

Nucleus

- Structure and function

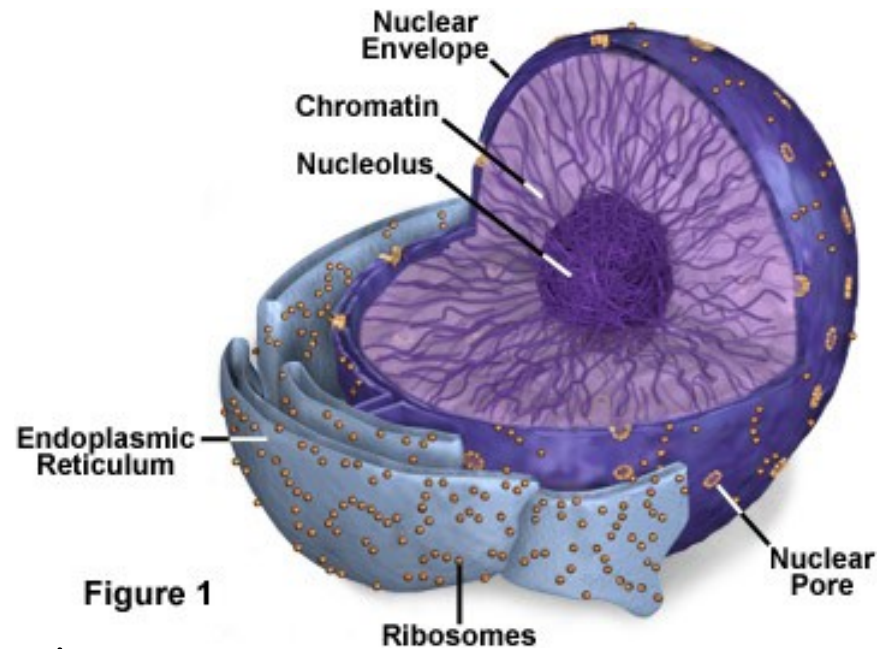
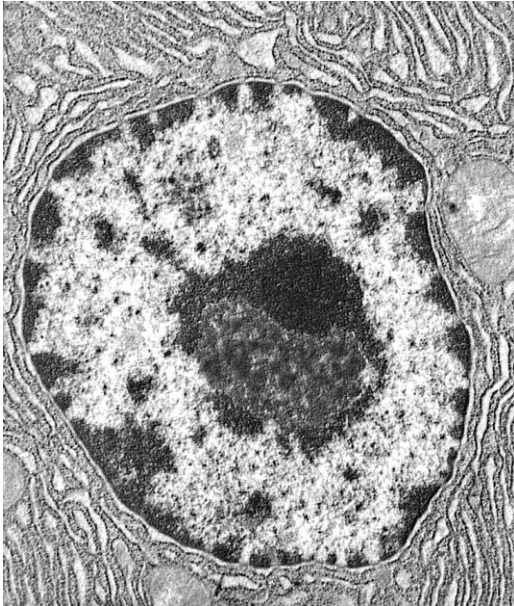


Figure 1

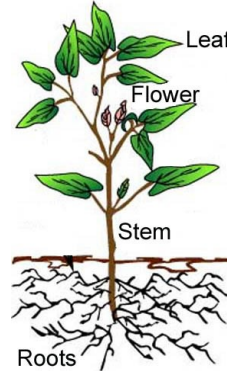
Prof. Dr. László Kőhidai
Dept. Genetics, Cell- and Immunobiology
Semmelweis University
2023.

Founders of „Cell Theory“(1838-39)

Descriptor for nucleus (1833)



**Robert Brown
(1773-1858)**



**Matthias Schleiden
(1804-1881)**

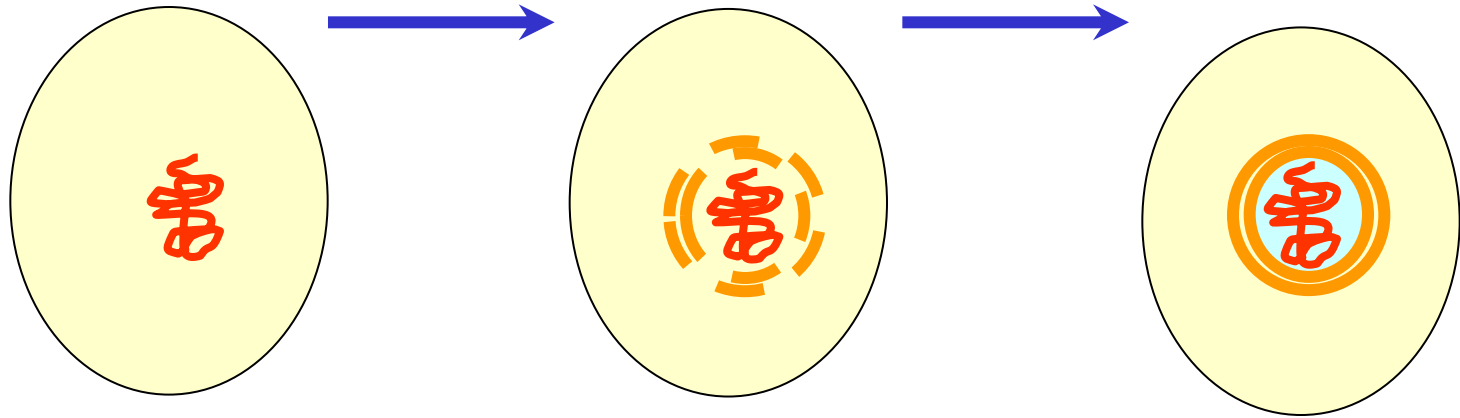


**Theodor Schwann
(1773-1858)**

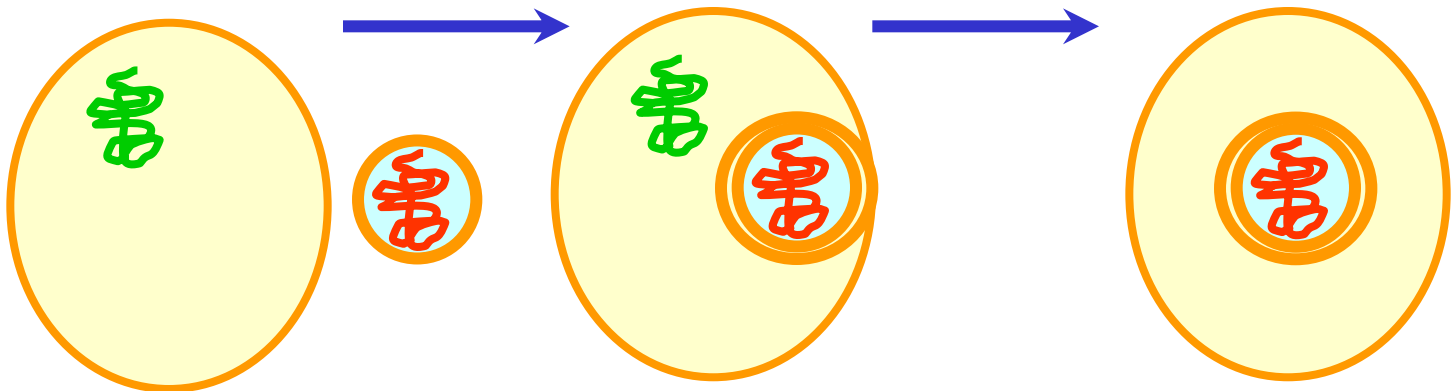


Development of nucleus - Theories

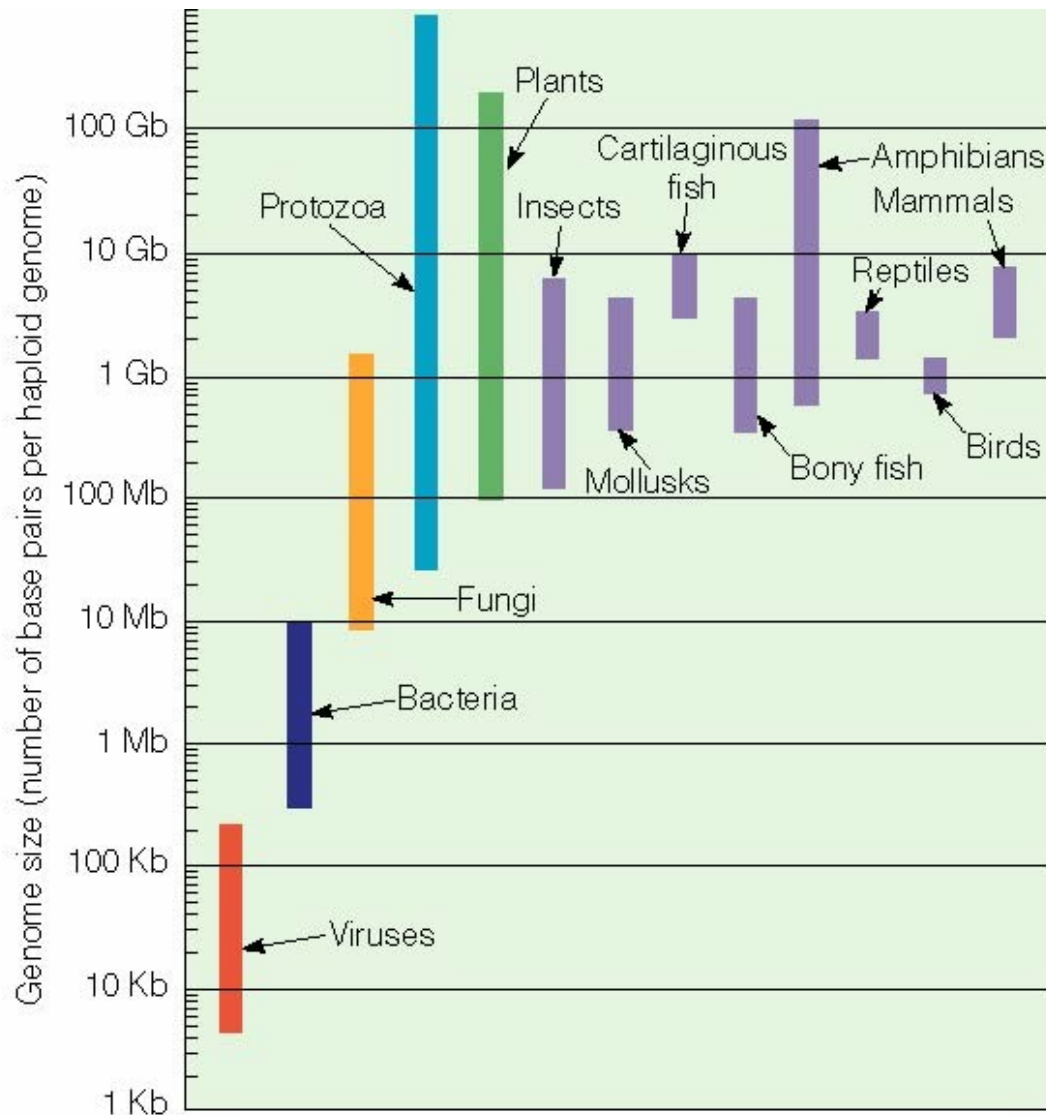
Karyogene theory



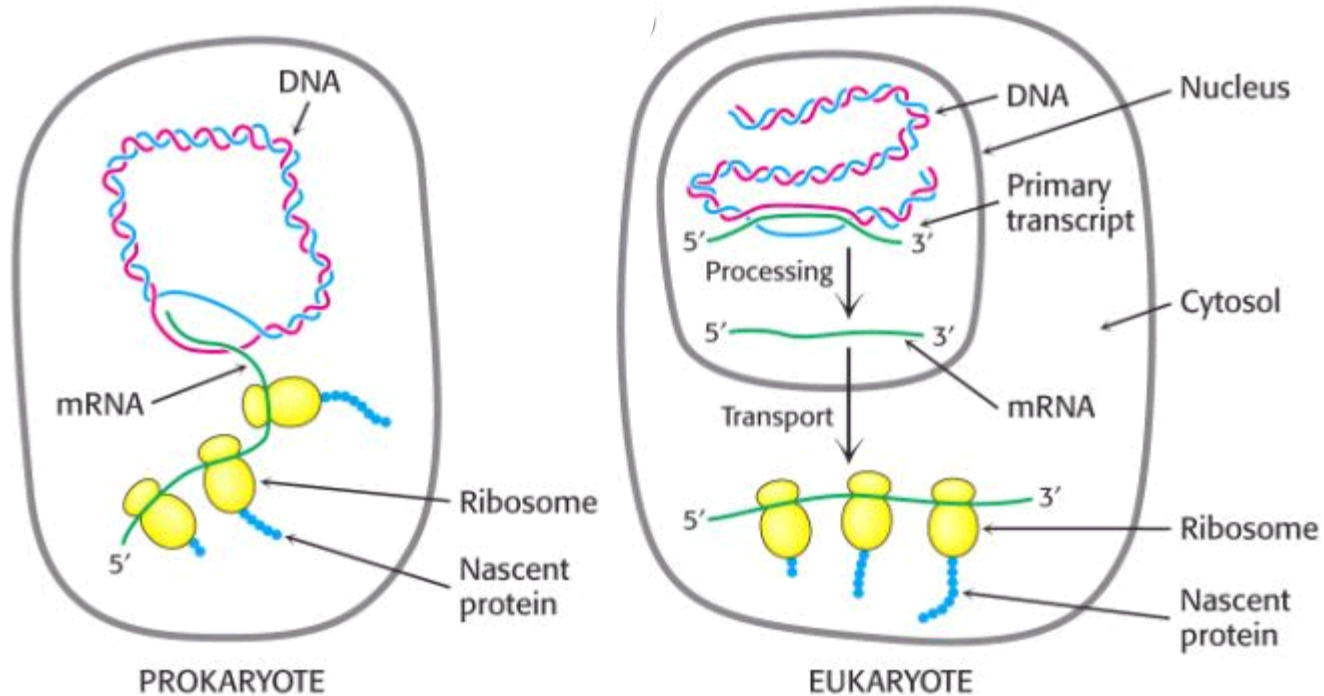
Endokaryotic etheory



Genome size change during evolution



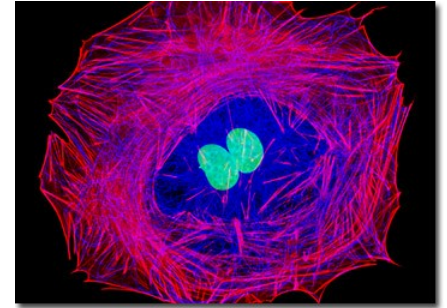
Compartmentalization



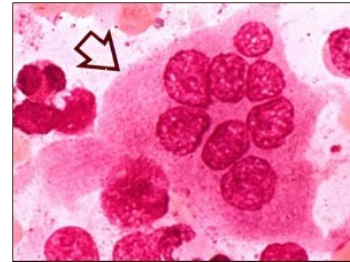
- Transcription and translation isolated
- Complex regulatory mechanisms isolated in time appear in the cell

Structure of nucleus 1

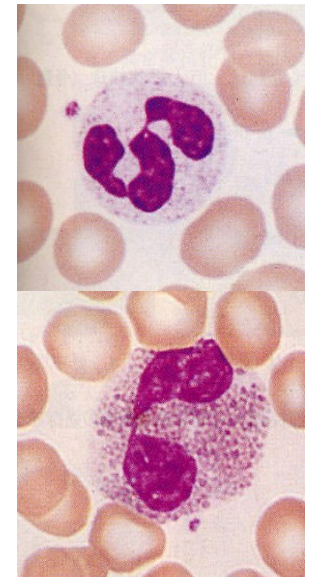
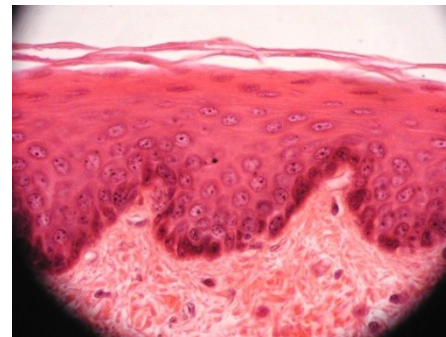
Localization: - in the centre of cell
- follow the function
(e.g. glands, skeletal m.)



Number: - 1 - 2 - many

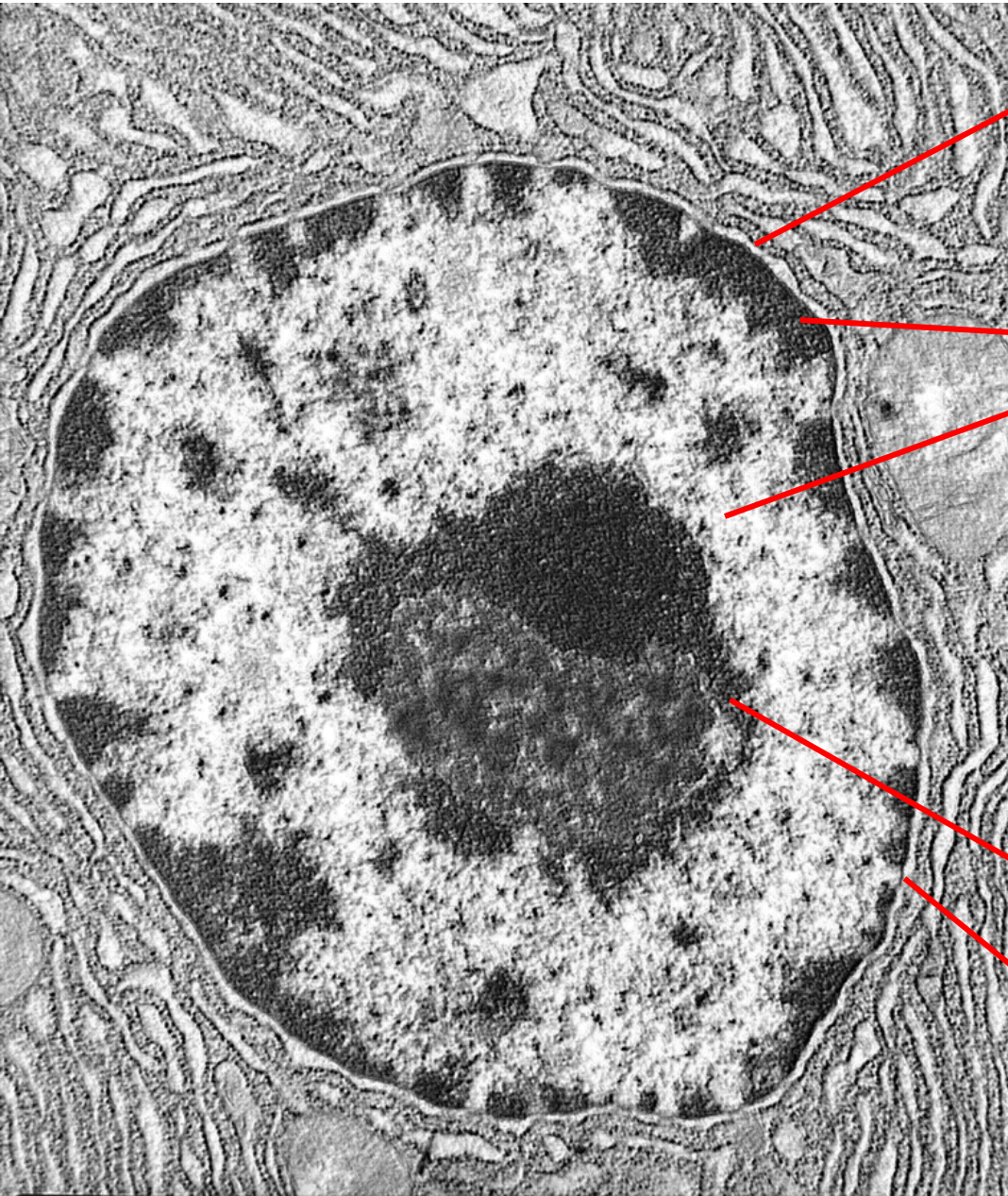


Shape: - spheric
- flattened
- rod
- segmented
- „garland“



Size: 5-10 μm

Structure of nucleus 2



Nuclear membrane

Chromatin

(DNA+proteins)

- heterochromatin
- euchromatin

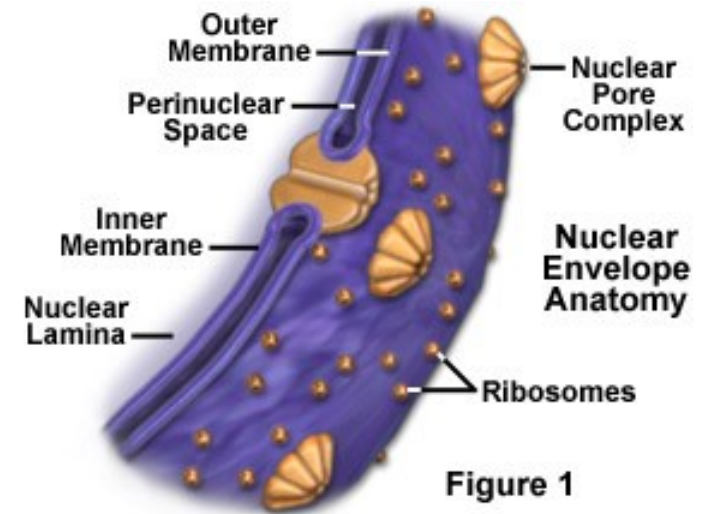
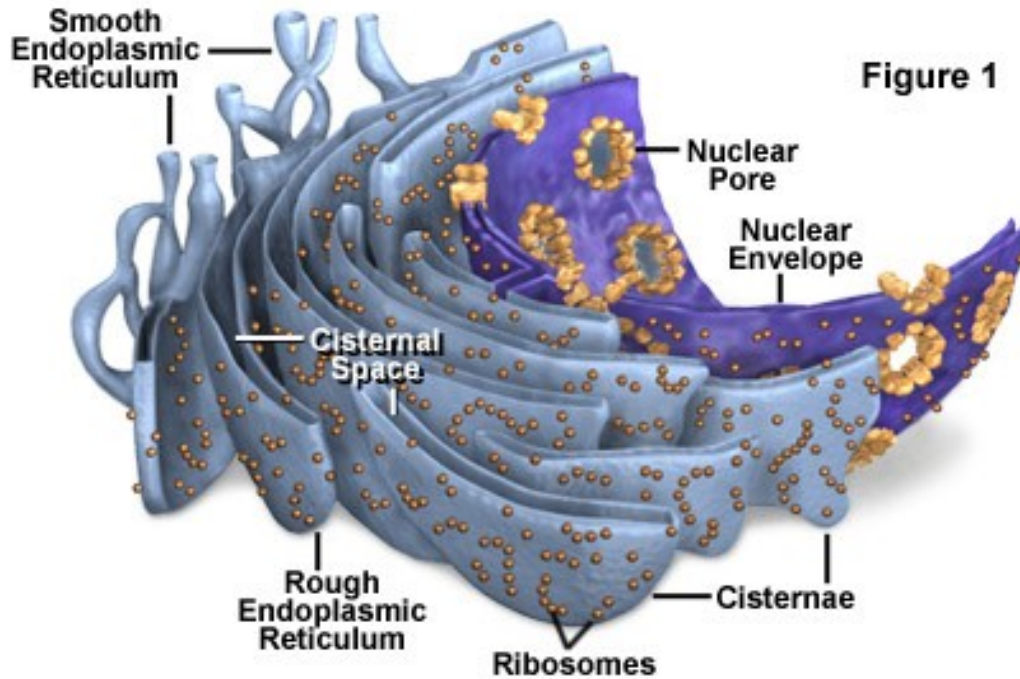
Interchromatin

- nuclear matrix-fibrillar elements
- nucleoplasm (karyolymph)
- nucleoskeleton
- macromolecular complexes

Nucleolus

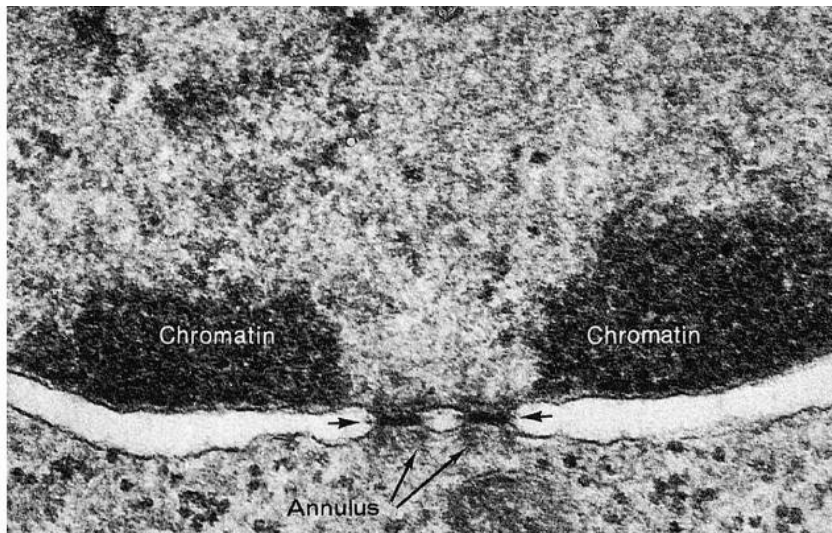
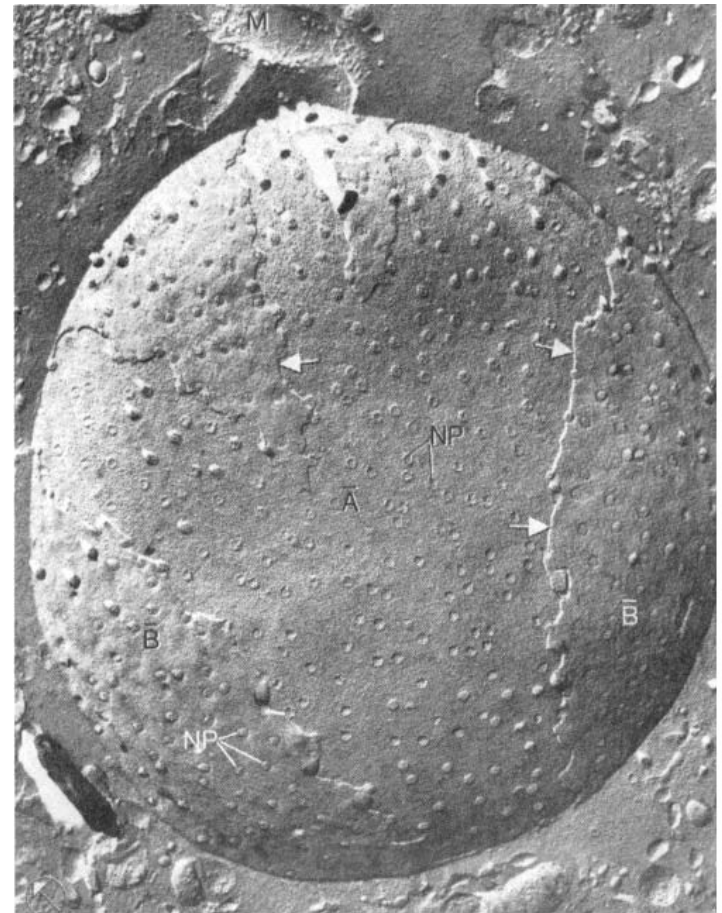
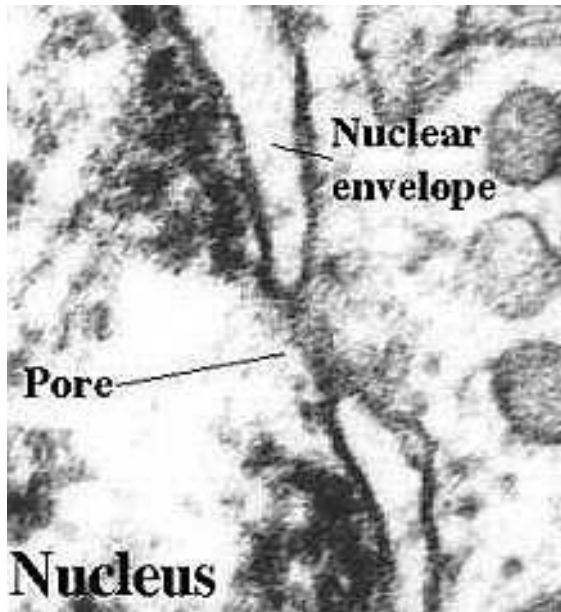
Nuclear pore complexes

