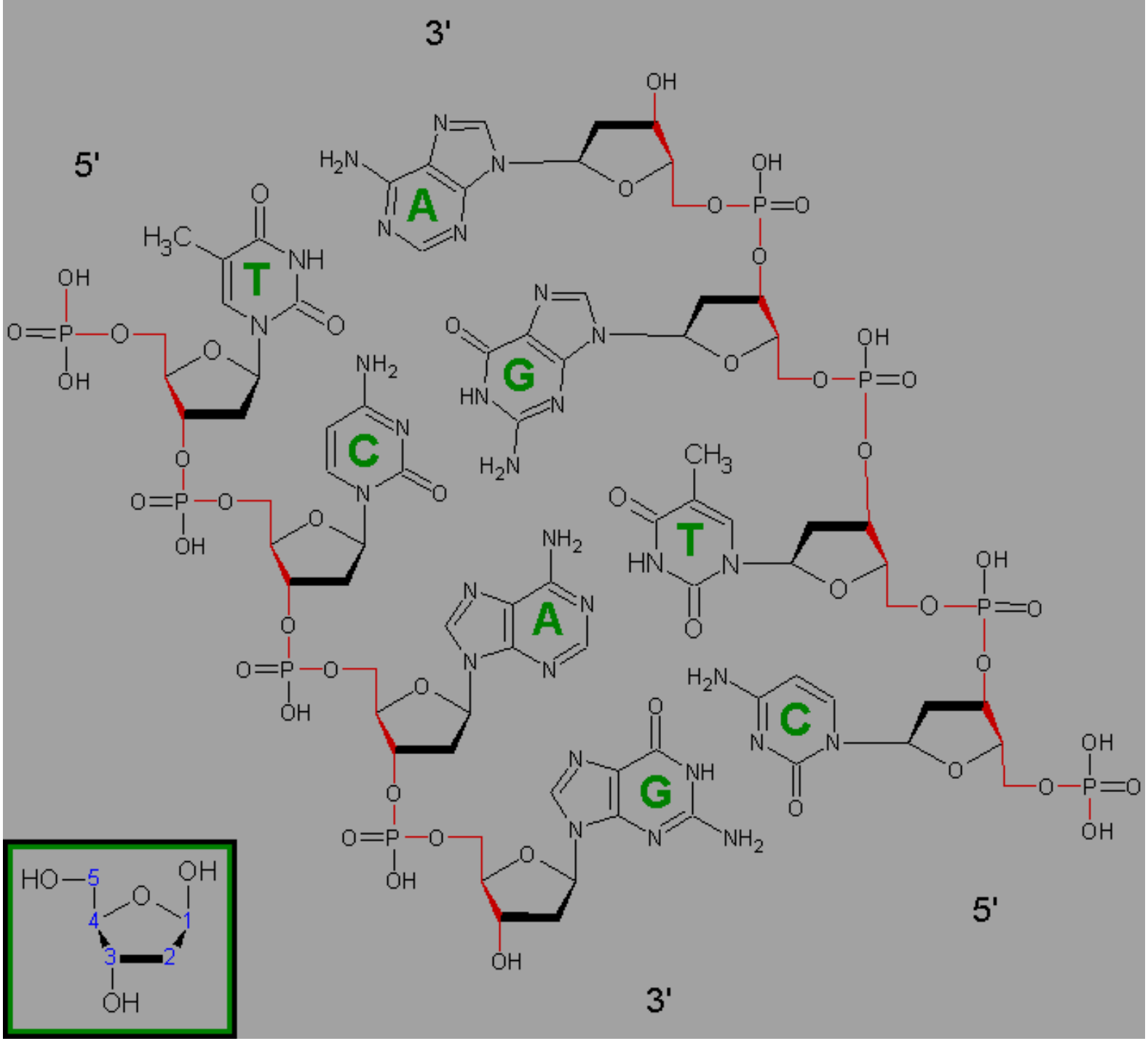
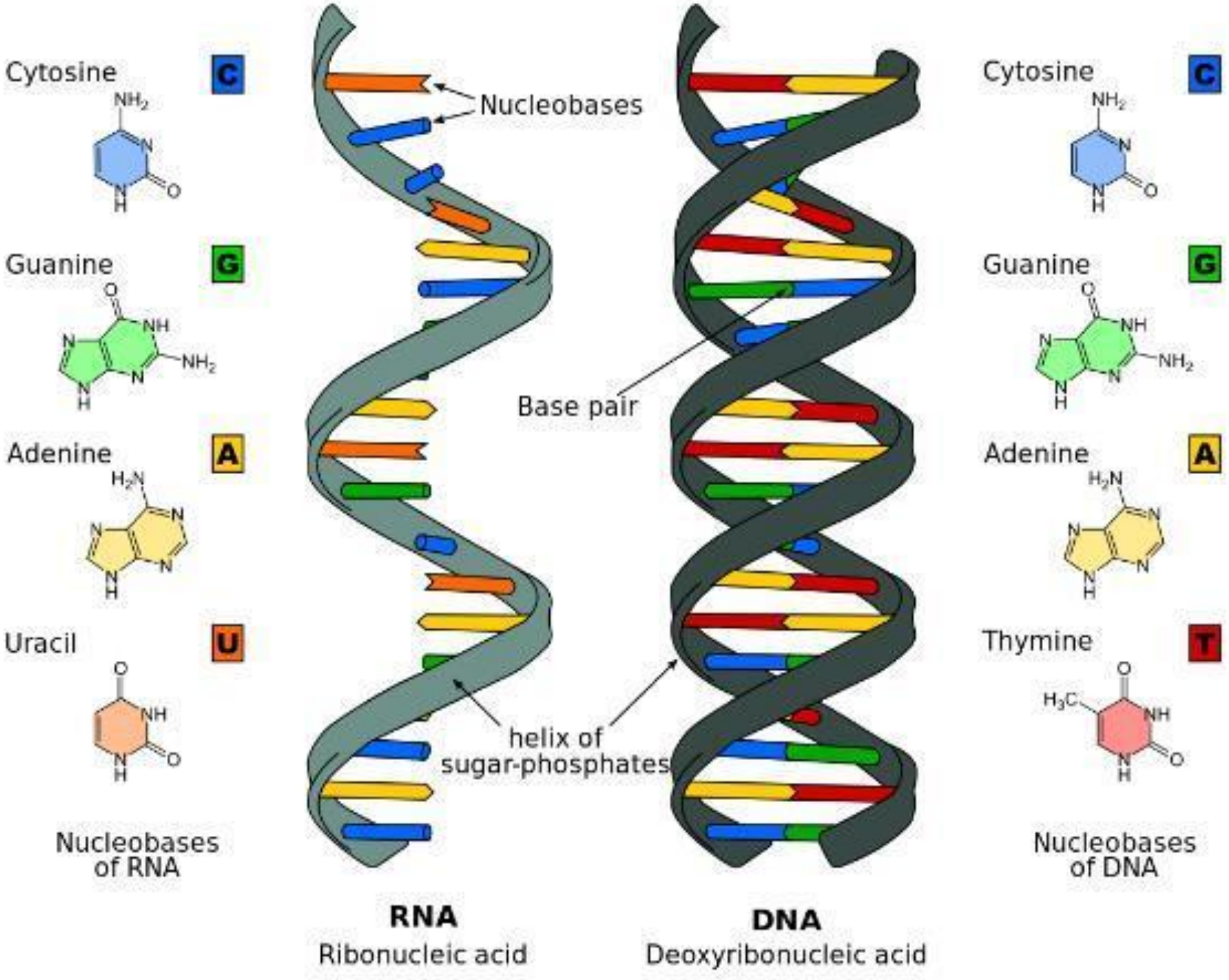
Практическая работа №1

# Строение и свойства нуклеиновых кислот. Репликация. База данных Genebank.

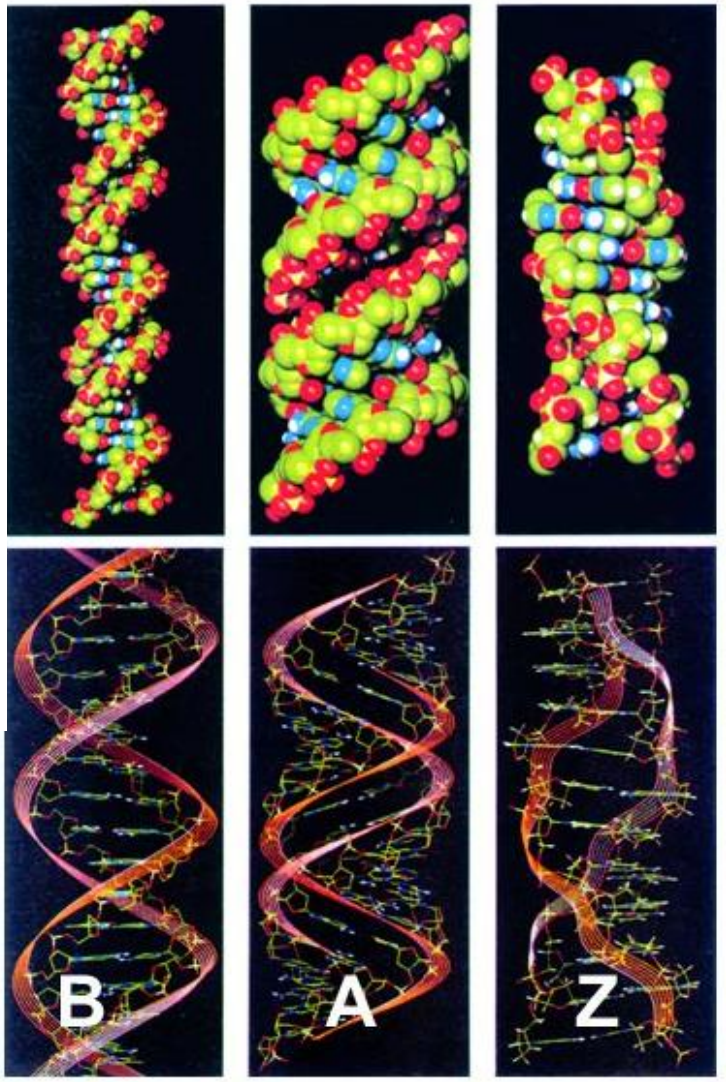

3'
5'
A
T
G
C
A
T
C
G
5'
3'
HO
OH
OH
1
2
3
4
5




https://www.youtube.com/watch?v=o_-6JXLYS-k

# ДНК – материальный носитель наследственности

| Geometry attribute | A-form | B-form | Z-form |
|---|---|---|---|
| Helix sense | right-handed | right-handed | left-handed |
| Repeating unit | 1 bp | 1 bp | 2 bp |
| Rotation/bp | 32.7° | 34.3° | 30° |
| bp/turn | 11 | 10 | 12 |
| Inclination of bp to axis | +19° | −1.2° | −9° |
| Rise/bp along axis | 2.3 Å (0.23 nm) | 3.32 Å (0.332 nm) | 3.8 Å (0.38 nm) |
| Pitch/turn of helix | 28.2 Å (2.82 nm) | 33.2 Å (3.32 nm) | 45.6 Å (4.56 nm) |
| Mean propeller twist | +18° | +16° | 0° |
| Glycosyl angle | anti | anti | C: anti,<br>G: syn |
| Sugar pucker | C3'-endo | C2'-endo | C: C2'-endo,<br>G: C3'-endo |
| Diameter | 23 Å (2.3 nm) | 20 Å (2.0 nm) | 18 Å (1.8 nm) |




