Structure of the Nucleus and nuclear membrane

Function of the Nucleus

HUGO- HUman GenOme project

Eukaryotic Nucleus - Structure

* Major components
  * An apparent amorphous mass enclosed by a *nuclear envelope*
  * Within:
    * *Chromosomes*
      * *Chromatin*
    * *Nuclear matrix*
      * Fibrillar network
   * *Nucleoli*
      * r-RNA and ribosomes
   * *Nucleoplasm*
      * Fluid of the nucleus

Eukaryotic Nucleus - Structure

*The nuclear envelope

*Components:

*Two cellular membranes

*Barrier to ions, solutes, macromolecules

*Membranes fused to form pores

*Complex assemblies of proteins

*Outer membrane

*Ribosomes

*Continuous with RER

Nucleus

Nuclear pores

1 μm

3
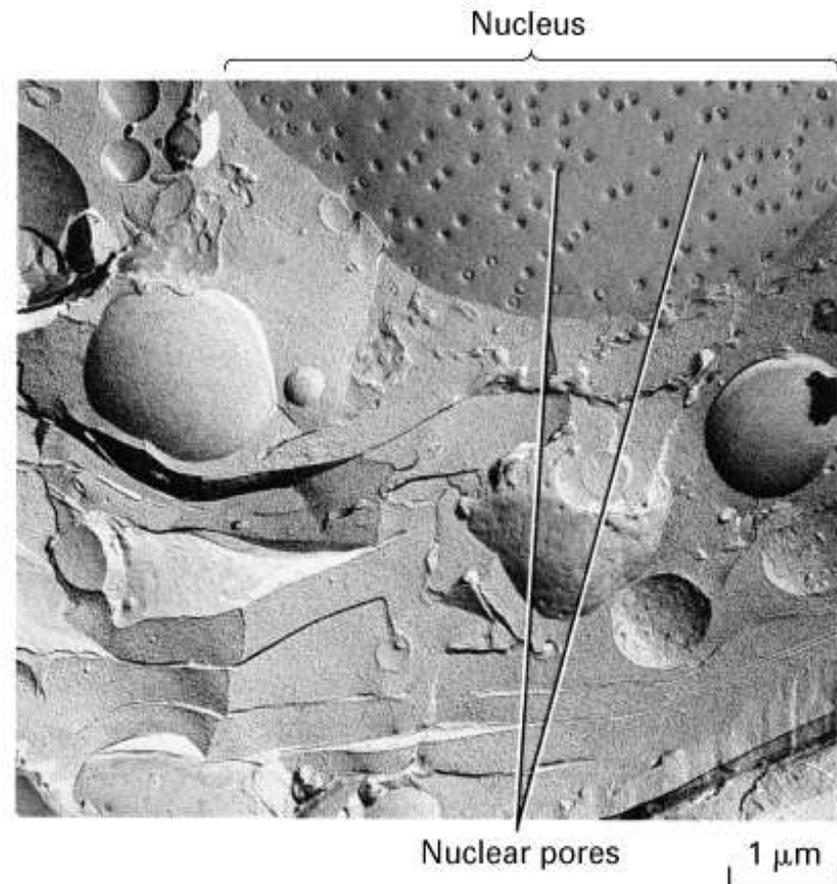
Eukaryotic Nucleus - Structure

*The nuclear envelope

  *Components:

    *Inner membrane

      * Bound to *nuclear lamina*

        * Filamentous network

    * Nuclear lamina

        * Supports envelope

        * Attachment of chromatin

    * Nuclear lamina fibers

        * Proteins – *lamins*

   superfamily – Intermediate filaments of cytoplasm.
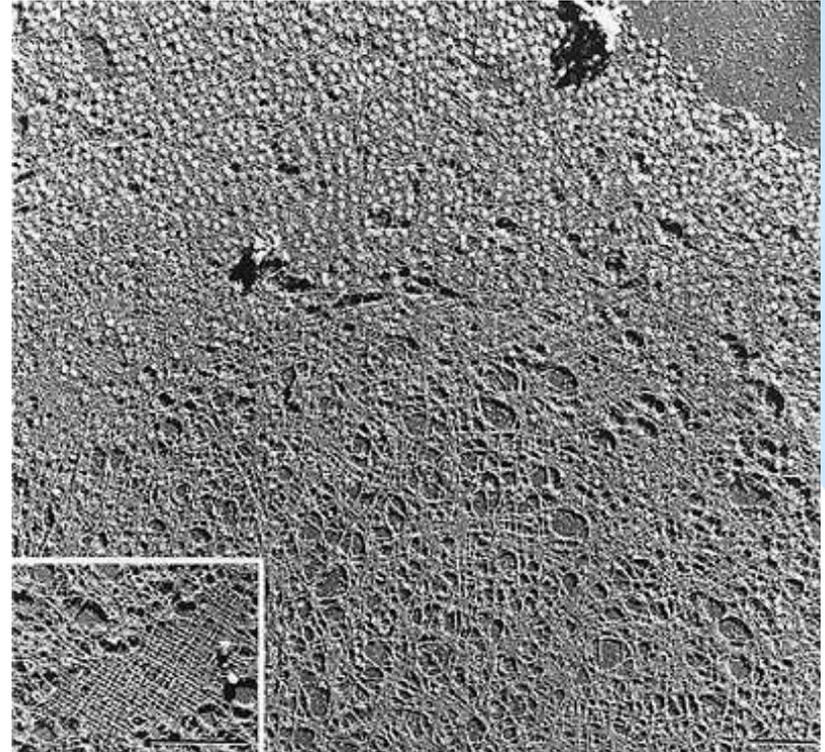
Reprinted with permission from Ueli Aebi, et al., Nature
323:561, 1986; © copyright 1986 Macmillan Magazines Limited.

*Lamins are filamentous proteins in the intermediate filament family

* Lamin phosphorylation in prophase disassembles the nuclear lamina & allows for nuc. envel. breakdown

* Lamin<u>ins</u> are extracellular proteins, unrelated
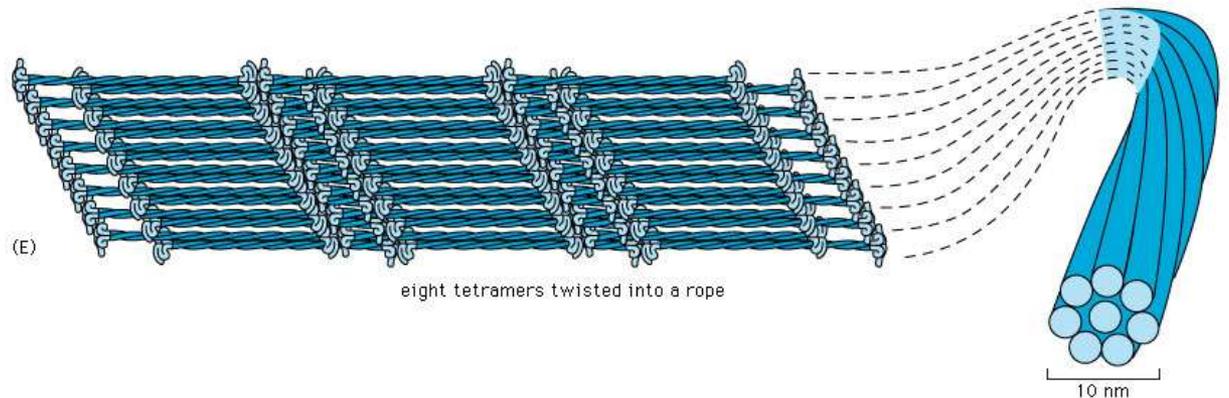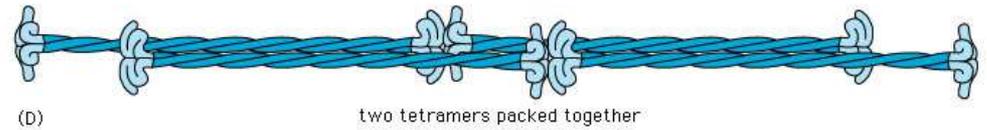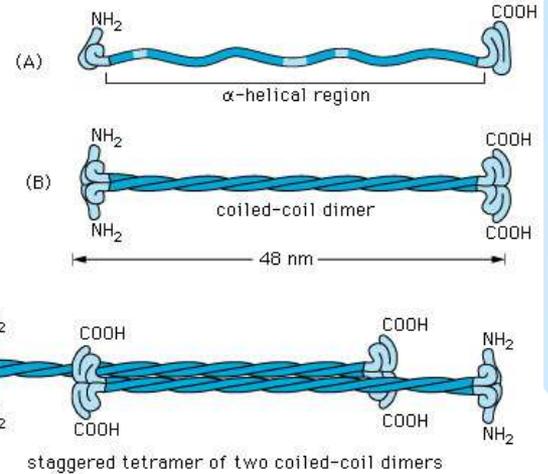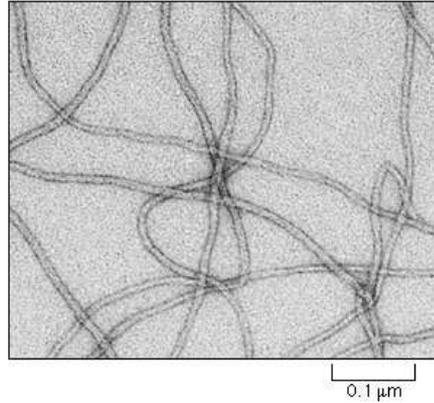
(A) α-helical region

(B) coiled-coil dimer — 48 nm

(C) staggered tetramer of two coiled-coil dimers

(D) two tetramers packed together

(E) eight tetramers twisted into a rope

10 nm

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Eukaryotic Nucleus - Structure

*The nuclear envelope

   *Components:

Perinuclear space · Outer membrane · Inner membrane · Smooth endoplasmic reticulum · Nuclear pore complex · Nucleolus · Chromatin · Rough endoplasmic reticulum · Nuclear lamina · Ribosomes

6

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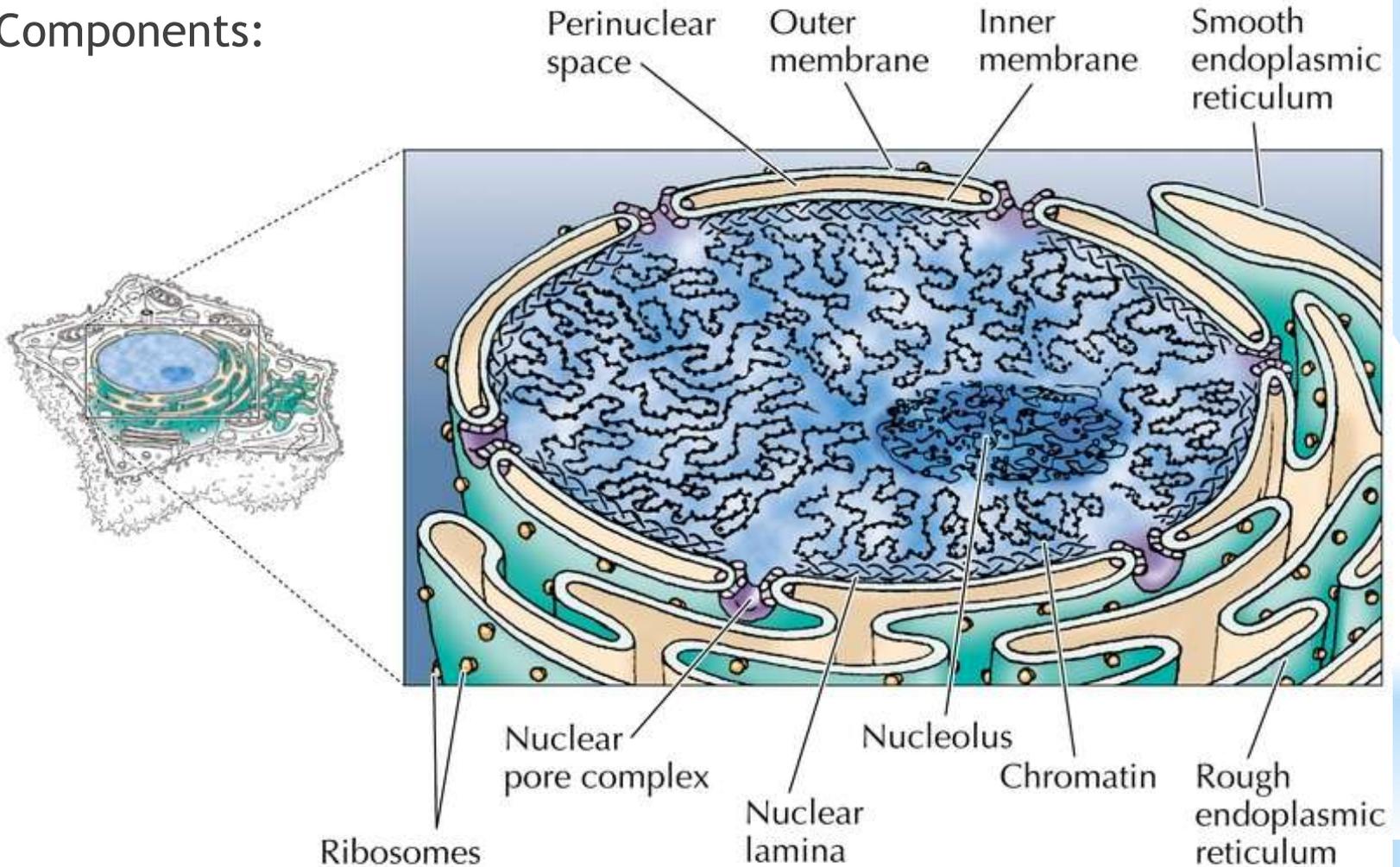
Eukaryotic Nucleus - Structure

