Popravljanje DNA napak

Literatura:

Predavanje

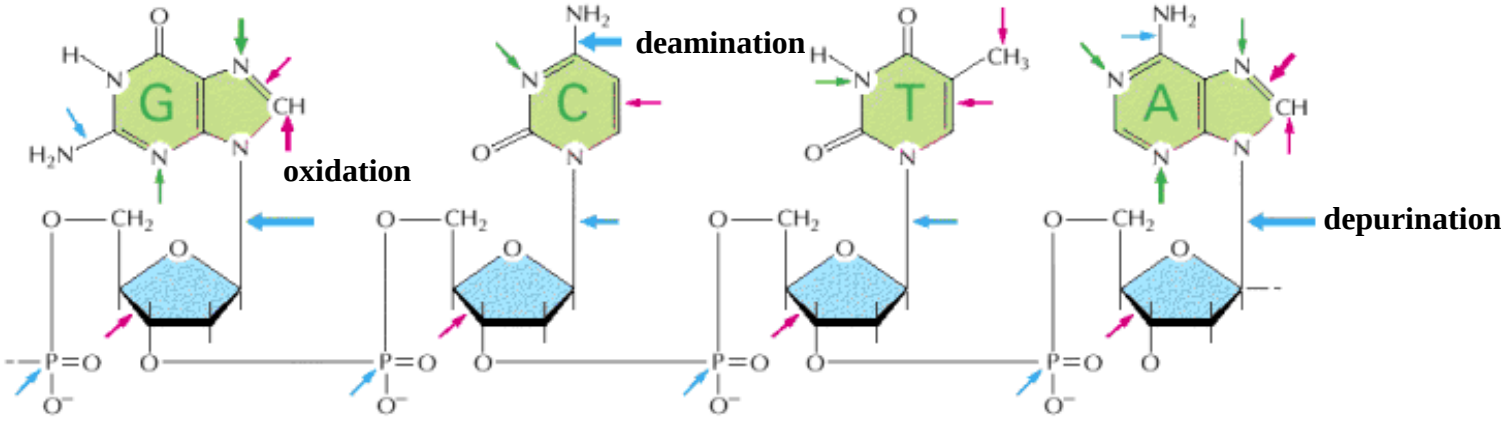
Lodish in sod., Molecular Cell Biology, 2013, od str. 151 naprej

Priloženi članki

Poškodbe DNA

- Nastanejo lahko spontano ali pod vplivom okolja.
- Eksogeni dejavniki (UV, ionizirajoča sevanja, težke kovine, onesnaženje zraka, kemoterapija, vnetni procesi)
- Endogeni dejavniki: v celičnih procesih (prenos elektronov v mitohondrijih, oksidacija lipidov v peroksisomih) nastanejo spojine (radikali, superoksid), ki poškodujejo DNA.
- Substitucije, delecije, insercije
- Korelcija med kopičenjem mutacij in rakom pri človeku.
- Pri človeku v 24h nastane več tisoč poškodb DNA v celici (10^4-10^6), a zaradi popravljalnih mehanizmov manj kot 1 od 1000 postane mutacija.
- Poškodbe povzročajo:
  - genomsko nestabilnost
  - bolezni (rak, nevrološke bolezni, imunska pomankljivost, prezgodnje staranje)

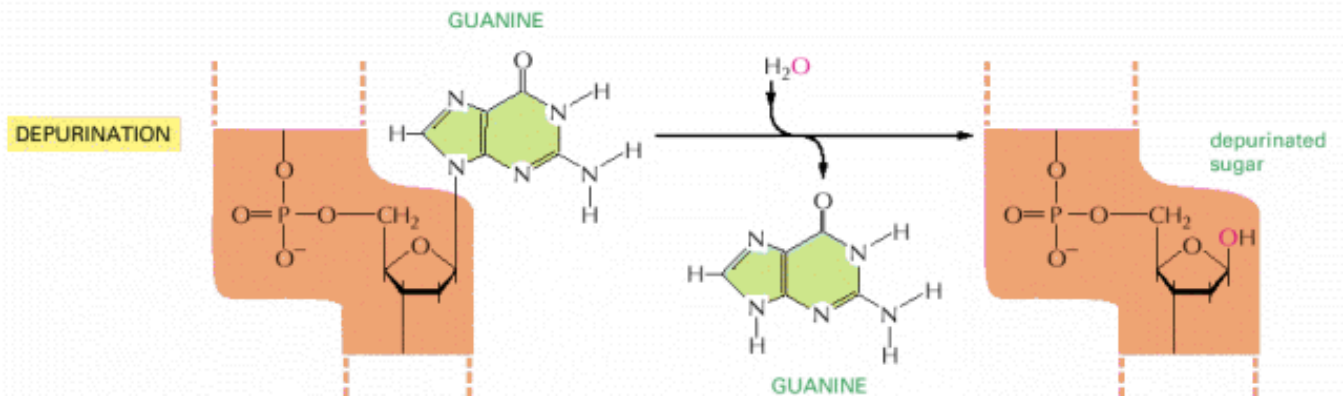
Spontane spremembe, ki spremenijo strukturo DNA



from Alberts *et al*., Molecular Biology of the Cell, 4th ed., Fig 5-46

Spontane mutacije so večinoma točkovne mutacije (tihe, nesmiselne, drugačnosmiselne, stop kodon).

Hidroliza glikozidne vezi

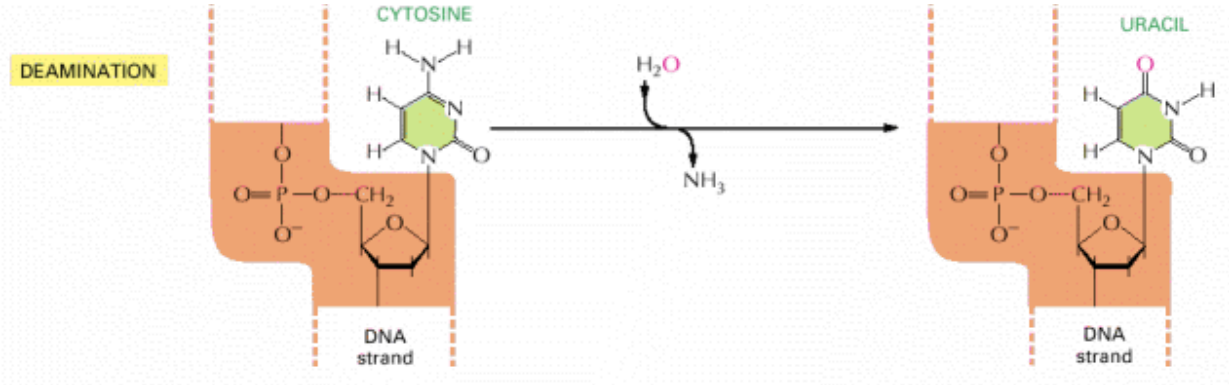


from Alberts *et al.*, Molecular Biology of the Cell, 4th ed., Fig 5-47

Pri spontani depurinaciji se zgubi 10.000 baz/celico/dan.

Nastane AP mesto.

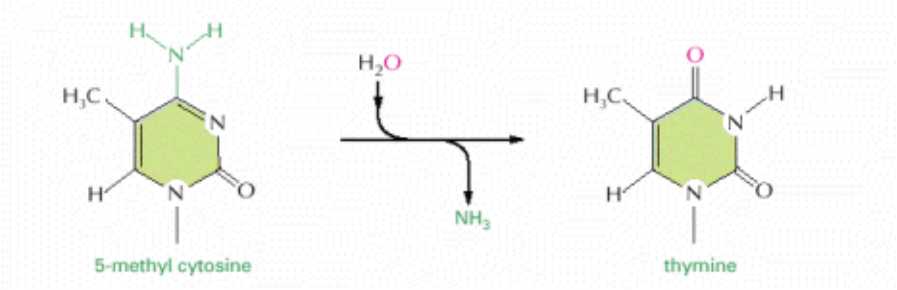
Deaminacija citozina v uracil



from Alberts *et al*., Molecular Biology of the Cell, 4th ed., Fig 5-47

Citozin se deaminira v uracil s hitrostjo 100-500/celico/dan.

Deaminacija 5-metil-citozina je zelo mutagena



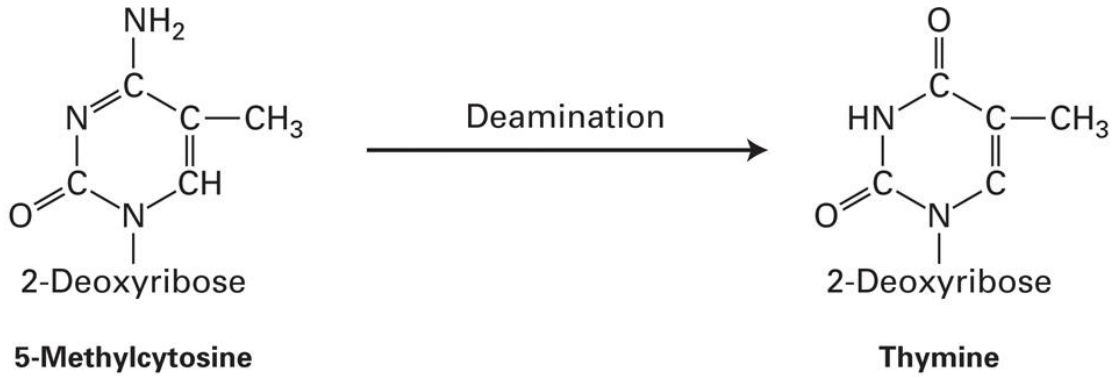
Deamination of 5-methyl cytosine to T occurs rapidly - base pairs with A

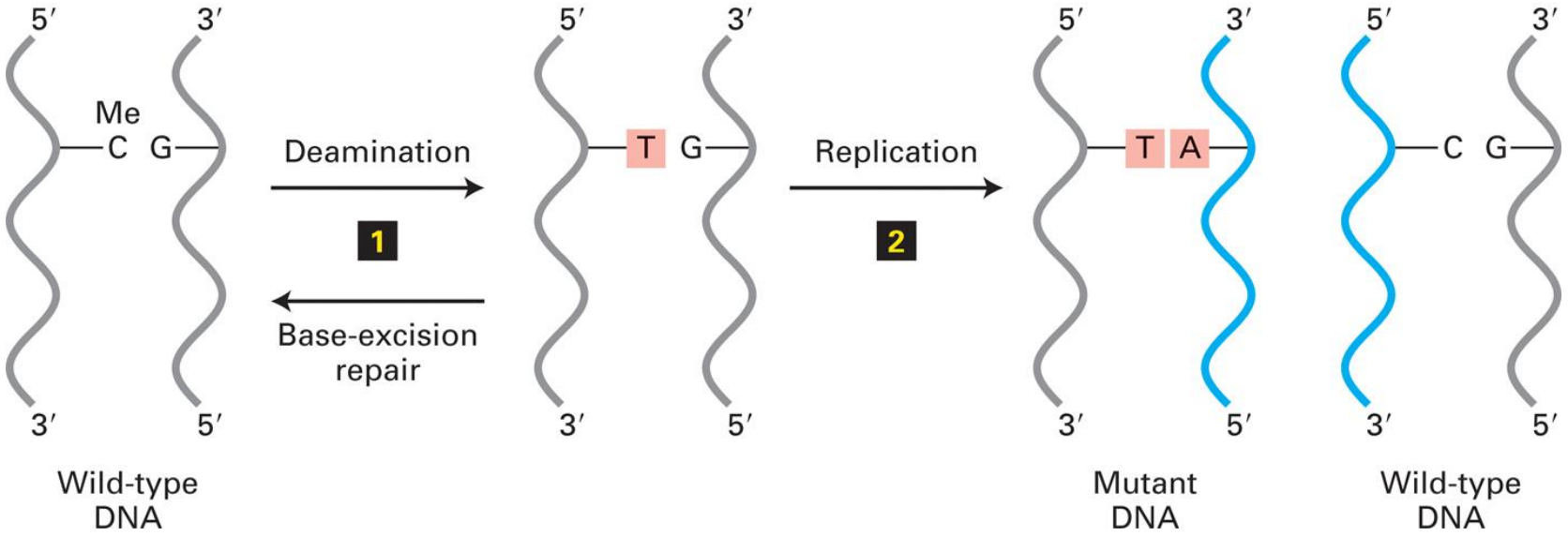
5-me-C is a target for spontaneous mutations

from Alberts *et al.*, Molecular Biology of the Cell, 4th ed., Fig 5-52

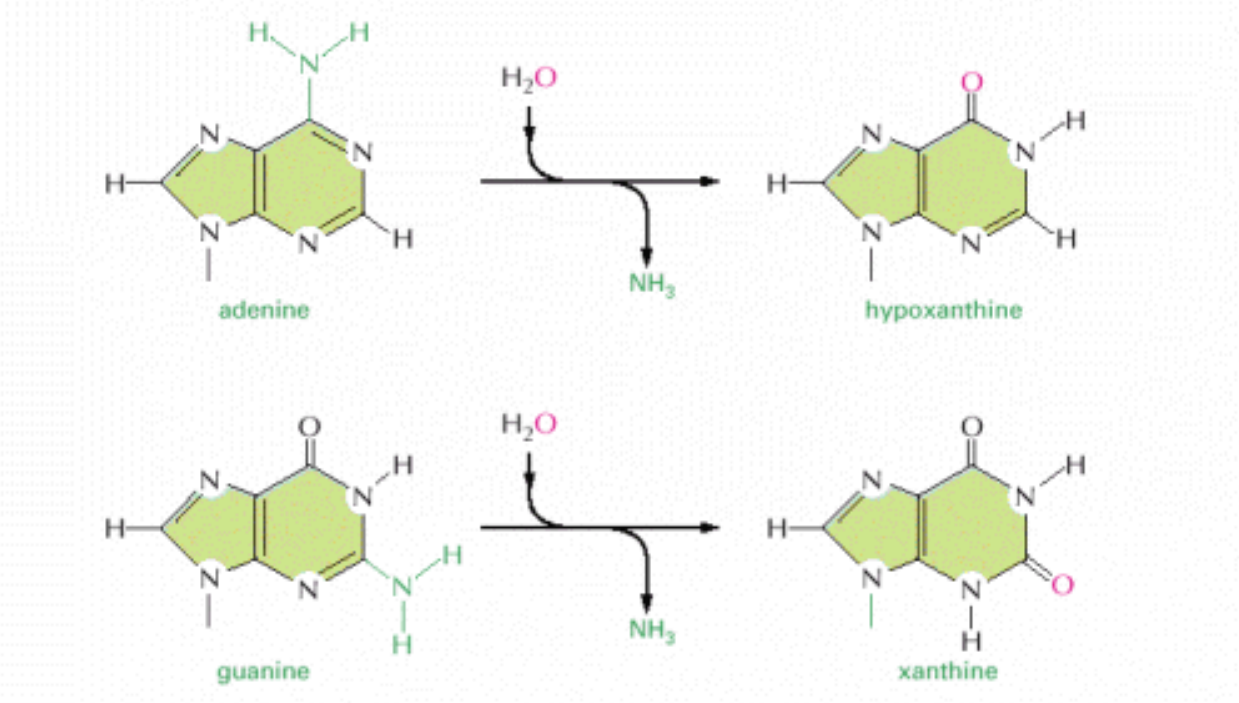
Je najpogostejša točkovna mutacija. Nastane spontano ali z deaminazami.

