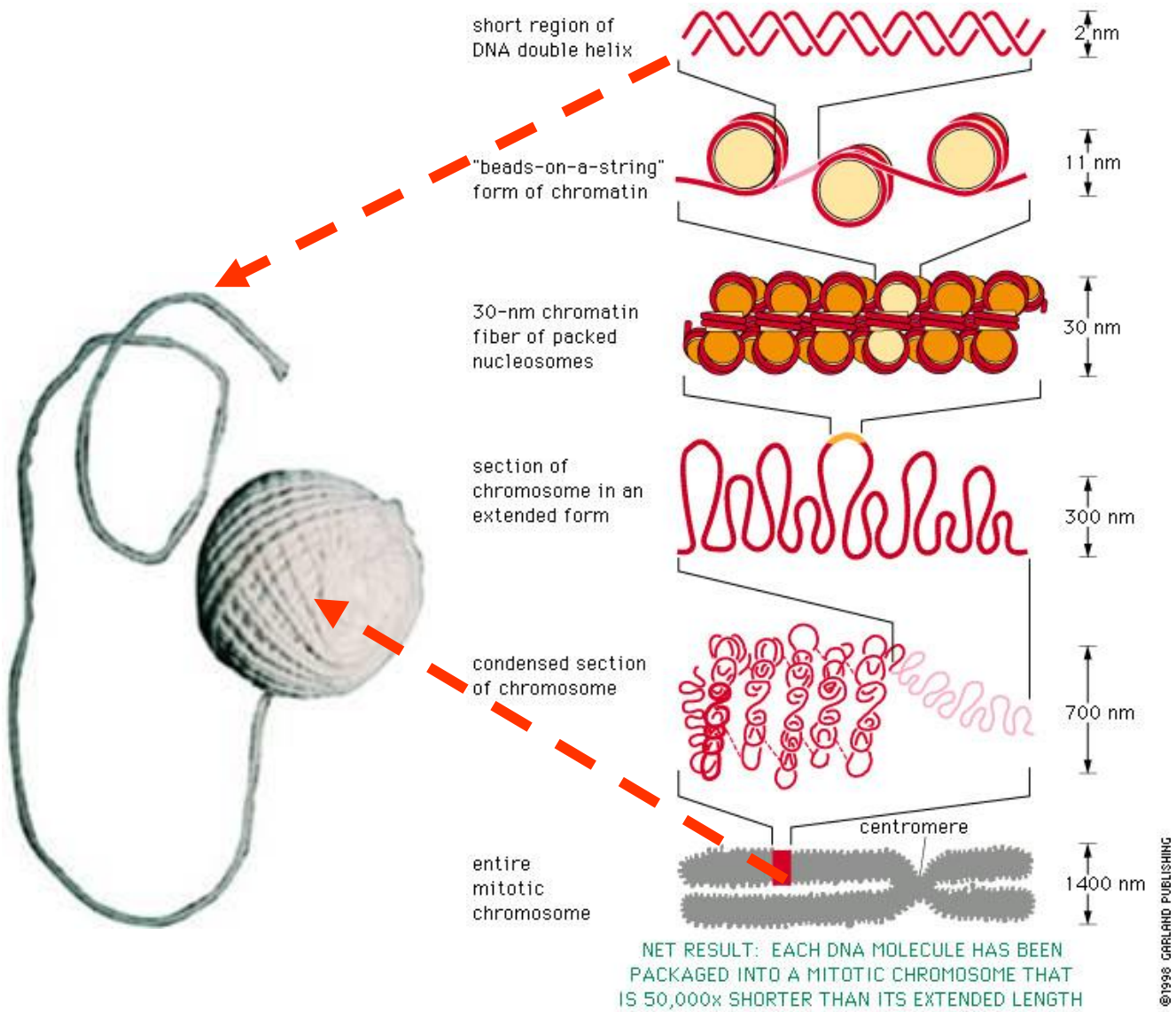




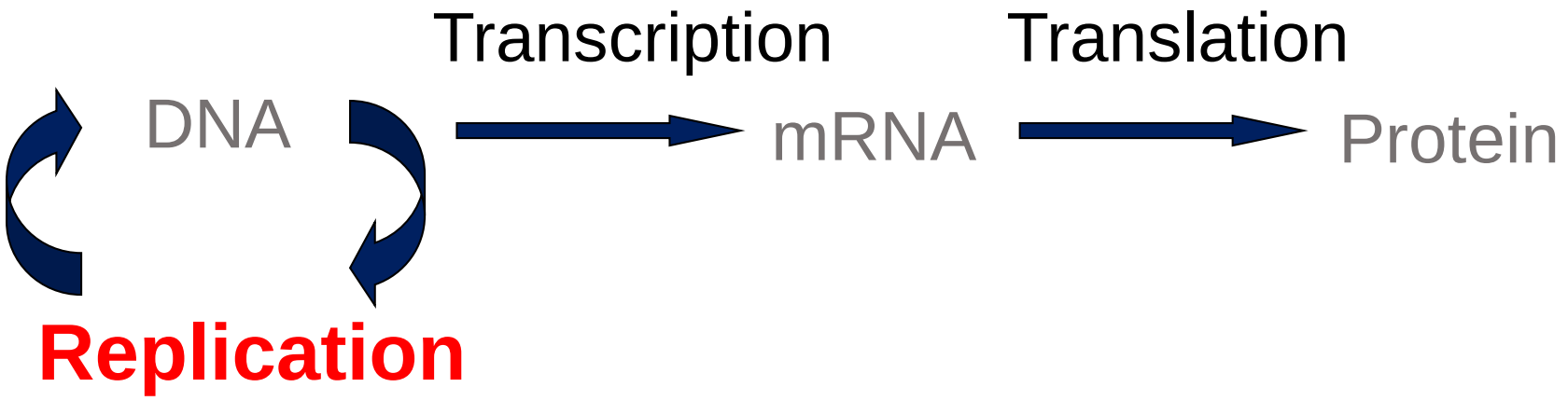
Cristal structure of nucleosome core particle consisting of histones (= proteins: H2A , H2B , H3 en H4) and DNA

