

- **дезаминирование оснований**, при котором цитозин превращается в урацил, аденин — в гипоксантин, а гуанин — в ксантин. Чаще всего дезаминируется цитозин;
- **депуринизация**, или **депиримидинизация**, результатом которой является появление в ДНК остатков дезоксирибозы, лишенных основания;
- образование под действием ультрафиолета (УФО) **пиримидиновых димеров** между рядом расположенными в цепи основаниями;
- **разрыв нуклеотидных цепей**;
- появление **ковалентных сшивок** между цепями или цепями и гистонами;
- возникновение **ошибок репликации**;
- образование **продуктов алкилирования ДНК** (6-метилгуанина, 7-метилгуанина, 3-метиладенина) под воздействием некоторых химических веществ.

# Репарация ДНК

**Как ДНК сохраняет стабильность?**

**Причины ошибок:**

- Химические агенты
- Излучение
- Ошибки репликации

**Системы репарации в клетке (исправление ошибок в ДНК)**

**Восстановление поврежденной цепи по неповрежденной матрице**

системы  
репарации



Повреждения ДНК приводят к нарушению Уотсон-Криковской структуры, локальной денатурации, блокированию репликации



Контрольные точки  
check-points

## **Сигналы для репарации ДНК:**

Непосредственно повреждение ДНК

События в цитоплазме, например окислительный стресс

**Репарация поврежденной ДНК – часть общей адаптивной реакции клетки на повреждающие воздействия**

# Системы репарации

- Прямая репарация (фотореактивация)
- Эксцизионная репарация

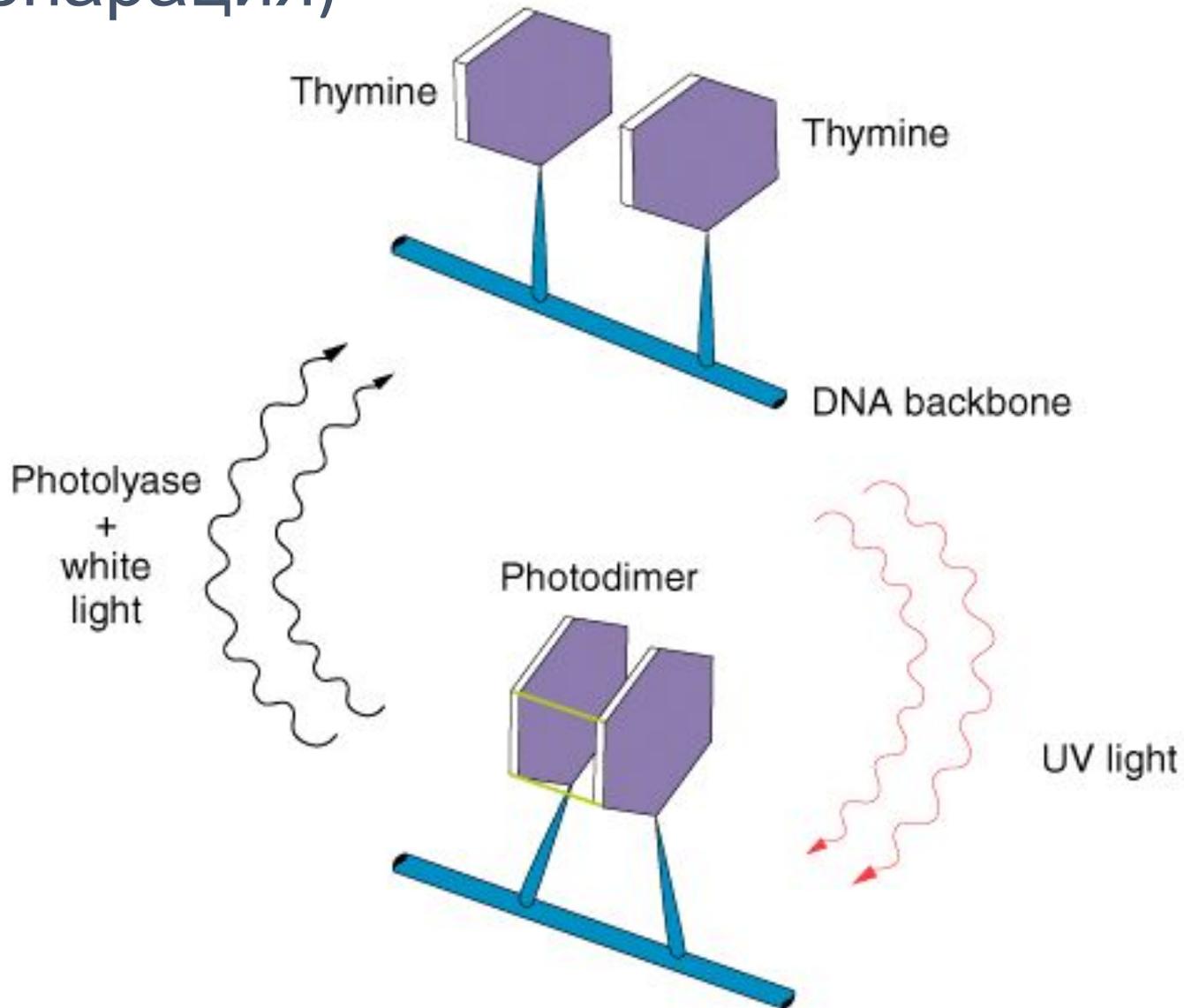
Mismatch repair

Base excision repair (BER)

Nucleotide excision repair (NER)

- Пострепликативная (рекомбинационная) репарация
- SOS-репарация

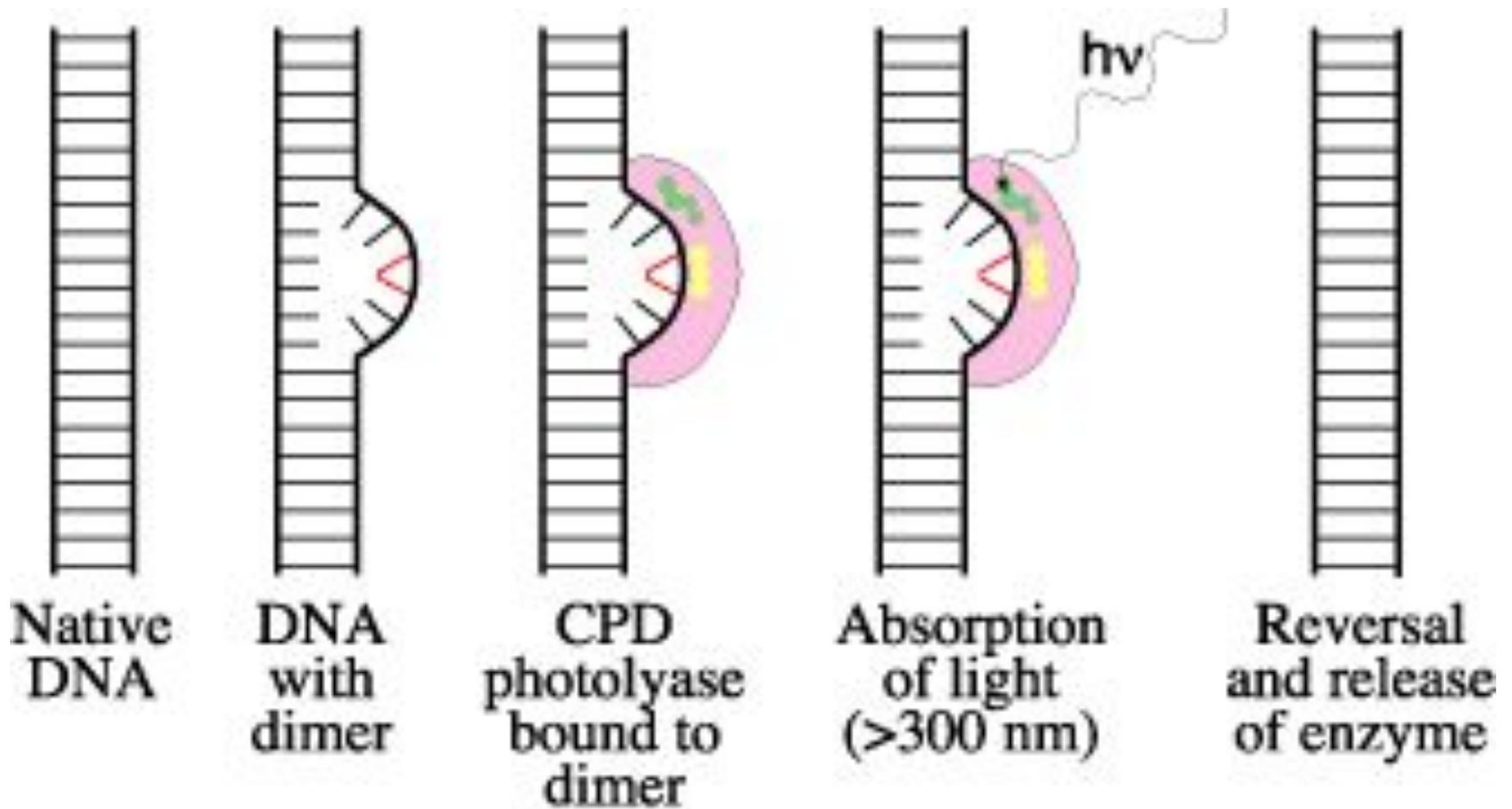
# Фотореактивация (прямая репарация)



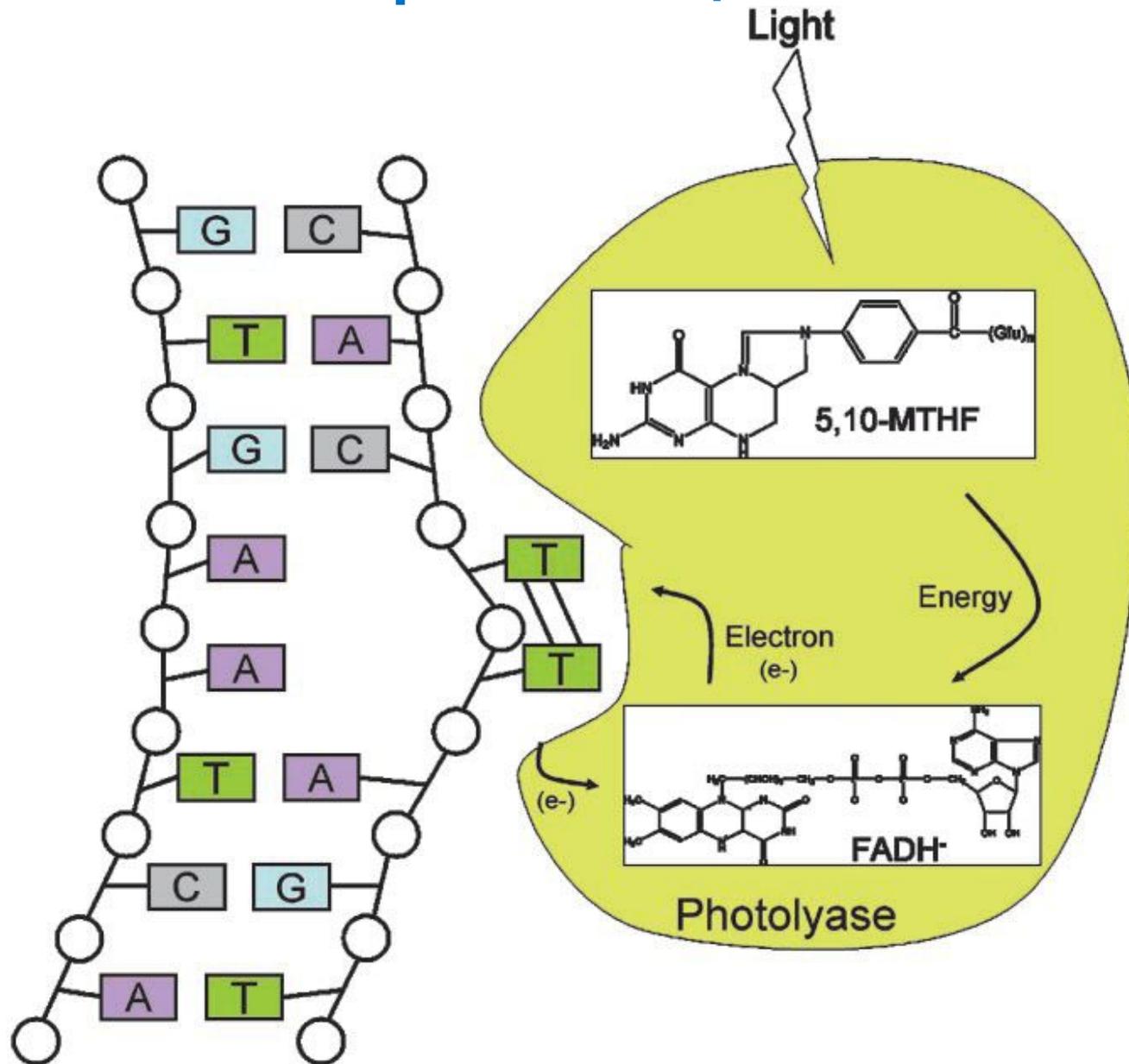
# Фотореактивация (1963г)

- Гены PHR/PRE
- Кодировать фермент фотолиазу, мономерный флавин-зависимый фермент
- Кофакторы : FADH- и 5,10-метенилтетрагидрофолат (5,10-MTHF)
- Связывается в темноте с димерами ТТ
- На свету кофактор поглощает фотон
- Используя эту энергию фотолиаза расщепляет ТТ димер
- Фотолиаза освобождает ДНК