**Ген** — структурная и функциональная единица наследственности живых организмов. Ген представляет собой участок ДНК, кодирующий последовательность определённого полипептида либо функциональной РНК.

Таблица 17.1. Номенклатура оснований

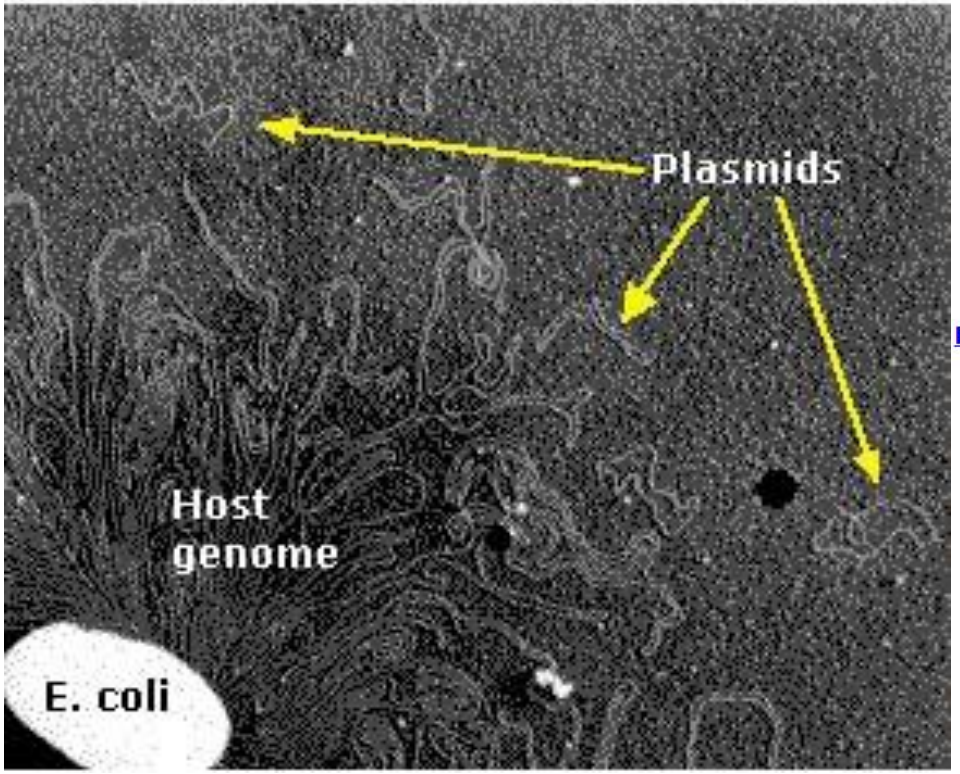
| **Основание** | **Рибонуклеотид** | **Рибонуклеозид** |
|---|---|---|
| Аденин (A) | Аденилат (AMP) | Аденозин |
| Цитозин (C) | Цитидилат (CMP) | Цитидин |
| Гуанин (G) | Гуанилит (GMP) | Гуанозин |
| Урацил (U) | Уридилат(иMP) | Уридин |
| **Основание** | **Дезоксирибо-нуклеотид** | **Дезоксирибо-нуклеозид** |
| Аденин (A) | Дезоксиаденилат (dAMP) | Дезоксиаденозин |
| Цитозин (C) | Дезоксицитидилат (dCMP) | Дезоксицитидин |
| Гуанин (G) | Дезоксигуанилат (dGMP) | Дезоксигуанозин |
| Тимин (T) | Дезокситимидилат **(dTMP)** | Дезокситимидин |

ACCT
pApCpG (положение фосфатной группы)
dAdCdG (дезоксирибоза)
rArCrG (рибоза)

# Прокариотическая клетка

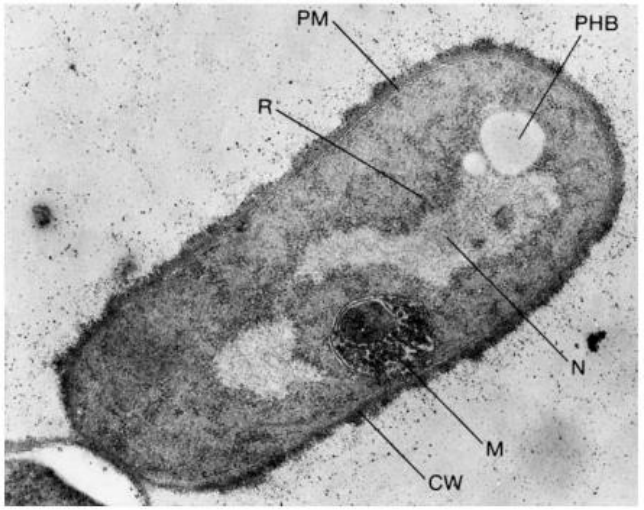
Нуклеоид – компартмент, содержащий кольцевую ДНК бактерии (геном бактерии), связанную с белками и РНК

Плазмида – внехромосомные автономно-реплицирующиеся молекулы дцДНК




Electron microscopic image of a typical rod-shaped bacterial cell






PM = plasma membrane
CW = cell wall
M = mesosome
R = ribosome
N = nucleoid
PHB = inclusion body

# Эукариотическая клетка

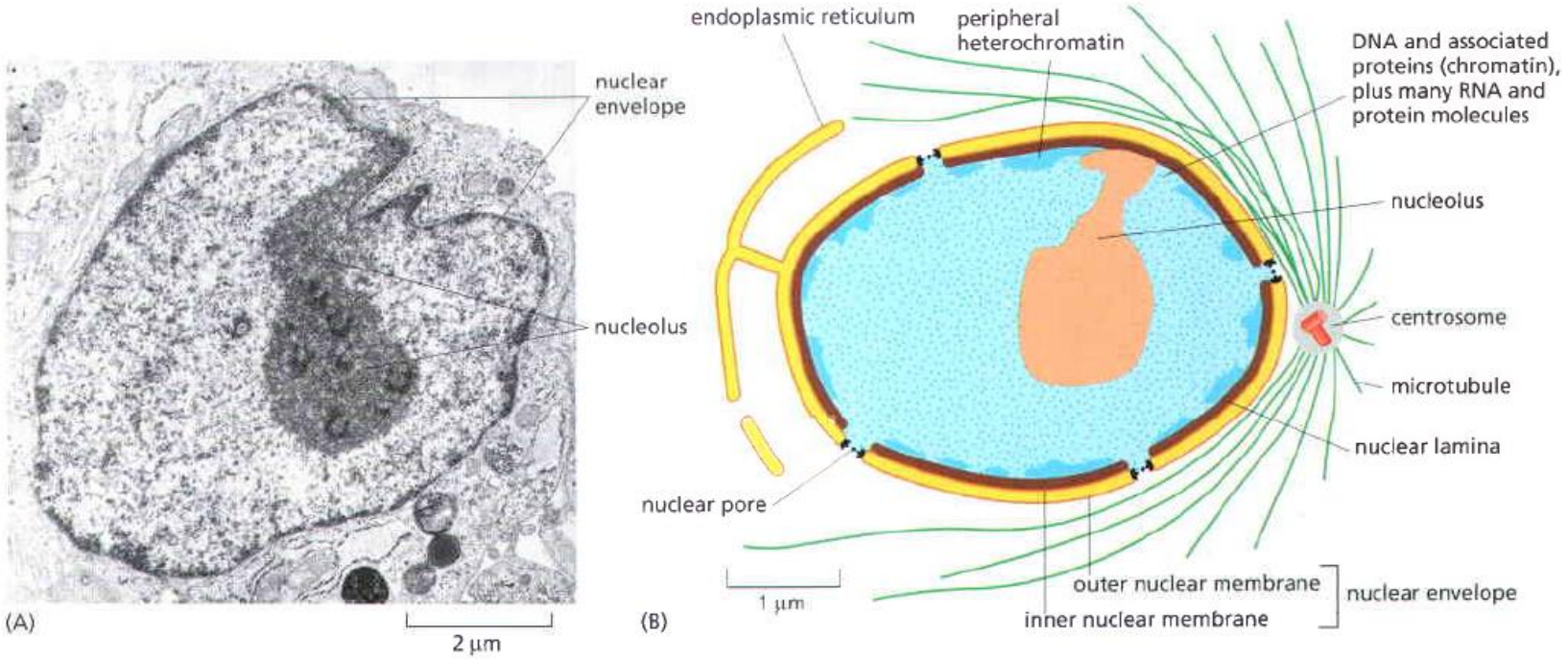
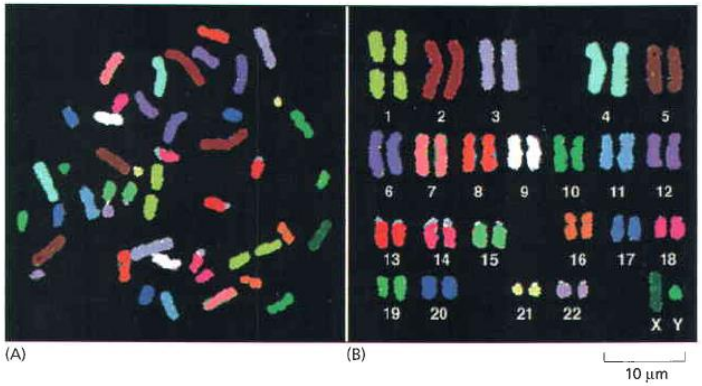