Fig.: Wikipedia

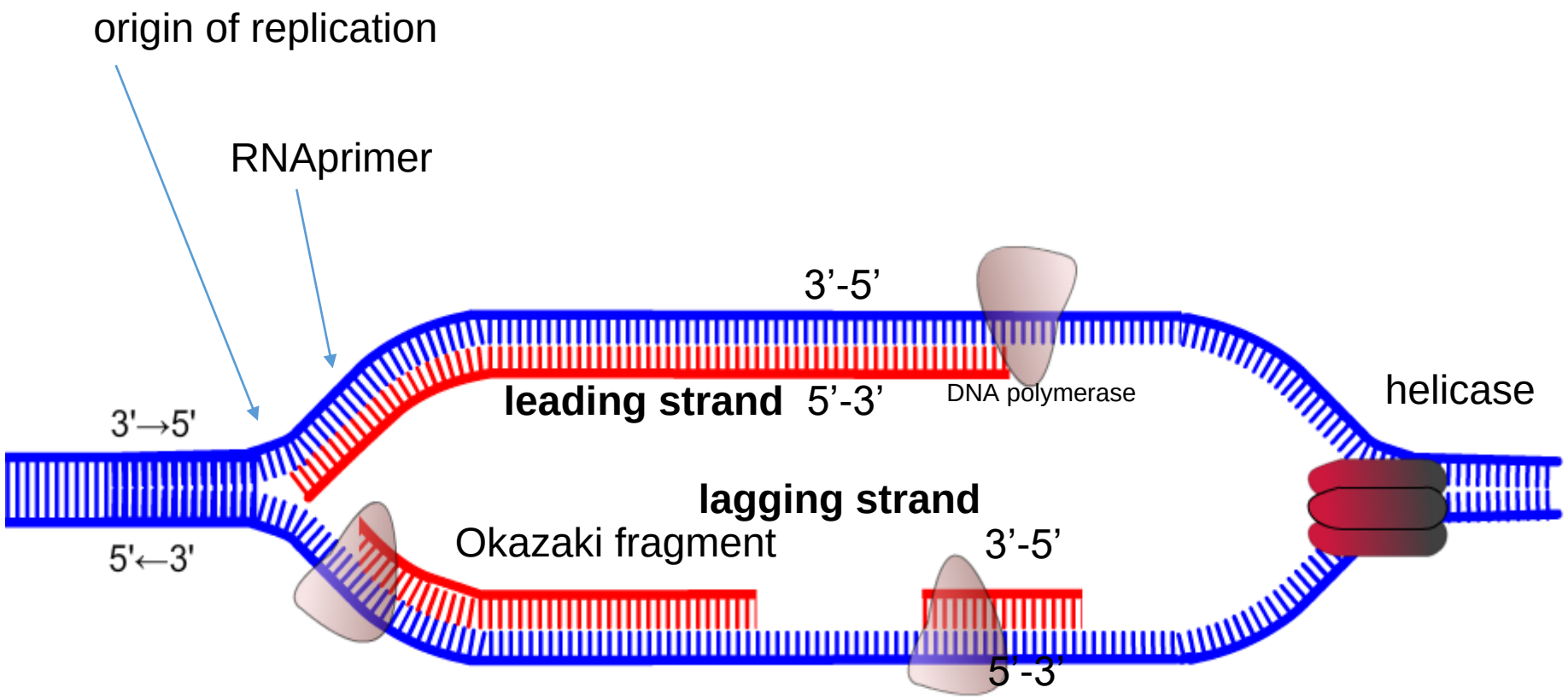
Chromatin structure



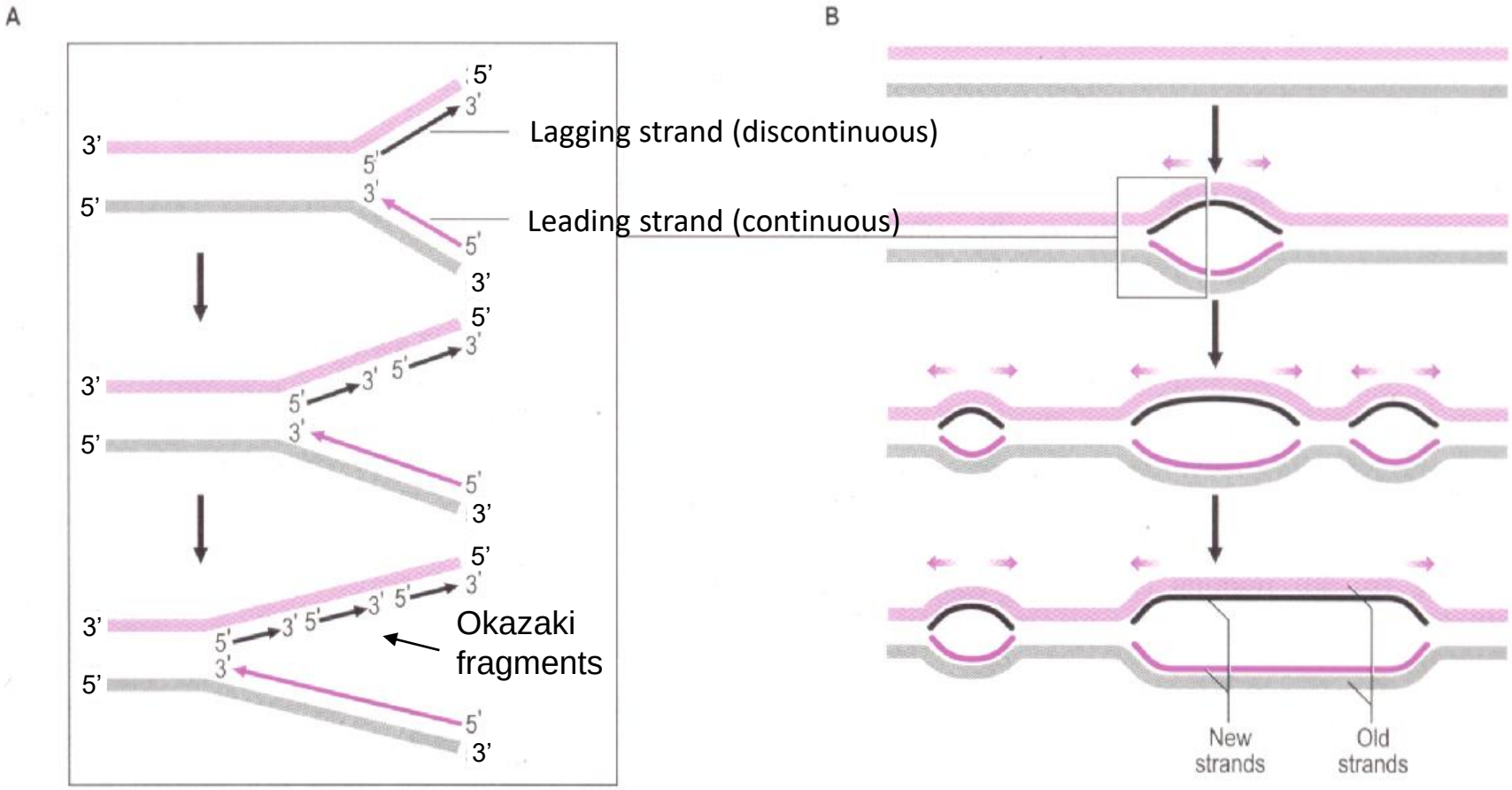
central dogma of molecular biology



DNA replication



DNA replication

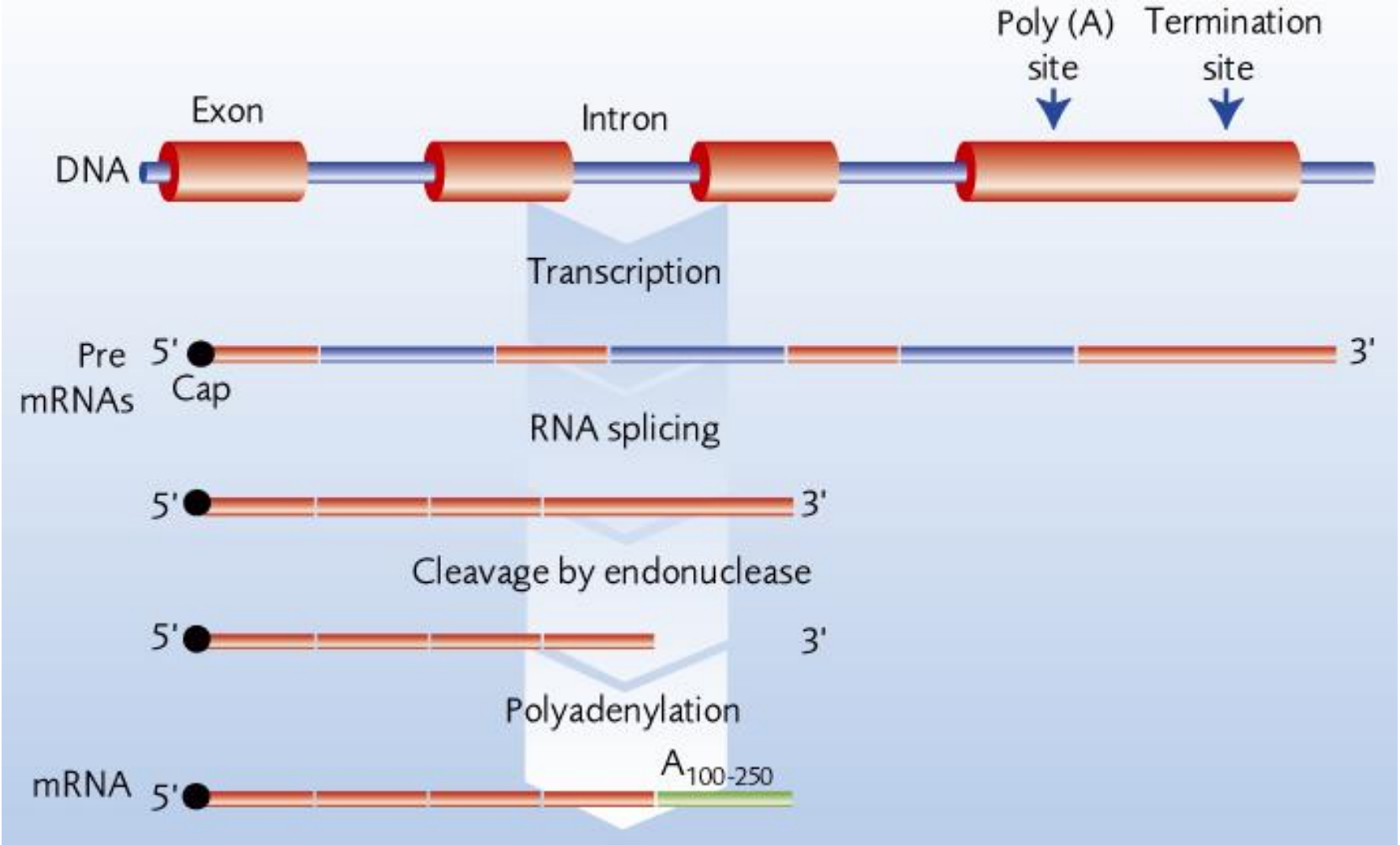


DNA replication

- During the S fase of the cell cycle DNA is copied and transmitted to 2 daughter cells
- complementary DNA strands open up at the *origins of replication* with use of DNA helicase
- DNA polymerase makes new strand
- DNA polymerase assist in 3'-5' phosphodiesterbounds
  - On 1 strand: continous process (leading strand)
  - On other strand: Okazaki fragments (lagging strand)
- DNA ligase unites Okazaki fragments