*The nuclear pore complex (NPC)

  *Large numbers of proteins – synthesized in cytoplasm and transported into the nucleus

  *RNAs manufactured in nucleus – transported to cytoplasm
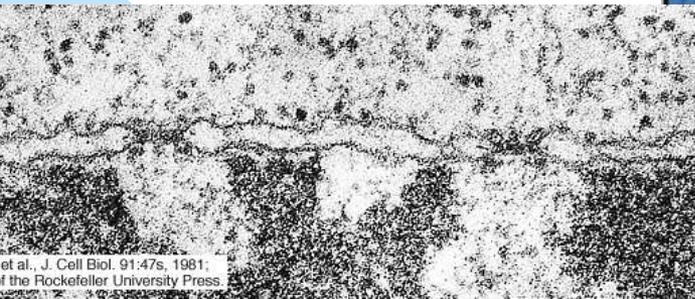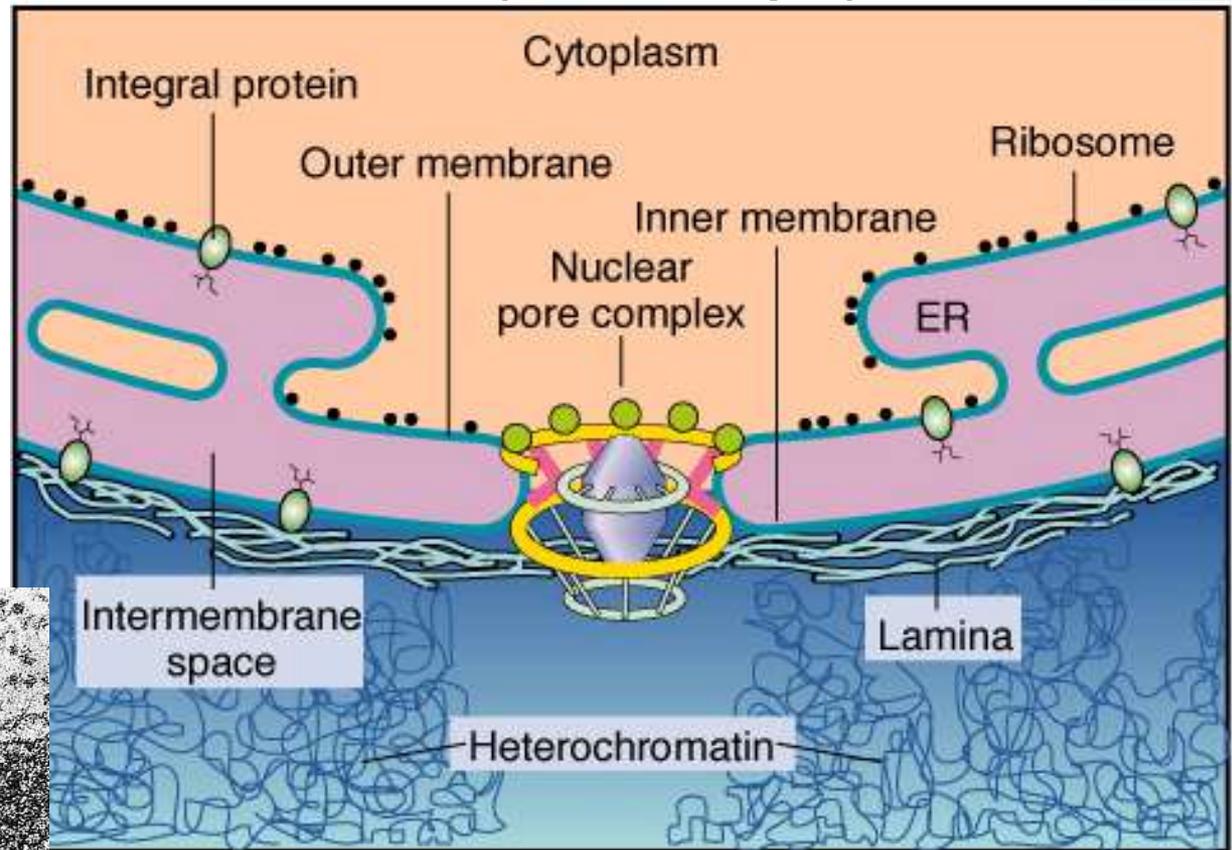
Eukaryotic Nucleus - Structure

*The nuclear pore complex (NPC)

  *Huge macromolecular complex

  *Octagonal symmetry
   * 8-fold repetition of subunits
    * 30-50 proteins – *nucleoporins*
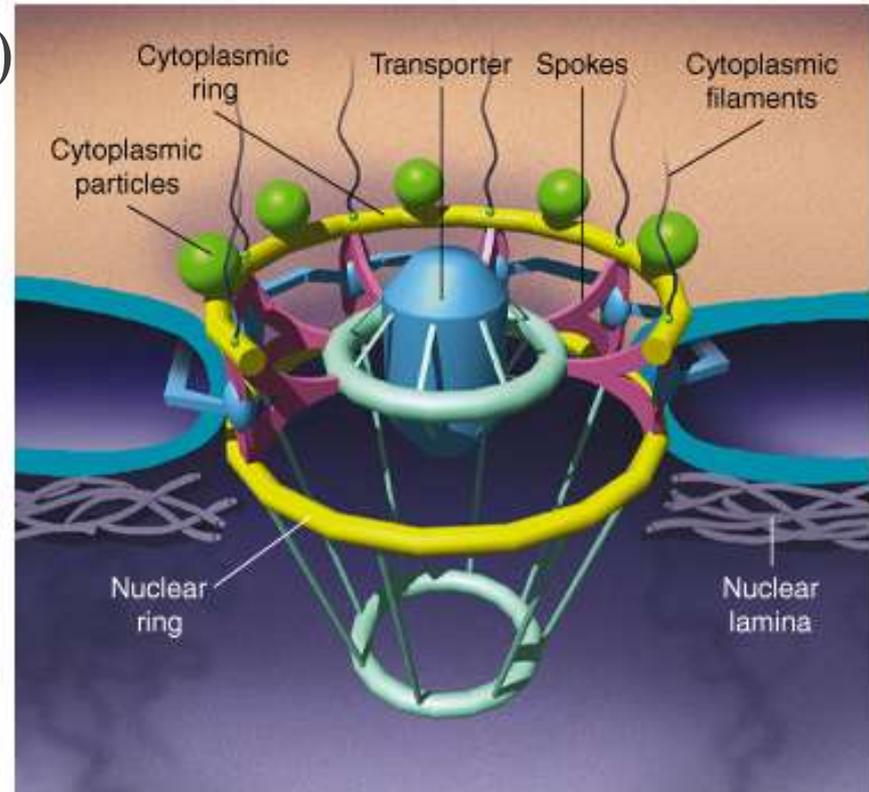
*Nucleoporins
  * Symmetrical – on both cytoplasmic and nuclear sides

# Eukaryotic Nucleus - Structure

*The nuclear pore complex (NPC)

Cytoplasm

Cytoplasmic filament

Cytoplasmic ring

Outer nuclear membrane

Spoke-ring assembly

Nuclear ring

Nuclear basket

Inner nuclear membrane

Central transporter
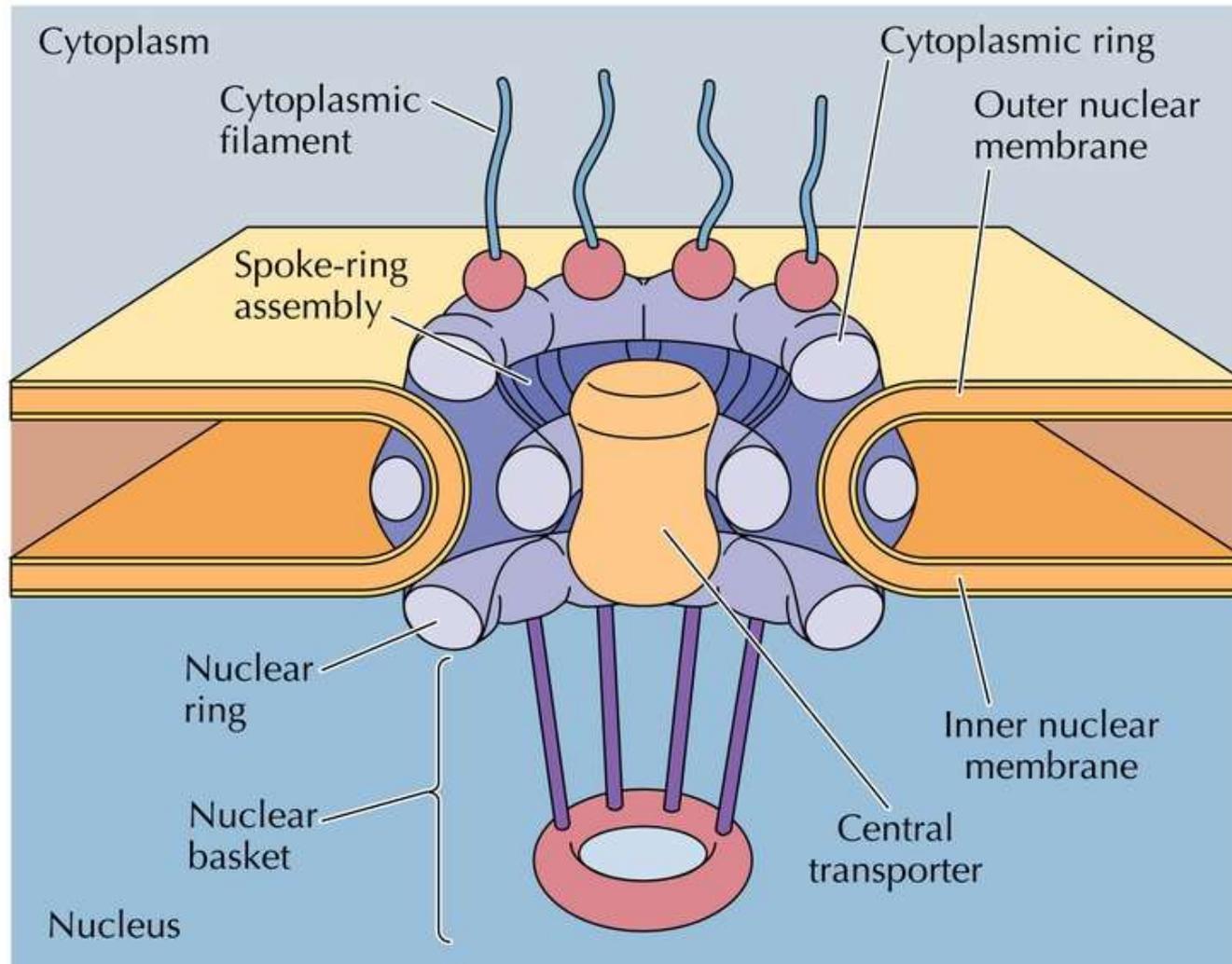
Nucleus

Eukaryotic Nucleus – Structure/Function

*The nuclear pore complex (NPC)

* *Low molecular wt solutes

* *Diffuse freely

* *Macromolecules

* *Regulated

* *Protein import to nucleus

* *Nuclear localization signal (NLS)

* *Best-studied – 1 or 2 sequences of +ve charged $\alpha\alpha$

T antigen

...⬤⬤⬤(Pro)(Lys)(Lys)(Lys)(Arg)(Lys)(Val)⬤⬤⬤...