# Фотореактивация



# Фотореактивация



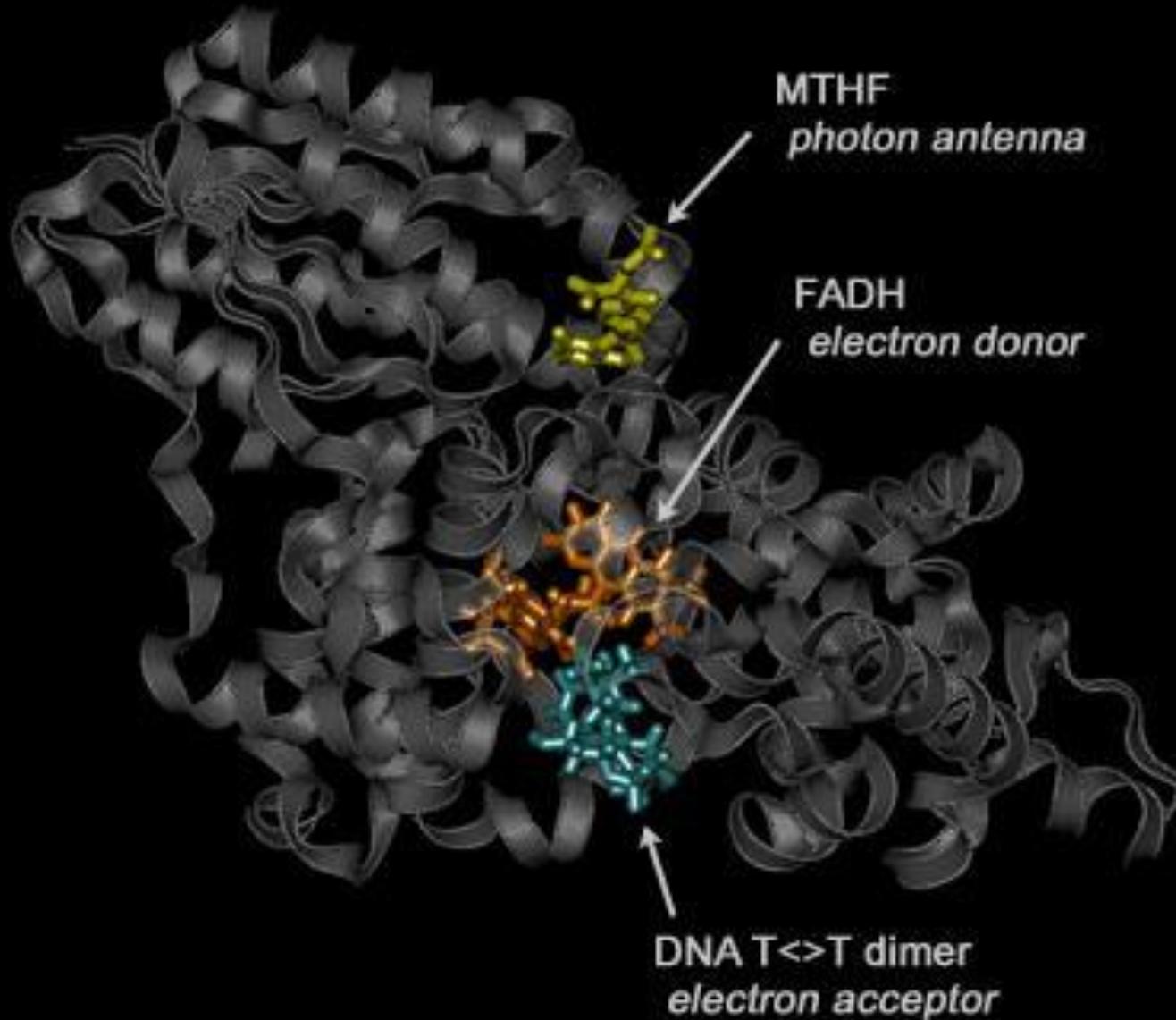
# Фототиазы

- Принадлежат большому семейству фототиаз-криптохромов.
- Представители этого семейства широко распространены во всех царствах
- В соответствии с их функцией:
  - CPD-фототиазы - репарируют CPDs,
  - (6-4)PP- фототиазы – репарируют (6-4) фотопродукты
  - Криптохромы. Не участвуют в репарации ДНК. У растений криптохромы регулируют рост, регулируемый синим светом, а у животных – циркадные ритмы.

# Фотолиазы имеют два типа хромофоров

- FADH (флавинадениндинуклеотид) и МТНФ (метенилтетрагидрофолат) .
- Каталитический кофактор FADH – непосредственно взаимодействует с субстратом –(ТТ димером) в фоторепарирующей реакции.
- Светоуловитель МТНФ– действует как антенна, улавливает энергию и передает ее каталитическому ко-фактору.

# DNA Photolyase from *E. coli*



PLAY

# Системы репарации

- Фотореактивация (прямая репарация)
- Эксцизионная репарация

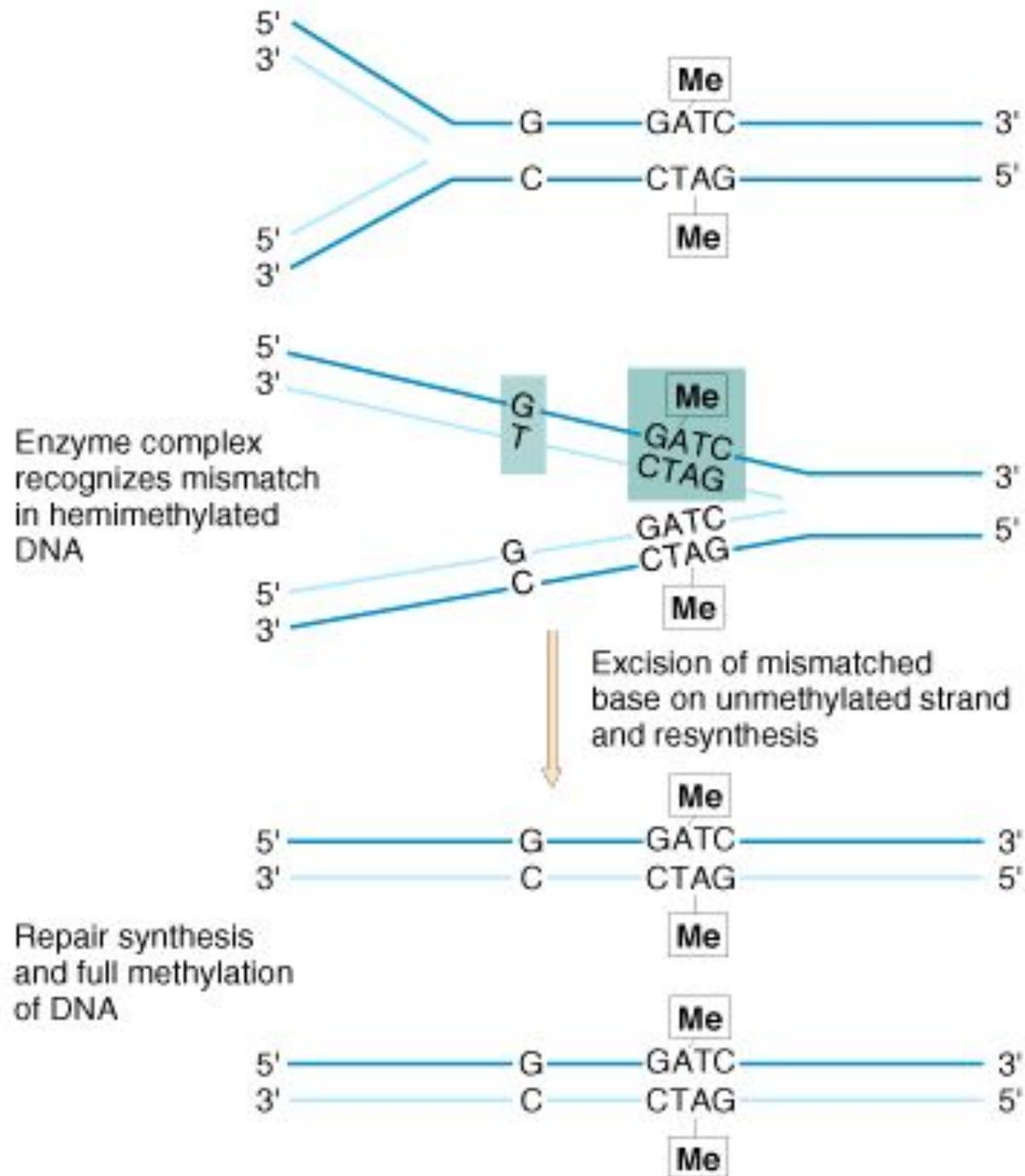
Mismatch repair

Base excision repair (BER)

Nucleotide excision repair (NER)

- Пострепликативная (рекомбинационная) репарация
- SOS-репарация

# Mismatch repair (MMR)

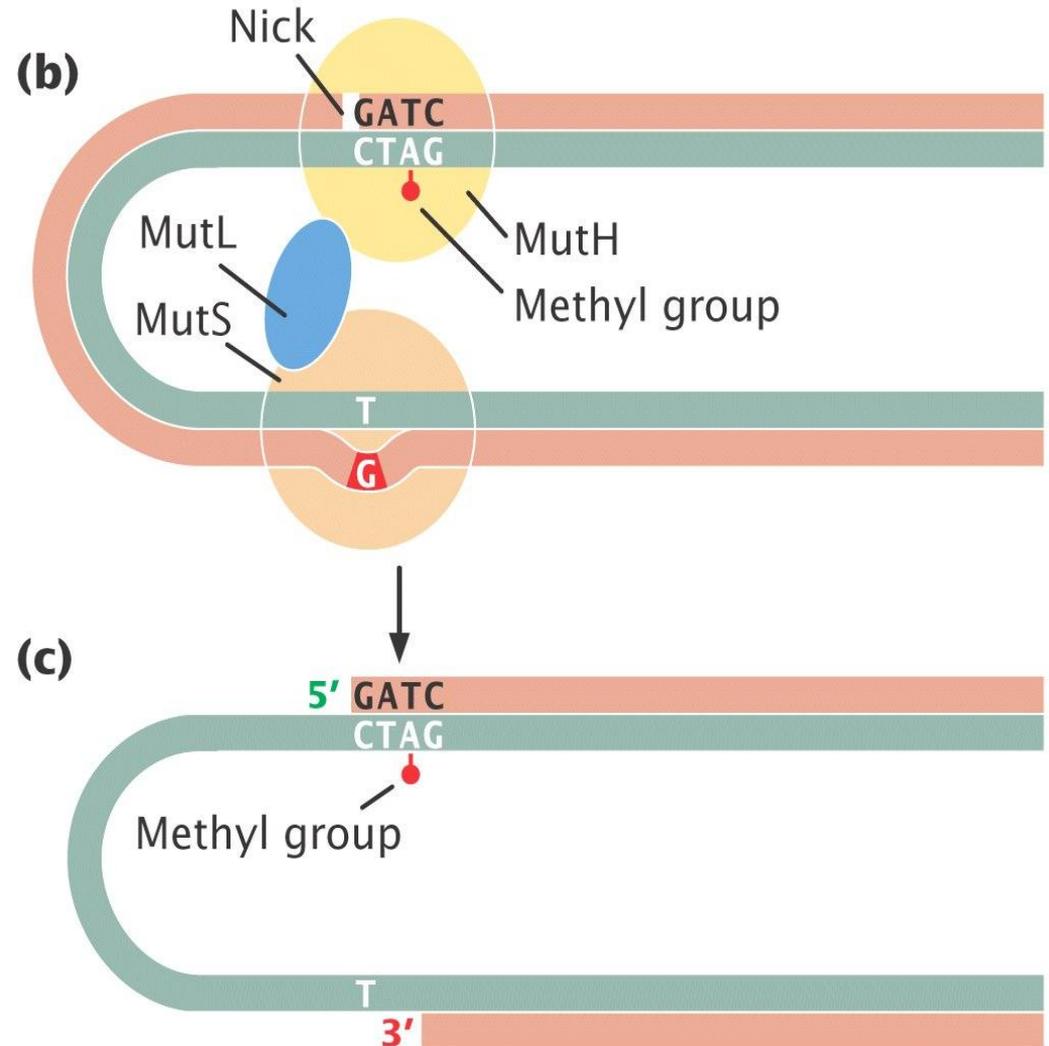


# Пути коррекции ошибочно спаренных оснований

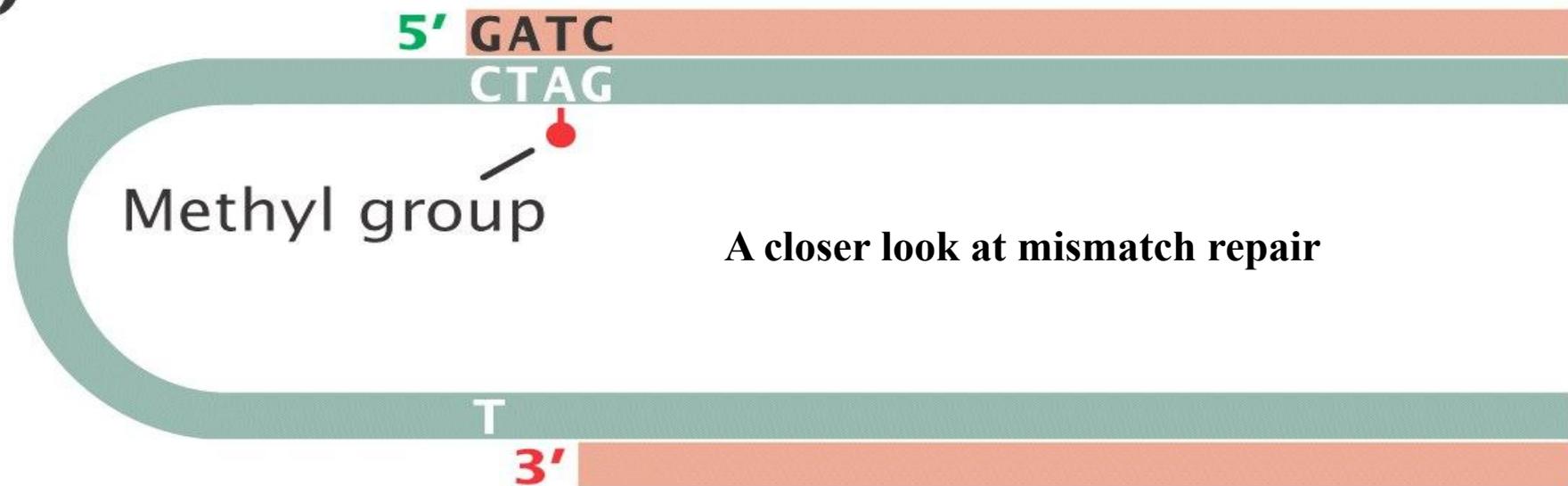
1. Коррекция с помощью 3'-5' экзонуклеазной активности полимераз
2. Мисмэтч репарация: выявляет некомплементарную пару **только** на дочерней цепи ДНК и производит замену неправильного основания **только** на дочерней цепи.

