Learning outcomes (Lecture 3a)
Replication of damaged DNA

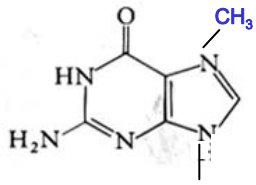
Understanding:

- Basic mechanism of damage avoidance by recombination repair in *E. coli*
- Concept of translesion synthesis
- Y-family polymerases and XP variants
- Polymerase switching

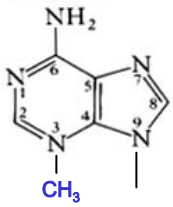
Effects of DNA damage on replication

1. No effect, eg 7Me-G.
2. Misreplication, eg O6-MeG
3. Lesion obstructs fork progression
4. Lesion stops initiation
5. Lesion arrests cell cycle.

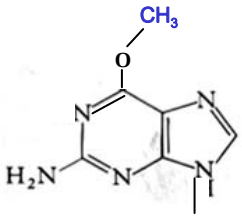
Methylated purines



7-methylguanine

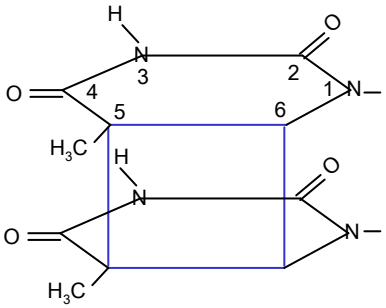


3-methyladenine

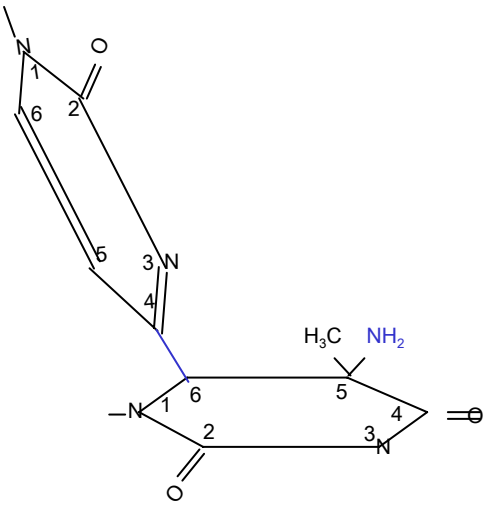


O-6-methylguanine

Major UV photoproducts



Cyclobutane thymine dimer



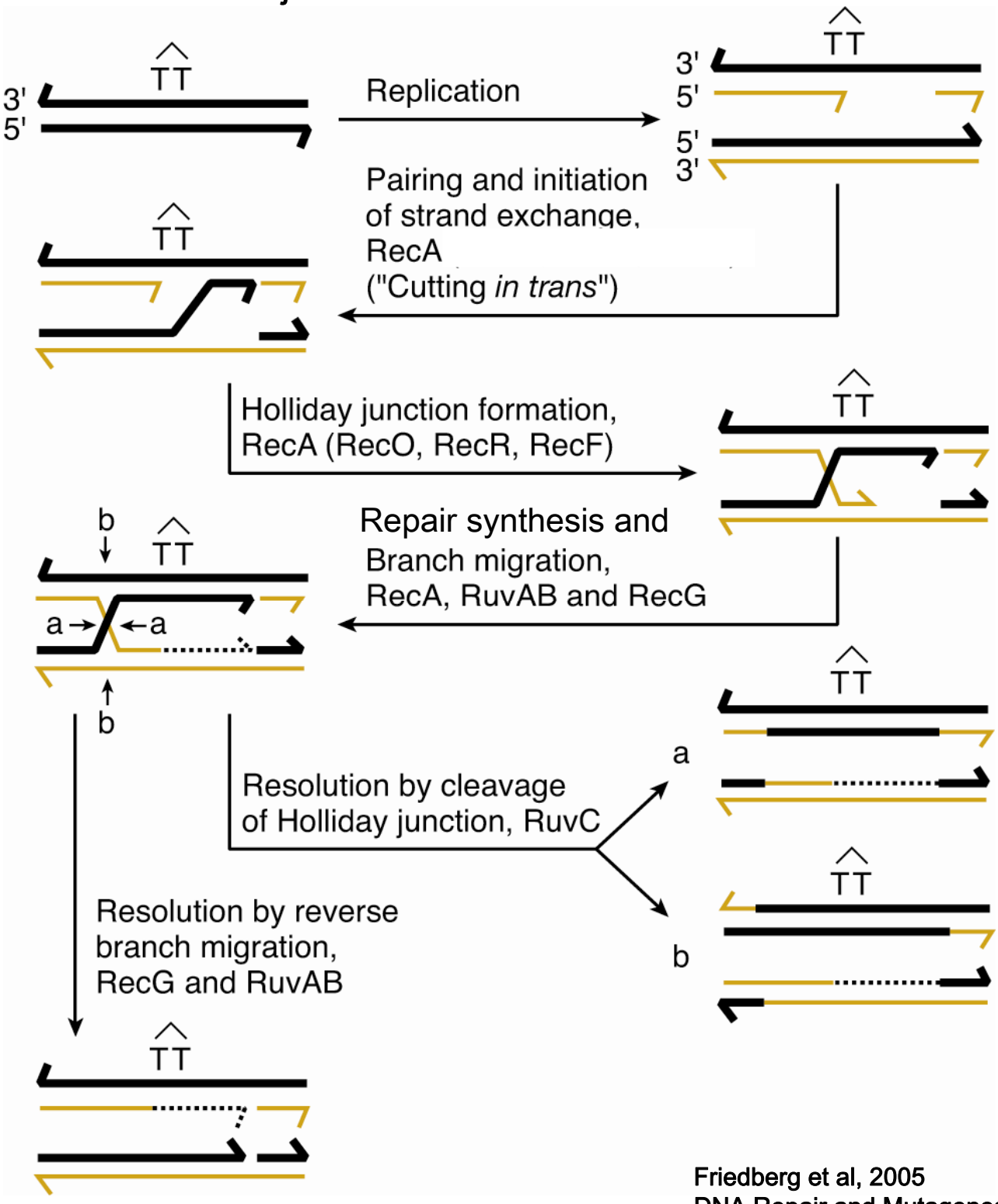
TC (6-4) photoproduct

3.2

Model for recombination repair of daughter-strand gaps

uvrA$^-$ strains tolerate 50 CPD per genome
New DNA is small, gets bigger.