Nucleoplasmin

...⬤(Lys)(Arg)⬤⬤⬤⬤⬤⬤⬤⬤⬤(Lys)(Lys)(Lys)(Lys)⬤...
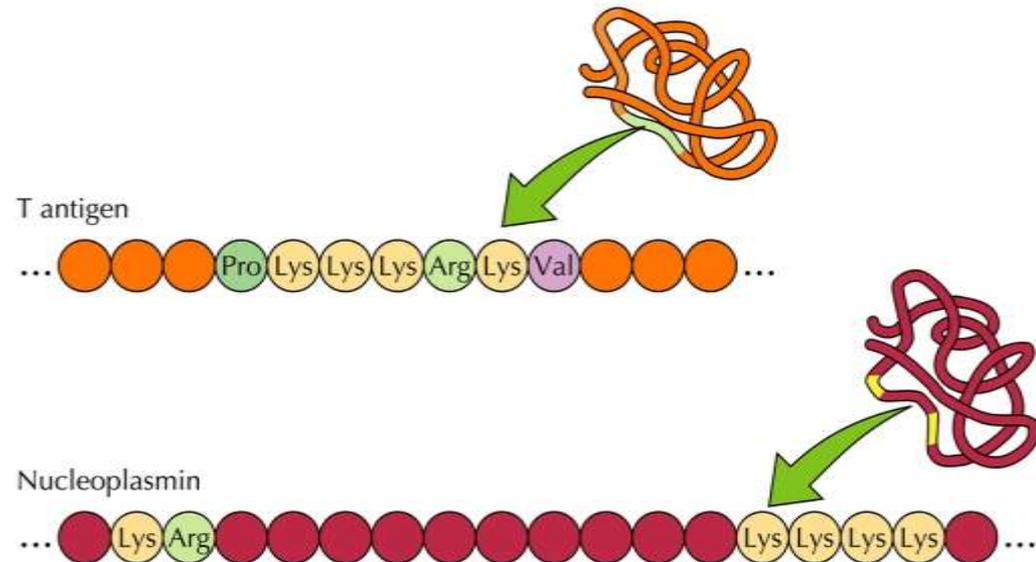
Eukaryotic Nucleus – Structure/Function

*The nuclear pore complex (NPC)
  *Transport receptors – *karyopherins*
    * Soluble

    * Importins
      * Cytoplasm to nucleus
   * Exportins
      * Nucleus to cytoplasm

Eukaryotic Nucleus – Structure/Function

*The nuclear pore complex (NPC)

*Import example - nucleoplasmin

*Step 1. NLS – attached protein binds soluble NLS receptor

*NLS receptor = importin α/β

*Step 2. NLS transports complex to cytoplasmic filaments
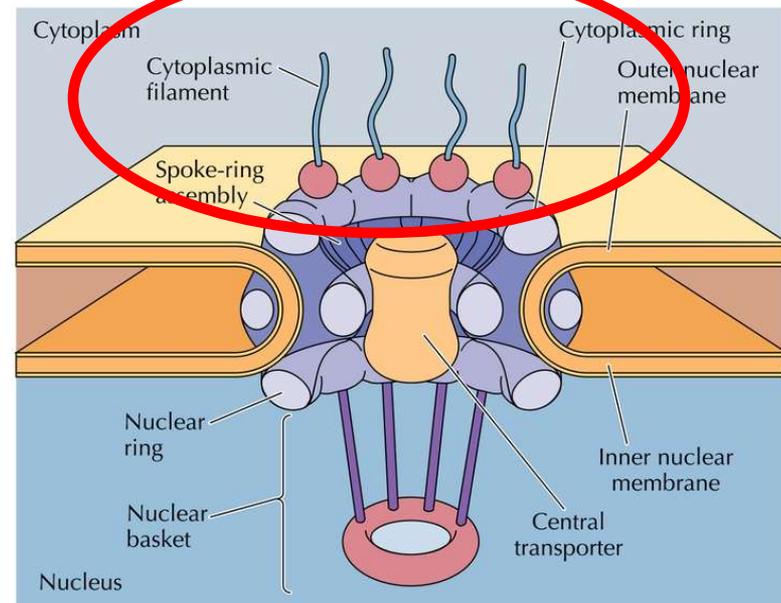
Eukaryotic Nucleus – Structure/Function

*Import example - nucleoplasmin

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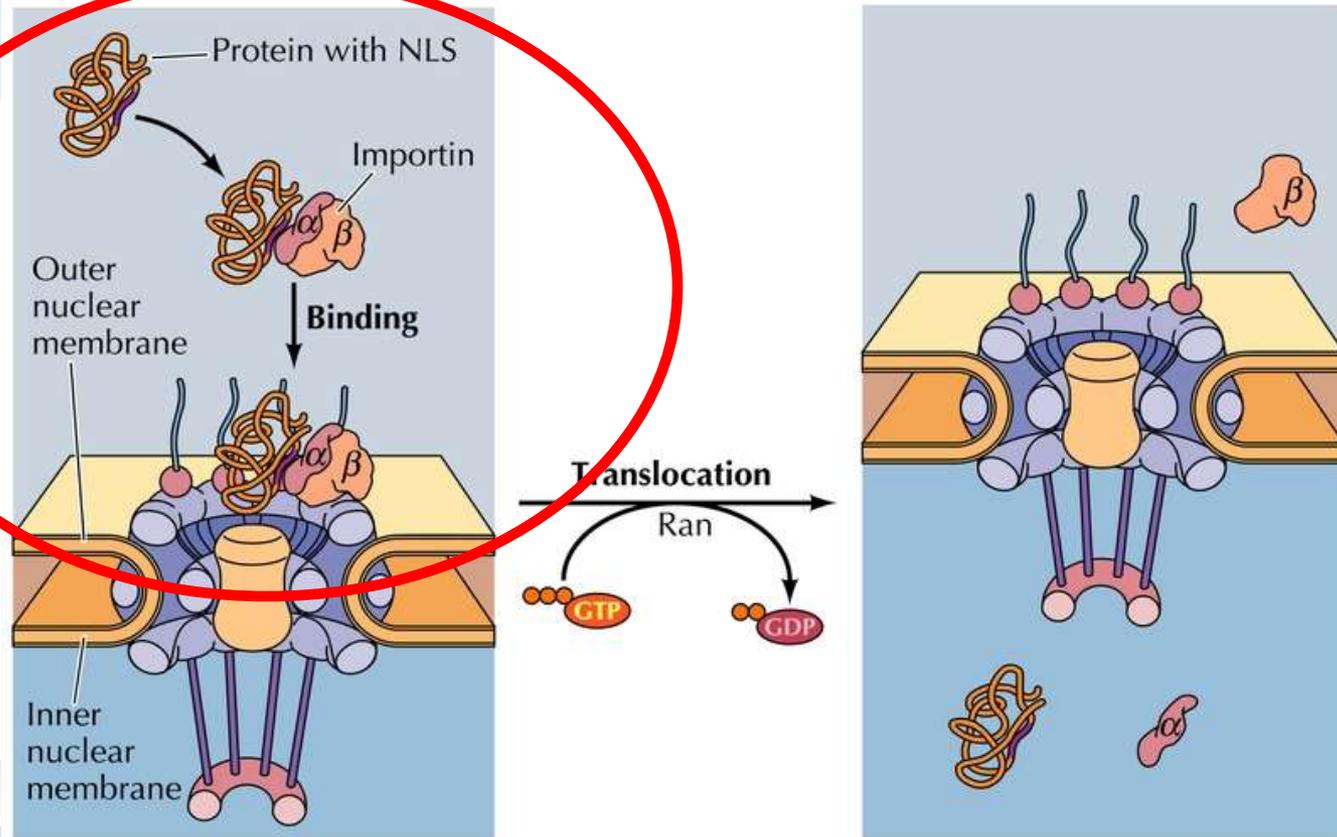
Eukaryotic Nucleus – Structure/Function

*Import example – nucleoplasmin

    *Step 3. Cytoplasmic filaments bend toward nucleus

    *Step 4. Change in conformation of transporter
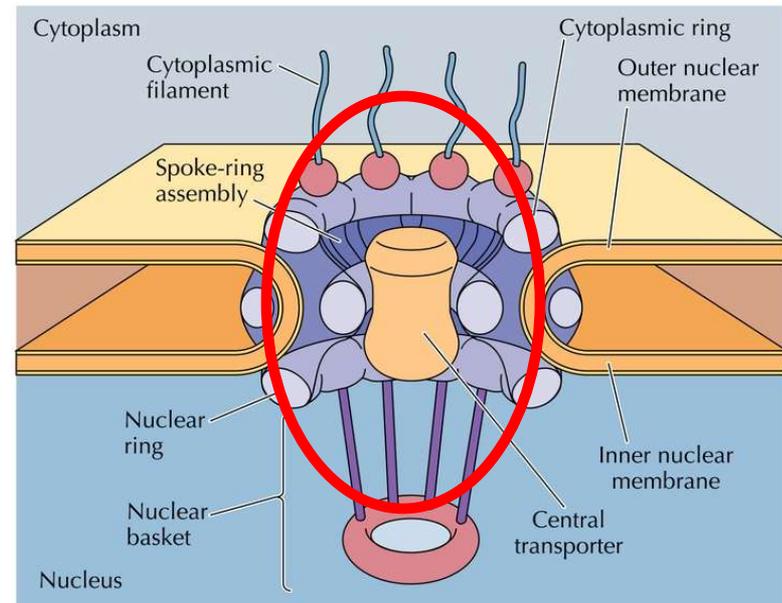
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Eukaryotic Nucleus – Structure/Function

* Import example – nucleoplasmin
  * Role of the GTP-binding protein – *Ran*
  * Active form – *Ran-GTP*
  * Inactive form – *Ran-GDP*
  * High concentration of GTP-Ran in nucleus
  * Low concentration in cytoplasm
    * Low concn. in cytoplasm – accessory protein – *Ran-GAP1* – converts GTP form to GDP form
    * High concn. In nucleus – accessory protein – RCC1 – conversion of GDP form to GTP form