# Основные белки метил-направляемой MMR *E. coli*

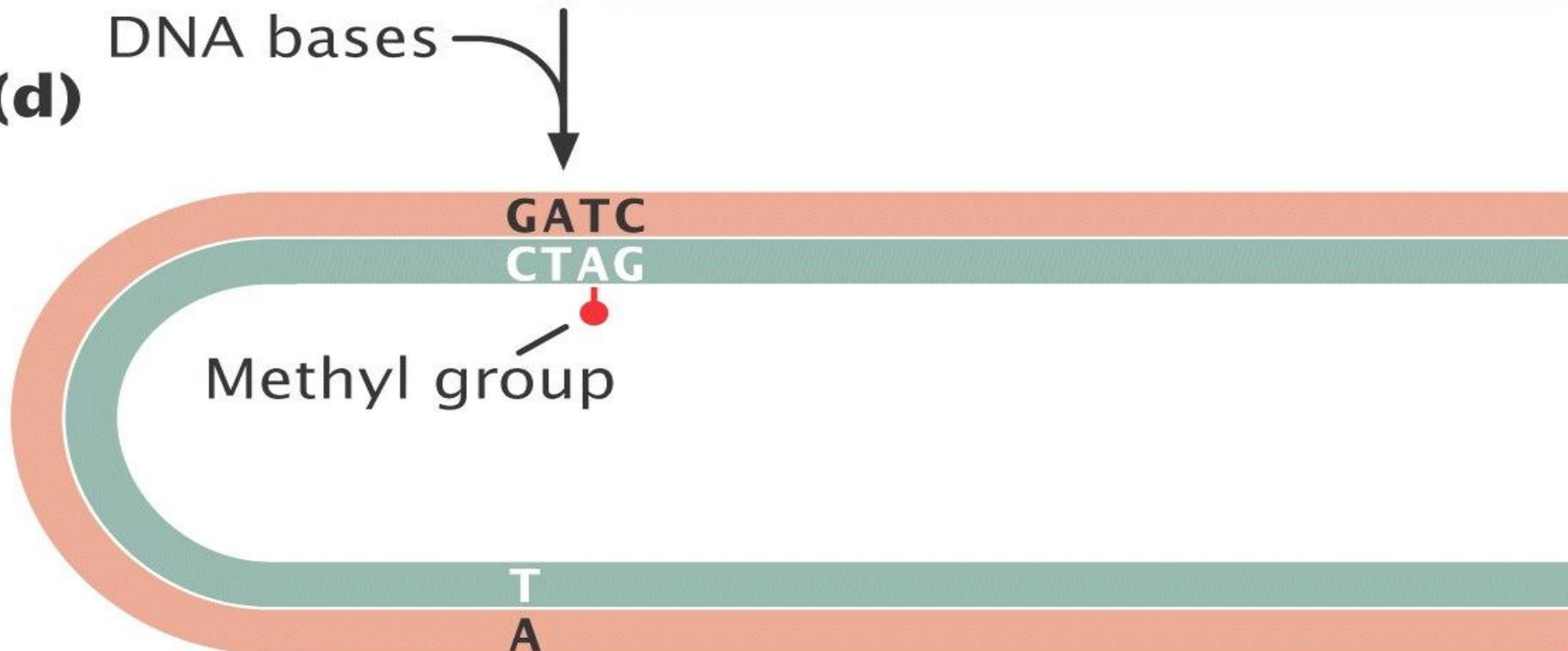
- Mut S и Mut L узнают ММ
- Mut H - узнает полуметилированный сайт GATC и делает надрез
- MutU (UvrD) – геликаза II раскручивает дуплекс и освобождает надрезанную область

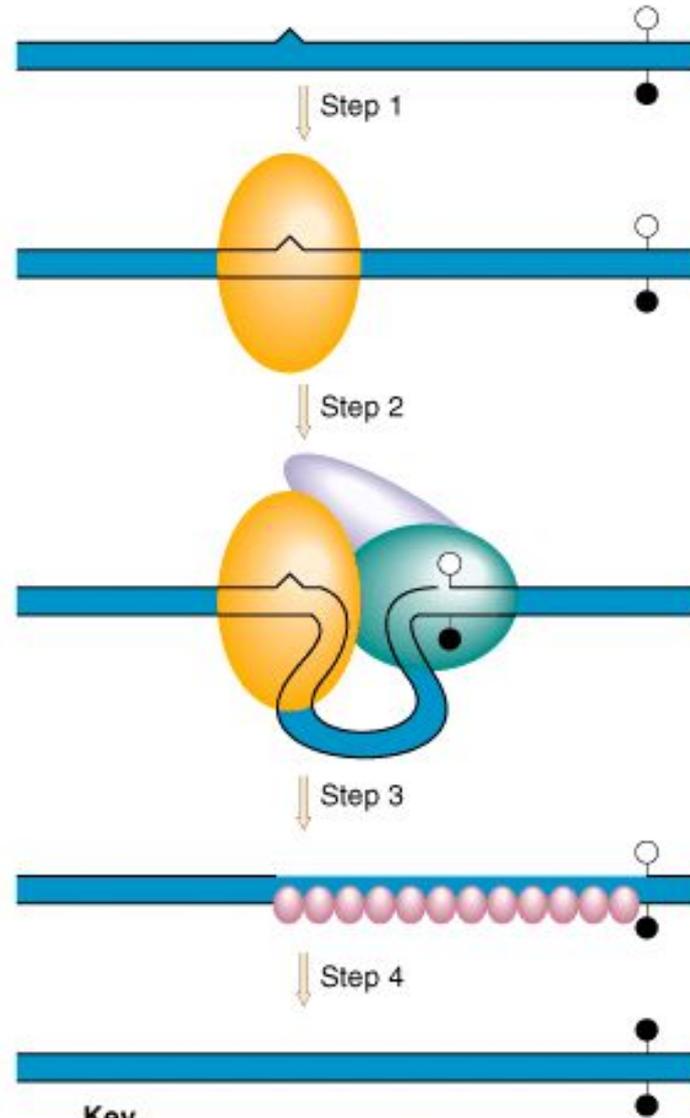
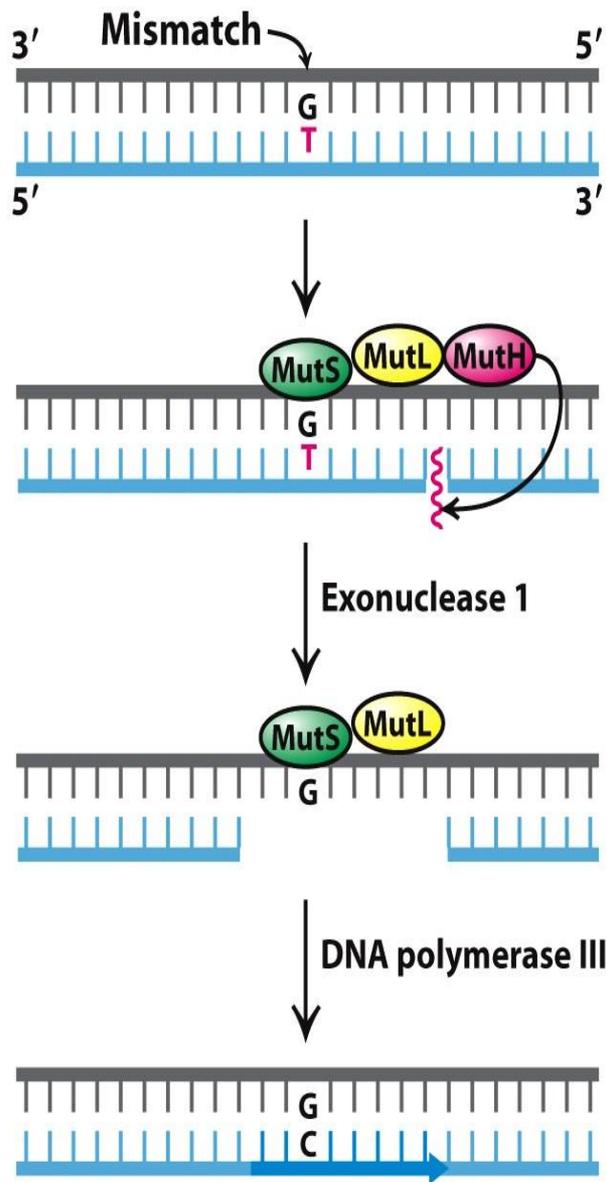


**(c)**



**(d)**





Key



GATC ○ unmethylated  
● methylated

Figure 28.36

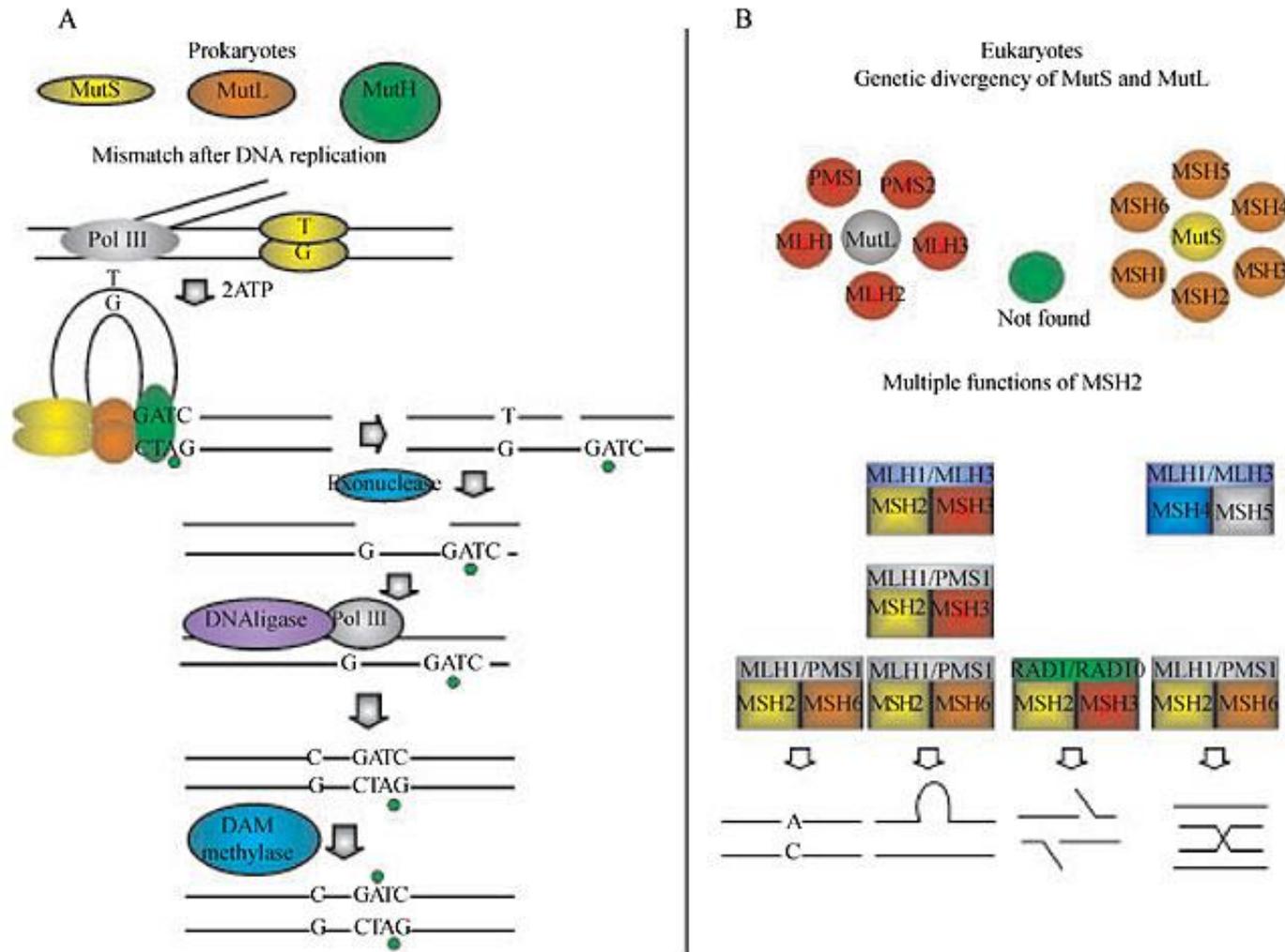
Biochemistry, Seventh Edition

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## Гомологи генов MutS, MutL у эукариот

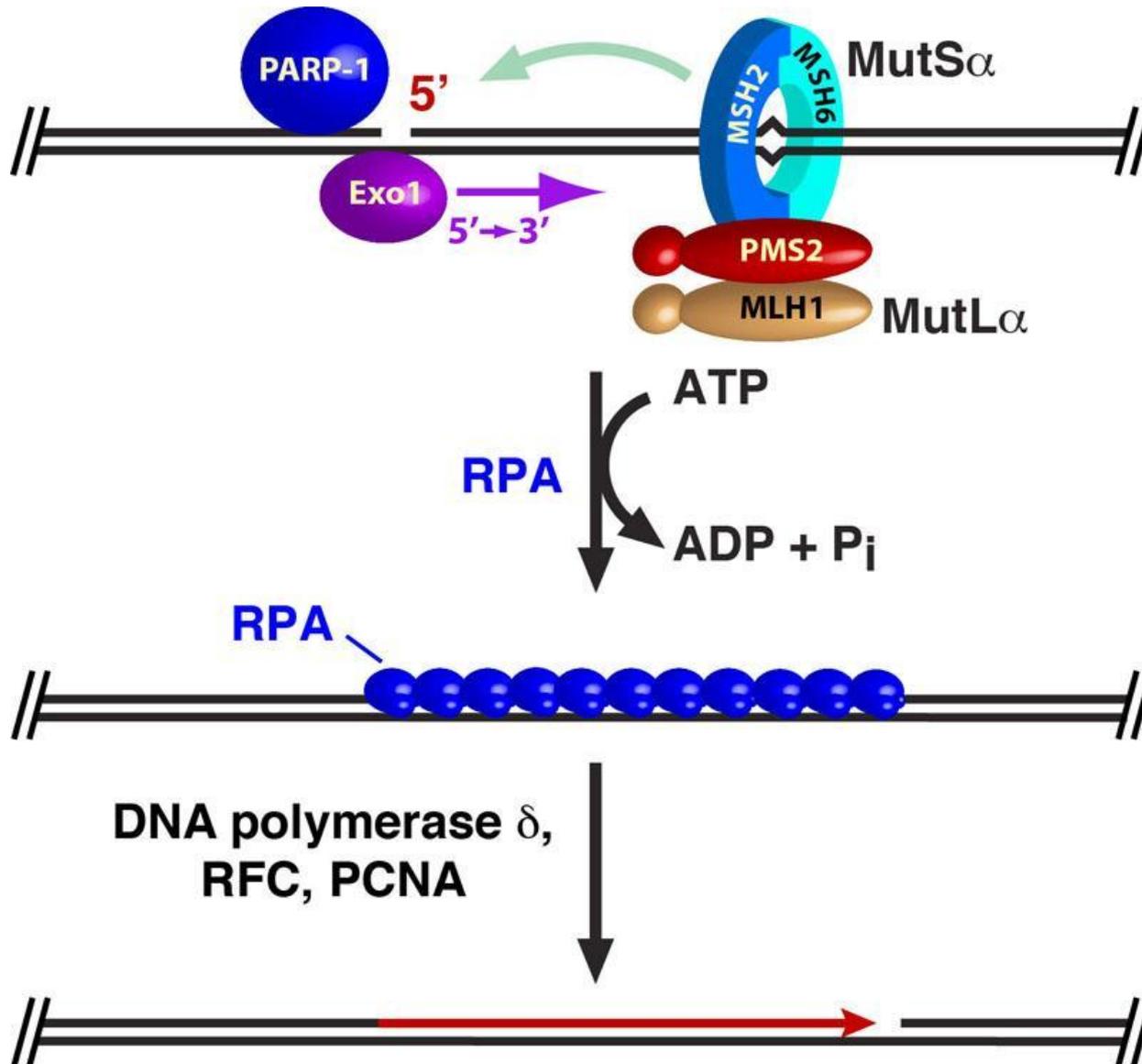
<i>E.coli</i>	<i>S.cerevisiae</i>	<i>S.pombe</i>	<i>C.elegans</i>	<i>D.melano gaster</i>	<i>A.thaliana</i>	<i>Human</i>
MutS	MSH2 MSH3 MSH6	MSH2 SWI4 MSH6	MSH2  MSH6	SPEL1  MSH6	MSH2 MSH3 MSH6 MSH7	MSH2 MSH3 MSH6
MutL	MLH1 PMS1 MLH2 MLH3	MLH1 PMS1	MLH1 PMS1	MLH1 PMS2	MLH1 PMS1  MLH3	MLH1 PMS2  MLH3 PMS1
MutH	-	-	-	-	-	-