Z deaminacijo metil-citozina nastane točkovna mutacija





Deaminacija adenina in gvanina so redke



from Alberts *et al*., Molecular Biology of the Cell, 4th ed., Fig 5-52

Ksantin in hipoksantin se parita s citozinom.

Poškodbe DNA z oksidacijo

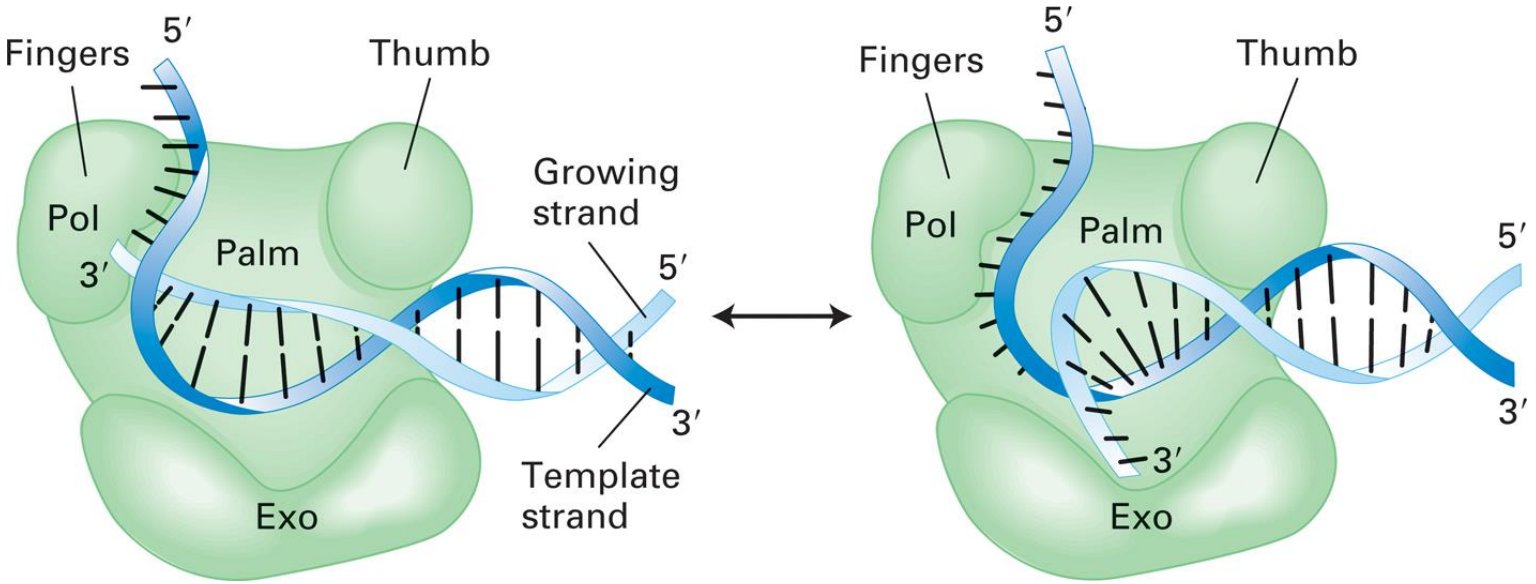
Poškodbe DNA z oksidacijo nastanejo z aerobnim metabolizmom, toksini iz okolja, aktiviranimi makrofagi, signalnimi molekulami (NO).

Mehanizmi popravljanja DNA napak

- Kontrolno branje DNA polimeraze
- Neposredno popravljanje alkiliranih baz
- Popravljanje z izrezovanjem baz
- Popravljanje neujemanja
- Popravljanje z izrezovanjem nukleotidov
- Popravljanje prelomov dvoverižne DNA
  - Popravljanje, podvrženo napakam, z združevanjem nehomolognih koncev
  - Homologna rekombinacija
  - Popravljanje prelomov ds DNA s prileganjem enojnih verig
- Človeški genom kodira 130 proteinov, ki sodelujejo pri popravljanju DNA. Nefunkcionalni proteini povzročijo nestabilnost genoma in vodijo v razvoj rakastih celic.

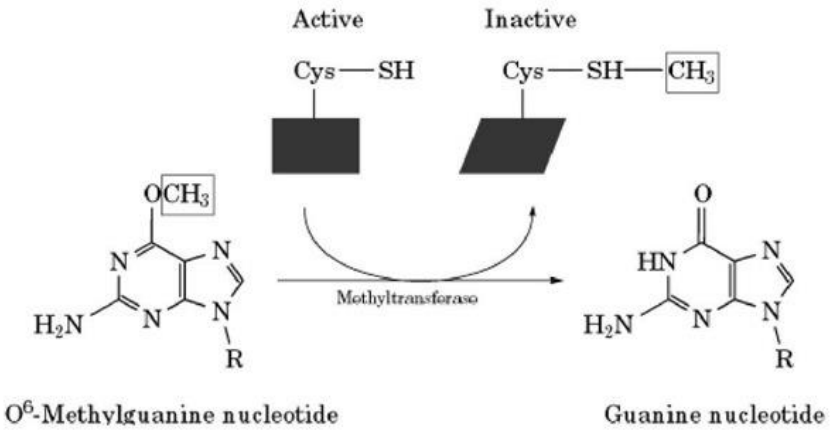
Kontrolno branje DNA polimeraz

- Polimeraze lahko vgradijo nepravilen nukleotid.
- DNA polimerazi Pol δ in Pol ε imata 3‘-5‘ eksonukleazno aktivnost.
- Polimerza se ustavi, če ni parjenja med novo-vgrajenim nukleotidom in nukleotidom na matrični verigi.
- Nastajajoča veriga se pomakne na eksonukleazno mesto v polimerazi, nepravilni nukleotid se izreže.
- Nato se 3‘ konec te verige pomakne nazaj na polimerazno mesto.



Neposredno popravljanje alkiliranih baz

- O^6-metilgvanin, pari se s timinom
- Endogeni in eksogeni povzročitelji
- Ena glavnih mutagenih poškodb DNA
- Popravljanje:
  - S Polimerazo η (translesion synthesis – TLS)
  - Z O^6-metilgvanin metiltransferazo



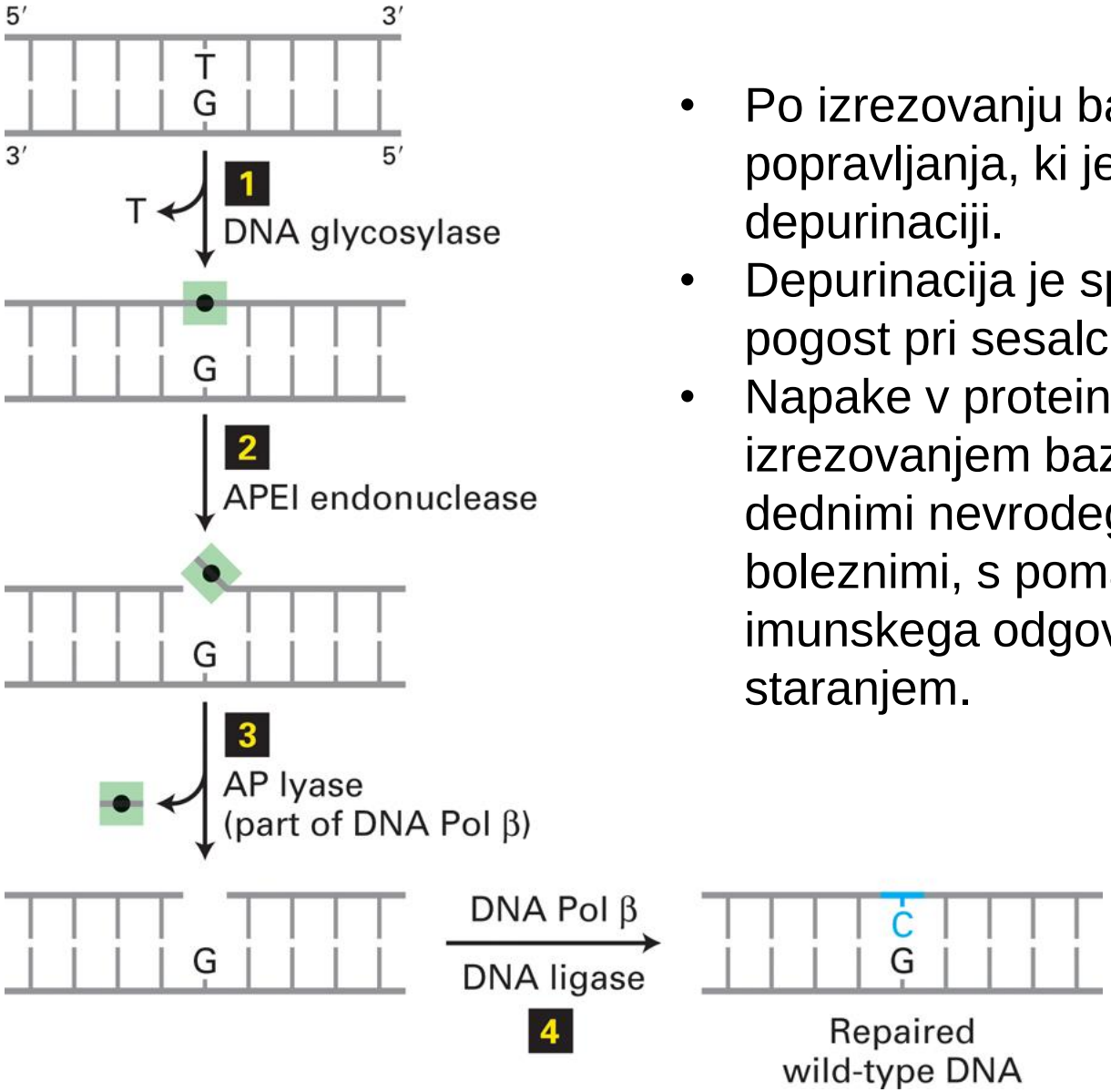
O^6-Methylguanine nucleotide

Guanine nucleotide

Popravljanje z izrezovanjem baz (base excision repair)

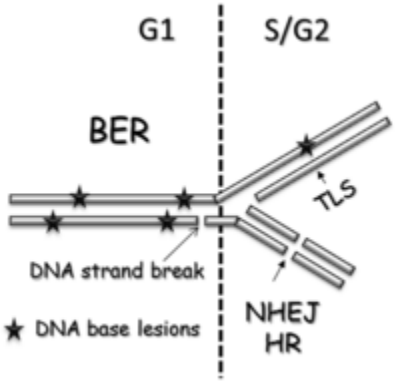
- Poteka pred DNA replikacijo.
- Popravlja napake, ki nastanejo z alkilirajočimi reagenti, oksidacijo (>100 vrst modifikacij), deaminacijo.
- Pri človeku je najpogostejša točkovna mutacija Met-C v T.
- Katera je normalna in katera je mutirana veriga?
- Evolucija popravljalnega mehanizma je šla v smeri, da se T zamenja s C.
- Neujemanje prepozna DNA glikozilaza, ki cepi N-glikozidno vez med deoksiribozo in bazo. Nastane abazično mesto (AP mesto).
- Pri človeku je veliko DNA glikozilaz, ki prepoznajo določen substrat, npr:
  - 4 uracil DNA glikozilaze
  - DNA glikozilaza za 8-oksigvanin (180 oksidacij gvanina/dan)
  - DNA glikozilaza za alkilirane baze

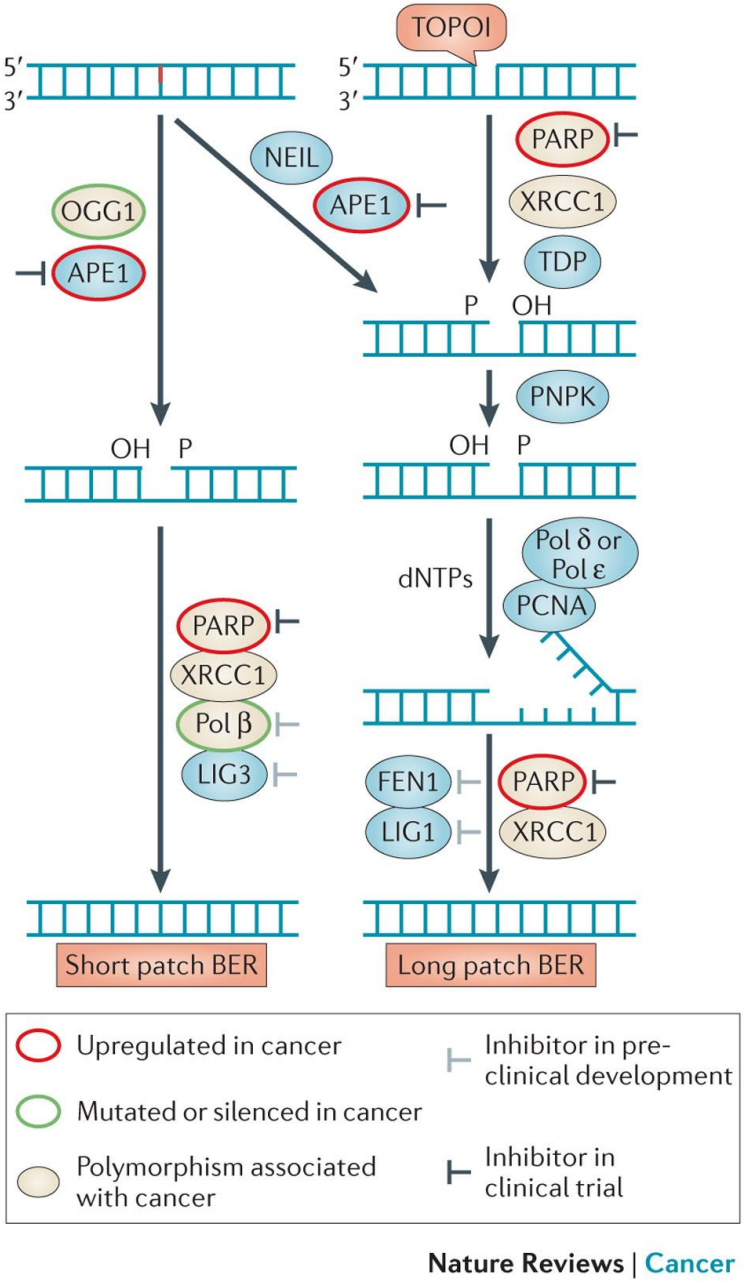
Popravljanje z izrezovanjem baz (base excision repair)



- Po izrezovanju baz sledi mehanizem popravljanja, ki je podoben tistemu pri depurinaciji.
- Depurinacija je spontan proces, pogost pri sesalcih.
- Napake v proteinih popravljanja z izrezovanjem baz so povezane z dednimi nevrodegenerativnimi boleznimi, s pomanjkanjem imunskega odgovora, z rakom, s staranjem.

