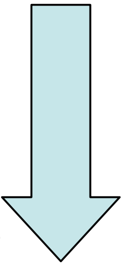
Overview

A. Introduction

B. Review of molecular microbiology

1. bacterial physiology
2. genomic information
3. flow of genomic information
4. quantitative/physical aspects
5. comparison to eukayrotes



cellular (biology)

molecular (chemistry/physics)

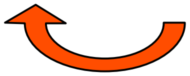
C. Systems biology

1. scope and focus
2. circuit as system-level descriptor
3. scope of this course

Bacterial physiology

- growth

  E. coli (minimal medium): glucose + NH_3 → biomass

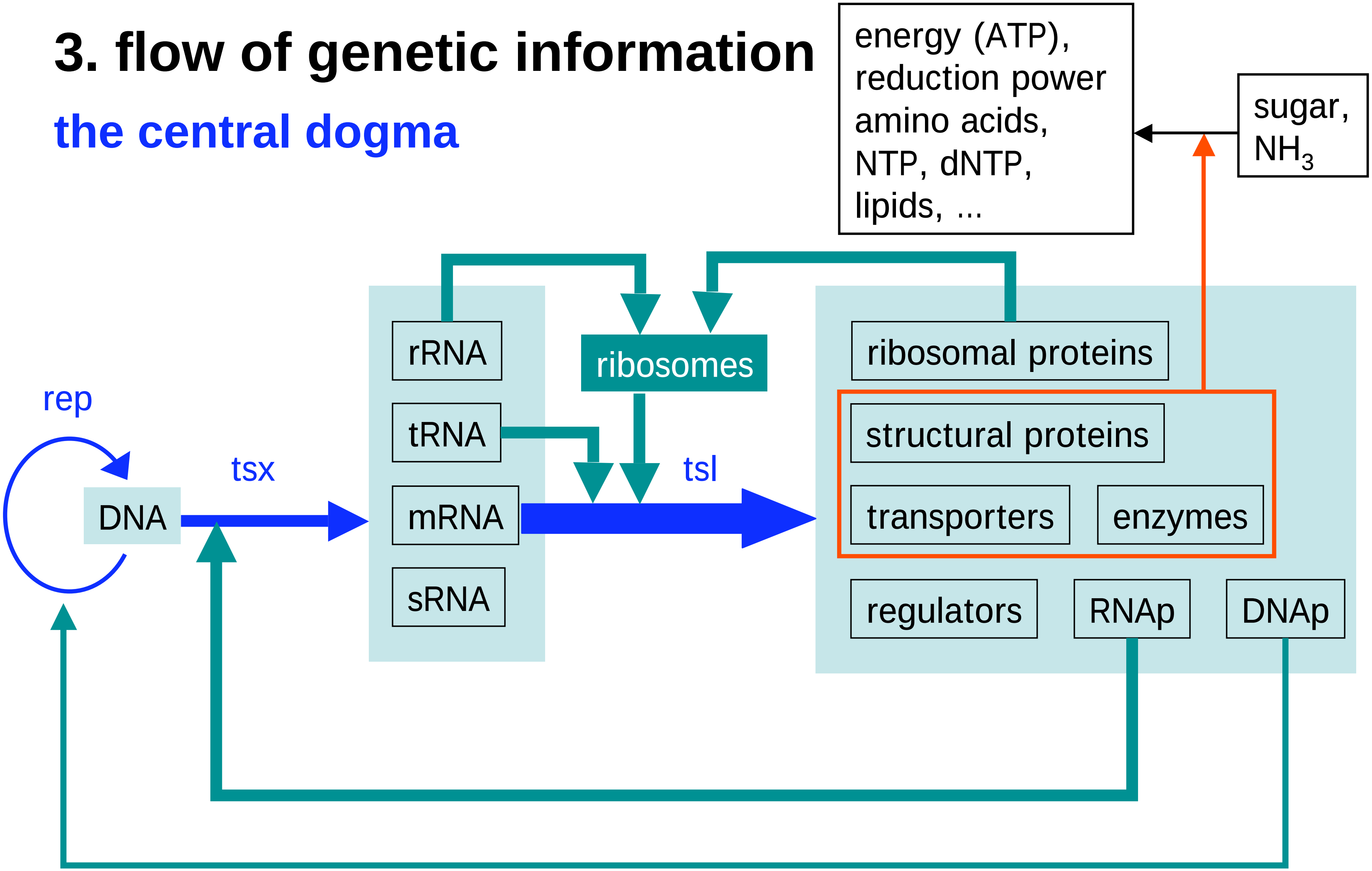


- survival
  - exponential growth:

    bacteria can sense the environment and adjust its “growth program” according to nutrients provided by the medium
  - coping with stressful conditions:
    - motility: flagella synthesis and chemotaxis
    - osmotic response: porin synthesis
    - heat shock response: chaperons
    - quorum sensing, biofilms, bacterial community
    - SOS response (e.g., to DNA damage)
  - non-growth condition
    - stationary phase (*E. coli* can be dormant for > 10 years)
    - sporulation (e.g., *B. subtilis*)
    - competence, conjugation (exchange of genetic materials)

3. flow of genetic information

the central dogma



energy (ATP),
reduction power
amino acids,
NTP, dNTP,
lipids, ...

sugar,
NH_3

rep

DNA

tsx

rRNA

tRNA

mRNA

sRNA

ribosomes

tsl

ribosomal proteins

structural proteins

transporters

enzymes

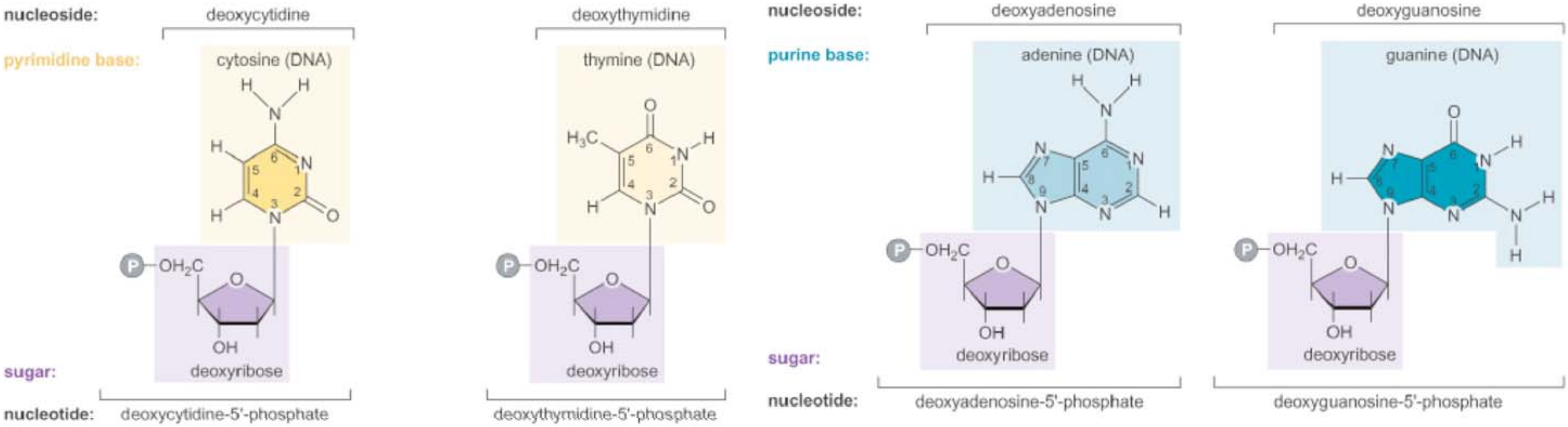
regulators

RNAp

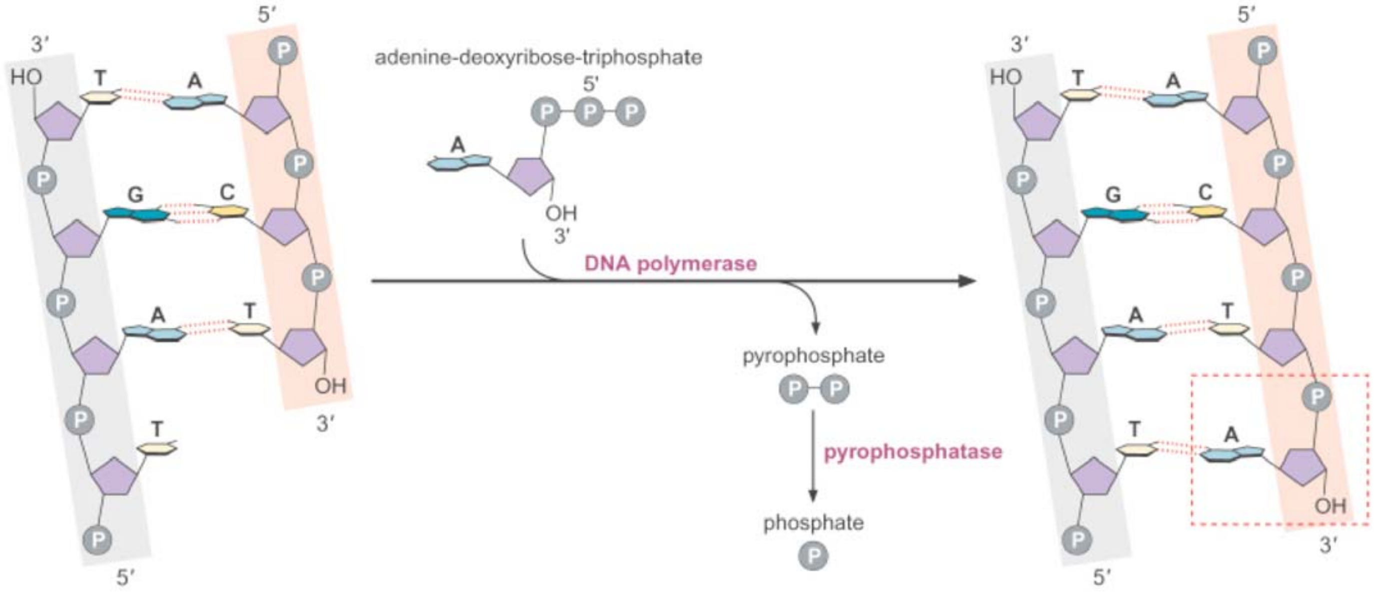
DNAp

❖ DNA replication

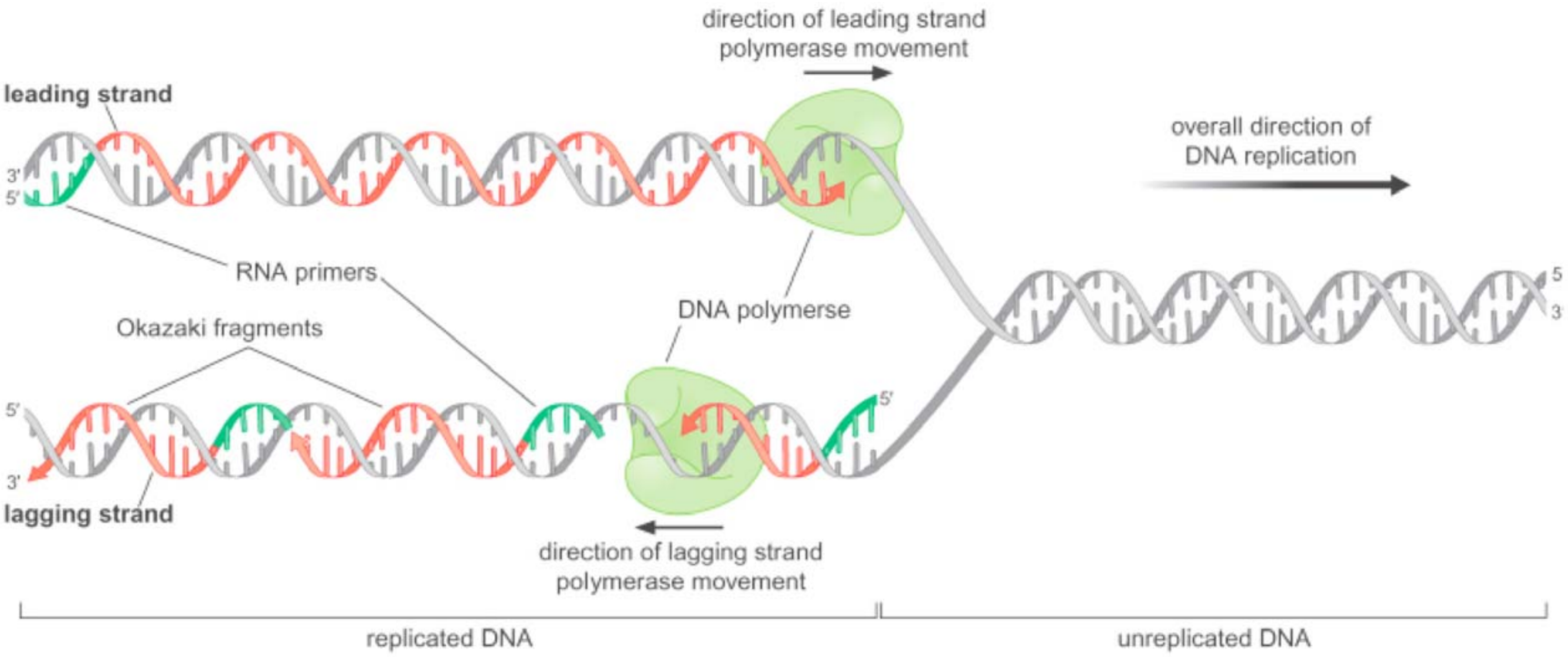
- the four “bases” of DNA: pyrimidines (C, T) and purines (A, G)