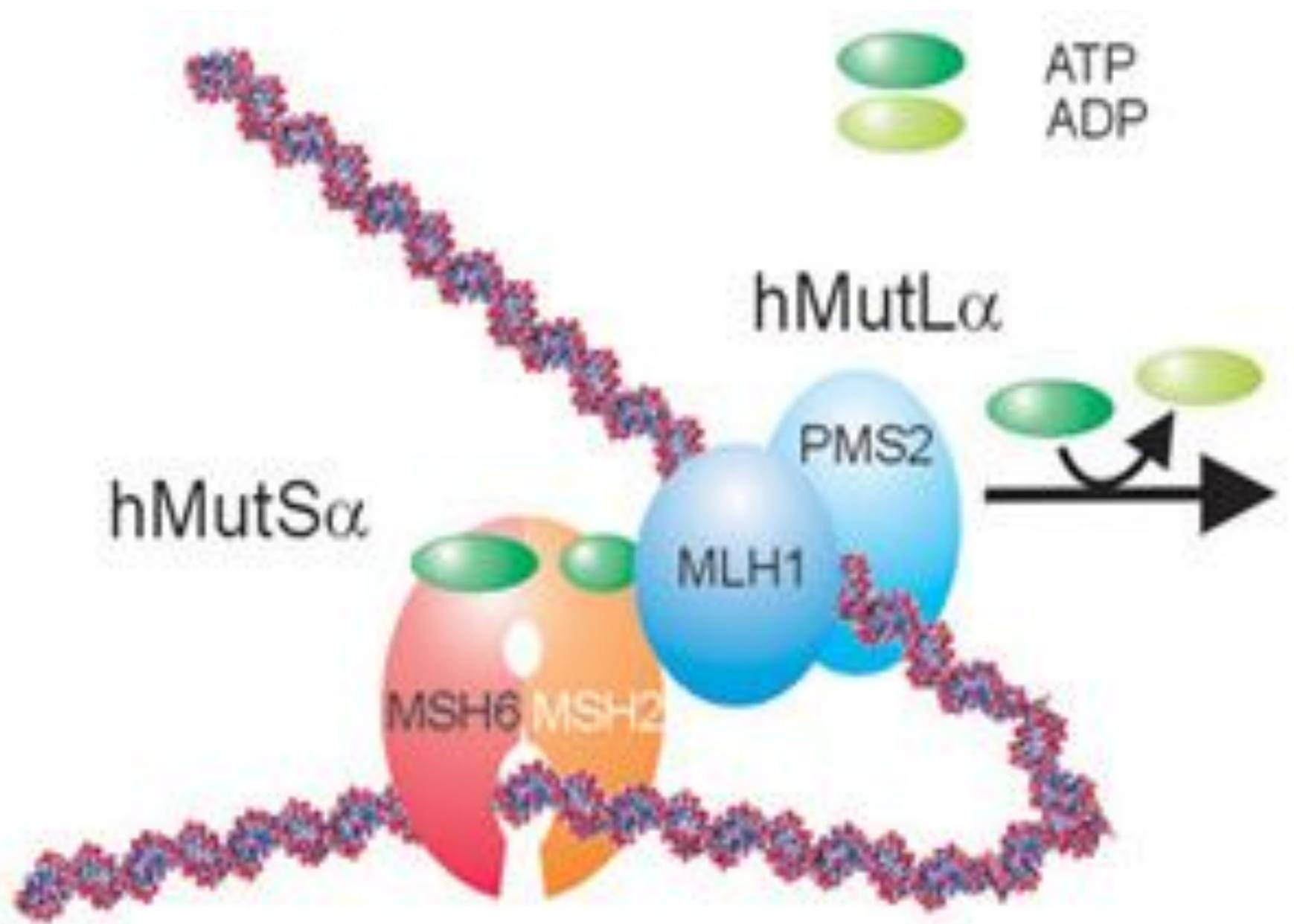
EXO1 вносит разрыв вместо  
MutH



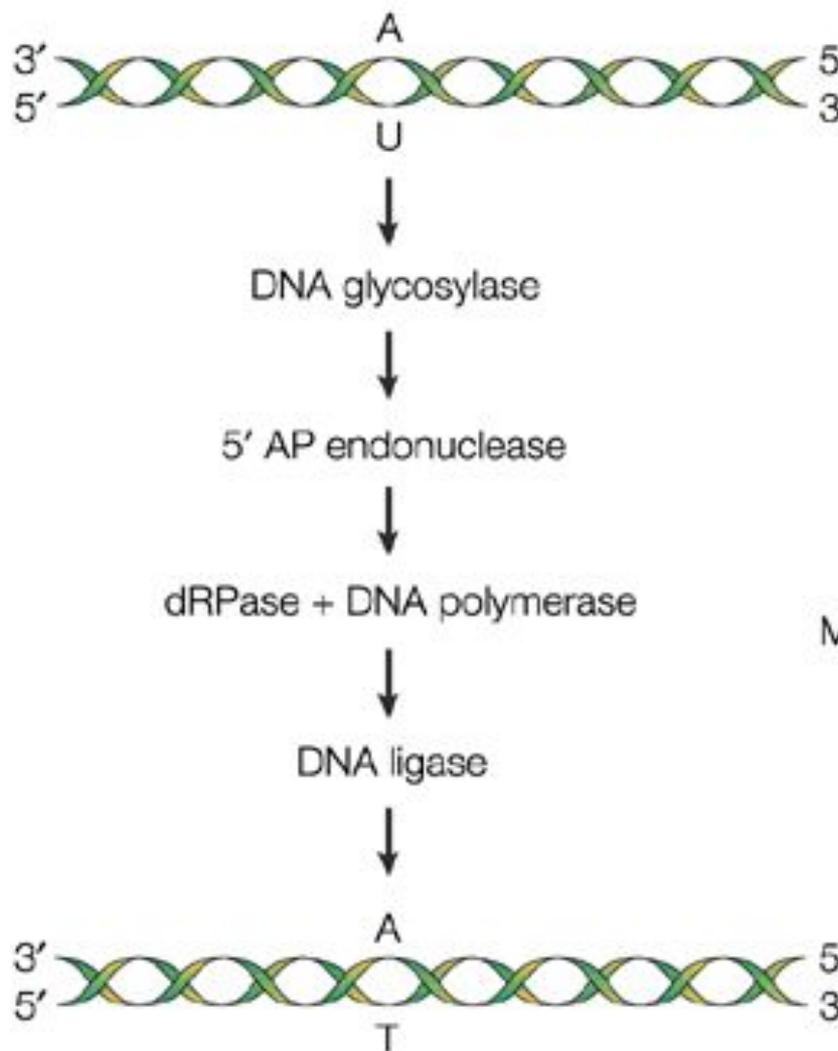
**Figure 3. A,** Schematic representation of mismatch repair in prokaryotes. The MutS homodimer protein binds to the DNA mismatch and makes a loop in DNA using the energy of hydrolysis of two ATP molecules. The MutL homodimer protein then associates with the bottom of this loop and activates the endonuclease MutH. The MutH protein only nicks the unmethylated strand, which contains the incorrect base. Afterwards, the cleaved strand is submitted to exonuclease activity, DNA resynthesis and ligation. **B,** Schematic representation of the eukaryotic mismatch repair system. The MutS proteins diverged into six orthologous genes, while MutL diverged into five other genes; these are denominated MSH and MLH, respectively. The MutH protein is not found in eukaryotes. The MSH and MLH proteins interact as a functional heterocomplex and repair several types of substrates, such as mismatches, single-strand loops generated during microsatellite replication, DNA double-strand breaks, and holiday junctions from meiotic crossing-over.