Popravljanje z izrezovanjem baz (base excision repair)





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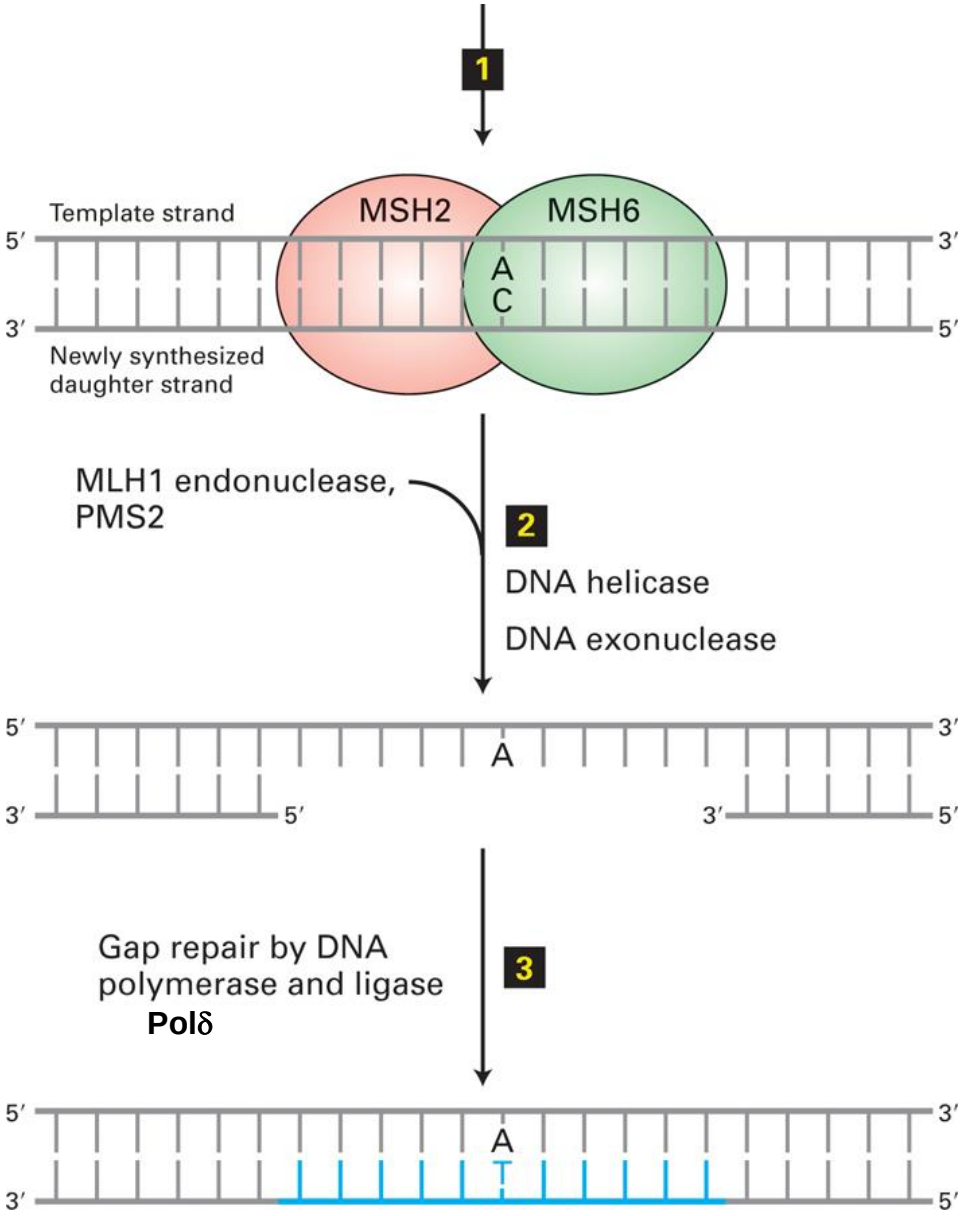
Popravljanje neujemanja (mismatch excision repair)

- Poteka po DNA replikaciji, saj popravlja napake, ki nastanejo med replikacijo.
- Proteinski kompleks loči hčerinsko in matrično verigo, a mehanizem ni znan.
- Popravlja neujemanja pri parjenju ali insercijske/delecijske zanke, ki nastanejo z deaminacijo, oksidacijo, metilacijo, reaktivnimi kisikovimi spojinami, rekombinacijskimi intermediati, replikacijskimi napakami.

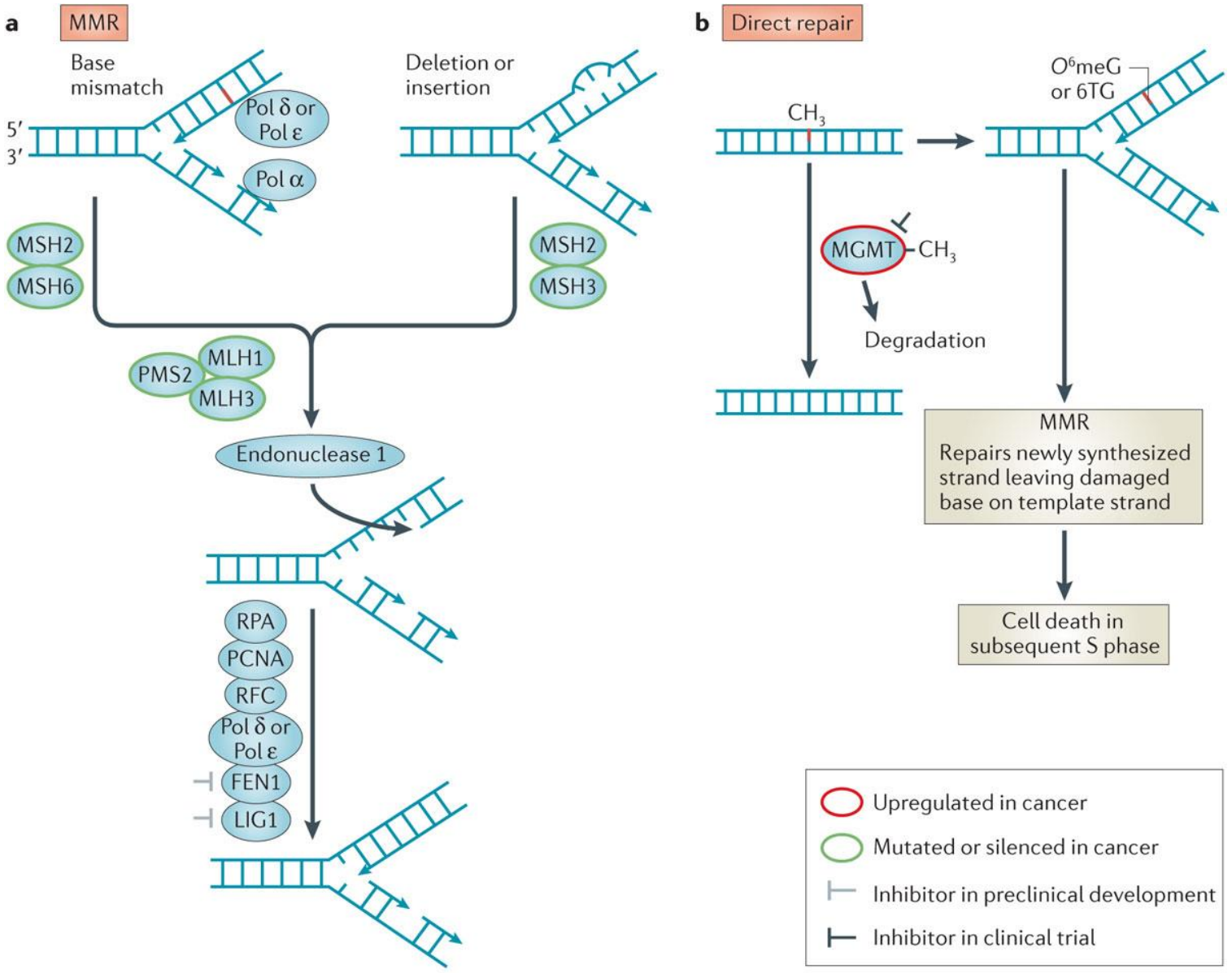
HNPCC-hereditary nonpolyposis colorectal cancer

- Predispozicijo za raka na črevesju imajo ljudje, ki so podedovali mutacijo v enem od genov MLH1 (MutL homolog1) ali MSH2 (MutS homolog2), ki sta del proteinskega kompleksa, ki sodeluje pri popravljanju neujemanja.
- Mutacija v drugem genu povzroči nastanek tumorskih celic.

Popravljanje neujemanja (mismatch excision repair)



Popravljanje neujemanja (mismatch excision repair)

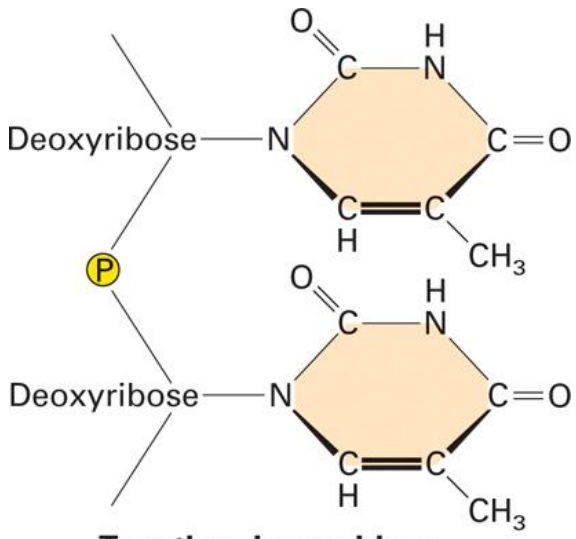


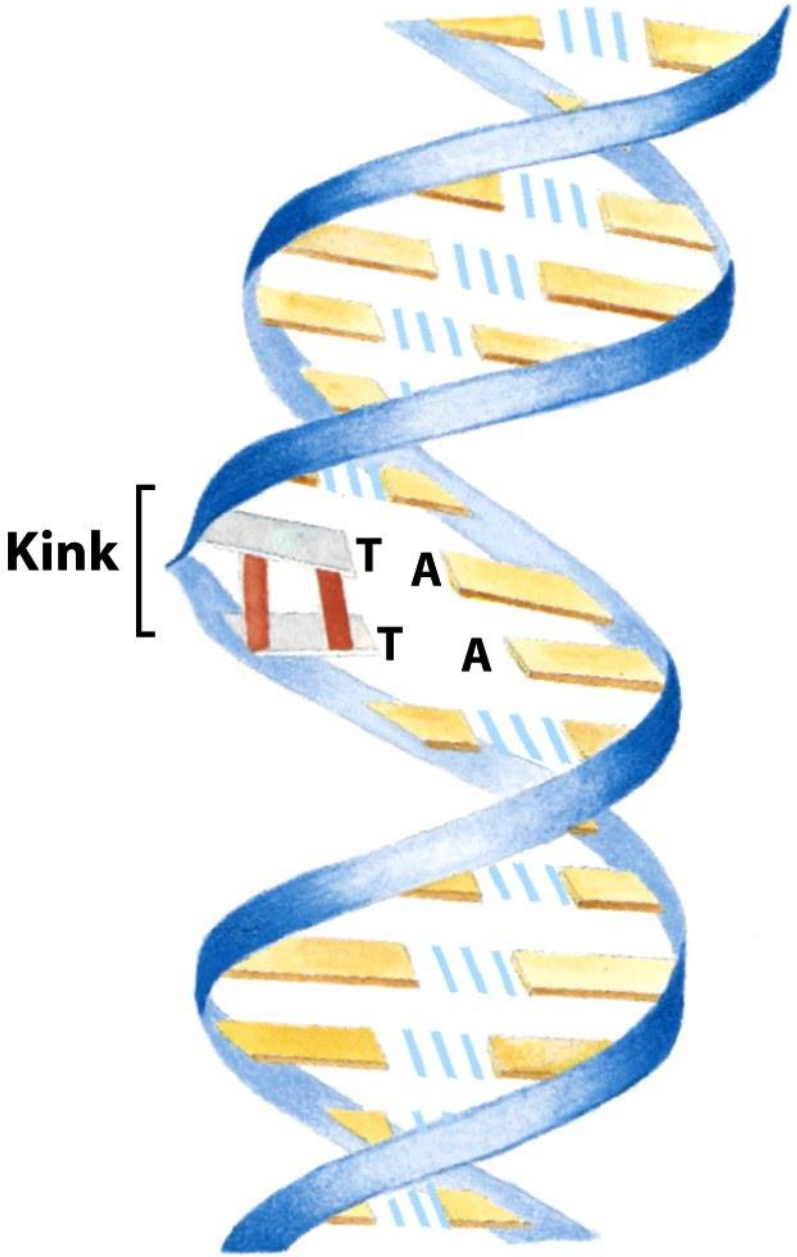
Popravljanje z izrezovanjem nukleotidov (nucleotide excision repair)

- Popravlja kemično modificirane baze, ki povzročijo lokalno spremembo strukture DNA.
- Proteini drsijo po dsDNA in iščejo manjše strukturne spremembe (npr. mehurje).
- Značilna napaka je timidinski dimer, ki nastane z UV, ciklopurini z reaktivnimi kisikovimi spojinami (ROS).
- Ubikvitinilacija histonov destabilizira nukleosom. Boljši dostop do poškodovanega kromatina.