- DNA synthesis



- the replication fork



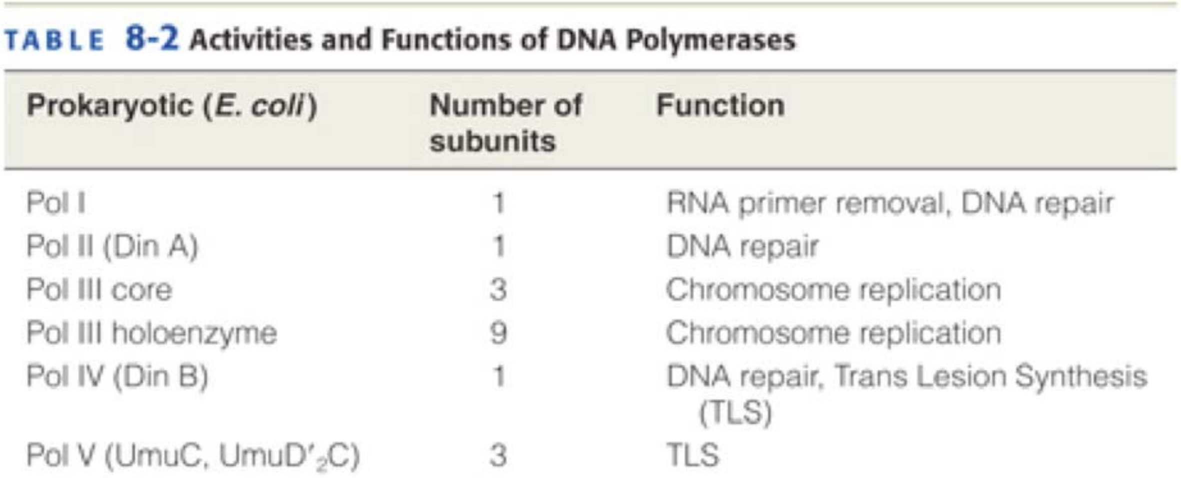


TABLE 8-2 Activities and Functions of DNA Polymerases

Prokaryotic (*E. coli*)	Number of subunits	Function
Pol I	1	RNA primer removal, DNA repair
Pol II (Din A)	1	DNA repair
Pol III core	3	Chromosome replication
Pol III holoenzyme	9	Chromosome replication
Pol IV (Din B)	1	DNA repair, Trans Lesion Synthesis (TLS)
Pol V (UmuC, UmuD'$_2$C)	3	TLS

- initiation of DNA replication
  - doubling time of *E. coli* can vary over 10x [fastest doubling time: ~20 min]
  - 40 min required to replicate chromosome
  - fixed time of 20 min between completion of one round of replication and cell division

➔ doubling time > 60 min: waiting time between division & replication

➔ doublint time < 60 min: multiple replication forks

➔ one replication origin every 1.7 µm (length of unit cell):

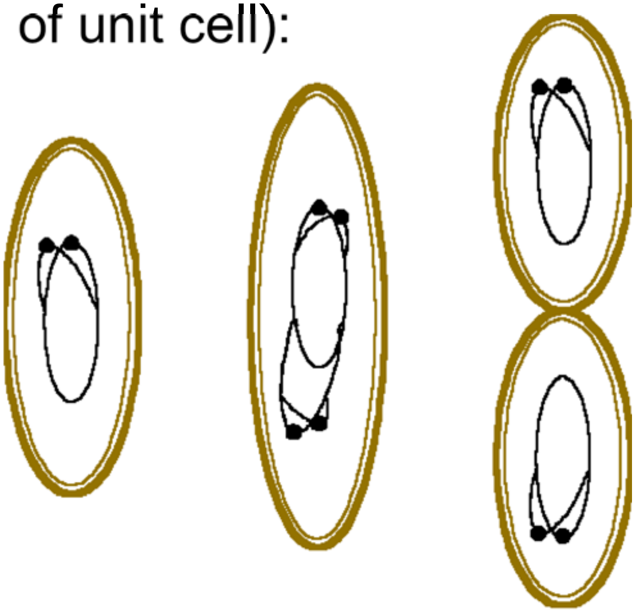
fast growing cells are larger!

Questions:

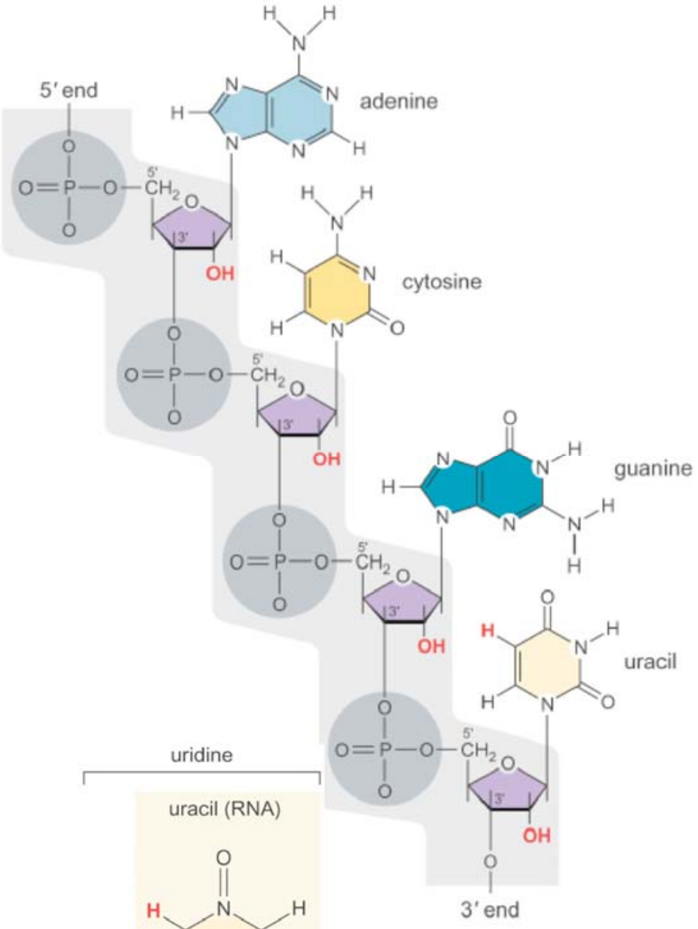
- mechanism of replication initiation control?

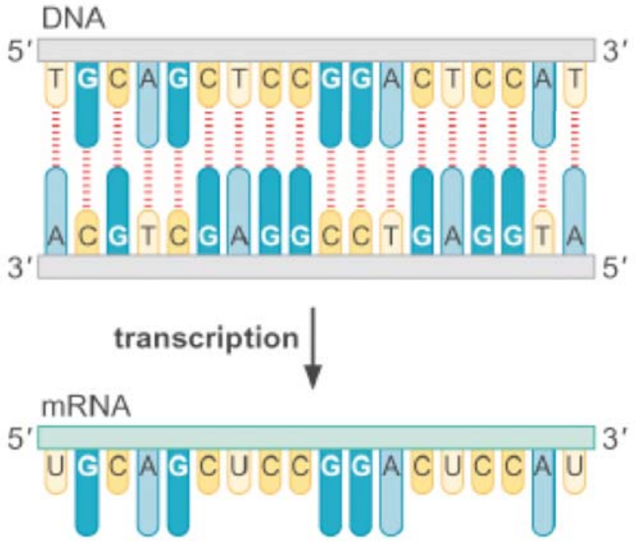
- how does the cell “measure” its volume?

- effect of gene doubling on protein level?

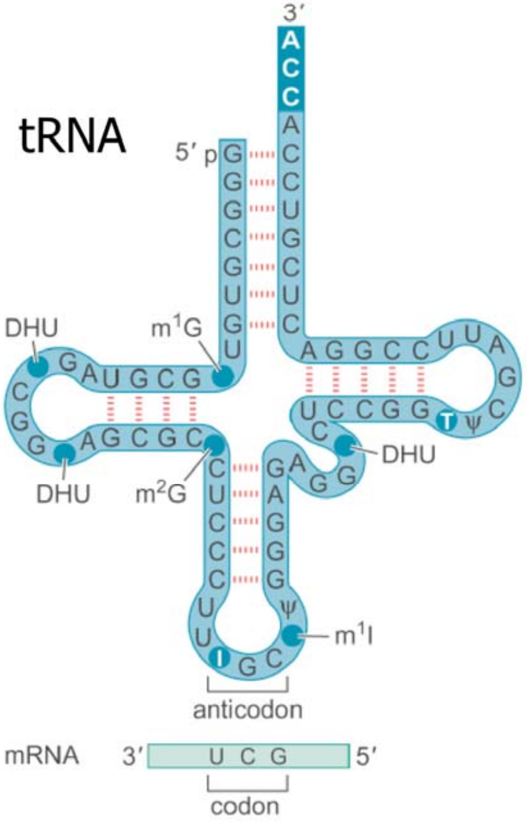


- ❖ transcription: DNA to RNA
  - RNA

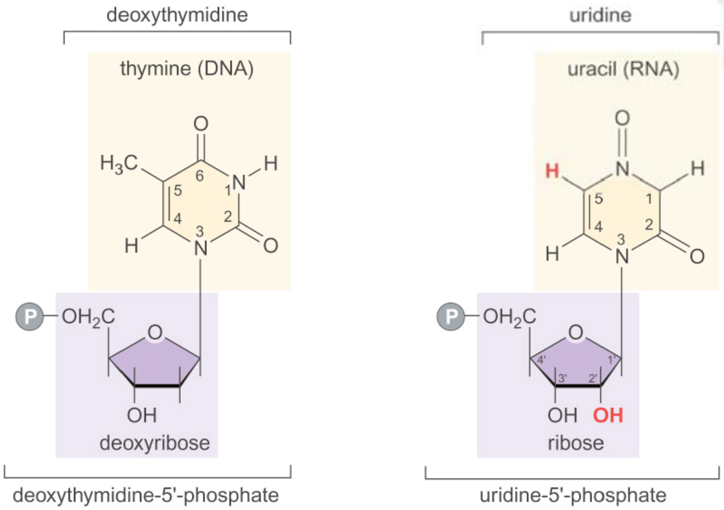




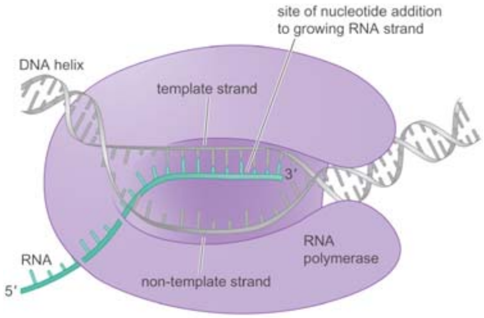
tRNA



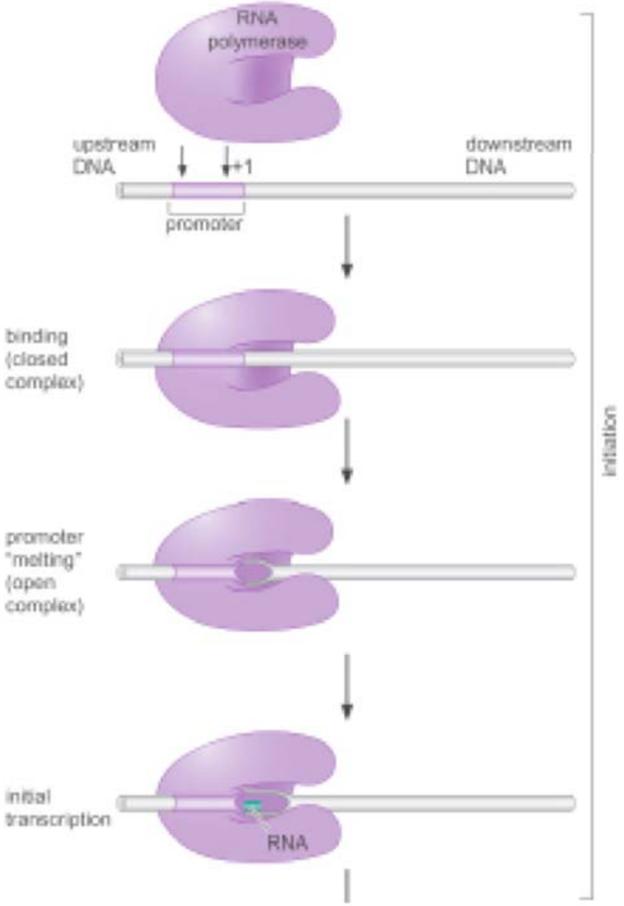
DNA vs RNA:



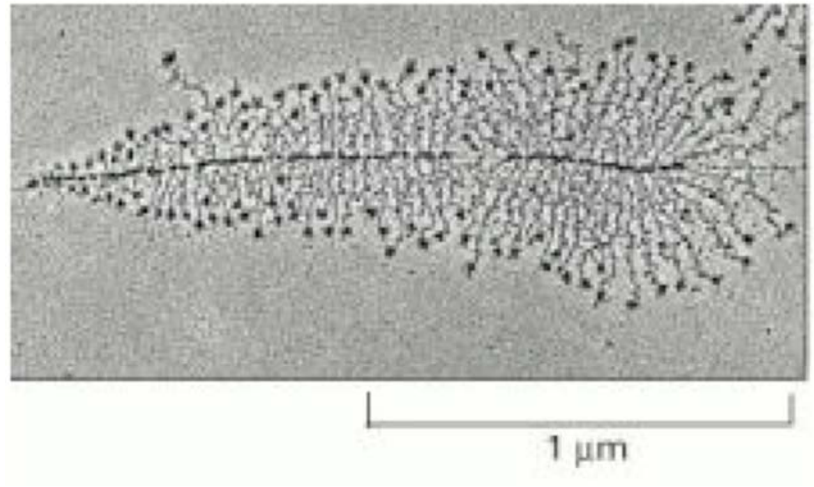
- RNA synthesis:



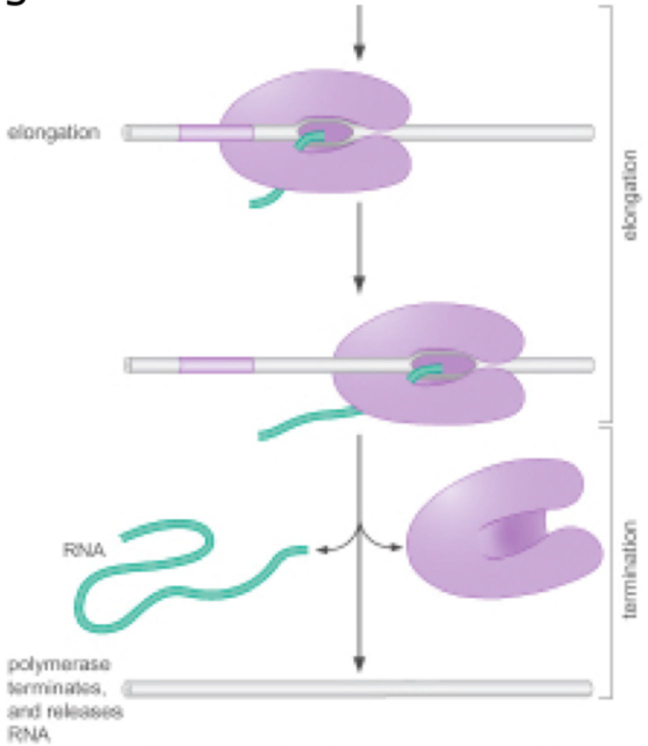
transcriptional initiation



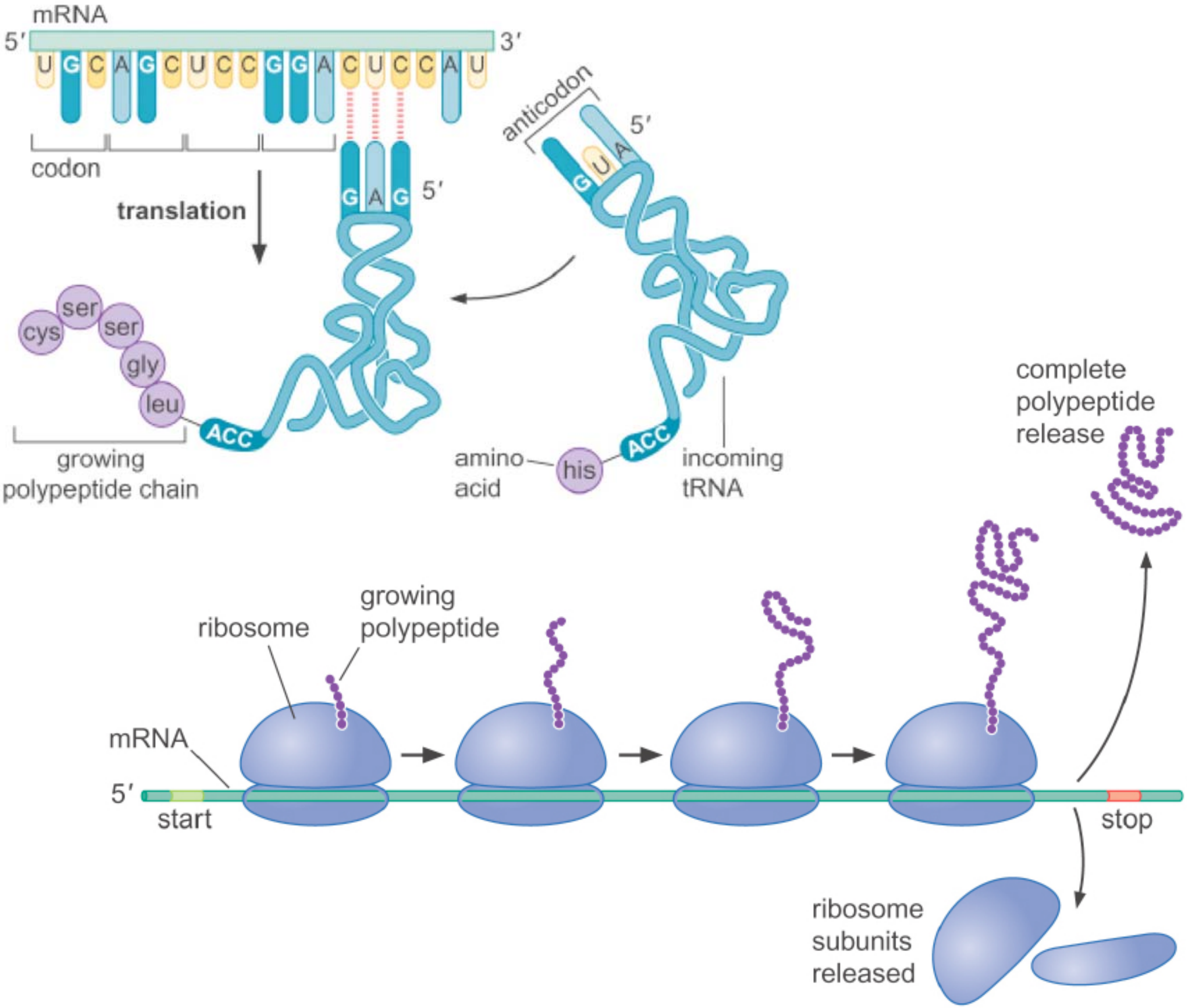
heavily transcribed genes coding ribosomal RNA