Nuclear membrane



- double membrane
- diverse composition
- outer membrane \Rightarrow rER (ribosomes)
- perinuclear space
- inner membrane \Rightarrow skeletal components
anchored chromatine

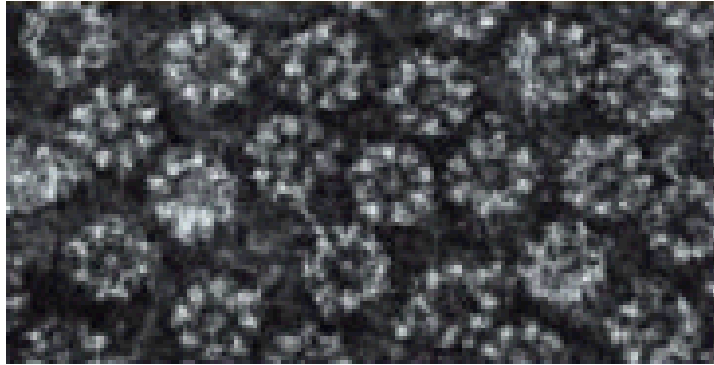
Nuclear pores



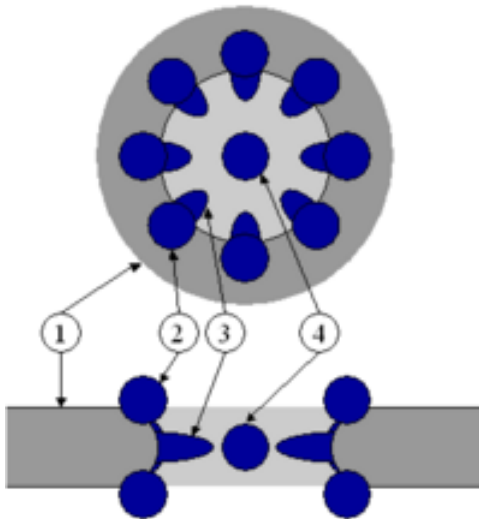
cytochemistry.net/cell-biology/Medical/03_005.jpg
(TEM)

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(freeze fracturing = FF)



Nuclear pores



Channels:
1 central
8 eripheral

Composition of nuclear pores



More than 100 nucleoporin proteins (Nups)

Ring structure

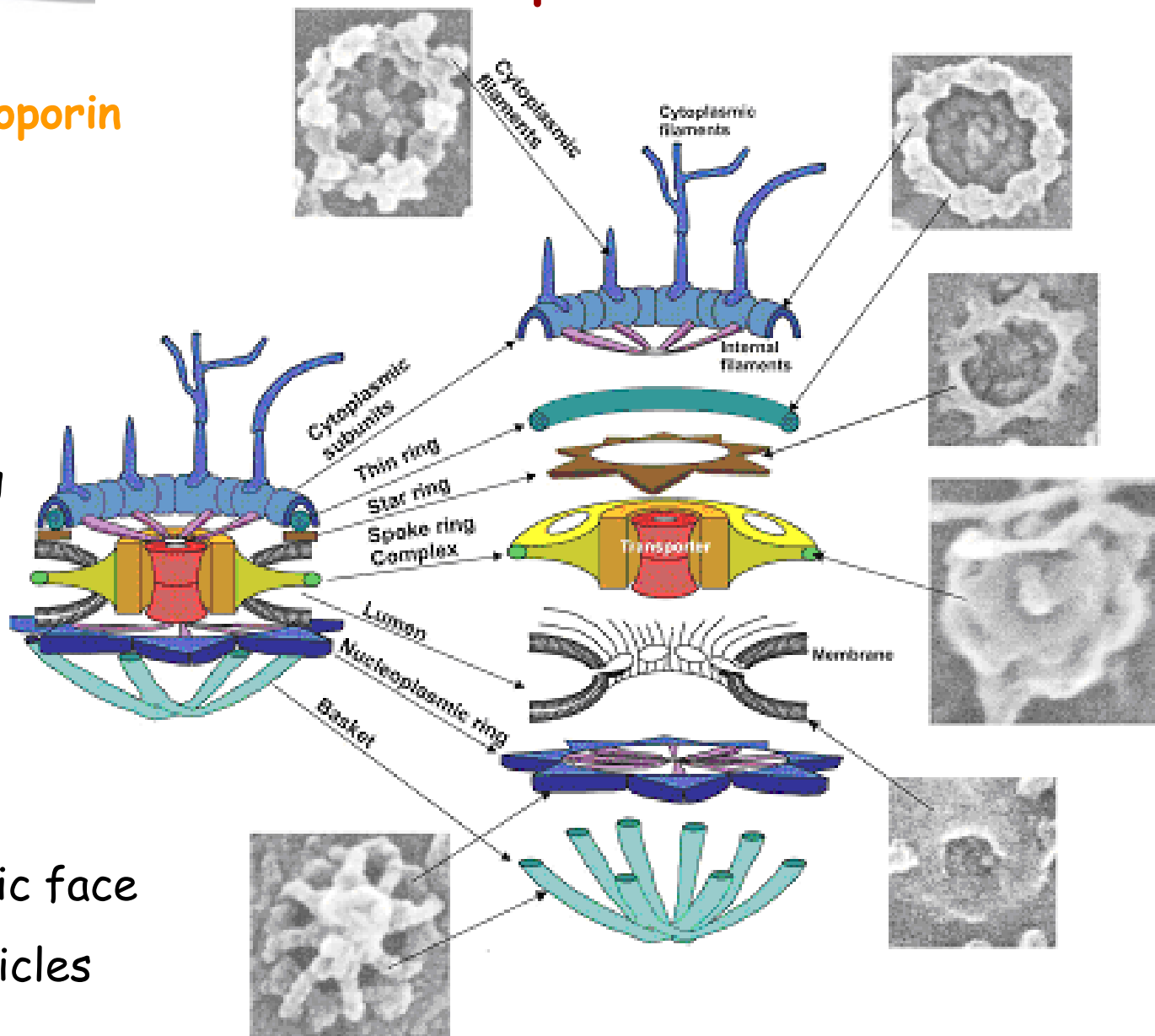
- cytoplasmic ring
- nuclear ring
- (+ inner spoke ring and luminal ring)

On the nuclear face

- cage/basket like structure

On the cytoplasmic face

- Filaments and particles



Nuclear transport

Selective (**size and chemical character**) gated transport

- <5 kDa - rapid entry
- 17 kDa - takes about 2 min..
- >60 kDa - can not enter

BUT

Enters: RNA polymerase, DNA polymerase (300 kD about 100 histones /min /per pore

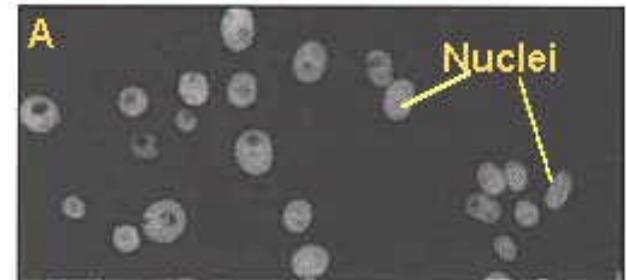
Released: ribosome subunits (30 nm) 10 min/pore
Some molecules crossed in complex form, others are elongated.

Participants of the transport:

- Transporter molecules- **karyoferins**
- The cargo molecules have **recognition signal** (NLS or NES)
- The **nucleoporines** of nuclear pore
- **Ran** protein

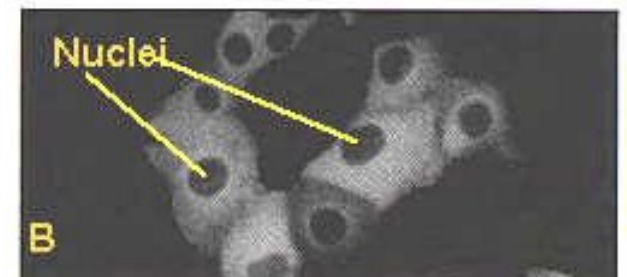
(A) LOCALIZATION OF T-ANTIGEN CONTAINING WILD-TYPE NUCLEAR IMPORT SIGNAL

Pro — Pro — Lys — Lys — Lys — Arg — Lys — Val —

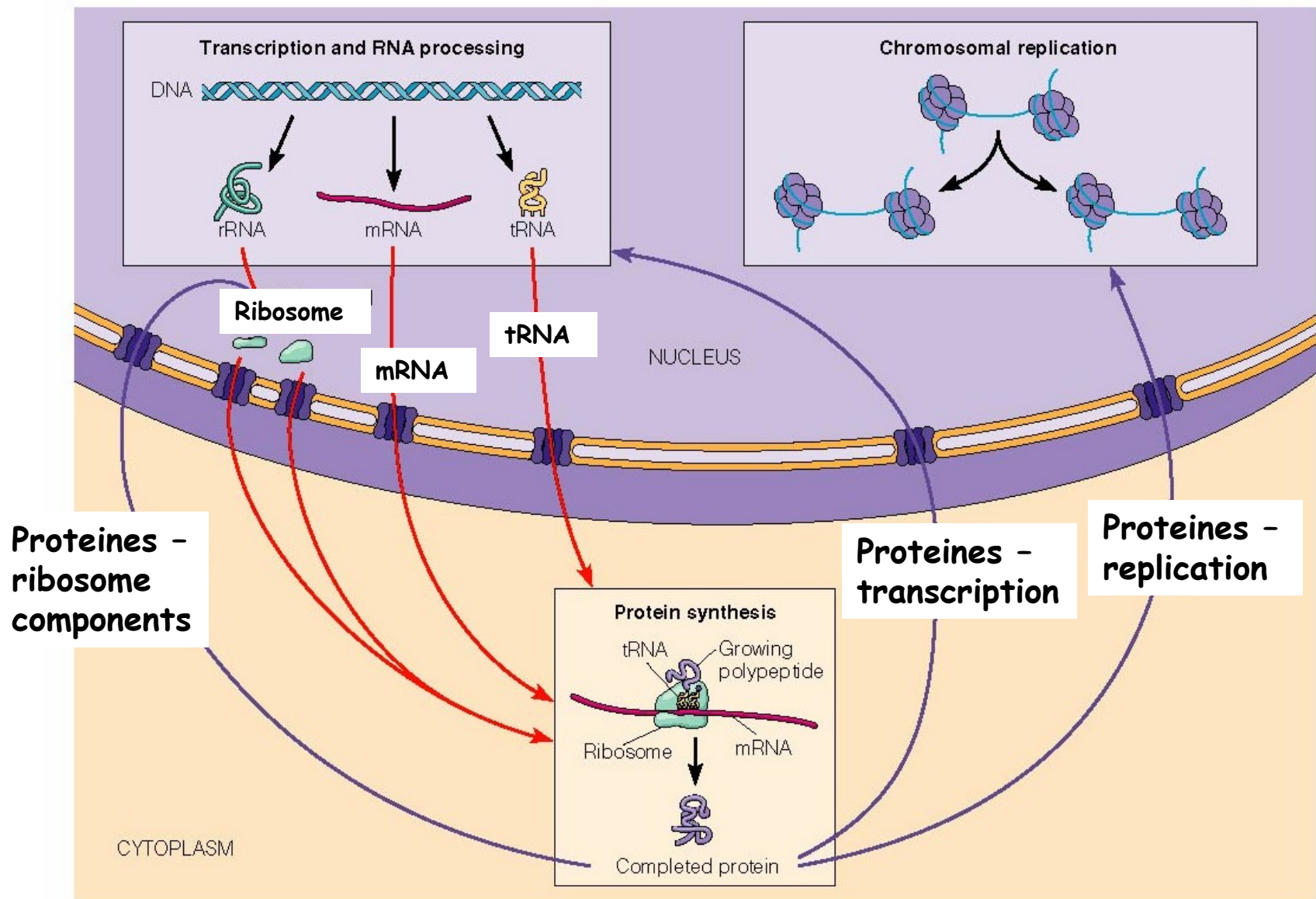


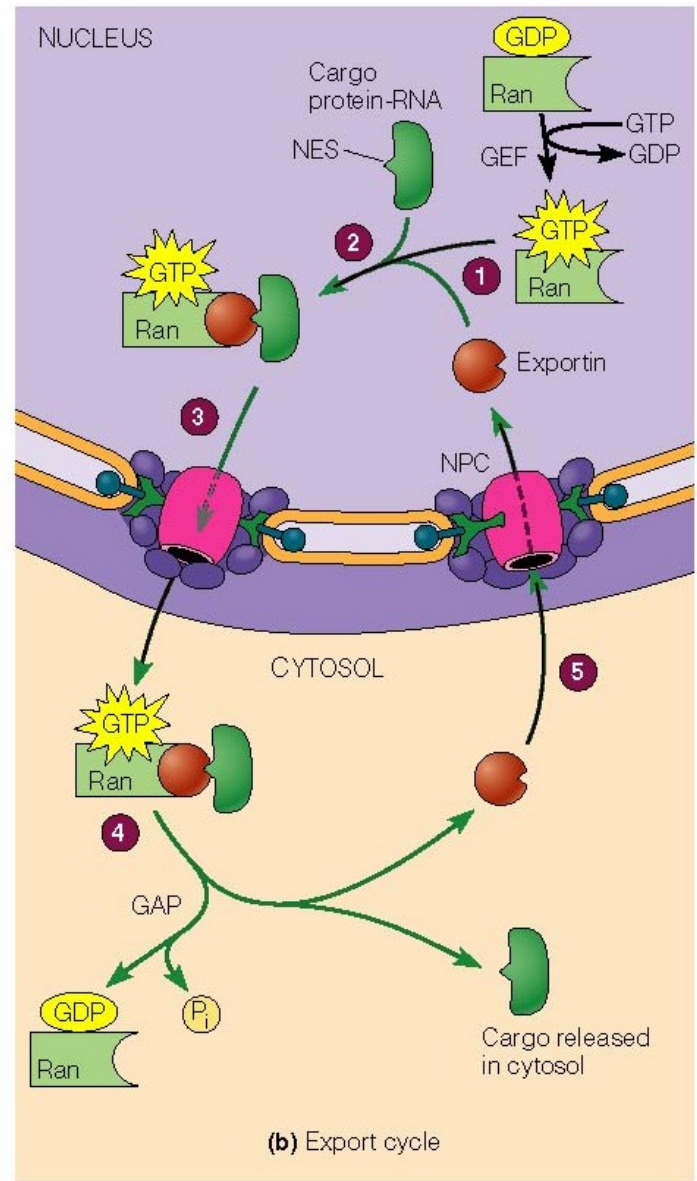
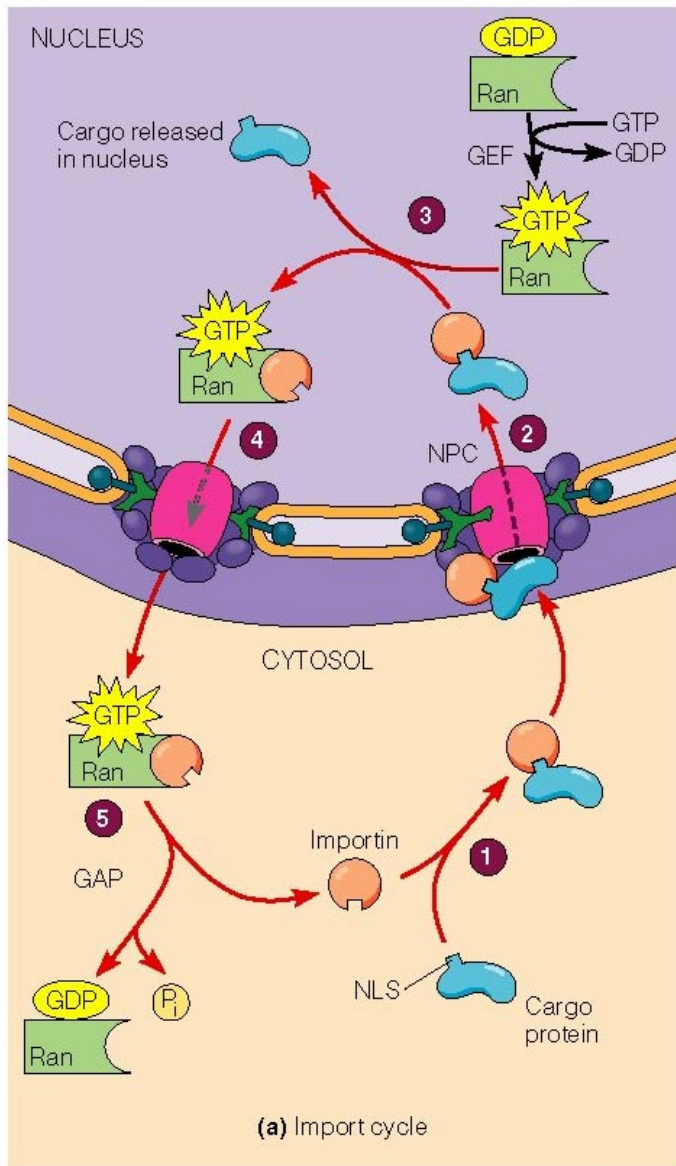
(B) LOCALIZATION OF T-ANTIGEN CONTAINING A MUTATED NUCLEAR IMPORT SIGNAL

Pro — Pro — Lys — Thr — Lys — Arg — Lys — Val —

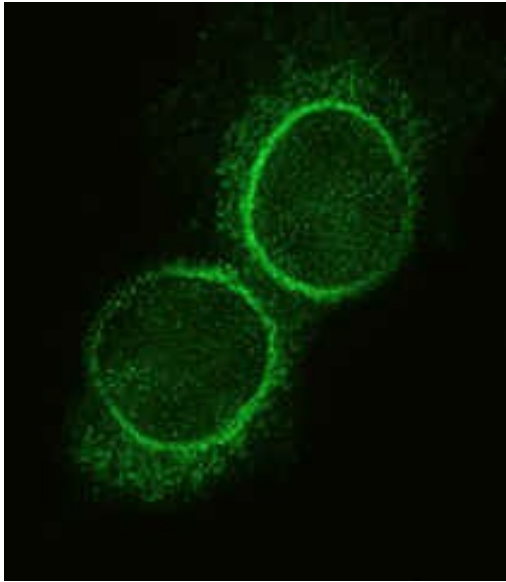


Nuclear membrane crossing transported molecules

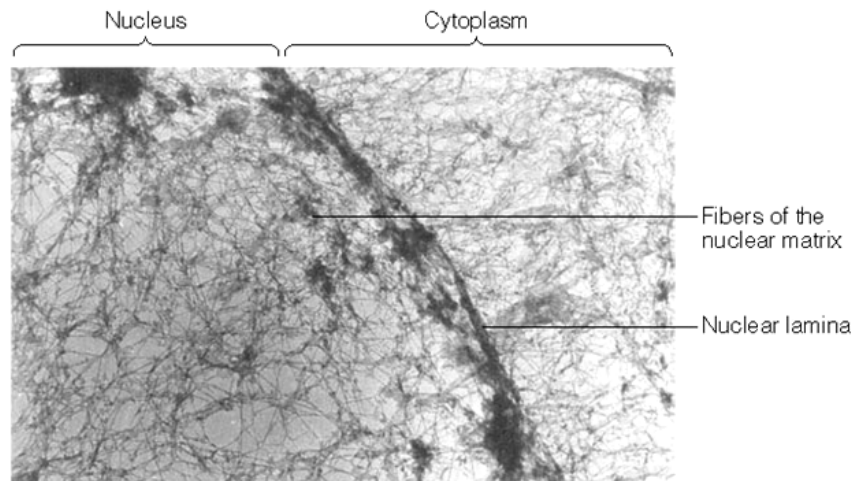
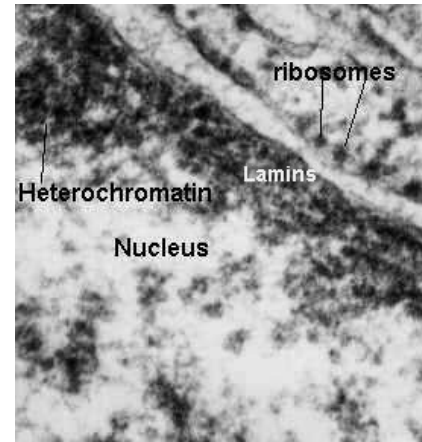




Nuclear lamina (fibrous lamina, lamina fibrosa)