# MMR у человека

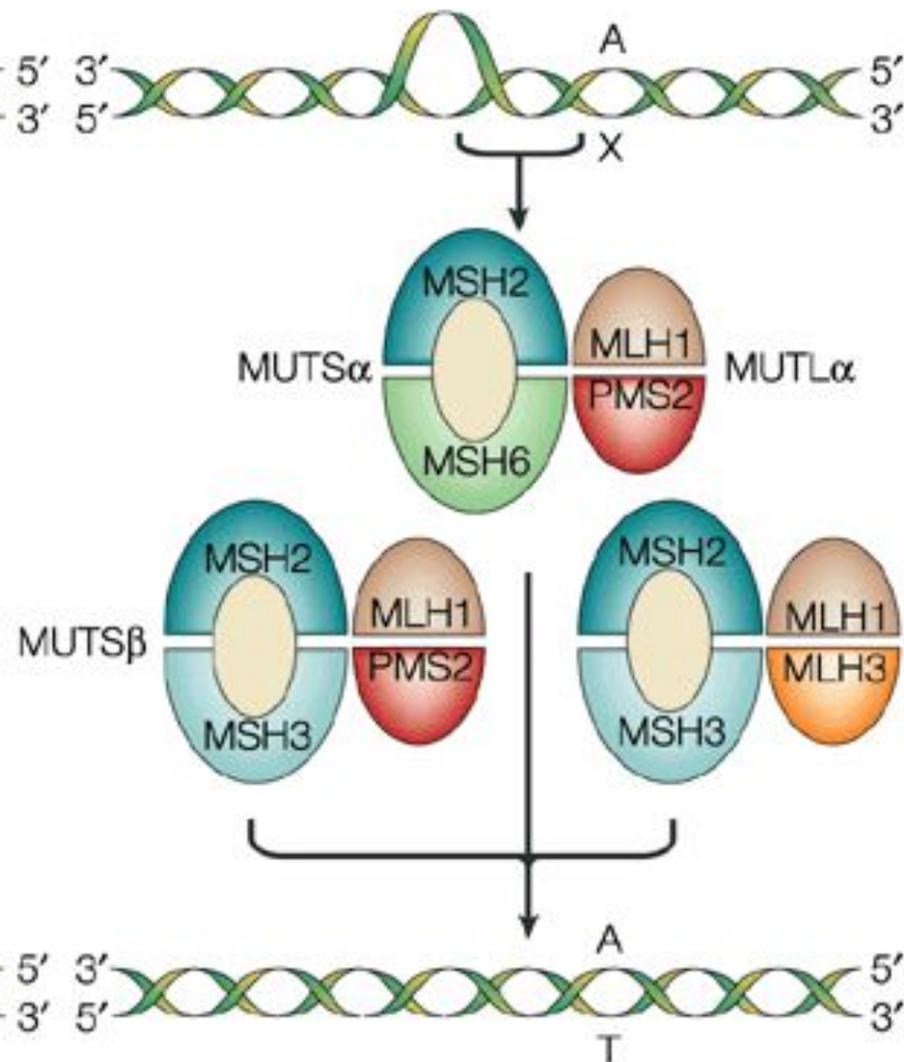




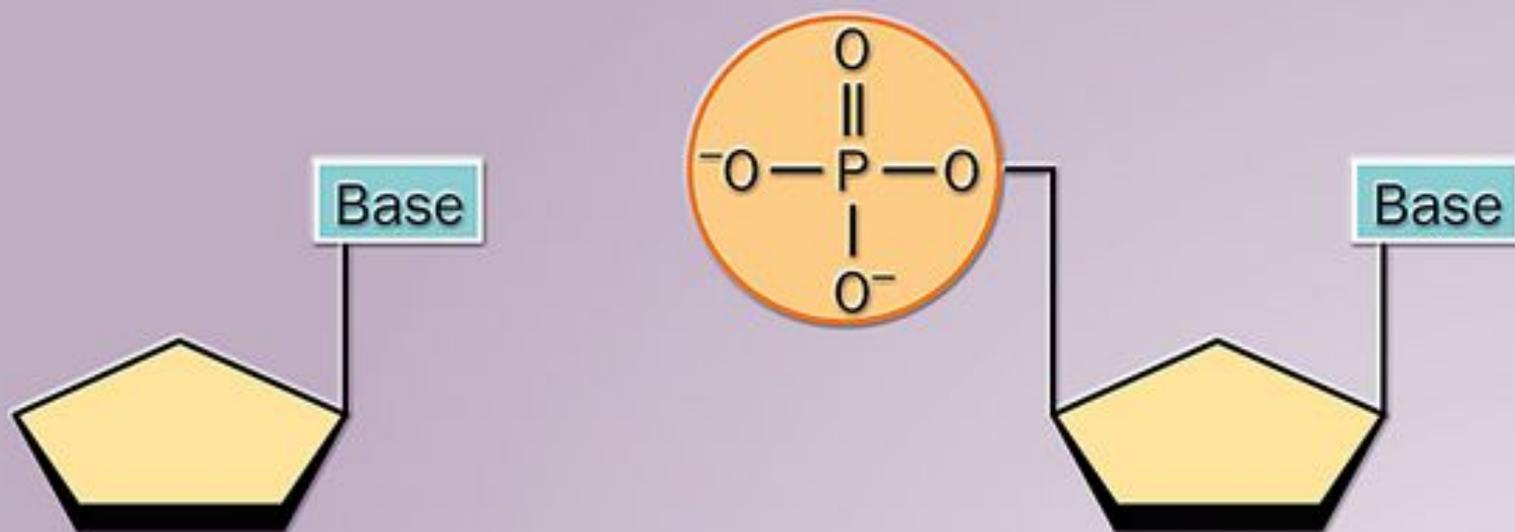
### a Base excision repair



### b Mismatch repair

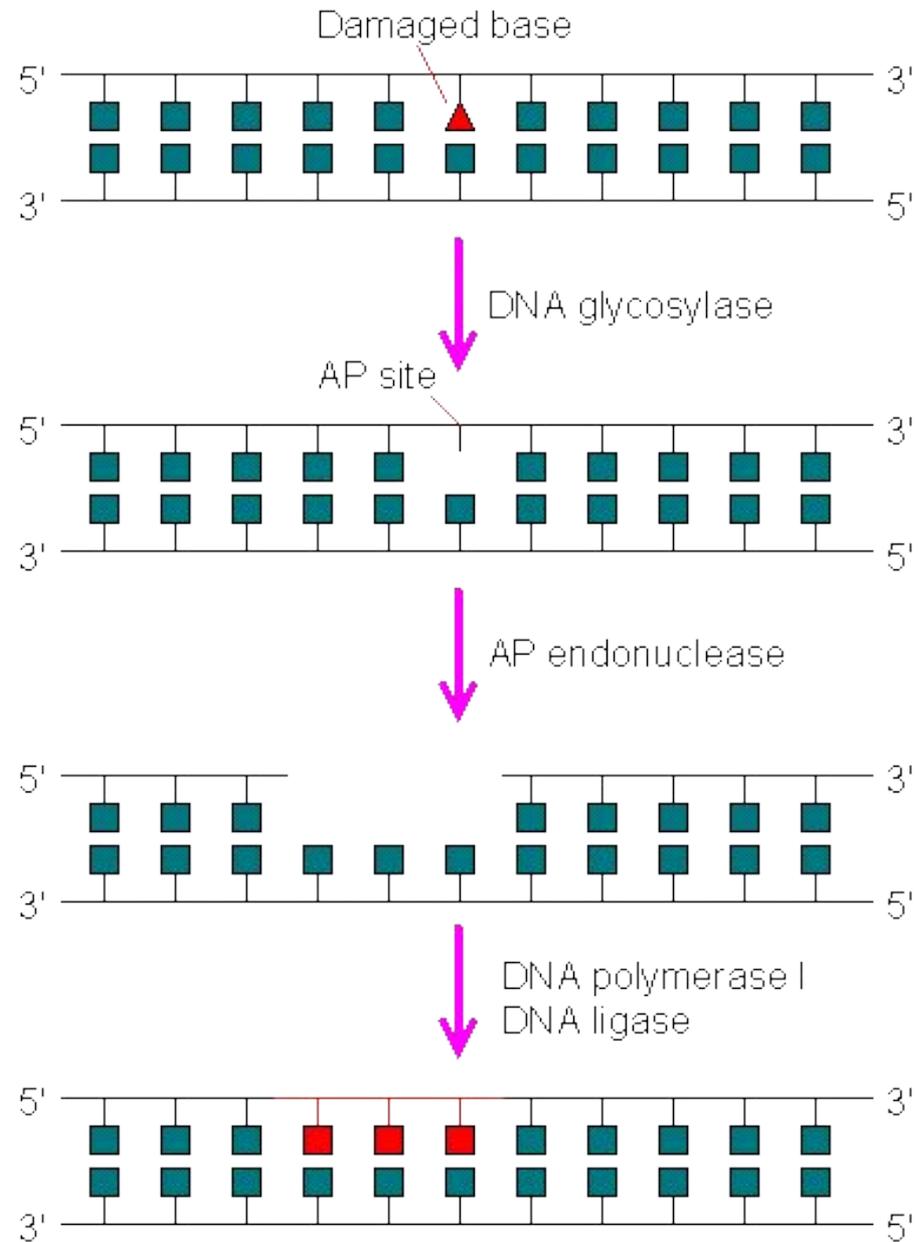


## Эксцизионная репарация оснований (BER) и нуклеотидов (NER)

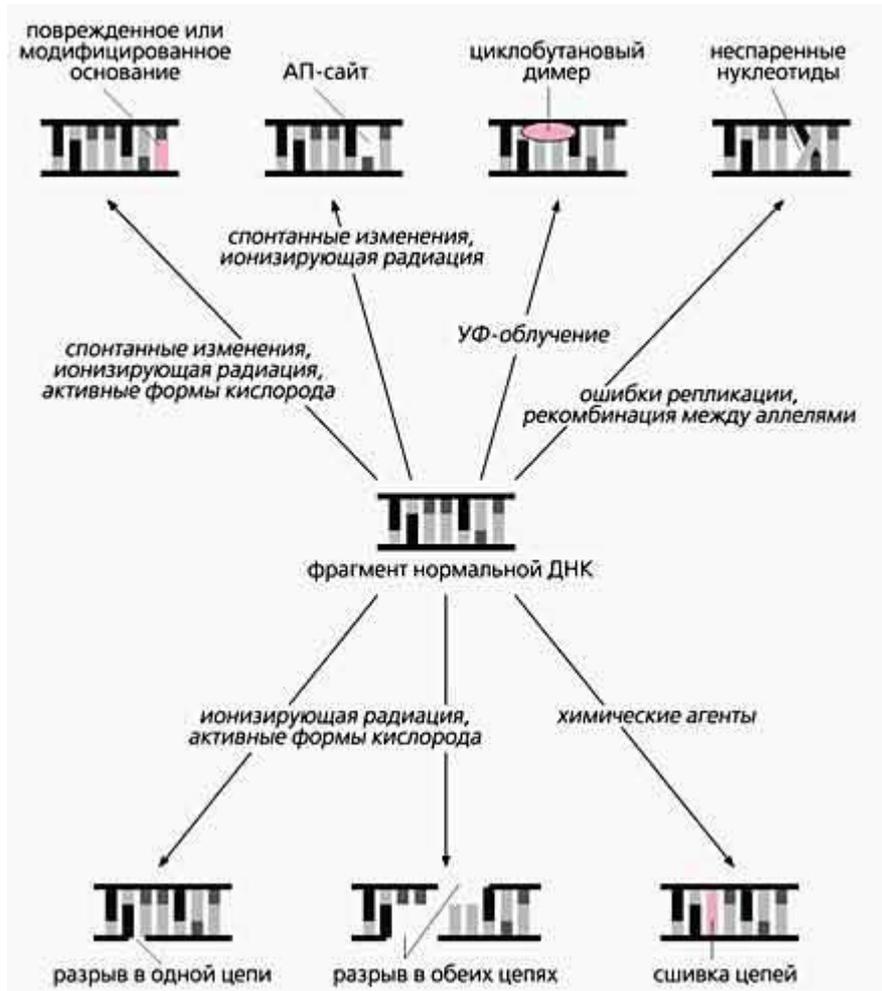
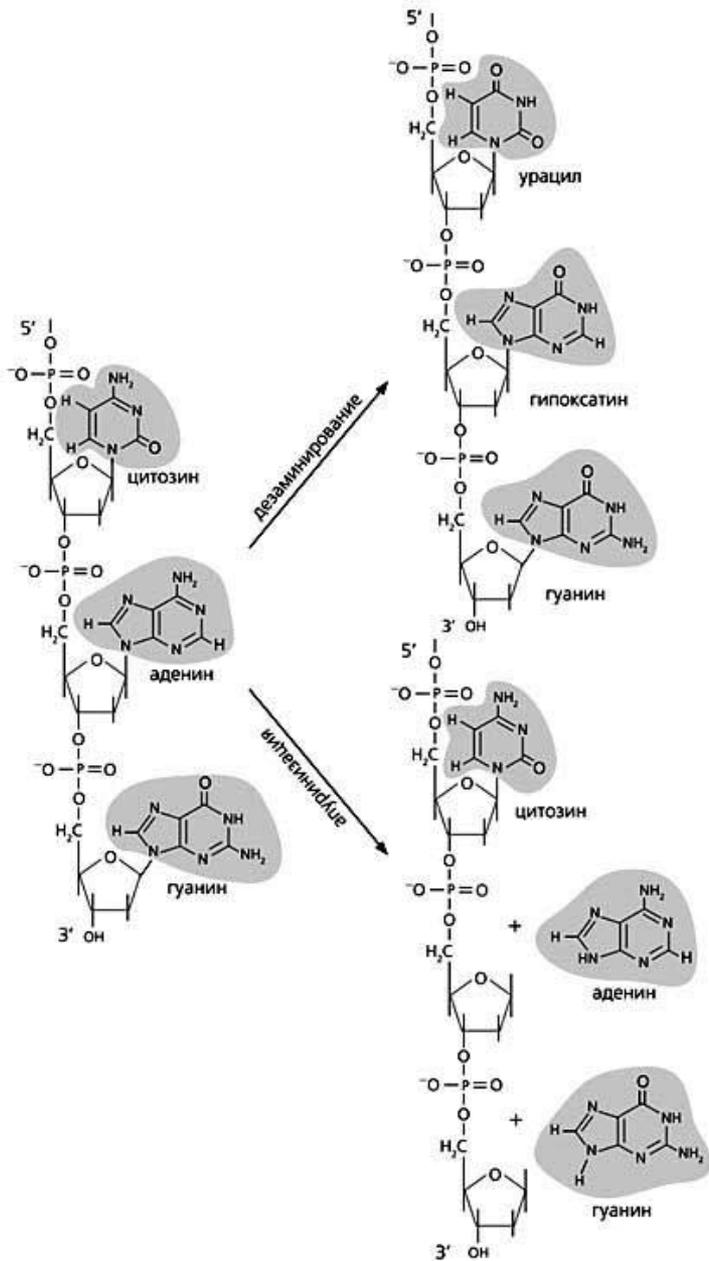


Нуклеозид

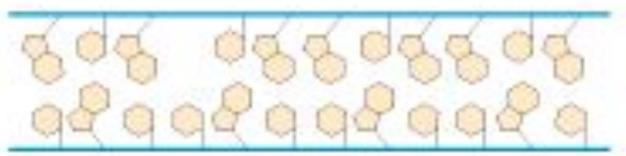
Нуклеотид (монофосфат)



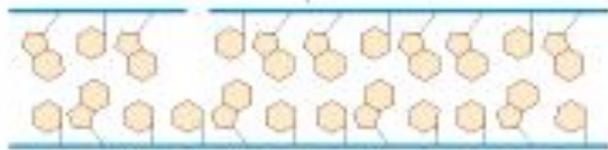
## Base excision repair



# Repairing apurinic and apyrimidinic sites



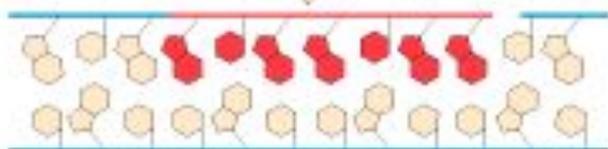
AP endonuclease  
makes cut



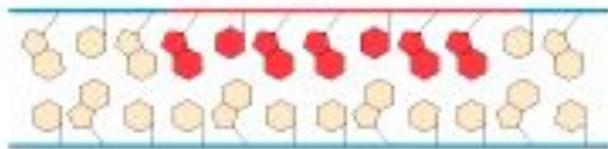
Excision  
exonuclease removes  
stretch of DNA



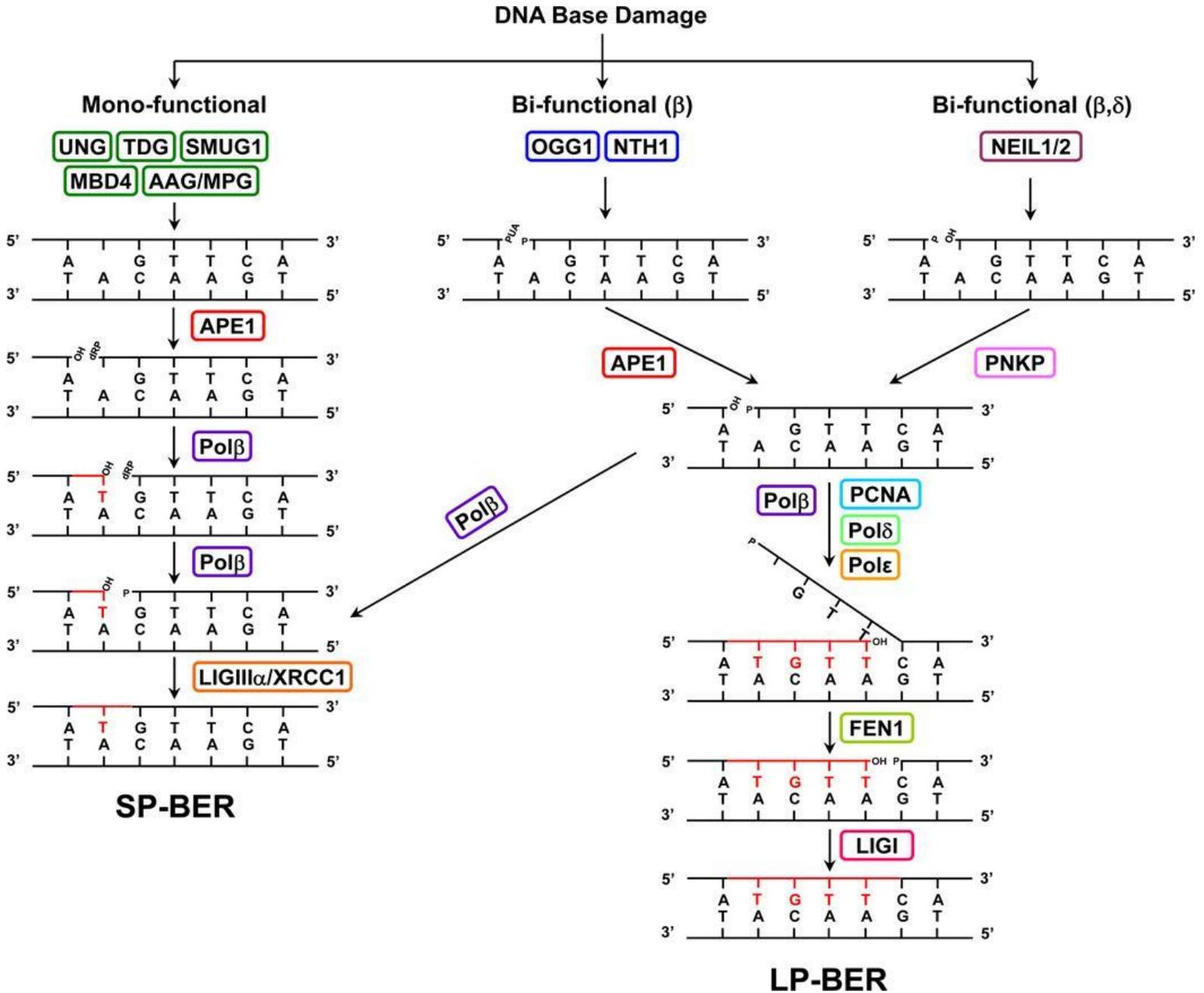
Polymerase  
synthesizes  
new DNA



Ligase  
seals  
nick



# Human BER pathways



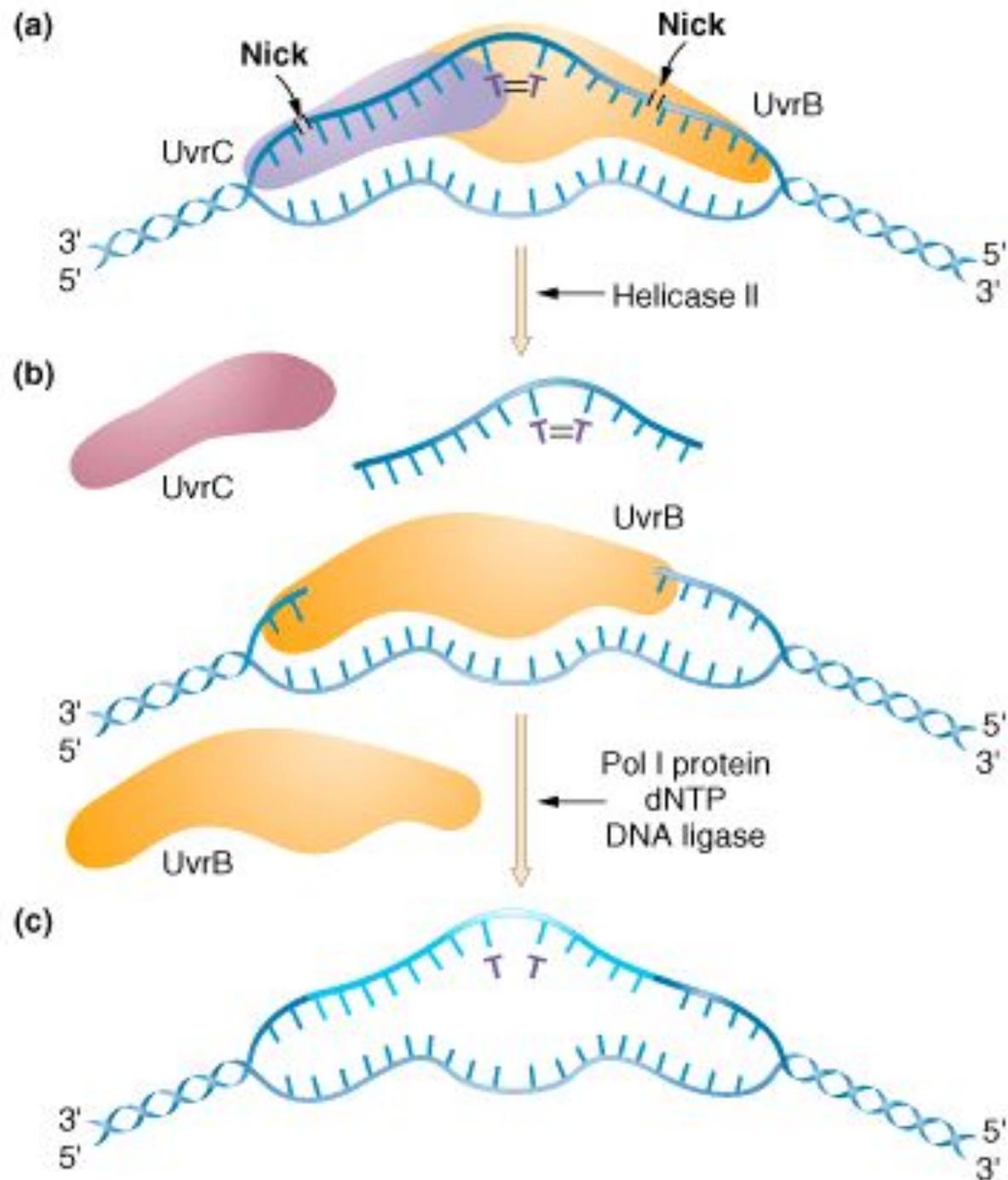
**Table II** - Base excision repair related proteins and their homologues in different organisms.

