DNA Oxidation: Basics of Repair

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Overview

- DNA damage
- base excision repair (BER)
- global genome (GG) & transcription coupled (TC) nucleotide excision repair (NER)
- sanitization of the deoxynucleotide pool

Consequences of DNA damage

DNA REPAIR MECHANISMS

SHORT-TERM CONSEQUENCES

PHYSIOLOGICAL DYSFUNCTION | **CELL DEATH** | **ABNORMAL GROWTH & METABOLISM**

Decreased cellular proliferation | ***Genomic instability*** | ***Defective signalling pathways*** | ***Impaired protein/ gene expression***

LONG-TERM CONSEQUENCES

Ageing | **Cancer** | **Disease**

Types of oxidative DNA damage

- purine & pyrimidine oxidation products
- abasic (AP) sites
- strand breaks

- see Schafer, SFRBM Education Program presentation:

  http://www.medicine.uiowa.edu/frrb/VirtualSchool/Virtual.html

BER

- non-bulky lesions, abasic sites & strand breaks are repaired by BER
- mono- or bifunctional lesion-specific glycosylases may initiate repair by the cleavage of the N-C1′ glycosidic bond between the base & deoxyribose-phosphate backbone leaving an abasic site
- following the removal of abasic sites, short or long-patch BER resumes resulting in the repair of a single or 2-8 nucleotide gap, respectively

BER enzymes: *e.g.* functional diversity I

Oxidized & ring opened purines: *e.g.* 8-oxoG, fapyG		
E.coli	***S. cerevisiae***	***H. sapiens***
MutM (fpg)	**yOGG1&2**	**hOGG1**
endo VIII (Nei)	_ _ _ _	**hNEIL1**
AlkA ***(not oxoG)***	_ _ _ _	**hMPG**

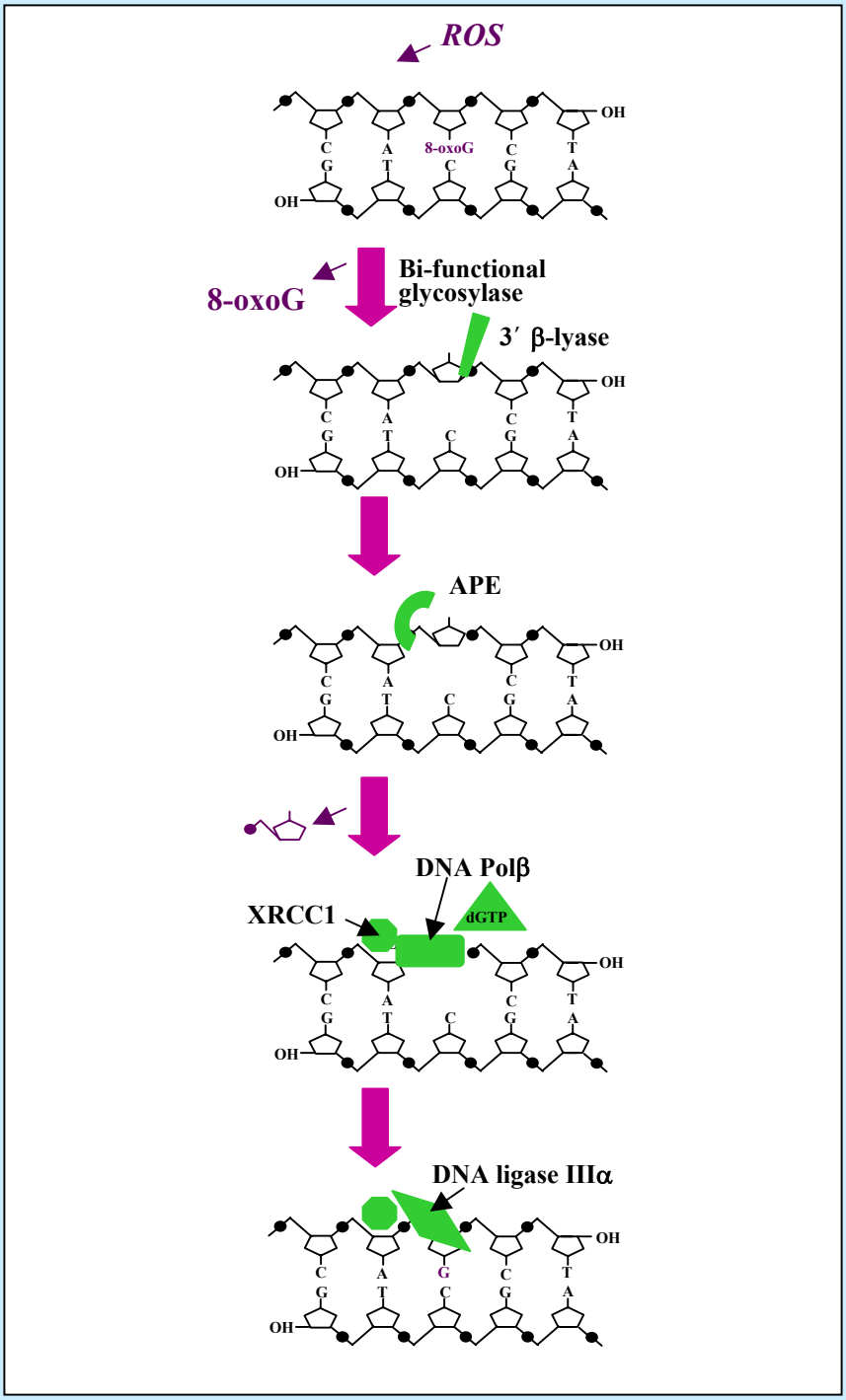
MutM/fpg: formamidopyrimidine glycosylase
endo VIII/Nei: endonuclease VIII
OGG: 8-oxoguanine DNA glycosylase
NEIL: Nei endonuclease VIII-like
MPG: N-methylpurine-DNA glycosylase
AlkA: 3-methyladenine DNA glycosylase II