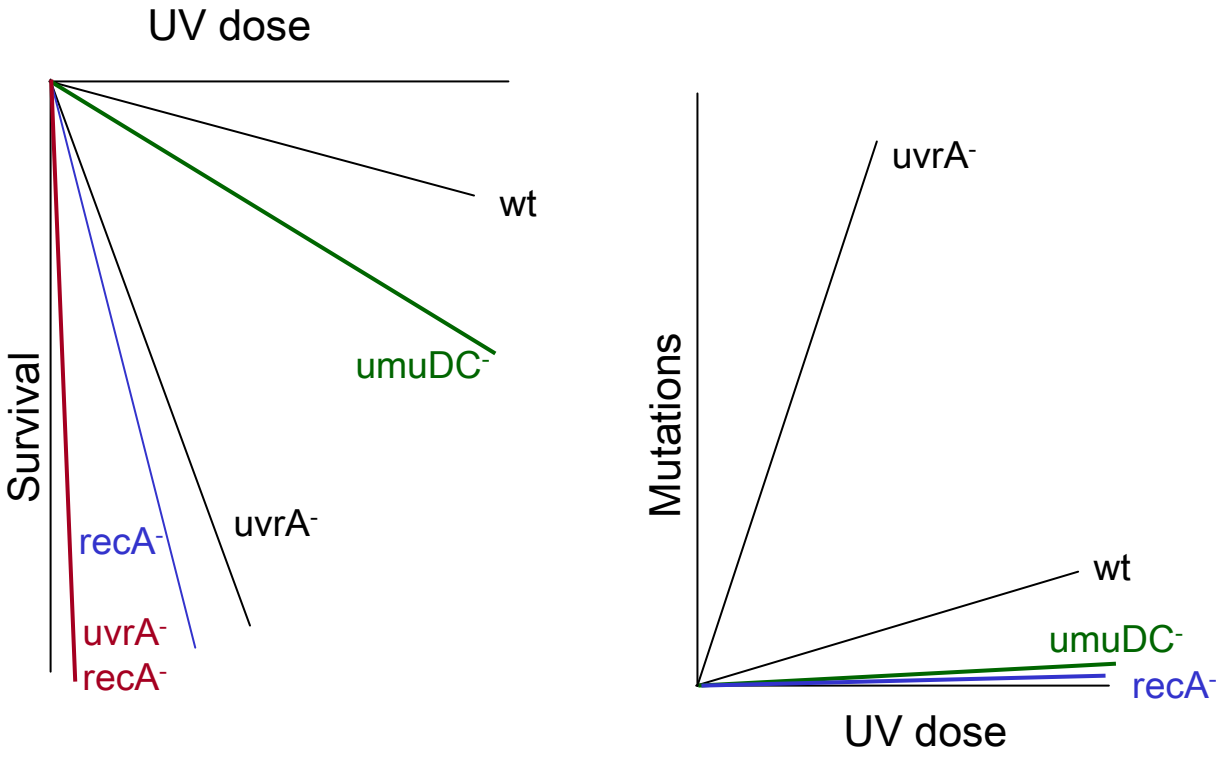
Major mechanism in *E. coli*



Friedberg et al, 2005
DNA Repair and Mutagenesis

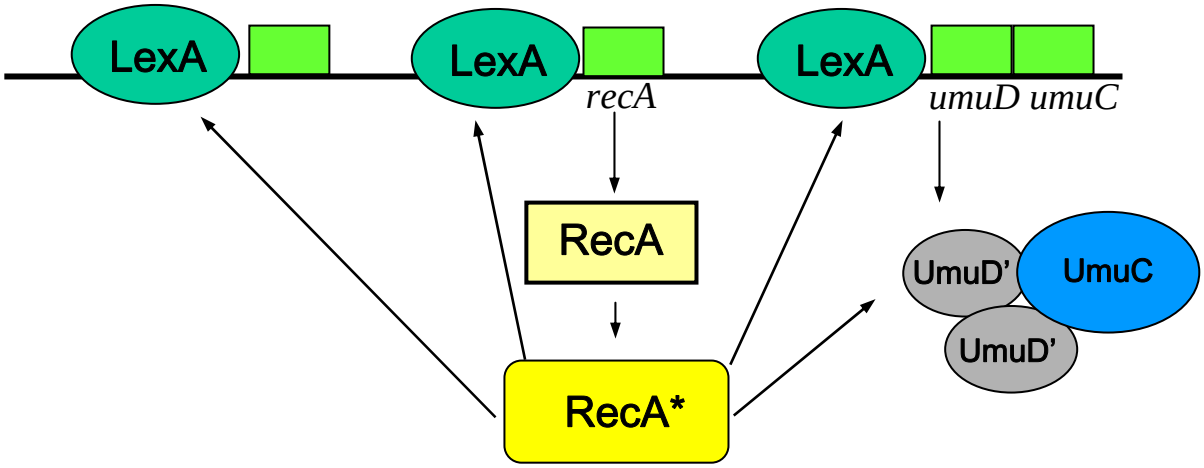
Genetics of UV mutagenesis

A. *E. coli*



SOS Response

In *E.coli, recA, umuCD* mutants are not mutable by UV light. *LexA* is a repressor of about 30 genes including *recA, umuCD* (as well as NER genes).



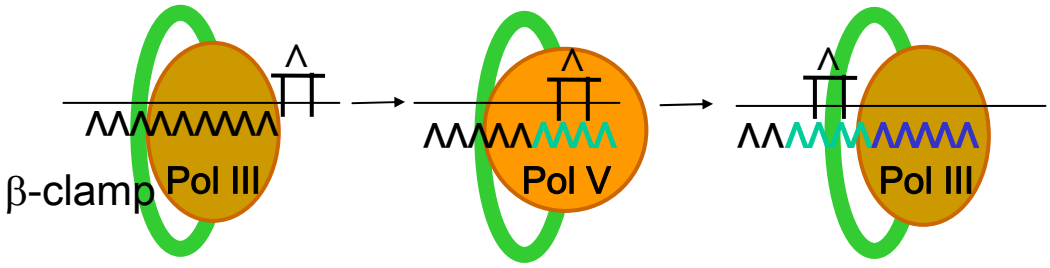
RecA is activated by ssDNA, exposed at replication fork when it encounters DNA damage (RecA*).