Enzyme (prototype)	Yeast	Human	<i>A. thaliana</i>	Sugarcane cluster (e-value <sup>a</sup> )
AP endonuclease ( <i>E. coli</i> Exonuclease III)	Apn2 (Eth1)	Apel (Ref1, Hap1), Ape2	Arp, two other homologues	SCEZAM2032F04.g (e-96)
AP endonuclease ( <i>E. coli</i> Endonuclease IV)	Apn1	-	-	-
Uracil DNA glycosylase ( <i>E. coli</i> Ung)	Ung1	Udg1	Ung1	SCEQFL5048B07.g (e-42)
3-Methyladenine glycosylases ( <i>E. coli</i> TagI and AlkA, human Aag)	Mag (AlkA family)	Aag	Aag, Mag, several TagI homologues	SCJLRT2049G09.g (Mag family, e-14), SCMCRT2104F02 (TagI family, e-37 <sup>b</sup> )
8-oxoguanine/formamidopyrimidine glycosylases ( <i>E. coli</i> MutM, yeast Ogg1)	Ogg1	Ogg1	Ogg1, MutM1, MutM2	SCVPRZ2036B05.g (Ogg1 family, e-66) SCCCLR2C01B12 (MutM family, e-111)
8-oxoguanine : adenine mispair glycosylase ( <i>E. coli</i> MutY)	-	Myh	MutY	-
Thymine glycol glycosylases ( <i>E. coli</i> Endonuclease III)	Ntg1, Ntg2	Ntg1	Nth1, other homologue	SCACSB1036F12 (e-85)
G:T mismatch glycosylase (human Tdg)	-	Tdg	-	-

<sup>a</sup>BLASTn e-values for the best hit obtained probing with *A. thaliana* proteins.

<sup>b</sup>There are several Tag homologues in *A. thaliana*, as well as several clusters in sugarcane. Therefore, the e-value shown was obtained probing with *E. coli* TagI.

# Nucleotide excision repair

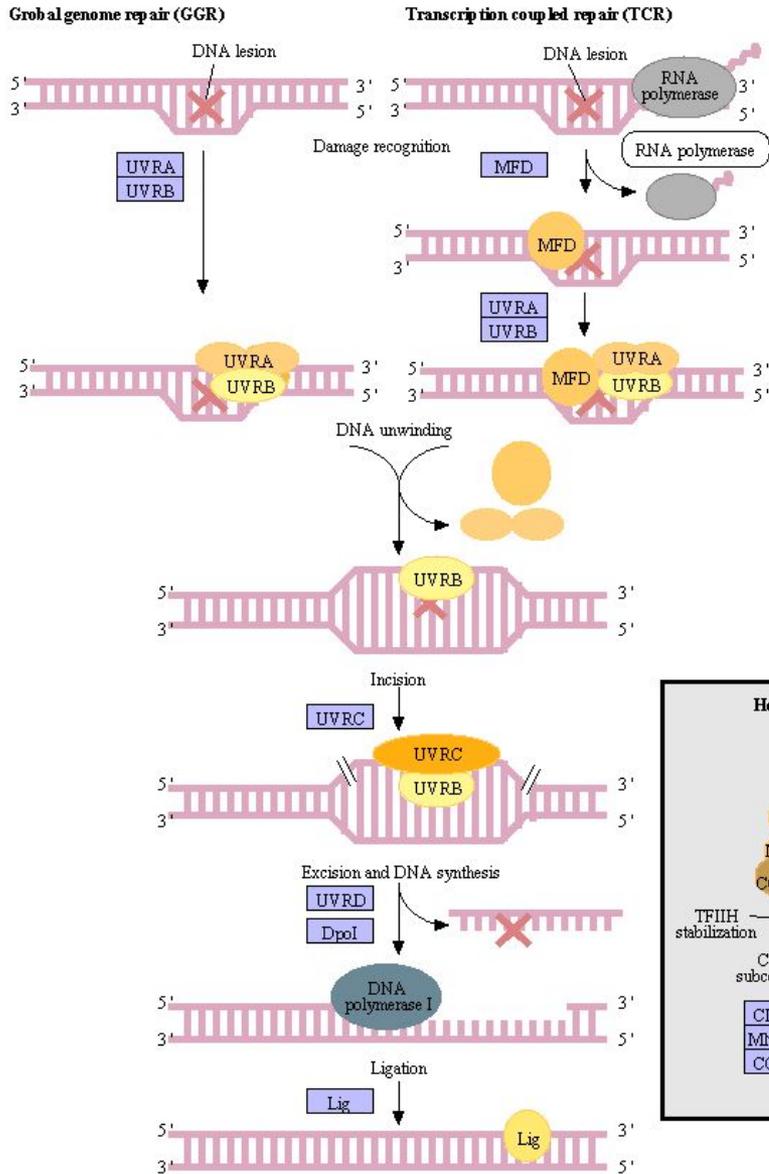


У всех живых организмах NER состоит из этапов:

- Узнавание повреждений
- Связывание мультисубъединичного комплекса с поврежденным сайтом
- Двойное надрезание поврежденной цепи на несколько нуклеотидов от поврежденного сайта в обоих направлениях 5' и 3'
- Освобождение олигонуклеотида, содержащего повреждение между двумя надрезами
- Заполнение образовавшейся брешки ДНК полимеразой
- Лигирование

# NUCLEOTIDE EXCISION REPAIR

## Prokaryotic type



## Eukaryotic type

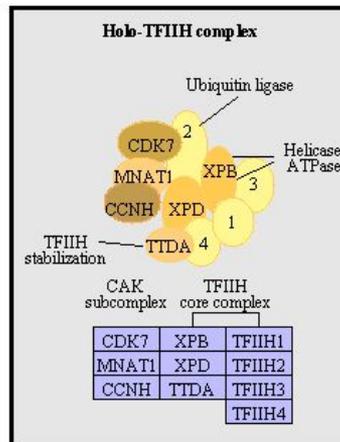
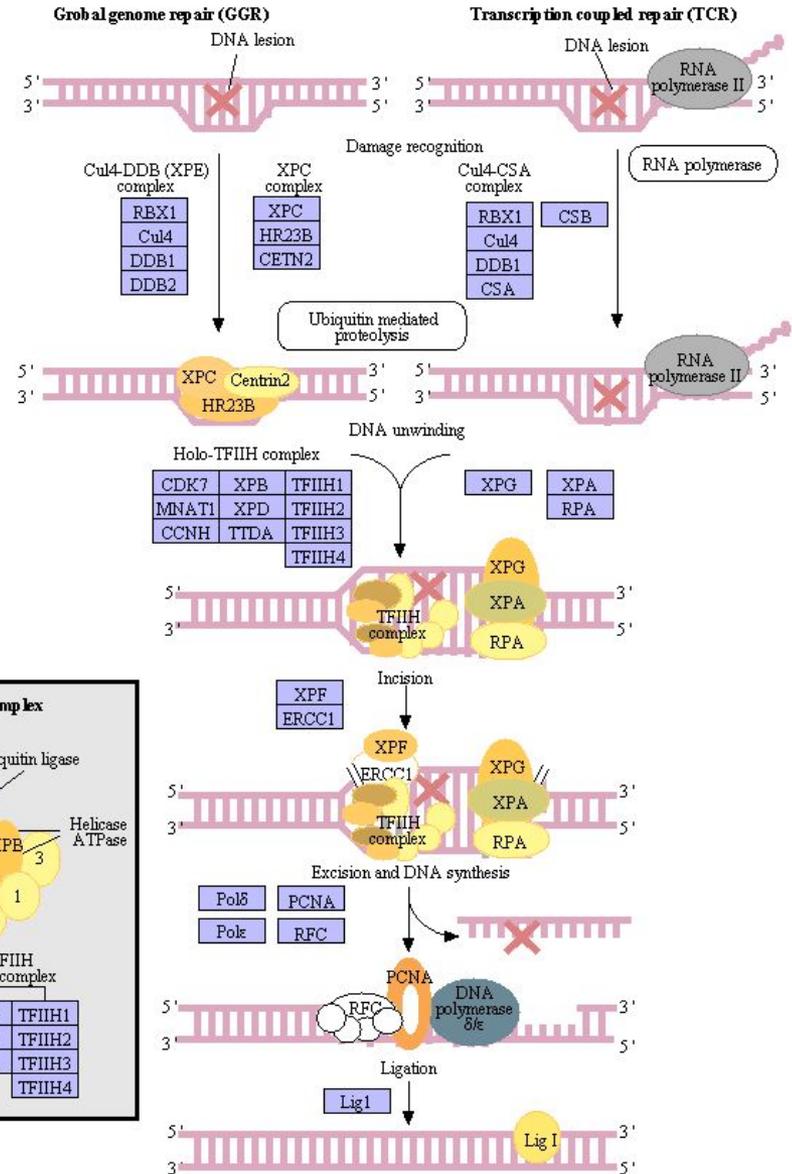


Table 1 | **Mammalian genes involved in nucleotide excision repair**

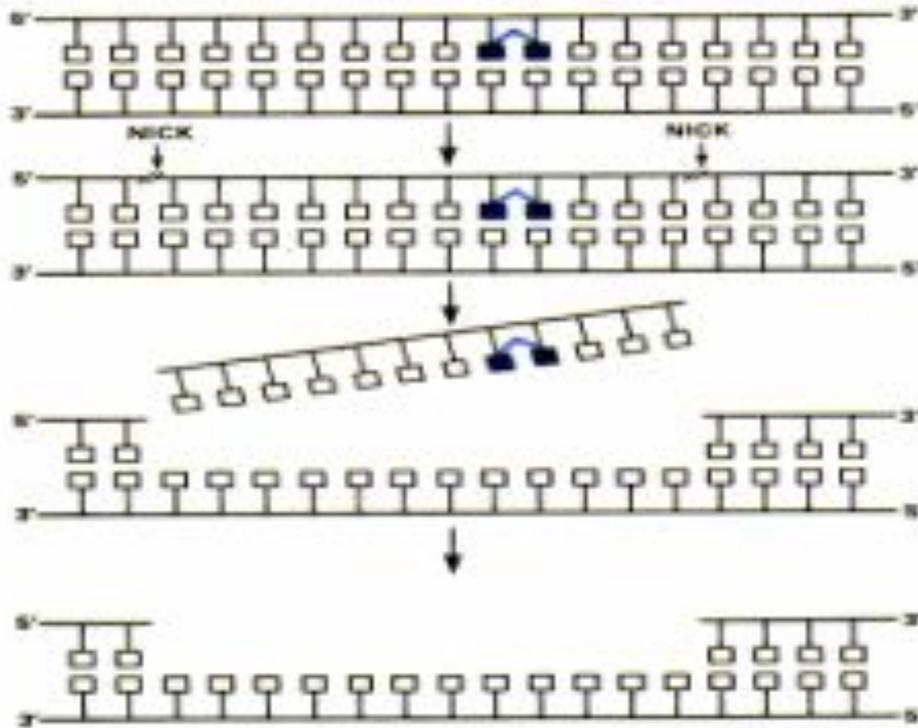
Human gene/protein	Function in NER	Mouse gene	Mouse mutant phenotype
<i>XPC/XPC</i>	Involved in damage recognition. Not required for TCNER. Represented in human XP.	<i>Xpc</i>	Defective in NER of the non-transcribed strand of transcriptionally active genes after UV radiation. Skin cancer after UV irradiation. Heterozygous mutants also prone to skin cancer. Liver and lung tumours after exposure to chemicals (AAF).
<i>RAD23B (HHR23B)/HRAD23B</i>	Binds to XPC. Involved in damage recognition. No human mutants known.	<i>Rad23B</i>	No NER-defective phenotype observed. Mice viable but small.
<i>RAD23A (HHR23A)/HRAD23A</i>	Can substitute for HRAD23B. No human mutants known.	<i>Rad23A</i>	Not NER defective. Mice viable and develop normally. <i>Rad23A/Rad23B</i> double mutation is lethal.
<i>CENT2/centrin2 (or caltacin 1)</i>	Stabilizes XPC in the presence of HRAD23. No human mutants known.	<i>Cent2</i>	Not available.
<i>XPA/XPA</i>	Involved in damage recognition. Represented in human XP.	<i>Xpa</i>	NER defective. Skin cancer after UV radiation or exposure to chemicals (benz[a]pyrene and DMBA).
<i>RFA1/RFA1</i>	Subunit of trimeric RFA complex. Involved in damage recognition. No human mutants known.	<i>Rfa1</i>	Not available.
<i>RFA2/RFA2</i>	Subunit of RFA complex. Involved in damage recognition. No human mutants known.	<i>Rfa2</i>	Not available.
<i>RFA3/RFA3</i>	Subunit of RFA complex. Involved in damage recognition. No human mutants known.	<i>Rfa3</i>	Not available.
<i>XPB/XPB</i>	Subunit of core TFIIH complex. 3'→5' DNA helicase. Promotes bubble formation. Represented in human XP/CS syndrome.	<i>Xpb</i>	Embryonic lethal.
<i>XPB/XPB</i>	Subunit of core TFIIH. 5'→3' DNA helicase. Promotes bubble formation. Represented in human XP, XP/CS syndrome and TTD.	<i>Xpd</i>	Embryonic lethal. An allele that mimics a mutation in human TTD is viable. These mice are NER defective and have TTD. They also manifest skin cancer after UV irradiation.
<i>GTF2H1/p62</i>	Core TFIIH subunit. Promotes bubble formation. No human mutants known.	<i>Gtf2h1</i>	Not available.
<i>GTF2H2/p44</i>	Core TFIIH subunit. Promotes bubble formation. No human mutants known.	<i>Gtf2h2</i>	Not available.
<i>GTF2H3/p34</i>	Core TFIIH subunit. Promotes bubble formation. No human mutants known.	<i>Gtf2h3</i>	Not available.