Xeroderma pigmentosum:

- Bolniki s to boleznijo imajo predispozicijo za nastanek raka.
- Bolniki nimajo funkcionalnega mehanizma za popravljanje z izrezovanjem nukleotidov, ker imajo mutacije v genih XP (od A do G). Njihova funkcija je bila raziskana v celičnih kulturah bolnikov.
- V stiku s sončno svetlobo (UV) pogosto nastane melanom ali karcinom luskavih celic.


Deoxyribose
Deoxyribose
P
Two thymine residues
UV
irradiation
Deoxyribose
Deoxyribose
P
Thymine-thymine dimer residue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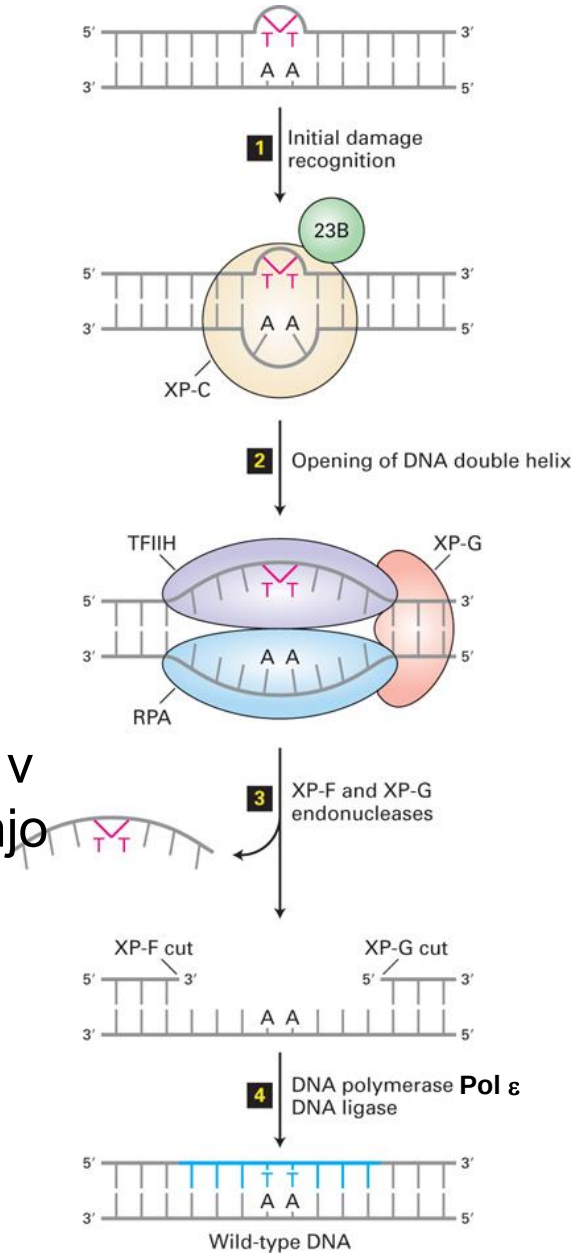
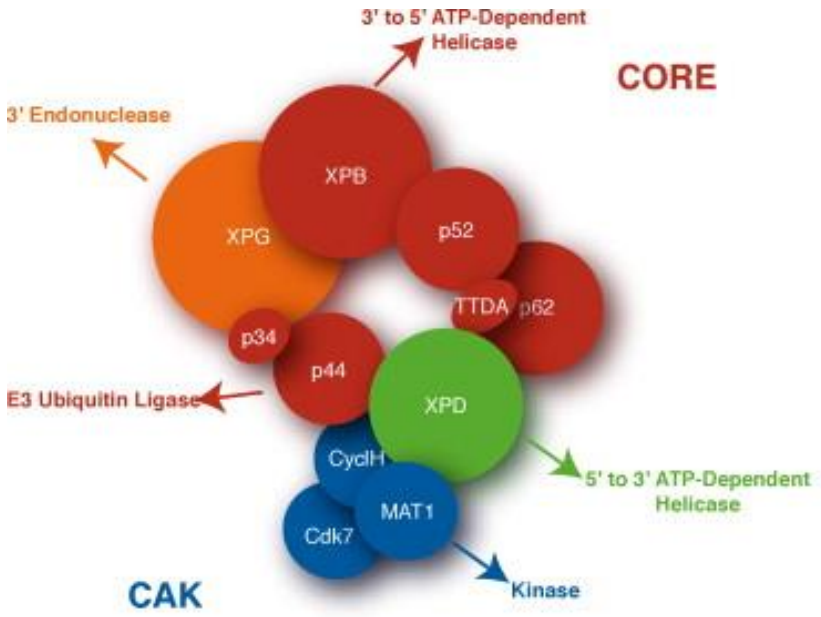
Popravljanje z izrezovanjem nukleotidov (nucleotide excision repair)

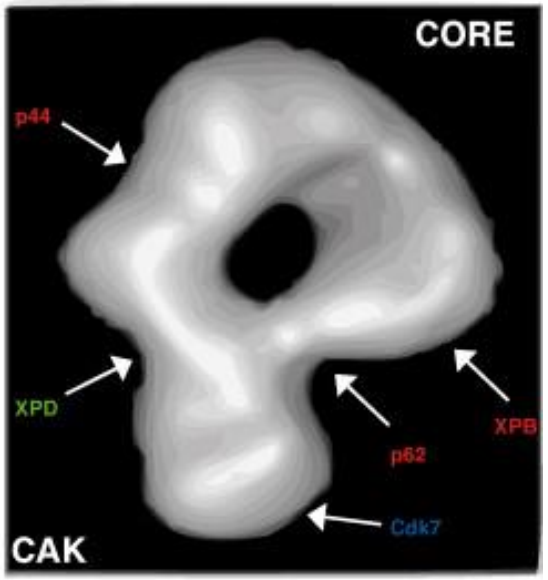
- Del proteinskega kompleksa je splošen transkripcijski faktor TFIIH, 2 podenoti imata helikazno aktivnost (hidroliza ATP).
- Izreže se 25-32 nt.
- S transkripcijo združeno popravljanje: DNA popravljanje je hitrejše v regijah, ki se prepisujejo v RNA. RNA polimeraza se ustavi pri poškodbi, nanjo se veže protein CSB, kar sproži odprtje DNA heliksa in vezavo TFIIH.



CSB - Cockayne syndrome group B protein
XP – xeroderma pigmentosum
RPA-replication protein A

Kompleks TFIIH





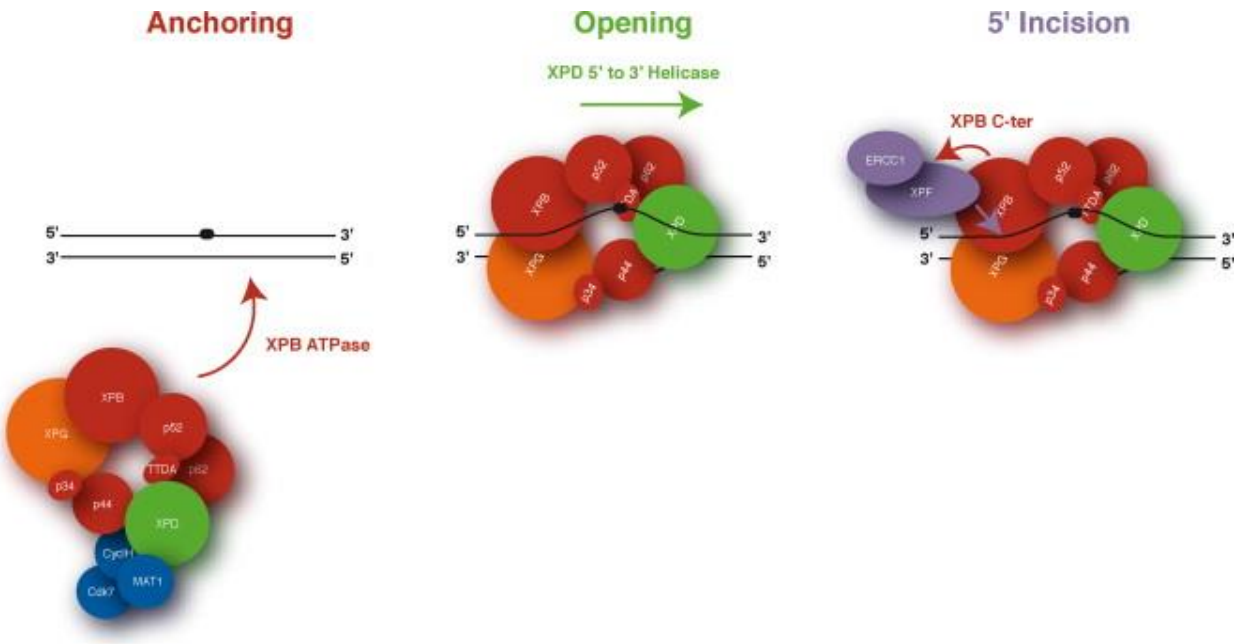
A ten or eleven TFIIH complex.
TFIIH is a ten/eleven-subunit complex composed of a core (in red; XPB, p62, p52, p44, p34 and p8/TTDA) associated to the CAK (in blue; Cdk7, Cyclin H and MAT1) through the XPD subunit (in green). We propose that XPG is a bona fide component of TFIIH, the eleventh subunit. Four enzymatic activities are found in TFIIH; XPB and XPD are 3′ to 5′ and 5′ to 3′ helicases respectively, Cdk7 is a kinase and p44 has been described as an E3 ubiquitin ligase in yeast. The electronic microscopic structure of human TFIIH and the localization of the subunits are shown.

Jean-Marc Egly , Frédéric Coin

A history of TFIIH: Two decades of molecular biology on a pivotal transcription/repair factor

DNA Repair, Volume 10, Issue 7, 2011, 714 - 721

Funkcija TFIIH podenot XPB in XPD pri popravljanju DNA z izrezovanjem nukleotidov



Function of the XPB and XPD subunits of TFIIH in NER.
XPB ATPase is required to anchor TFIIH to the damaged DNA (left panel) while XPD helicase opens damaged DNA (middle panel) to allow incision by ERCC1-XPF and XPG. The C-terminal end of XPB is necessary for the incision generated in 5′ by ERCC1-XPF in a mechanism that is not known (right panel).

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TFIIH pri človeku

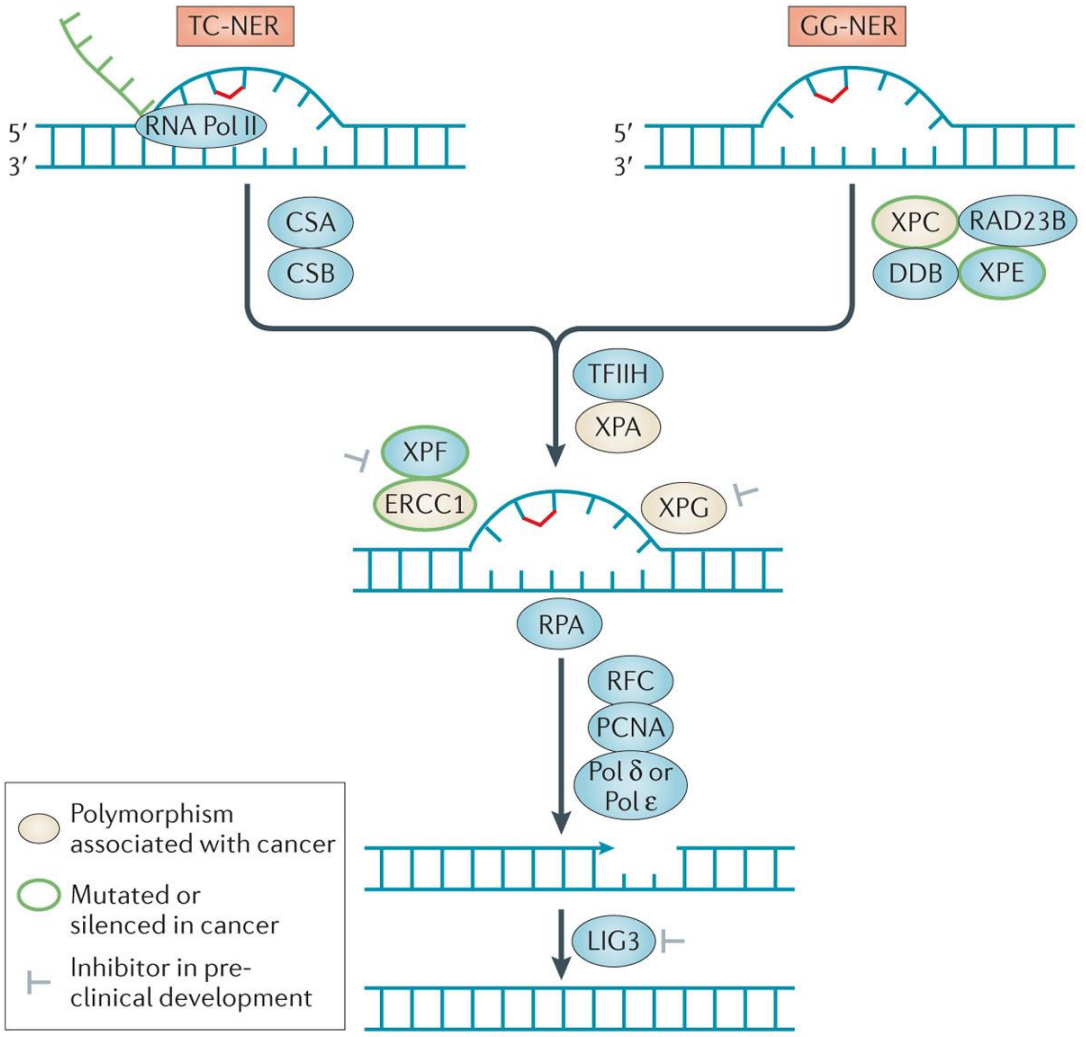
TFIIH composition in human.			
Sub-complexes	Human	Function	Human genetic disorder(s)
Core	XPG	3′ Endonuclease	XP, XP/CS
	XPB	3′ to 5′ Helicase	XP/CS, TTD
	XPD	5′ to 3′ Helicase	XP, XP/CS, TTD
	p62		
	p52		
	p44		
	p34		
	TTDA		TTD
Kinase	CyclinH		
	MAT1		
	Cdk7	Kinase	
XP - xeroderma pigmentosum			
CS - Cockayne syndrome			
TTD - trichothiodystrophy			

Jean-Marc Egly , Frédéric Coin

A history of TFIIH: Two decades of molecular biology on a pivotal transcription/repair factor

DNA Repair, Volume 10, Issue 7, 2011, 714 - 721

Popravljanje z izrezovanjem nukleotidov (nucleotide excision repair)