Eukaryotic Nucleus – Structure/Function

*Import example – nucleoplasmin

*Role of the GTP-binding protein – *Ran*
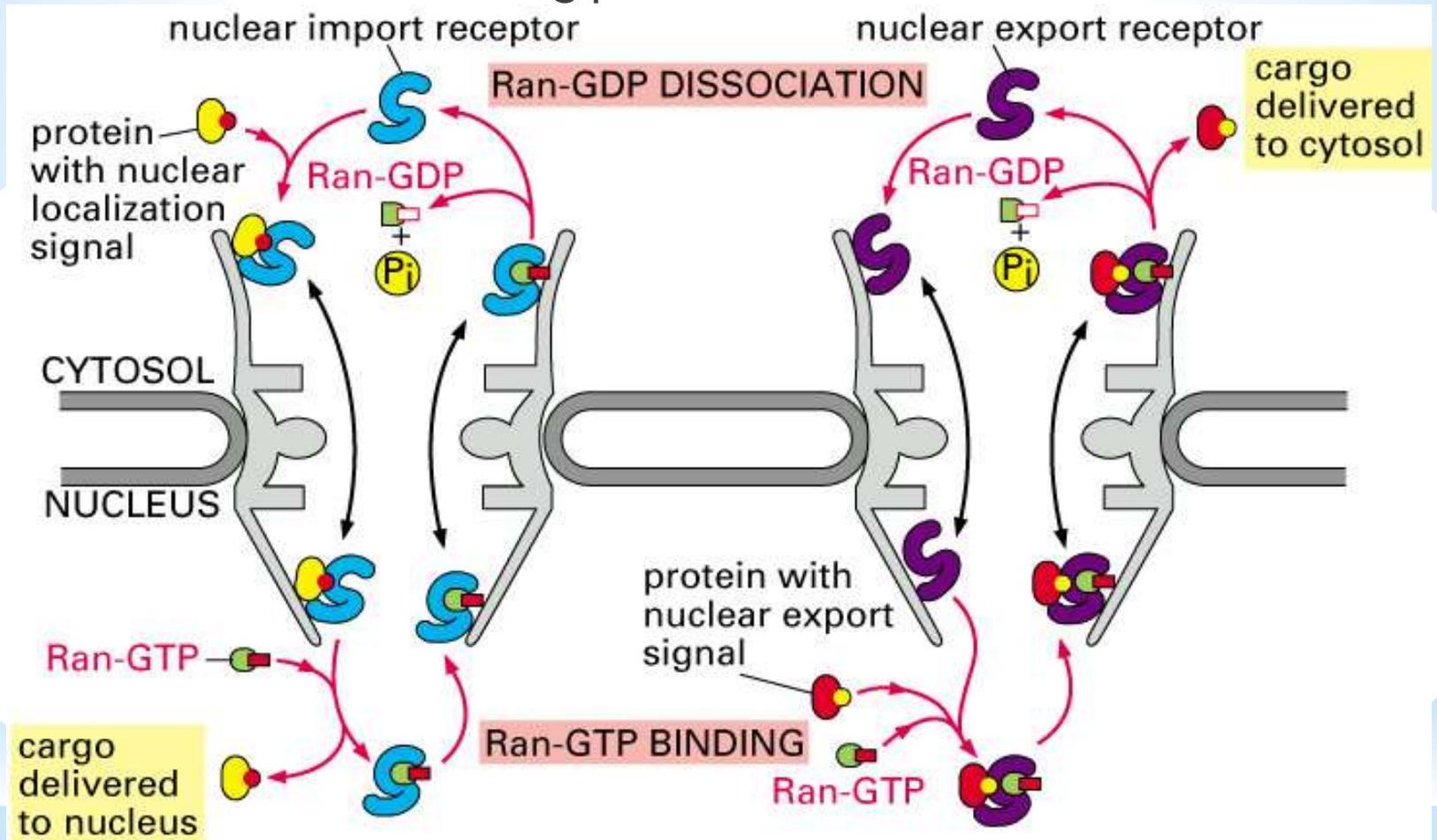
Figure 12–16. Molecular Biology of the Cell, 4th Edition.

Eukaryotic Nucleus – Structure/Function

*Import example – nucleoplasmin

  *Step 5. Importin – NLS protein complex binds to Ran-GTP and importin dissociates

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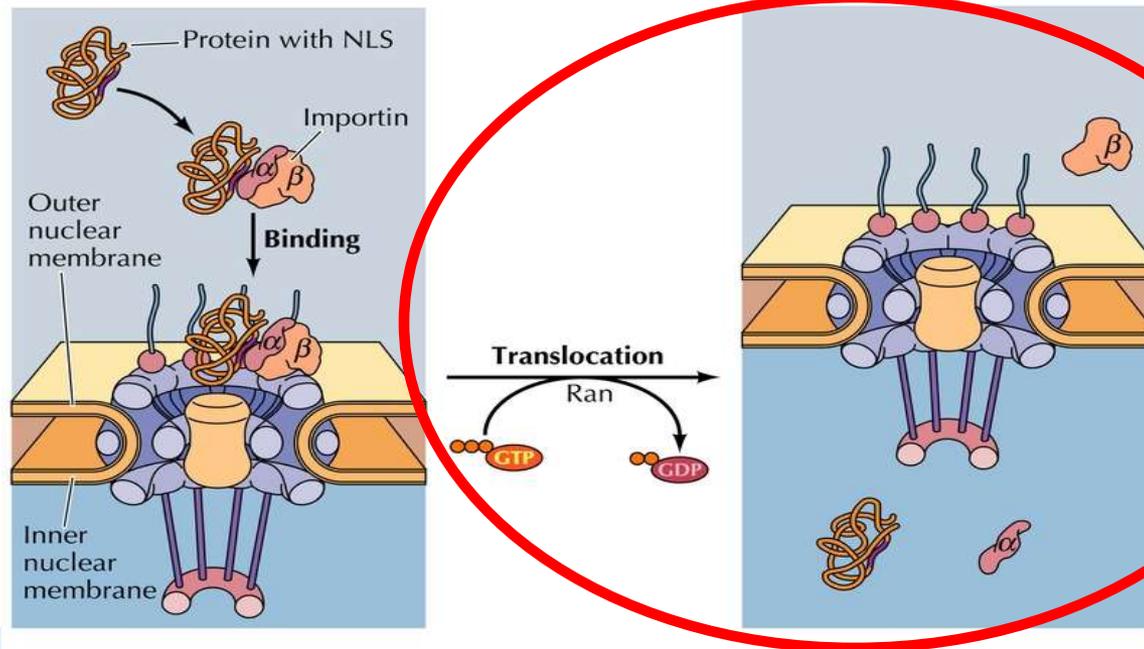
Eukaryotic Nucleus – Structure/Function

*Import example – nucleoplasmin

*Step 6. Ran-GTP-importin β shuttled back to cytoplasm

*Step 7. Importin α subunit transported by an exportin


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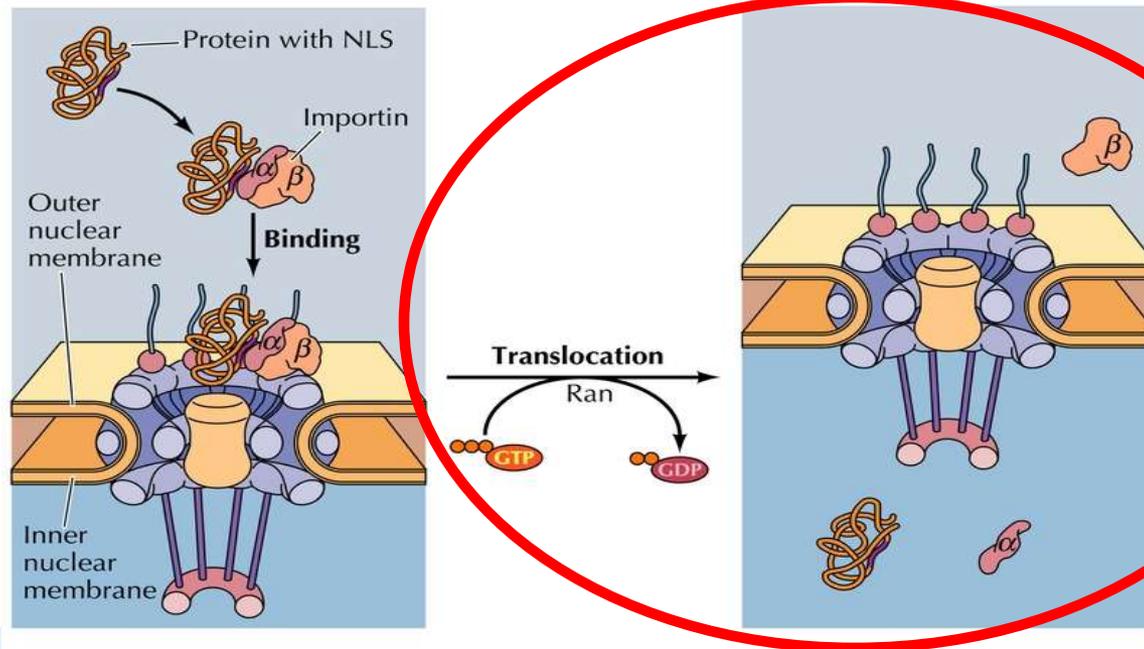
Eukaryotic Nucleus – Structure/Function

* Export – RNAs
    * Move as ribonucleoproteins (RNPs)
        * Except t-RNA – direct transport by exportin-τ
    * Protein component contains nuclear export signal (NES)
    * Exportins recognize NES
    * Binds Ran-GTP – stabilizes complex
    * Carried to cytoplasm
    * Ran-GAP1 converts Ran-GTP to Ran-GDP

Eukaryotic Nucleus – Structure/Function

*Export – RNAs
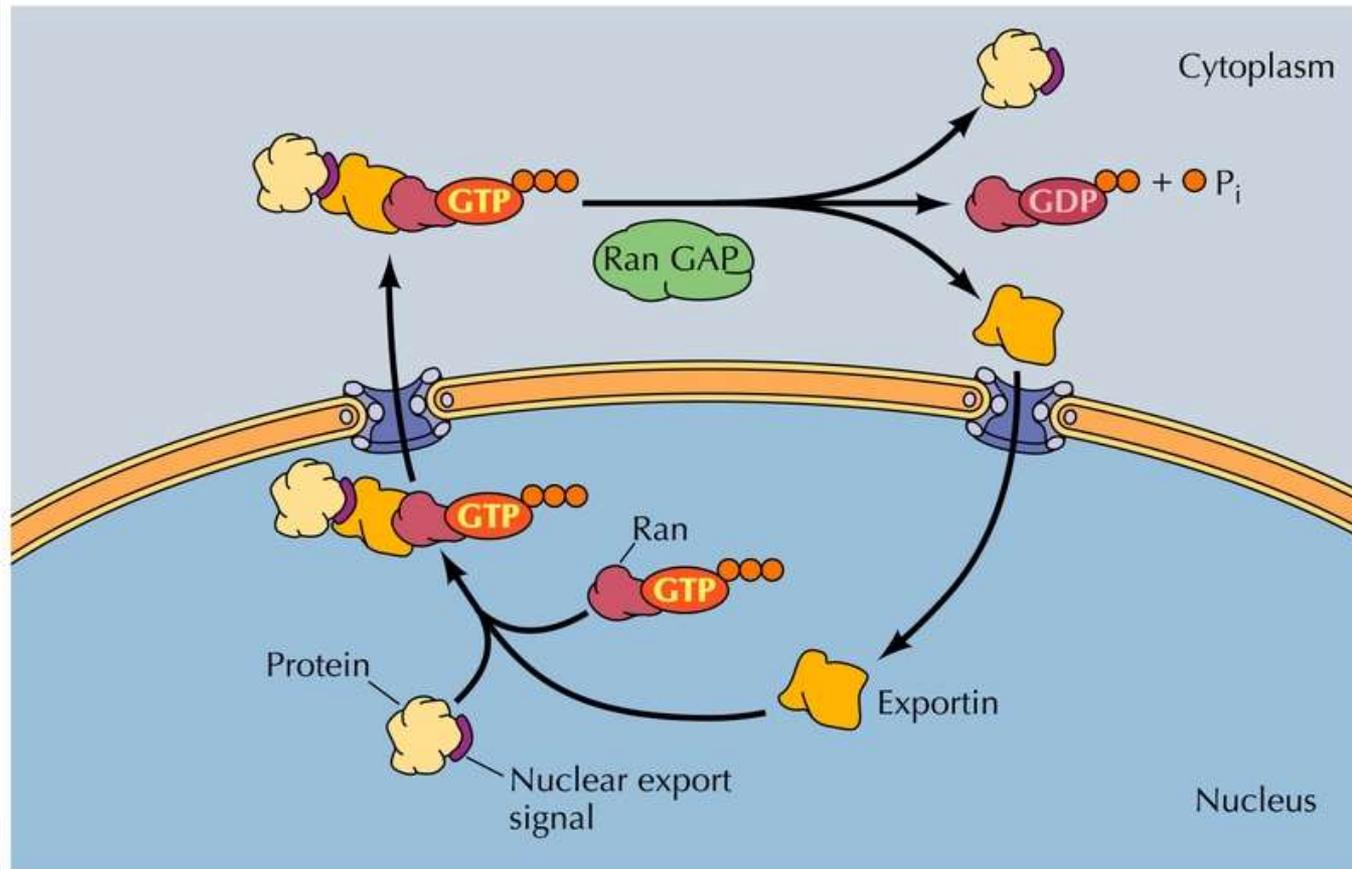
Eukaryotic Nucleus –Function

Eukaryotic Nucleus –Function: Gene expression

Gene in DNA

(a) Mammalian gene

up to −50 kb   ~200   ~30   +10 to +50 kb

(b) S. cerevisiae gene   +1

~−90

- Exon
- Intron
- TATA box
- Promoter-proximal element
- Enhancer; yeast UAS

mRNA

(a)