RecA* catalyses cleavage and inactivation of lexA repressor.

Results in increased levels of RecA* and UmuDC.

RecA* also catalyses cleavage of N-terminal 24aa from UmuD → UmuD'

UmuD'$_2$C is DNA Pol V, which, unlike Pol III, can synthesise past DNA damage – but it makes errors

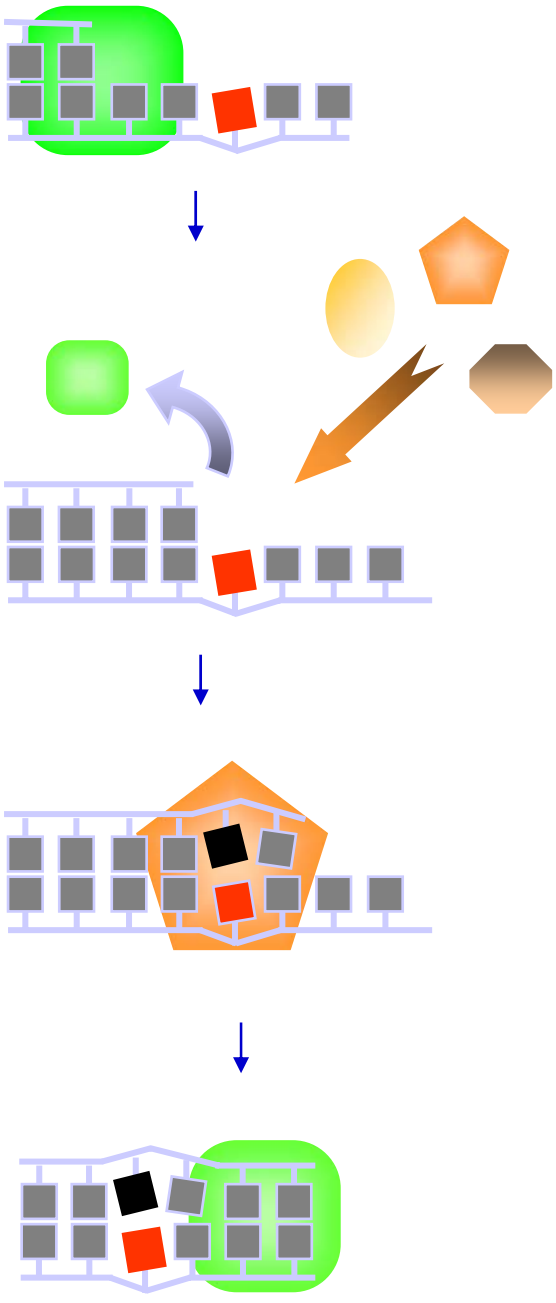


3.5

Translesion synthesis (TLS) (A8, A10)

Quantitatively minor, but v important

Translesion Synthesis (TLS)