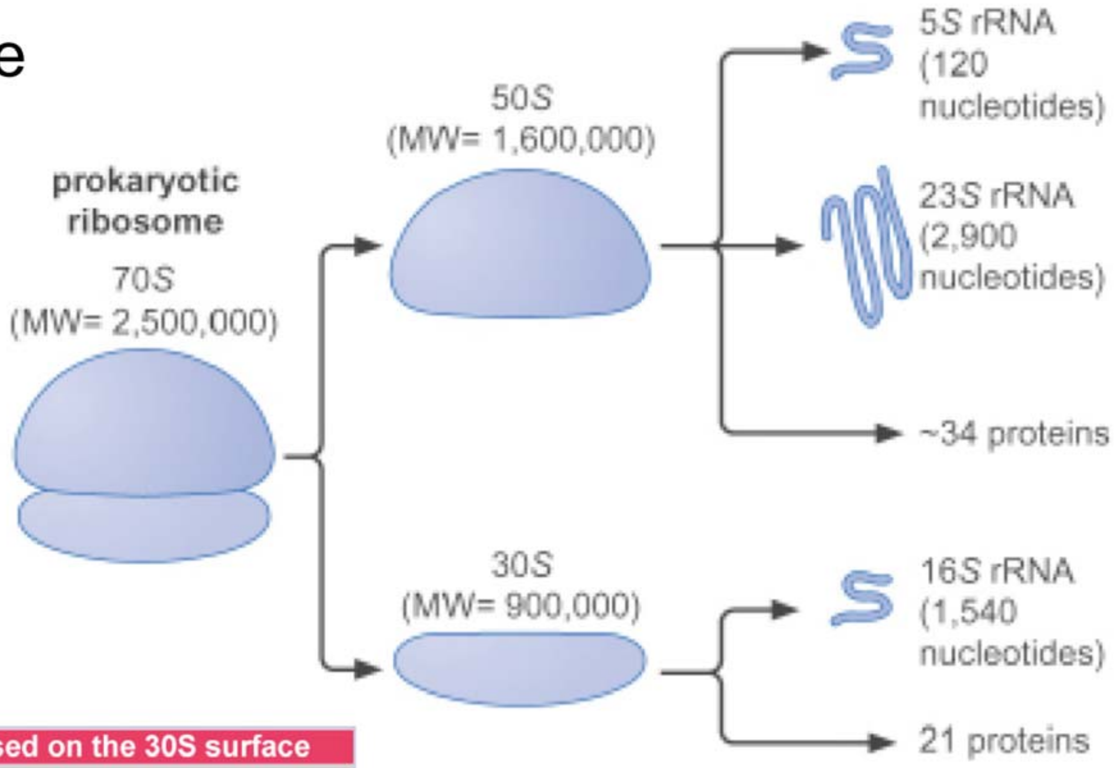
elongation and termination



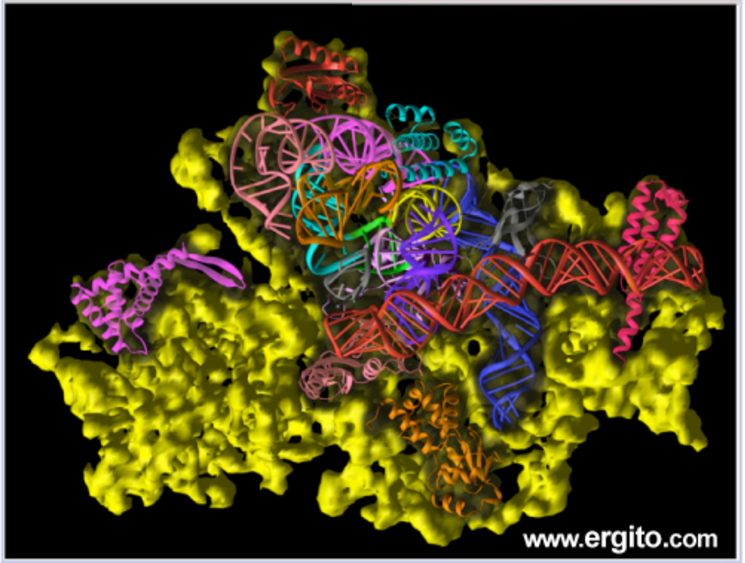
- translation: RNA to protein



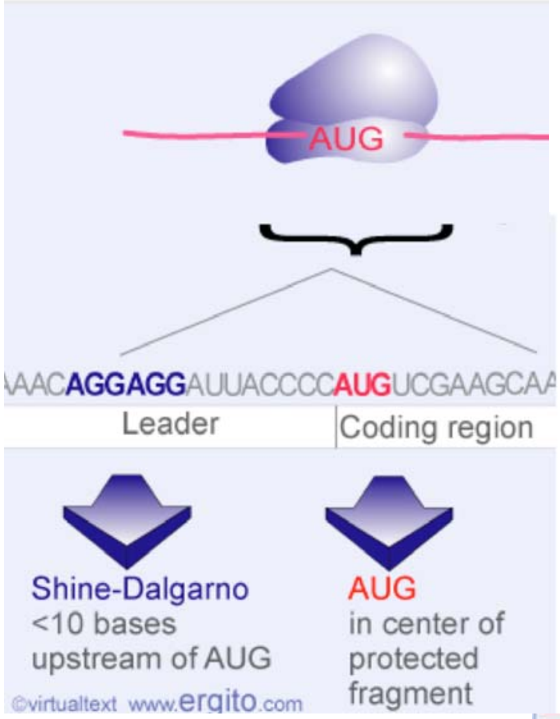
- the ribosome



Ribosomal RNA is exposed on the 30S surface



- translational initiation, elongation and termination



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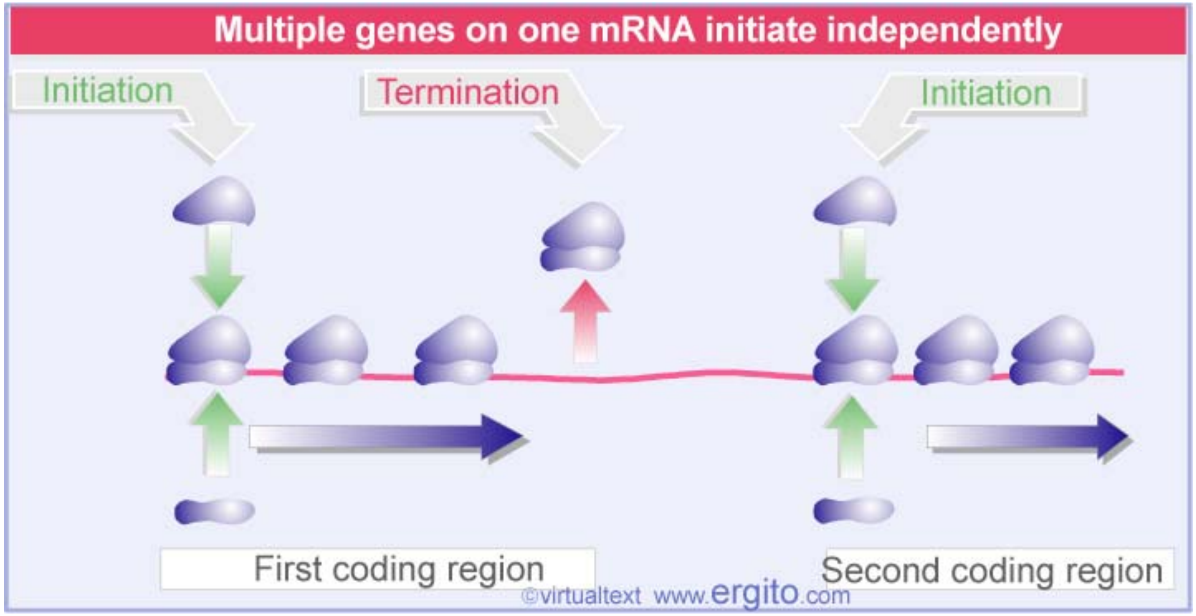

Multiple genes on one mRNA initiate independently

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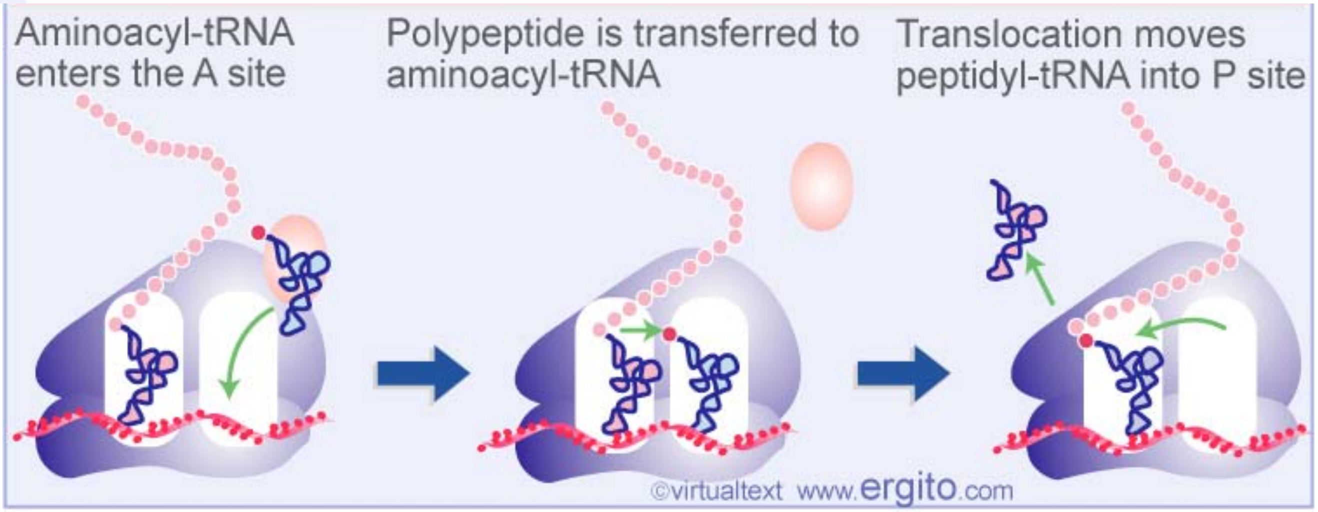

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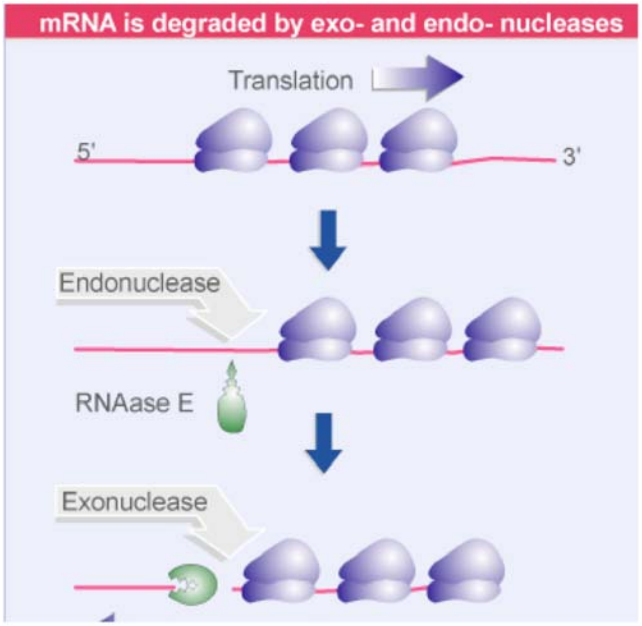
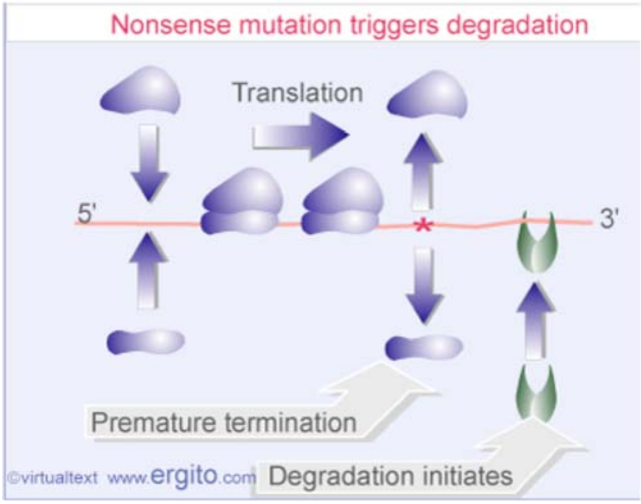
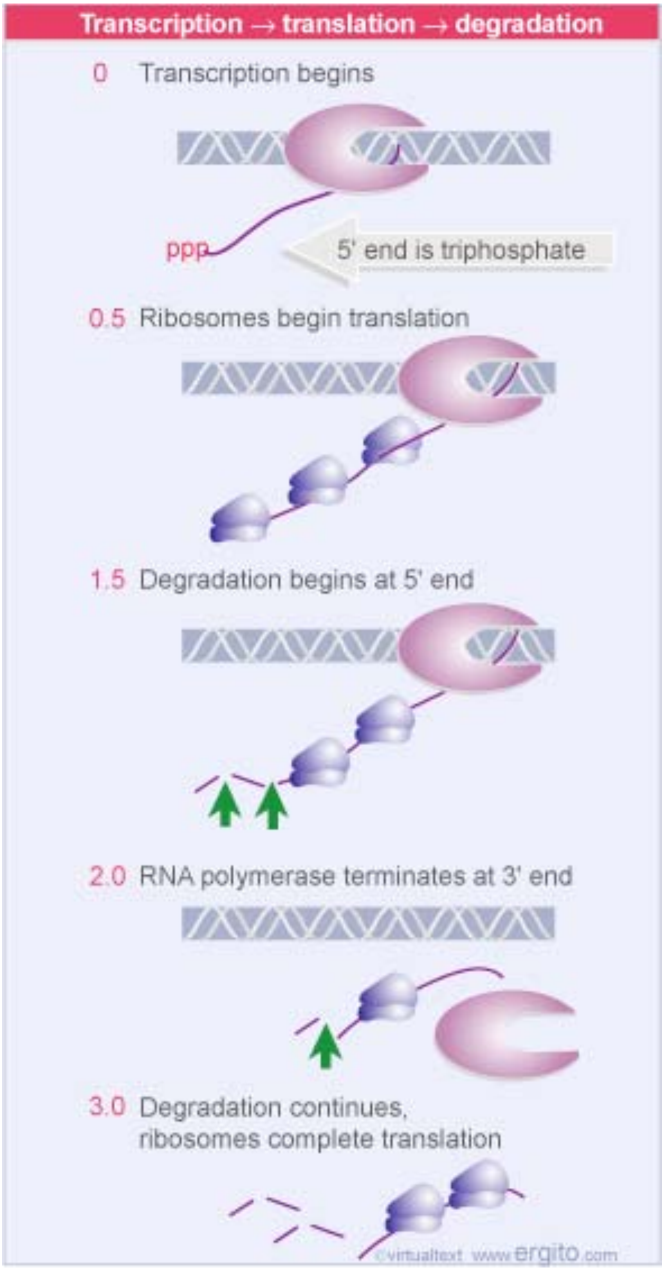
❖ rapid mRNA degradation -- use it or get rid of it !