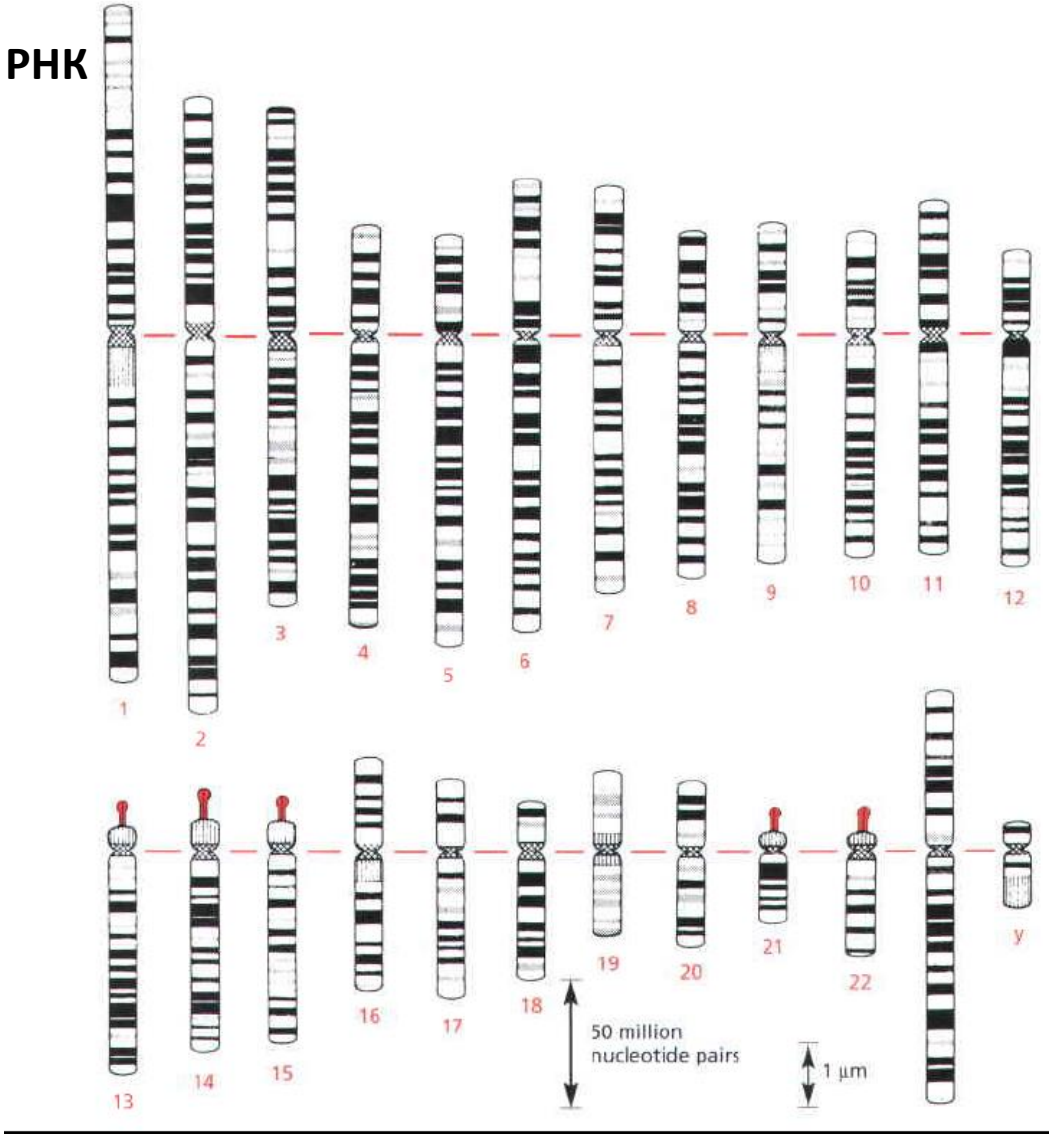

**Figure 4–9 A cross-sectional view of a typical cell nucleus.** (A) Electron micrograph of a thin section through the nucleus of a human fibroblast. (B) Schematic drawing, showing that the nuclear envelope consists of two membranes, the outer one being continuous with the endoplasmic reticulum membrane (see also Figure 12–8). The space inside the endoplasmic reticulum (the ER lumen) is colored *yellow;* it is continuous with the space between the two nuclear membranes. The lipid bilayers of the inner and outer nuclear membranes are connected at each nuclear pore. A sheet-like network of intermediate filaments *(brown)* inside the nucleus provides mechanical support for the nuclear envelope, forming a special supporting structure called the nuclear lamina (for details, see Chapter 12). The heterochromatin near the lamina contains specially condensed regions of DNA that will be discussed later.

**ДНК сосредоточена в ядре**

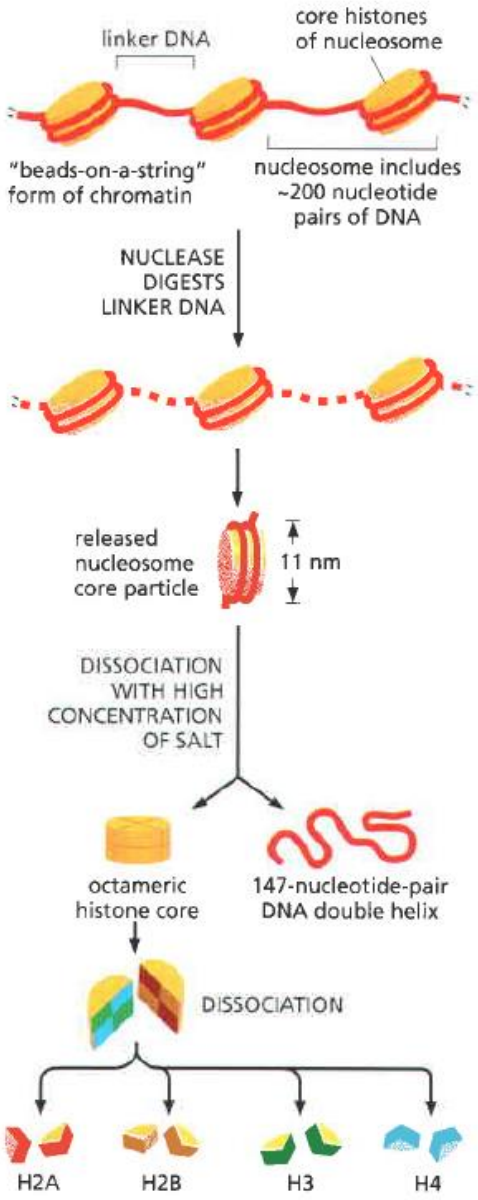
# Хромосомы

**ДНК эукариот в комплексе с белками и РНК – хроматин**



# Гистоны. Структурная организация нуклеосом



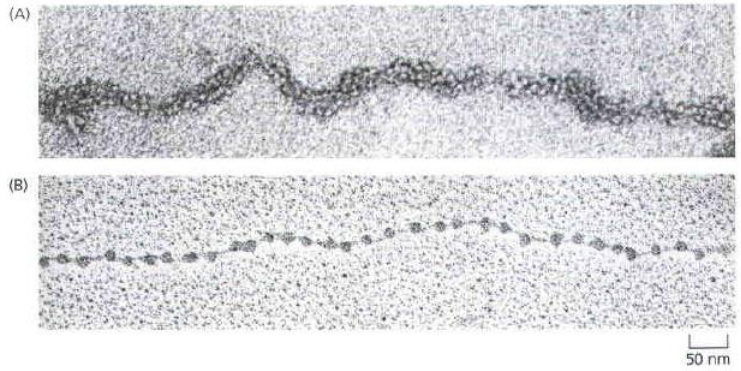




**Figure 4–22 Nucleosomes as seen in the electron microscope.** (A) Chromatin isolated directly from an interphase nucleus appears in the electron microscope as a thread 30 nm thick. (B) This electron micrograph shows a length of chromatin that has been experimentally unpacked, or decondensed, after isolation to show the nucleosomes. (A, courtesy of Barbara Hamkalo; B, courtesy of Victoria Foe.)