Replicative DNA synthesis is blocked by DNA lesion

Replacement of by TLS DNA polymerase

TLS polymerase can incorporate either correct or incorrect nucleotide

Replication restart

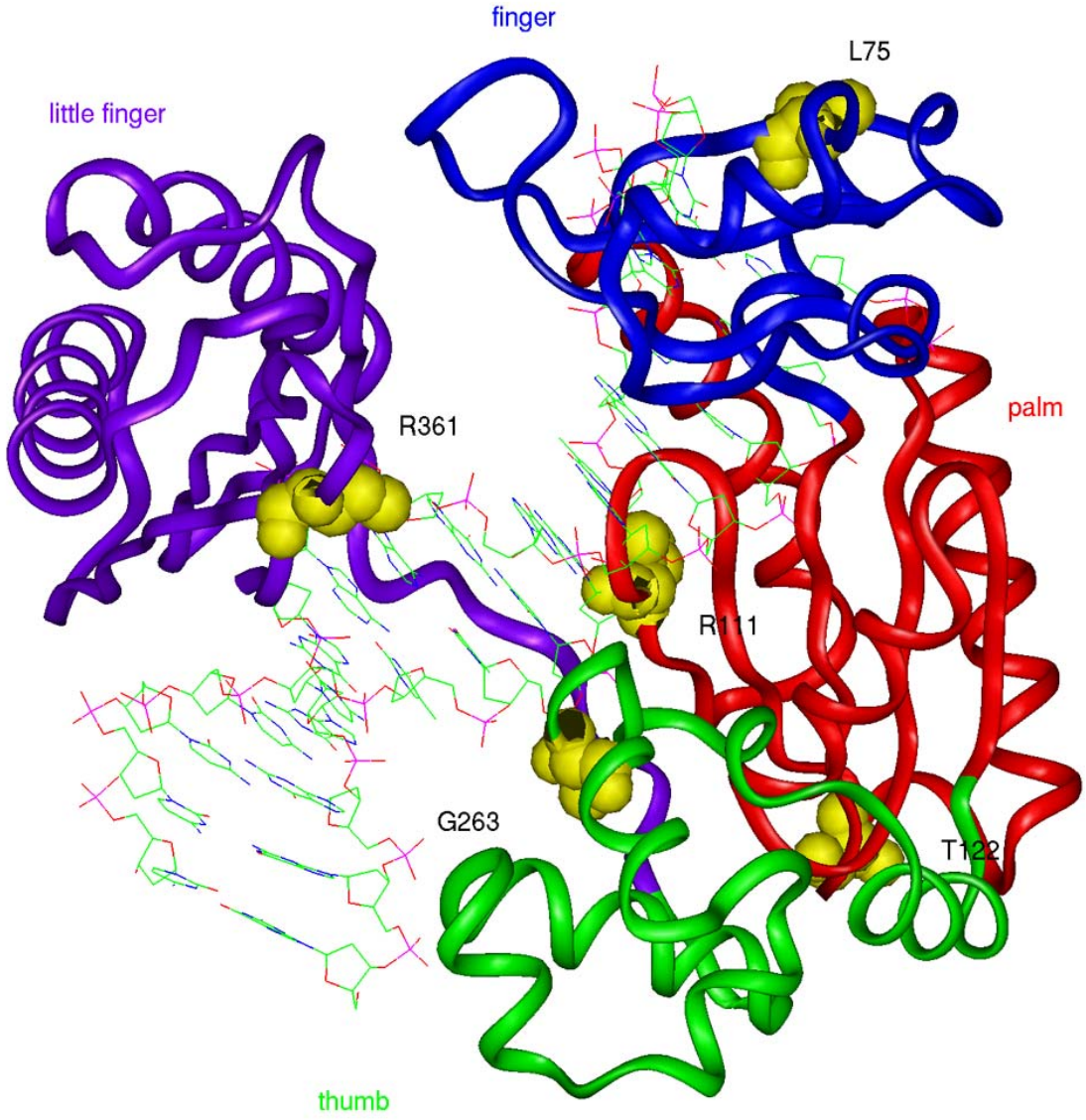
DNA Polymerases

	Name	Function	3'-5' Exonuclease Proofreading	Processivity	Fidelity	TLS
E. coli	Pol I	Removal of RNA primers; Repair synthesis: BER and NER	Yes	High	High	No
	Pol II	TLS	Yes (Weak)		High	Yes
→	Pol III	Replication, MMR	Yes	V. high	V. high	No
	*Pol IV	TLS	No	Low	Low	Yes
→	*Pol V	TLS	No	Low	Low	Yes
Mammalian	Pol α	RNA-DNA priming during replication	No	Low	High	No
	Pol β	BER	No	Moderate	Moderate	Poor
	Pol δ	Replication, NER	Yes	V. high	V. high	No
	Pol ε	Replication, NER	Yes	V. high	V. high	No
	Pol ζ	TLS	No	Low	Low	Yes
	*Rev1	TLS	No	Low		Yes
	*Pol η	TLS (CPD)	No	Low	Low	Yes
	*Polι	TLS	No	Low	Low	Yes
	*Pol κ	TLS	No	Low	Low	Yes