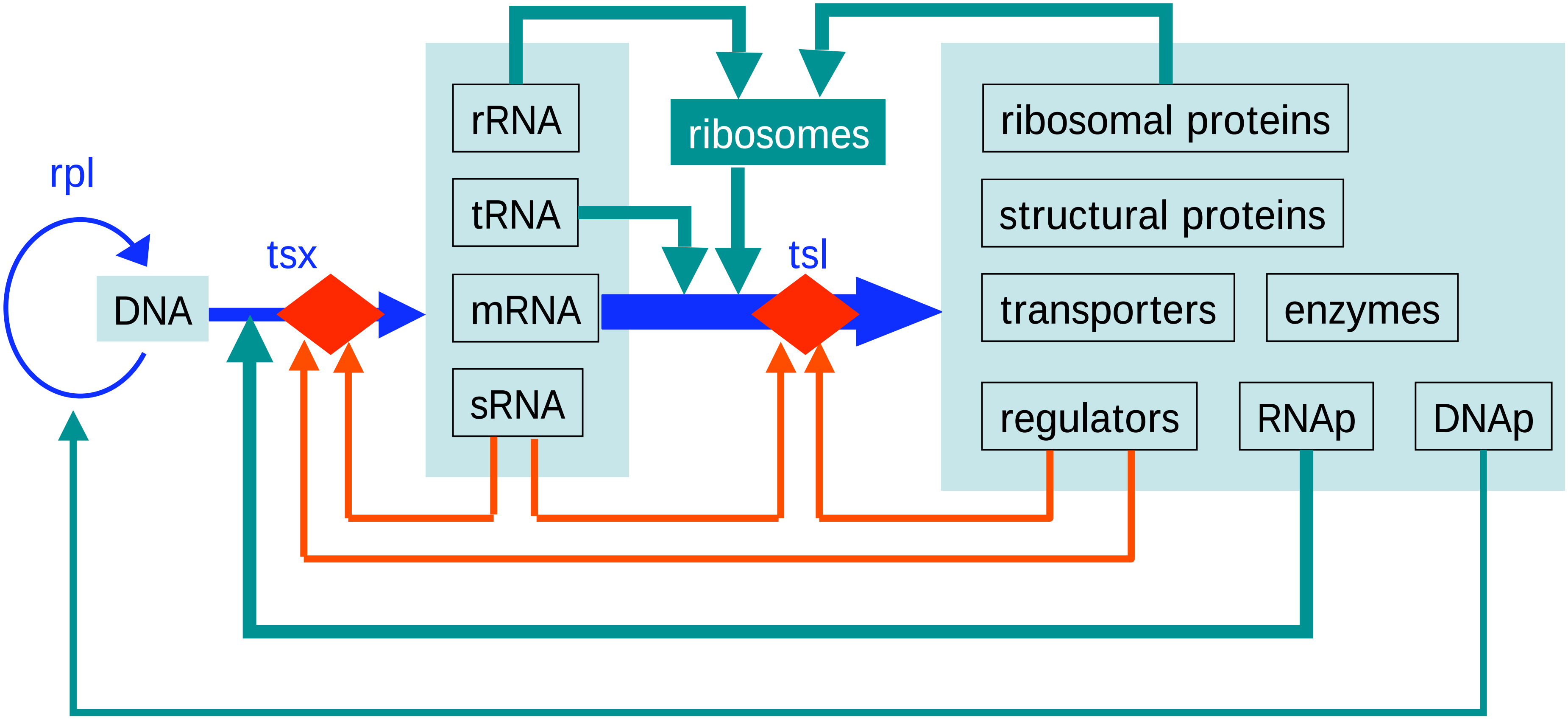


- premature termination





❖ central dogma + regulation

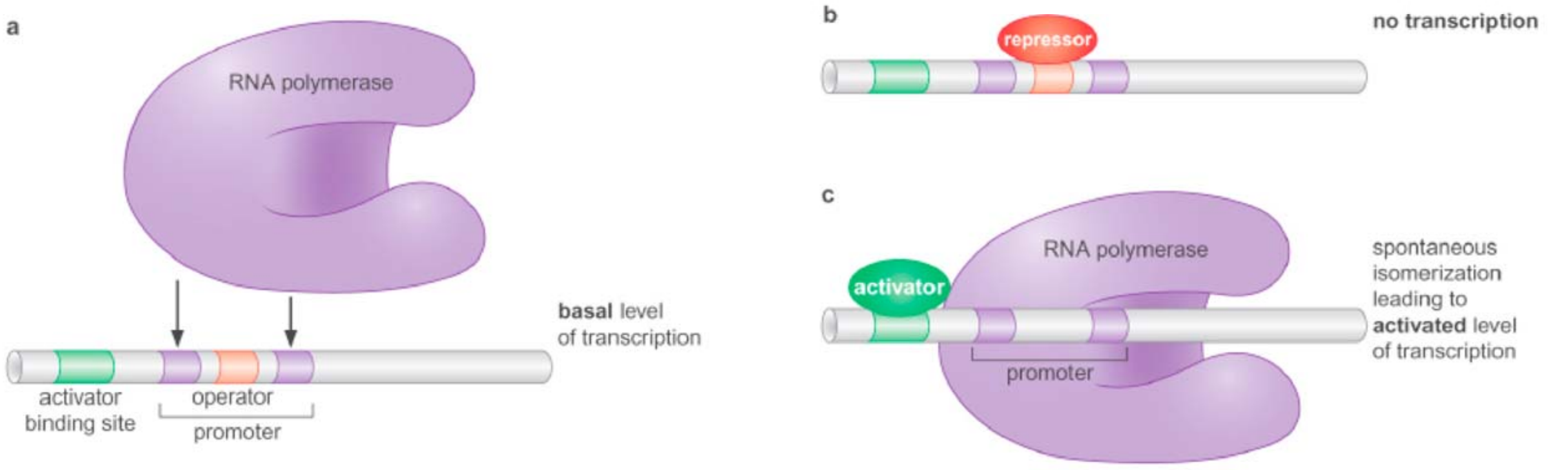


- tsx initiation control by transcription factors (TF)
- tsl initiation control by sRNA and RNA-binding proteins
- tsx termination control by sRNA and anti-terminators
- control of mRNA and protein degradation

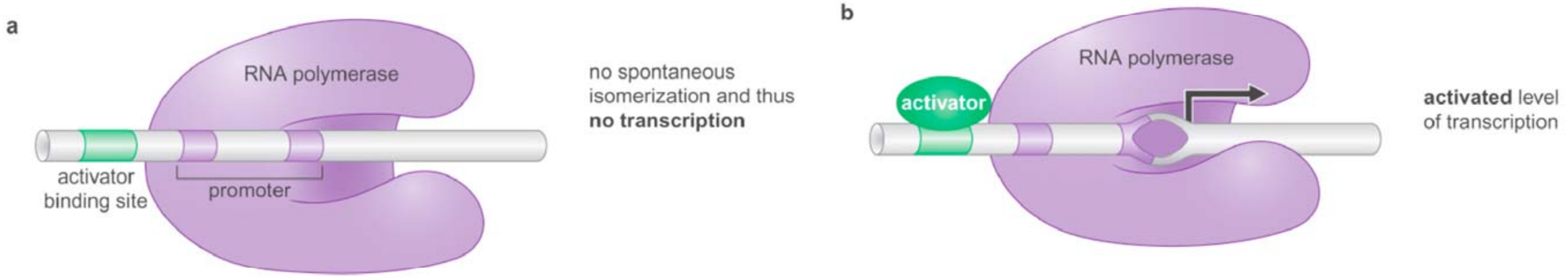
} coupled to environmental signals

❖ transcriptional initiation control

- modulation of RNAp-promoter affinity
  via activators and repressors

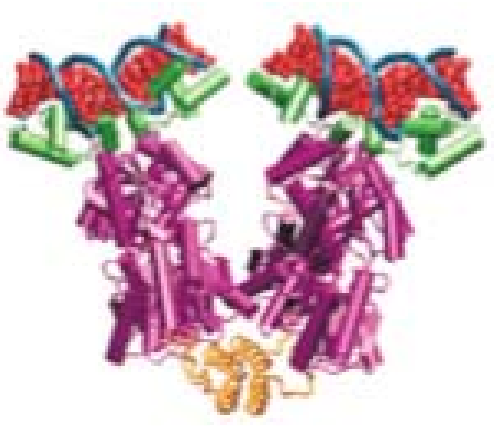


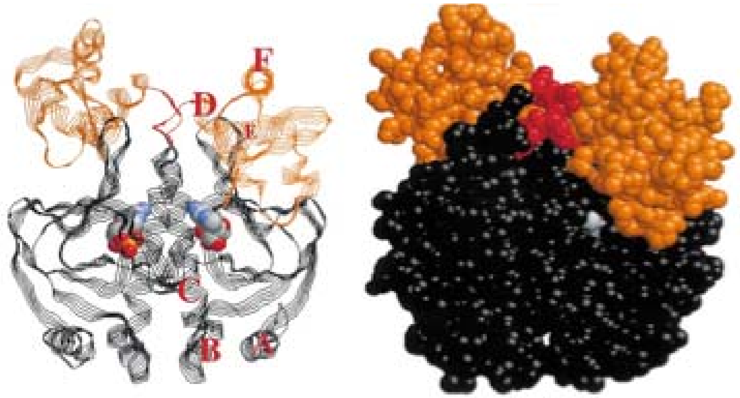
- allosteric activation of RNAp



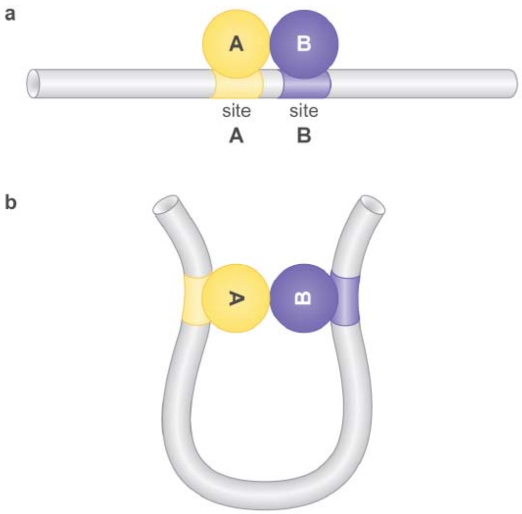
➔ net result: rate of tsx init dependent on cellular conc of activators/repressors
controlled by, e.g., inducer molecules

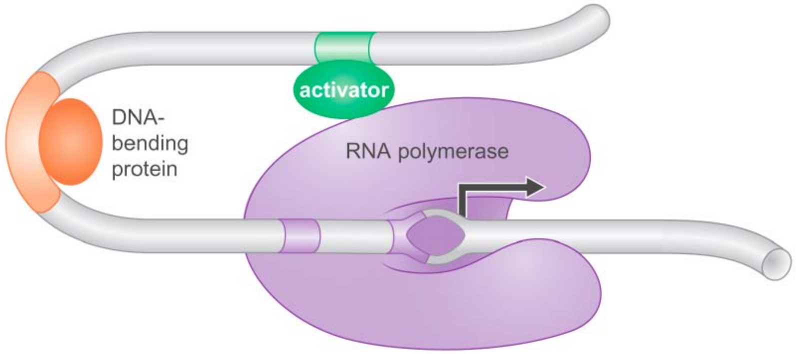
- Molecular determinants of transcriptional initiation control
  - protein-DNA interaction
  - protein-ligand interaction



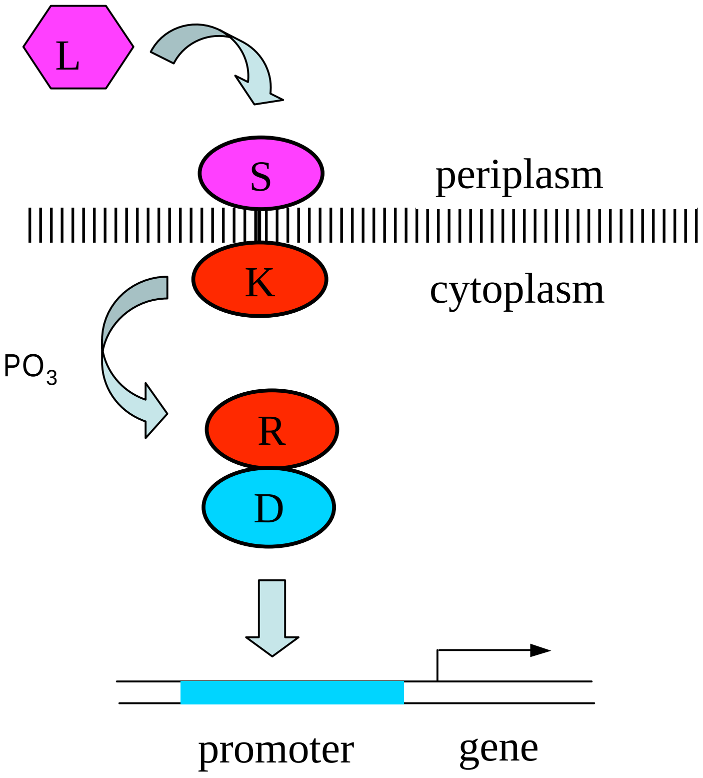


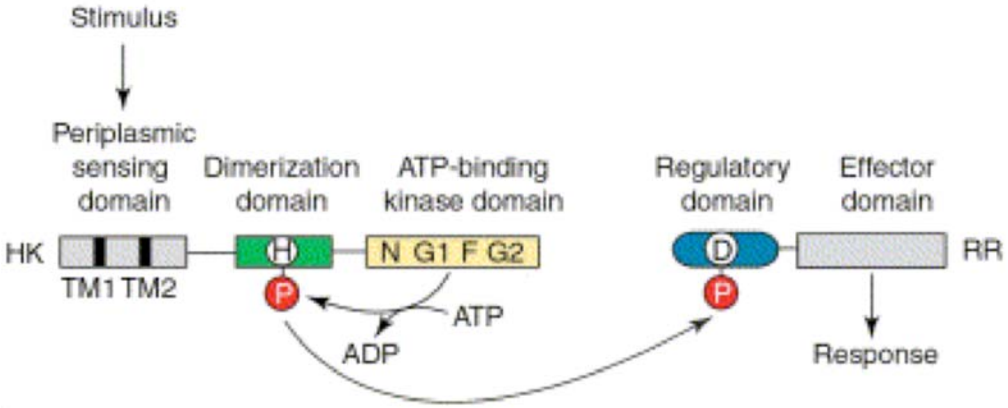
  - protein-protein interaction
  - necessity of DNA looping