BER enzymes: *e.g.* functional diversity II

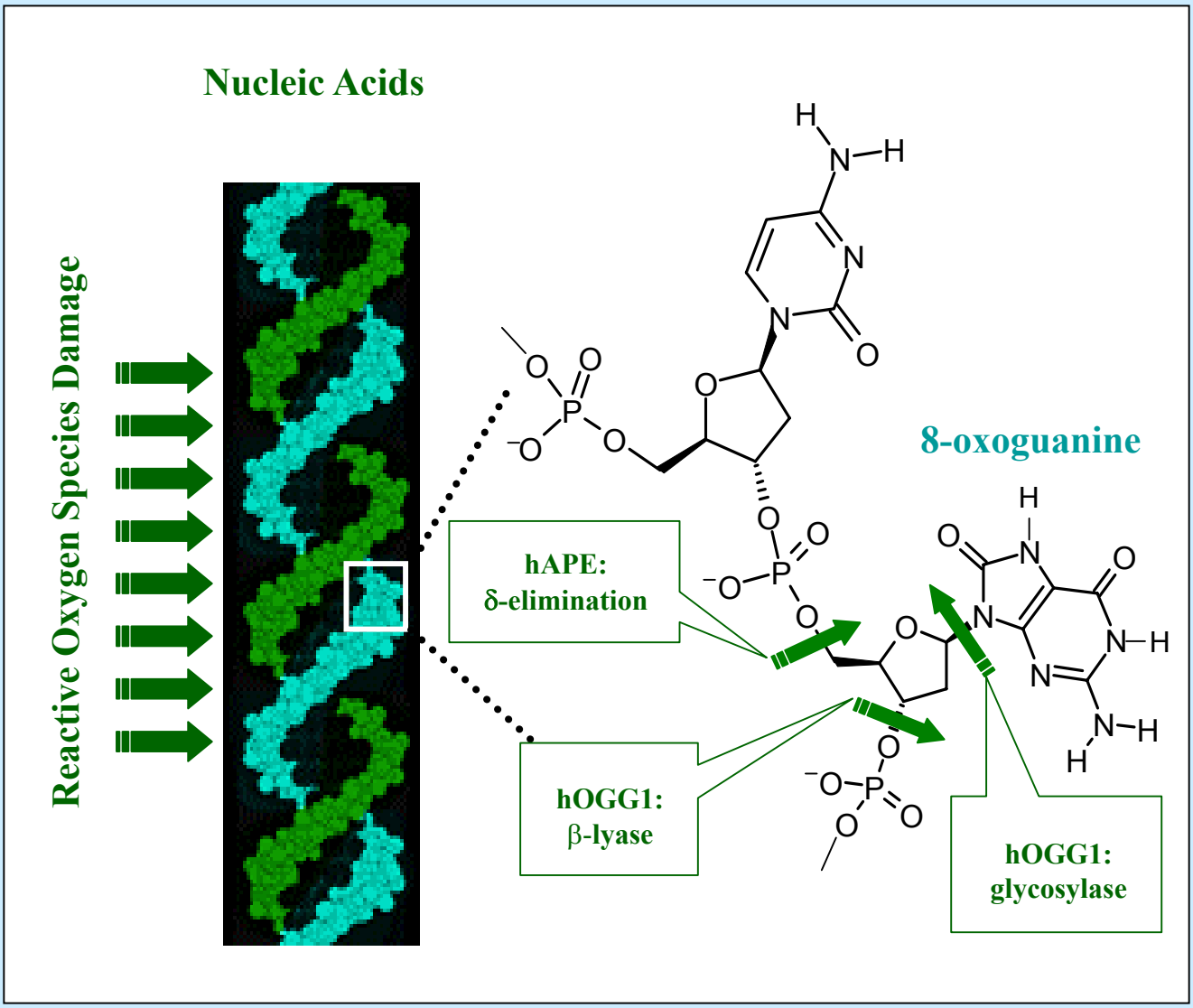
Oxidized & ring opened pyrimidines: *e.g.* T-glycol, fapy		
E.coli	***S. cerevisiae***	***H. sapiens***
endo III (Nth)	**Ntg1&2**	**hNTHL1**
endo VIII (Nei)		**hNEIL1-3**
AlkA		
		hTDG

endo III : endonucleases III
Ntg: endonuclease III-like glycosylase
NTHL1: Nth endonuclease III-like 1
TDG: thymine-DNA glycosylase