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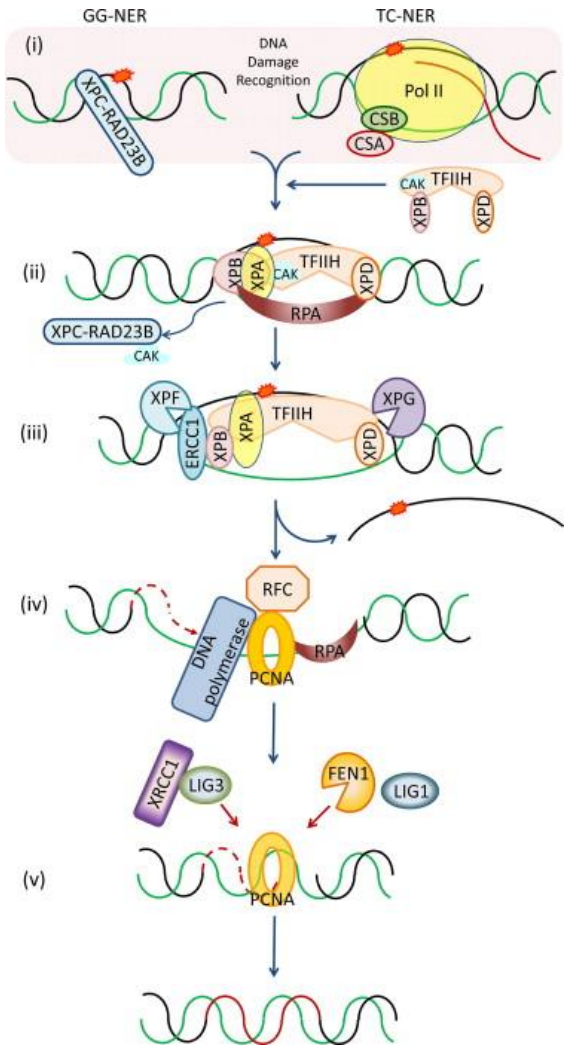
Pol ε

Popravljanje z izrezovanjem nukleotidov (nucleotide excision repair)

Nucleotide excision repair pathways. Two subpathways of mammalian NER: GG-NER and TC-NER. (i) XPC-RAD23B recognizes DNA damage-induced structural change as the initiation step of GG-NER. TC-NER is initiated by stalling of an elongating RNAP at a blocking lesion on the transcribed strand within an active gene. After these initial recognition steps, GG-NER and TC-NER pathways involve many of the same protein components. (ii) Following recognition, the TFIIH complex is recruited. Through the activity of the helicase subunits, XPB and XPD, TFIIH promotes opening of the DNA duplex around the lesion, facilitating recruitment of XPA and RPA. (iii) The XPF–ERCC1 complex is recruited to the lesion via a direct interaction with XPA, while XPG is specifically engaged through an interaction with TFIIH. The two endonucleases, XPF–ERCC1 and XPG, are responsible for carrying out incision 5′ and 3′, respectively, to the DNA damage. (iv) After dual incision and removal of the damage-containing oligonucleotide fragment, a DNA polymerase carries out gap-filling repair synthesis in cooperation with RFC and PCNA. (v) Finally, the nick is sealed by either XRCC1–LIG3α or a FEN1–LIG1 complex. CAK, the cyclin-dependent kinase (CDK)-activating kinase; GG-NER, global genome-NER; RFC, replication factor C; RPA, replication protein A; TC-NER, transcription-coupled NER; TFIIH, transcription factor II H.



Teruaki Iyama , David M. Wilson III, **DNA repair mechanisms in dividing and non-dividing cells**

DNA Repair, Volume 12, Issue 8, 2013, 620 - 636

Prelomi ds DNA

- Preprečijo, da bi celica vstopila v mitozo.
- Prelomi se lahko popravijo – celični cikel
- Prelomi se napačno popravijo – kromosom ima nepravilno strukturo
- Prelom se ne popravi – celica gre v programirano smrt.

Popravljanje prelomov ds DNA

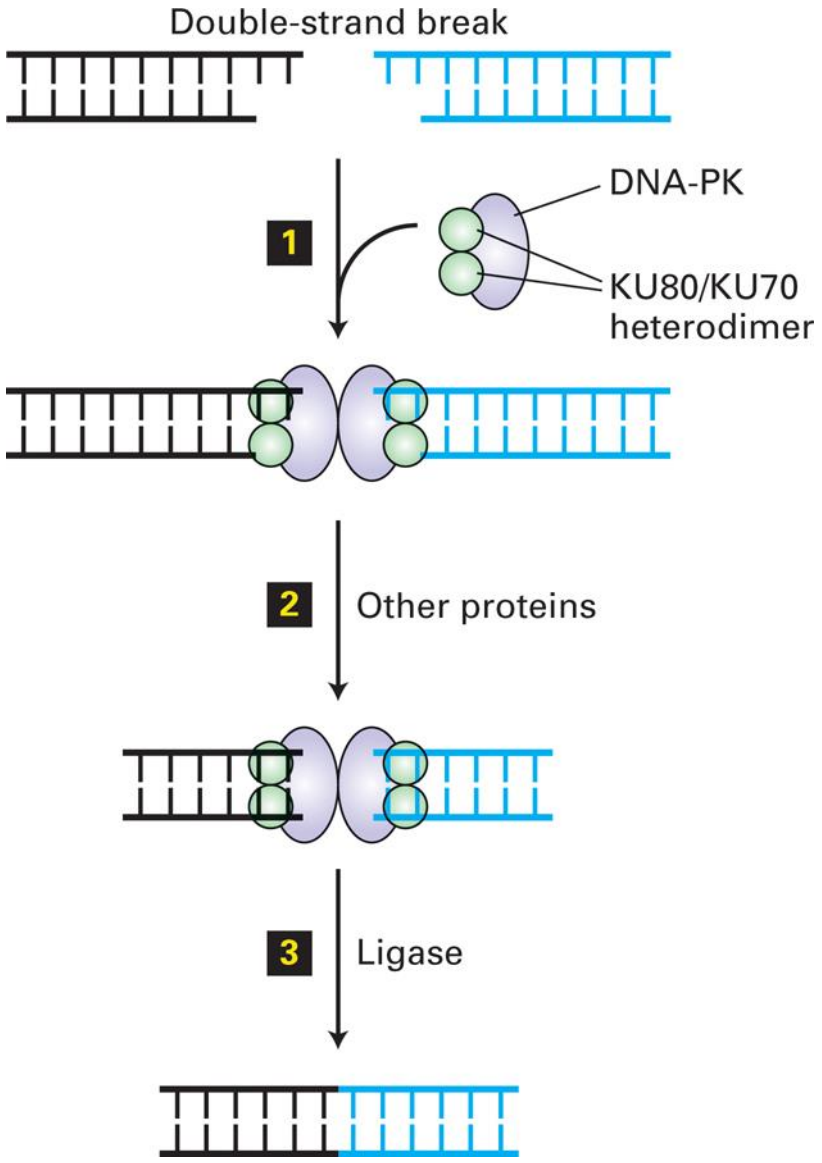
- Povzročajo jih ionizirajoča sevanja in zdravila proti raku (eksogena dejavnika) ter ROS (endogeni dejavnik).

- Huda poškodba, ker lahko povzroči reorganizacije genoma in spremeni izražanje genov.

- 3 mehanizmi:
  - homologna rekombinacija
  - nehomologno združevanje koncev
  - popravljanje prelomov ds DNA s prileganjem enojnih verig

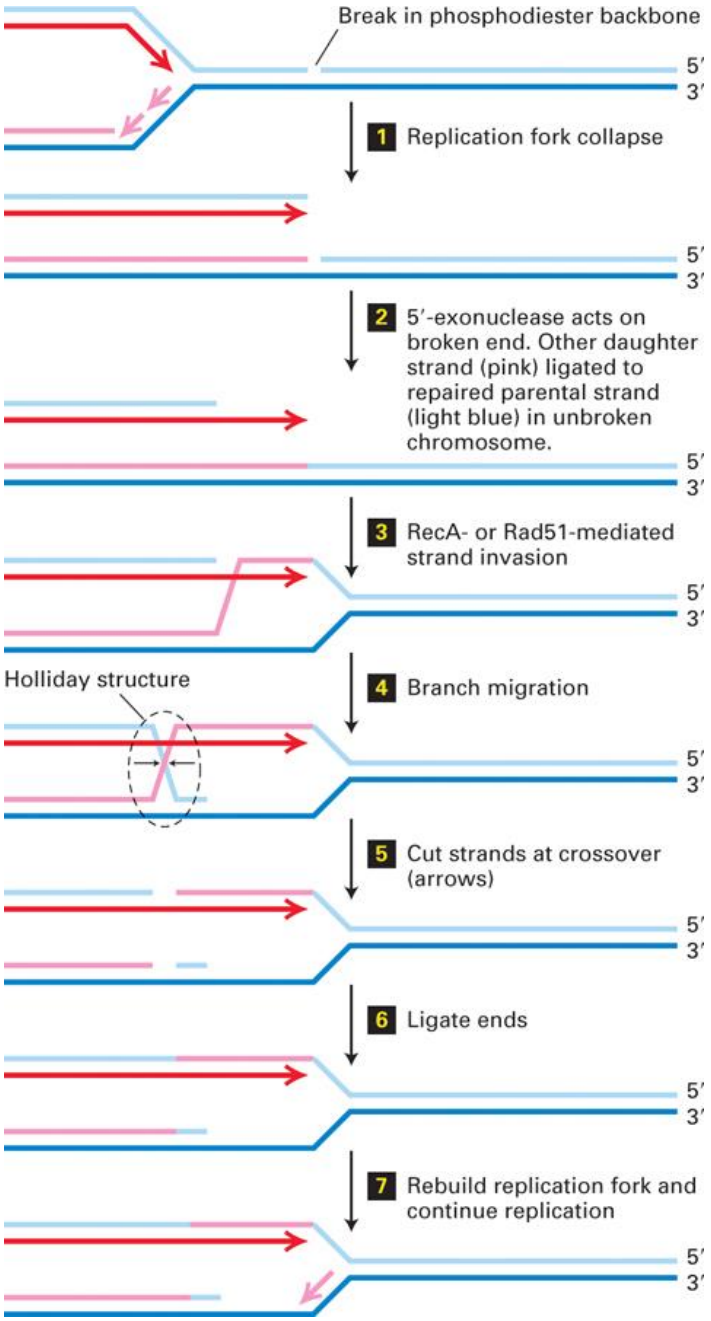
Nehomologno združevanje koncev

- Popravljanje, podvrženo napakam (error-prone repair)
- Glavni način popravljanja prelomov dsDNA, če v bližini ni sestrske kromatide.
- Pri zdrževanju nehomolognih koncev se izgubi nakaj nukleotidov – popravljanje lahko povzroči mutacijo.
- Večinoma se združijo pravi konci, ker se v jedru molekule lahko le malo premikajo.
- Translokacije delov DNA iz enega kromosoma na drugega vodijo v nenormalno celično rast (rak).



Poprava poškodovanih vilic

- Če se poškodba DNA ne popravi pred replikacijo, se replikacijske vilice tam sesedejo.
- ‚branch' je 1 fosfodiesterska vez
- V tem procesu poteka rekombinacija, popravljanje napak in ponovna replikacija.



Homologna rekombinacija

- Popravljanje preloma dsDNA
- Izmenjava večjih delov dsDNA; zelo pogost se dogaja med mejozo
- Poveča genetsko raznolikost.