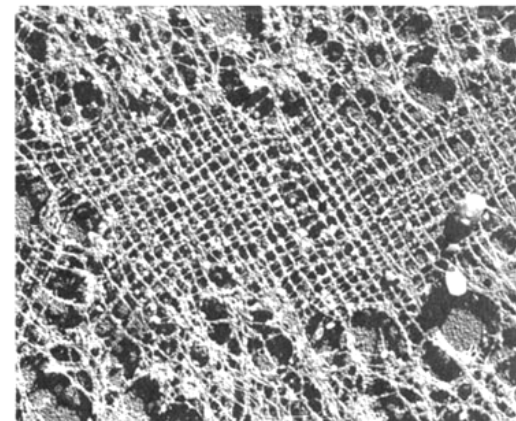


www.med.cam.ac.uk/.../principles/cs/cs.htm1.jpg



(a) Attachment of nuclear matrix fibers to the nuclear lamina

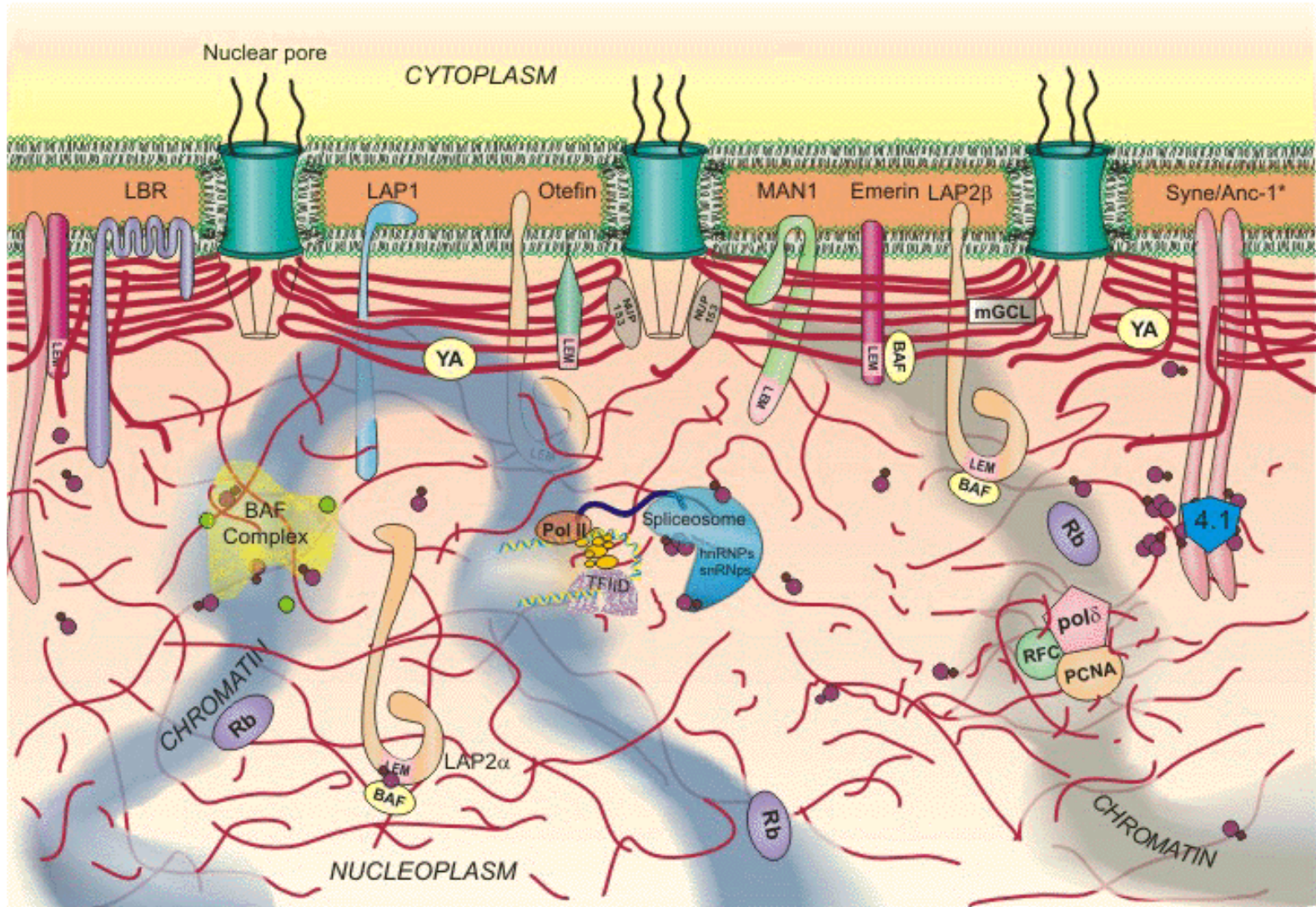
1 *mm*



(b) Surface view of nuclear lamina

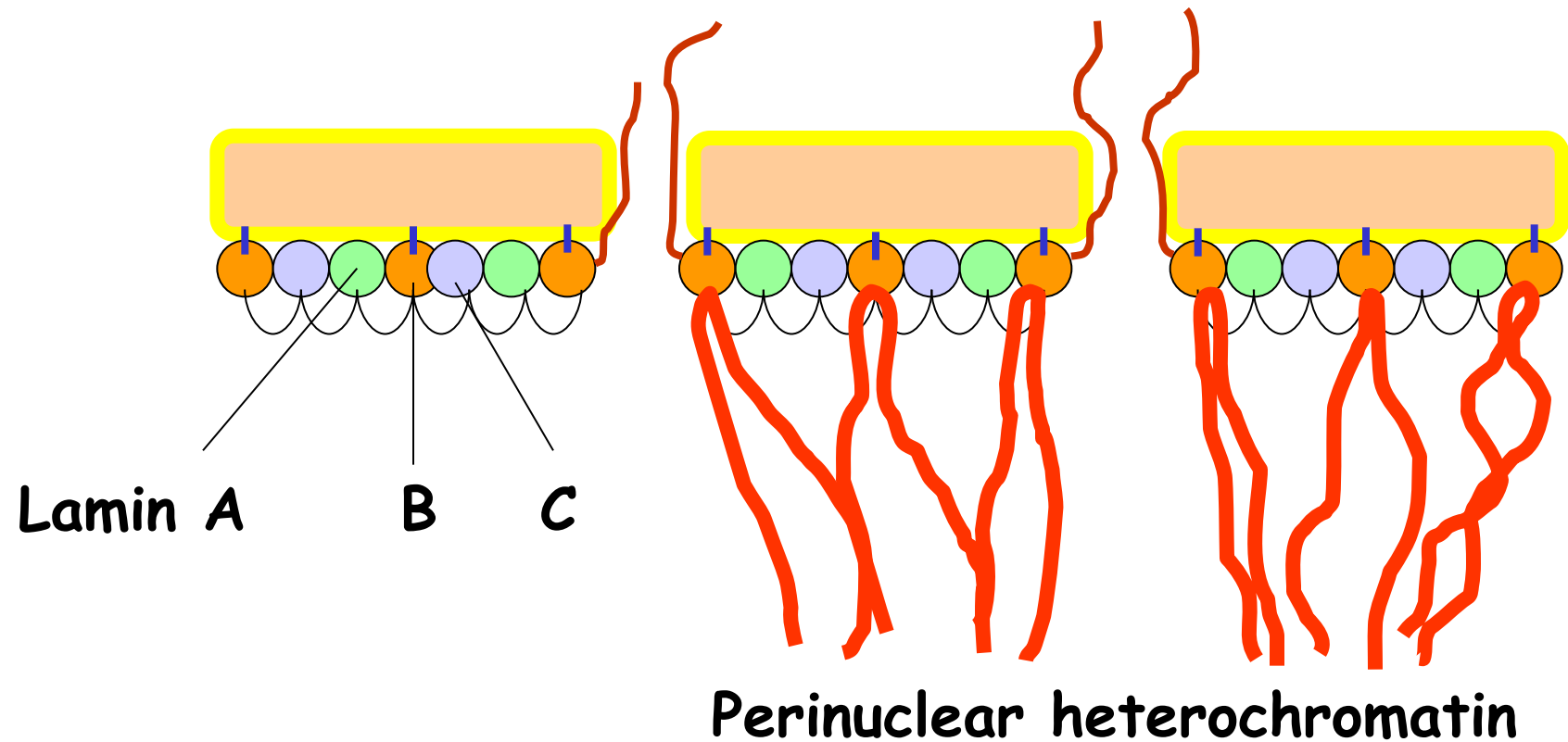
1 *mm*

Structure of the nuclear lamina

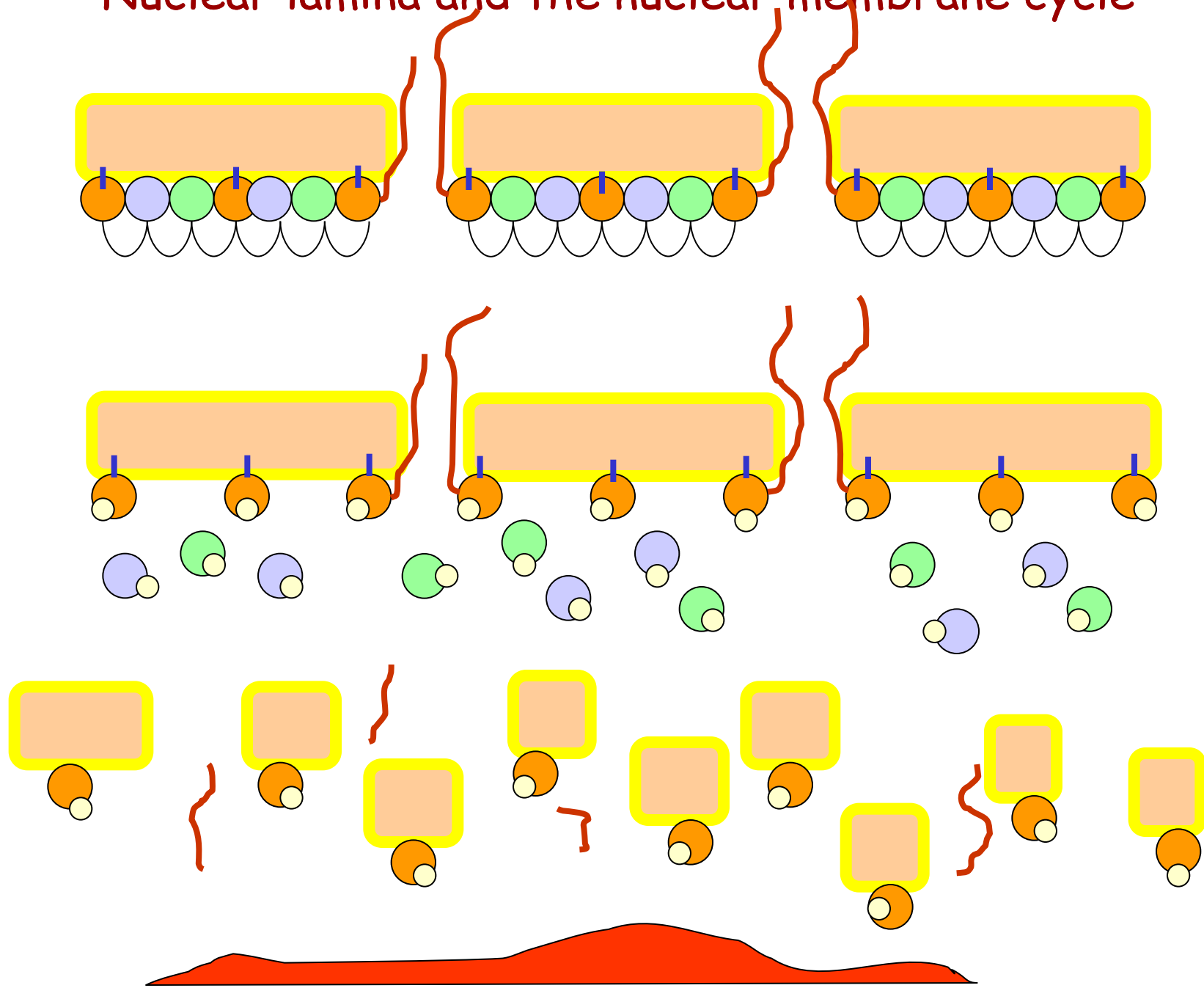


- | | | | |
|--|--|--|---|
|  Lamins |  Arps |  G-Actin |  Oligomeric Actin |
| |  mRNA |  Profilin |  Transcription factors |

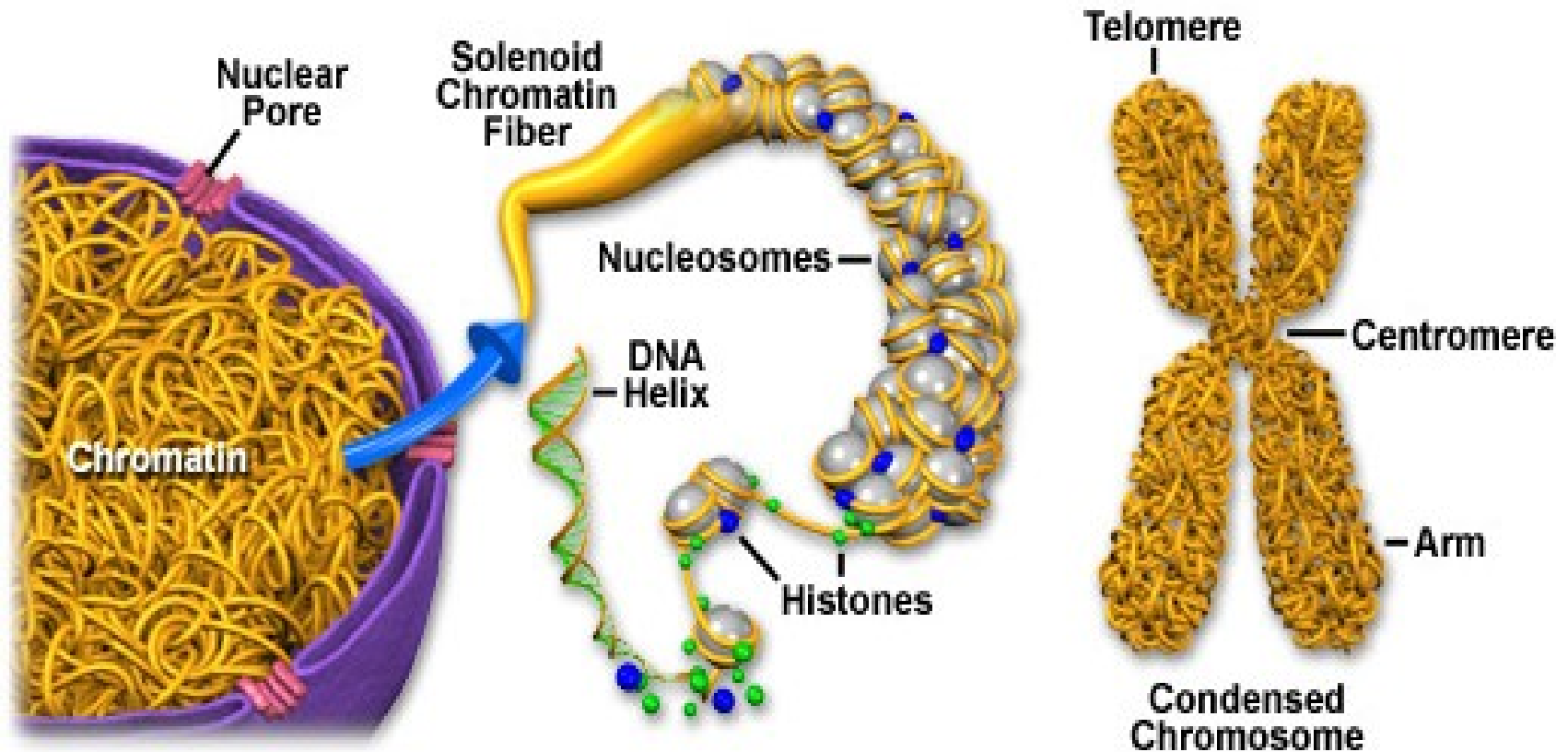
Role of lamins in building nuclear lamina



Nuclear lamina and the nuclear membrane cycle

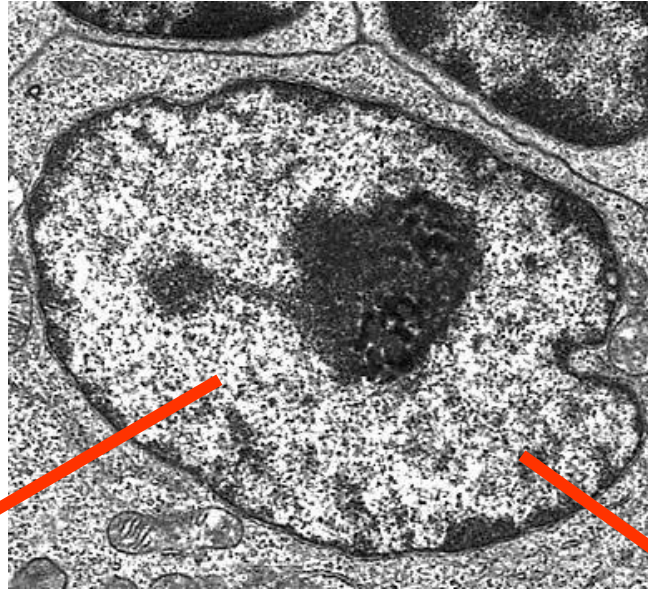


Chromatin and its organization



In human 1 nucleus contains about **2 meter** DNA

Chromatin organization



Euchromatin

- Loose structure
- Active
- Transcribed to mRNA

Heterochromatin

- Dense structure
- Inactive
- Perinuclear h.chr.
- Facultative
 - potentially transcribed
- Constitutive
 - no transcription

The basic structure of chromatin: nucleosome

- DNA double helix and histons
- Histones: basic proteins (arginin- and lysine-rich)
- 5 classes: **H1**, **H2A**, **H2B**, **H3** and **H4**

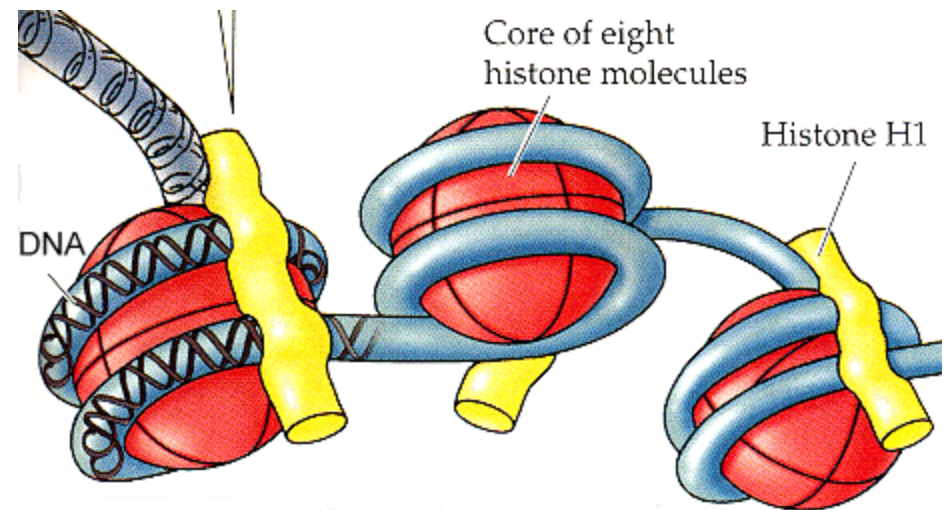
nucleosomal histones

Histone discs (octamer):

8 histone molecules (2*4)

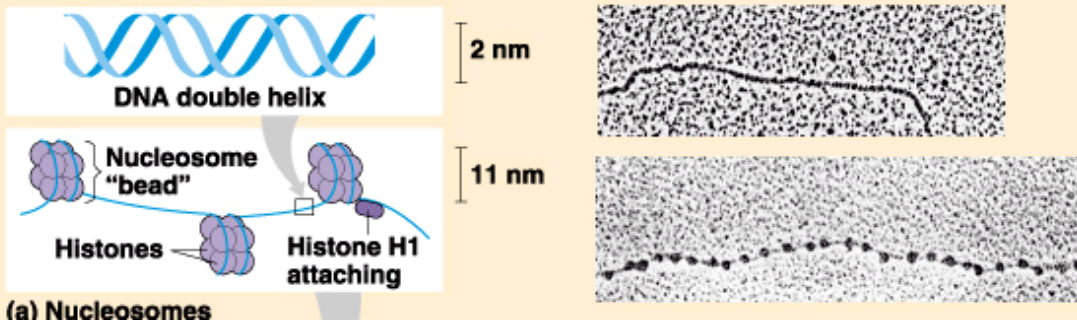
- 2 folds
- 146 basepair-long DNA rolls over

Between the 2 discs about 60 bp linker region + **H1 molecule**

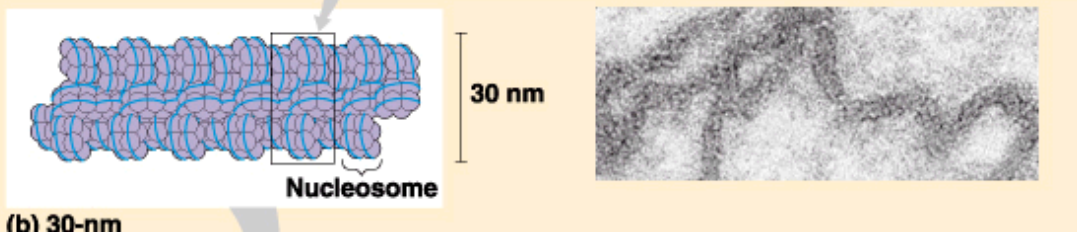


In 1 nucleus
about 25 million nucleosomes

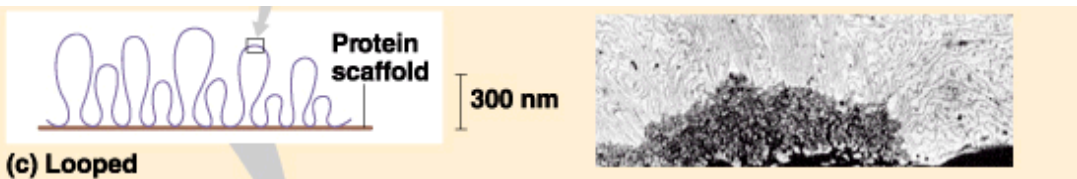
Organization of chromatin levels



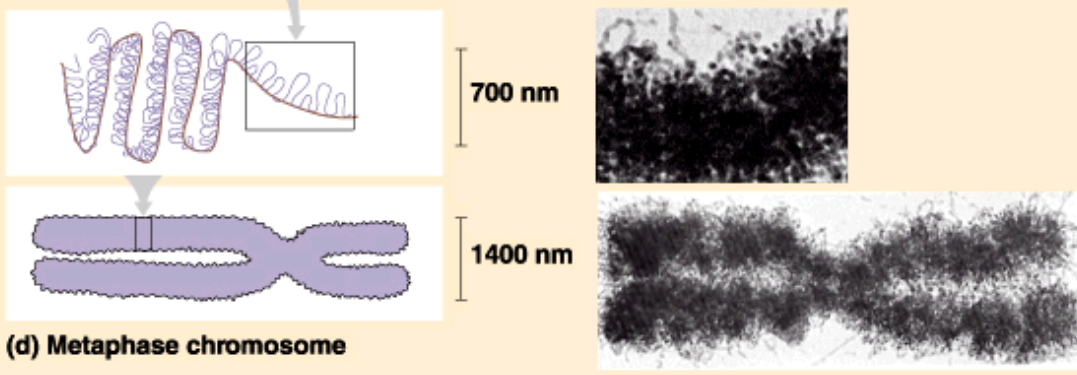
nucleosome



solenoid



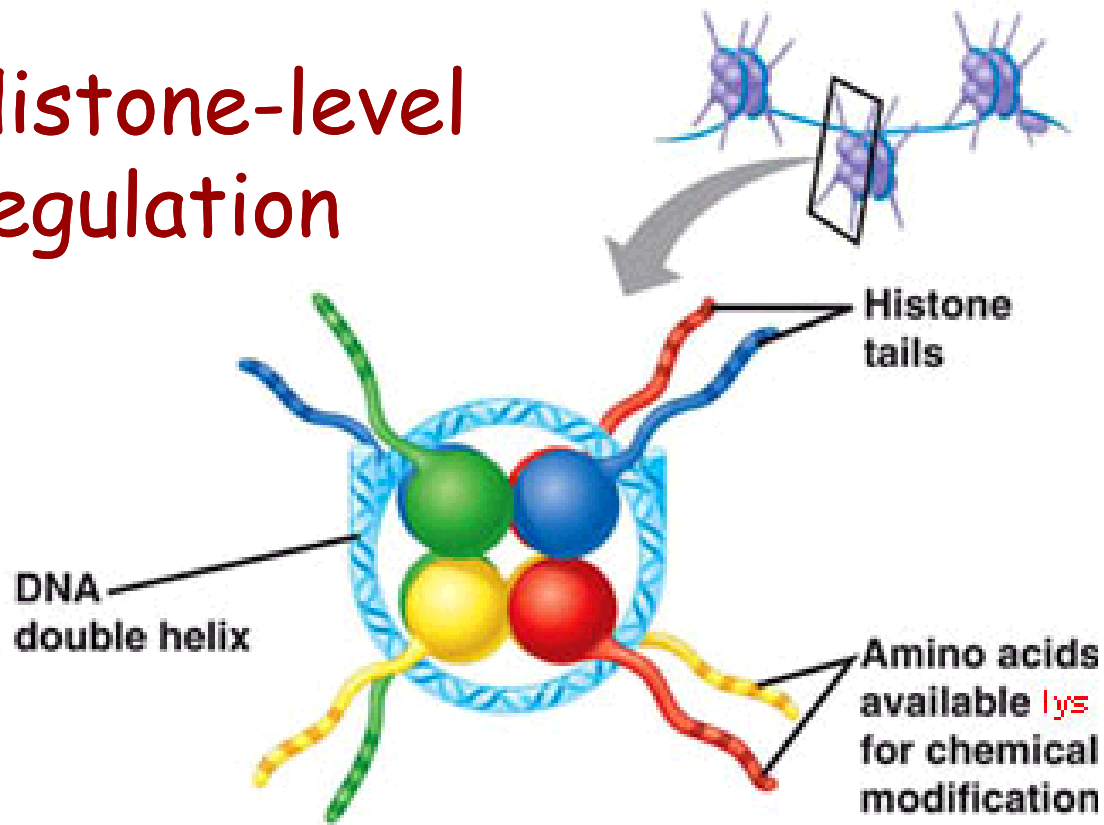
loop structure



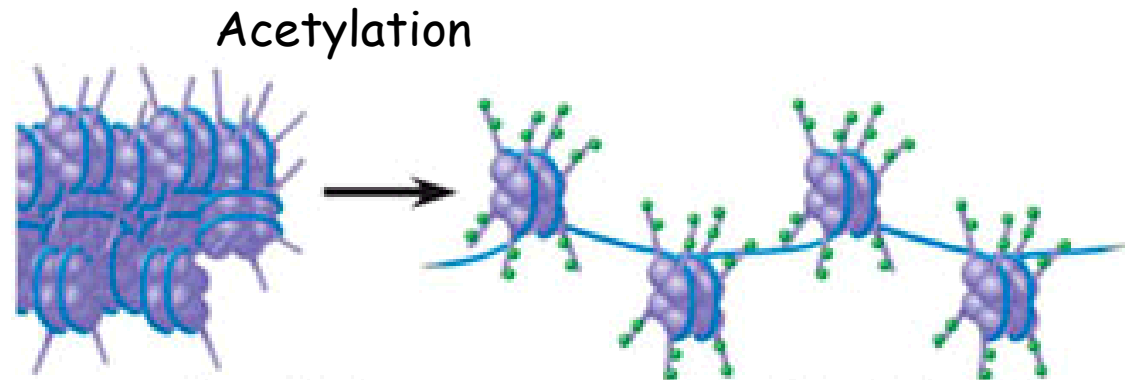
chromatin
fascicle

chromosome

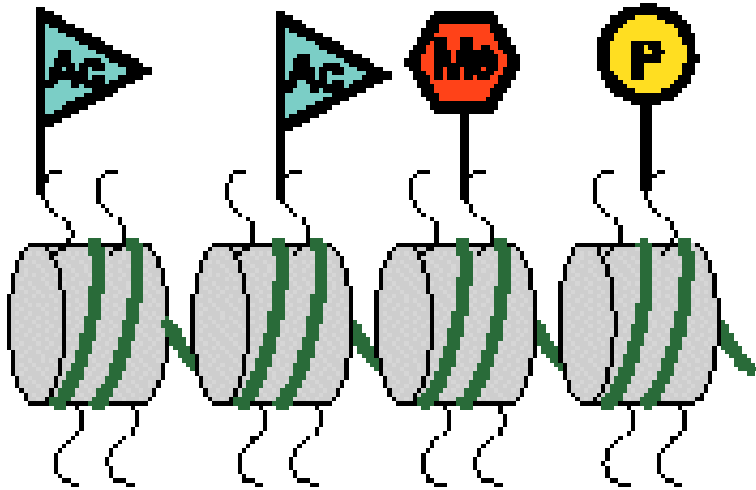
Histone-level regulation



Histone acetyl transferase= HAT

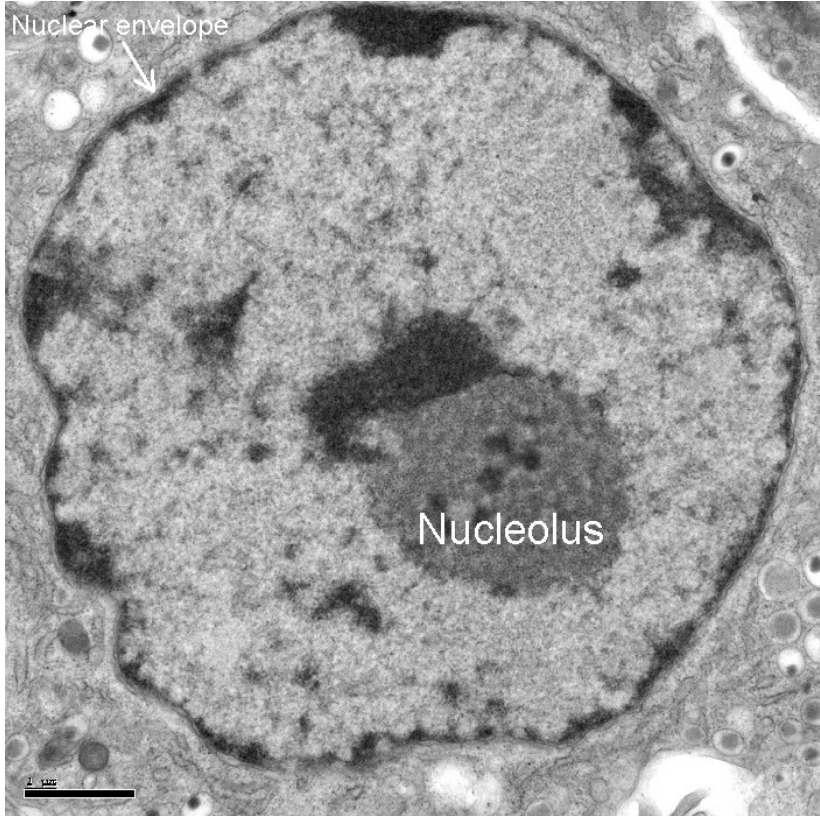
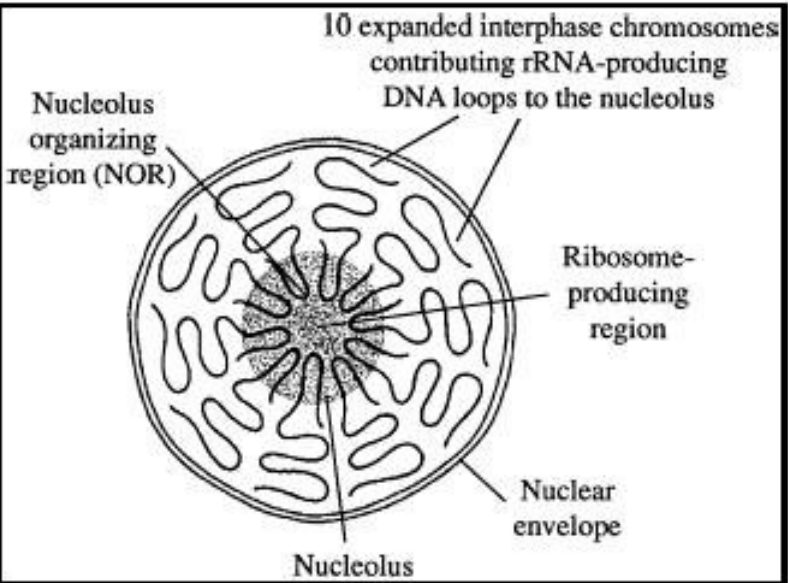
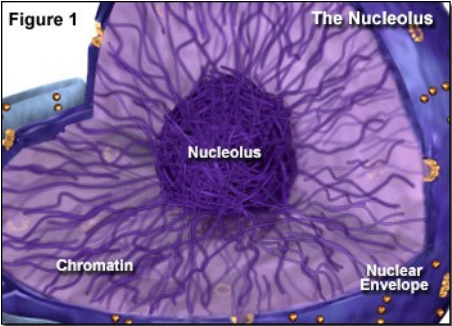