5′

E

P

A

5′

3′ mRNA

Polypeptide

30S
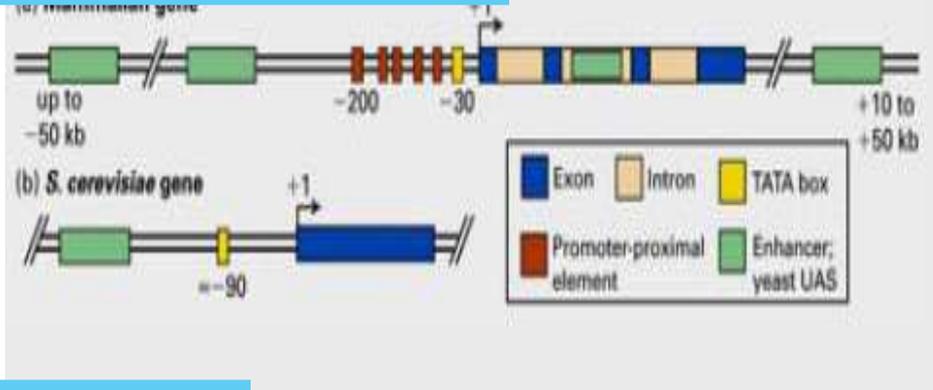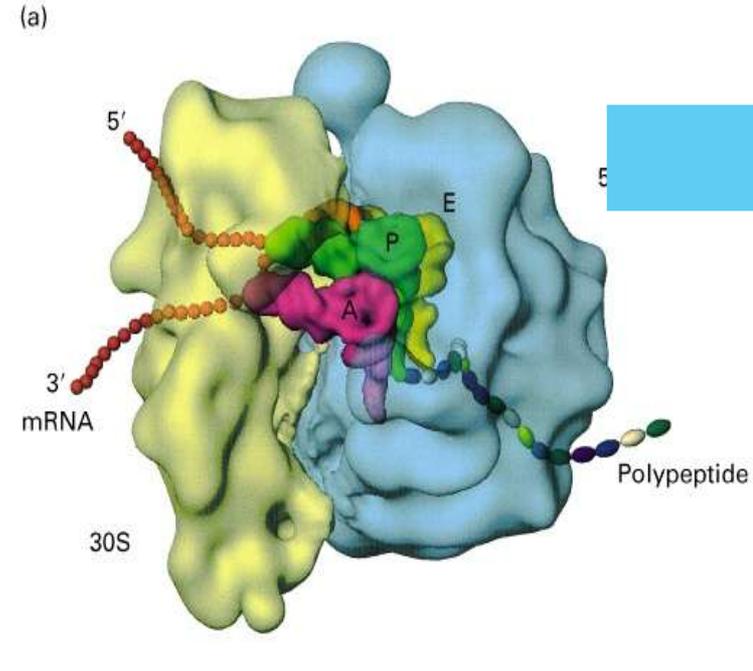
Proteins

# Different places of regulatory of the gene expression
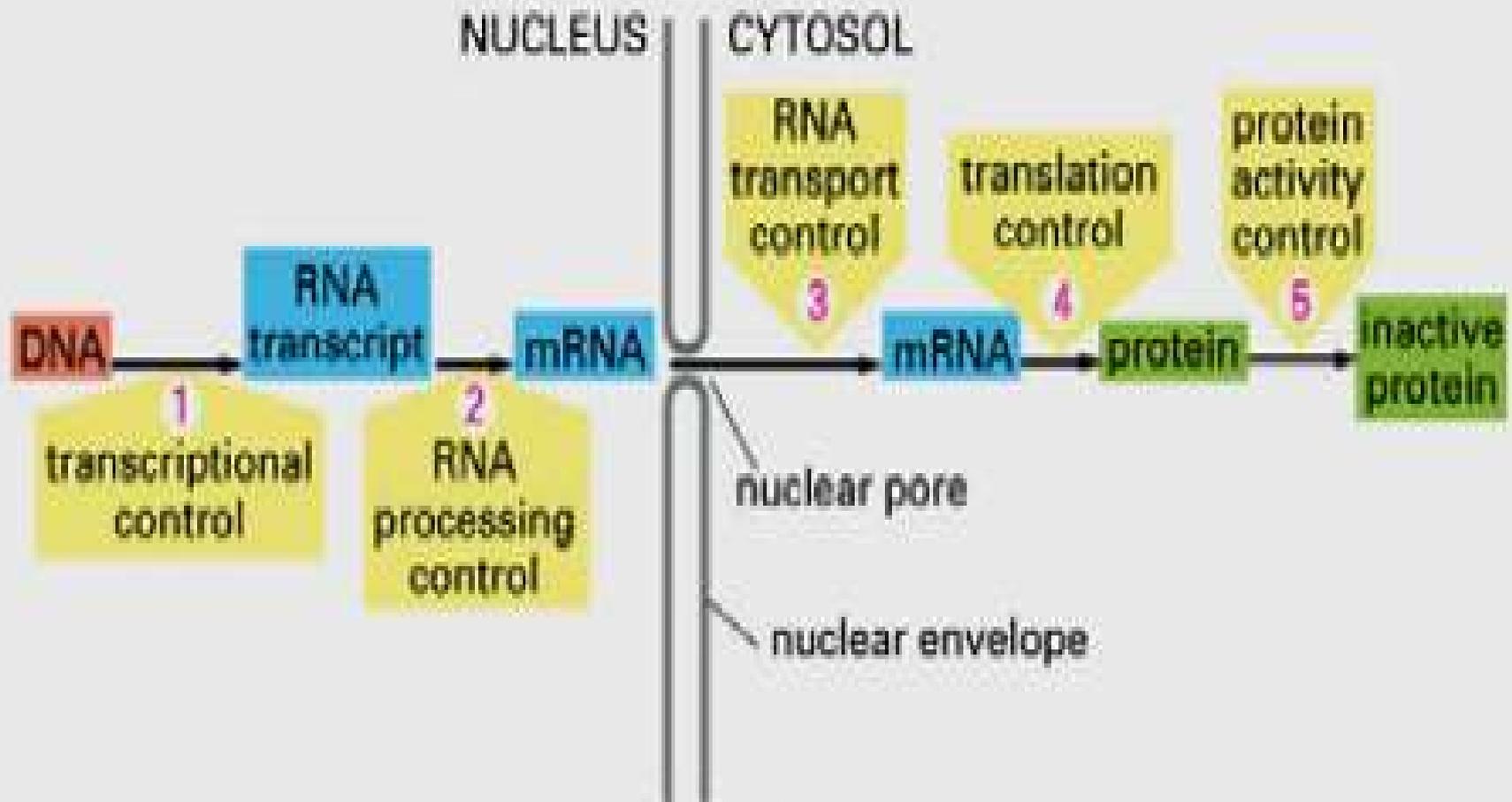
Gene expression
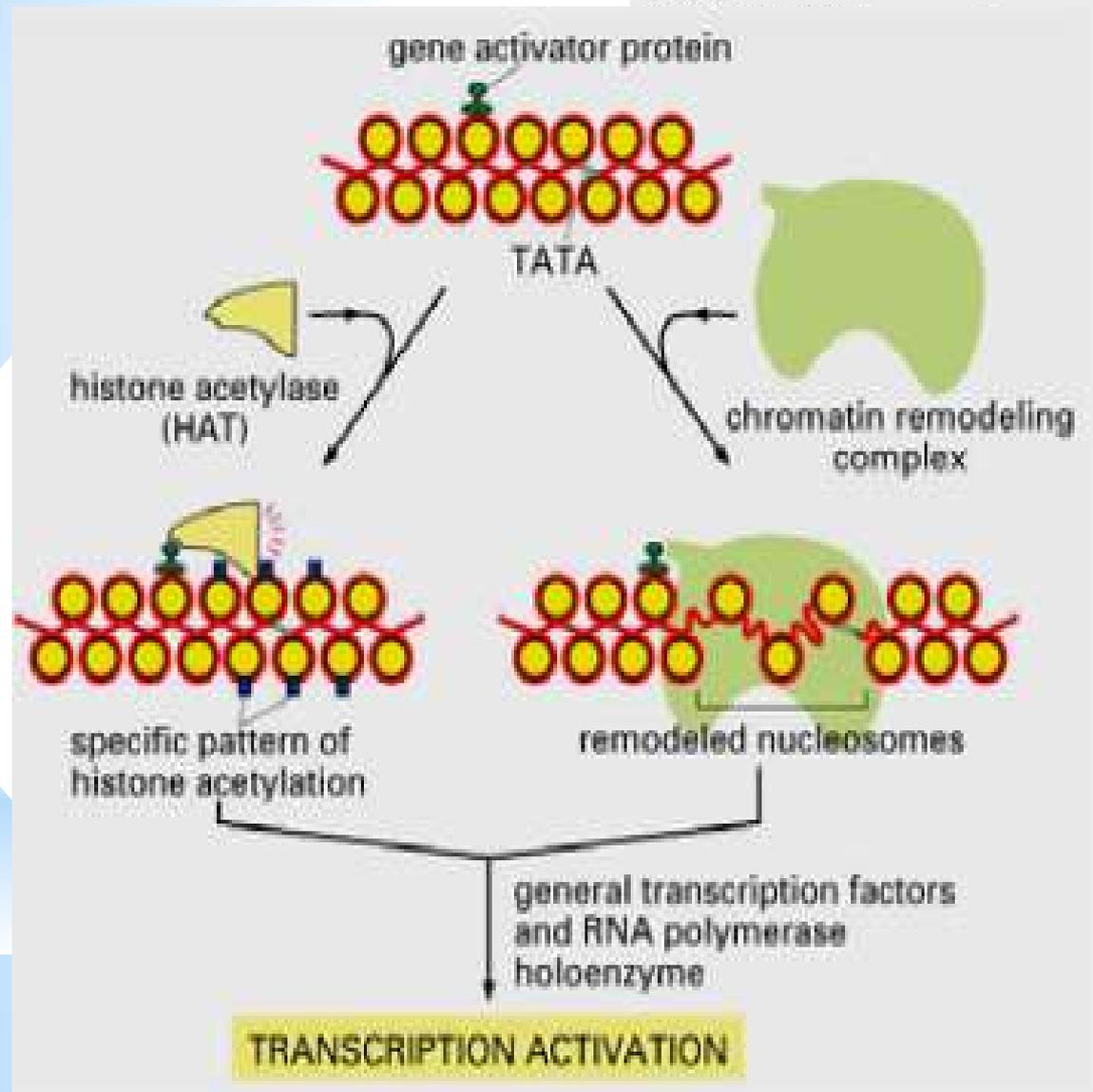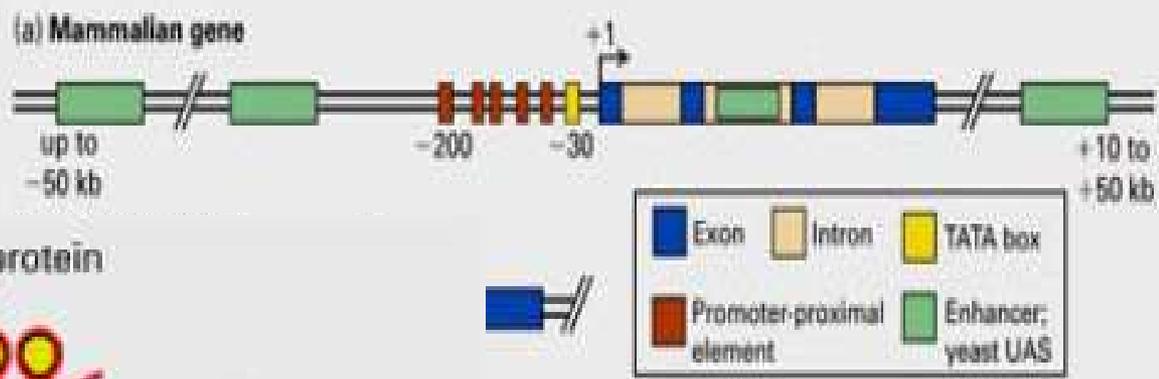
TATA homeobox