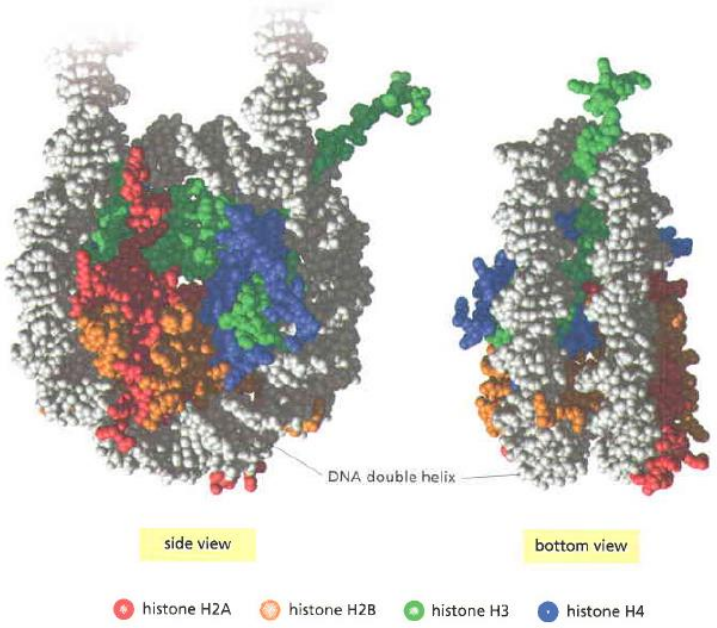
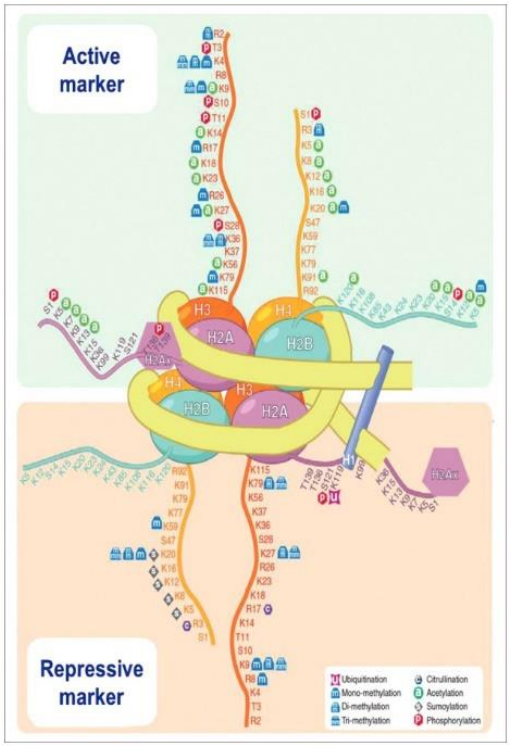



**Figure 4–24 The structure of a nucleosome core particle, as determined by x-ray diffraction analyses of crystals.** Each histone is colored according to the scheme in Figure 4–23, with the DNA double helix in *light gray*. (From K. Luger et al., *Nature* 389:251–260, 1997. With permission from Macmillan Publishers Ltd.)

# Гетеро- и эухроматин




Heterochromatin

***Silencing***

(DNA-methylation,
sRNAs, histone variants)

Euchromatin

**Histone modifications**

***Transcriptional regulation***

(transcription factors,
enhancers, repressors,
chromatin remodeling complexes)

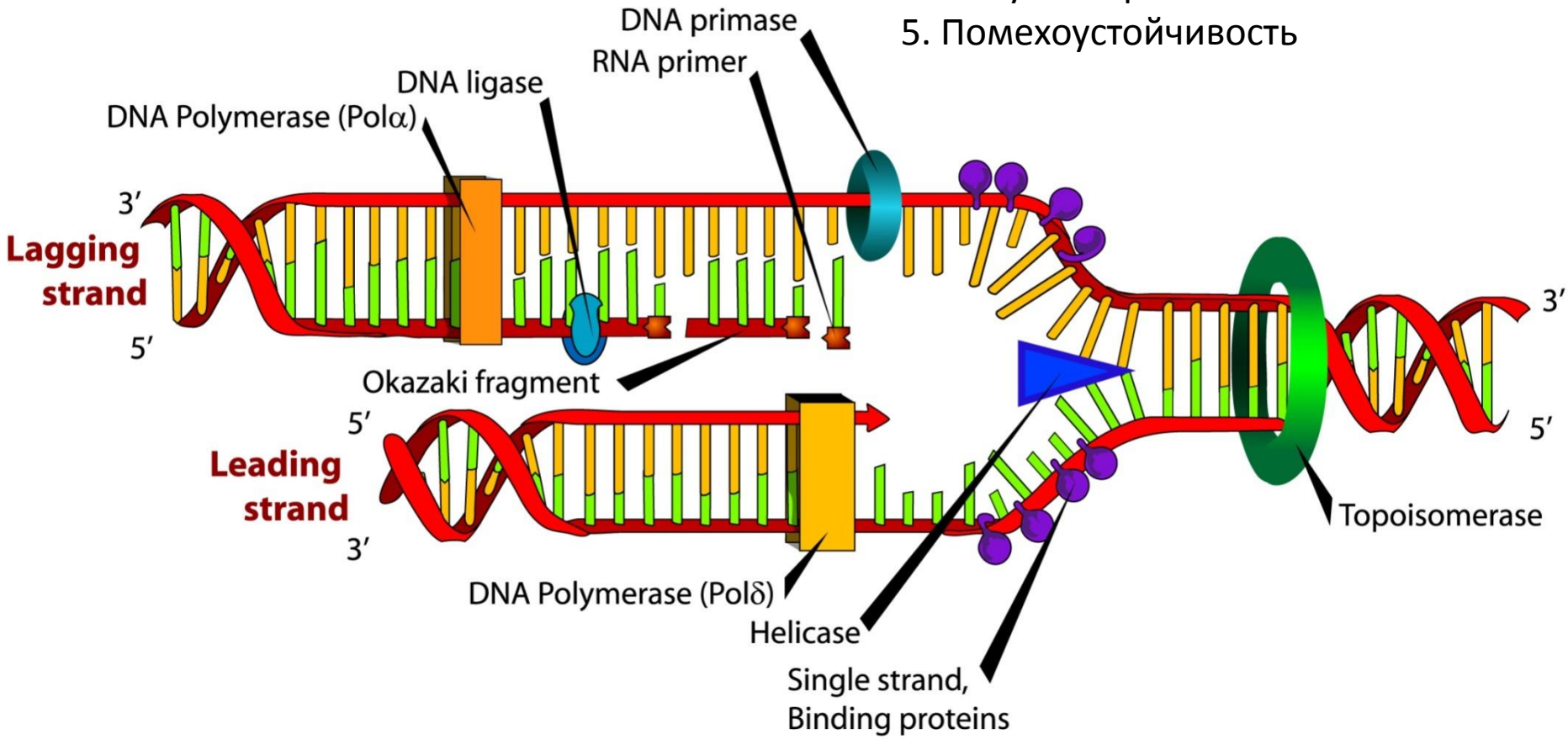
Induced

Repressed

***Abiotic Stress***

# Репликация

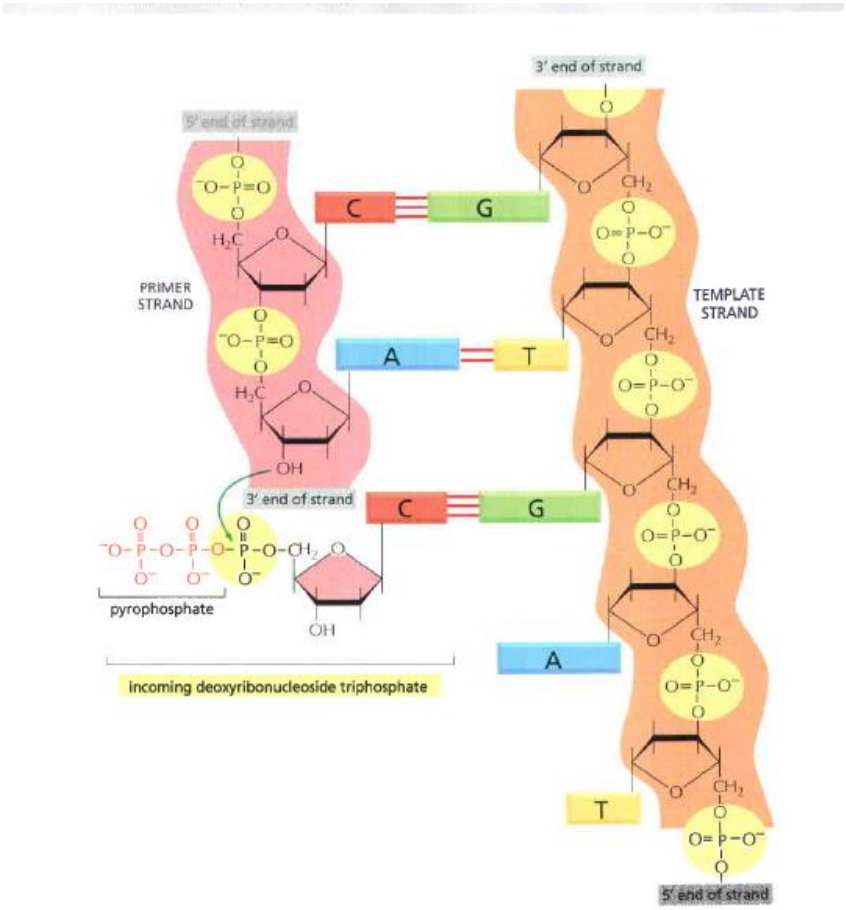
1. Комплементарность.
2. Двунаправленность
3. Асимметричность вилки репликации
4. Полуконсервативность
5. Помехоустойчивость