Histone-level regulation 3

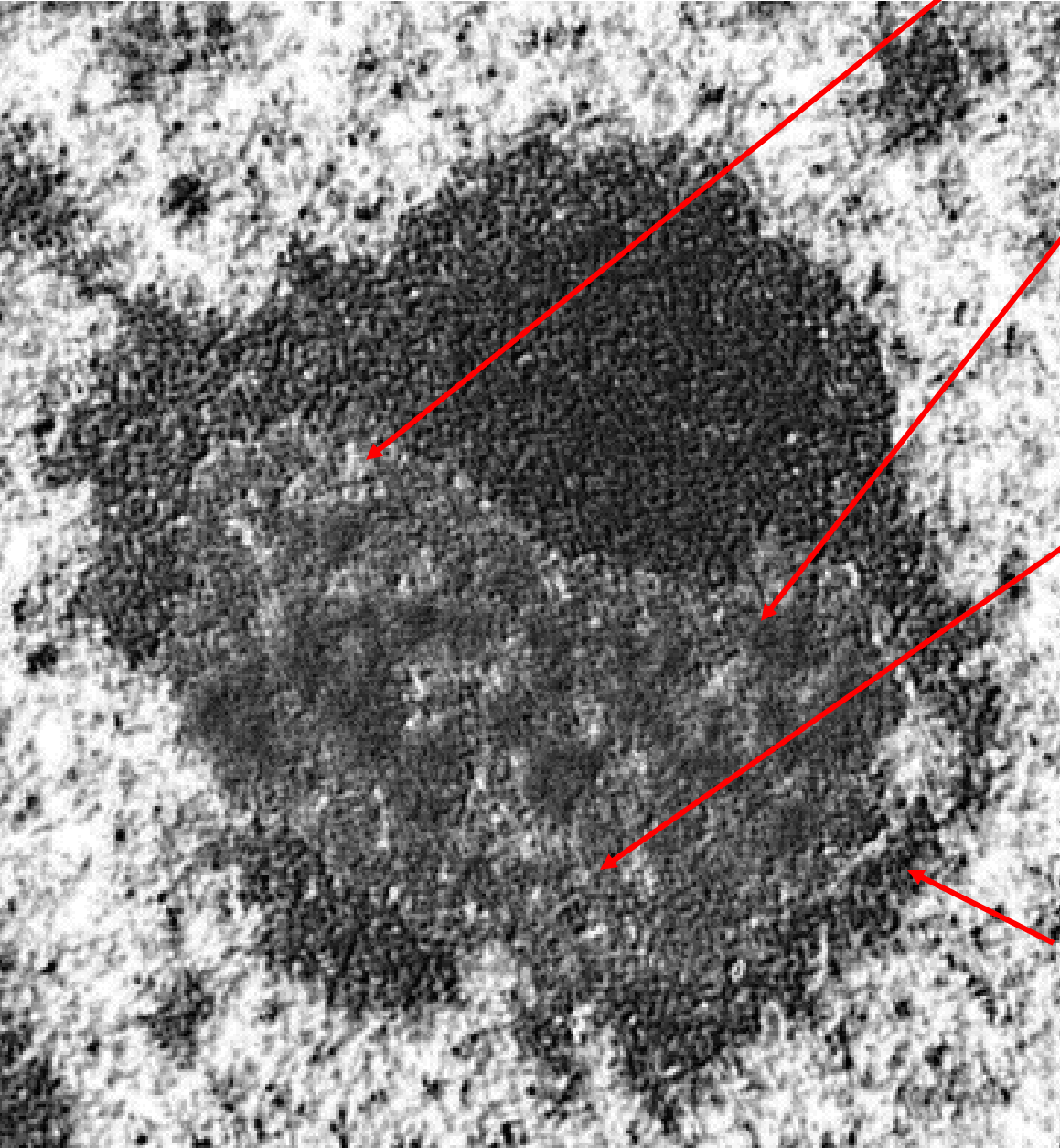


- **Methylation** - histones or DNA turning off
- **Acetylation** - histones turning on
- **Phosphorylation** - in genes the outcome is not unequivocal

Nucleolus



Nucleolus



FC/ pars amorpha

Less electron-dense

fibrillar center

rDNA: many copies, transcription

DFK/ pars fibrosa

Strongly electron-dense fibrillar component

Pre-rRNA modification

DGK/ pars granulosa

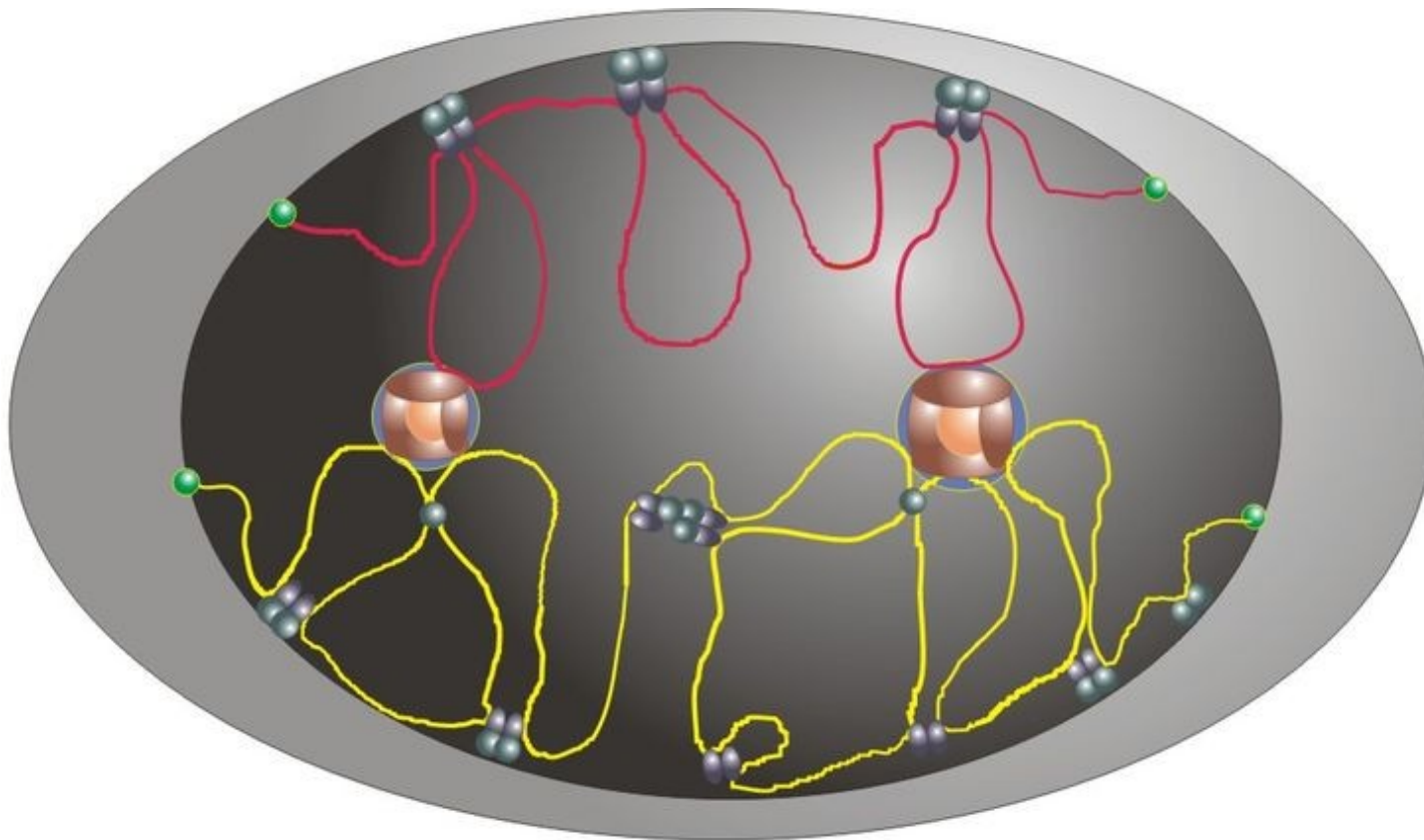
Moderately electron-dense granular component

Organization of rRNA and ribosomal proteins

Nac = nucleolar associated chromatin

(PCh = pars chromosoma)

Development of chromosomal territories



telomer

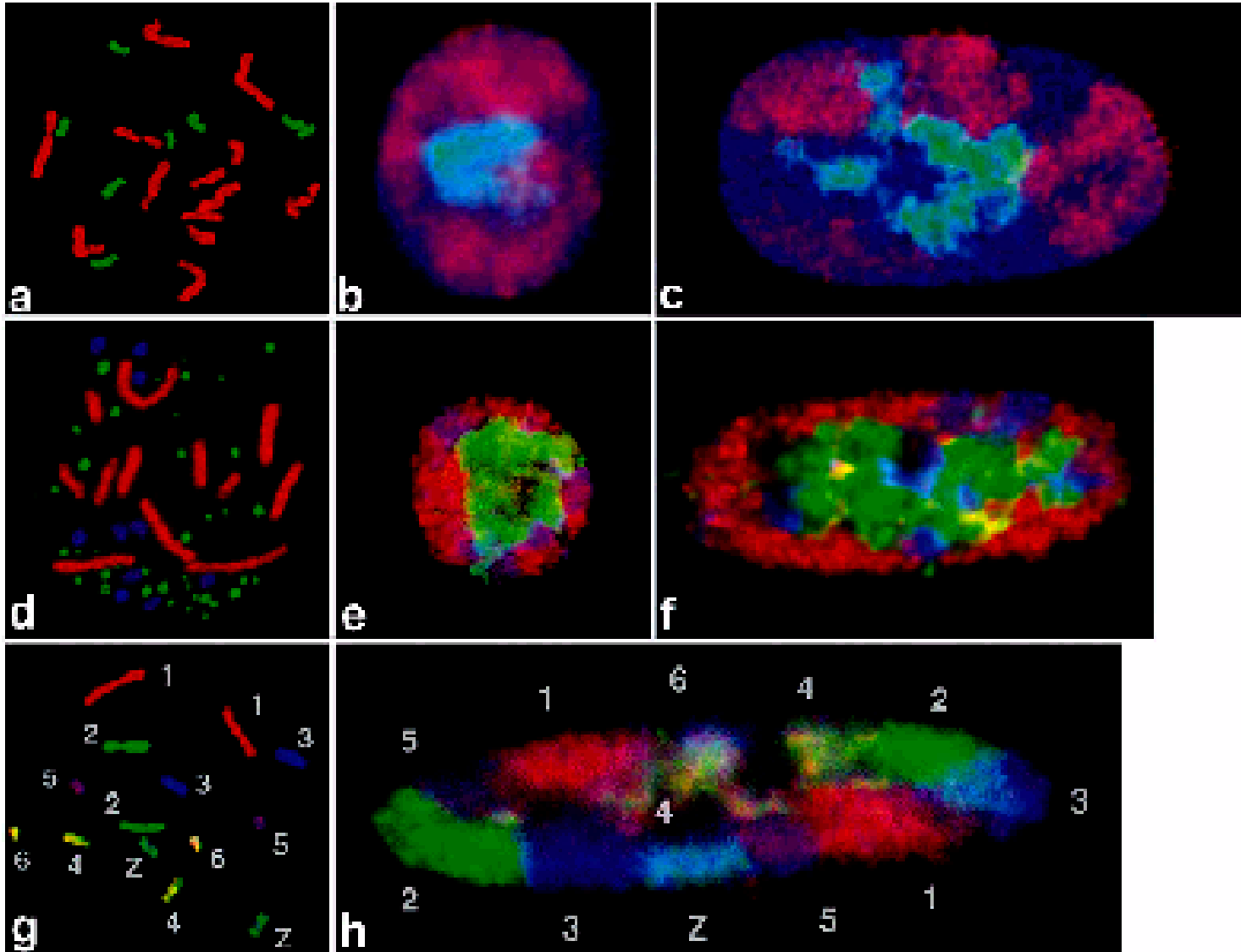


„insulator“ protein
complex

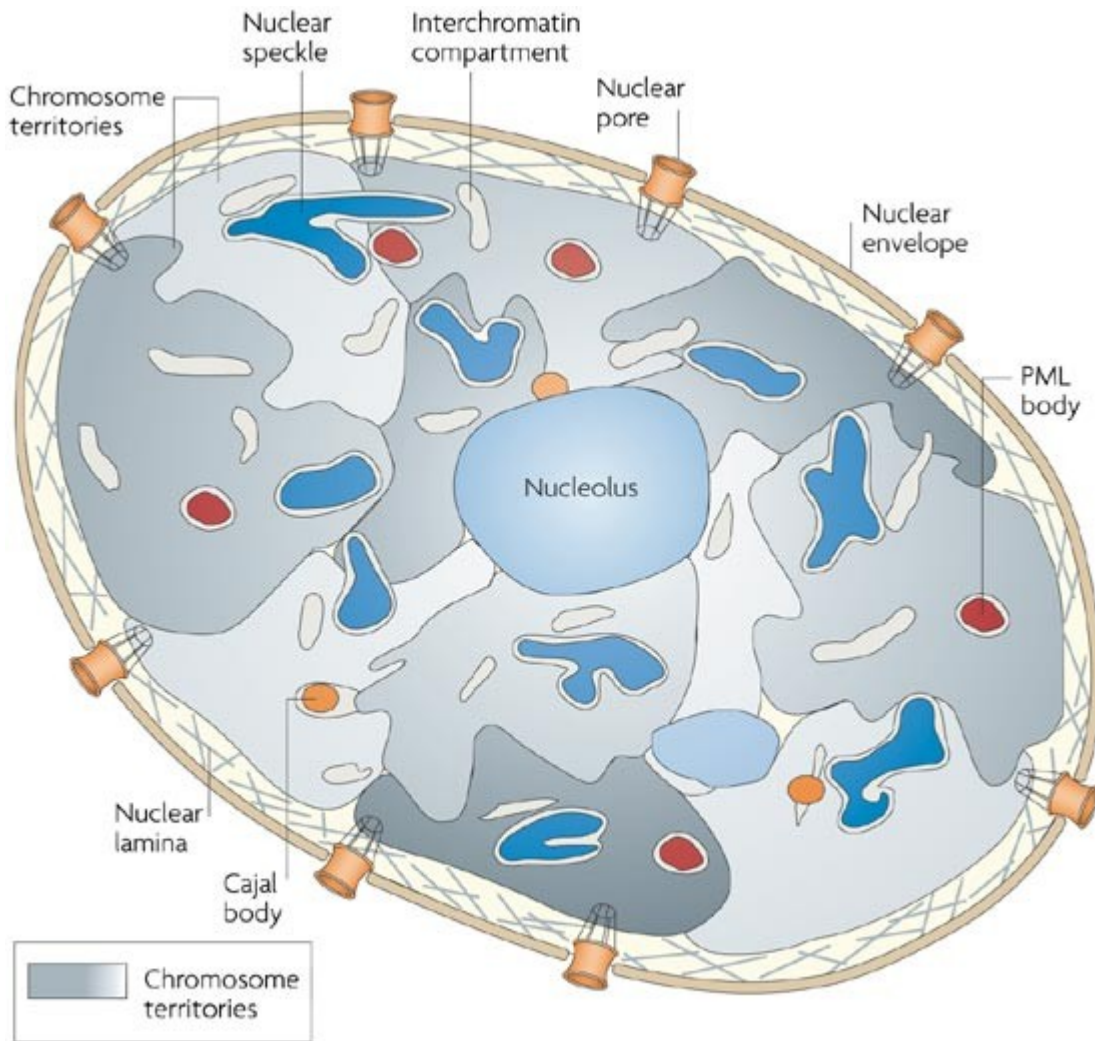


chromatin-fibre
organizing
multiprotein
complex

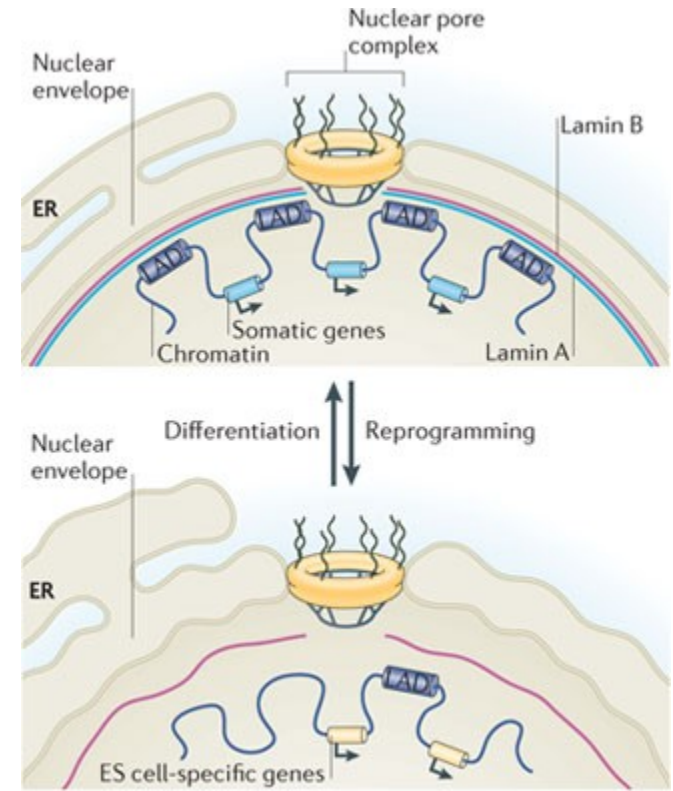
Chromosomal territories - a particular chromosome is located in a specific region of the nucleus



Linkage of chromosome territories to parts of the nucleus



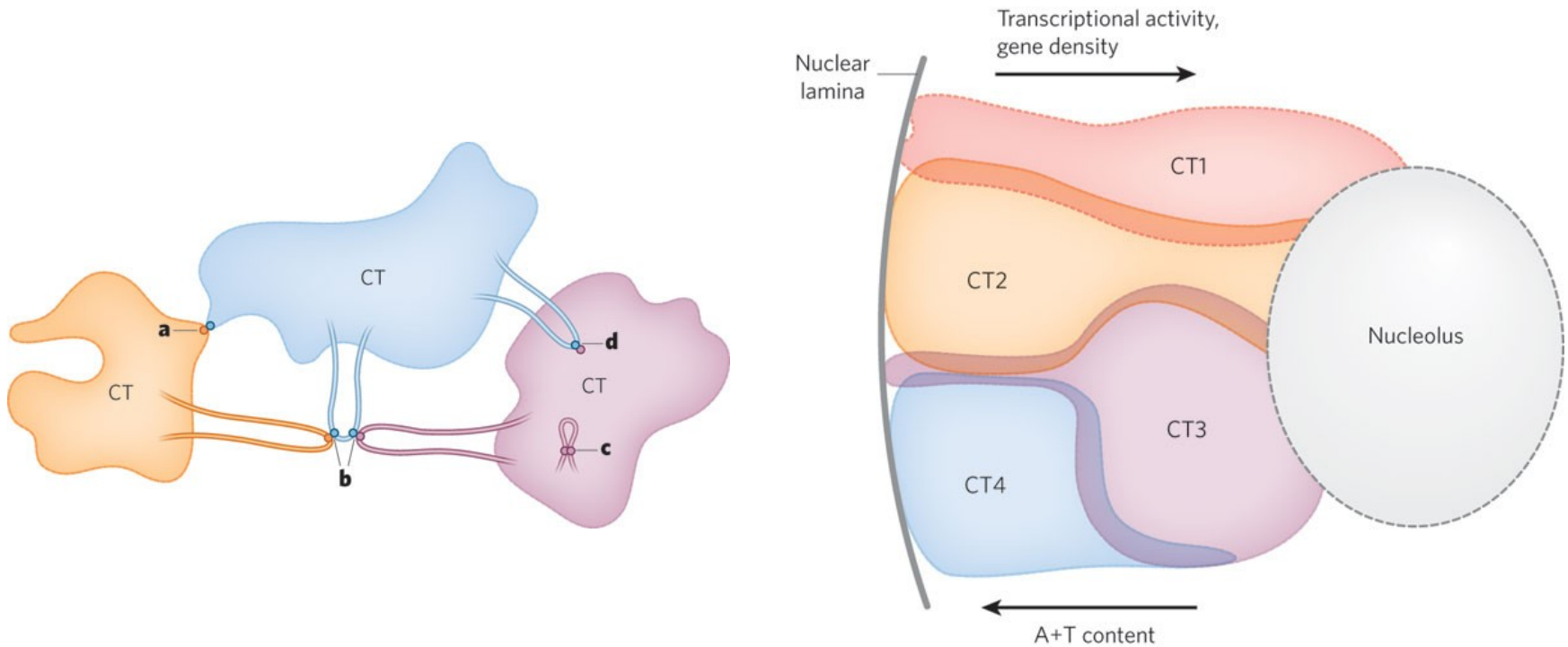
Nature Reviews | Genetics



Nature Reviews | Molecular Cell Biology

Chromosome territories

Relations of internal structure and activity

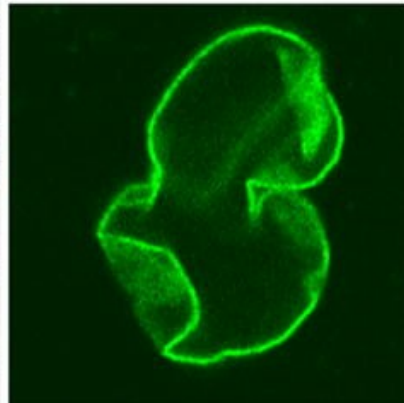
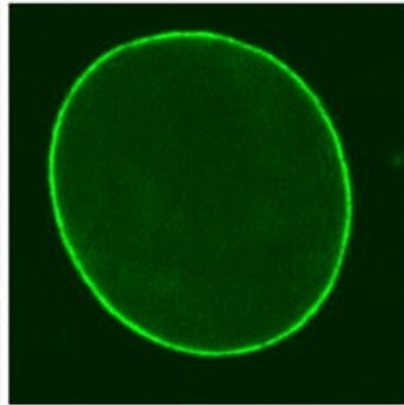


Chromatin rearrangement - remodelling

1. Nucleosome structure can be disrupted
 - proteins that **unwind the loops** eg HMG (high motility group)
 - with **transcription factors** that recognize and bind to these sites
2. Transcription
3. Nucleosome structure **repair**
 - Histone Acetylation (HAT)
 - Histone Deacetylation (HDA = Histone deacetylase)

Disease of nuclear lamina

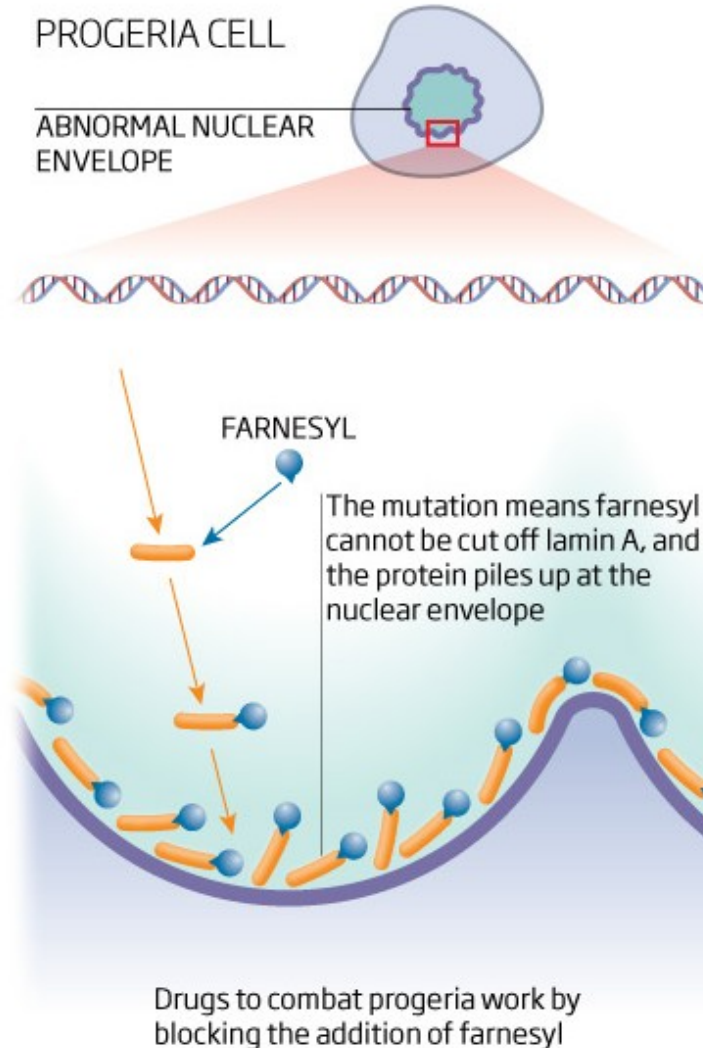
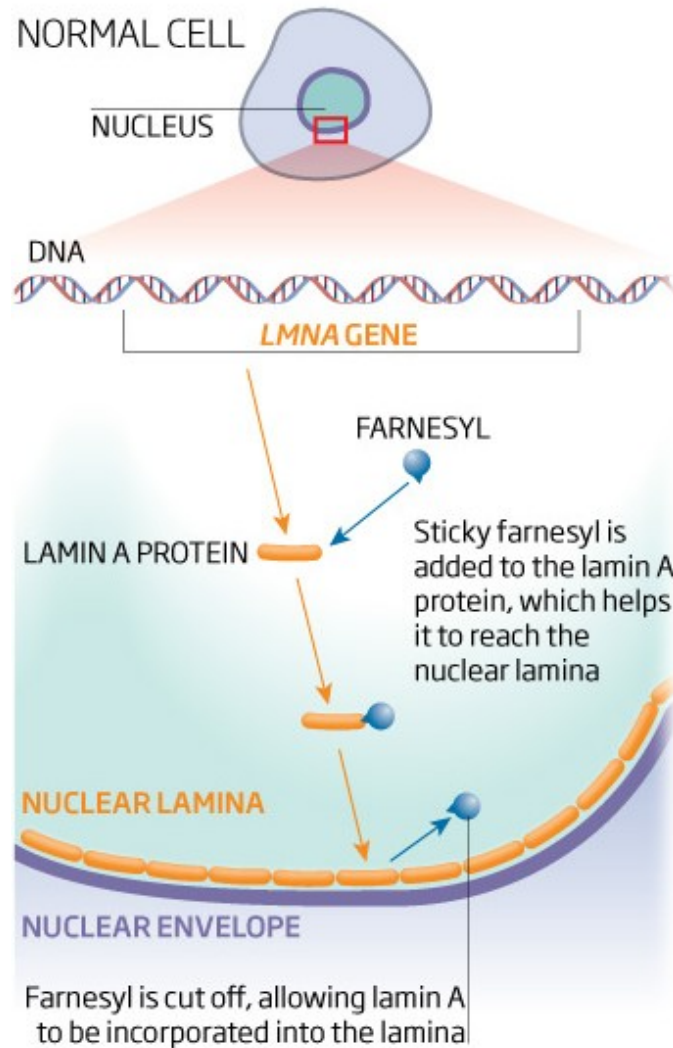
- Progeria ~ Hutchinson-Gilford syndrome -