Short-patch BER



BER of 8-oxoG

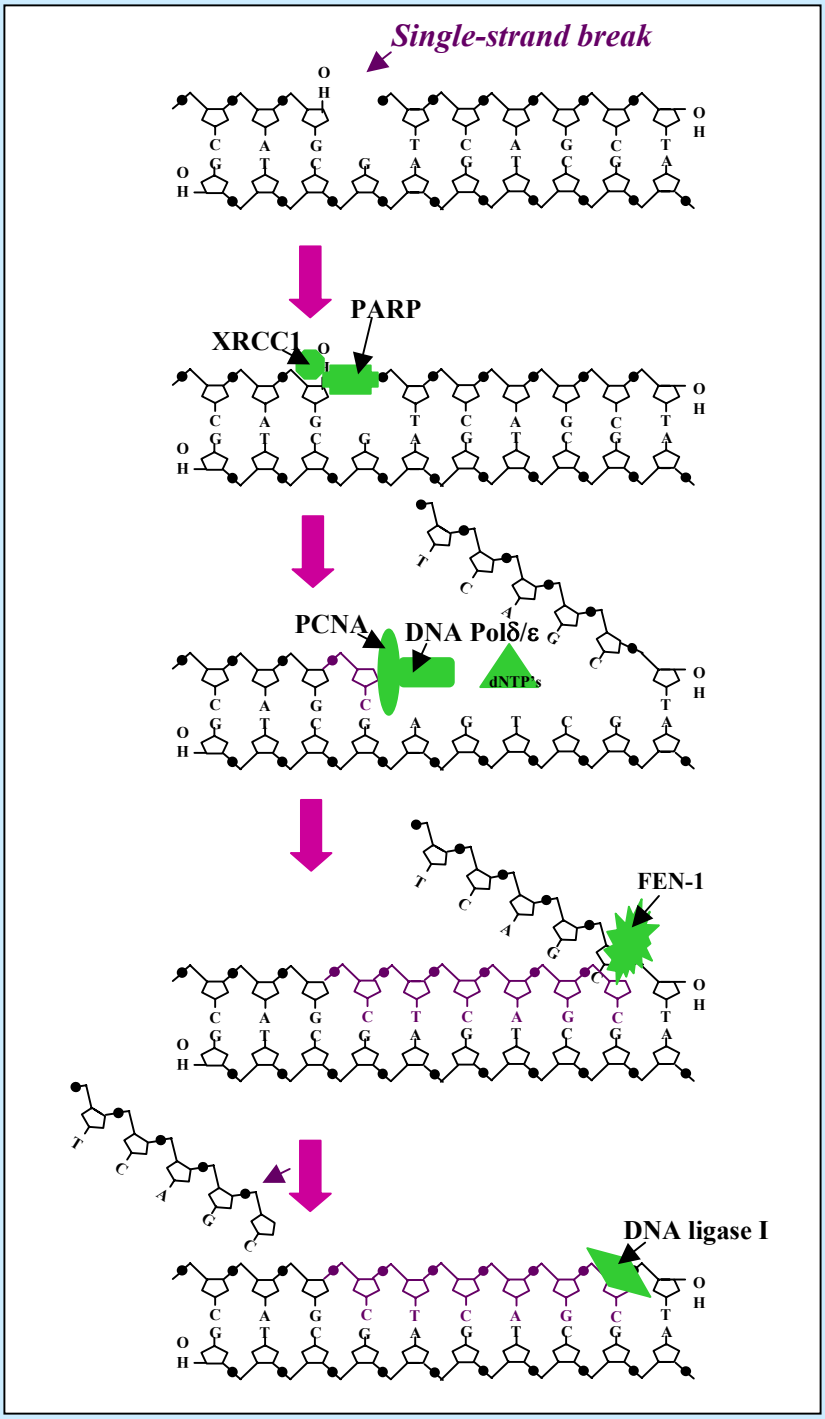


hAPE/HAP1/REF1: apurinic/apyrimidinic endonuclease

Other BER Proteins

Removal of abasic sites	
APE1 (HAP1, REF1)	AP endonuclease
Gap filling	
PCNA	Proliferating cell nuclear antigen
DNA pol	DNA polymerase
Sealing the nick	
XRCC1	X-ray cross-complementing protein & ligase accessory factor
LIG3	DNA ligase