https://www.youtube.com/watch?v=dKubyIRiN84

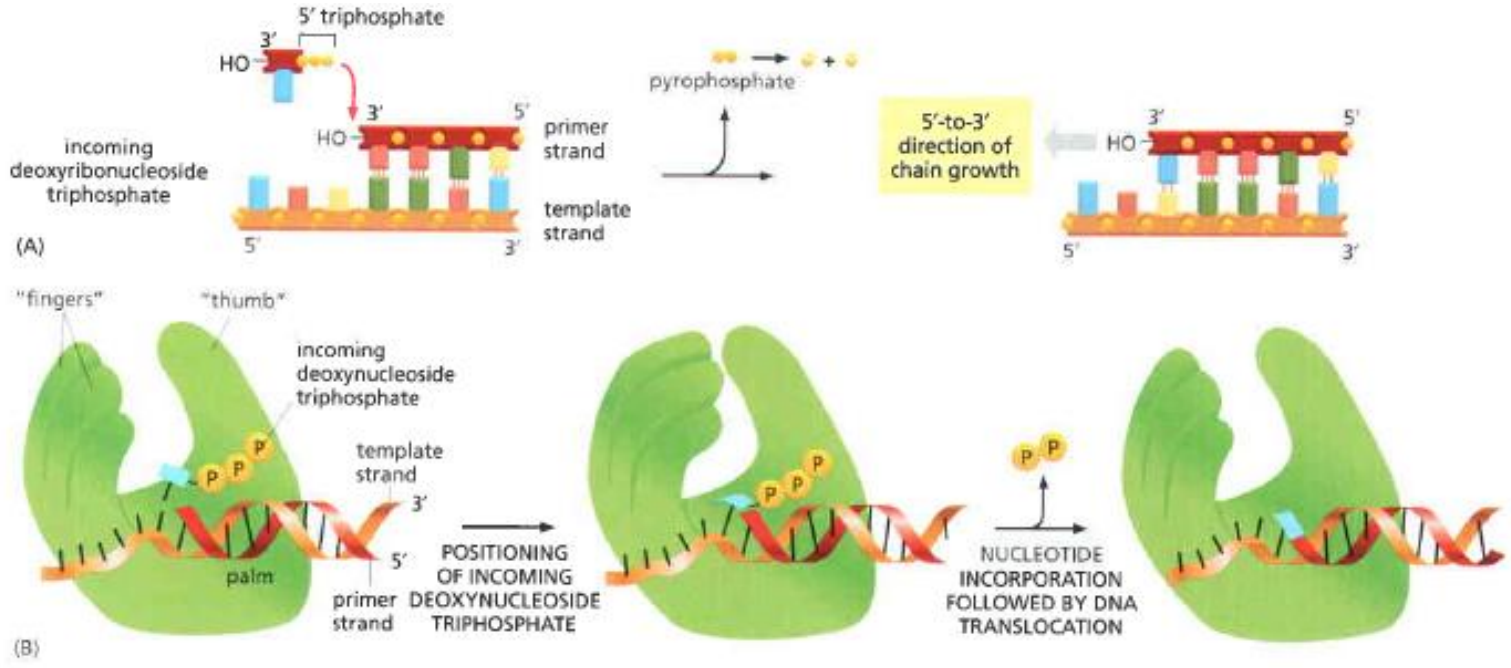
# Репликация

Полуконсервативность
Комплементарность



# ДНК-полимераза

Требуется праймер-затравка



# Почему 5’→3’?

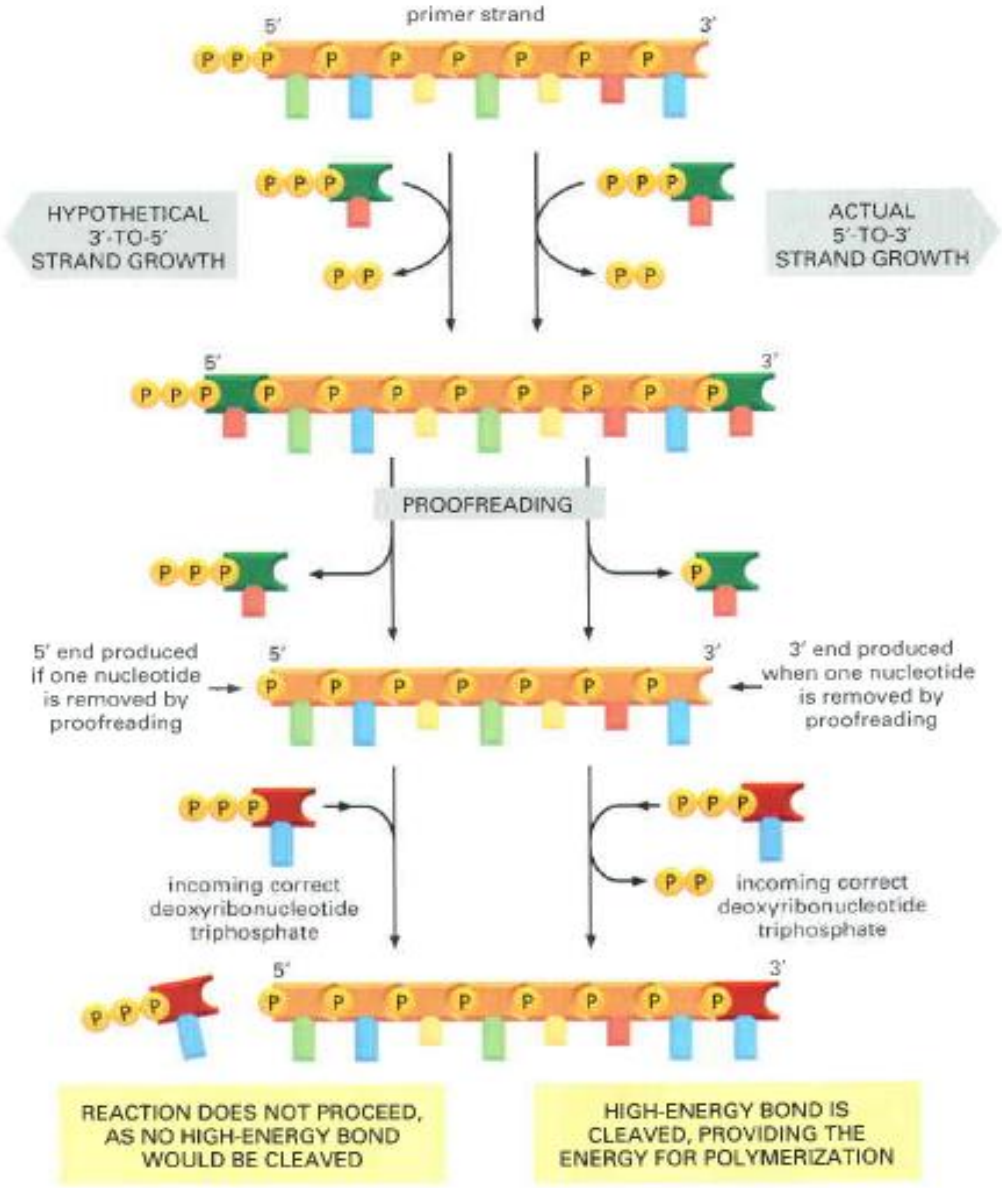



**Figure 5–10 An explanation for the 5′-to-3′ direction of DNA chain growth.** Growth in the 5′-to-3′ direction, shown on the right, allows the chain to continue to be elongated when a mistake in polymerization has been removed by exonucleolytic proofreading (see Figure 5–8). In contrast, exonucleolytic proofreading in the hypothetical 3′-to-5′ polymerization scheme, shown on the left, would block further chain elongation. For convenience, only the primer strand of the DNA double helix is shown.

# Помехоустойчивость

**Table 5–1 The Three Steps That Give Rise to High-Fidelity DNA Synthesis**

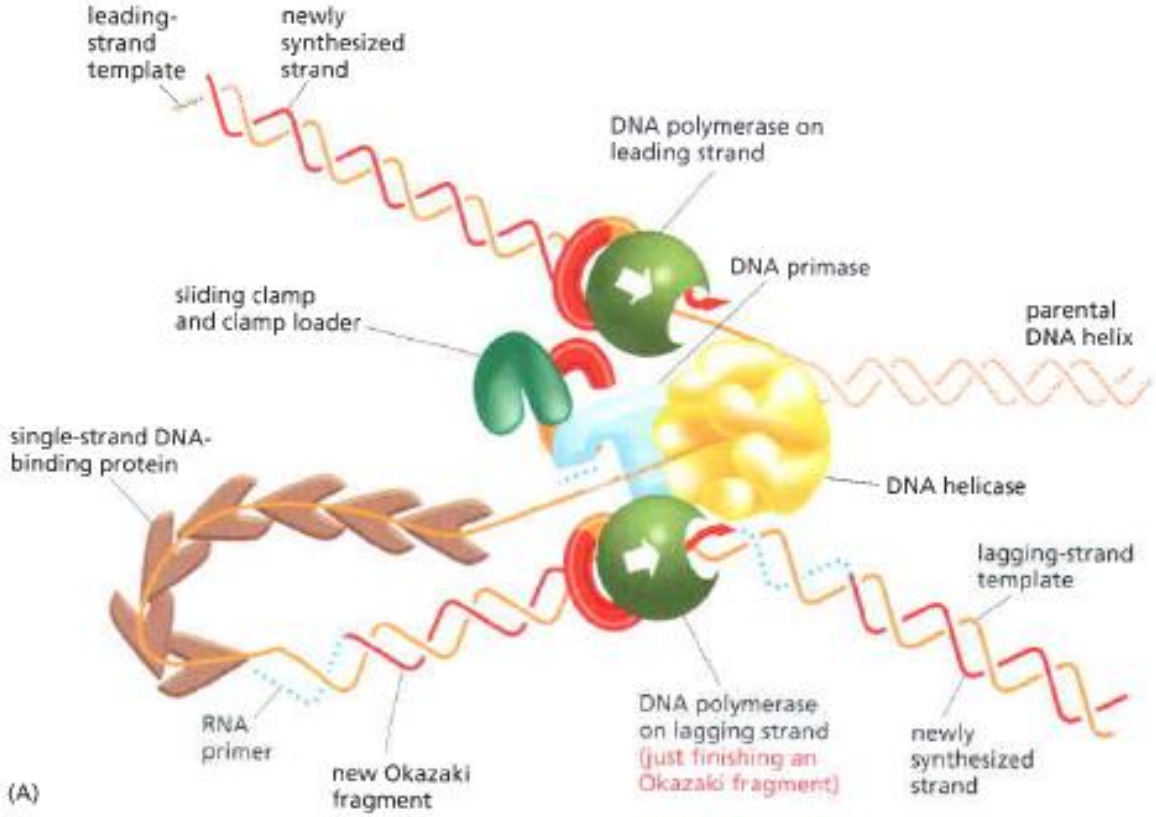
| REPLICATION STEP | ERRORS PER NUCLEOTIDE |
|---|---|
| 5′ → 3′ polymerization | 1 in $10^5$ |
| 3′ → 5′ exonucleolytic proofreading | 1 in $10^2$ |
| Strand-directed mismatch repair | 1 in $10^2$ |
| Combined | 1 in $10^9$ |

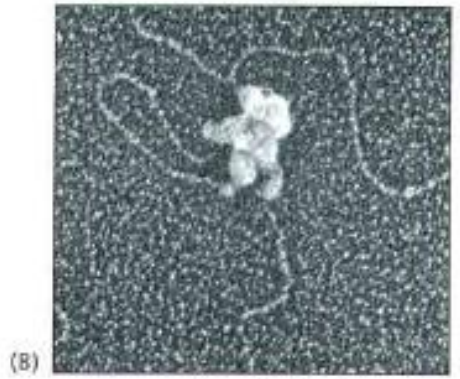
The third step, strand-directed mismatch repair, is described later in this chapter.