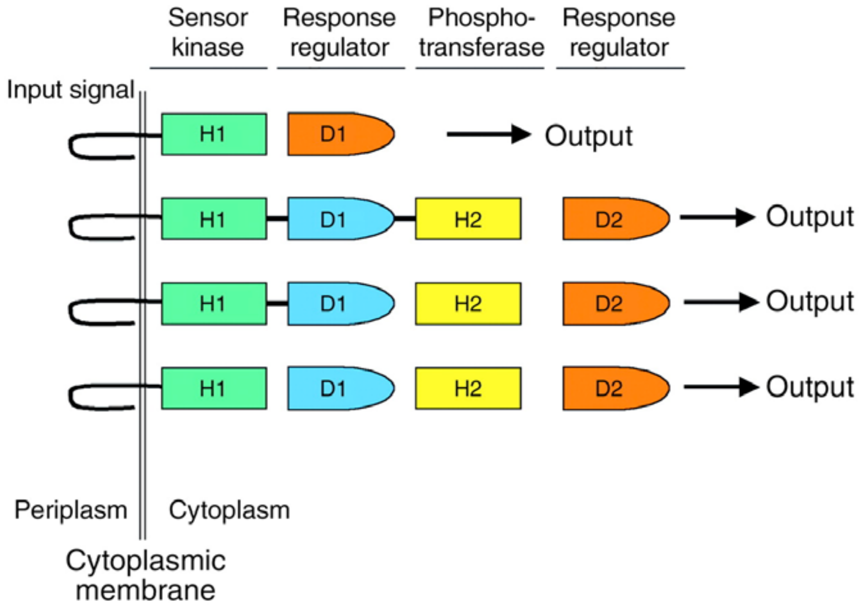




❖ two-component signaling systems

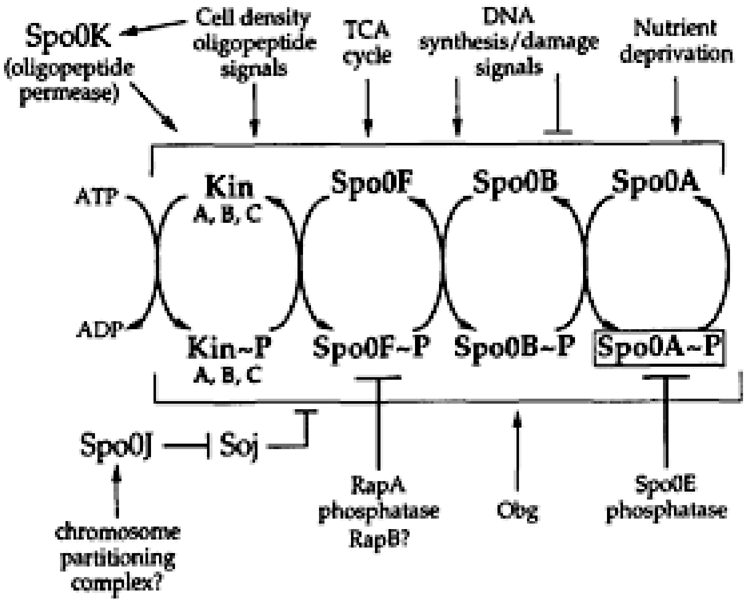


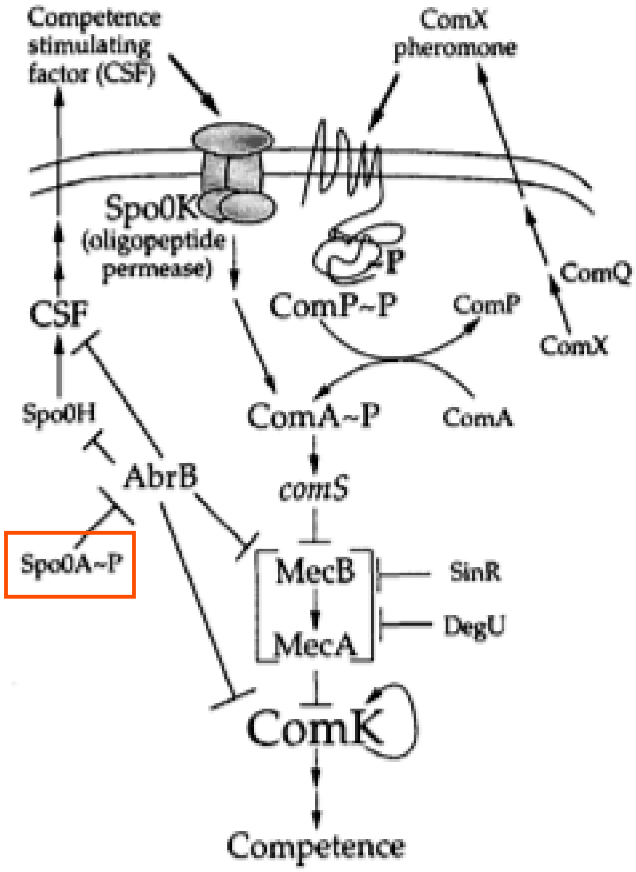




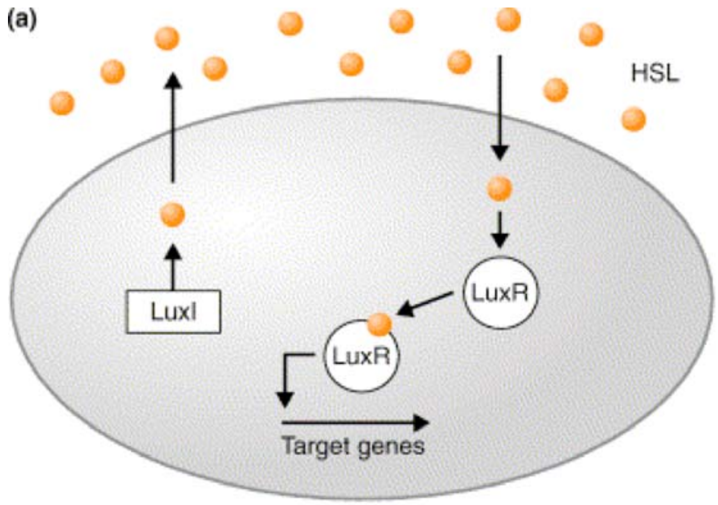
sporulation and competence control in *B. subtilus*

[A. Grossman, 95]

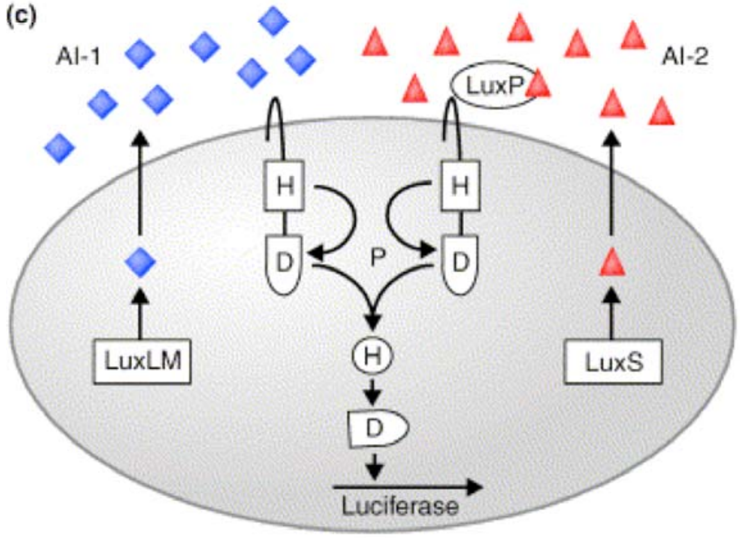




quorum sensing: inter-cellular communication



– density-dependent gene expression

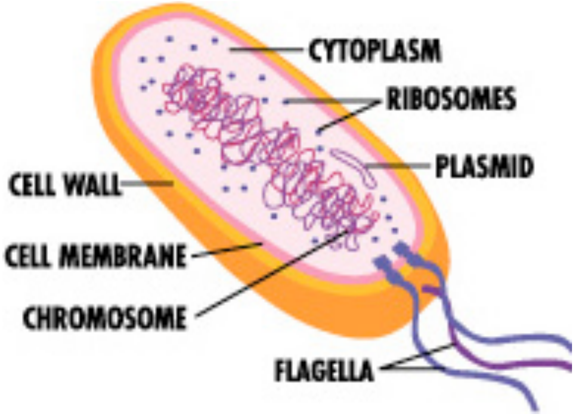


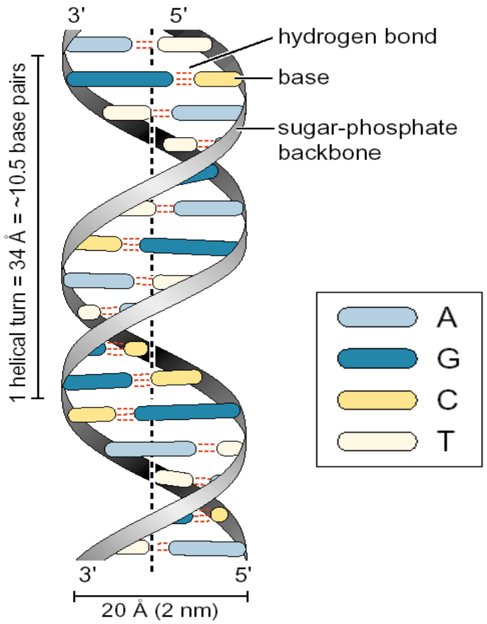
– can detect multiple signaling molecules

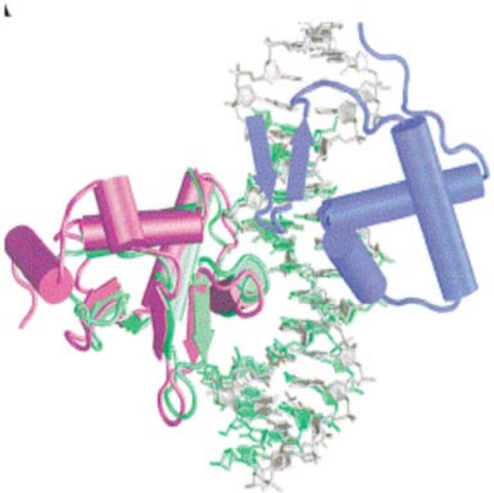
– potential for complex language

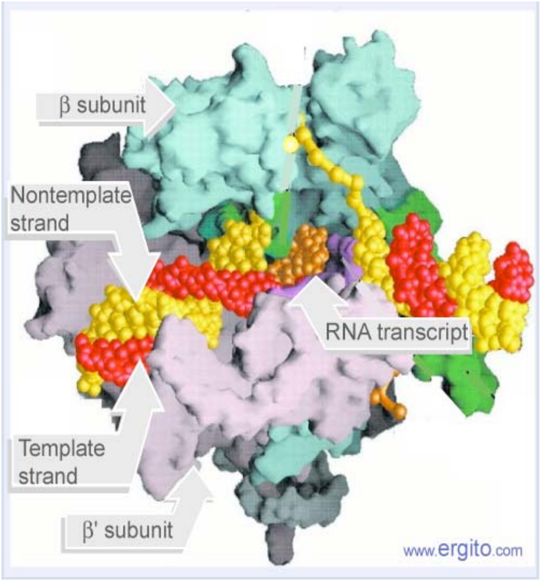
4. quantitative physical aspects

- dimensions
  - DNA: 2 nm x 2 nm x 3.4 nm/turn
  - small proteins: (few nm)^3 or ~10nt
  - protein complexes, (10-20 nm)^3 or 30 ~ 60nt
  - cell size: 1 um^2 x 3 um
  - concentration: 1 molecule/cell ~ 1nM
  - intracellular diffusivity: ~10 um^2/sec