Long-patch BER



DNA repair pathways & the prevention of mutations

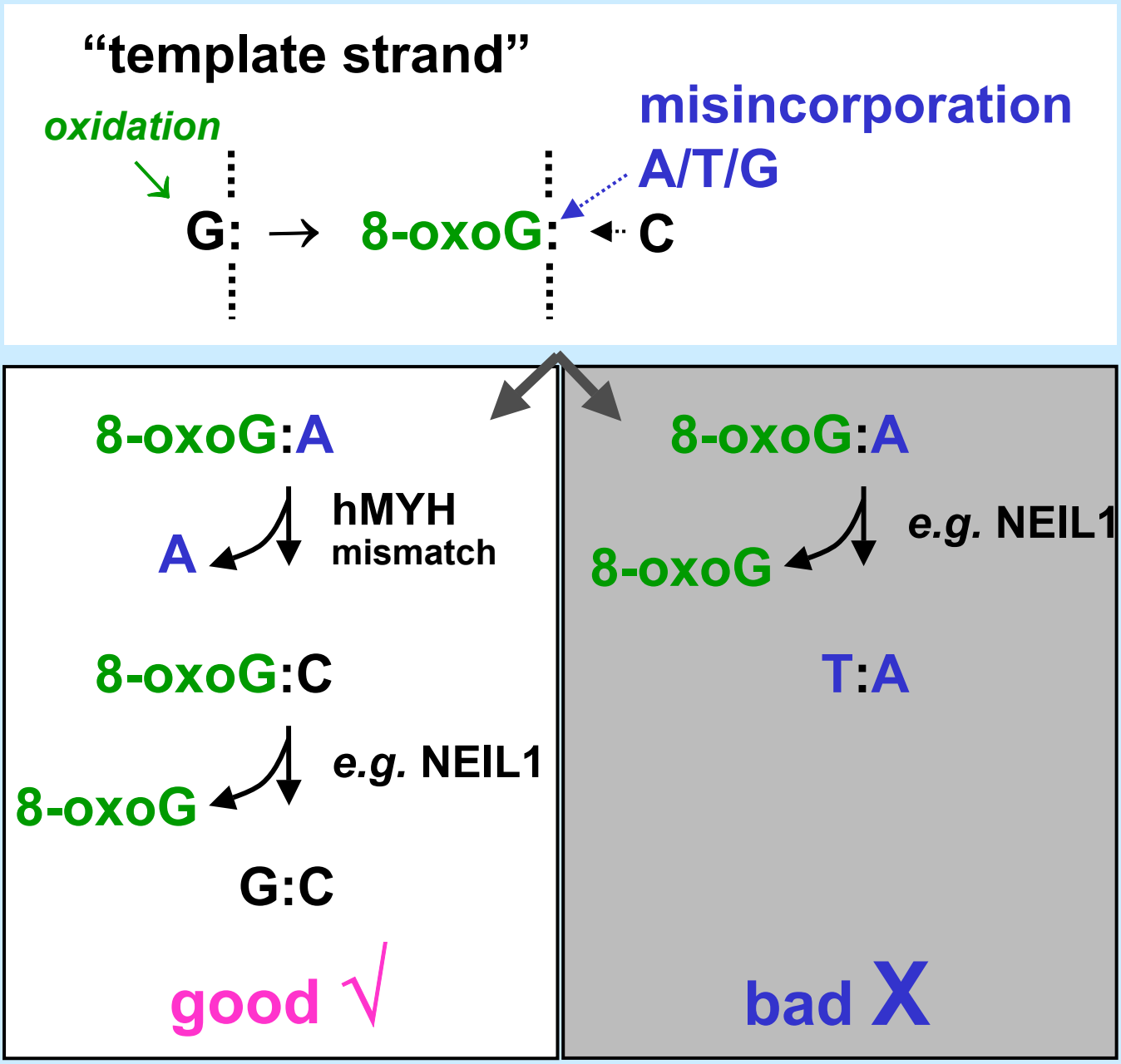
Complexities of DNA repair pathways & replication…

..specific enzymes prevent incorrect base pairing opposite DNA lesions during DNA replication

Sanitization of the deoxynucleotide pool...

..specific enzymes prevent the incorporation of damaged deoxynucleotides opposite a template strand during DNA replication

Complexities of repair pathways & replication..



Sanitization of the deoxynucleotide pool..