* Y-family of DNA polymerases

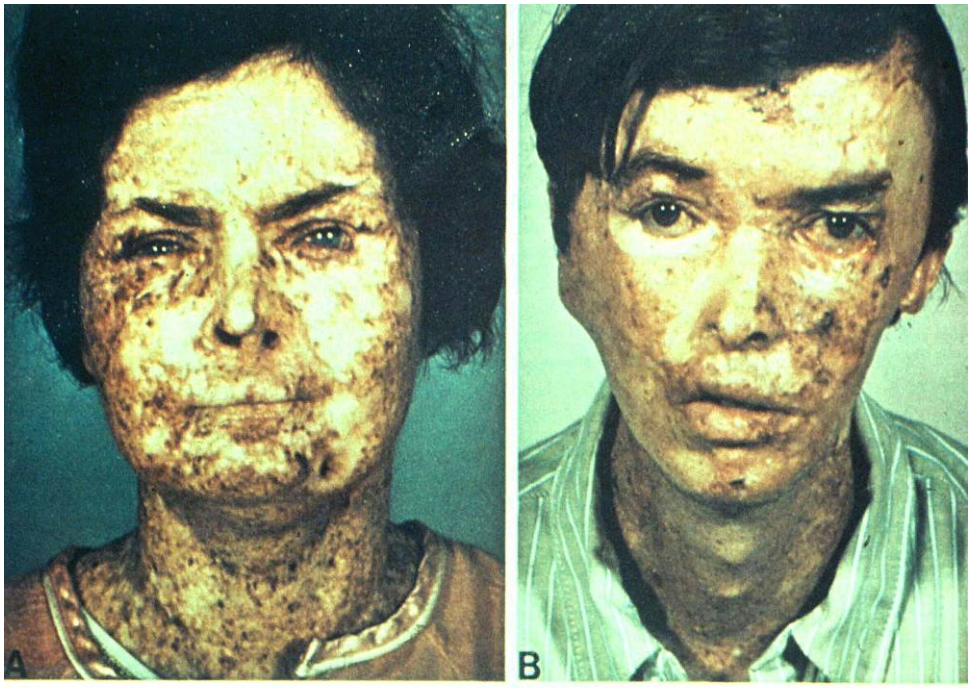
Properties of Y-family polymerases

- **Conserved catalytic domain at N-terminus**
- **Finger, palm and thumb domains characteristic of DNA polymerases**
- **Extra Little finger domain**
- **C-terminal third involved in protein-protein interactions**
- **Catalytic domains have more open structure**
- **Can accommodate damaged bases in active sites**
- **Error-prone on undamaged DNA**
- **Poor processivity**



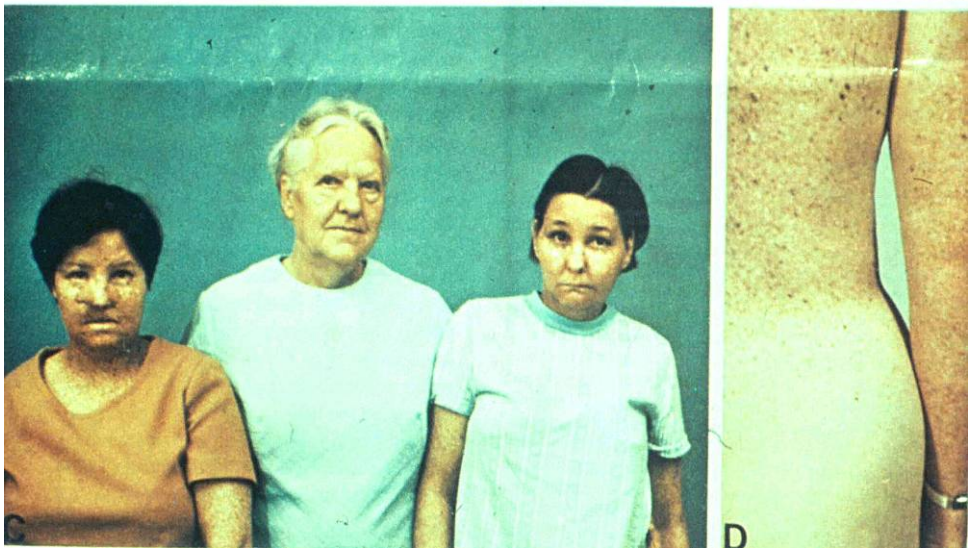
Xeroderma Pigmentosum Patients

XP-C



XP variant

XP-D



Robbins et al 1974

Properties of XP, CS and TTD complementation groups

Clinical features				Repair characteristics		
Group	Skin Cancer	Neurological abnormalities	Relative frequency of occurrence	UV-sensitivity	Residual UDS*	Remarks
XP-A	+	++	high	+++	<5	
XP-B	+/-	+++/+	very rare	++	<10	Combined XP/CS or TTD
XP-C	+	-	high	+	15-30	Deficient in 'global genome' repair. Normal transcription-coupled repair
XP-D	+	++/-	intermediate	++	15-50	Includes patients with TTD and patients with XP/CS
XP-E	+/-	-	rare	±	>50	
XP-F	+/-	-	rare/ intermediate	+	15-30	Repair slow but prolonged
XP-G	+/-	+++/+	rare	++	<10	Includes patients with XP/CS
XP-V	+	-	high	+	100	Defective in post-replication repair. Normal NER
CS-A	-	++	rare	+	100	Defective in transcription-coupled repair 'Global genome' repair normal
CS-B	-	++	high	+	100	Defective in transcription-coupled repair 'Global genome' repair normal
TTD-A	-	+	very rare	+	15	TTD