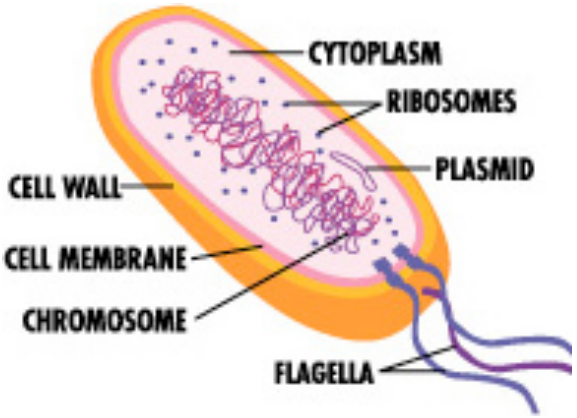


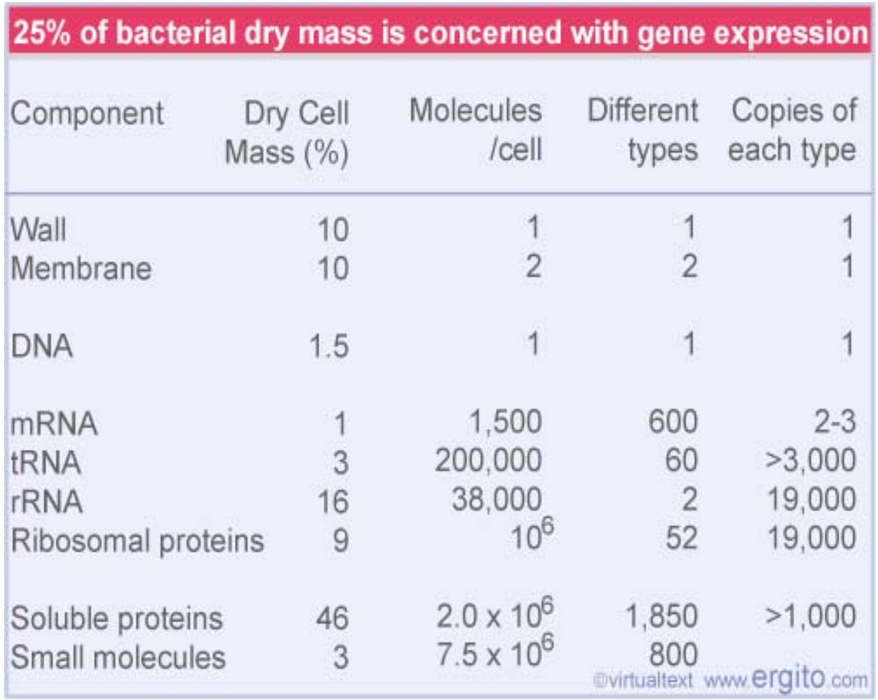






- abundance
  - ribosomes: ~ 20,000 (52 proteins each)
  - RNAp ~ 1,000 (a few pct available)
  - proteins: 2×10^6 (TF: 10 ~ 1,000 / type)
  - mRNA: 0.1 ~ 100/cell;
    peaked at 2 ~ 3 copies / cell





25% of bacterial dry mass is concerned with gene expression

Component	Dry Cell Mass (%)	Molecules /cell	Different types	Copies of each type
Wall	10	1	1	1
Membrane	10	2	2	1
DNA	1.5	1	1	1
mRNA	1	1,500	600	2-3
tRNA	3	200,000	60	>3,000
rRNA	16	38,000	2	19,000
Ribosomal proteins	9	10^6	52	19,000
Soluble proteins	46	2.0×10^6	1,850	>1,000
Small molecules	3	7.5×10^6	800	

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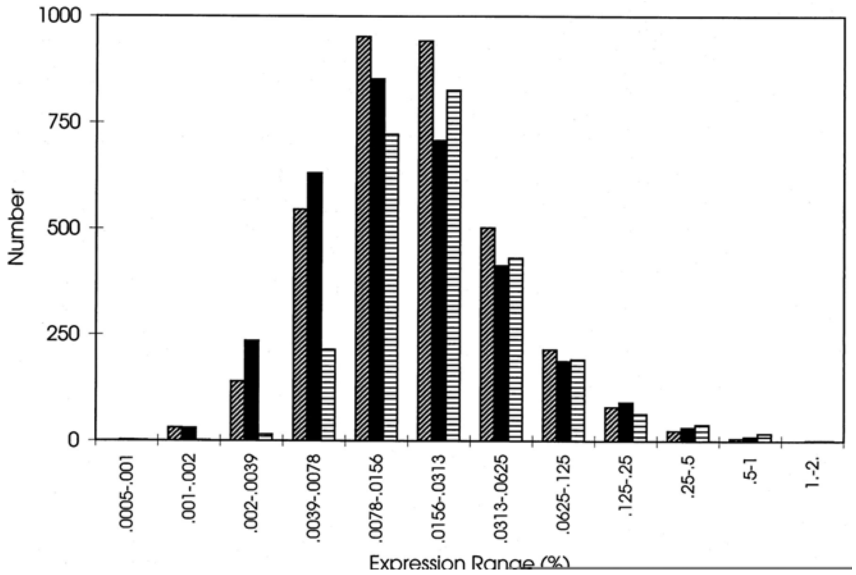
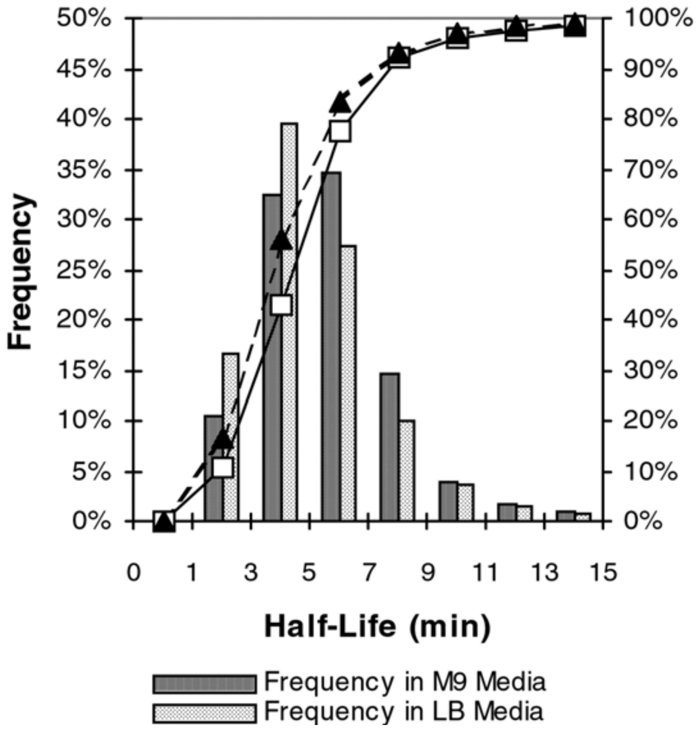
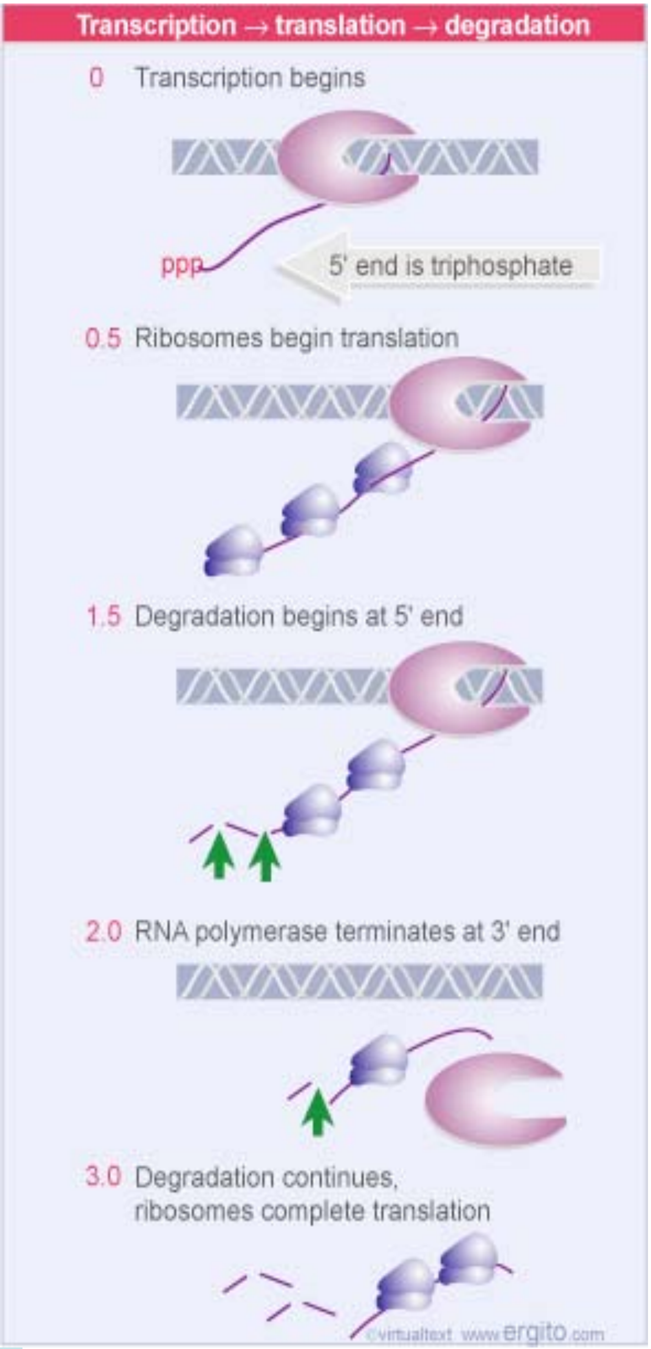


- rates
  - transcription: elongation ~40 nt/s
  - translation: ~ 15 aa/s
  - mRNA half-life: < 5 min
  - protein half-life:
    from cell-doubling time (passive decay)
    down to a few min (active proteolysis)



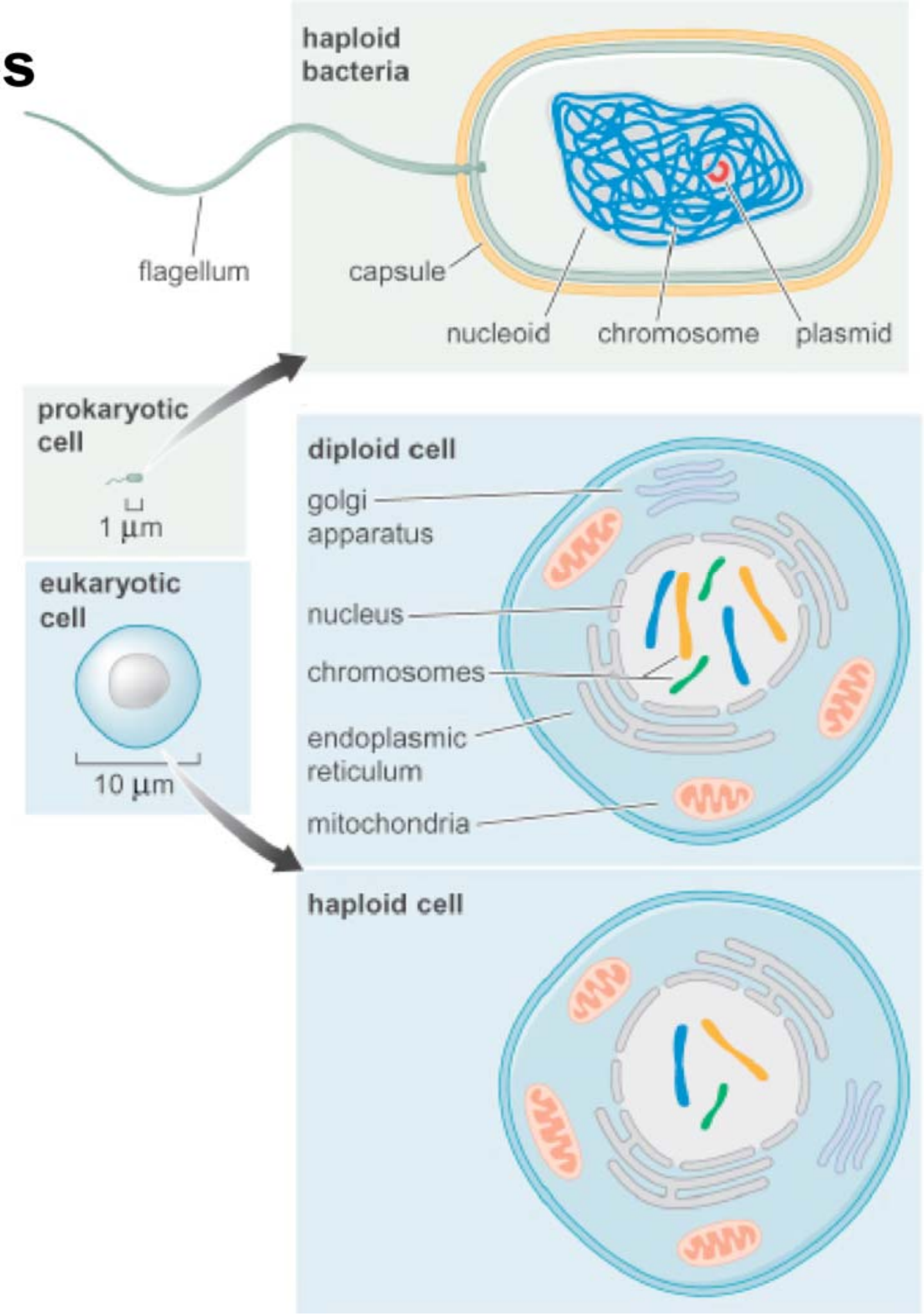
Question: which of these numbers are related?