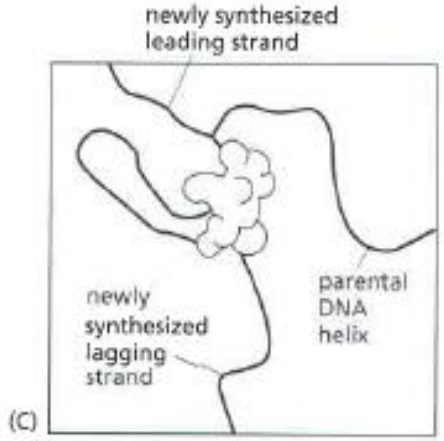
# Вилка репликации

Двунаправленность

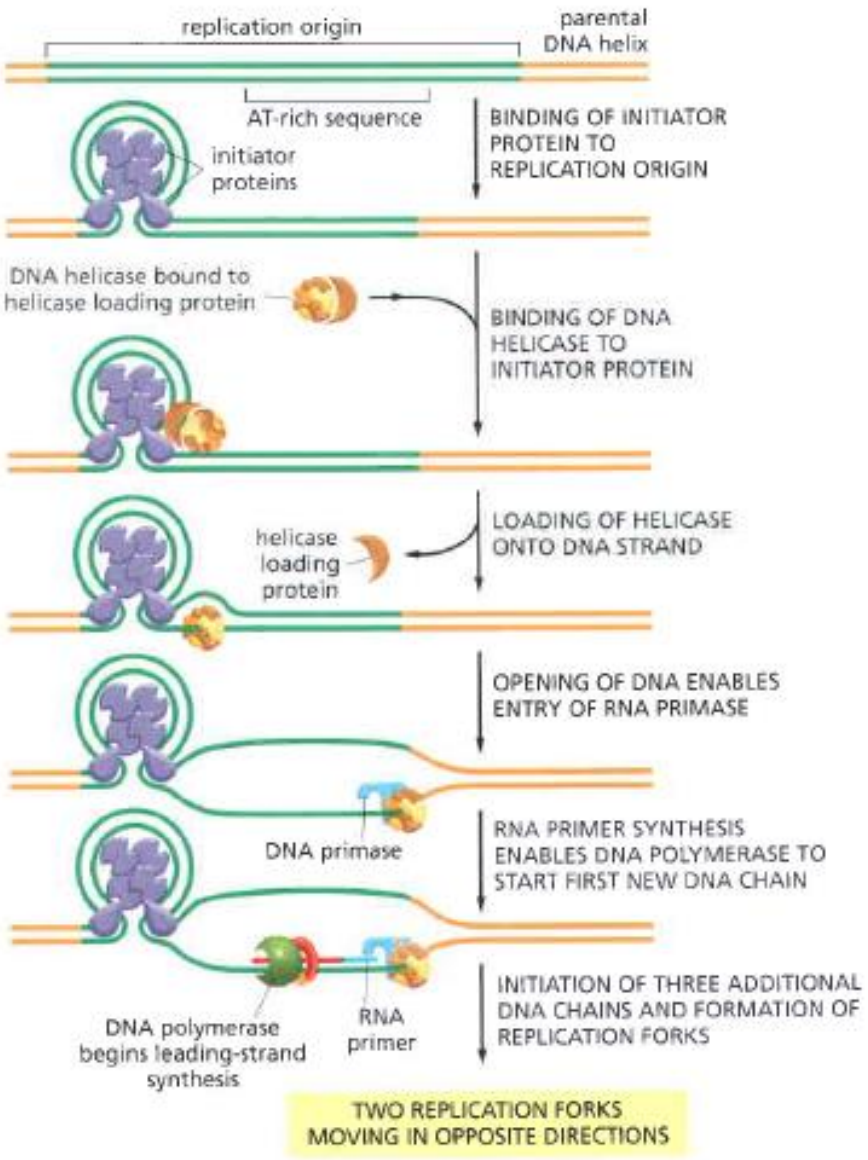
Асимметричность вилки репликации

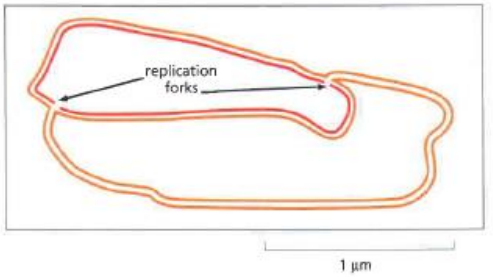


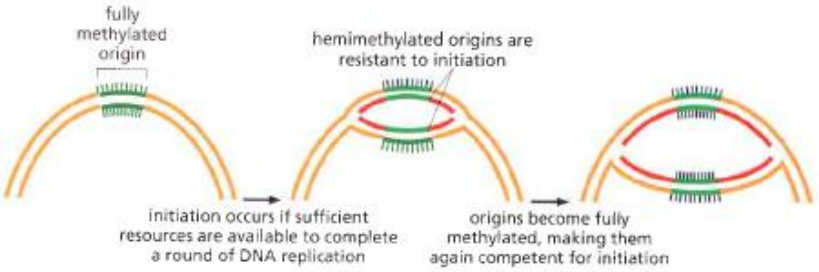



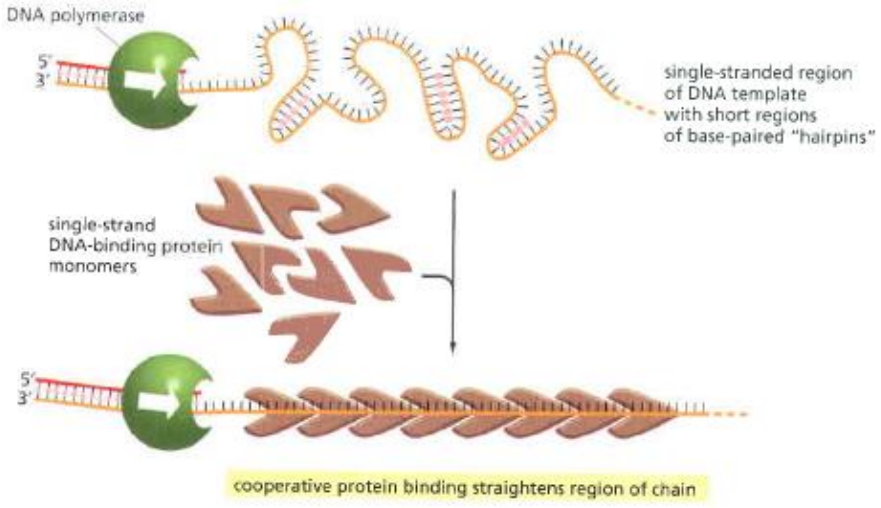
# Репликация у прокариот

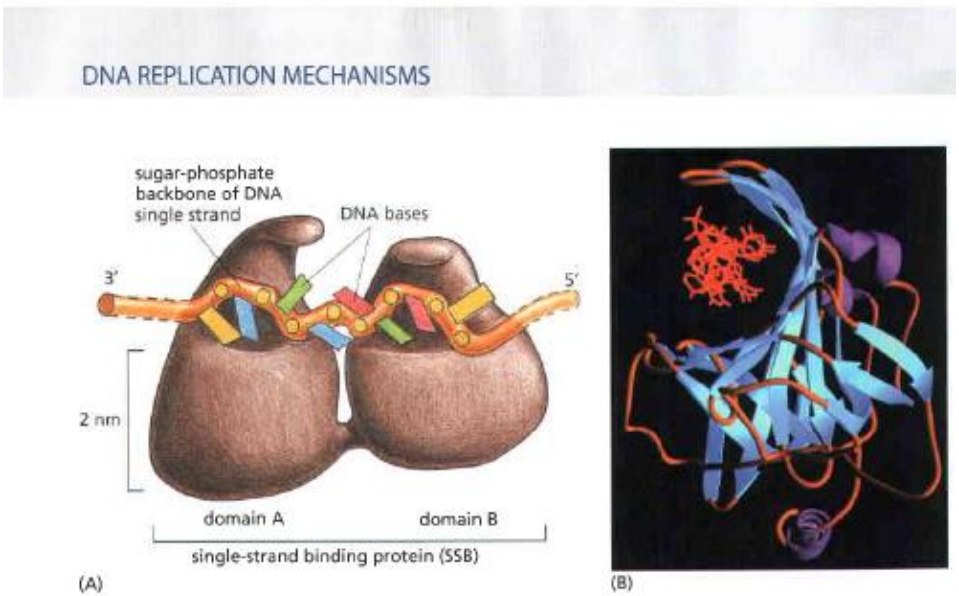


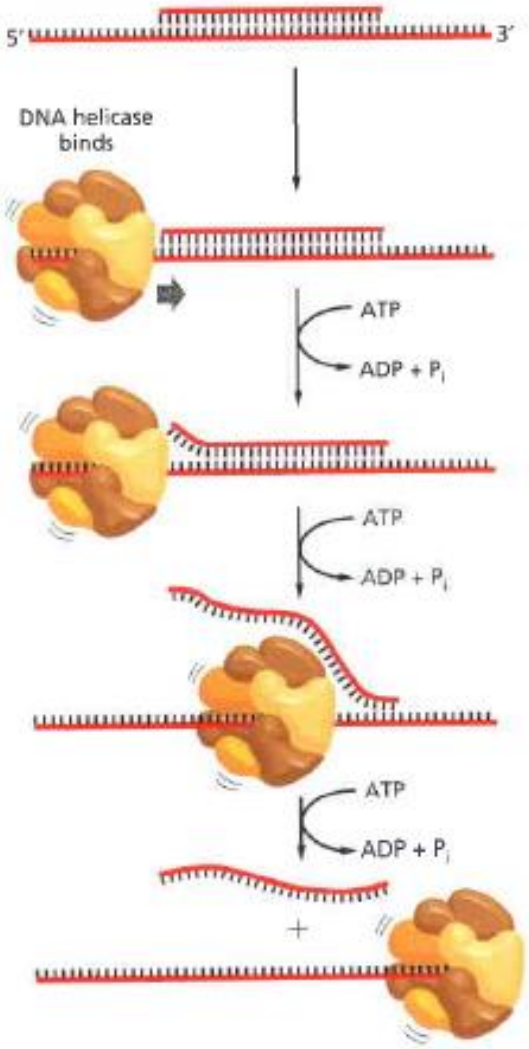



# SSB-белки и хеликаза



# Топоизомеразы

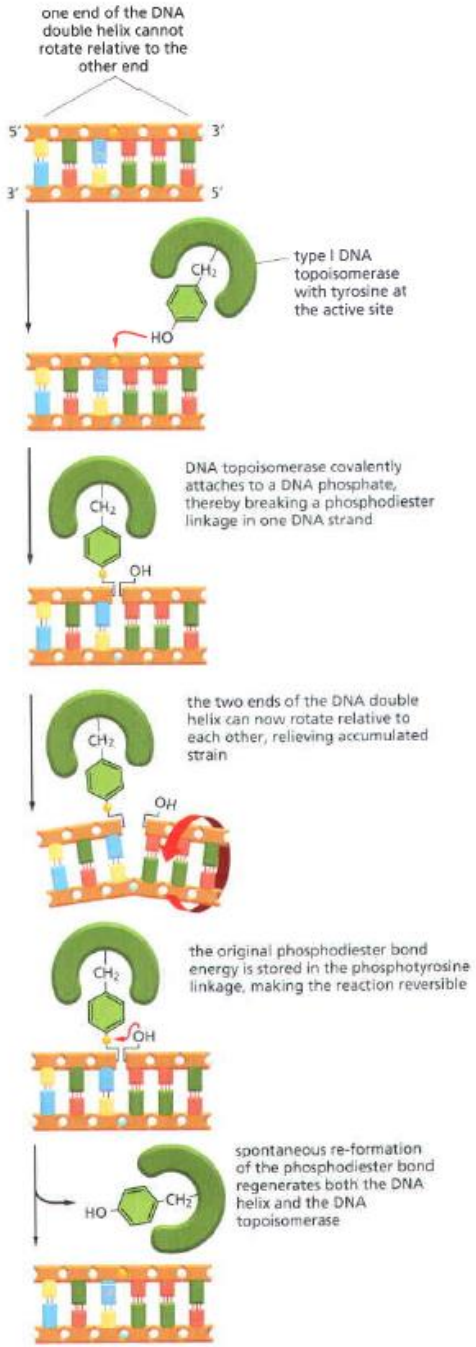



Figure 5–22 **The reversible DNA nicking reaction catalyzed by a eucaryotic DNA topoisomerase I enzyme.** As indicated, these enzymes transiently form a single covalent bond with DNA; this allows free rotation of the DNA around the covalent backbone bonds linked to the *blue* phosphate.

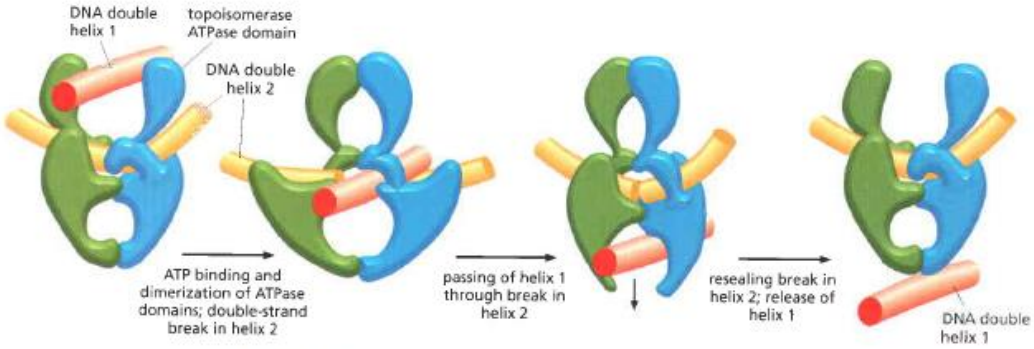



Figure 5–23 **A model for topoisomerase II action.** As indicated, ATP binding to the two ATPase domains causes them to dimerize and drives the reactions shown. Because a single cycle of this reaction can occur in the presence of a non-hydrolyzable ATP analog, ATP