*Unscheduled DNA synthesis as a percentage of wild-type activity

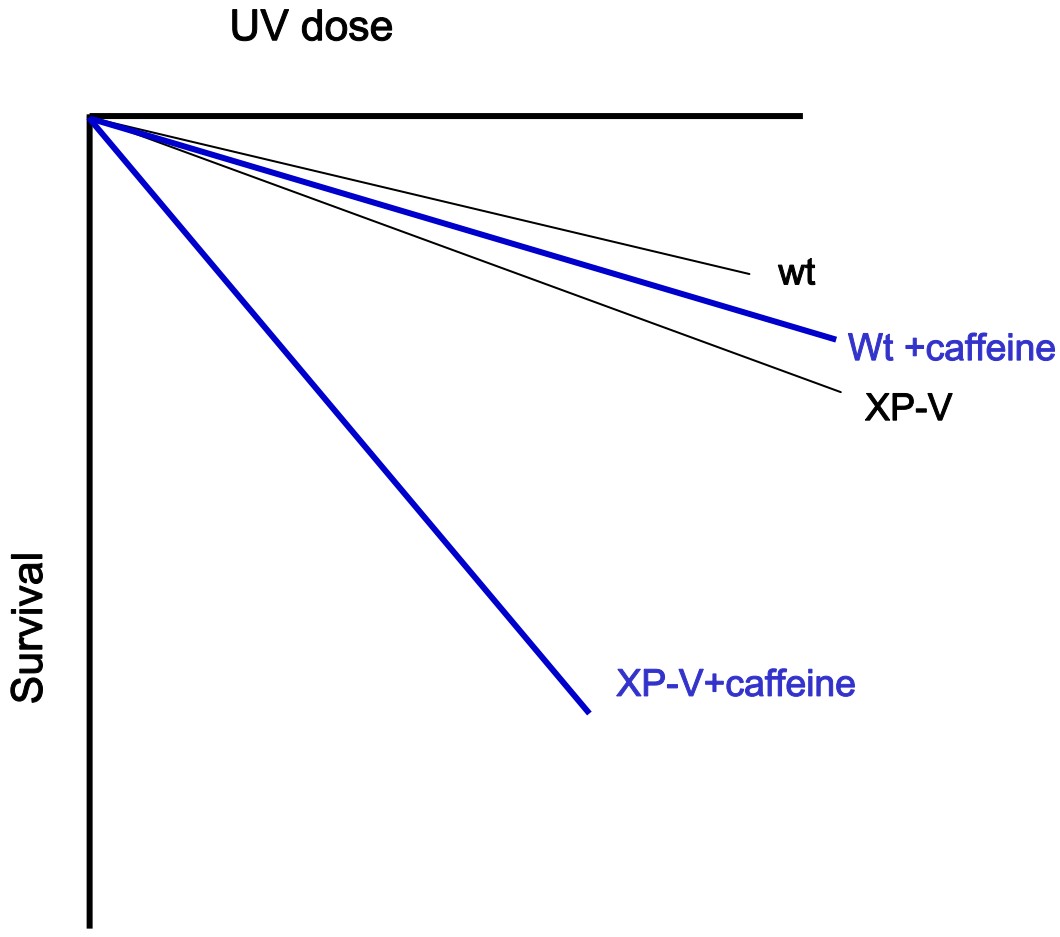
---

XP variants

- XP-Variant patients are hypersensitive to sunlight
- XP-V cells carry out normal nucleotide excision repair but are defective in their replication of UV-damaged DNA (postreplication repair)
- The cells are only mildly sensitive to killing by UV
- This sensitivity can be increased with caffeine (diagnostic test)
- They are hypermutable with UV light
- They are defective in Polη

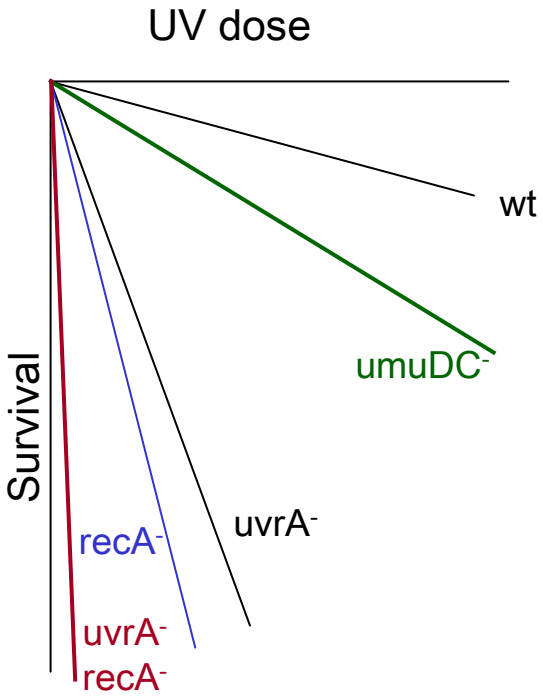
Diagnostic test for XP Variant Patients

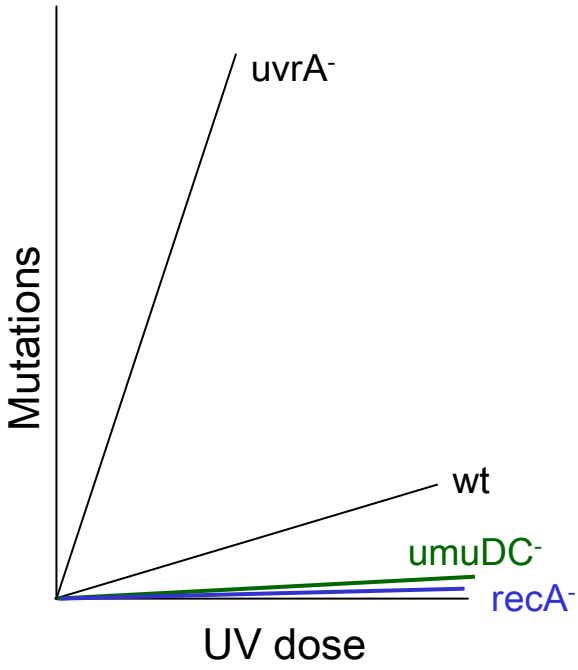
- **UDS is normal**
- **Cell survival after UV is close to normal**
- **Cell survival after UV is reduced by caffeine**