Binging site of RNA polymerase

Gene expression – regulatory by transcription factors

spacer DNA

gene regulatory sequences

gene regulatory proteins

general transcription factors

RNA polymerase

upstream

TATA box

start of transcription

promoter

25
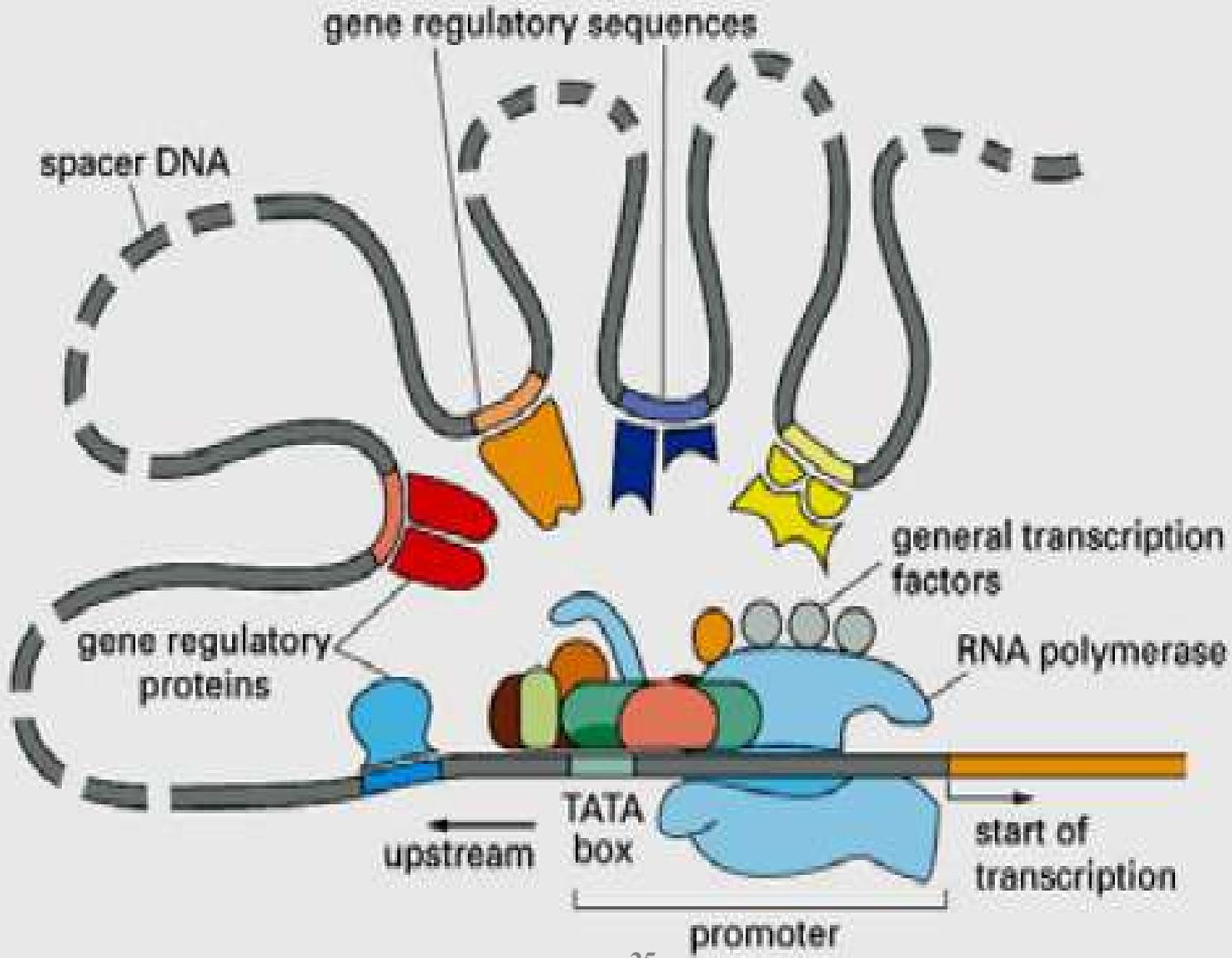
Eukaryotic Nucleus –Function: Gene expression
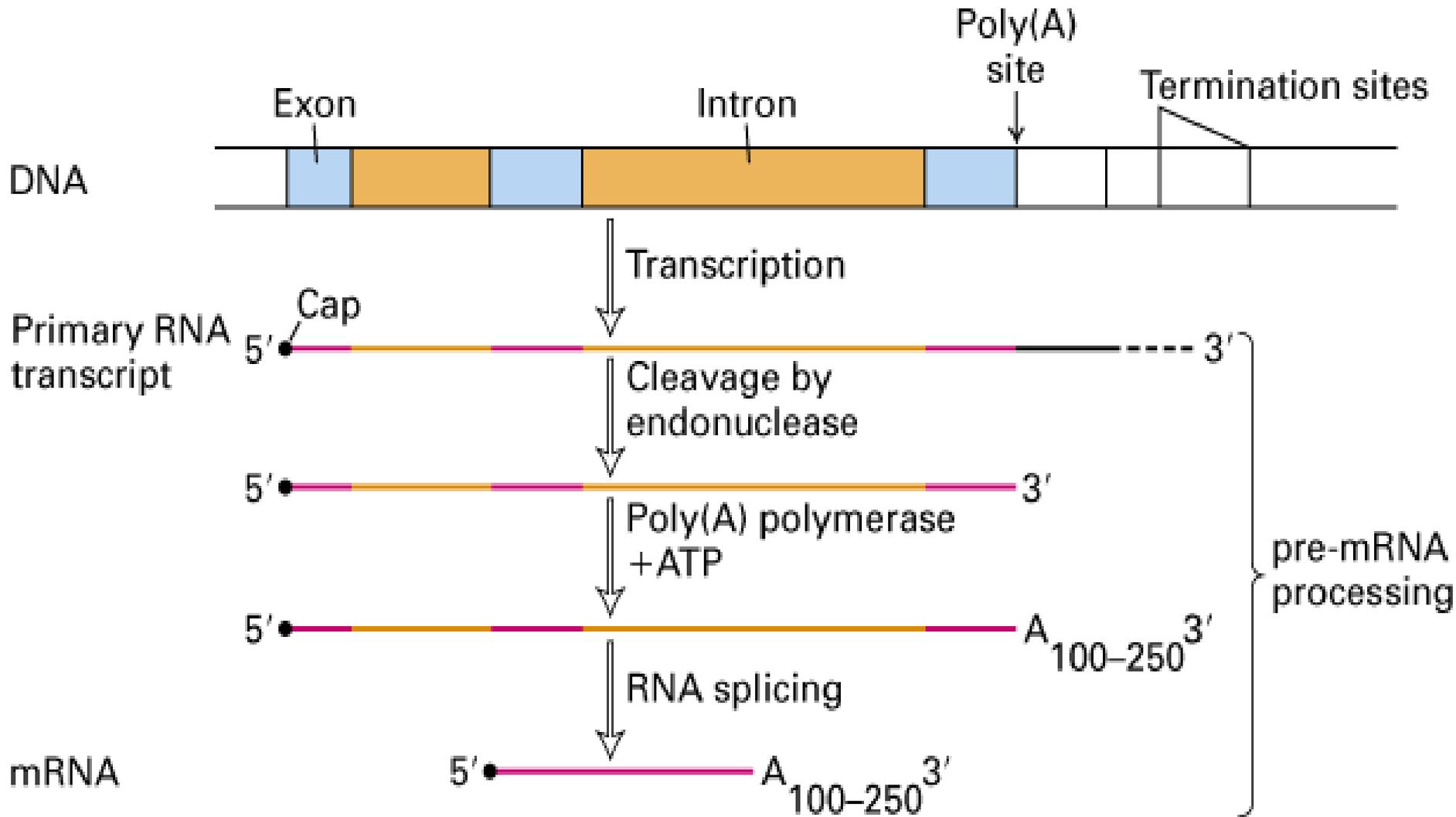
What is gene ?

Part of DNA – sequence of nucleotides
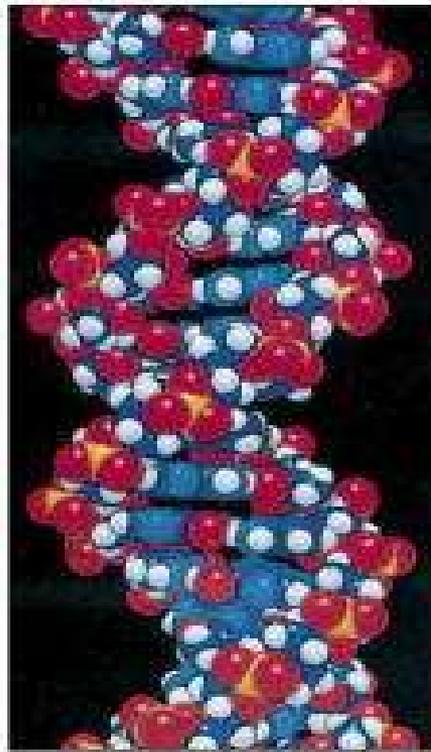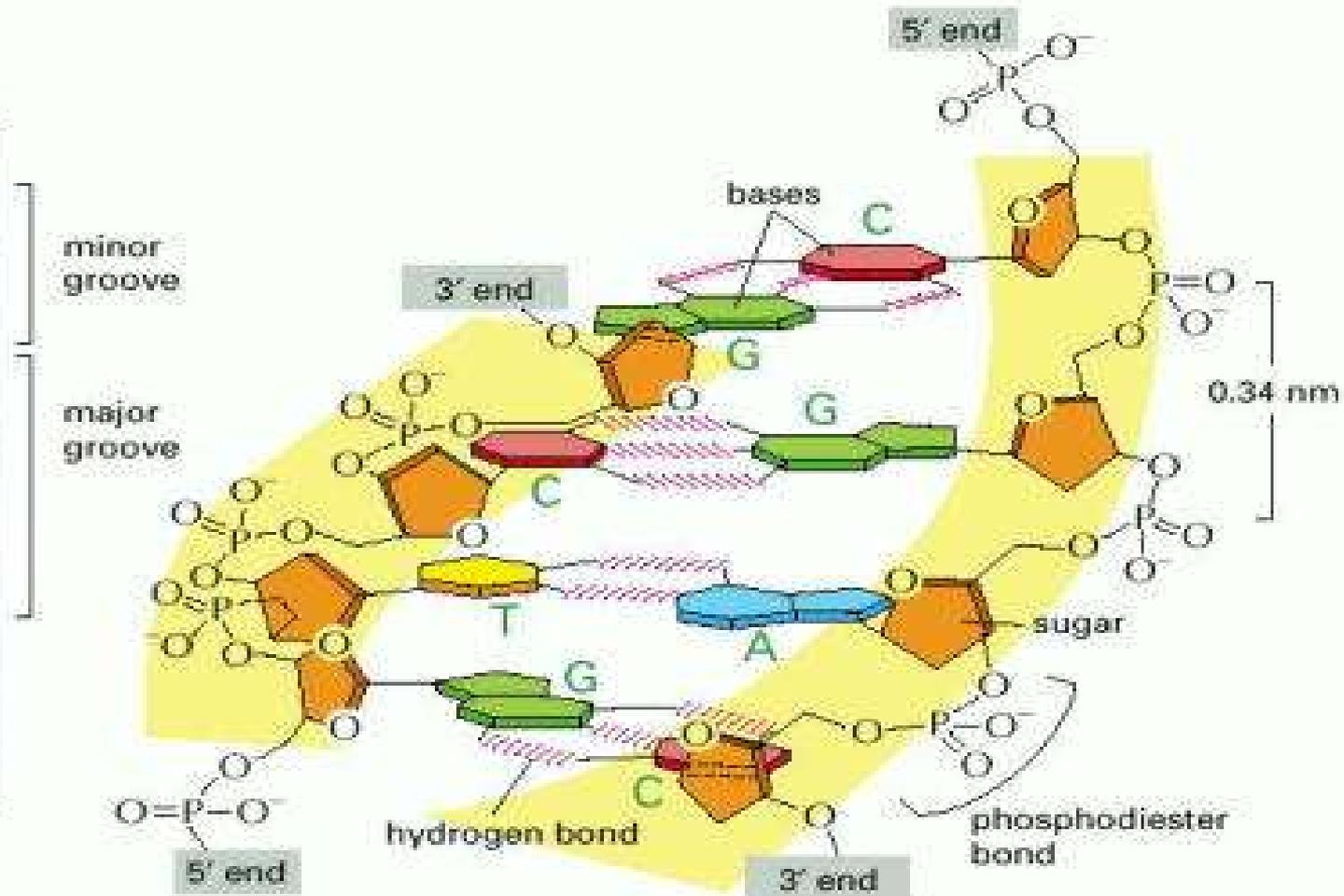
Ribosomal RNA

MgPar MgPar Repeat

Transfer RNA

1 kb

Amino acid biosynthesis

Biosynthesis of cofactors, prosthetic groups, carriers

Cell envelope

Cellular processes

Central intermediary metabolism

Energy metabolism

Fatty acid and phospholipid metabolism

Purines, pyrimidines, nucleosides and nucleotides

Regulatory functions

Replication

Transport/binding proteins

Translation

Transcription

Other categories

Hypothetical

Unknown

How is stored gene?