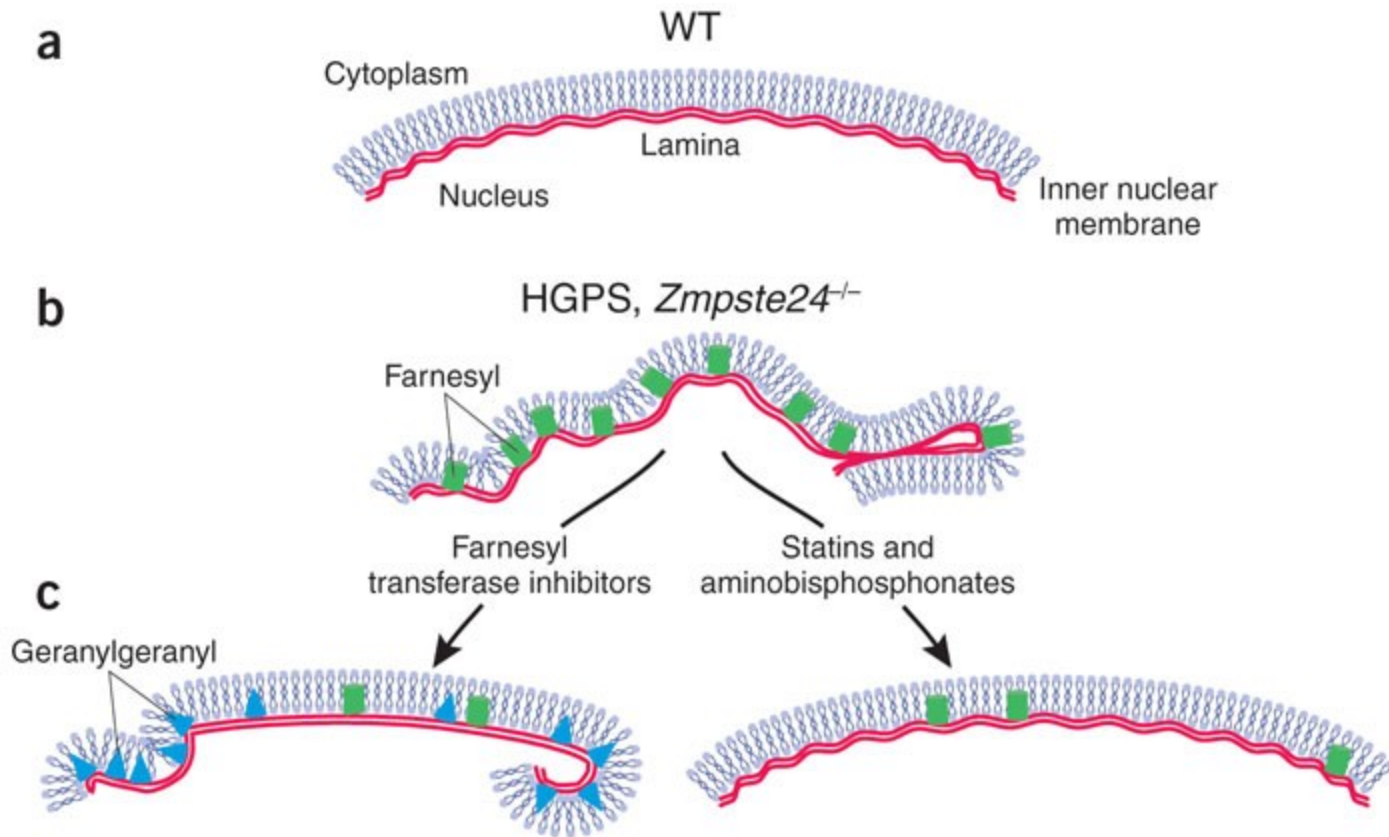
- Mutation of Lamin A gene
- Lasting farnesylation of Lamin A-CAAX
- abnormal morphology of nucleus
- heterochromatin disorganization
- DNA repair is abnormal

Accelerated aging

Role of farnesyl group - 1



Role of farnesyl group - 2



Data on the human genome

3,000,000,000 basepairs ~ 500,000,000 codon

If 1 codon = 1 word and 1 page = 850 words

THIS MEANS

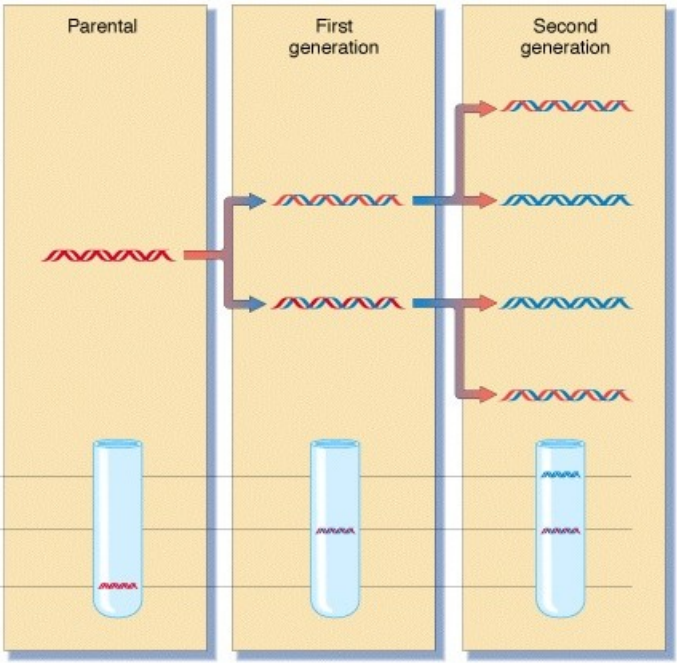
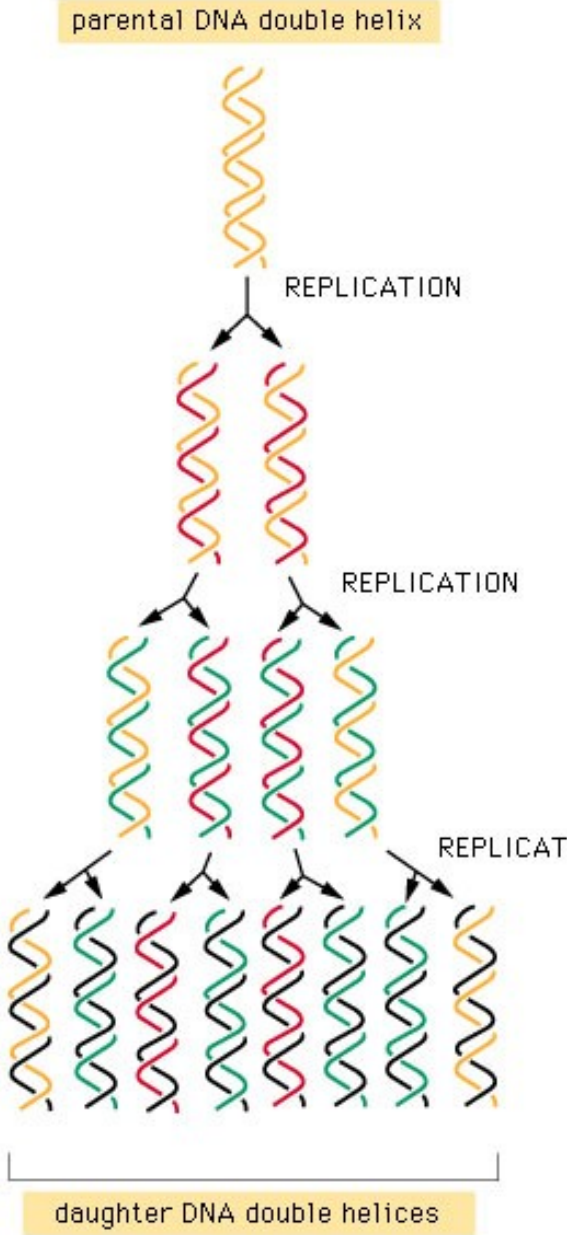
The human genome corresponds to **590,000** pages.

If we read the above book at 3 bp/min

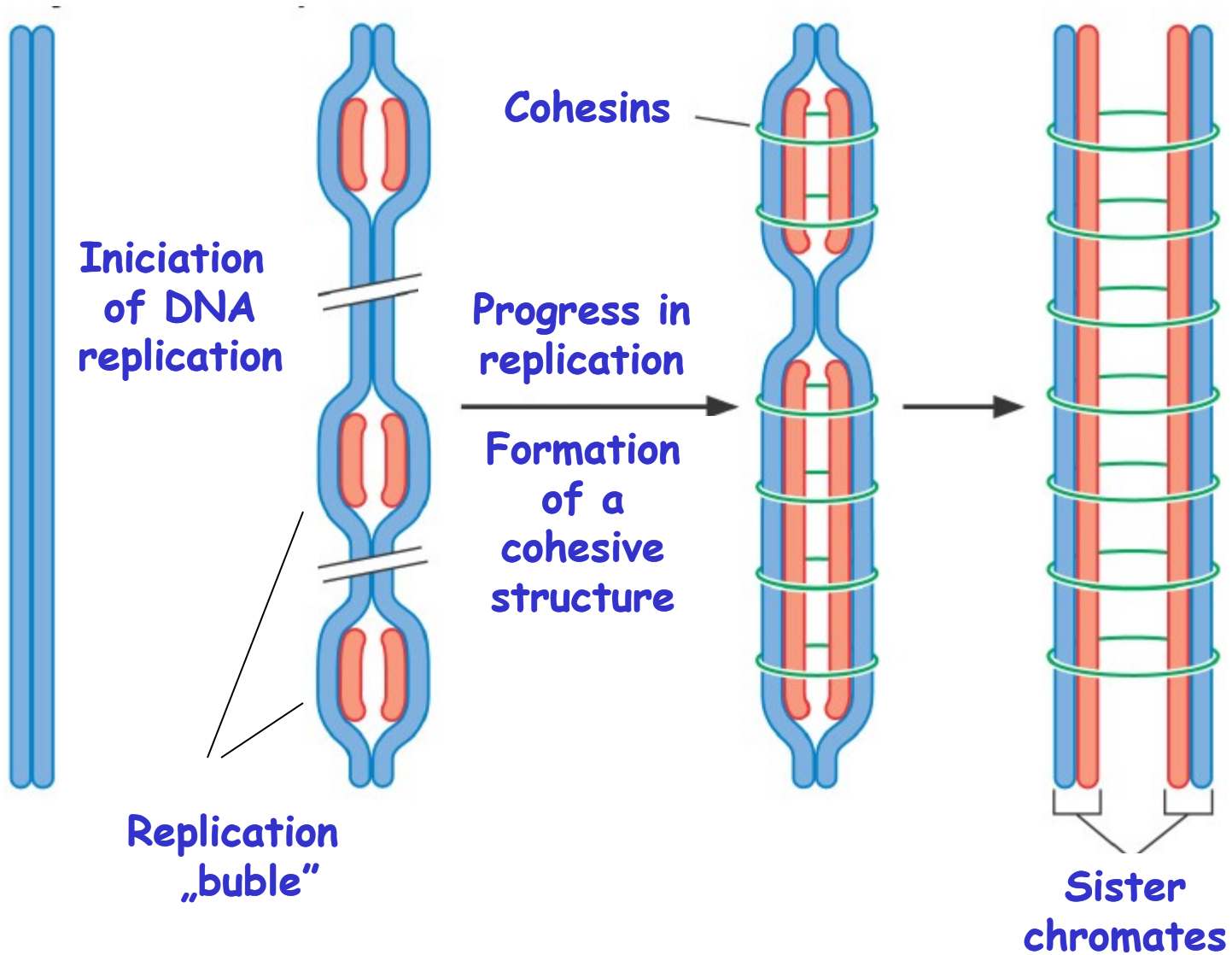
THIS MEANS

It would take **47.6** years to read the book.

Doubling of DNA semiconservative principle



Duplication of DNA 1



**Central
dogma**



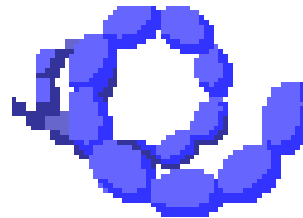
DNA

Transcription



RNA

Translation
Synthesis of
polypeptide
based on
mRNA template

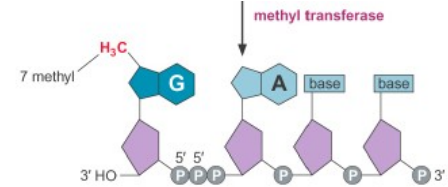


Protein

mRNA maturation and editing

hnRNS - (heterogeneous nuclear RNA) - primary transcribed RNA - in eukaryotes

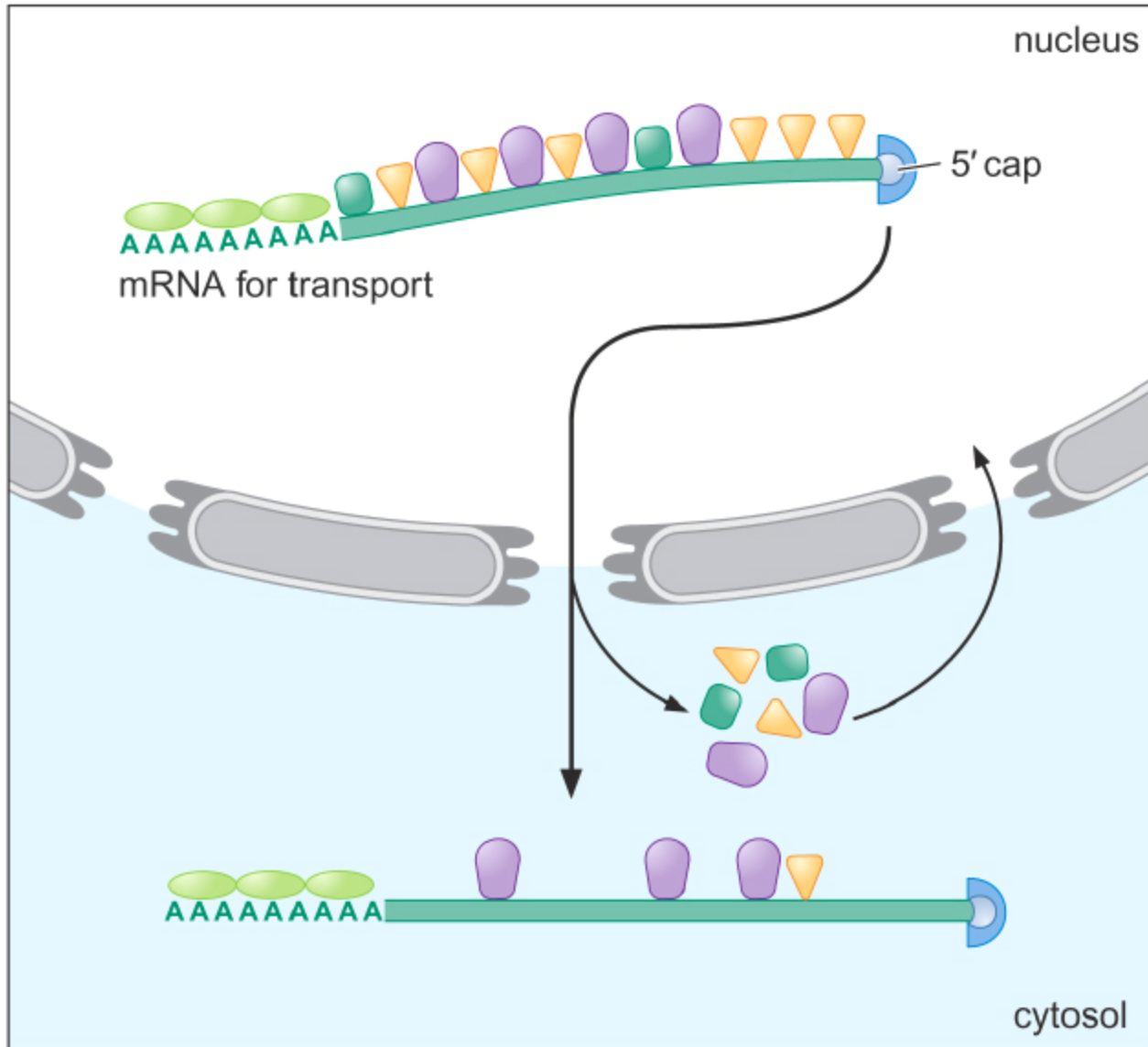
5' cap - 7-methylguanosine on the mRNA at the 5' end makes 3' like the molecule; protects mRNA from degradation



Poly-adenine tail - 20-200 adenine containing sequence
poly-A-polymerase adds
increases the lifespan of mRNA

pre-mRNA - 100-400 nucleotid RNA

Nucleus - cytoplasm transport of the mature mRNA

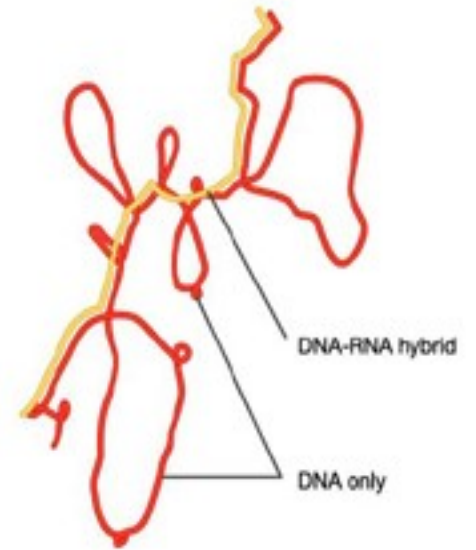
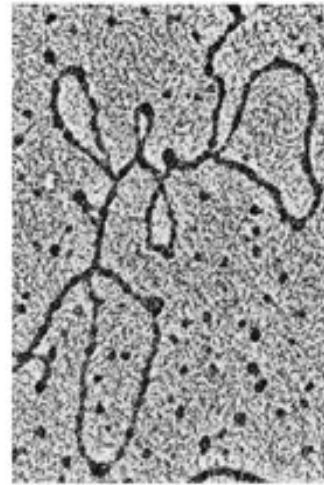




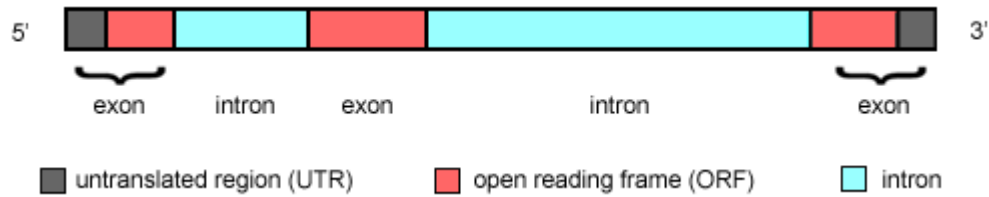
Roberts,
Richard J.



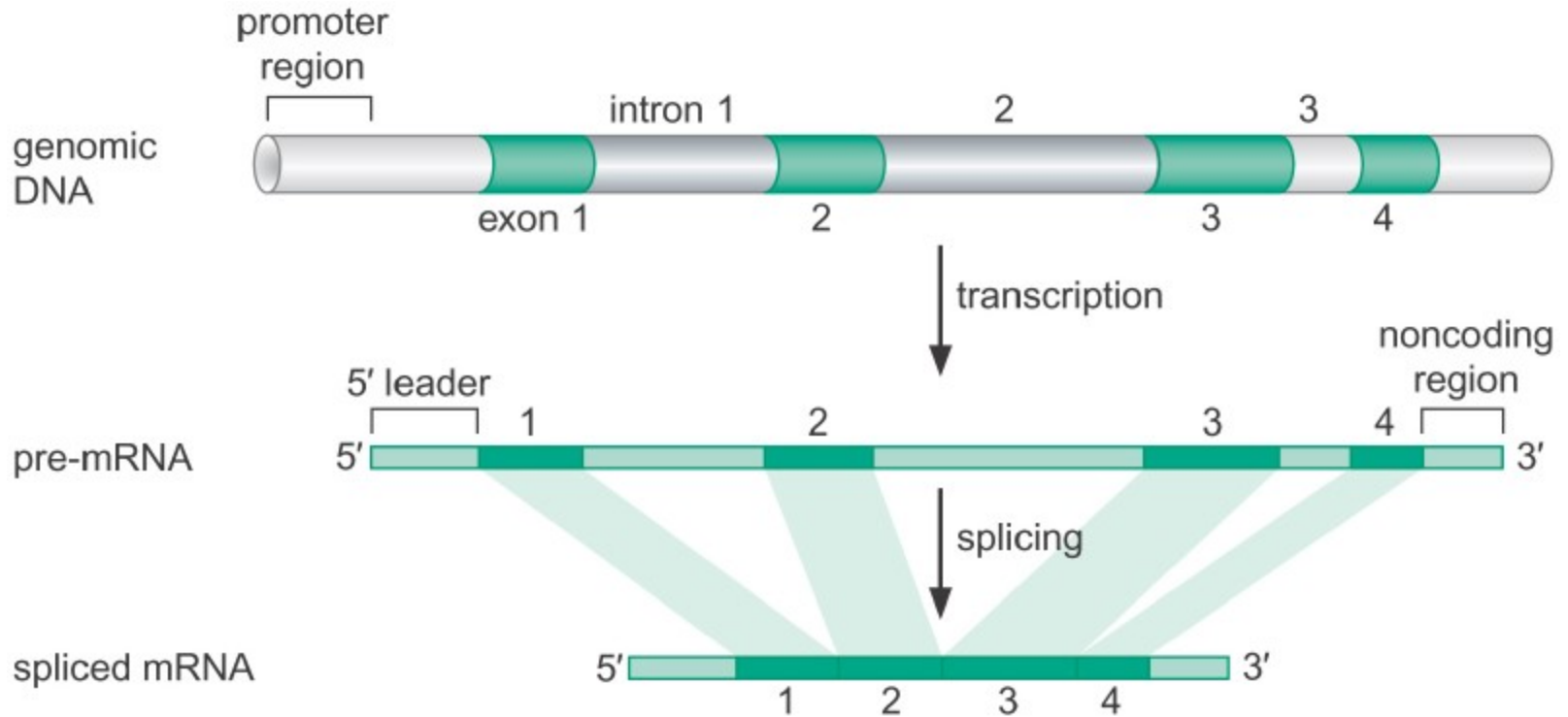
Sharp,
Phillip A.