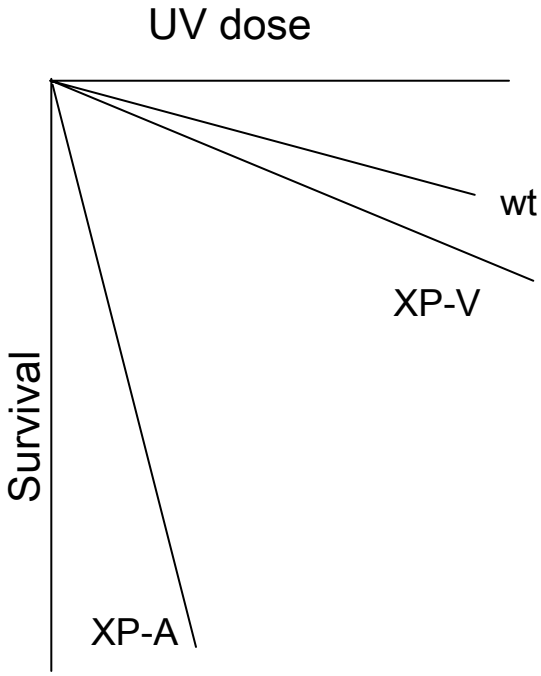
Genetics of UV mutagenesis

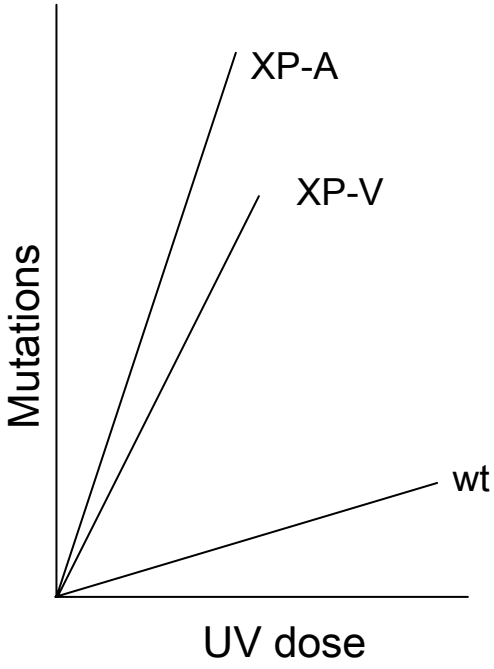
A. *E. coli*





B. Human cells





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→	Pol III	Replication, MMR	Yes	V. high	V. high	No
	*Pol IV	TLS	No	Low	Low	Yes
→	*Pol V	TLS	No	Low	Low	Yes
Mammalian	Pol α	RNA-DNA priming during replication	No	Low	High	No
	Pol β	BER	No	Moderate	Moderate	Poor
	Pol δ	Replication, NER	Yes	V. high	V. high	No
	Pol ε	Replication, NER	Yes	V. high	V. high	No
→	Pol ζ	TLS	No	Low	Low	Yes
	*Rev1	TLS	No	Low		Yes
→	*Pol η	TLS (CPD)	No	Low	Low	Yes
	*Polι	TLS	No	Low	Low	Yes
	*Pol κ	TLS	No	Low	Low	Yes

* Y-family of DNA polymerases

DNA polymerase η

- Member of Y-family
- Can carry out TLS past CPDs
- Puts correct bases opposite CPD!
- Can carry out TLS past other lesions inefficiently
- Inaccurate on undamaged template