- DNA replication is *semiconservative*: 1 new strand and 1 old strand, 1 error in 10^9 copied bases

Central dogma of molecular biology

From DNA to RNA: transcription



Further: http://www.youtube.com/watch?v=WsofH466lqk

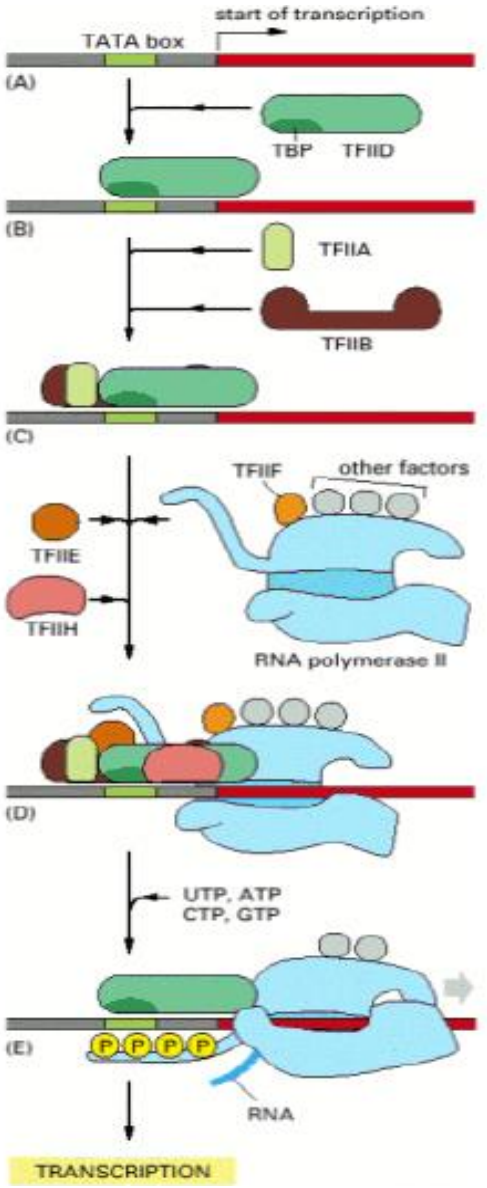
Transcription

1) binding of transcription factors in promotor region

2) binding of RNA polymerase with initiation of transcription

3) the "anti-sense strand" is read from 3'→ 5'. The complementary, non-copied DNA strand is called "sense strand" because of the resemblence with the mRNA strand

4) the RNA strand is produced in 5'→ 3' direction



Génétique moléculaire humaine – Strachan et Read 4th ed 2012

Promotor

=promotor region contains following sequences:

- TATA box (TATAAA) on position -25 à -30
- CCAAT box on position -80

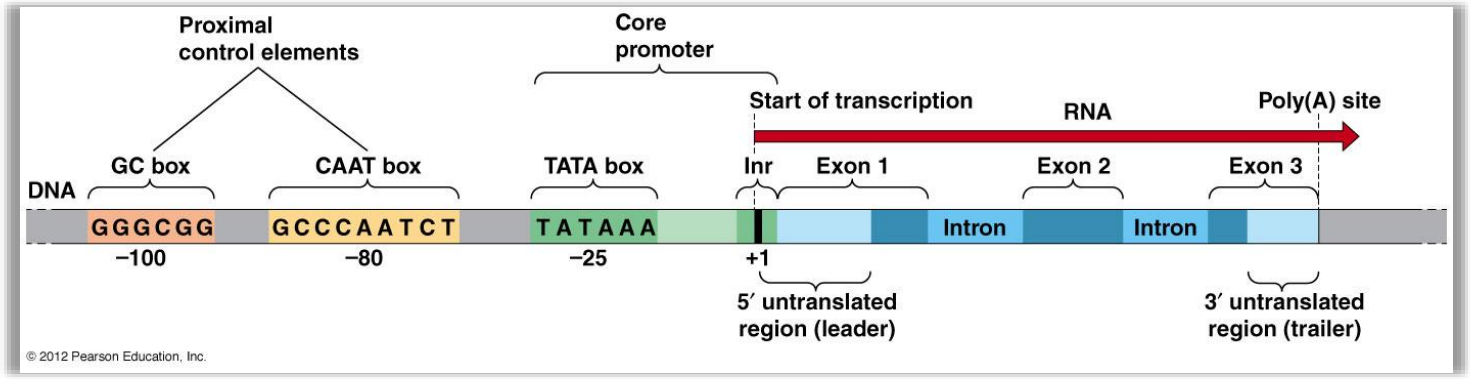
} Tissue specific genes

- GC box (GGGCGG) -100 à 150

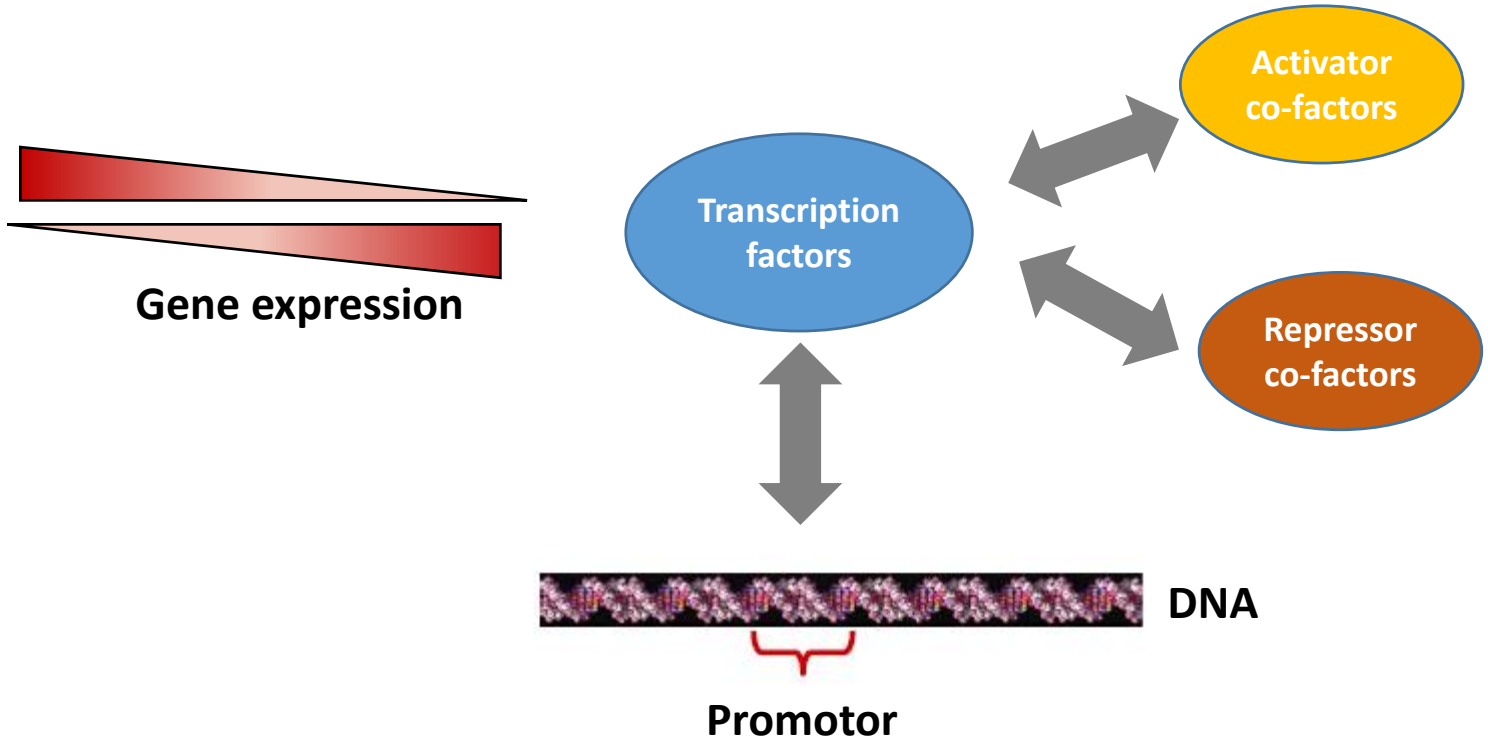
} “housekeeping” genes

- other enhancers further upstream

cis-acting elements ⟺ Transcription factors
trans-acting elements

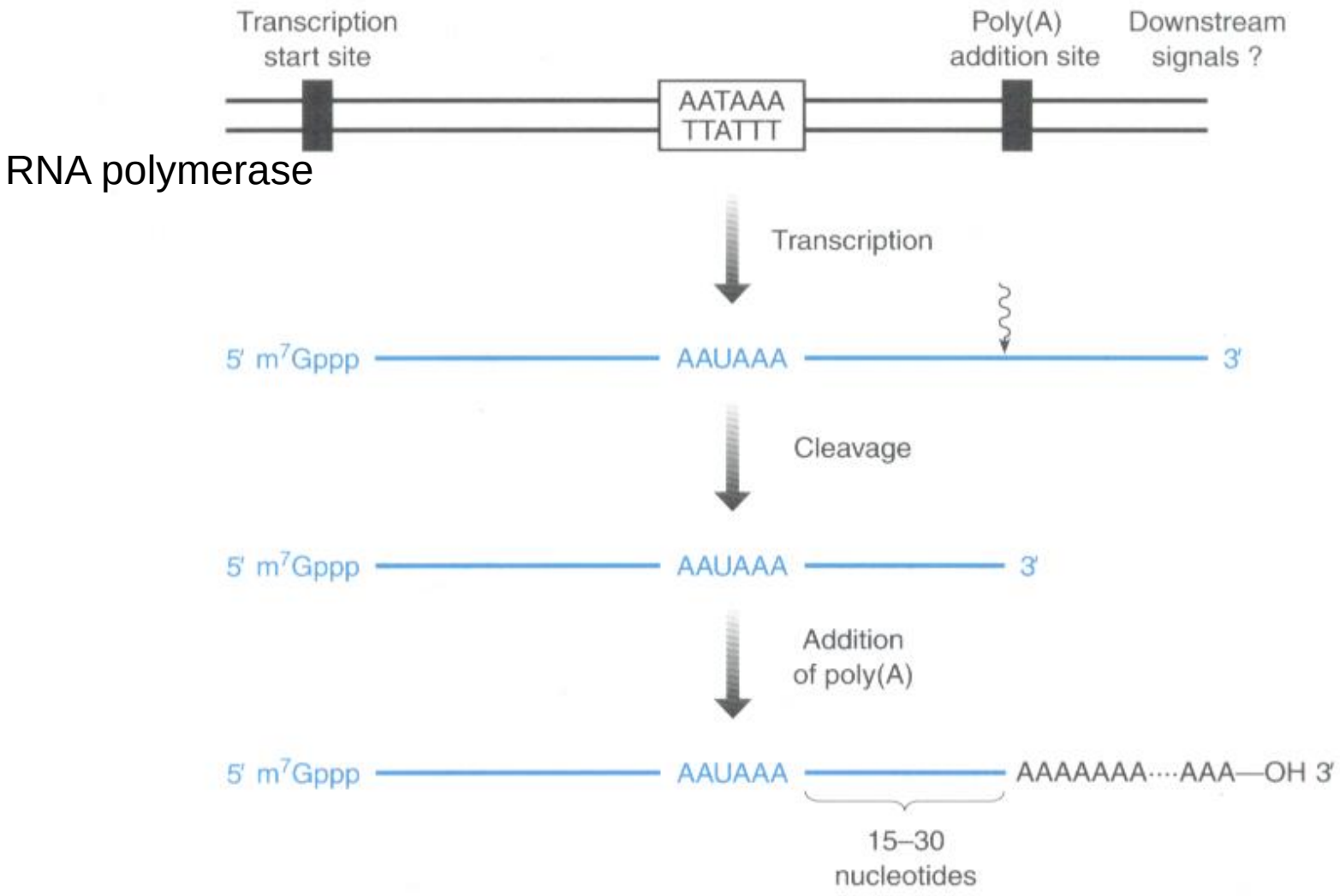


Gene expression is a tightly regulated process



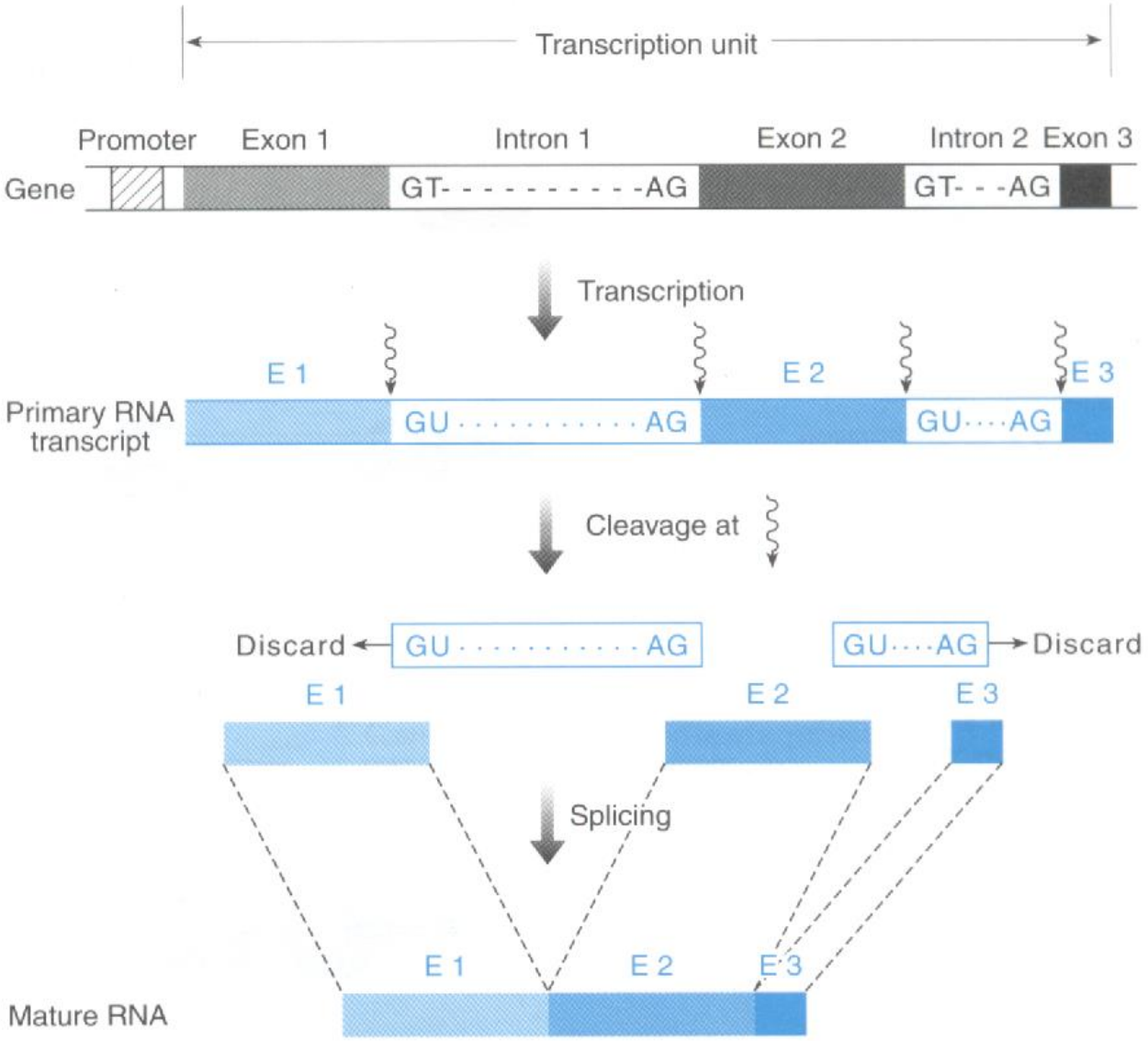
Post-transcriptional modifications of mRNA