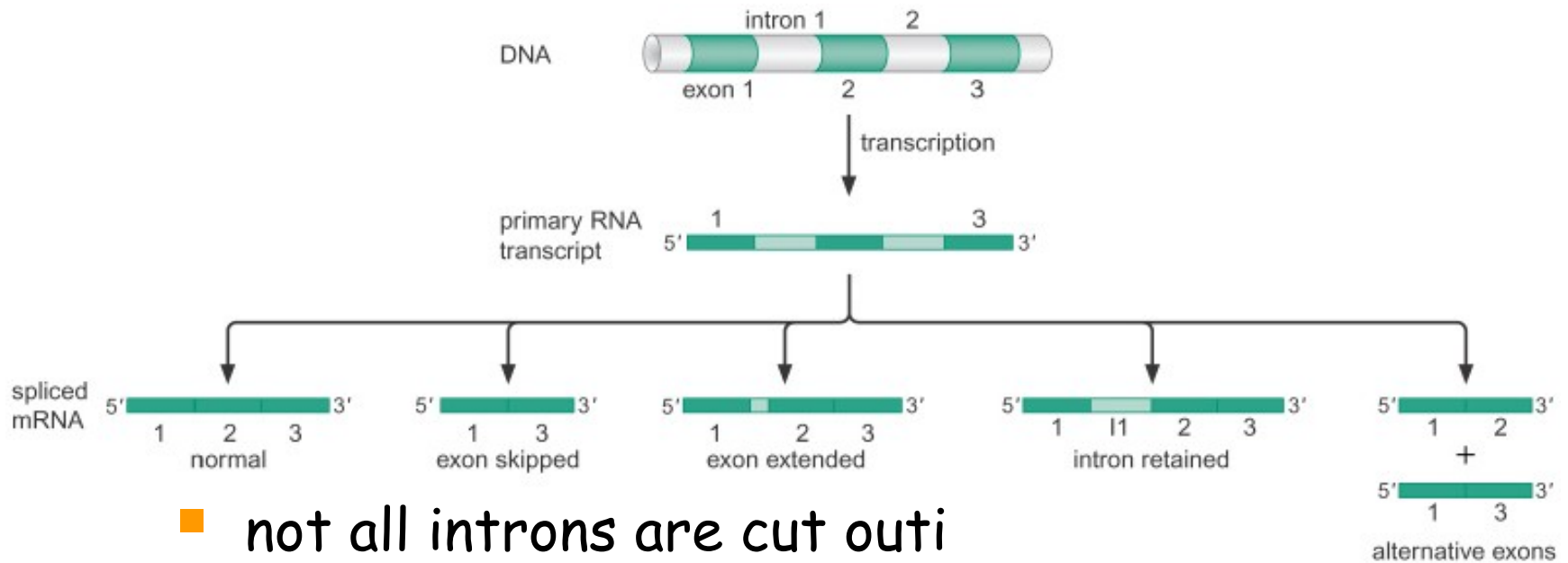
1993



Sequences coding RNA



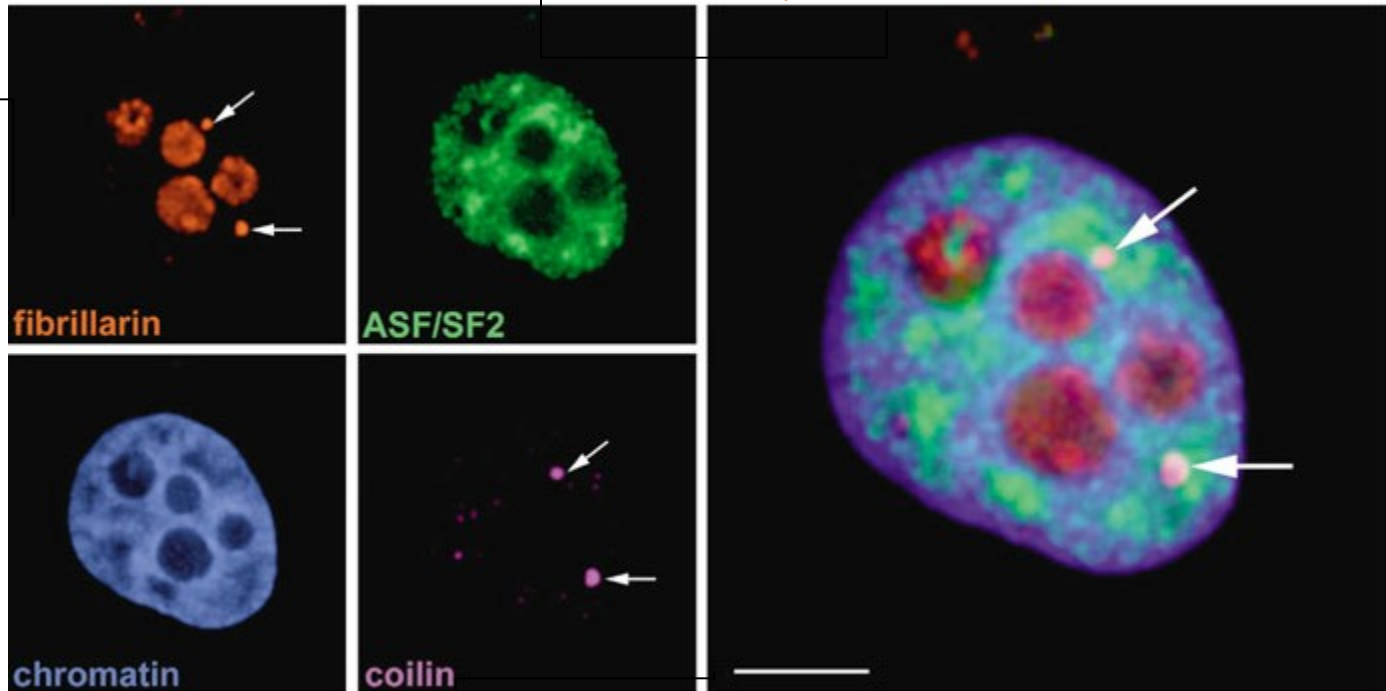
Alternative splicing



- not all introns are cut out
- exons are also cut out
- result: formation of **protein families**

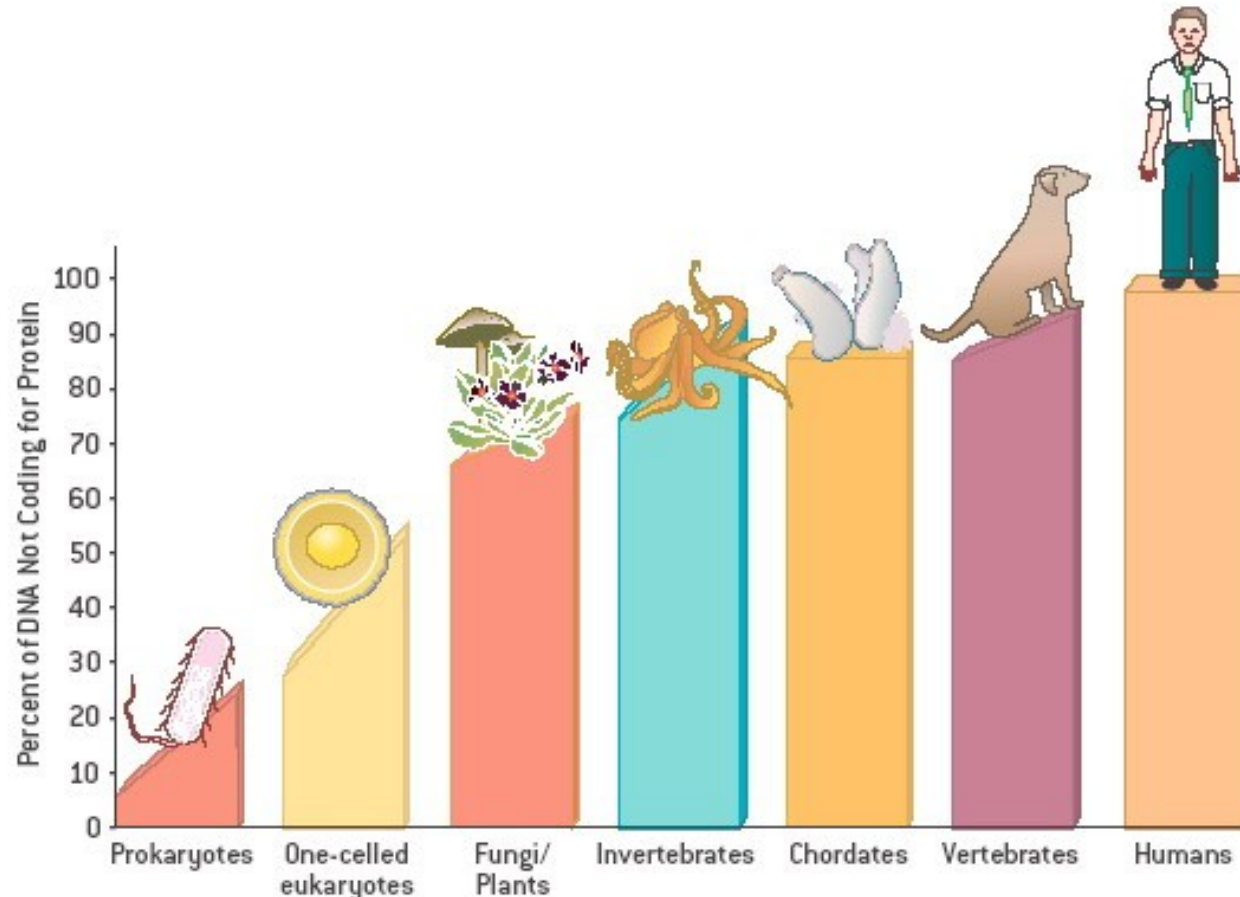
Small nuclear ribonucleoprotein (snRNP) - splicing
pre-rRNA - DFC

Alternative splicing factor/splicing factor 2
- pre mRNA splicing;
RNA export, translation

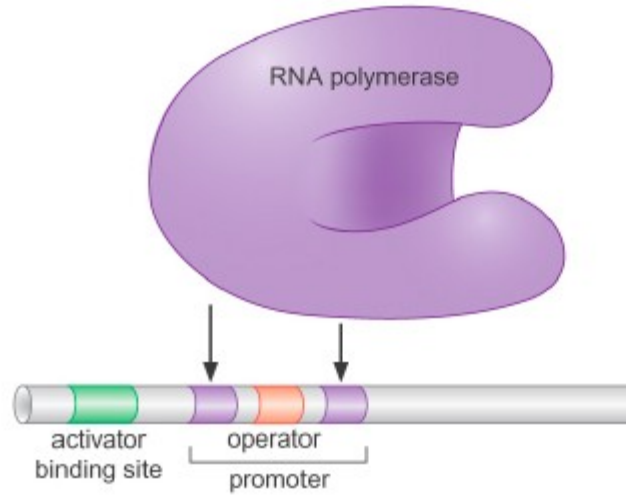


Main composing molecule of Cajal bodies - fixes to the nucleolus, posttranslation modification of RNA, associates with NOR and determines the association of individual nucleoli

Ratio of *non-protein coding sequences* at different stages of phylogenesis



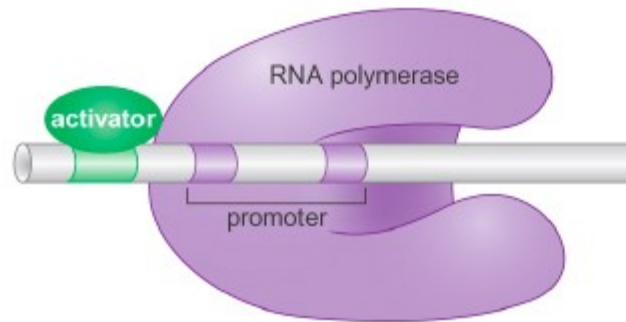
NONPROTEIN-CODING SEQUENCES make up only a small fraction of the DNA of prokaryotes. Among eukaryotes, as their complexity increases, generally so, too, does the proportion of their DNA that does not code for protein. The noncoding sequences have been considered junk, but perhaps it actually helps to explain organisms' complexity.



Basic level
transcription



No
transcription



Spontaneous
isomerization
activated transcription

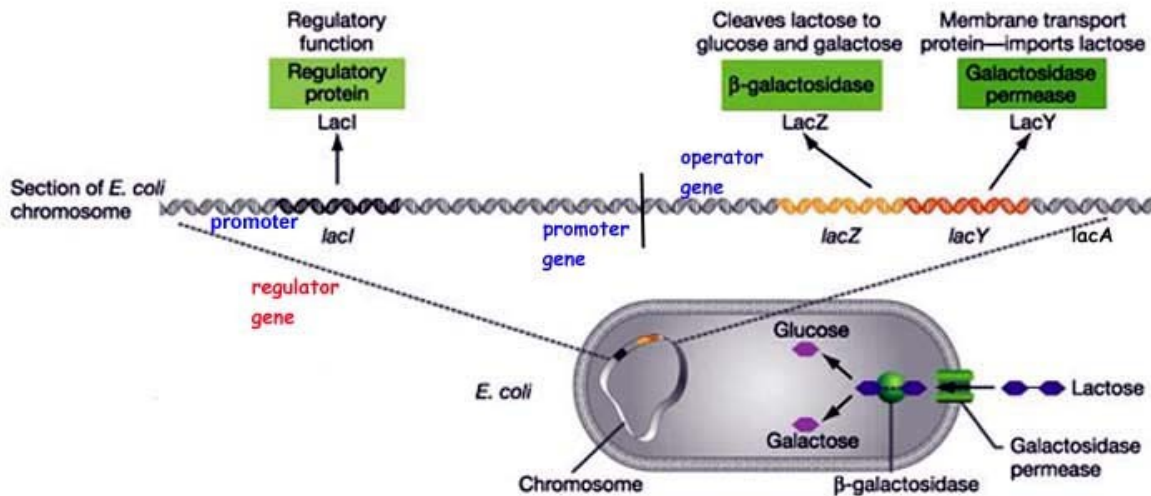


**François
Jacob**

**André
Lwoff**

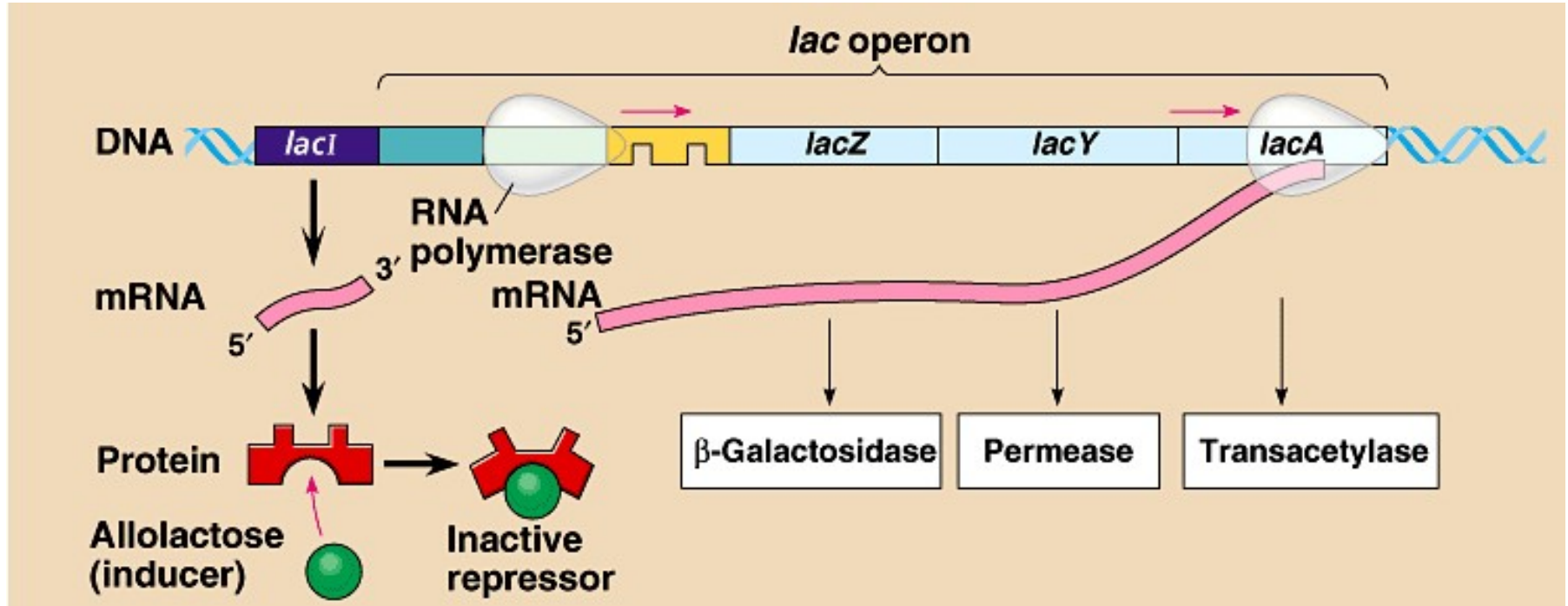
**Jacques
Monod**

"for their discoveries concerning genetic control of enzyme and virus synthesis"



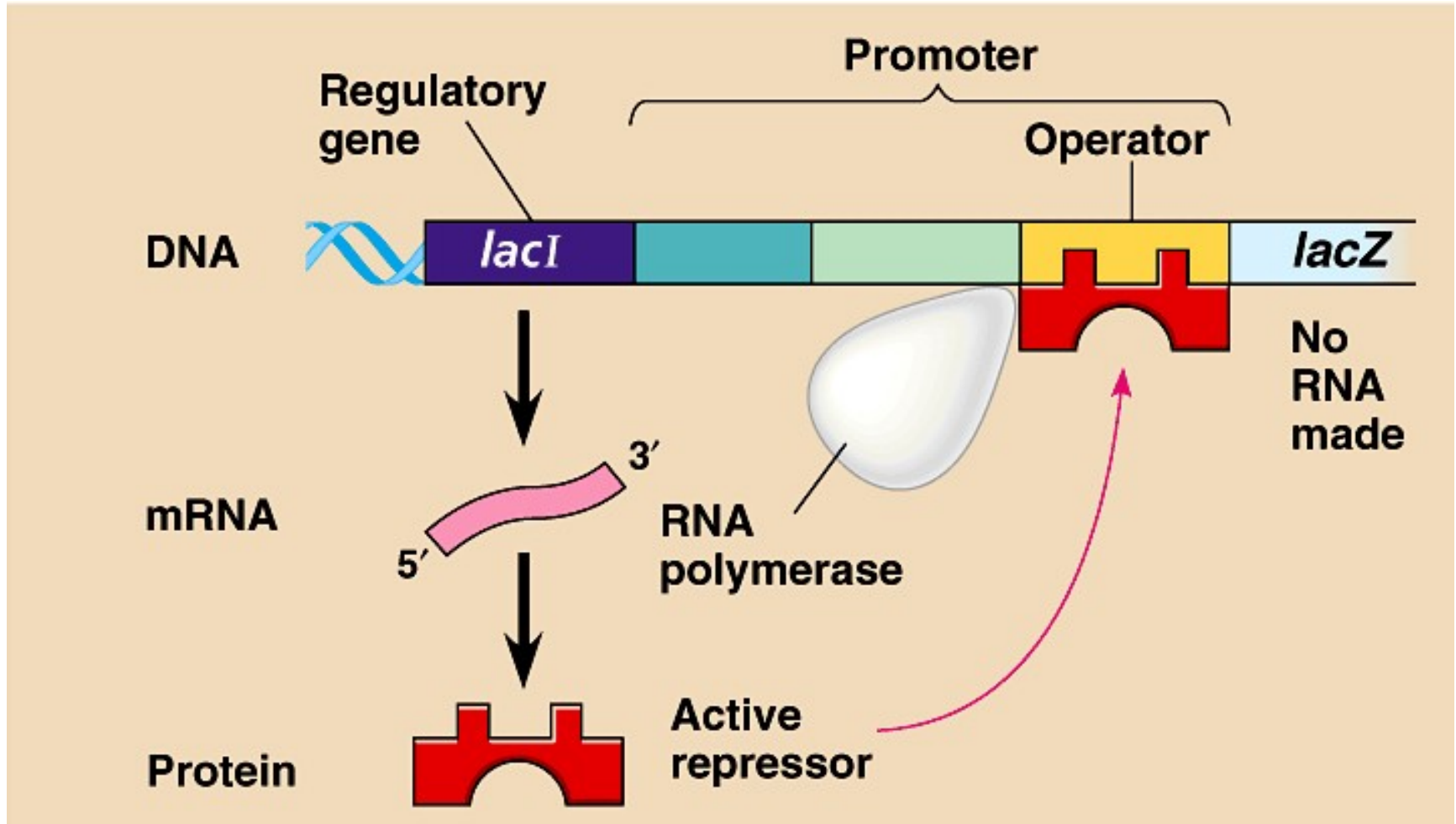
Regulator - repressor
Promoter - RNA polym.
Operator - repressor-bind.
Structure genes - enzymes

Gene Level Regulation 1 - Prokaryotes



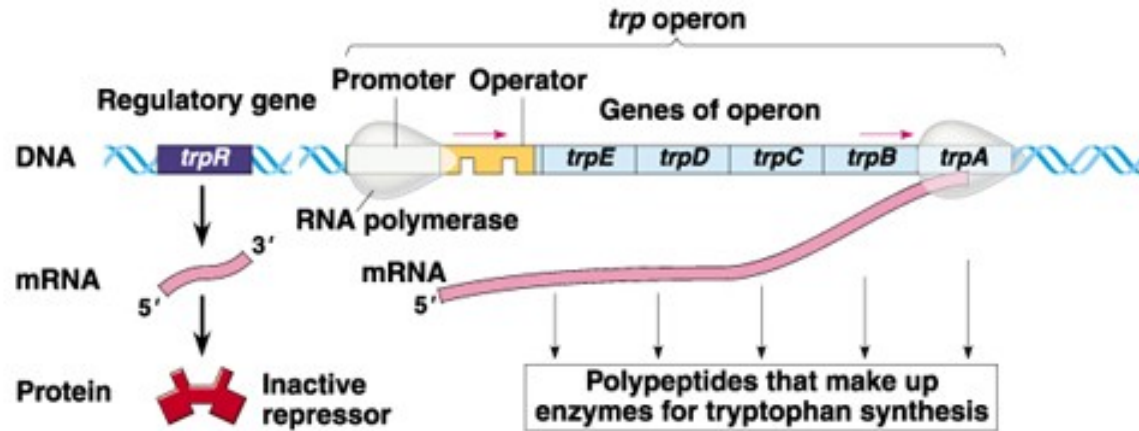
Presence of lactose - **blocked** repressor - **active** protein synth.

Gene Level Regulation 2 - Prokaryotes

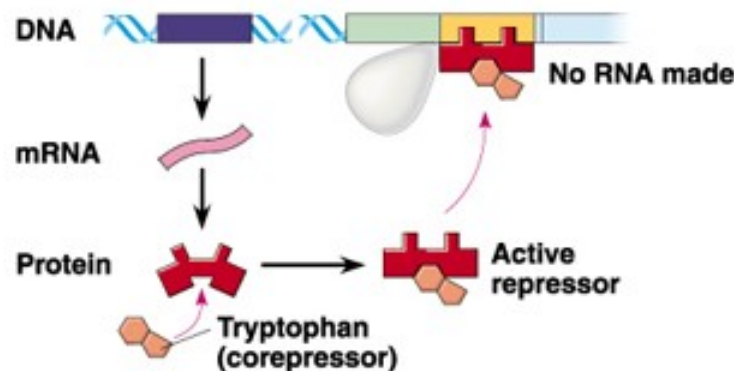


Lactose deficiency - active repressor - blocked prot. synth.

Gene Level Regulation 3 - Prokaryots

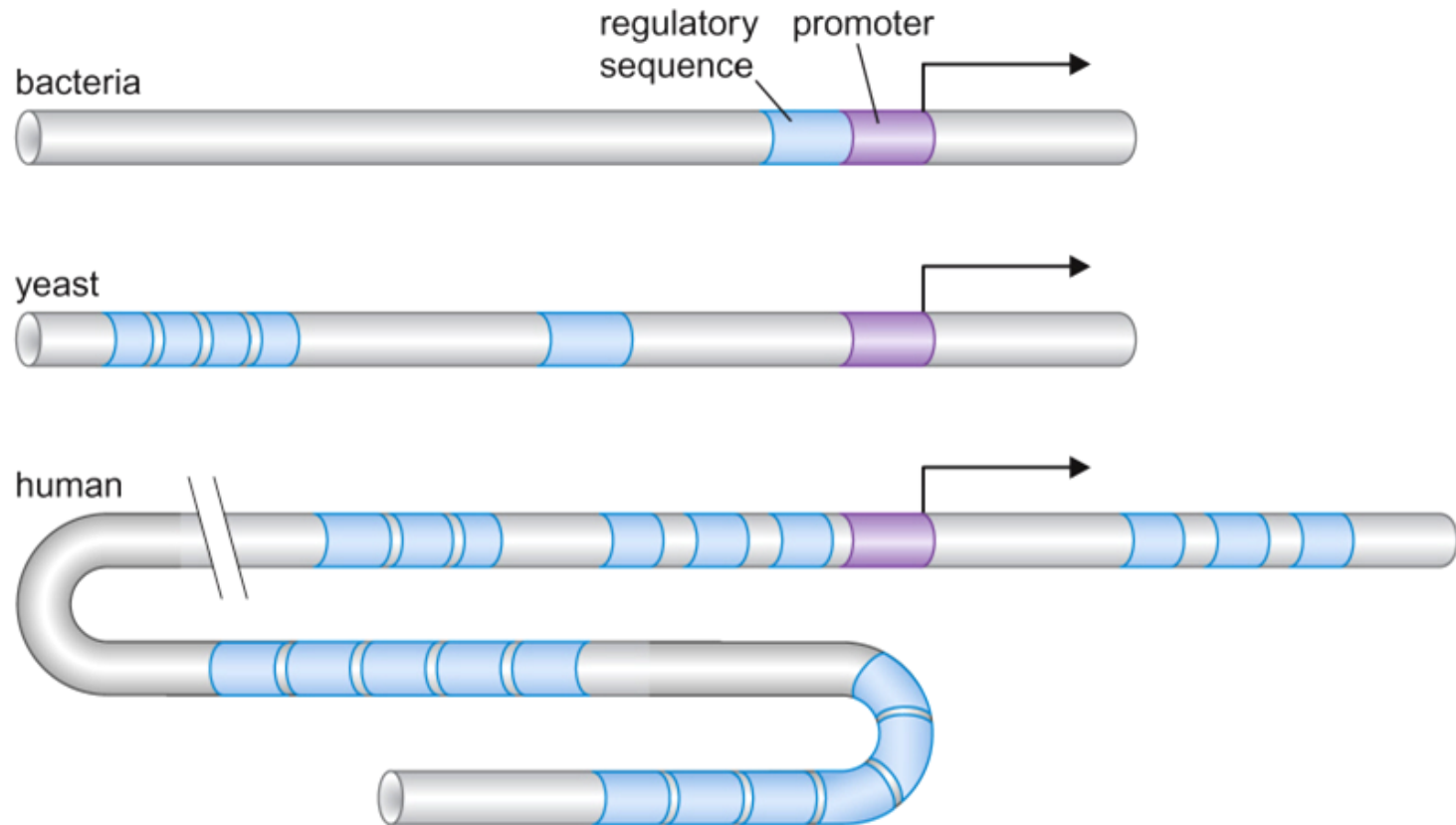


Trp deficiency - **blocked** repressor - **active** prot. synth.



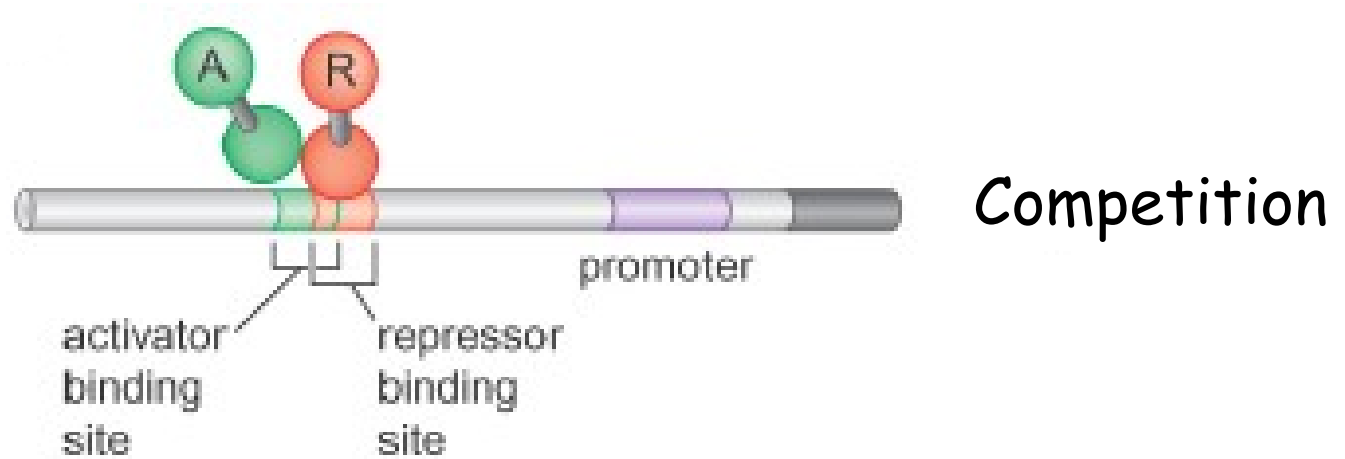
Presence of Trp - **active** repressor - **blocked** prot. synth.