hydrolysis is thought to be needed only to reset the enzyme for each new reaction cycle. This model is based on the structure of enzyme in combination with biochemical experiments. (Modified from J.M. Berger, *Curr. Opin. Struct. Biol.* 8:26–32, 1998. With permission from Elsevier.)

# Репликация у эукариот

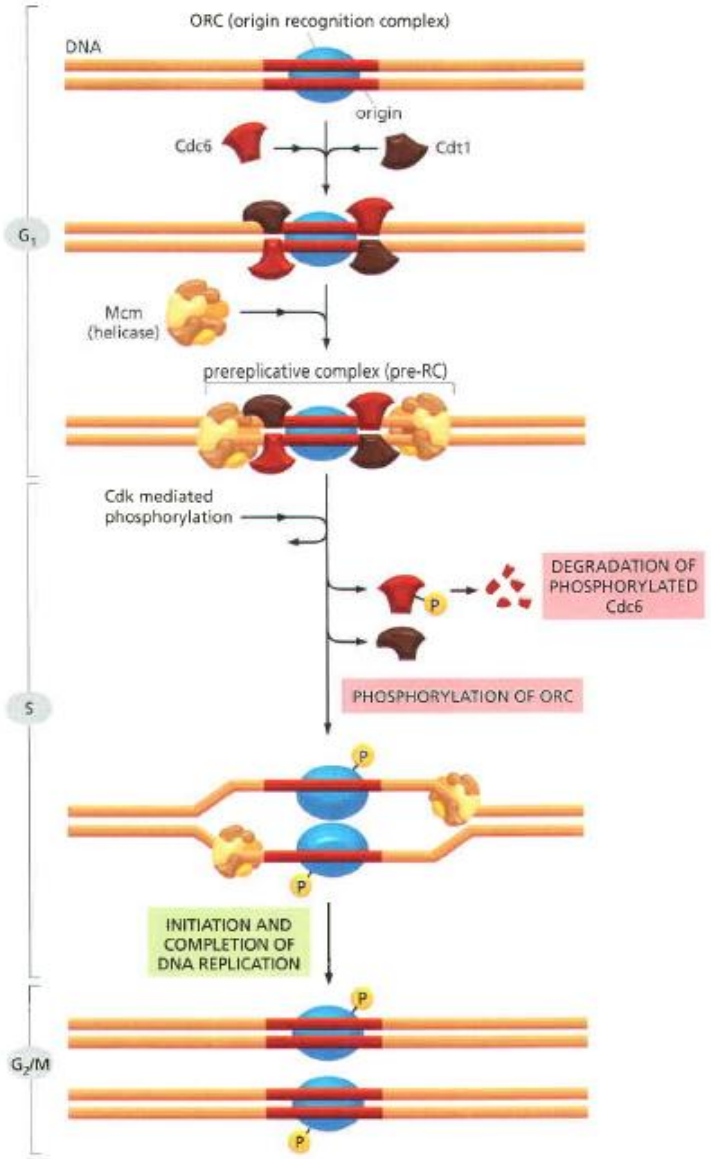
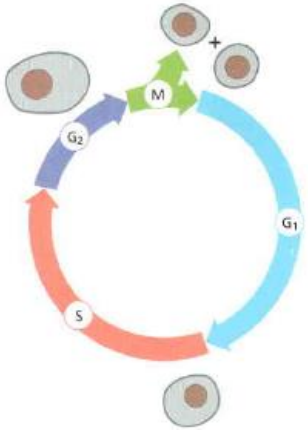
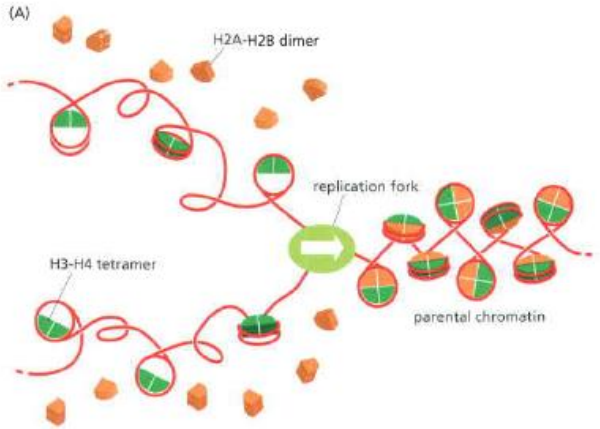
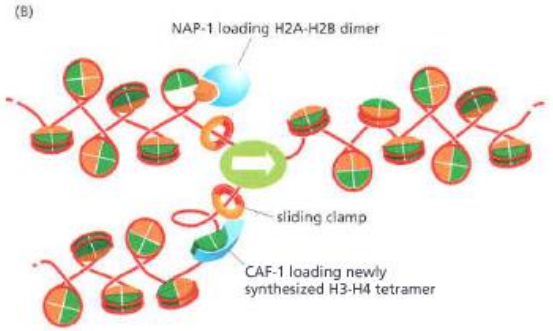


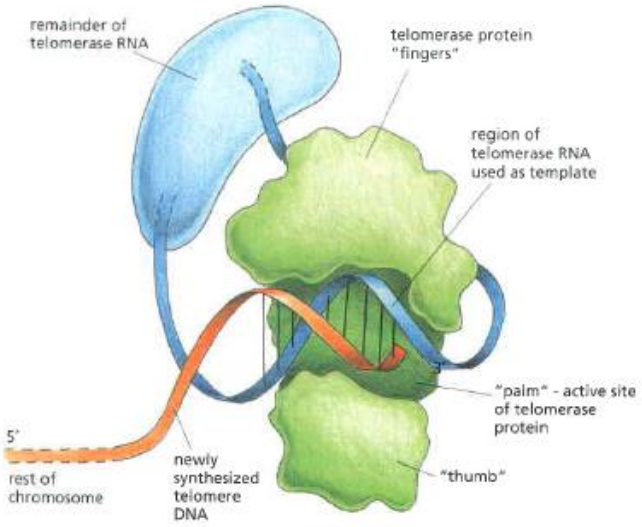

**Figure 5–36 The mechanism of DNA replication initiation in eucaryotes.** This mechanism ensures that each origin of replication is activated only once per cell cycle. An origin of replication can be used only if a prereplicative complex forms there in $G_1$ phase. At the beginning of S phase, cyclin-dependent kinases (Cdks) phosphorylate various replication proteins, causing both disassembly of the prereplicative complex and initiation of DNA replication. A new prereplicative complex cannot form at the origin until the cell progresses to the next $G_1$ phase.