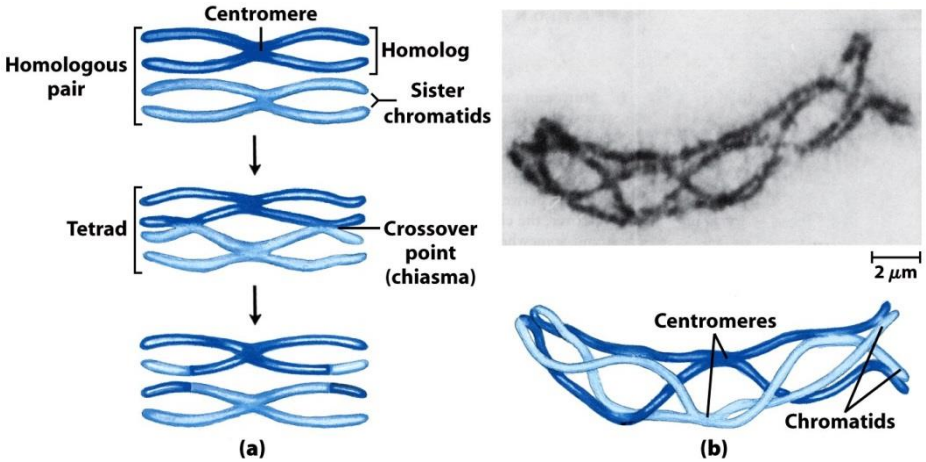


Figure 25-32
Lehninger Principles of Biochemistry, Fifth Edition

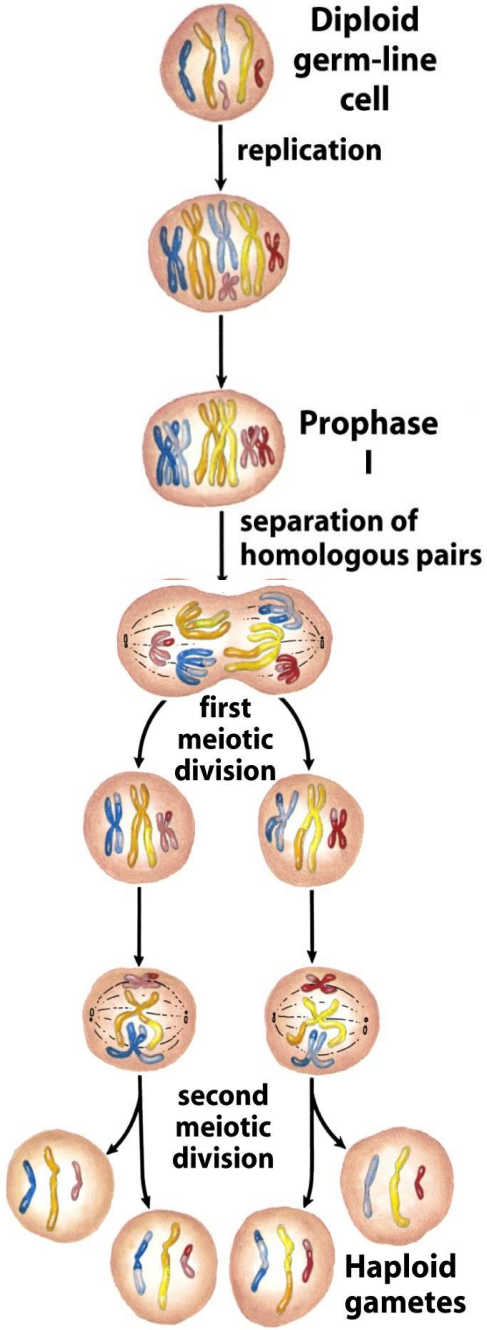
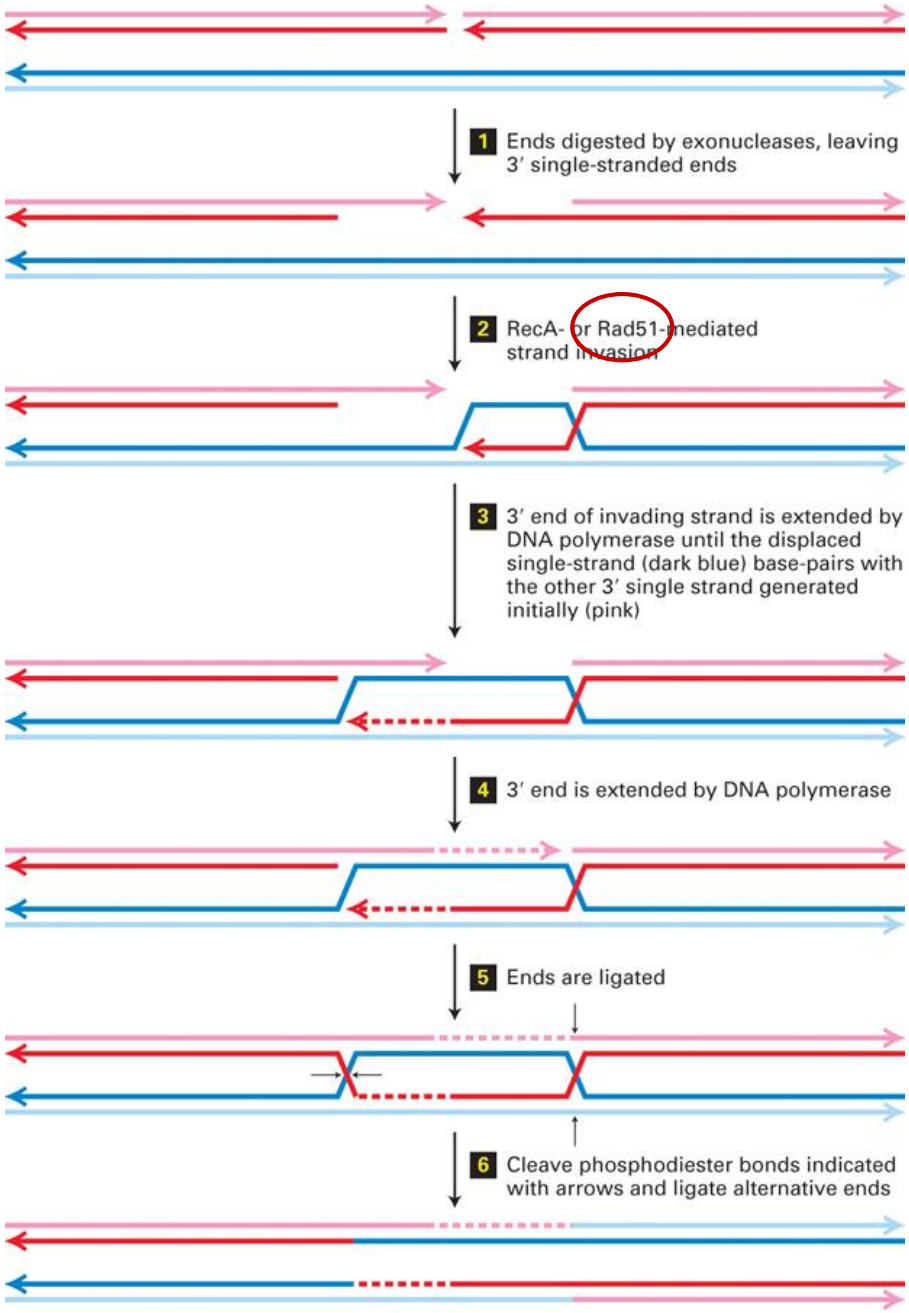
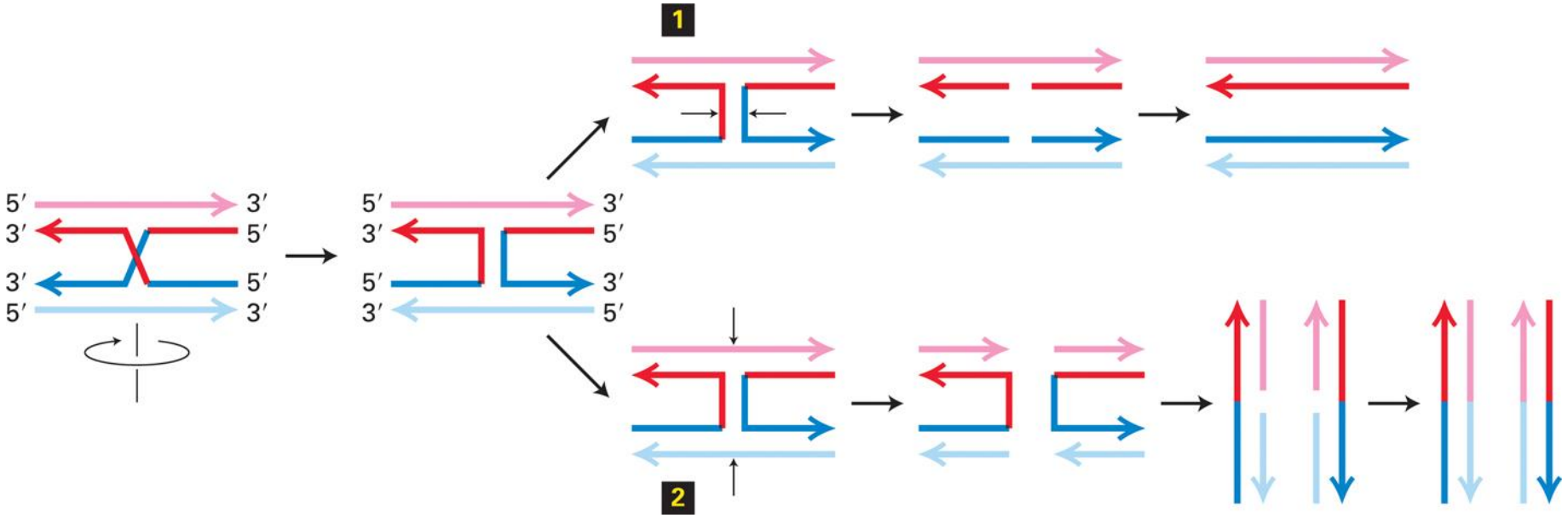


Figure 25-31 part 2
Lehninger Principles of Biochemistry, Fifth Edition

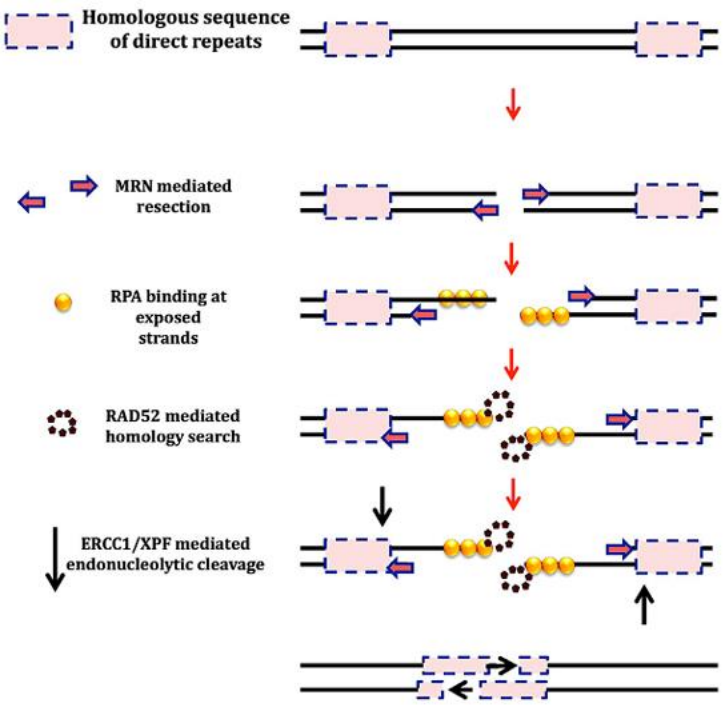
Homologna rekombinacija



Alternativni razpleti Hollidayevih struktur



Popravljanje prelomov dsDNA s prileganjem enojnih verig repetitivnih elementov

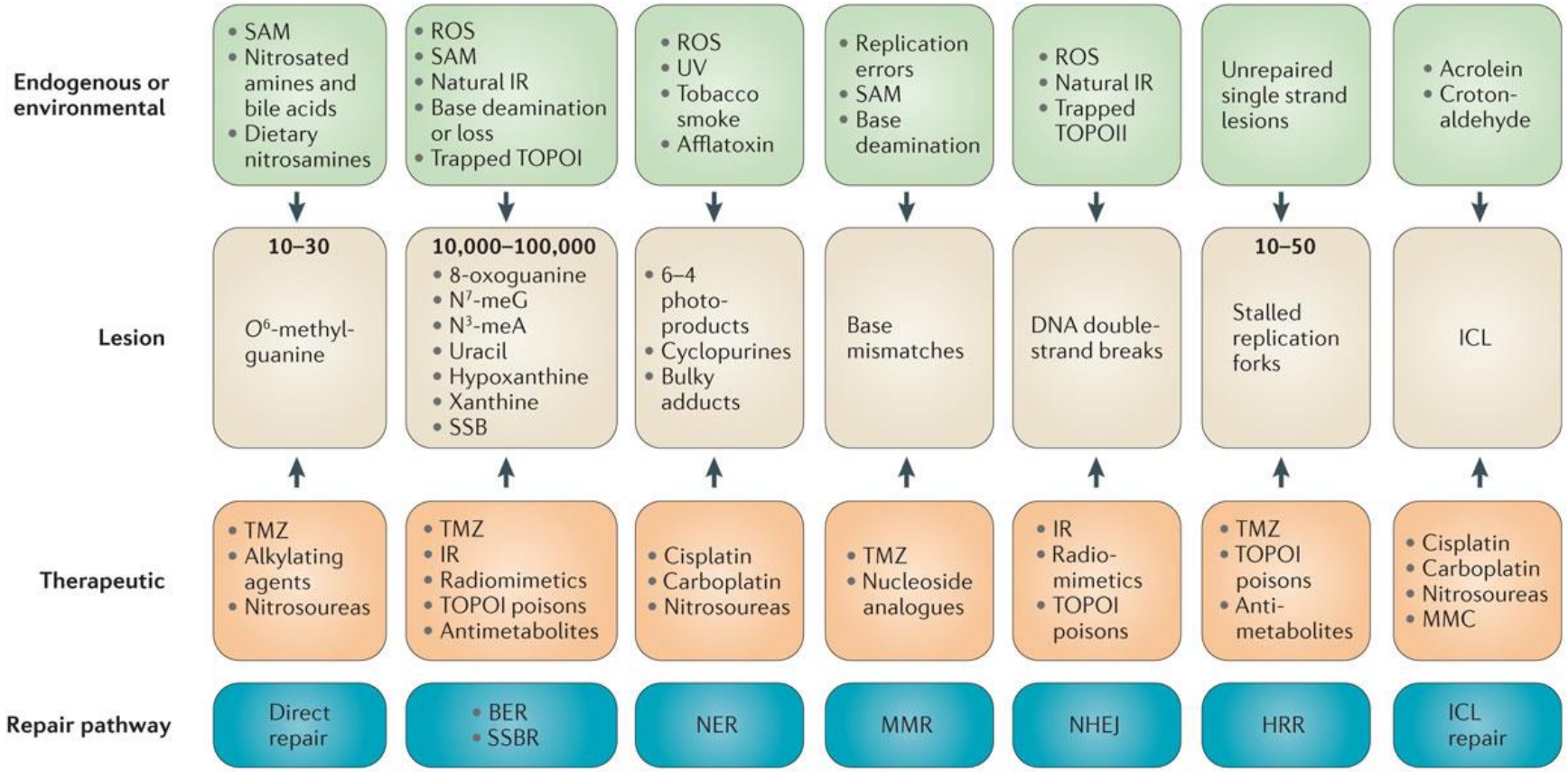


Double DNA break repair via Single Strand Annealing (SSA). After DNA undergoes DSBs, it may be repaired in a non-conservative mechanism via SSA. MRN mediated resection of damaged DNA ends causes recruitment of RPA, which coat the resulting single strands and recruits Rad52 that commences search for homology sequence in the direct repeats. ERCC1 causes endonucleolytic cleavage following by gap filling and ligation. This mode of repair results in loss of DNA sequence and hence known as non-conservative DNA repair. (Khalil in sod, Biodiscovery 2012).

Homologna rekombinacija in rak

- BRCA1 in BRCA2 sodelujeta pri transkripciji, ohranjevanju kromosomov, popravljanju DNA in celičnem ciklu.
- BRCA2 sodeluje pri popravljanju prelomov dsDNA
- Mutacije v vsaj enem genu povzročijo več kot 80 % verjetnost za razvoj raka na dojkah (tudi raka na jajčnikih).

Viri DNA poškodb in njihovo popravljanje



BER-base excision repair, HRR-homologous excision repair, ICL-interstrand crosslink, IR-ionizing radiation, MMC-mitomycin C, NER-nucleotide excision repair, NHEJ-non-homologous end joining, ROS-reactive oxygen species, SAM-S-adenosylmethionine, SSB-single-strand break, SSBR-SSB repaire, TMZ-temozolomide, TOPO-topoizomerase.