…prevents incorporation of 8-oxoG into DNA

E.coli	*H. sapiens*	substrate
MutT	**NUDT1 (hMTH1)**	**8-oxodGTP**

MutT: nucleoside triphosphate pyrophosphohydrolase

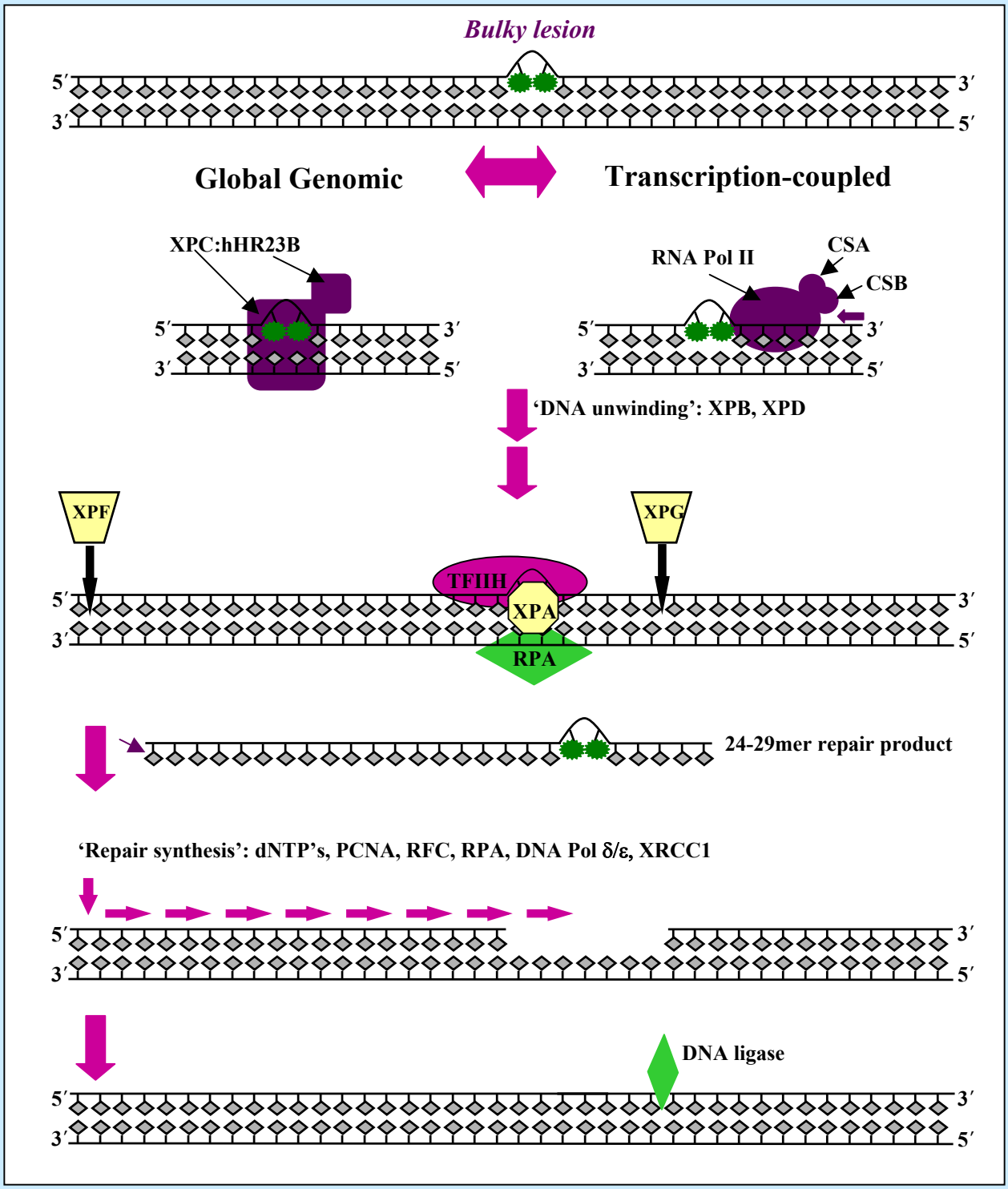
NUDT1: nudix (nucleoside diphosphate linked moiety X)-type motif 1

hMTH1: human homolog of MutT

GG & TC NER

- bulky & small lesions causing differing degrees of DNA distortion are removed by NER
- NER is implicated as a back-up system for BER
- different complexes initiate NER in transcribed & non-transcribed DNA
- NER involves the dual incision of unwound DNA either side of the lesion by a multisubunit ATP-dependent nuclease & the release of a 24-29 oligomer
- the resultant gap is filled & sealed by the action of various components including DNA polymerases & ligases

GG & TC NER


Bulky lesion
5′
3′
3′
5′
Global Genomic
Transcription-coupled
XPC:hHR23B
RNA Pol II
CSA
CSB
'DNA unwinding': XPB, XPD
XPF
XPG
TFIIH
XPA
RPA
24-29mer repair product
'Repair synthesis': dNTP's, PCNA, RFC, RPA, DNA Pol δ/ε, XRCC1
DNA ligase

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