Structure of DNA
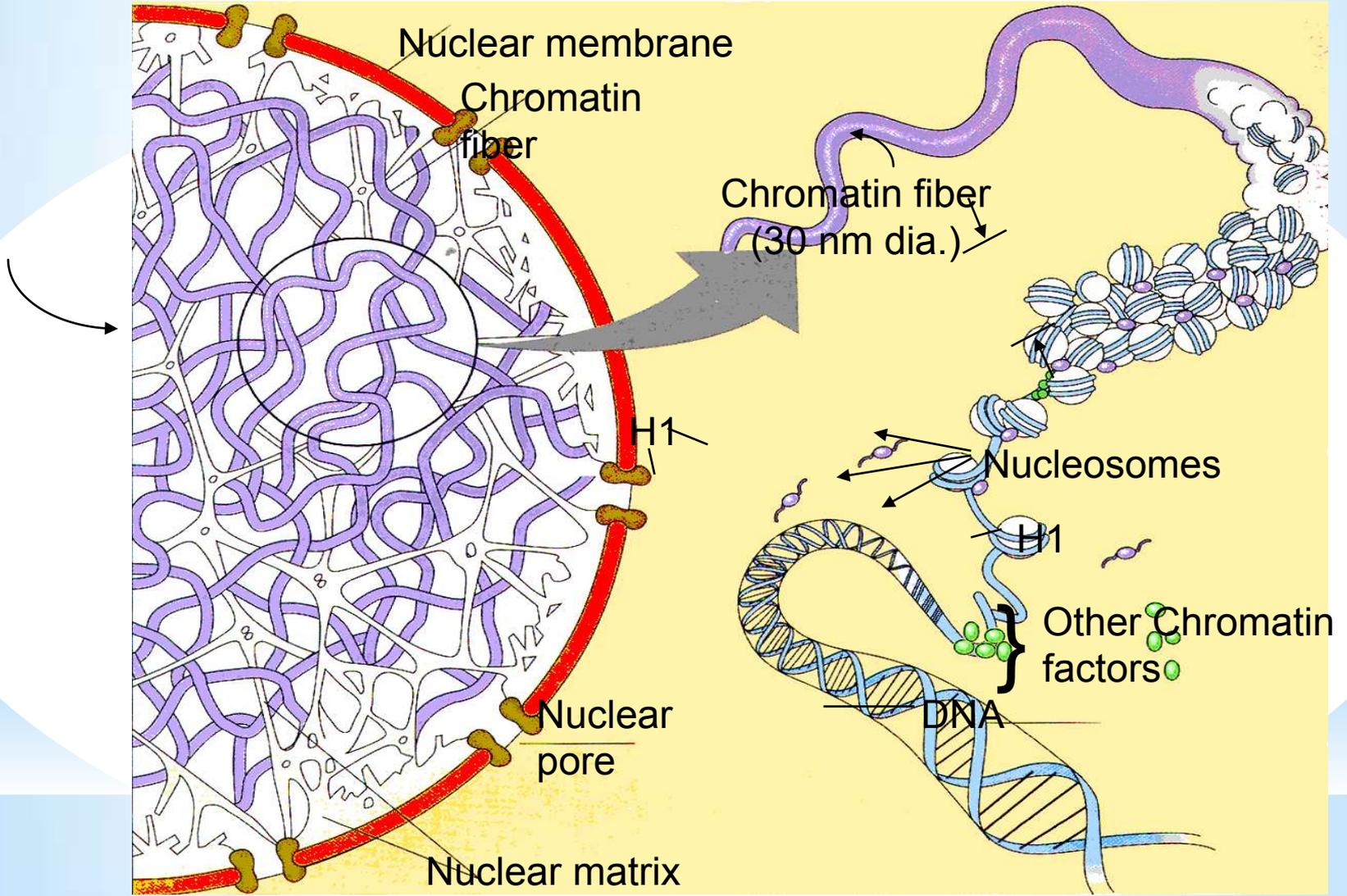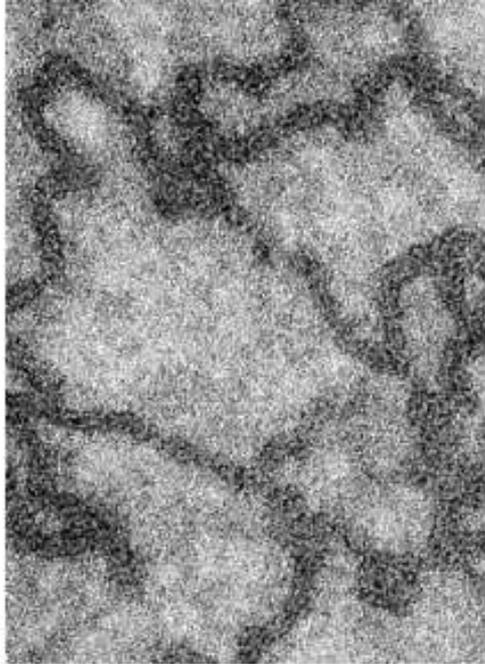
(b)
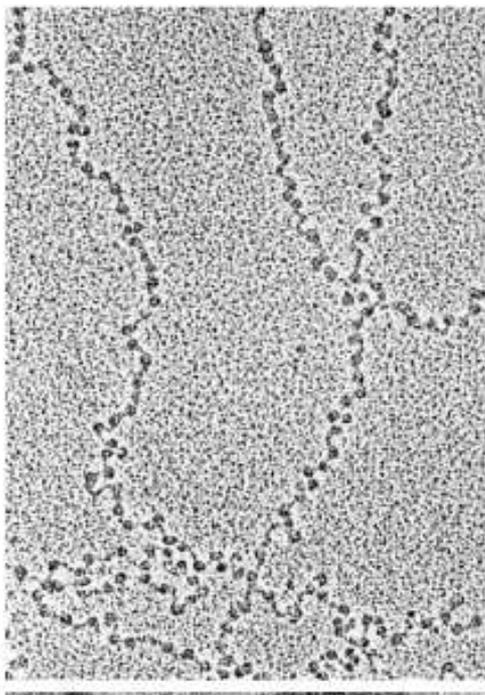
(a)
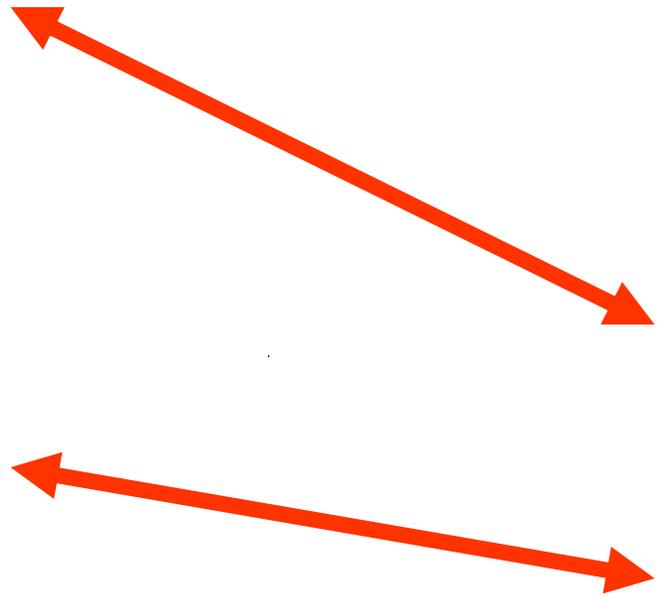
Metaphase chromosome — 1400 nm

Condensed scaffold-associated form — 700 nm
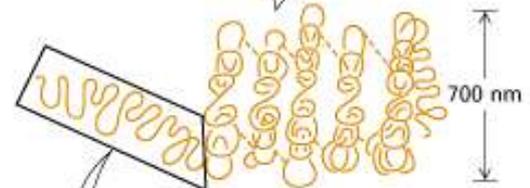
Extended scaffold-associated form — 300 nm

Chromosome scaffold
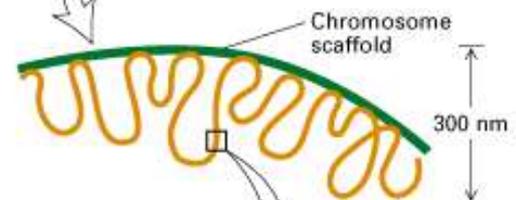
30-nm chromatin fiber of packed nucleosomes — 30 nm
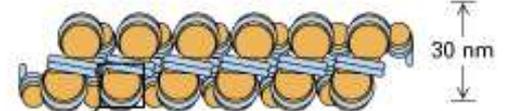
"Beads-on-a-string" form of chromatin — 11 nm
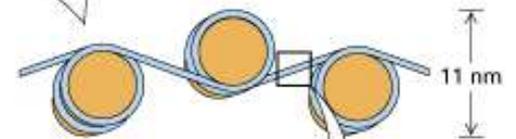
Short region of DNA double-helix — 2 nm

Nucleosome
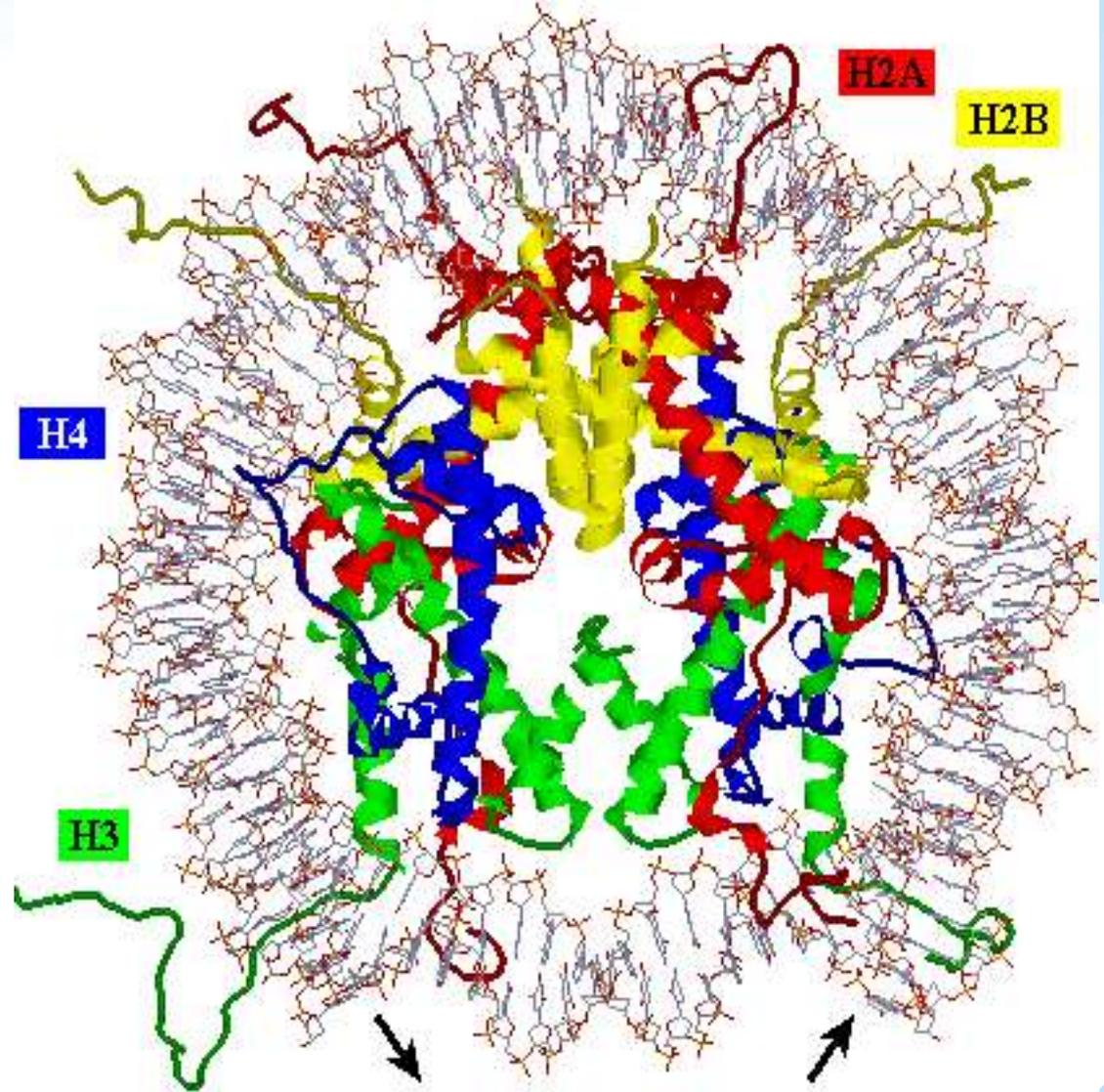
- regulation of transcription
- integration of specific locus
- „body guards" of gene expression

What is gene?