1) capping and 2) polyadenylation

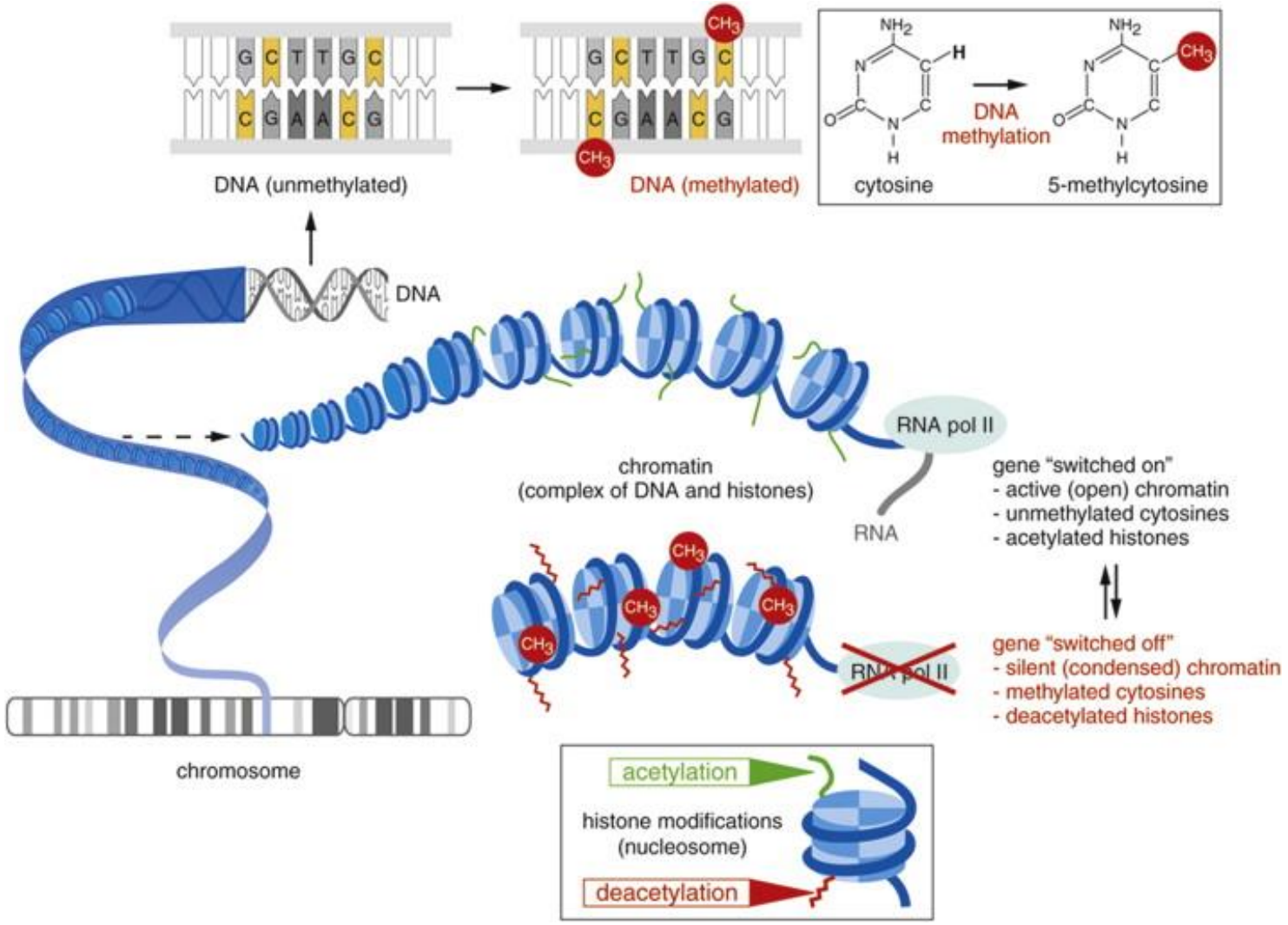


Posttranscriptional modifications of mRNA

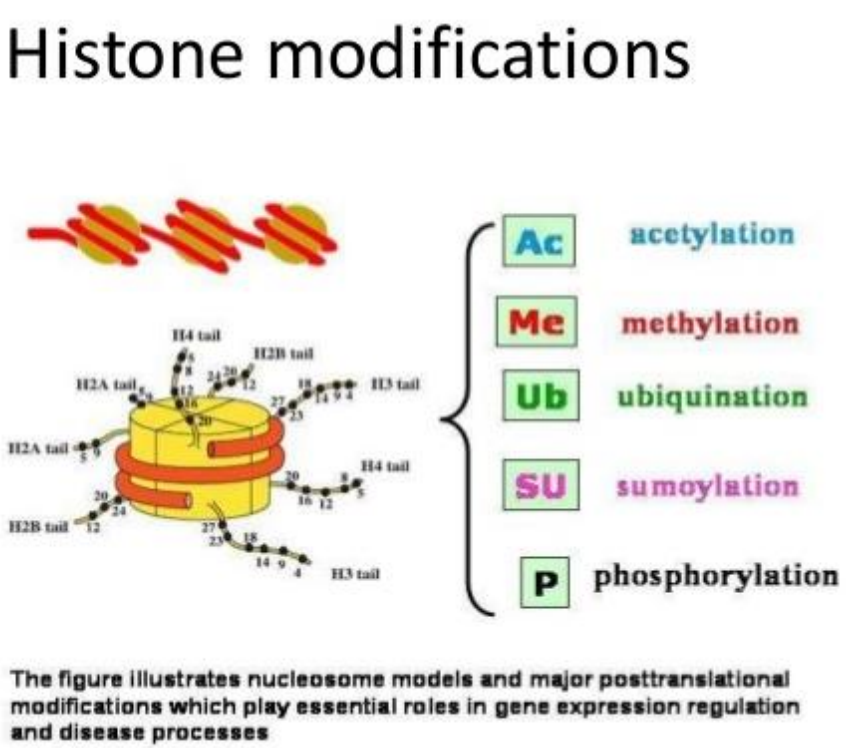
3) RNA splicing



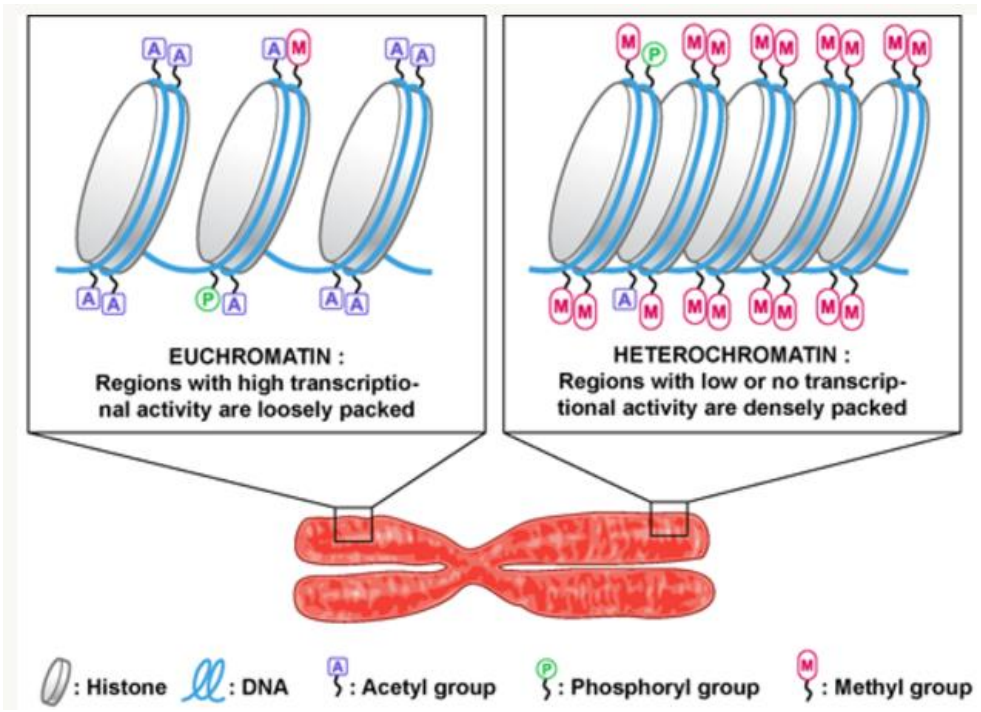
Epigenetic alterations



Epigenetic alterations



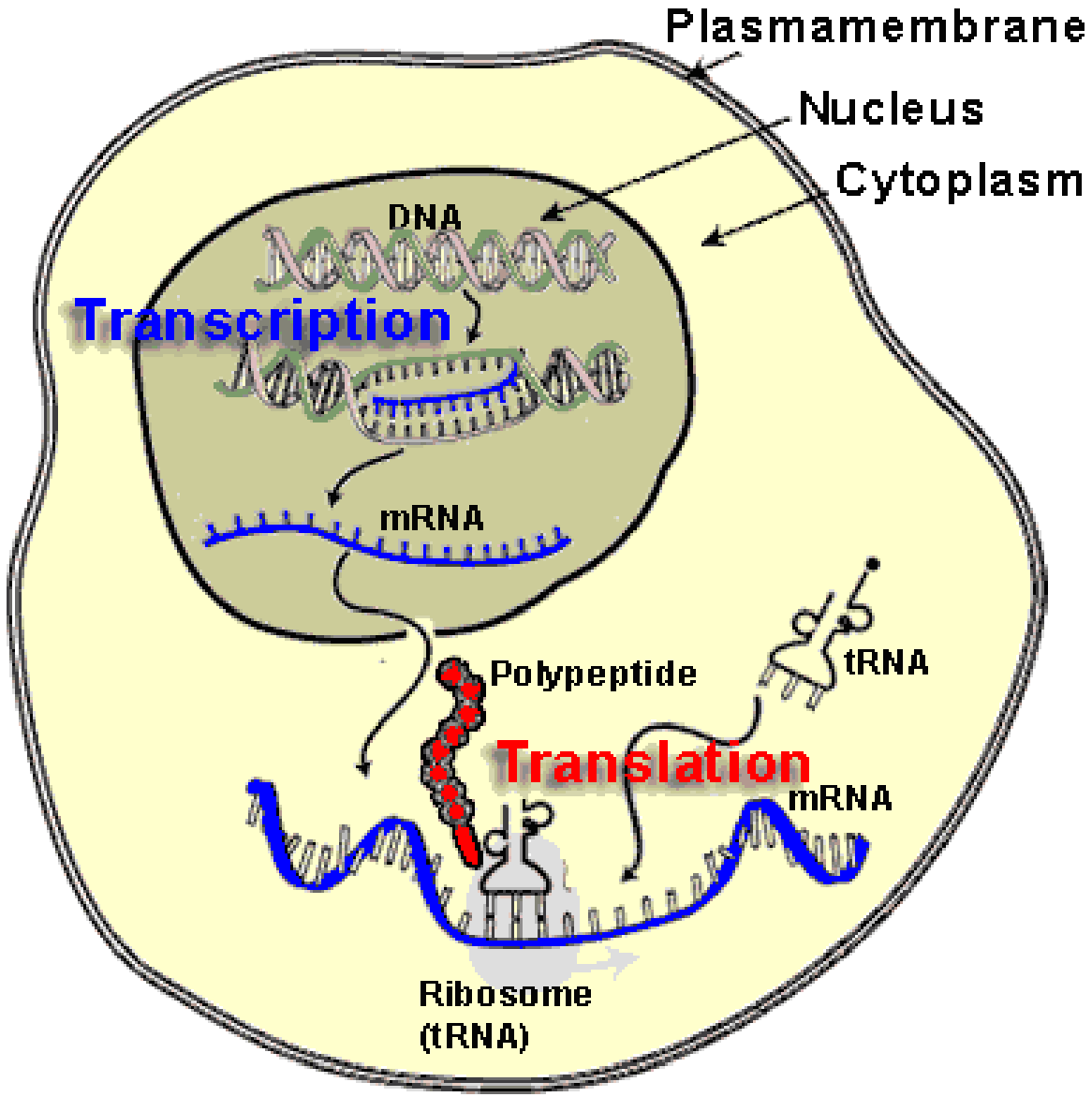