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TABLE 24-2 Some Human Hereditary Diseases and Cancers Associated with DNA-Repair Defects

Disease	DNA-Repair System Affected	Sensitivity	Cancer Susceptibility	Symptoms
PREVENTION OF POINT MUTATIONS, INSERTIONS, AND DELETIONS				
Hereditary nonpolyposis colorectal cancer	DNA mismatch repair	UV irradiation, chemical mutagens	Colon, ovary	Early development of tumors
Xeroderma pigmentosum	Nucleotide excision repair	UV irradiation, point mutations	Skin carcinomas, melanomas	Skin and eye photosensitivity, keratoses
REPAIR OF DOUBLE-STRAND BREAKS				
Bloom's syndrome	Repair of double-strand breaks by homologous recombination	Mild alkylating agents	Carcinomas, leukemias, lymphomas	Photosensitivity, facial telangiectases, chromosome alterations
Fanconi anemia	Repair of double-strand breaks by homologous recombination	DNA cross-linking agents, reactive oxidant chemicals	Acute myeloid leukemia, squamous-cell carcinomas	Developmental abnormalities including infertility and deformities of the skeleton; anemia
Hereditary breast cancer, BRCA-1 and BRCA-2 deficiency	Repair of double-strand breaks by homologous recombination		Breast and ovarian cancer	Breast and ovarian cancer

SOURCES: Modified from A. Kornberg and T. Baker, 1992, *DNA Replication*, 2d ed., W. H. Freeman and Company, p. 788; J. Hoeijmakers, 2001, *Nature* **411:**366; and L. Thompson and D. Schild, 2002, *Mutation Res.* **509:**49.

Pathway	Proteins	Importance in cancer	Refs
Direct repair	MGMT	Higher levels in tumours than in normal tissue confers resistance to DNA-alkylating agents. Methylation of the *MGMT* promoter is associated with better response to BCNU and TMZ in brain tumours	14,150, 151
BER	OGG1	Truncating mutations and R46Q variant (which has reduced activity) observed in renal cancers. *OGG1* methylation is seen in various cancers and loss of expression is associated with poor prognosis in breast cancer. The polymorphism OGG1-S326C is associated with reduced activity and increased risk of developing lung cancer	22,23
	APE1	High APE1 expression in several tumour types is associated with drug and radiotherapy resistance	24
	XRCC1	The polymorphism XRCC1-R194W increased the efficiency of BER and is protective against cancer. The polymorphism XRCC1-R399Q reduced the efficiency of BER and predisposes to cancer	23,152
	PARP1	Higher levels of expression occur in tumours. The polymorphism PARP1-V762A confers reduced activity and predisposes to various cancers	152,153
	Pol β	30% of tumours have Pol β mutations that are not found in normal tissue, including frameshifts, del208-236, and K289M and I260M dominant-negative, transforming mutations	23
Global NER	XP proteins	XP (which is caused by defective global NER) is characterized by UV radiation sensitivity and skin cancer. *XPC* methylation occurs in bladder cancer. SNPs in XPA and XPC have been associated with lung and bladder cancer, and SNPs in XPG have been associated with sensitivity to chemotherapy	42,154
	ERCC1	ERCC1 polymorphisms are associated with skin and lung cancer. *ERCC1* methylation occurs in glioma	42
TLS	Pol H and Pol Q	Aberrant expression observed in several tumour types	45
MMR	MSH2 and MLH1	MMR defects cause Lynch syndrome and HNPCC, which are associated with colorectal, stomach, ovarian and endometrial cancers. *MLH1* promoter methylation is associated with spontaneous tumours in these tissues. MMR defects confer resistance to the DNA-methylating agents 6-thioguanine and cisplatin	48,51
DSB repair	NBS1	Nijmegen breakage syndrome (which is caused by defective NBS1) is characterized by chromosome instability, immunodeficiency, ionizing radiation sensitivity and cancer predisposition, especially lymphoma; heterozygous mutants are also cancer prone	155,156
	MRE11	Point mutations are observed in ovarian cancers and shortening of the T(11) repeat microsatellite occurs in 93% of primary colorectal cancer probably as a result of MMR-induced microsatellite instability	50, 157–159
NHEJ	KU70	SNPs associated with breast cancer, and epigenetic silencing is associated with breast, colorectal and lung cancer	160
	KU80	Epigenetic silencing is associated with lung cancer	161
	DNA-PKcs, ligase 4 and XRCC4	SNPs associated with glioma. SNPs may protect against breast and lung cancer. MiR-101 (which is induced by NMYC) targets DNA-PKcs and thus reduces its expression	159, 162,163
HRR	BRCA1 and BRCA2	BRCA1 and BRCA2 mutation carriers have increased risk of breast, ovarian, prostate, pancreatic, melanoma and other gastrointestinal, gynaecological and haematological malignancies. Methylation of the *BRCA1* promoter is common in spontaneous breast, ovarian and lung cancers	128,164, 165
	XRCC2	XRCC2 is a RAD51 paralogue, frameshift mutation owing to microsatellite slippage that in MSI tumours confers sensitivity to crosslinking agents	166
	RAD50	Frameshift mutations in the *RAD50*-associated microsatellite, which results in a truncated protein, occur in 31% of gastrointestinal cancers	167
	FANC proteins	Fanconi's anaemia is associated with haematological malignancies, especially MDS and AML, HNSCC, and oesophageal and gynaecological cancer. Most mutations occur in FANCA (65%), FANCC (15%) or FANCG (10%). *FANCD1* (*BRCA2*), *FANCN* (*PALB2*) and *FANCJ* (*BACH1*; also known as *BRIP1*) are breast cancer susceptibility genes. Methylation of FANC genes is common in sporadic cancers; for example, *FANCF* is methylated in lung, ovarian and cervical cancer	69,168, 169
Cell cycle checkpoints	ATM and CHK2	Ataxia telangiectasia (which is caused by defects in ATM) is associated with radiosensitivity and 100-fold increased cancer predisposition. Heterozygous germline mutations in *ATM* are associated with leukaemia and breast and pancreatic cancer. Epigenetic silencing of *ATM* and *ATM* polymorphisms are associated with breast, lung and colorectal cancer. MiR-421 and miR-101, which are induced by NMYC, both target ATM. CHK2 is a candidate tumour suppressor gene, inactivation is observed in multiple human tumours	163,170, 171,172
	ATR	Frameshift mutations caused by deletions in the A(10) microsatellite as a consequence of MMR defects are associated with leukaemia, lymphoma and stomach and endometrial cancer	79

AML, acute myeloid leukaemia; APE1, AP endonuclease 1; ATM, ataxia-telangiectasia mutated; ATR, ataxia-telangiectasia and Rad3-related; BACH1, BRCA1-associated C-terminal helicase 1; BER, base excision repair; BRIP1, BRCA1-interacting protein C-terminal helicase 1; DDR, DNA damage response; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; DSB, DNA double-strand break; FANC, Fanconi anaemia group protein; HNPCC, hereditary nonpolyposis colorectal cancer; HNSCC, head and neck squamous cell carcinoma; HRR, homologous recombination repair; MGMT, O^6-methylguanine DNA methyltransferase; MDS, myelodysplastic syndrome; miR, microRNA; MMR, mismatch repair; MSI, microsatellite instability; NBS1, Nijmegen breakage syndrome protein 1; NER, nucleotide excision repair; NHEJ, non-homologous end joining; OGG1, 8-oxoguanine DNA glycosylase; PARP1, poly(ADP-ribose) polymerase 1; Pol, DNA polymerase; SNP, single nucleotide polymorphism; TLS, translesion synthesis; TMZ, temozolomide; UV, ultraviolet; XP, Xeroderma pigmentosum.

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Inhibitorji proteinov v popravljalnih mehanizmih

Pathway	Target	Inhibitor	Current stage
Direct repair	MGMT	O^6-benzylguanine and lomeguatrib	The first clinical trial of O^6-benzylguanine in combination with BCNU was reported in 1998 (REF. 10). Currently in Phase II clinical trials with toxicity issues and marginal benefit. Lomeguatrib plus TMZ combinations are in Phase II trials but no positive data have been reported (dose and tumour type issues?)[12]
BER	FEN1	NSC-281680	*In vitro* TMZ sensitization[173]
	Pol β	Pamoic acid, oleanolic acid and eicosapentaenoic acid	*In vitro* sensitization to IR and bleomycin[174,175]
	Ligase 1 and ligase 3	L67 and L189	*In vitro* sensitization to MMS and IR[176]
	APE1	Methoxyamine	Responses in combination with pemetrexed seen in Phase I trial. Phase II combinations with pemetrexed and with TMZ are underway[177]
		Lucanthone	Phase I trial of combination with TMZ induced radiosensitization (also undergoing testing with a topoisomerase II poison)[30]
		CRT0044876	Preclinical evidence of TMZ sensitization[31,178]
	PARP	AG014688 (also known as CO-338 and rucaparib)	The first PARPi in clinical trial. Phase I and II trials with TMZ in patients with melanoma showed profound PARP inhibition and some clinical responses but increased myelosuppression in Phase II trials, single agent Phase II trials in patients with breast and ovarian cancer with BRCA mutations[35,36]. Phase I trials with various cytotoxic combinations are underway
		AZD2281 (also known as olaparib)	Good single agent activity (40% response rate) demonstrated in BRCA mutation-associated ovarian cancer and 41% in breast cancer and 24% in patients with ovarian cancer without BRCA mutations at the MTD[103]. Phase II single agent trial in selected patients[104,179]. Several Phase I and II trials with a variety of drug combinations; however, there have been toxicity issues with topotecan combination and little benefit was observed with dacarbazine combination[180,181]
		ABT-888 (also known as veliparib)	Phase 0 trial to determine active dose. Phase I/II single agent and combination trials are ongoing in various solid and lymphoid tumours. Combinations with topotecan and cyclophosphamide were tolerated and evidence of inhibition of PARP and DNA repair was obtained[142,143]
		INO-1001	Phase II trial of TMZ combinations in melanoma and glioblastoma
		MK4827	Phase I trial of single agent in BRCA mutation-associated ovarian cancer
		CEP-9722	Phase I trial of TMZ combination in solid tumours
		GPI-21016 (also known as E7016)	Phase I/II TMZ combination in solid tumours and melanoma
		BMN673	Phase I trial single agent in solid and haematological malignancies
		BSI-201 (also known as iniparib	Phase II/III trial of combination with gemcitabine and carboplatin. This is no longer considered a PARP inhibitor[182]
NER	XPG	Cyclosporine	Preliminary *in vitro* studies[183]
	ERCC1–XPF	Cetuximab	Preliminary *in vitro* studies[184]
TLS	Pol	3-*O*-methylfunicone, aurintricarboxylic acid and ellagic acid	Preliminary *in vitro* studies. aurintricarboxylic acid and ellagic acid displayed potent nanomolar activity[46,47]
MMR	MLH1	DAC (reactivation)	Preclinical sensitization to cisplatin, TMZ and epirubicin[53]. Phase II trial was terminated because of adverse reaction. Clinical trials are ongoing in combination with carboplatin and with TMZ
NHEJ	DNA-PKcs	NU7026, NU7441, IC86621 and IC87361	Preclinical *in vitro* and *in vivo* enhancement of responses to IR and etoposide, *ex vivo* sensitization of patient-derived CLL cells to mitoxantrone[61–65]
		OK-135	Preclinical inhibition of DNA repair in radioresistant L5178Y cells[185]
		SU11752	Preclinical *in vitro* inhibition of DSB repair and radiosensitization[186]
		CC-115	Dual DNA-PK and mTOR inhibitor in Phase I clinical trial

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Nadaljevanje tabele

Inhibitorji proteinov v popravljalnih mehanizmih

HRR	MRE11	Mirin	*In vitro* radiosensitization[70,71]
	RAD51	B02, A03, A10 and imatinib	*In vitro* identification of RAD51 inhibition by a high-throughput screen of NIH compound library and inhibition of plasmid rejoining by HRR[187]. Imatinib inhibits RAD51 phosphorylation, DNA damage-induced RAD51 focus formation and sensitized cells to chlorambucil, MMC and IR[72,73]
	BRCA1	AG024322 and SCH727965 (CDK1 inhibitors)	CDK1 activates BRCA1. Preclinical *in vitro* and *in vivo* studies showed that AG024322 is synthetically lethal with PARP inhibition[115]. A clinical trial has been initiated with SCH727965 and ABT-888
Checkpoints	ATM	KU55933, KU60019 and CP466722	Preclinical *in vitro* sensitization to IR, etoposide and camptothecin[81,188,189]
	ATR	Caffeine, shisandrin B, NU6027 and VE821	Preclinical *in vitro* chemosensitization and radiosensitization[92–95]
	WEE1	MK-1775	Preclinical *in vitro* and *in vivo* chemosensitization and radiosensitization[190,191] and patient-derived sarcoma explants *ex vivo* and as a single agent[192]. Evidence of activity in clinical trials[193]
	CDC25	Several, including IRC-083864 (Debio 0931)	IRC-083864 has activity in pancreatic and prostate cancer xenografts[194] and has entered clinical trial under the name of Debio 0931 (REF. 195) but no data are available
	CHK1 and CHK2	UCN-01	CHK1 and CHK2 (UCN-01 is a pan-kinase inhibitor): Phase I/II trials as a single agent and in combinations, trials were stopped owing to toxicities[91]
		AZD7762	CHK1 and CHK2: Phase I combinations with gemcitabine and with irinotecan
		PF00477736	CHK1: Phase I combination with gemcitabine
		SCH900776	CHK1: Phase I various drug combinations in leukaemia and lymphoma
		XL9844	CHK1 and CHK2: Phase I in combination with gemcitabine
		LY2606368	CHK1: Phase I single agent trial
		PV1019	CHK2: *in vitro* sensitization of topoisomerase I poisons and IR[196]

APE1, AP endonuclease 1; ATM, ataxia-telangiectasia mutated; ATR, ataxia-telangiectasia and Rad3-related; BER, base excision repair; CDC25, cell division cycle 25; CDK1, cyclin-dependent kinase 1; CLL, chronic lymphocytic leukaemia; DAC, decitabine; DDR, DNA damage response; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; DSB, DNA double-strand break; FEN1, flap endonuclease 1; HRR, homologous recombination repair; IR, ionizing radiation; MGMT, O^6-methylguanine DNA methyltransferase; MMC, mitomycin C; MMR, mismatch repair; MMS, methyl methanesulphonate; MTD, maximum tolerated dose; NER, nucleotide excision repair; NHEJ, non-homologous end joining; NIH, US National Institutes of Health; PARP, poly(ADP-ribose) polymerase; PARPi, PARP inhibitor; Pol, DNA polymerase; TLS, translesion synthesis; TMZ, temozolomide; XP, Xeroderma pigmentosum. *Where no reference is given information may be found on the ClinicalTrials.gov website (see Further information).