Phylogeny of gene-level regulation



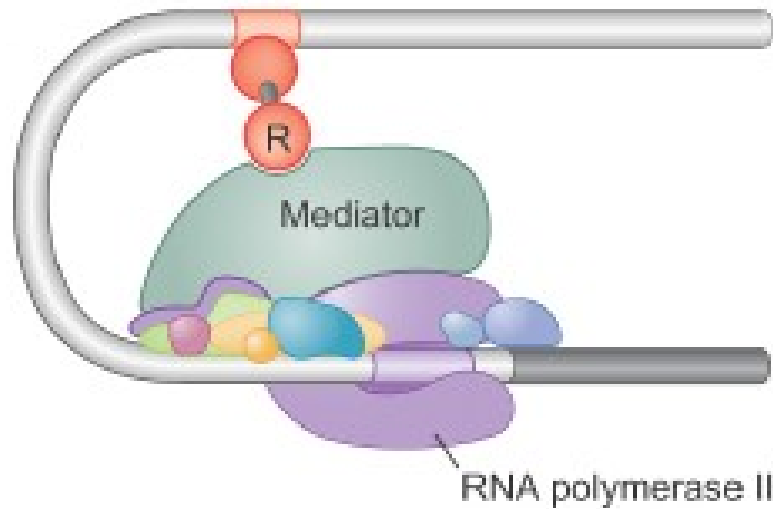
Transcriptional regulatory mechanisms 1

- Eukaryots -

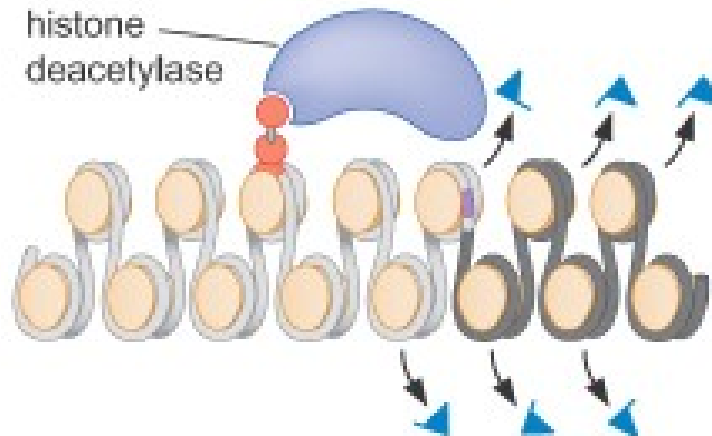


Transcriptional regulatory mechanisms 2

- Eukaryots -

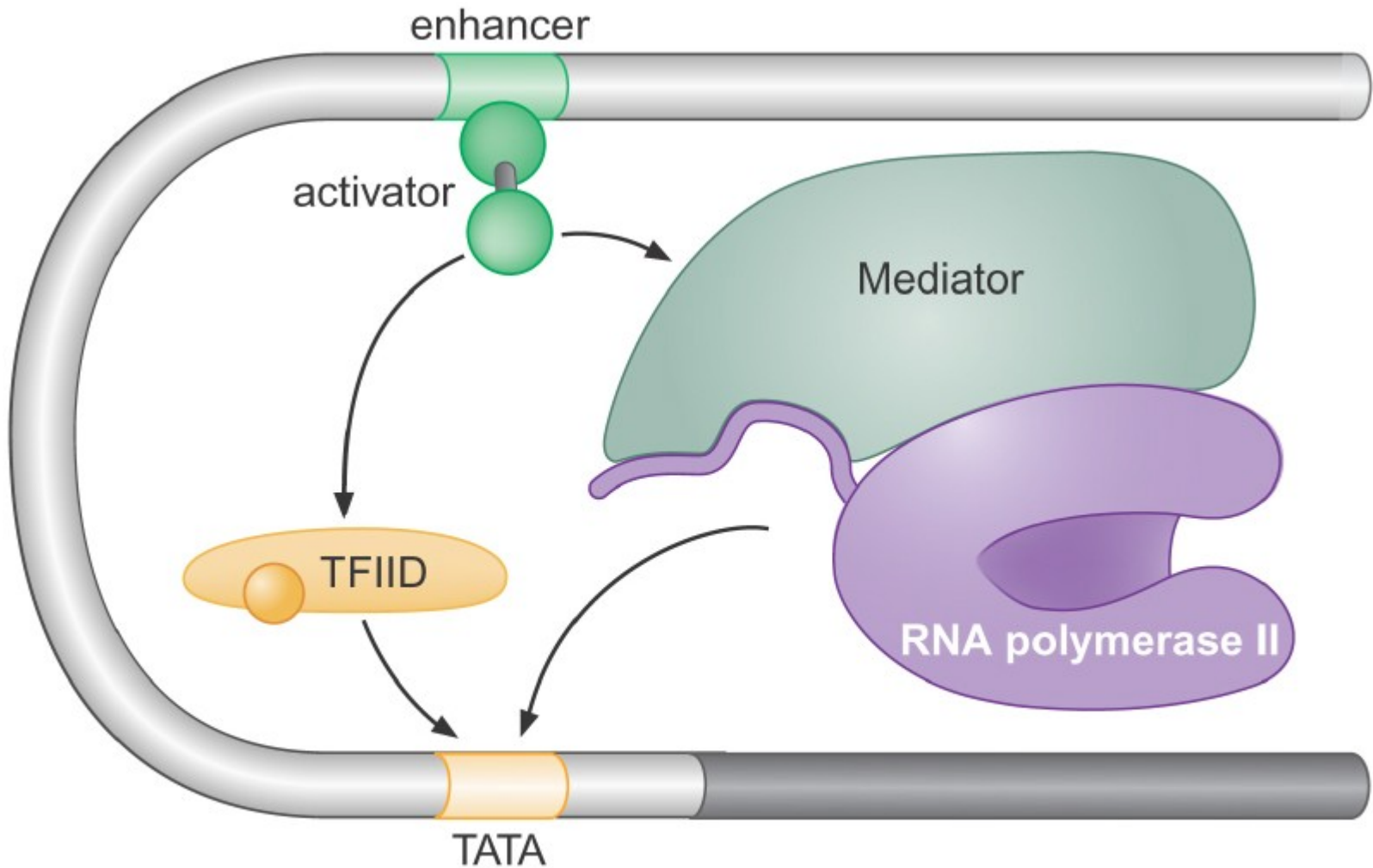


Direct
repression

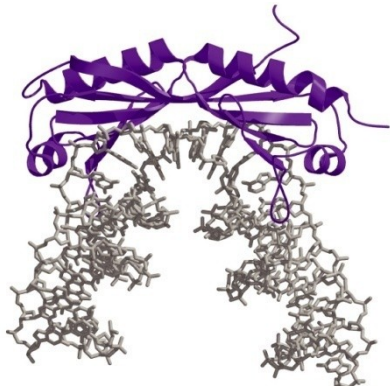


Indirect
repression

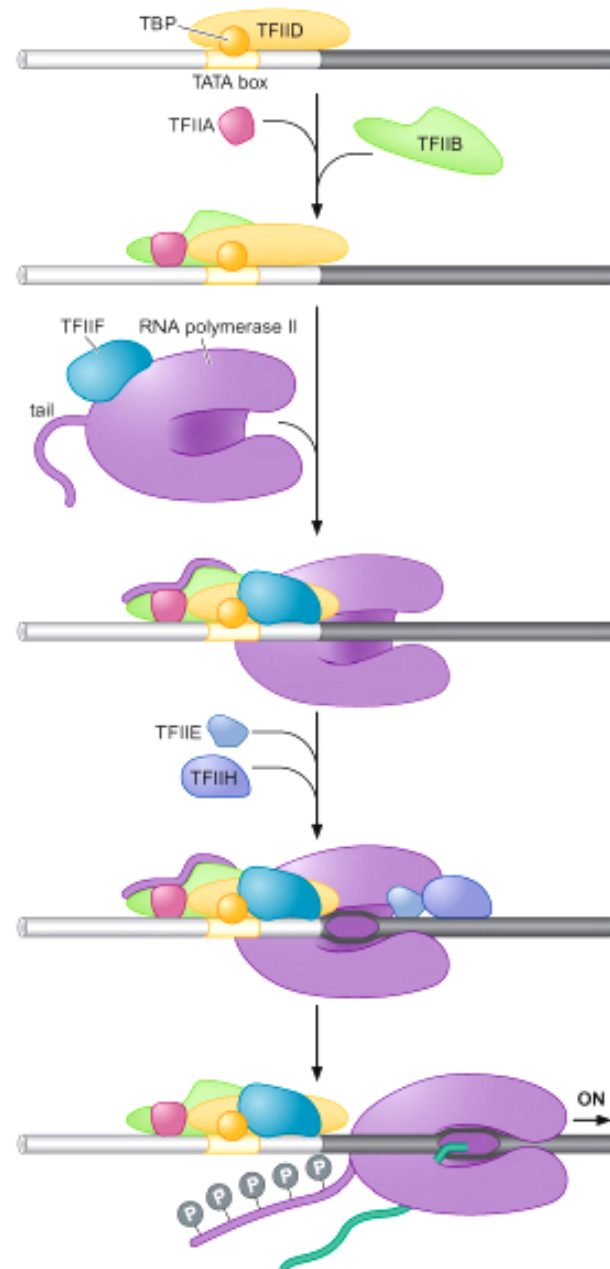
Transcriptional regulatory mechanisms 3 - Eukaryots -



Transcriptional regulatory mechanisms 4 - Eukaryots -



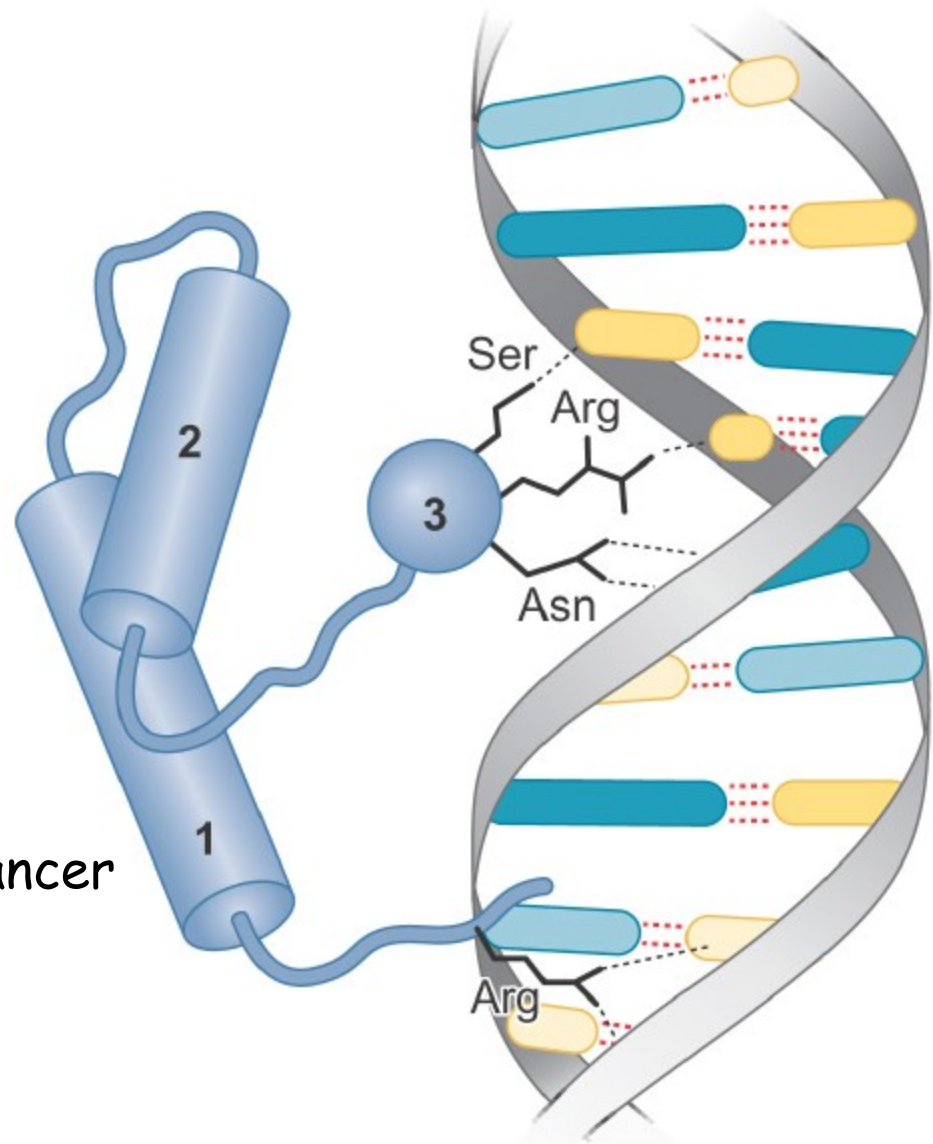
TBF = TATA binding protein
TF = transcription factor



Transcription factors

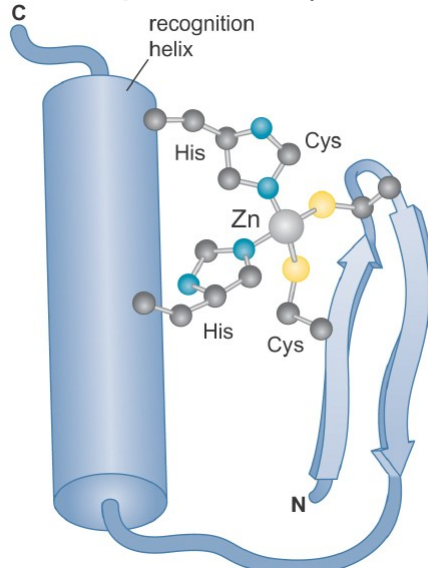
Homeo-domain structure

- 3 alpha-helical structures
- of which 1 regulates
- the other 2 stabilizer
- connected to: promoter, enhancer



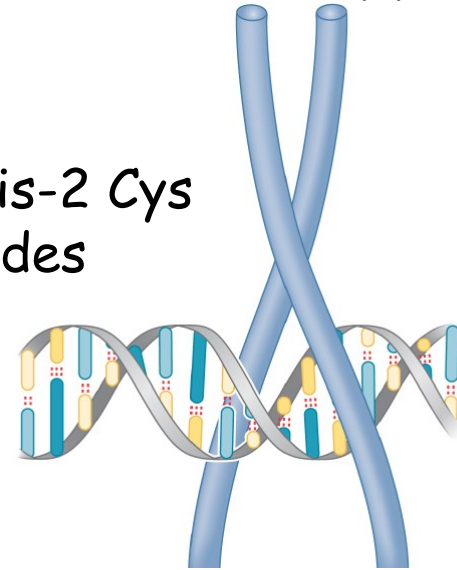
Transcription factors 2

Zn-fingered proteins

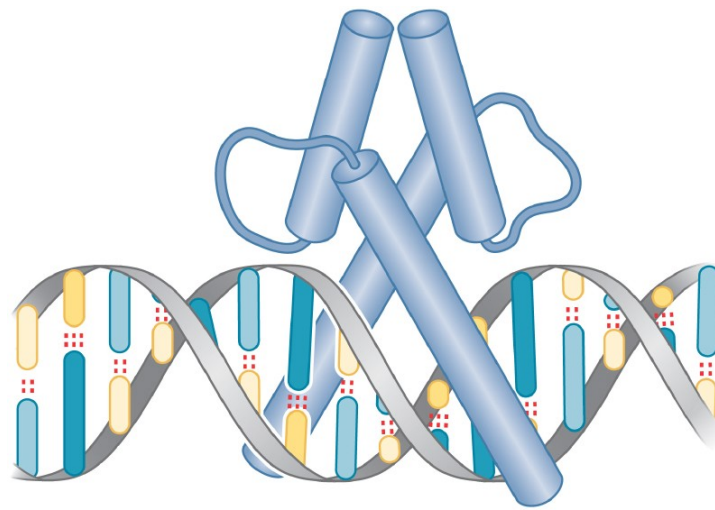


- 2 beta sheets+ 1 alpha helix
- the Zn^{2+} bound to 2 His-2 Cys
- linked to 3-4 nucleotides

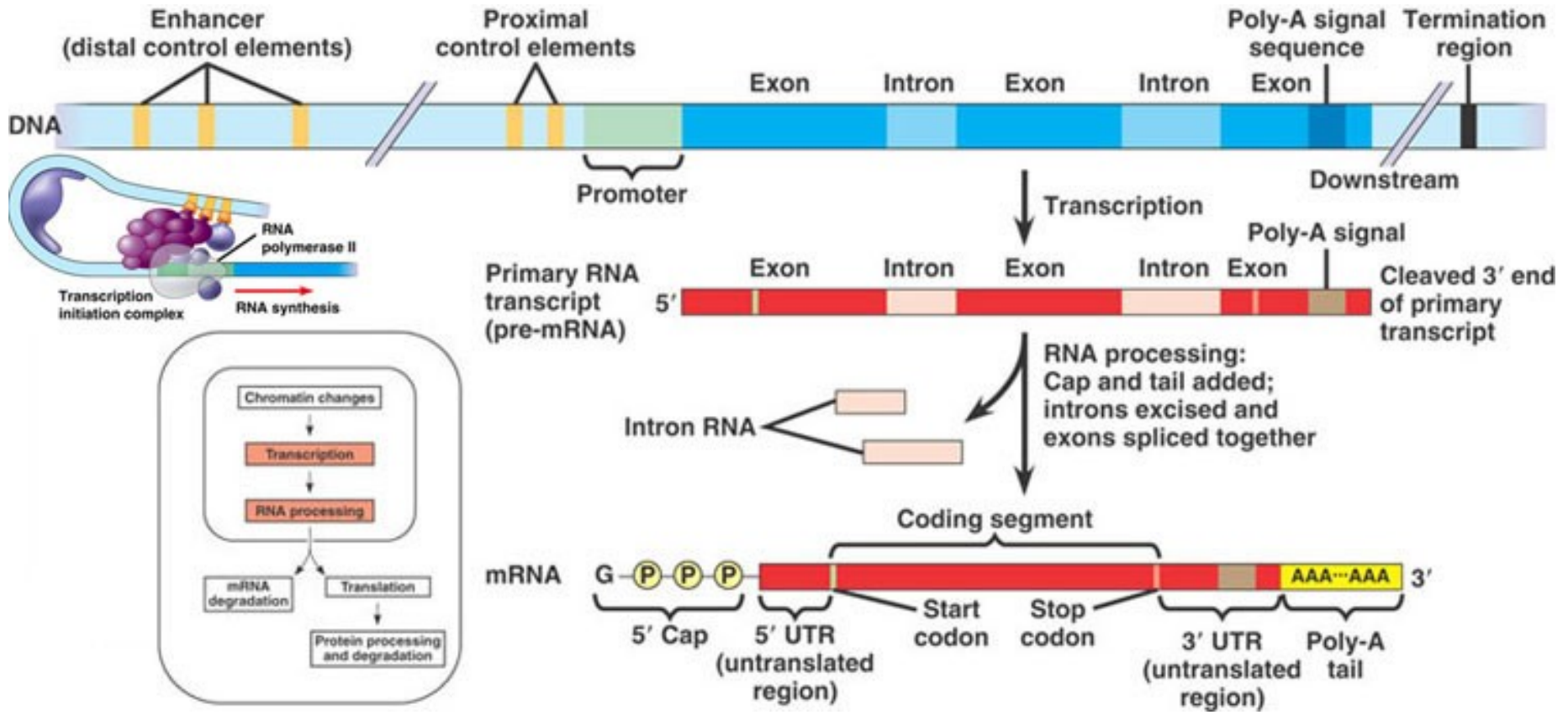
Leucine-zipper



Helix-loop-helix motive

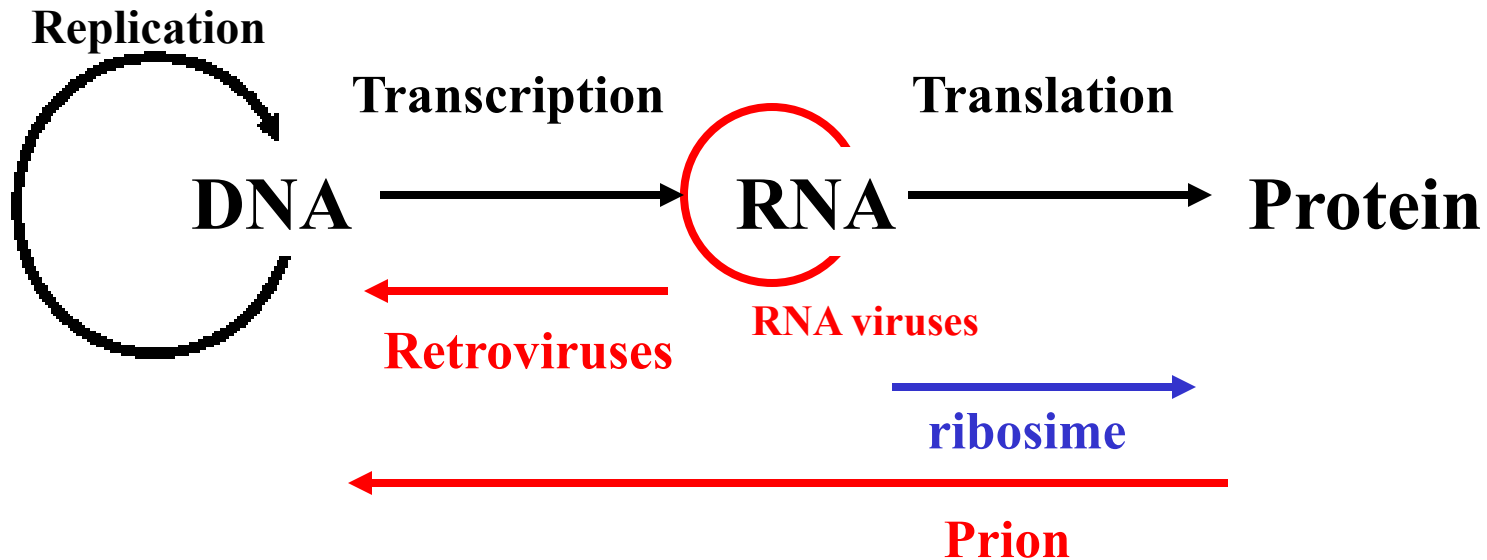


- 2 helical domains
- dimerization
- bound to consensus sequ.

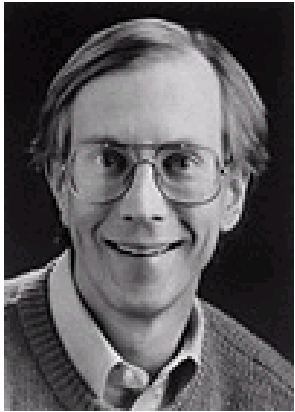


Appendix

The biological information flow - „The reality„

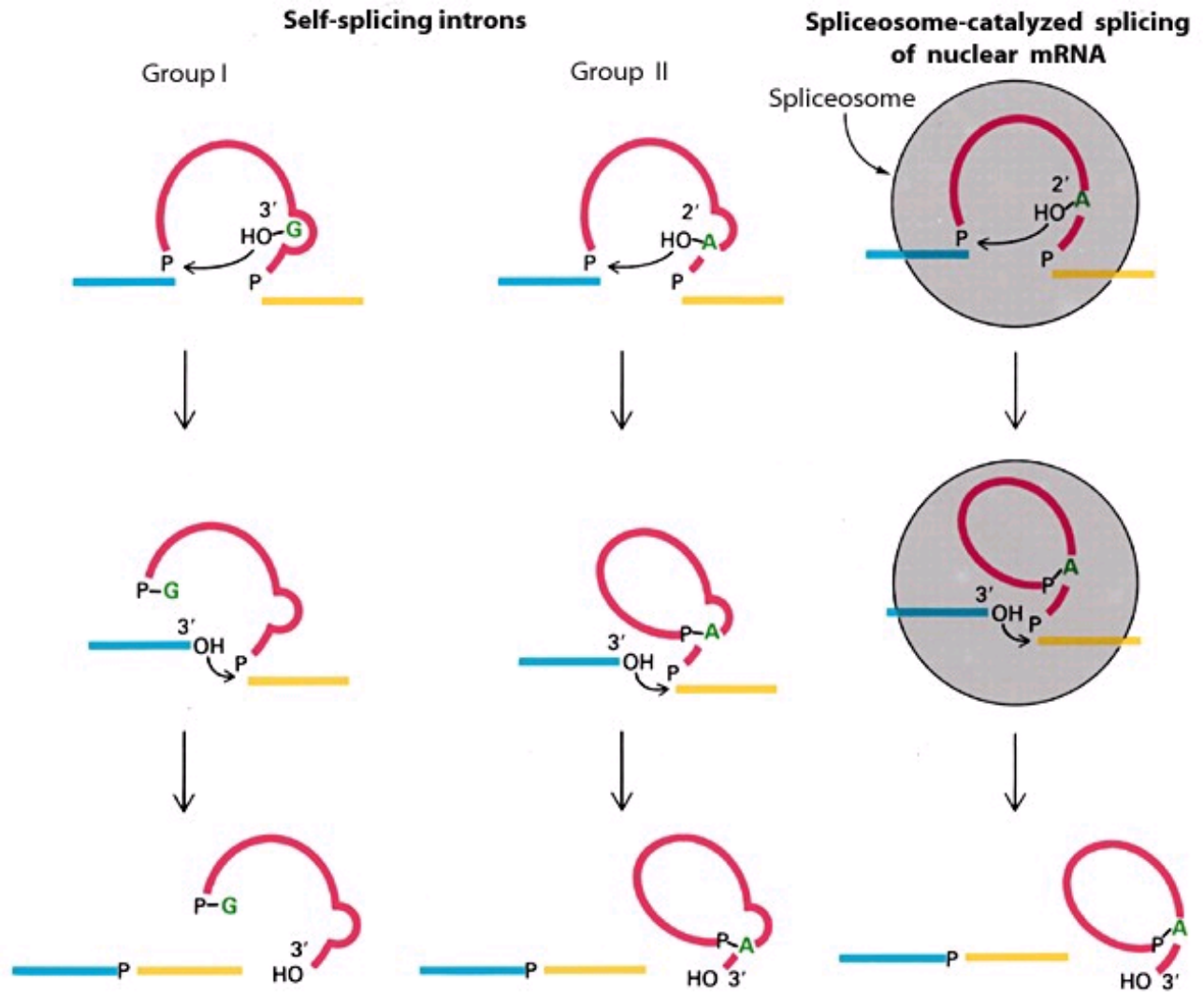
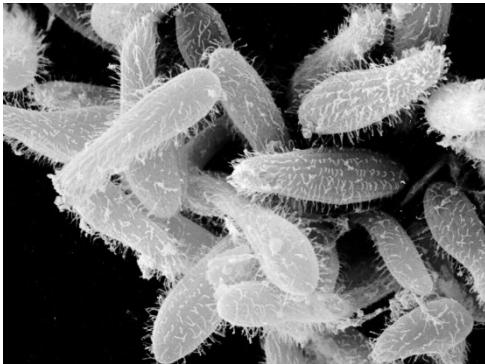