The figure illustrates nucleosome models and major posttranslational modifications which play essential roles in gene expression regulation and disease processes



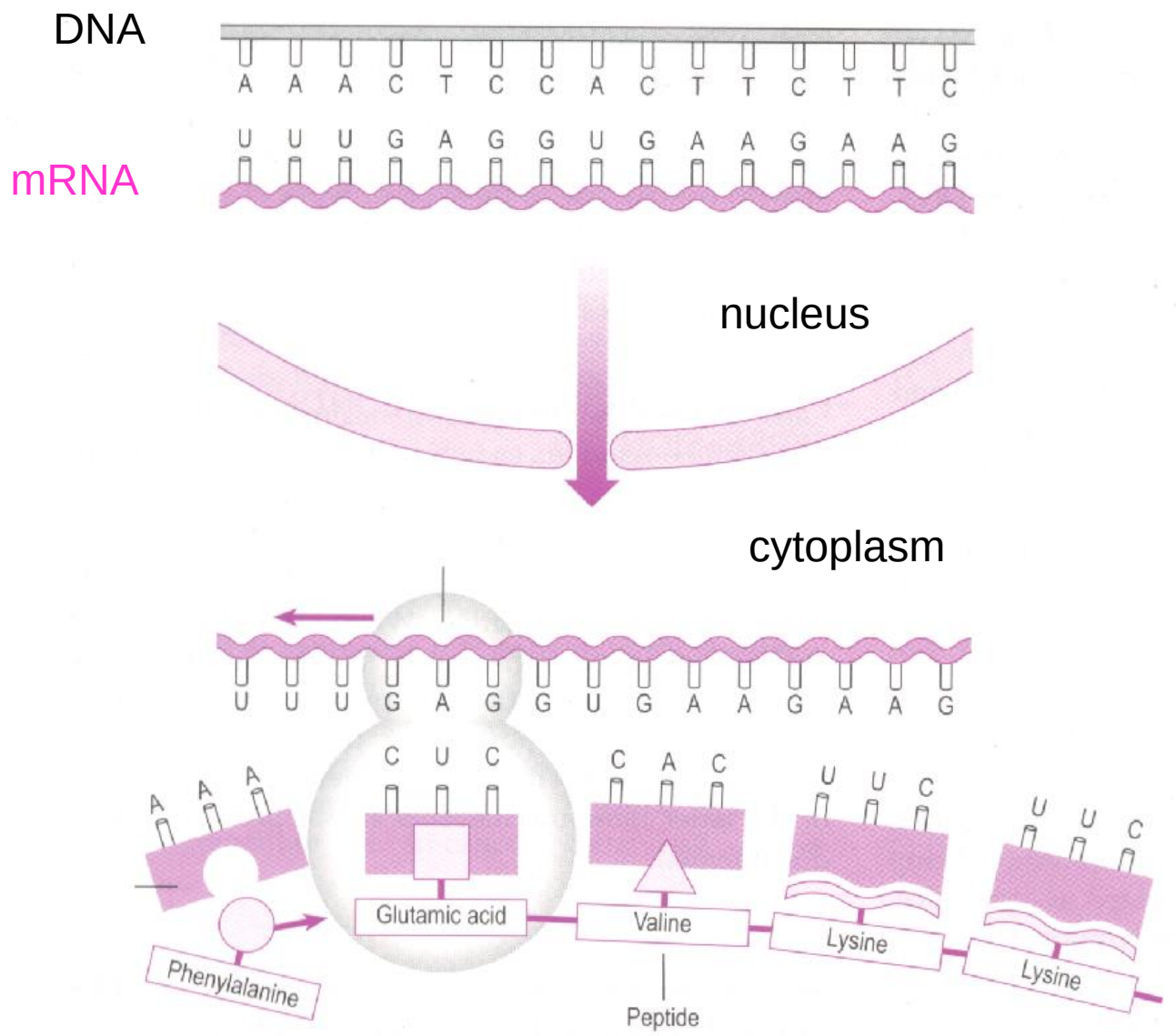
Modification	Structure	Charge	Effect
Methylation	R-CH_3	Neutral	↑ packaging
Acetylation	R-$COCH_3$	Negatif	↓ packaging
Phosphorylation	R-PO_4	Negatif	↓ packaging