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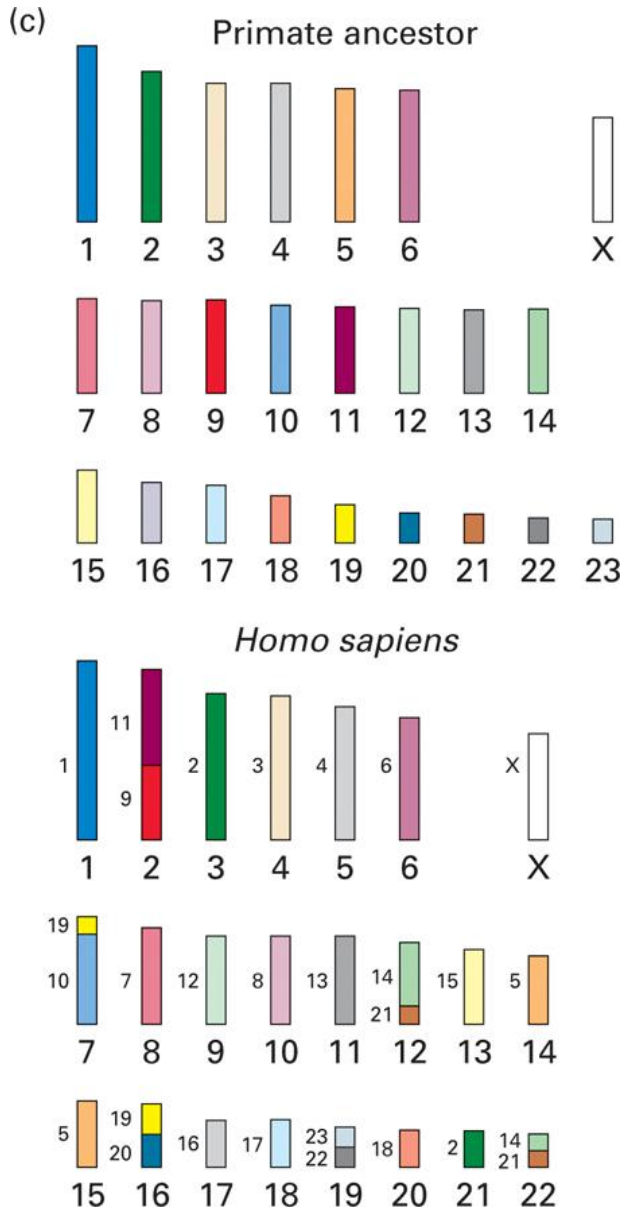
Figure 6.42 (c) **Evolution of primate chromosomes.**



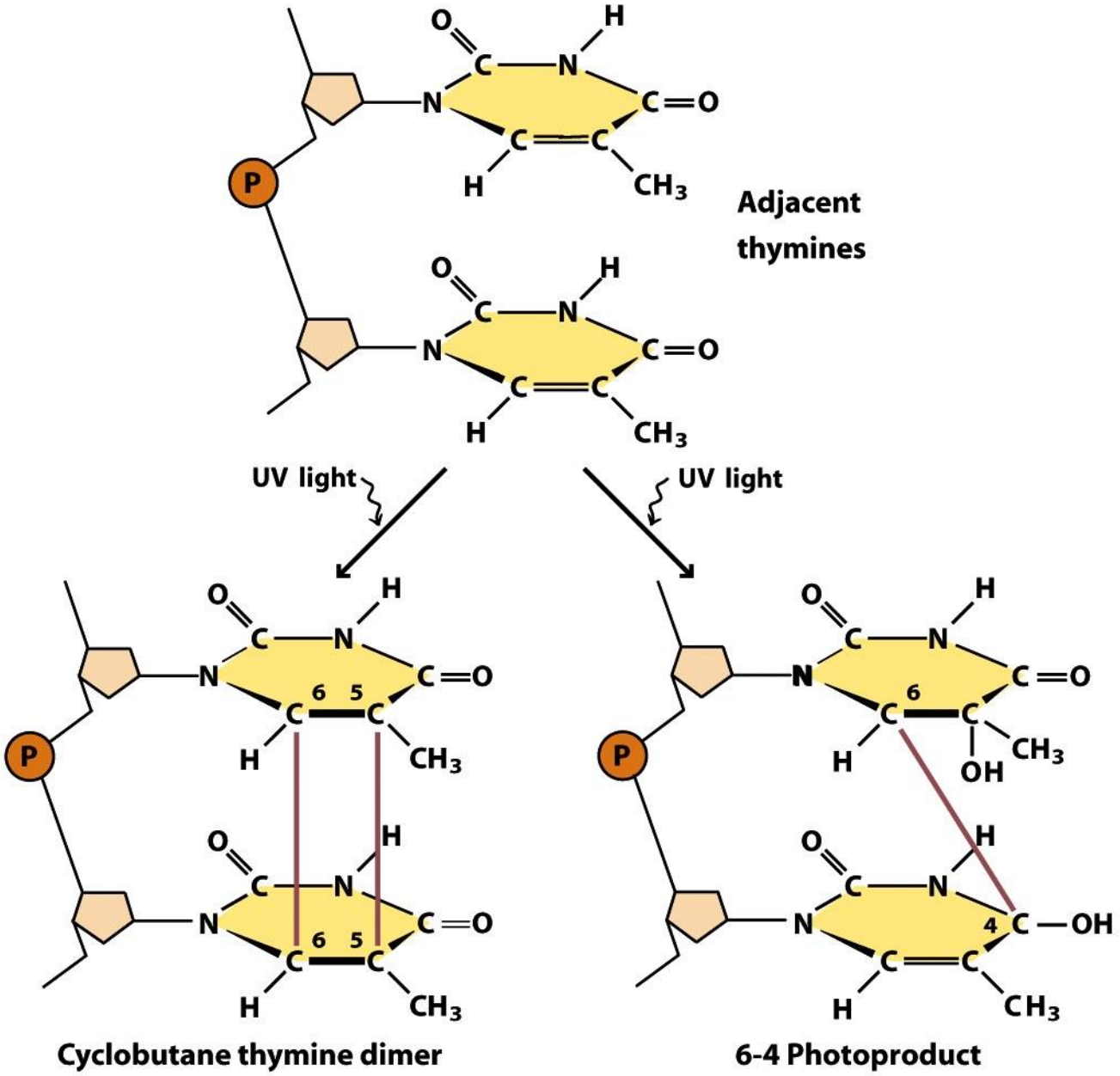


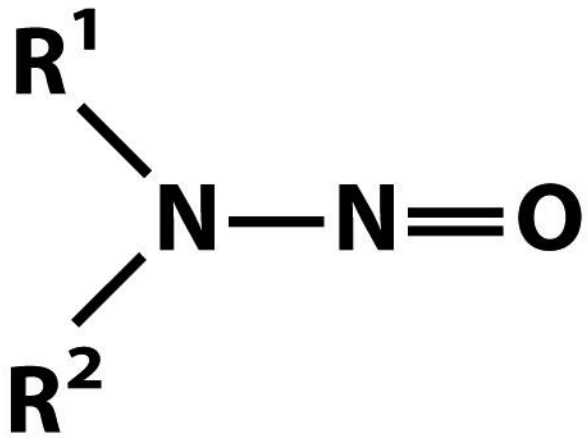
Figure 8-31a
Lehninger Principles of Biochemistry, Fifth Edition

$NaNO_2$

Sodium nitrite

$NaNO_3$

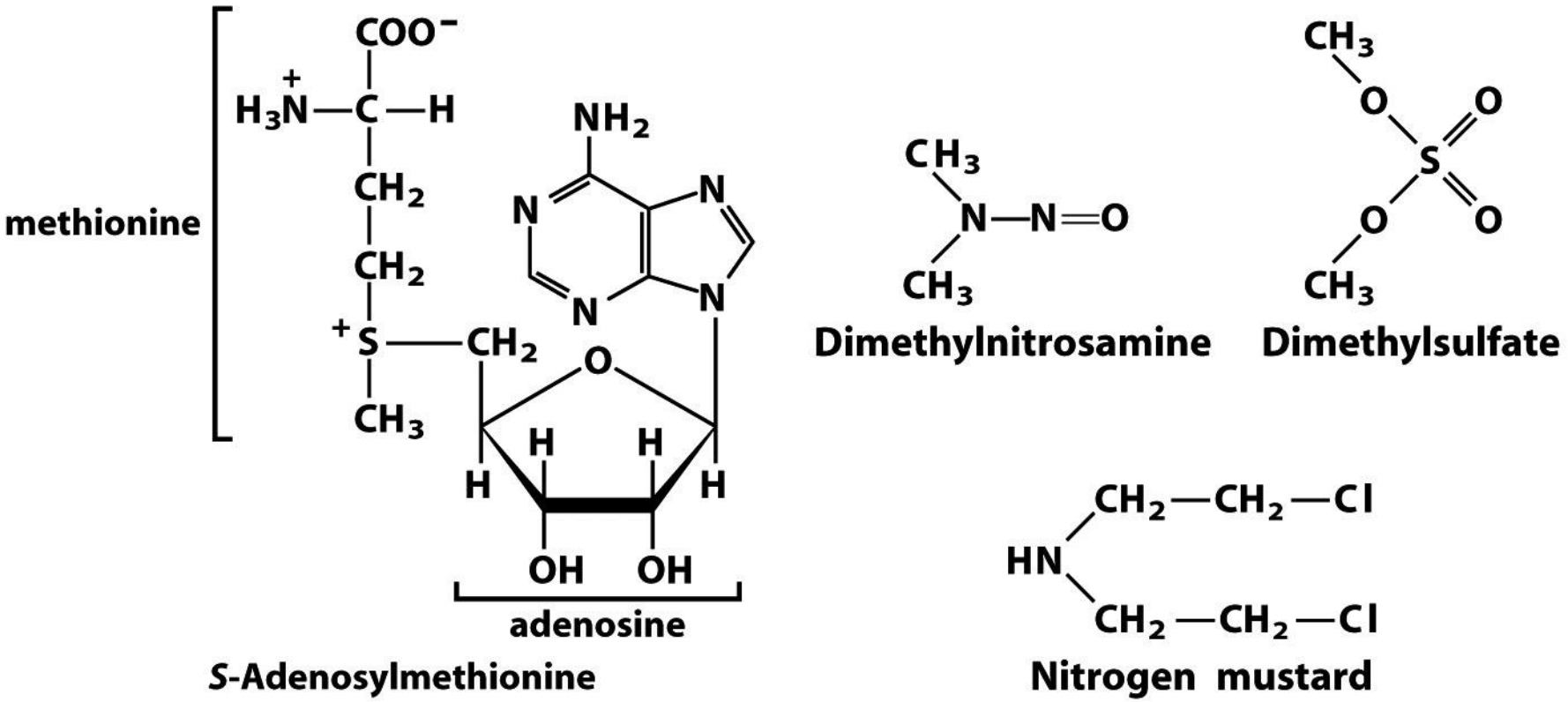
Sodium nitrate



Nitrosamine

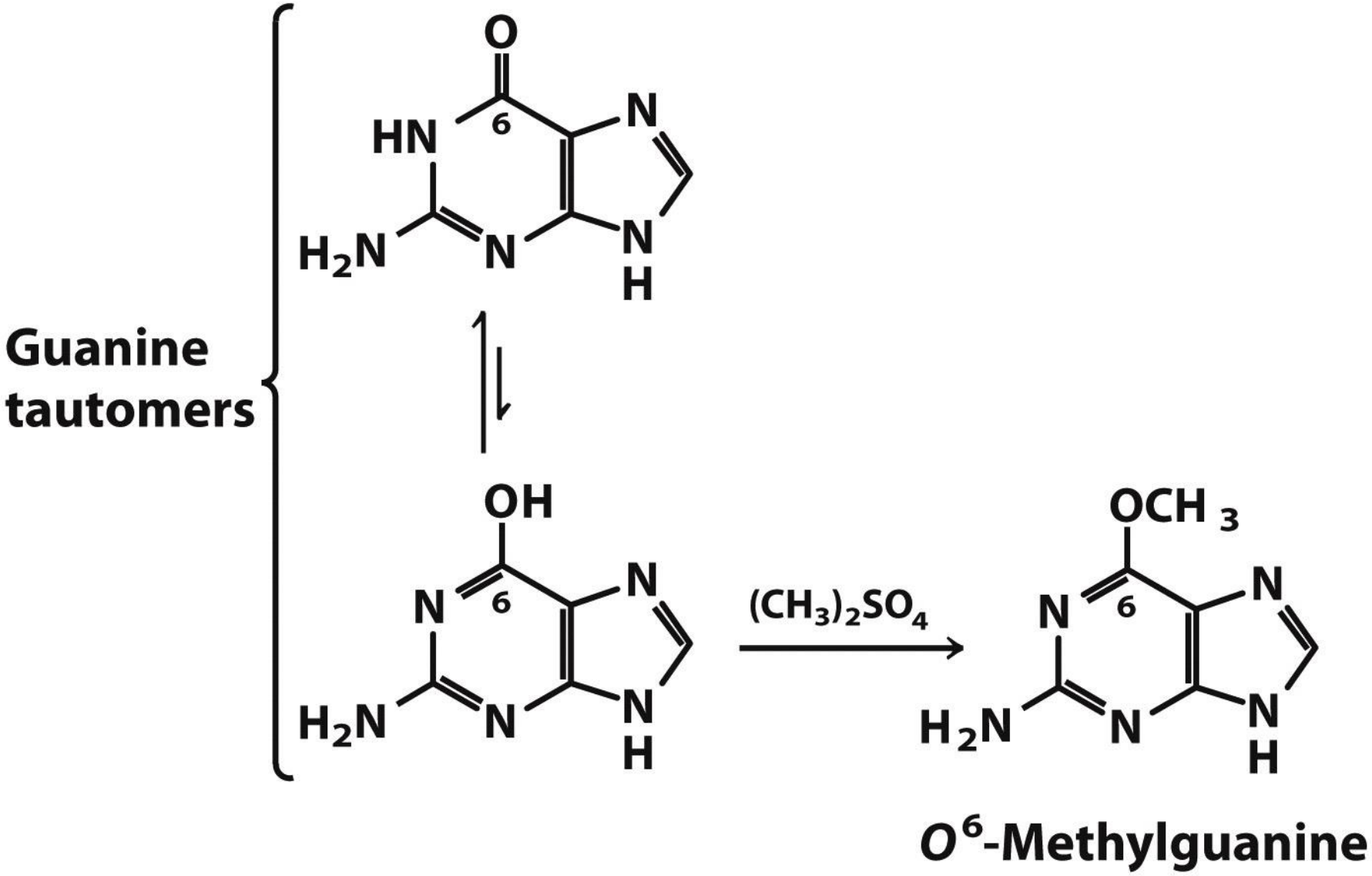
Nitrous acid precursors

Figure 8-32a
Lehninger Principles of Biochemistry, Fifth Edition



Alkylating agents

Figure 8-32b
Lehninger Principles of Biochemistry, Fifth Edition



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Lehninger Principles of Biochemistry, Fifth Edition