Ribosome

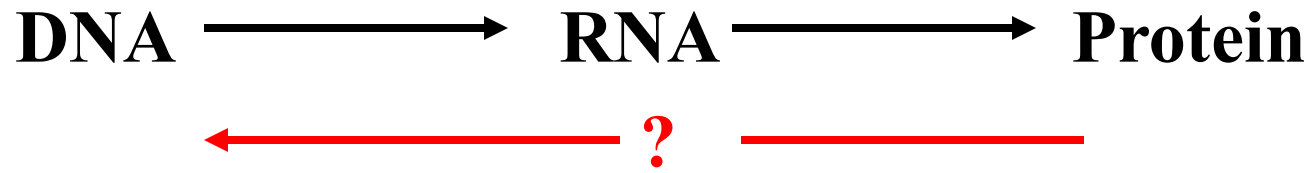


Thomas Cech

1989

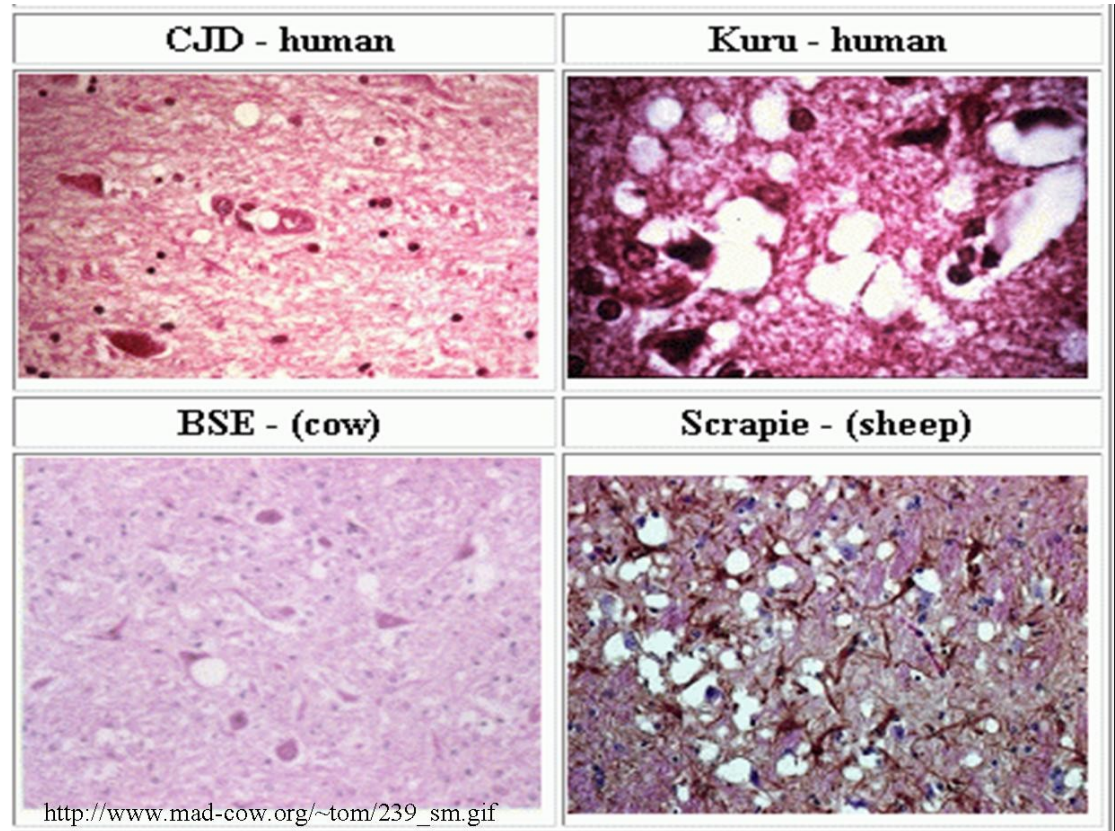


Prions



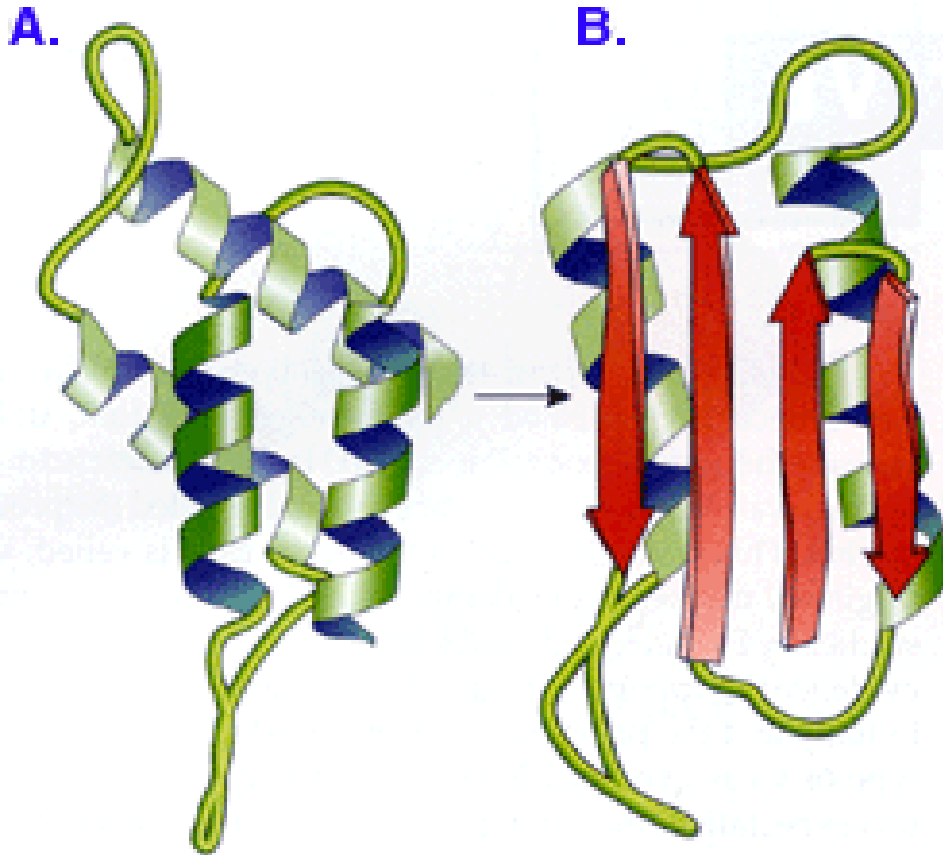
Stanley B. Prusiner

1997



Spongiform encephalopathia

Structure of prion



AS sequence is identical

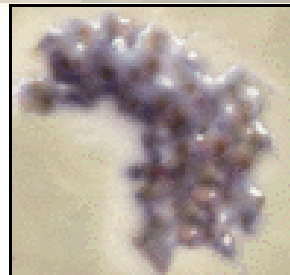
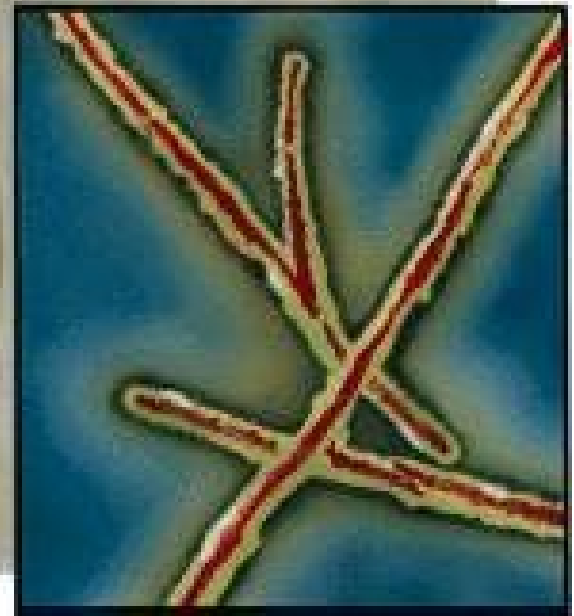
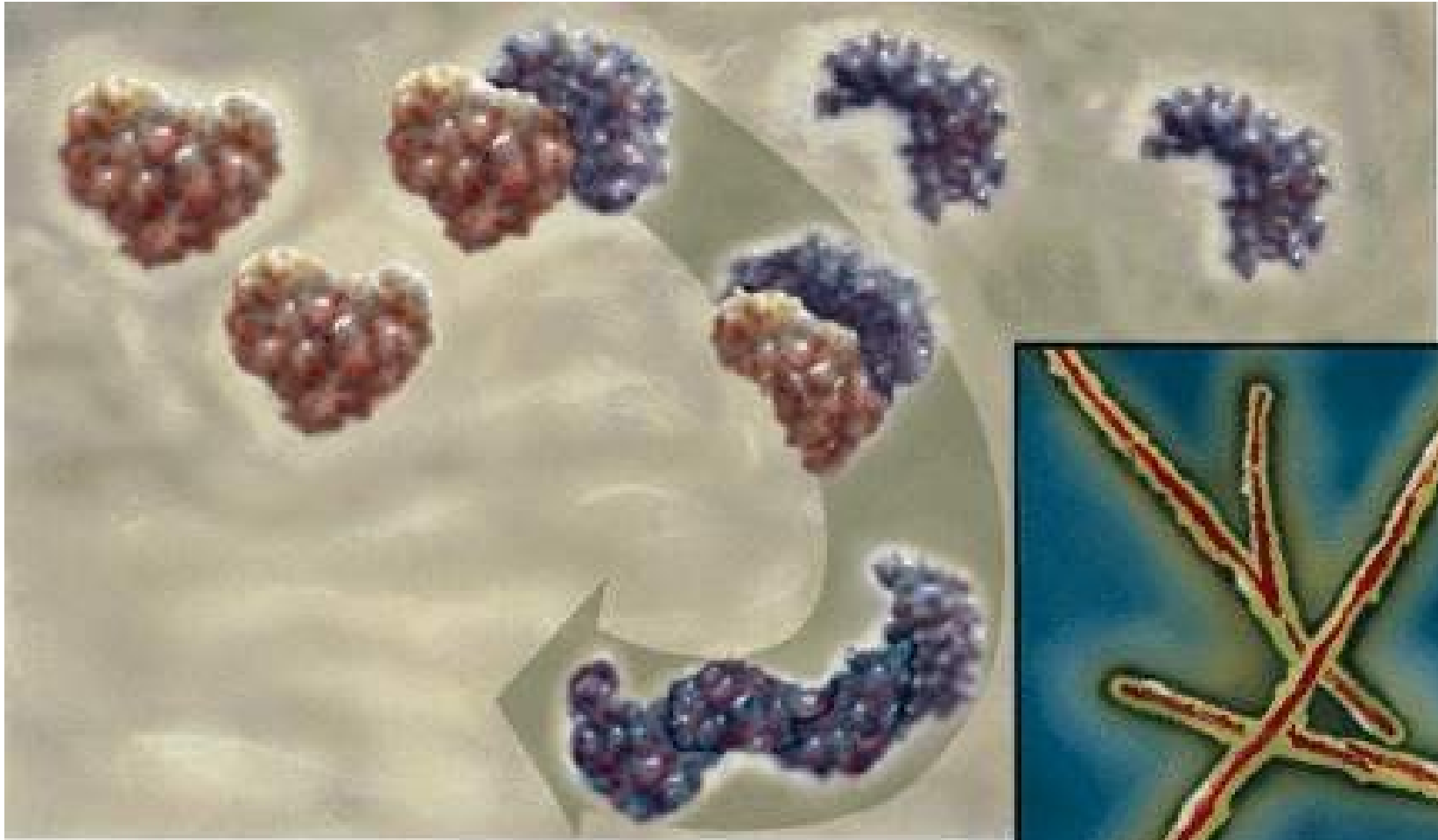
A. normal (PrP^c) protein
soluble α -helix

B. abnormal (PrP^{sc}) protein
45% β -sheet – insoluble

Thermostable,

Resistant to UV & proteases

Aggregates on the cell surface



PrPc

PrPSc

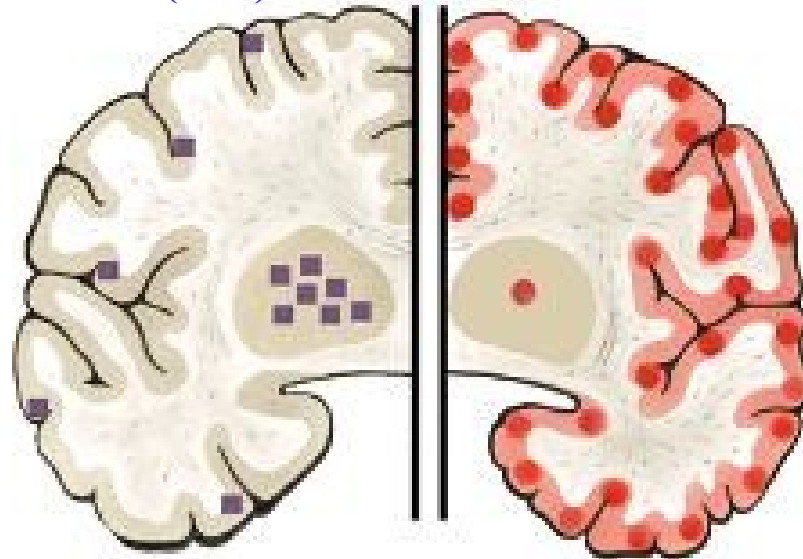
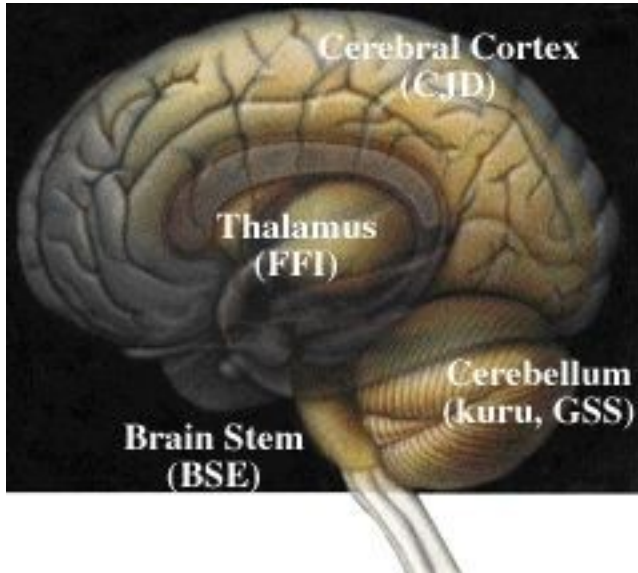
Aggregates to long filaments
=> neuron damages

Appearances - localization

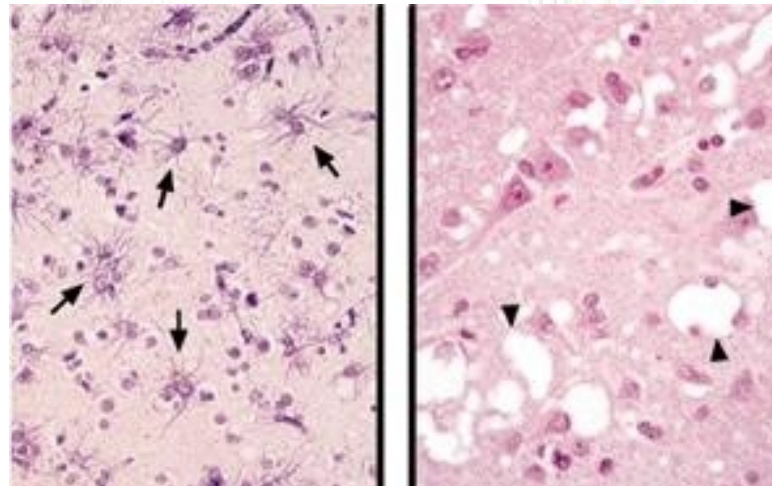
Fatal familial

<-> Creutzfeldt-Jakob disease

insomnia (FFI)

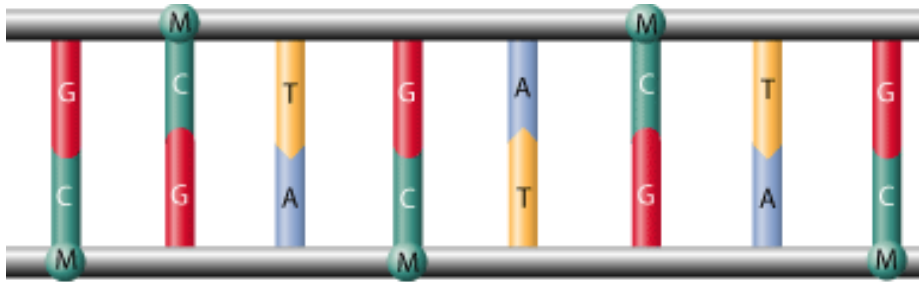


GSS= Gerstmann -Straussler-
Scheinker disease

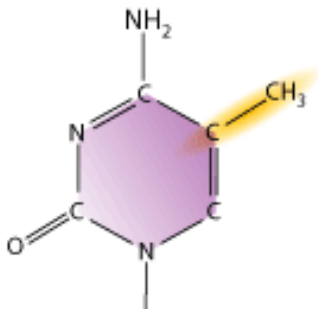


Other contradictions

- Inheritance of methylation pattern
=> Epigenetics
- Structural inheritance
- Sense - antisense strand?



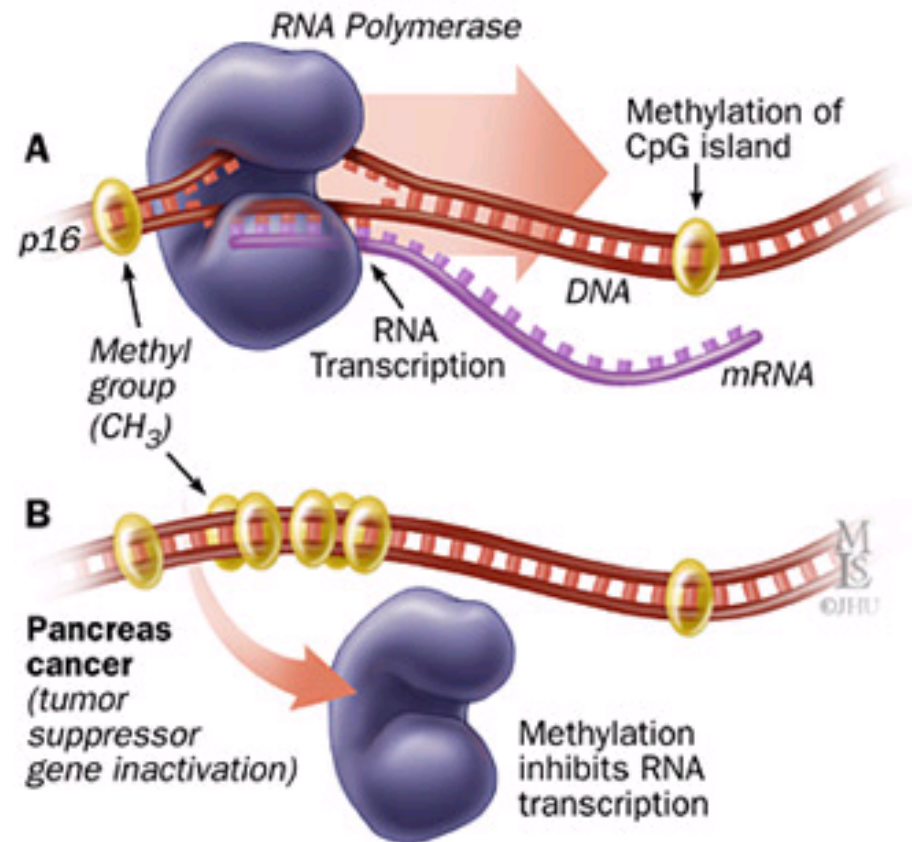
Methylation pattern



DNA methylation is the addition of a methyl group (M) to the DNA base cytosine (C).

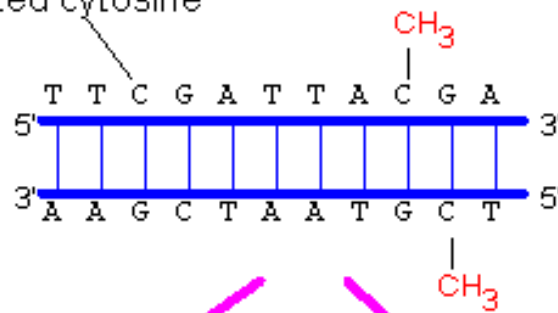
CpG island

Result: **gene silencing**

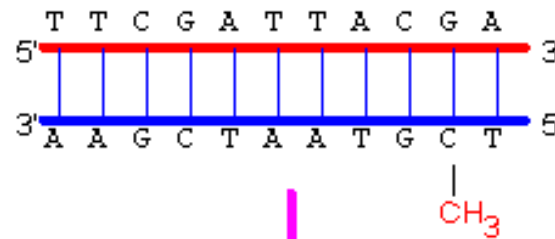
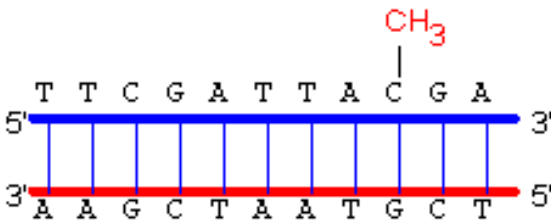


Inheritance of methylation pattern

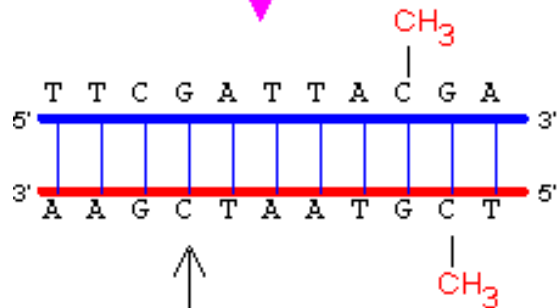
Unmethylated cytosine



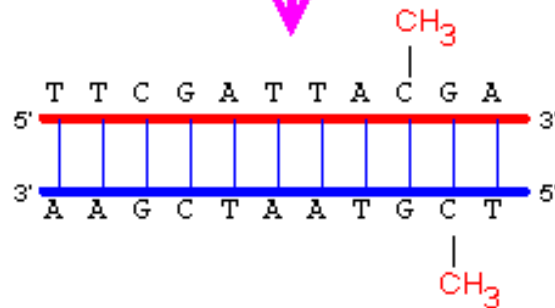
DNA replication



Methylation

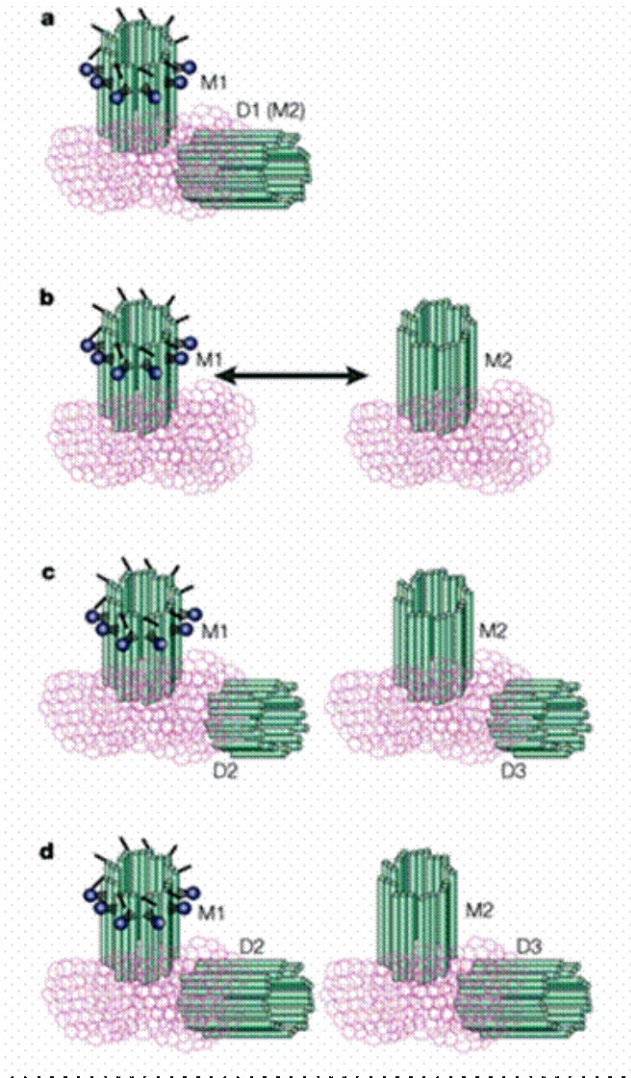


Methylation



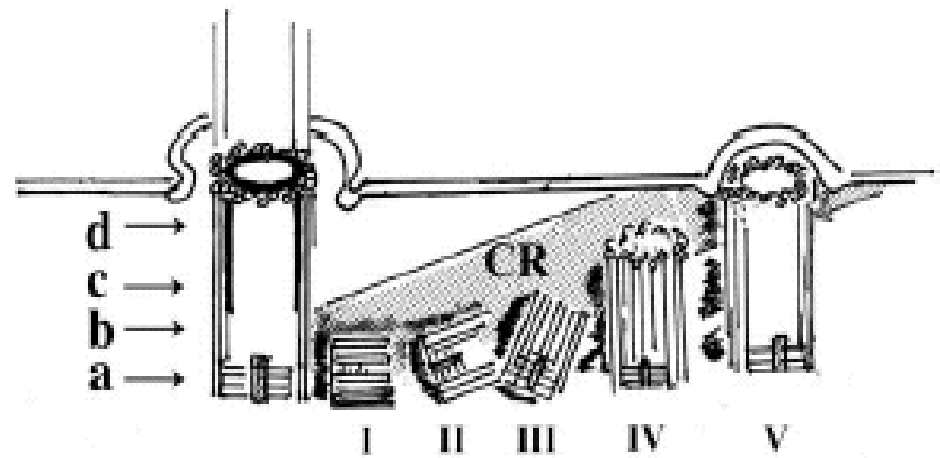
Not recognized by maintenance methylase

Duplication of cytocentre



Structural inheritance

Basal body of Paramecium cilia



„Sense“ in the antisense strand ???

Sense – the corresponding mRNA serves the code of the amino acid sequence of protein (e.g. protein A)

Antisense - mRNA serves the code of an other, functionally related protein to protein A