Central dogma of molecular biology

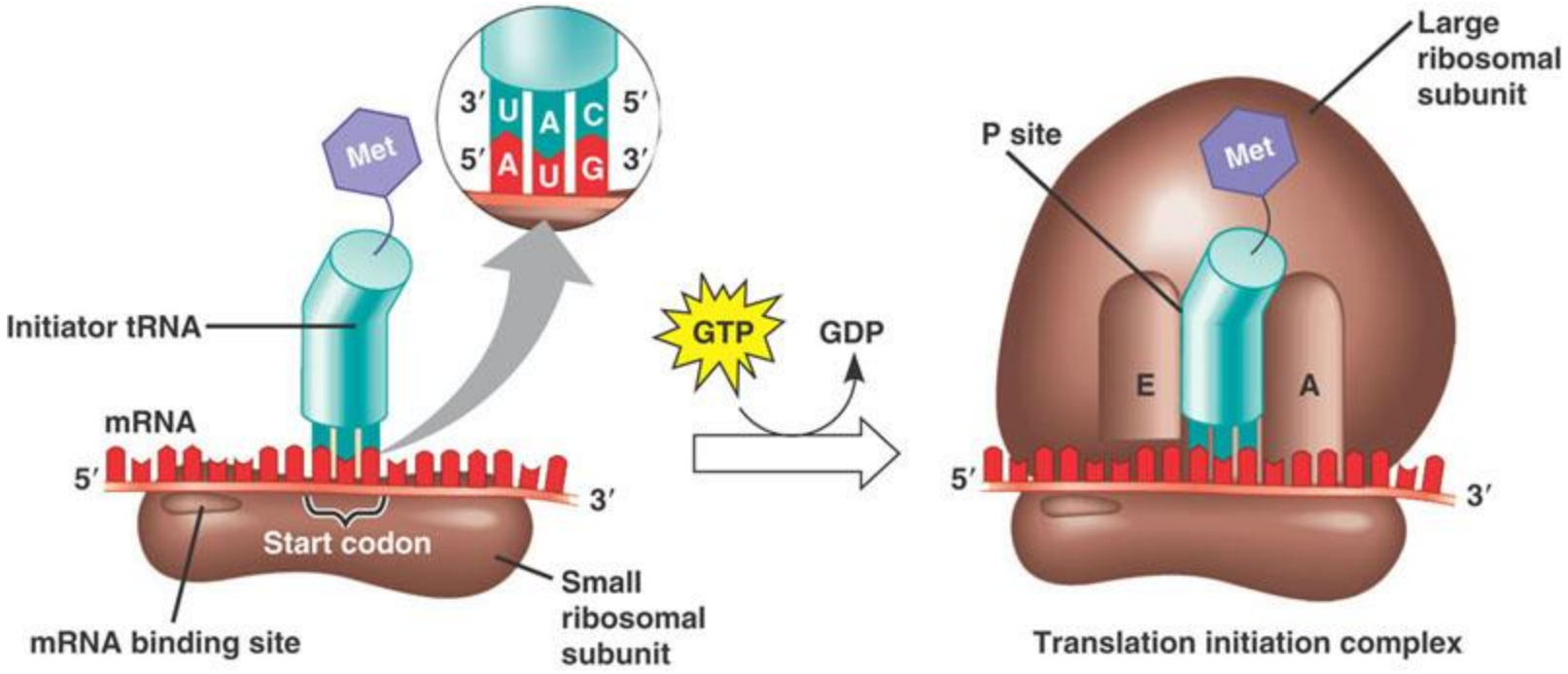
From RNA to protein: translation



Translation



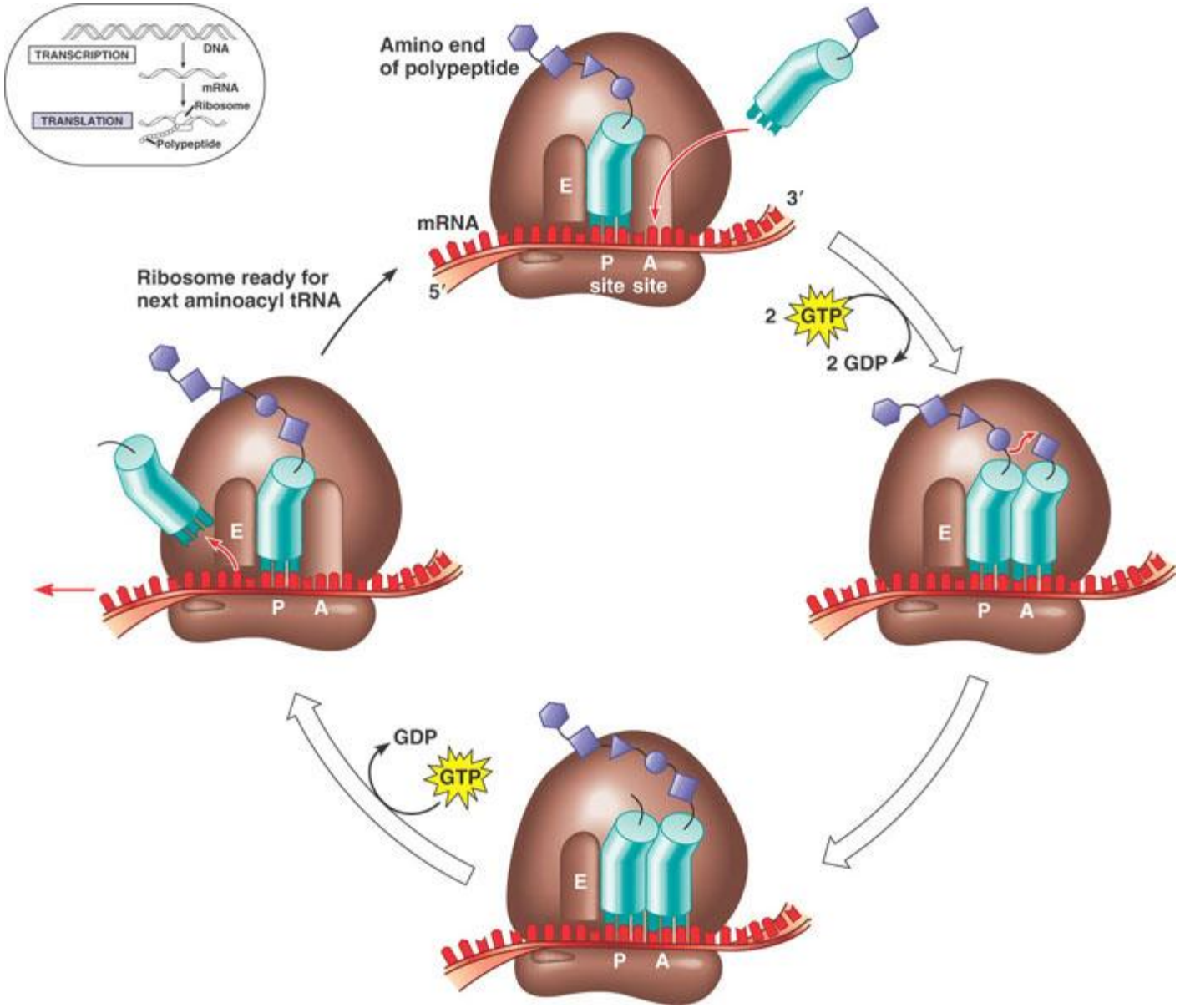
Initiation of translation



http://kvhs.nbed.nb.ca/gallant/biology/translation_initiation.html

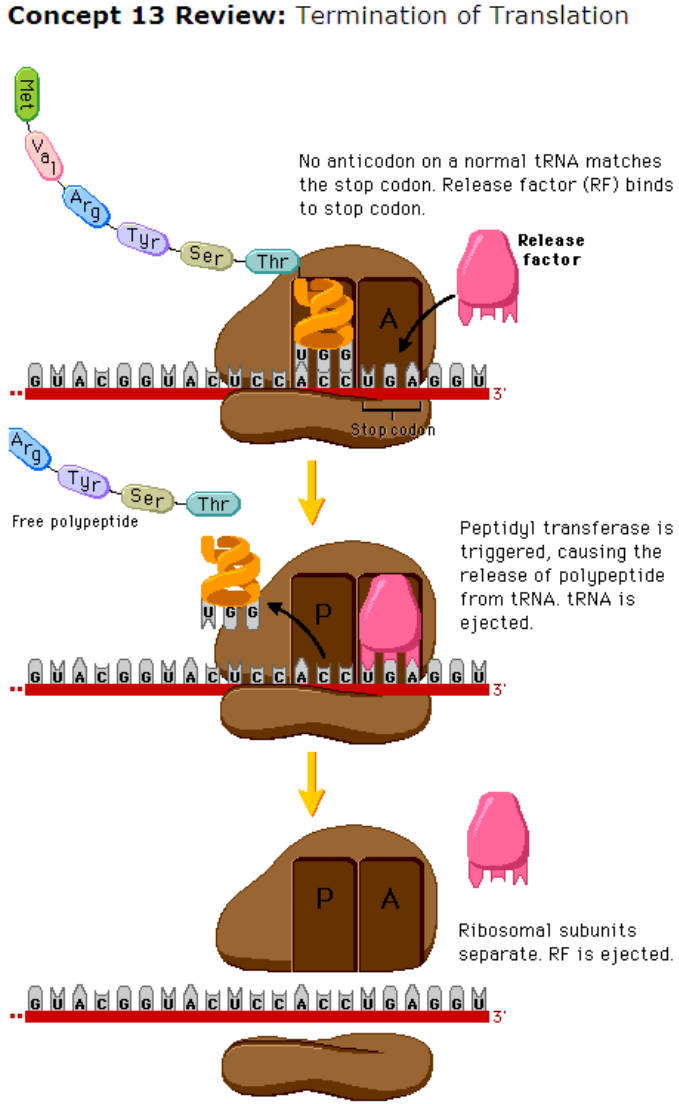
http://www.allesoverdna.nl/woordenboek/transcriptie.html

Elongation polypeptide chain



http://www.bio.miami.edu/dana/250/250SS13_9print.html

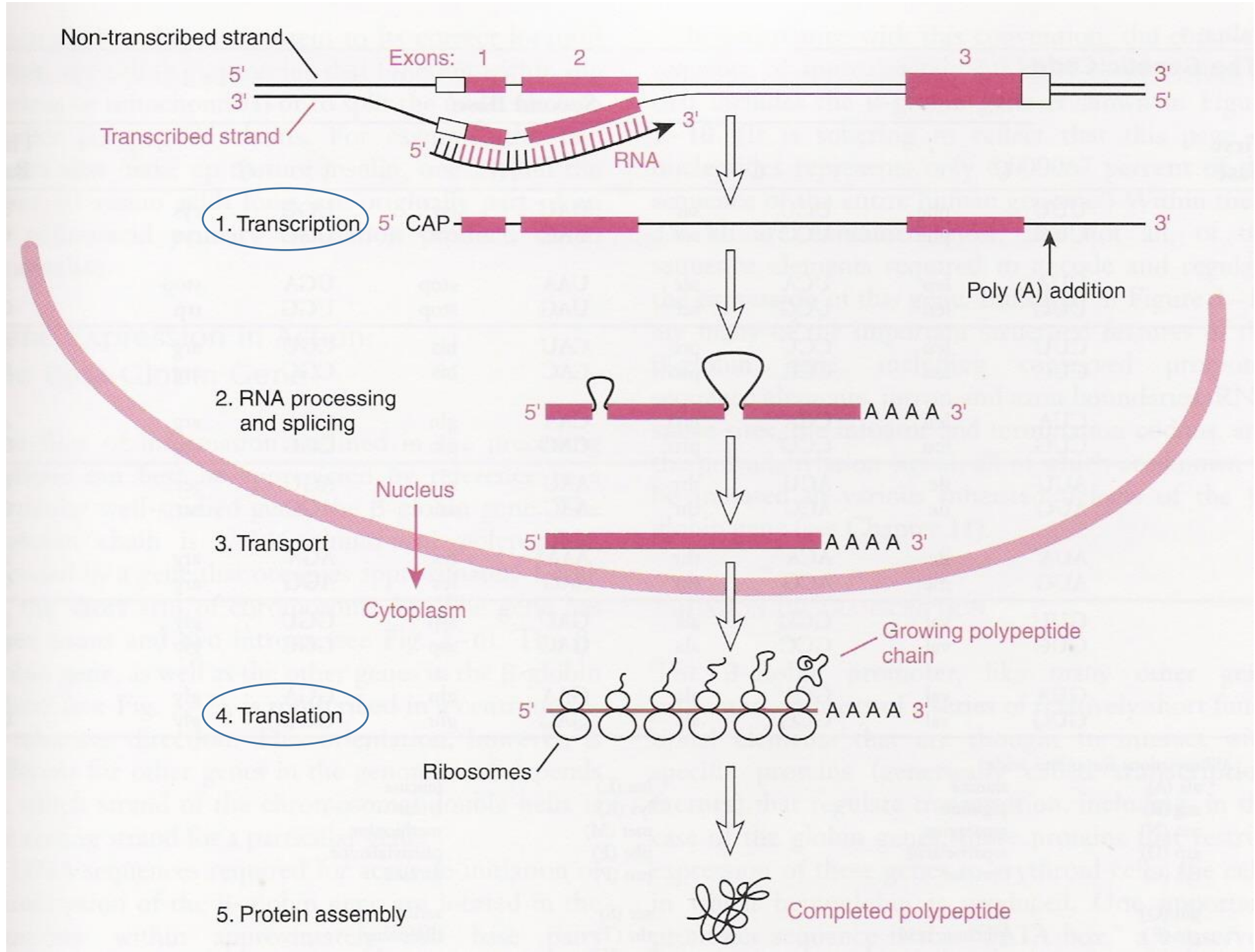
Termination of translation



http://www.phschool.com/science/biology_place/biocoach/translation/term.html

Translation

- mRNA is transcribed from 5’ to 3’ by ribosomes
- per codon (3 bases) an amino acid is incorporated into the carboxyterminal side of the growing protein chain
- codon on mRNA is recognized by anticodon on the tRNA
- the genetic alfabet consists of 4 letters (A;T;G;C); and there are 20 different amino acids (AA).
  Thus: 4^3 possible codons for 20 AA: ‘*the genetic code is degenerate*’
- 3 stopcodons (TAA, TAG, TGA)
- 1 startcodon (ATG = methionine)
- only 1 codon for tryptophan and methionine


Non-transcribed strand
5'
3'
Transcribed strand
Exons: 1
2
3
3'
5'
5'
3'
RNA
1. Transcription
5' CAP
3'
Poly (A) addition
2. RNA processing and splicing
5'
A A A A 3'
Nucleus
3. Transport
5'
A A A A 3'
Cytoplasm
Growing polypeptide chain
4. Translation
5'
A A A A 3'
Ribosomes
5. Protein assembly
Completed polypeptide

Take home messages

- The DNA is composed of 4 nucleotides
- Chromosomes are condensed (mitotic) state of the DNA
- Non-covalent chemical forces between the nucleotides and interaction with proteins determine the well-organized DNA structure
- 1-5% of the human genome code for proteins
- Each chromosome and gene comes in pairs
- Gene expression is tightly controlled either by DNA topology and/or proteins
- Cell fate/differentiation and function is governed by gene expression patterns
- Modification in the DNA structure or abnormal gene expression might lead to disease