Promoter | Protein coding sequence | Terminator

Genomic DNA

Type of gene:
>rRNA
>tRNA
>mRNA

*Number of genes

*Walter Gilbert [1980s] 100k

*Antequera & Bird [1993] 70-80k

*John Quackenbush et al. (TIGR) [2000] 20k

*Ewing & Green [2000] 30k

*Tetraodon analysis [2001] 35k

*Human Genome Project (public) [2001] ~ 31k

*Human Genome Project (Celera) [2001] 24-40k

*Mouse Genome Project (public) [2002] 25k -30k

*Lee Rowen [2003] 25,947

* ENCODE [2012] 20,687 protein-coding genes

Human genome by functions

PANTHER Classification System, 2011
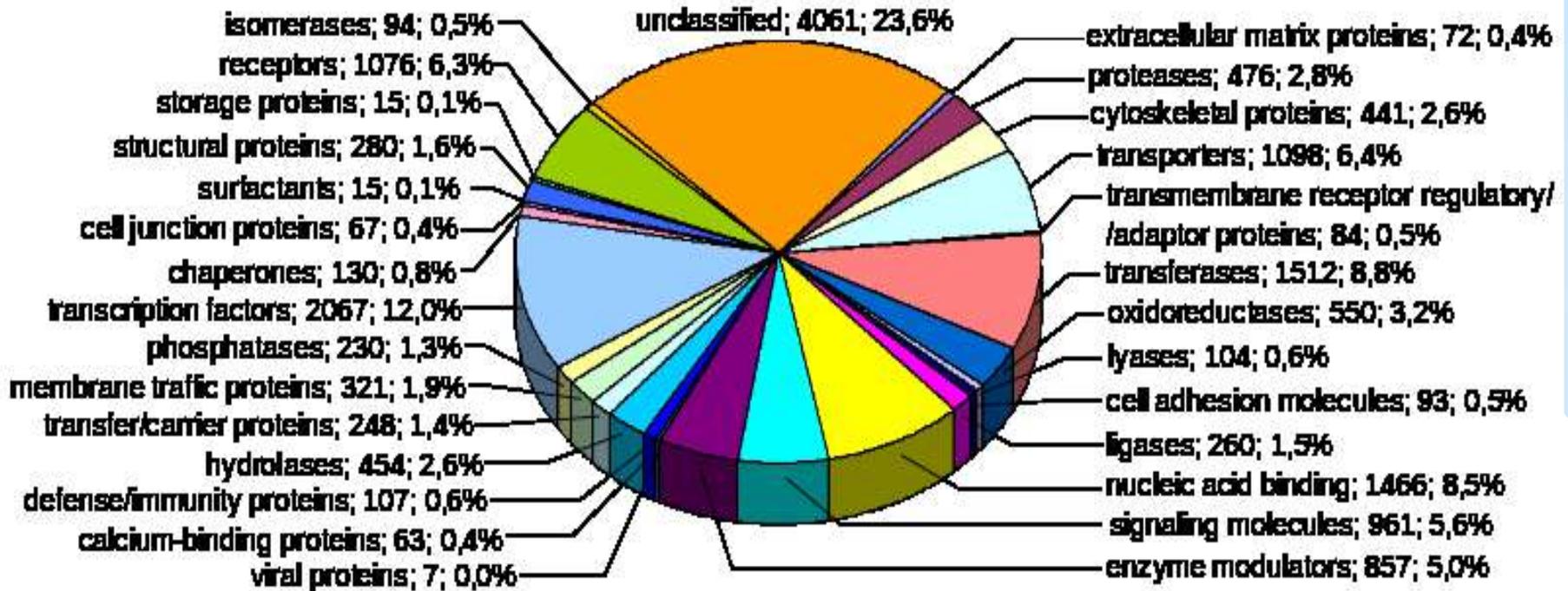
Examples of human protein-coding genes

Protein	Chrom	Gene	Length	Exons	Exon length	Intron length	Alt splicing
Breast cancer type 2 susceptibility protein	13	BRCA2	83,736	27	11,386	72,350	yes
Cystic fibrosis transmembrane conductance regulator	7	CFTR	202,881	27	4,440	198,441	yes
Cytochrome b	MT	MTCYB	1,140	1	1,140	0	no
Dystrophin	X	DMD	2,220,381	79	10,500	2,209,881	yes
Glyceraldehyde-3-phosphate dehydrogenase	12	GAPDH	4,444	9	1,425	3,019	yes
Hemoglobin beta subunit	11	HBB	1,605	3	626	979	no
Histone H1A	6	HIST1H1A	781	1	781	0	no
Titin	2	TTN	281,434	364	104,301	177,133	yes

* HUGO- Human Genome Project (HGP)

Results presented by Robert Krulwich (2001-04-17). *Cracking the Code of Life* (Television Show). PBS.

Start in October 1990 in U.S. Department of Energy's Office of Science and in US National Institutes of Health (NIH)- National Human Genome Research Institute

HGP originally aimed to map the nucleotides contained in a human haploid reference genome (> 3 billion)

Draft of results- February 2001 (Nature) 90%, complete in April 2003

http://www.genome.gov/12011238

Other similar project- hapliod:Celera Genomics (1998); diploid: International HapMap Project, Applied Biosystems, Perlegen, Illumina, J. Craig Venter Institute, Personal Genome Project, Roche-454.
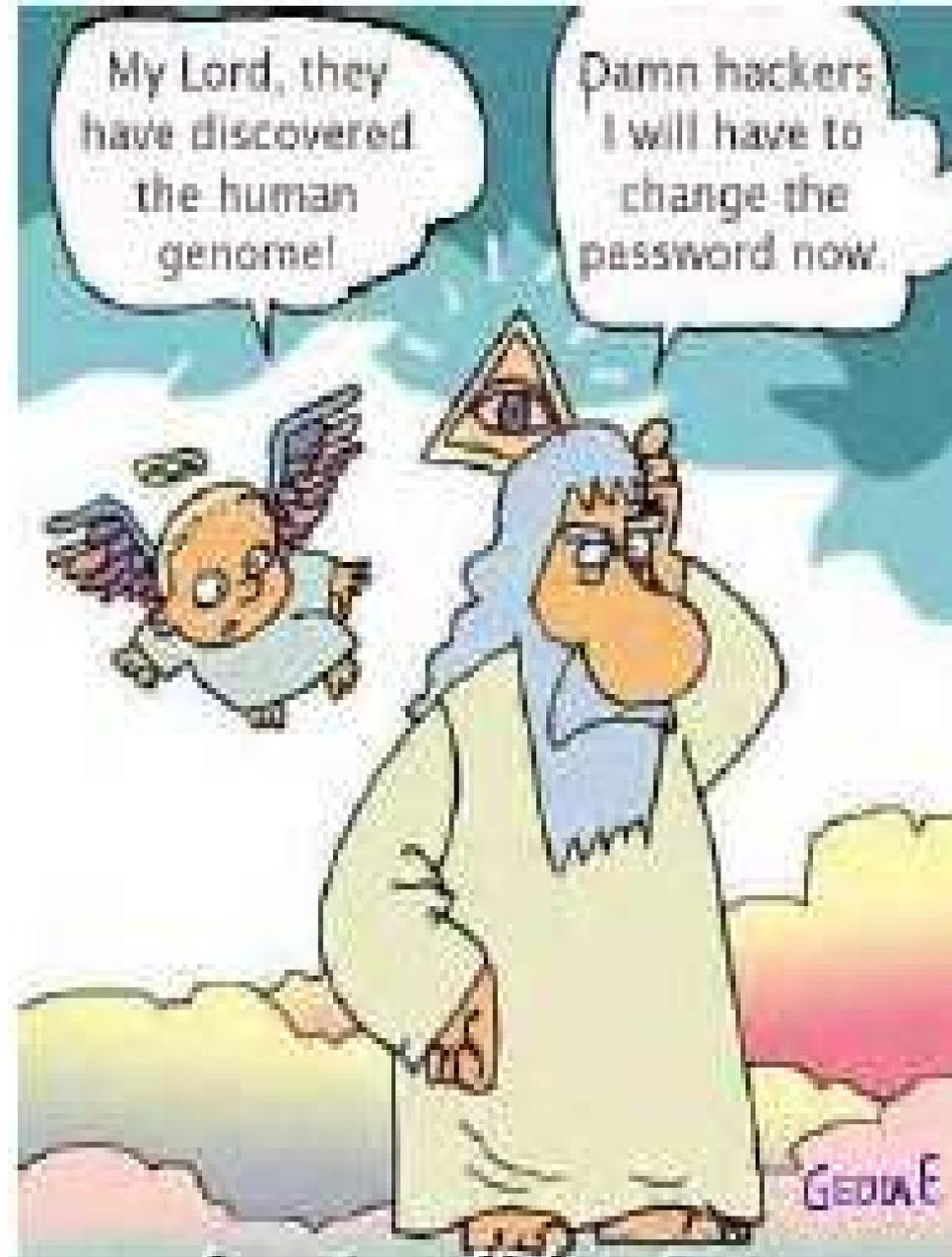
Chromosome	Length (cm)	Base pairs	Variations	Confirmed proteins	Putative proteins	Pseudogenes	miRNA	rRNA	snRNA	snoRNA	Misc ncRNA	Links
1	8.5	249,250,621	4,401,091	2,012	31	1,130	134	66	221	145	106	EBI
2	8.3	243,199,373	4,607,702	1,203	50	948	115	40	161	117	93	EBI
3	6.7	198,022,430	3,894,345	1,040	25	719	99	29	138	87	77	EBI
4	6.5	191,154,276	3,673,892	718	39	698	92	24	120	56	71	EBI
5	6.2	180,915,260	3,436,667	849	24	676	83	25	106	61	68	EBI
6	5.8	171,115,067	3,360,890	1,002	39	731	81	26	111	73	67	EBI
7	5.4	159,138,663	3,045,992	866	34	803	90	24	90	76	70	EBI
8	5.0	146,364,022	2,890,692	659	39	568	80	28	86	52	42	EBI
9	4.8	141,213,431	2,581,827	785	15	714	69	19	66	51	55	EBI
10	4.6	135,534,747	2,609,802	745	18	500	64	32	87	56	56	EBI
11	4.6	135,006,516	2,607,254	1,258	48	775	63	24	74	76	53	EBI
12	4.5	133,851,895	2,482,194	1,003	47	582	72	27	106	62	69	EBI
13	3.9	115,169,878	1,814,242	318	8	323	42	16	45	34	36	EBI
14	3.6	107,349,540	1,712,799	601	50	472	92	10	65	97	46	EBI
15	3.5	102,531,392	1,577,346	562	43	473	78	13	63	136	39	EBI
16	3.1	90,354,753	1,747,136	805	65	429	52	32	53	58	34	EBI
17	2.8	81,195,210	1,491,841	1,158	44	300	61	15	80	71	46	EBI
18	2.7	78,077,248	1,448,602	268	20	59	32	13	51	36	25	EBI
19	2.0	59,128,983	1,171,356	1,399	26	181	110	13	29	31	15	EBI
20	2.1	63,025,520	1,206,753	533	13	213	57	15	46	37	34	EBI
21	1.6	48,129,895	787,784	225	8	150	16	5	21	19	8	EBI
22	1.7	51,304,566	745,778	431	21	308	31	5	23	23	23	EBI
X	5.3	155,270,560	2,174,952	815	23	780	128	22	85	64	52	EBI
Y	2.0	59,373,566	286,812	45	8	327	15	7	17	3	2	EBI
mtDNA	0.00054	16,569	929	13	0	0	0	2	0	0	22	EBI