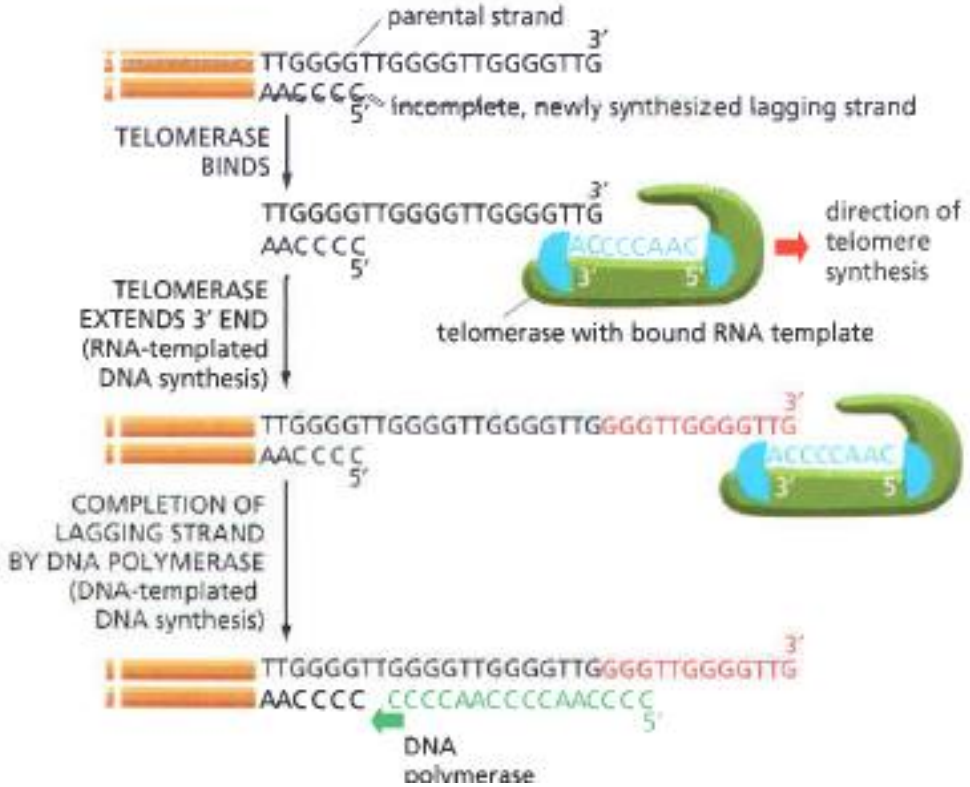


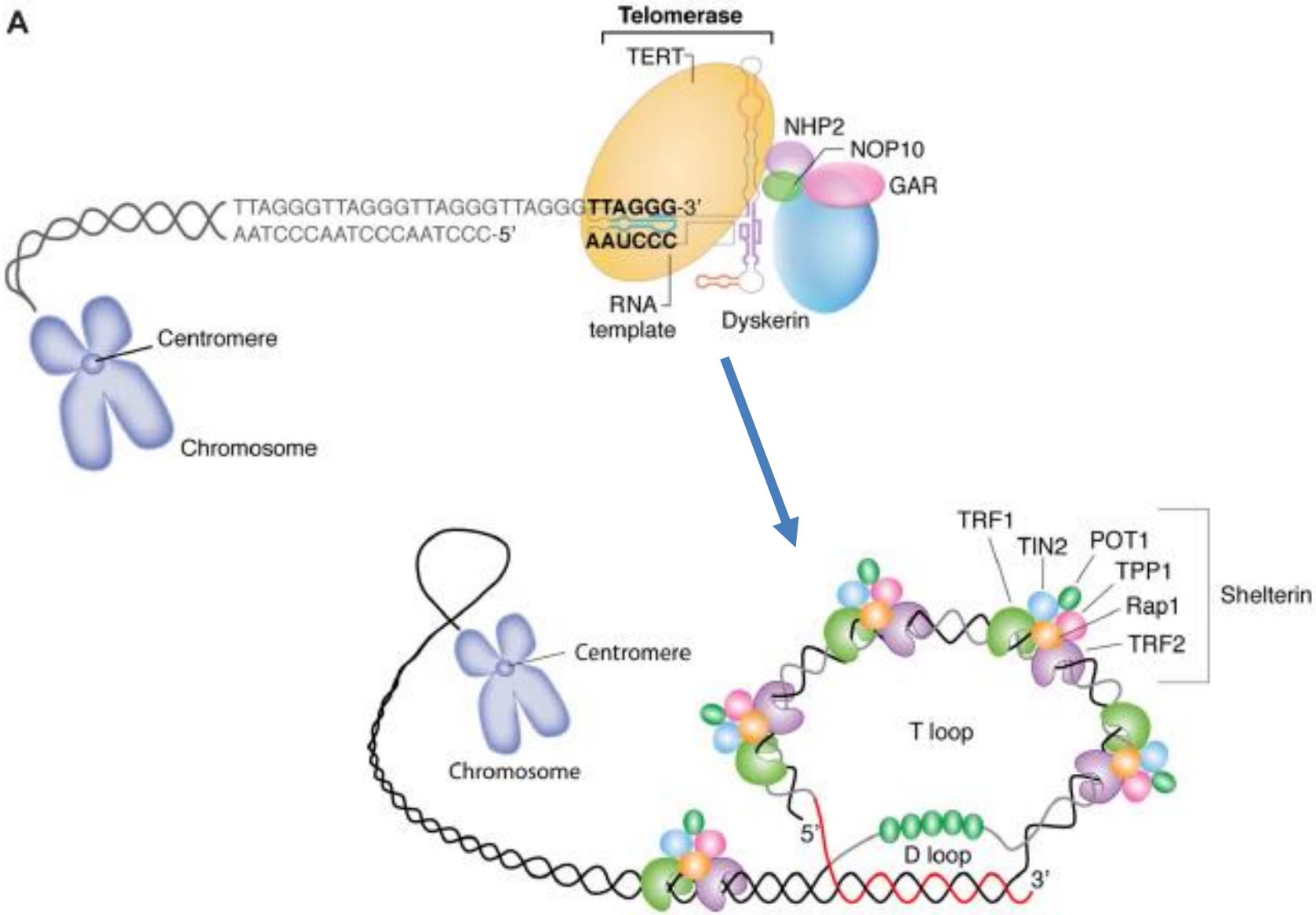



# Теломераза


remainder of telomerase RNA
telomerase protein "fingers"
region of telomerase RNA used as template
"palm" - active site of telomerase protein
"thumb"
5'
rest of chromosome
newly synthesized telomere DNA


parental strand
3'
TTGGGGTTGGGGTTGGGGTTG
AACCCC
5'
incomplete, newly synthesized lagging strand
TELOMERASE BINDS
TTGGGGTTGGGGTTGGGGTTG
AACCCC
direction of telomere synthesis
telomerase with bound RNA template
TELOMERASE EXTENDS 3' END (RNA-templated DNA synthesis)
TTGGGGTTGGGGTTGGGGTTGGGGTTGGGGTTG
COMPLETION OF LAGGING STRAND BY DNA POLYMERASE (DNA-templated DNA synthesis)
DNA polymerase

# Теломеры


A
Telomerase
TERT
NHP2
NOP10
GAR
TTAGGGTTAGGGTTAGGGTTAGGGTTAGGG-3'
AATCCCAATCCCAATCCC-5'
AAUCCC
RNA template
Dyskerin
Centromere
Chromosome
TRF1
TIN2
POT1
TPP1
Rap1
TRF2
Shelterin
Centromere
Chromosome
T loop
5'
D loop
3'

## Стандартные обозначения полиморфных позиций:

| | | |
|---|---|---|
| **Символ** | Обозначает | Объяснение |
| **R** | A или G | puRine |
| **Y** | C или T | pYrimidine |
| **M** | A или C | aMino |
| **K** | G или T | Keto |
| **S** | C или G | сильное /Strong/ взаимодействие - три водородные связи |
| **W** | A или T | слабое /Weak/ взаимодействие - две водородные связи |
| **H** | (A, C, T) но не G | H следует за G в алфавите |
| **B** | (C, G, T) но не A | B следует за A в алфавите |
| **V** | (A, C, G) но не T(U) | V следует за T(U) в алфавите |
| **D** | (A, G, T) но не C | D следует за C в алфавите |
| **N** | (A, G, C, T) | любое основание / Nucleotide |

http://molbiol.ru/scripts/01_12.html

# Genebank

## Задание для самостоятельной работы

Пользуясь сервисом Genebank для выбранного белка
http://www.ncbi.nlm.nih.gov/ → PROTEIN

Определить
http://www.ncbi.nlm.nih.gov/ →GENE

Название гена:
Организм хозяина:
Локализация гена (Location): номер хромосомы
Кодируемый белок (General protein information): название белка
Последовательность мРНК (mRNA and Protein(s)→ NM.XXXXXXXX→ FASTA): указать длину мРНК кодирующей данный белок