<i>RPA2/RFA2</i>	Subunit of the core RPA complex. Involved in damage recognition. No human mutants known.	<i>Rfa2</i>	Not available.
<i>RPA3/RFA3</i>	Subunit of RFA complex. Involved in damage recognition. No human mutants known.	<i>Rfa3</i>	Not available.
<i>XPB/XPB</i>	Subunit of core TFIIH complex. 3'→5' DNA helicase. Promotes bubble formation. Represented in human XP/CS syndrome.	<i>Xpb</i>	Embryonic lethal.
<i>XPD/XPD</i>	Subunit of core TFIIH. 5'→3' DNA helicase. Promotes bubble formation. Represented in human XP, XP/CS syndrome and TTD.	<i>Xpd</i>	Embryonic lethal. An allele that mimics a mutation in human TTD is viable. These mice are NER defective and have TTD. They also manifest skin cancer after UV irradiation.
<i>GTF2H1/p62</i>	Core TFIIH subunit. Promotes bubble formation. No human mutants known.	<i>Gtf2h1</i>	Not available.
<i>GTF2H2/p44</i>	Core TFIIH subunit. Promotes bubble formation. No human mutants known.	<i>Gtf2h2</i>	Not available.
<i>GTF2H3/p34</i>	Core TFIIH subunit. Promotes bubble formation. No human mutants known.	<i>Gtf2h3</i>	Not available.
<i>GTF2H4/p52</i>	Core TFIIH subunit. Promotes bubble formation. No human mutants known.	<i>Gtf2h4</i>	Not available.
<i>XPG/XPG</i>	3' DNA-structure-specific endonuclease. Required for bimodal incision. Represented in human XP and XP/CS syndrome.	<i>Xpg</i>	NER defective. Mice viable but runted, indicating a vital function.
<i>ERCC1/ ERCC1</i>	5' DNA-structure-specific endonuclease with XPF. Required for bimodal incision. No human mutants known.	<i>Ercc1</i>	NER defective. Mice runted or inviable, indicating a vital function.
<i>XPF/XPF</i>	5' DNA-structure-specific endonuclease with ERCC1. Required for bimodal incision. Represented in human XP.	<i>Xpf</i>	Not available.
<i>DDB1/DDB1</i>	Forms a complex with DDB2. Complex defective in individuals with XP-E.	<i>Ddb1</i>	Not available.
<i>DDB2/DDB2</i>	Forms a complex with DDB1. Complex defective in individuals with XP-E.	<i>Ddb2</i>	Not available.
<i>CSA/CSA</i>	Required for TCNER. Represented in human CS.	<i>Csa</i>	TCNER defective. No obvious CS phenotype. Skin cancer after UV irradiation.
<i>CSB/CSB</i>	Required for TCNER. Represented in human CS.	<i>Csb</i>	TCNER defective. No obvious CS phenotype. Skin cancer after UV irradiation.
<i>XAB2/XAB2</i>	Interacts with XPA, CSA and CSB. Involved in TCNER. No human mutants known.	<i>Xab2</i>	Not available.

AAF, *N*-acetoxy-acetylaminofluorene; CS, Cockayne syndrome; DMBA, 7,12-dimethylbenzanthracene; ERCC1, excision repair cross-complementing 1; HRAD23, human homologue of *RAD23*; NER, nucleotide excision repair; RPA, replication protein A; TCNER, transcription-coupled NER; TFIIH, transcription factor IIH; TTD, trichothiodystrophy; UV, ultraviolet; XP, xeroderma pigmentosum.

# Mechanism of Incision by the NER Pathway

---



*E. coli*

5' incision is 8 nuc. from lesion  
3' incision is 4 nuc. from lesion

Mammals

5' incision is 22 nuc. from lesion  
3' incision is 6 nuc. from lesion

# Genetics of NER in Humans

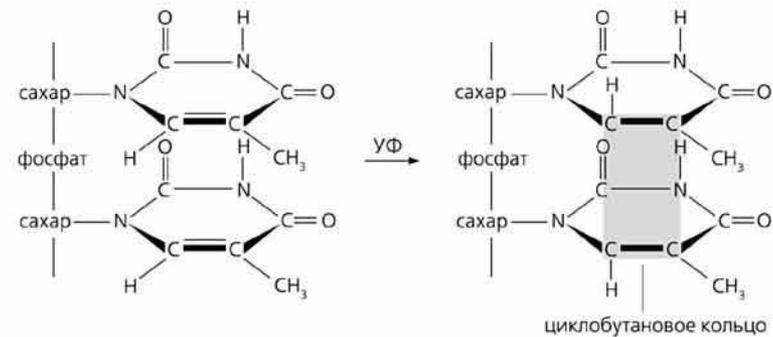
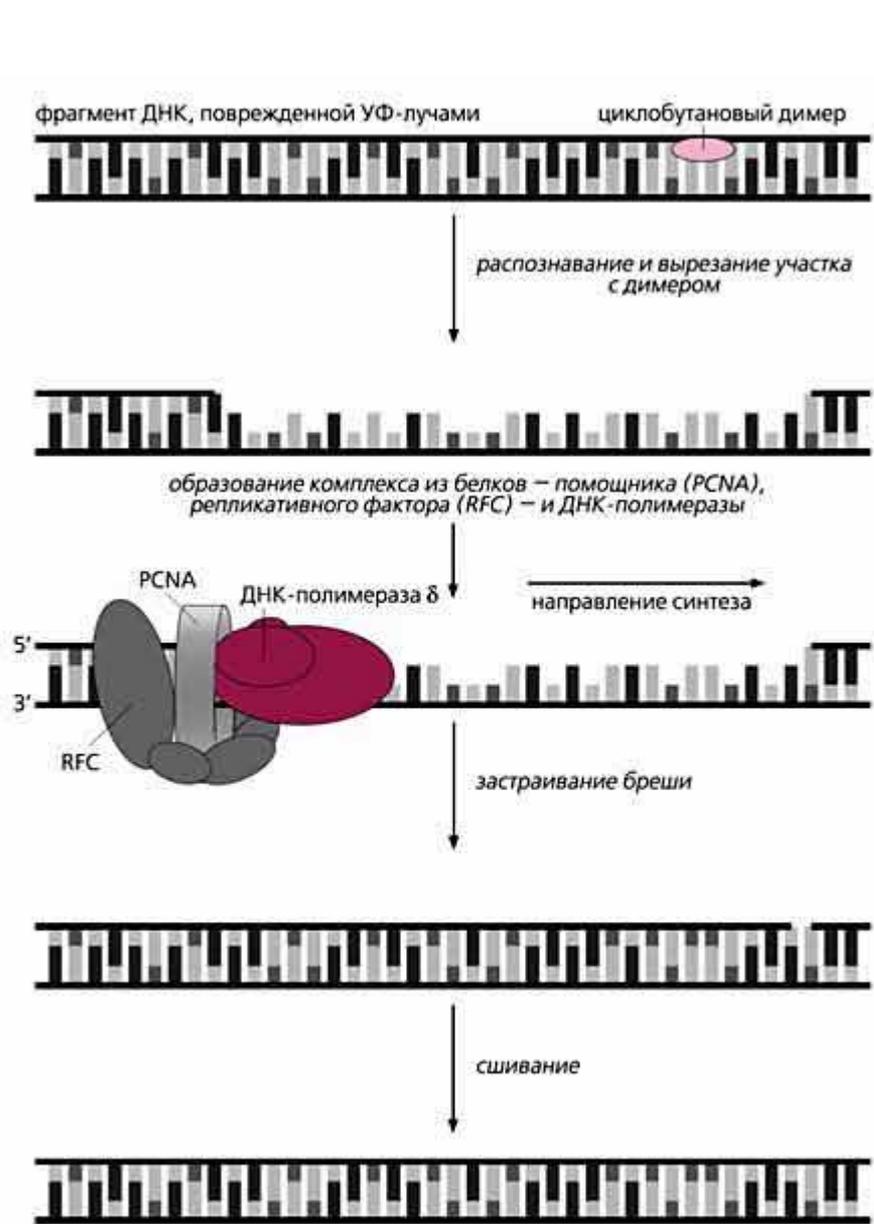
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## Xeroderma Pigmentosum (classical)

- Occurrence: 1-4 per million population
- Sensitivity: ultraviolet radiation (sunlight)
- Disorder: multiple skin disorders; malignancies of the skin; neurological and ocular abnormalities
- Biochemical: defect in early step of NER
- Genetic: autosomal recessive, seven genes (A-G)

## Xeroderma Pigmentosum (variant)

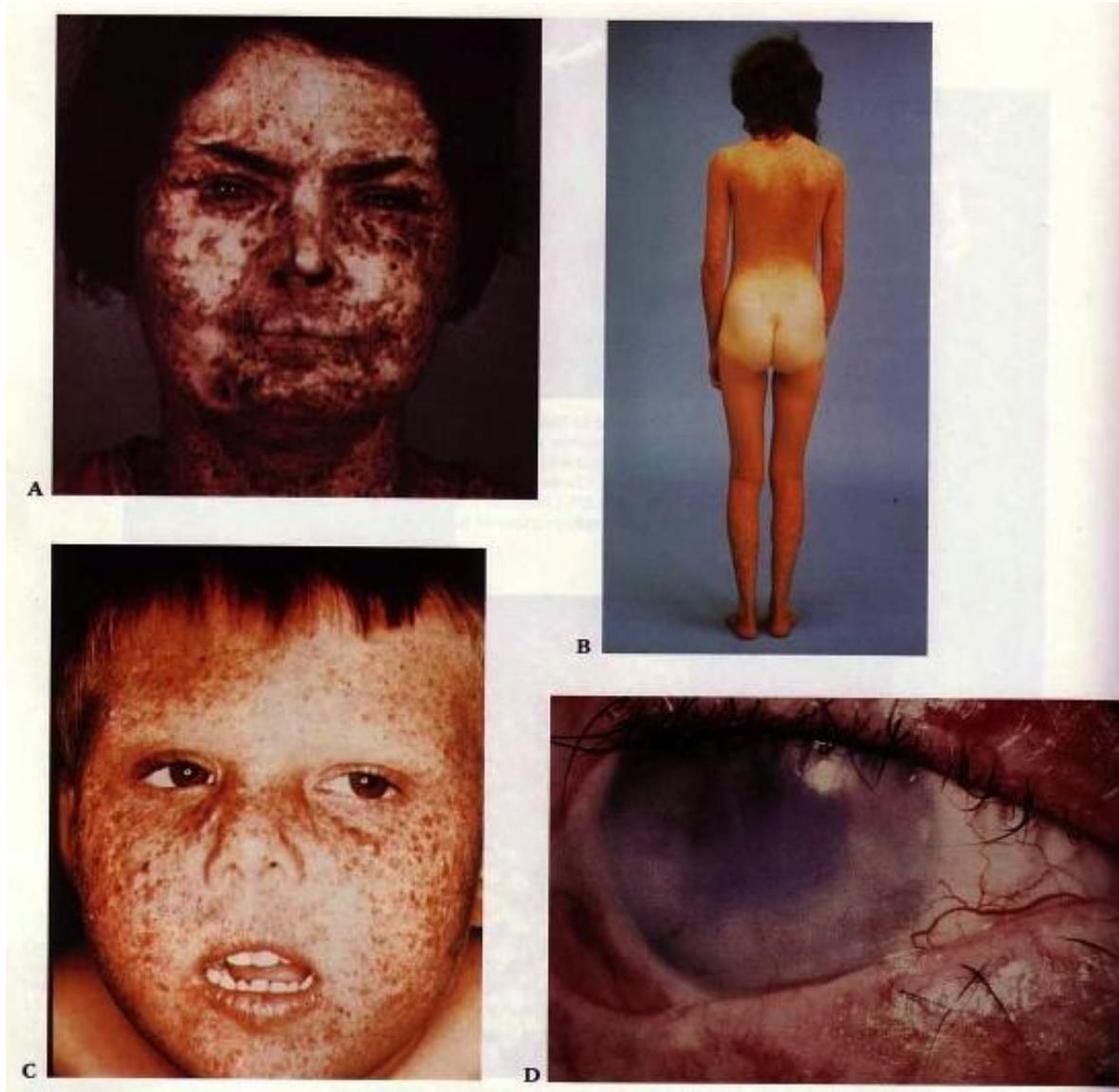
- Occurrence: same as classical
- Sensitivity: same as classical
- Disorder: same as classical
- Biochemical: defect in translesion bypass



Стадии восстановления ДНК, в которой образовался циклобутановый димер. Участок с этой структурой, мешающей копированию ДНК, распознают и вырезают эндонуклеазы семейства ХР (ксеродермы), а застраивает образовавшуюся брешь в 29 нуклеотидов полимераза  $\delta$  (или  $\epsilon$ ) при наличии фактора репликации (RFC) и белка-помощника (PCNA).

# Xeroderma Pigmentosum

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# Genetics of NER in Humans

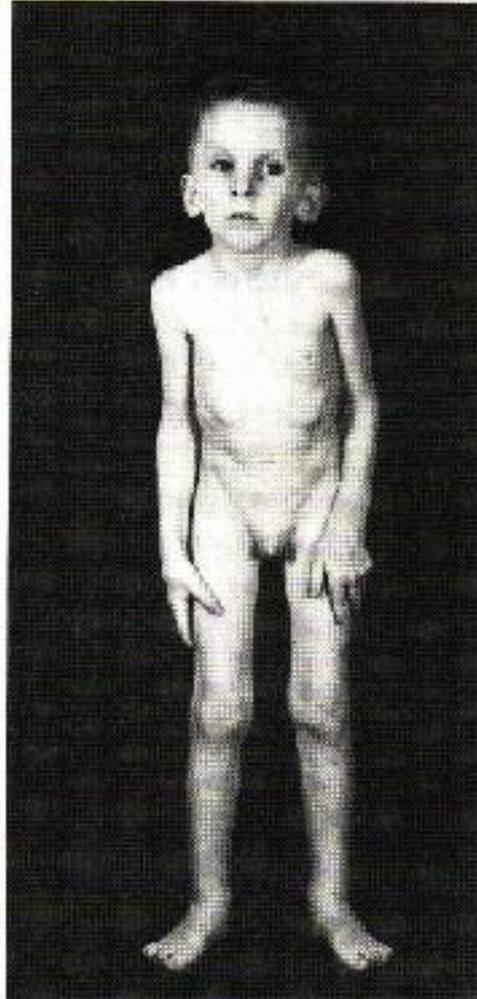
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## Cockayne's Syndrome

- Occurrence: 1 per million population
- Sensitivity: ultraviolet radiation (sunlight)
- Disorder: arrested development, mental retardation, dwarfism, deafness, optic atrophy, intracranial calcifications; (no increased risk of cancer)
- Biochemical: defect in NER
- Genetic: autosomal recessive, five genes (A, B and XPB, D & G)

# Cockayne's Syndrome

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# Genetics of NER in Humans

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## Trichothiodystrophy

- Occurrence: 1-2 per million population
- Sensitivity: ultraviolet radiation (sunlight) in subset of patients
- Disorder: sulfur deficient brittle hair, mental and growth retardation, peculiar face with receding chin, ichthyosis; (no increased cancer risk)
- Biochemical: defect in NER
- Genetic: autosomal recessive, three genes (TTDA, XPB, XPD)

# Trichothiodystrophy

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Human Diseases with DNA-Repair Defects

Disease	Sensitivity	Cancer susceptibility	Complementation groups	Symptoms
Ataxia telangiectasia	$\gamma$ irradiation	Lymphomas	5	Ataxia, dilation of blood vessels (telangiectases) in skin and eyes, chromosome aberrations, immune dysfunction
Bloom syndrome	Mild alkylating agents	Carcinomas, leukemias, lymphomas	1	Photosensitivity, facial telangiectases, chromosome alterations
Cockayne syndrome	UV light		2	Dwarfism, retinal atrophy, photosensitivity, progeria, deafness, trisomy 10
Fanconi anemia	Cross-linking agents	Leukemias	3	Hypoplastic pancytopenia, congenital anomalies
Xeroderma pigmentosum	UV light, chemical mutagens	Skin carcinomas and melanomas	8	Skin and eye photosensitivity, keratoses
HNPCC		Colon, ovary	4	Early development of tumors

**Note:** Other human hereditary disorders that may be related to DNA-repair defects include dyskeratosis congenita (Zinsser-Cole-Engman syndrome), progeria (Hutchinson-Gilford syndrome), and trichothiodystrophy. HNPCC, hereditary nonpolyposis colorectal cancer.