5. Comparison to eukaroyotes

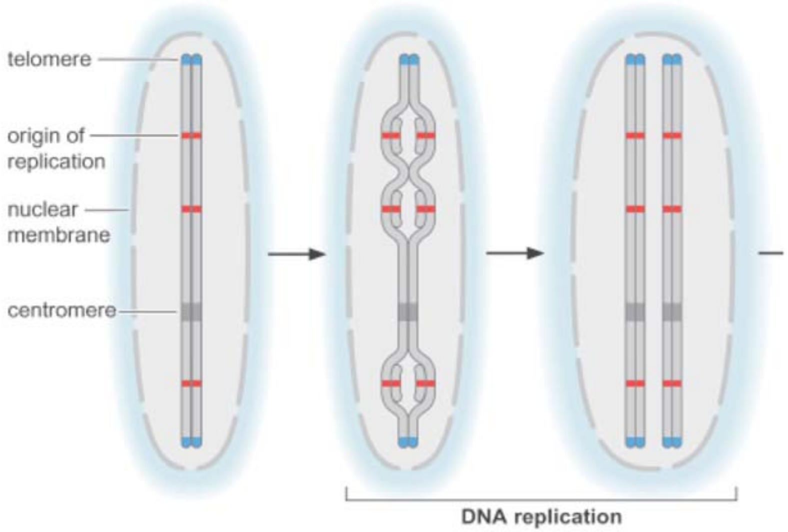
❖ size and structure

eukaryotes:
– cells ~1000x larger
– DNA in nucleus
– linear chromosomes
– haploids and diploids
– organelles
– vesicles
– microtule network
– …

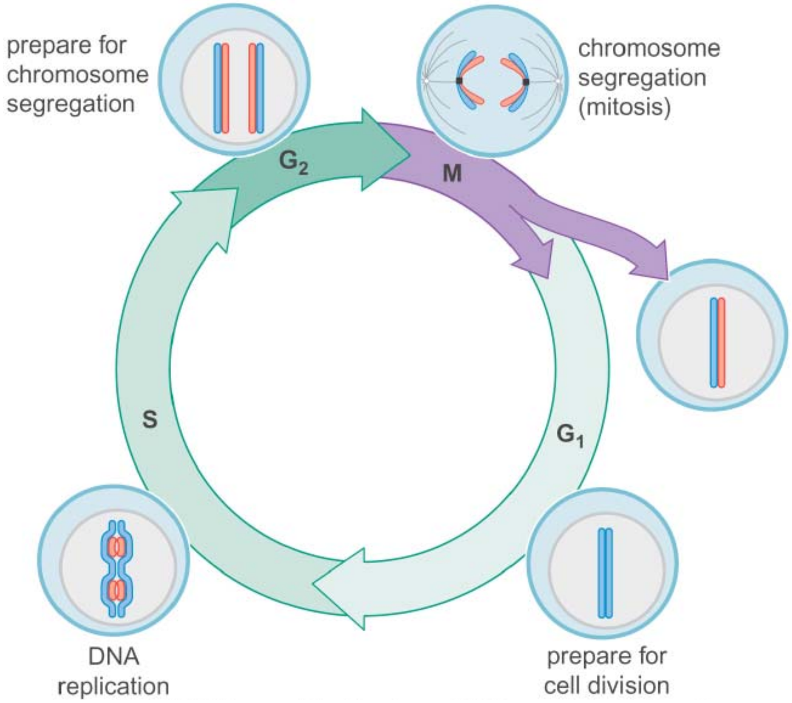


❖ Chromosome

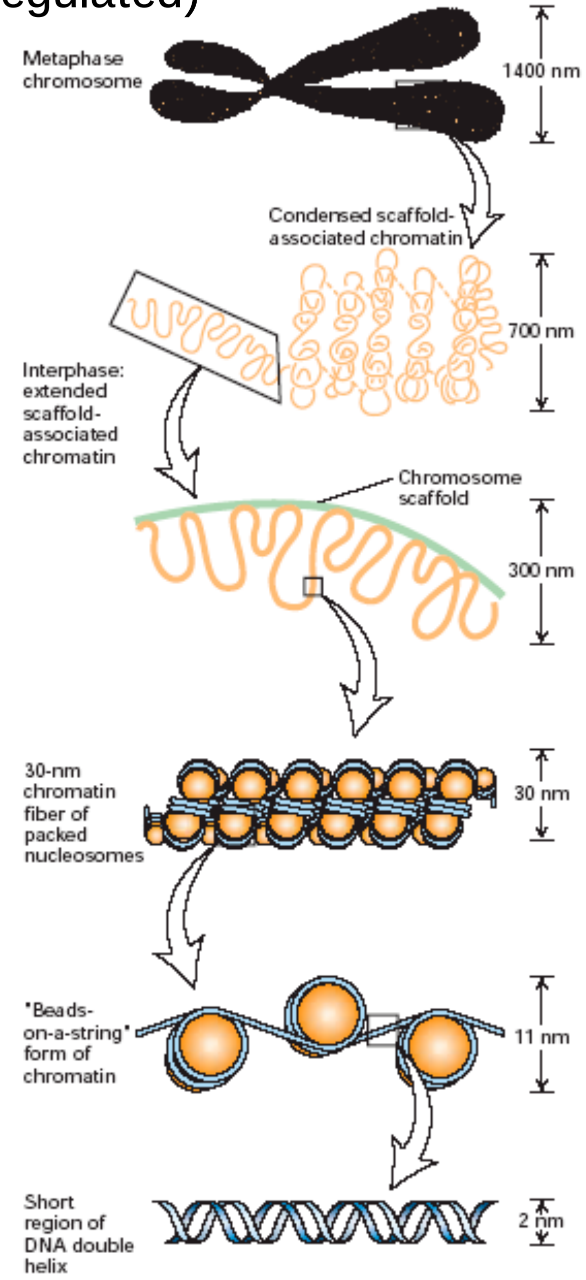
duplication



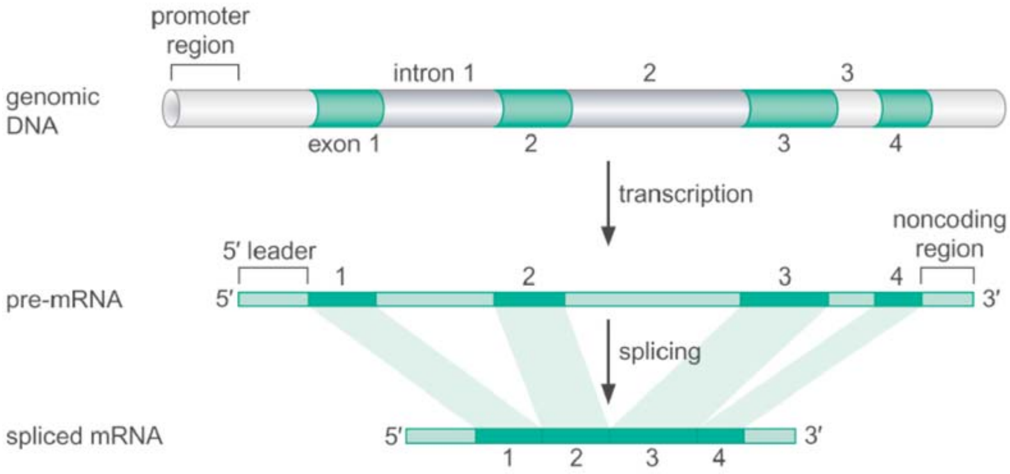
cell cycle



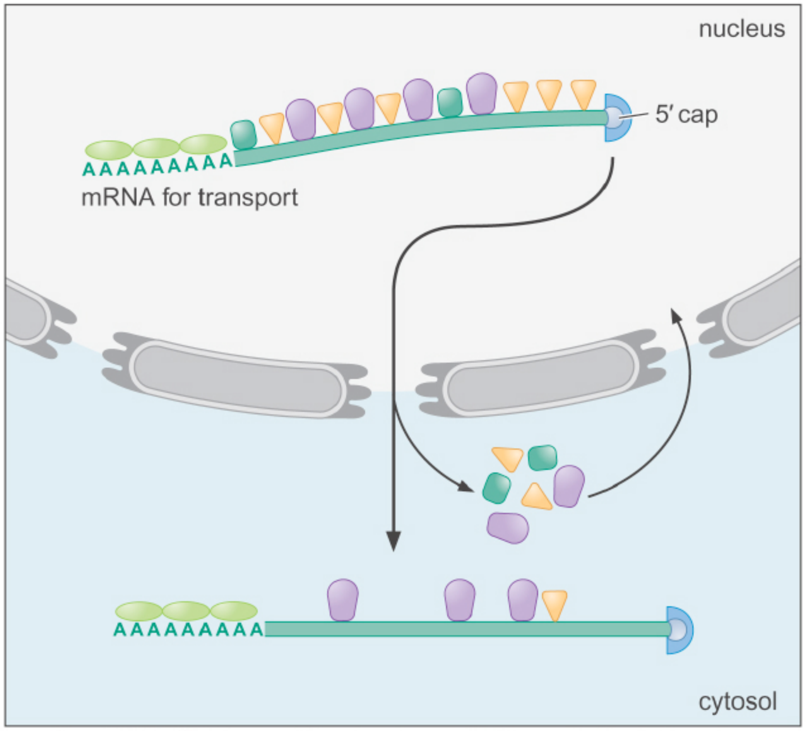
chromatin structure (regulated)



- RNA splicing and transport



only RNA with appropriate proteins bound are selected for transport out of the nucleus

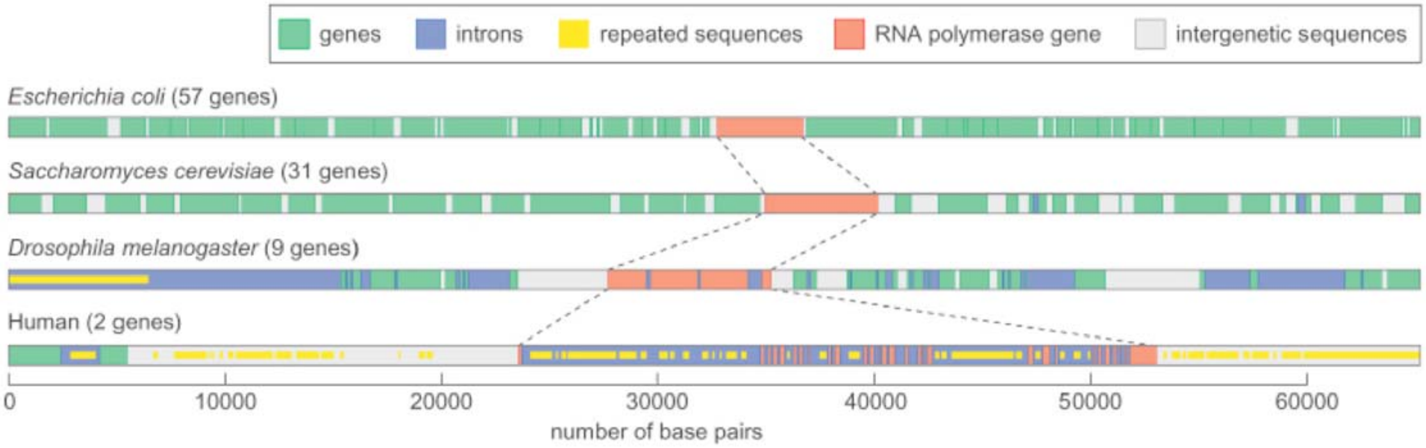


❖ genome and organization

- genome size

Organism	**Genome length**	**No. genes**
M. genitalium	0.5 Mb	500
E. coli	4.5 Mb	4,000
Yeast	12 Mb	6,000
Human	3,000 Mb	35,000
Rice	500 Mb	50,000
Lilly	90,000 Mb	?

- organization (human)
  - multiple replication origins
  - large intergenic separation: 3Gb/30,000 genes = 100kb (mostly transposable elements)



❖ Gene regulation in eukaryotes

- control of transcriptional initiation
  - direct activation by recruitment of RNAp
  - activation/repression by modifying chromatin structure
- control of entry of regulators into nucleus
- control of RNA splicing (e.g., alternative splicing)
- localization of mRNA
- control of mRNA life-time
- control of mRNA translation
- ubiquitination system to tag protein for degradation
- …