**Source:** After A. Kornberg and T. Baker, *DNA Replication*, 2d ed. Copyright © 1992 by W. H. Freeman and Company.

# Системы репарации

- Фотореактивация (прямая репарация)
- Эксцизионная репарация

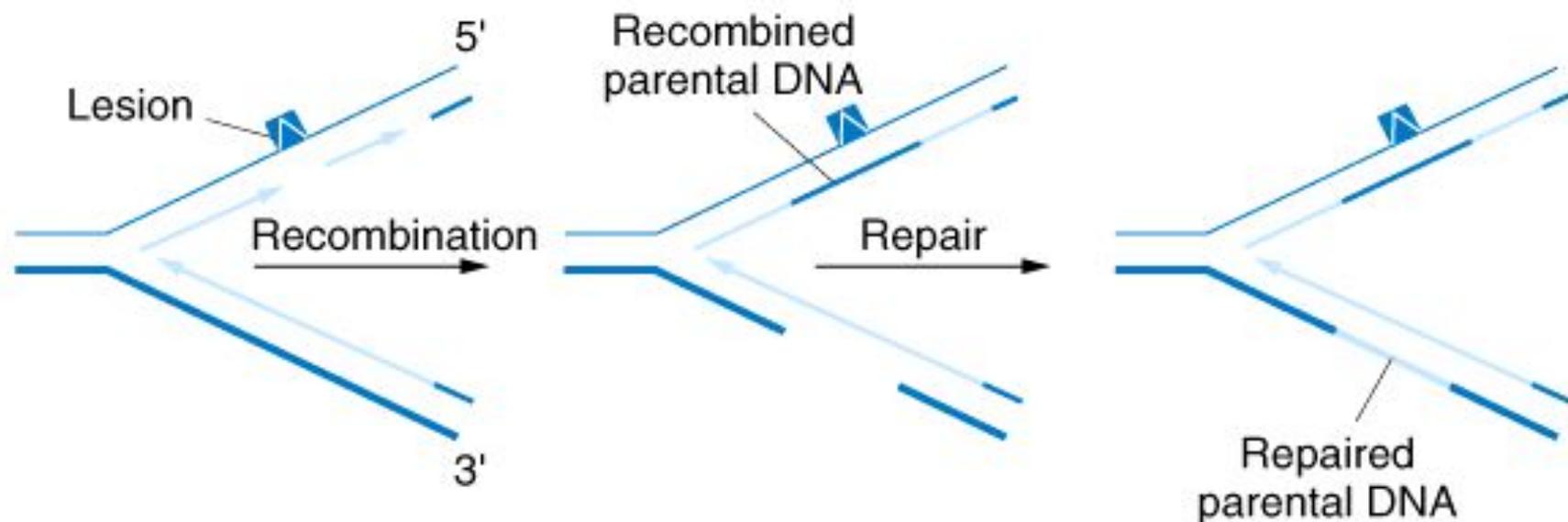
Mismatch repair

Base excision repair (BER)

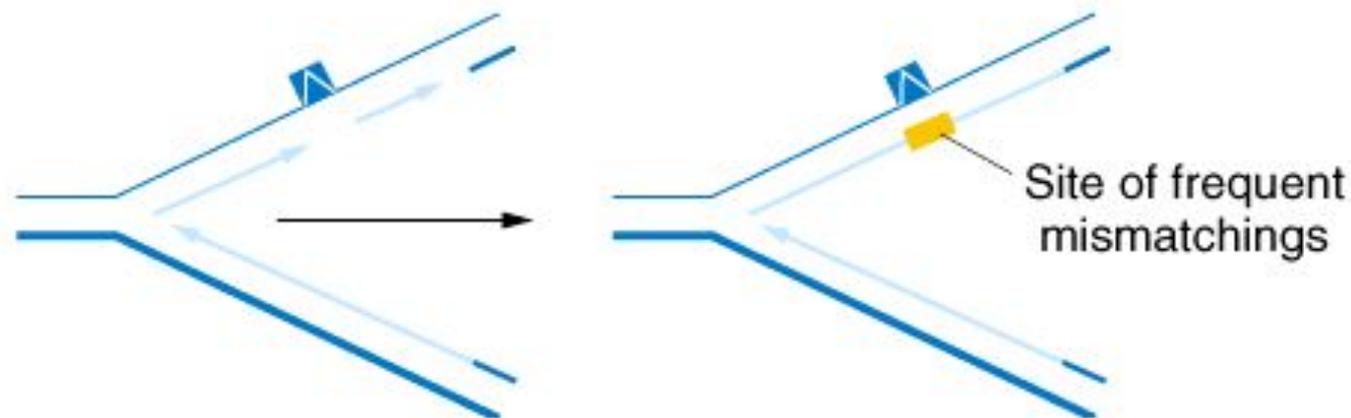
Nucleotide excision repair (NER)

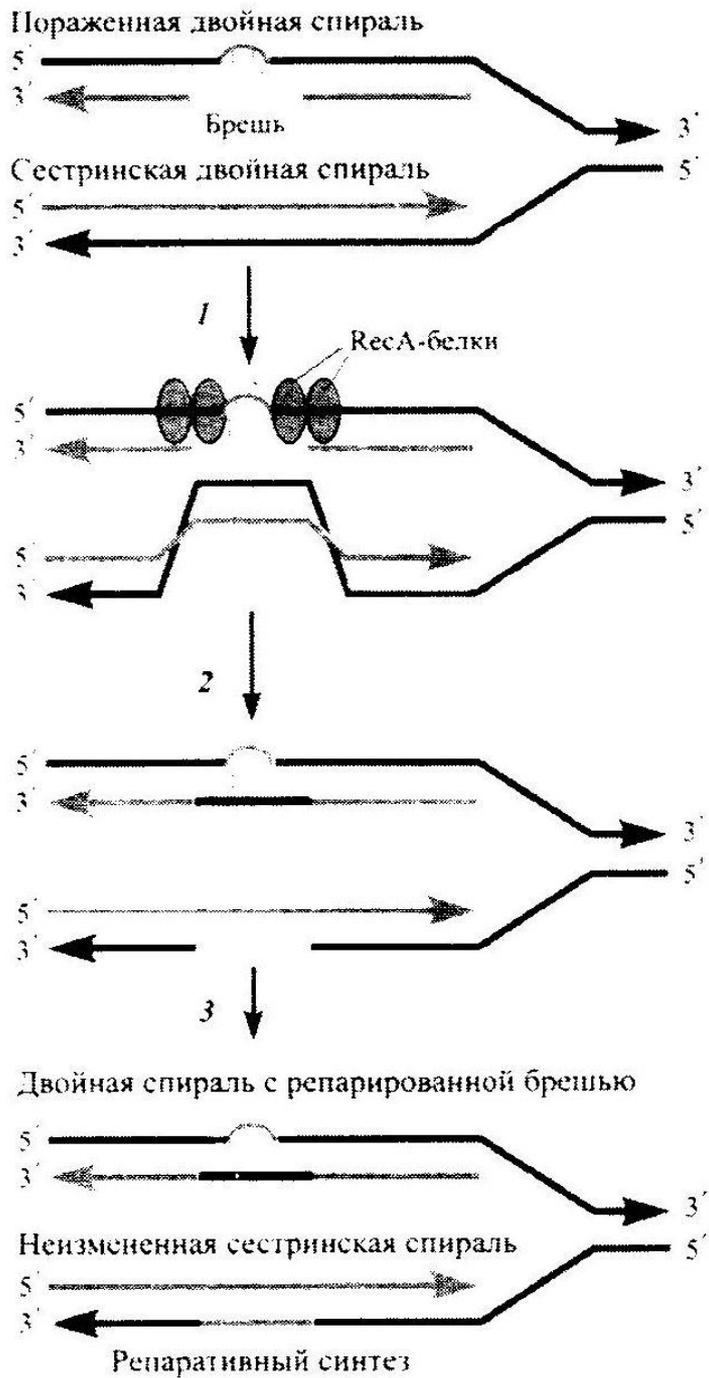
- Пострепликативная (рекомбинационная) репарация
- SOS-репарация

**(a) Postreplication recombination repair**



**(b) Error-prone (SOS) replication (lesion bypass)**





## Пострепликативная (рекомбинационная) репарация

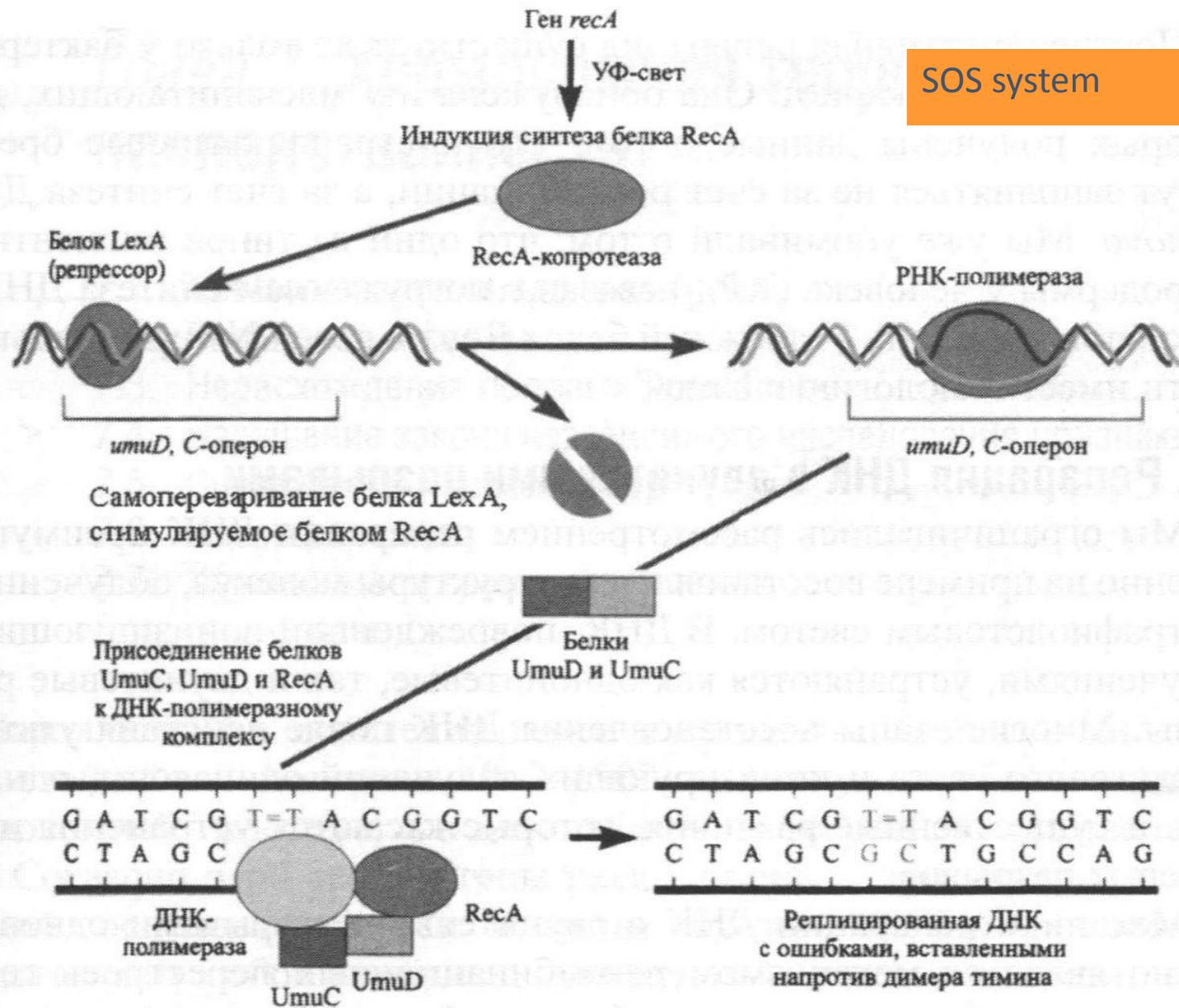
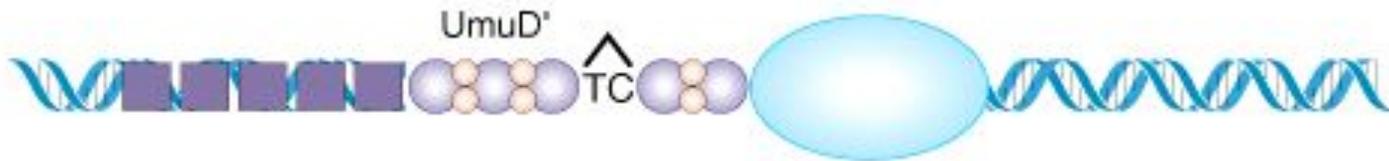
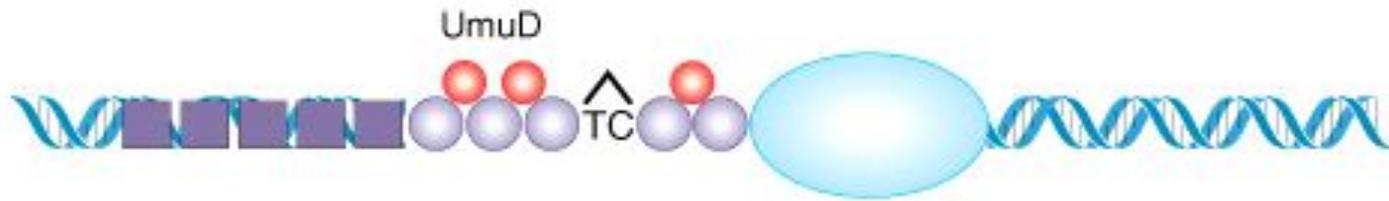
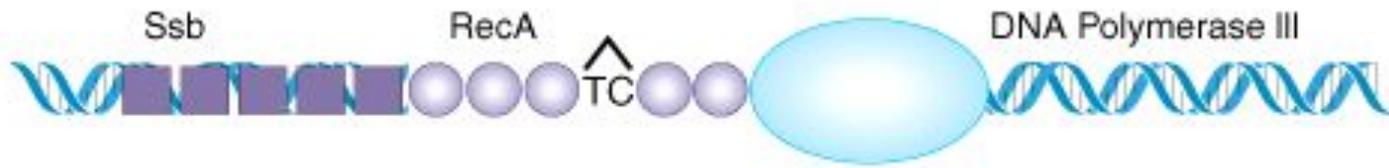


Рис. 6.8. SOS-репарация у *E. coli* (из Сойфера, 1997, с уточнениями)



SOS  
system

# Репарация двуцепочечных разрывов