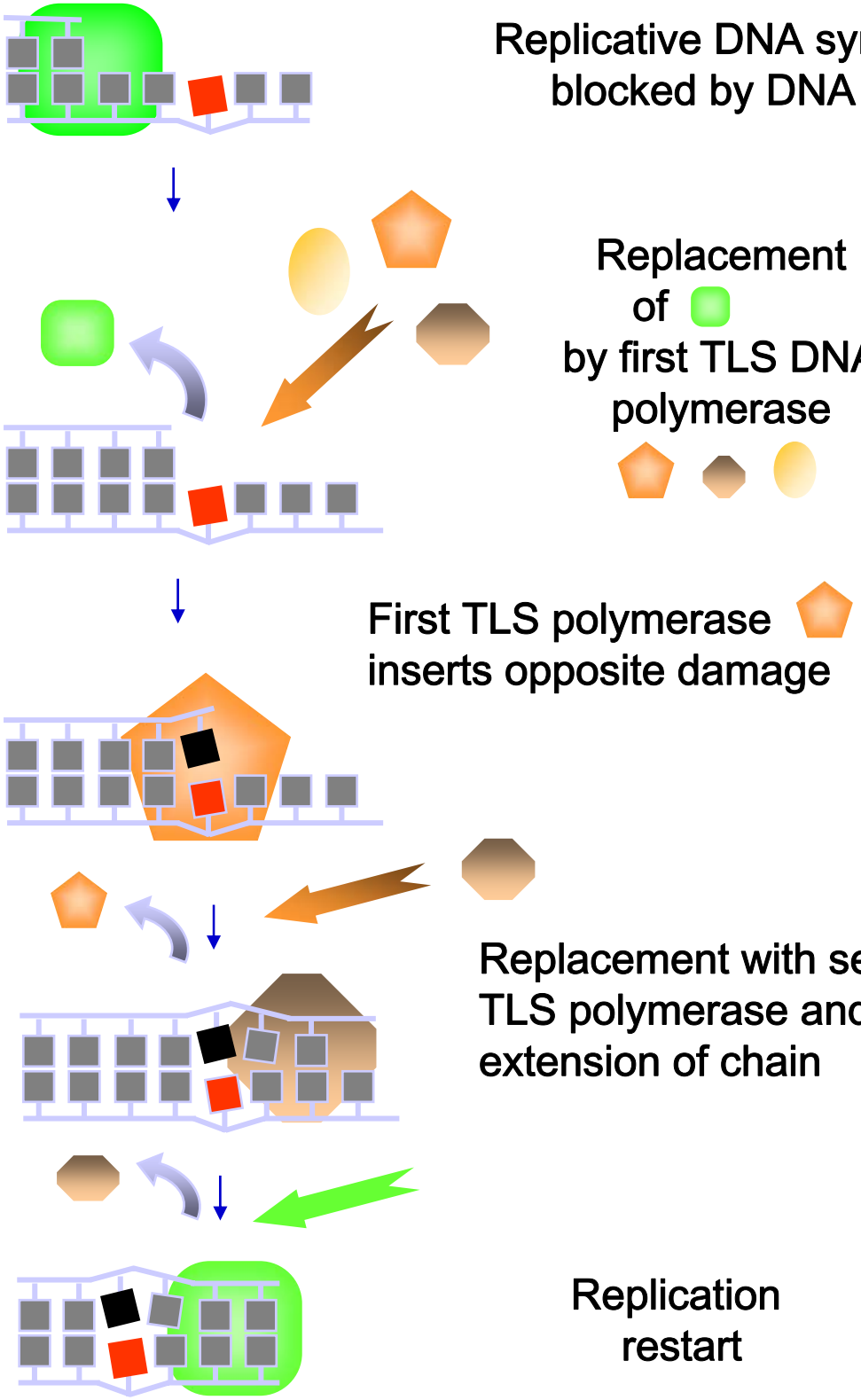
NB TLS is the major pathway in mammalian cells

What do the other Y-family pols do?

Different lesions

Insertion and extension?

Insertion and extension

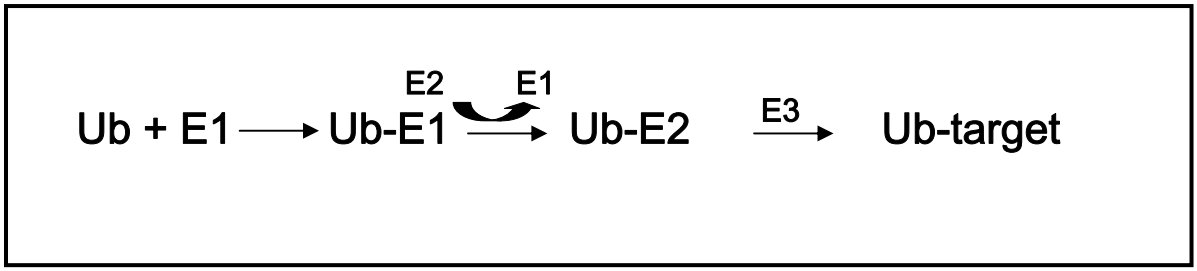


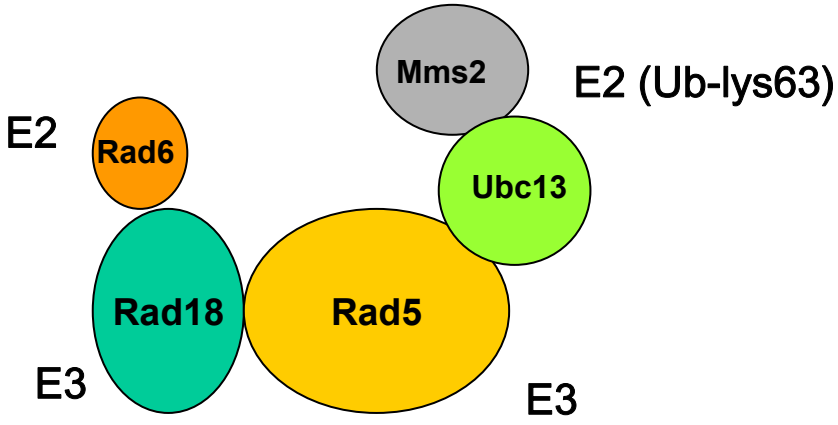
Polζ is a good extender: needed for replication past most lesions (except CPD – polη can do it all)

Polymerase Switch A9

Proteins involved in replication of DNA damage in *S. cerevisiae*

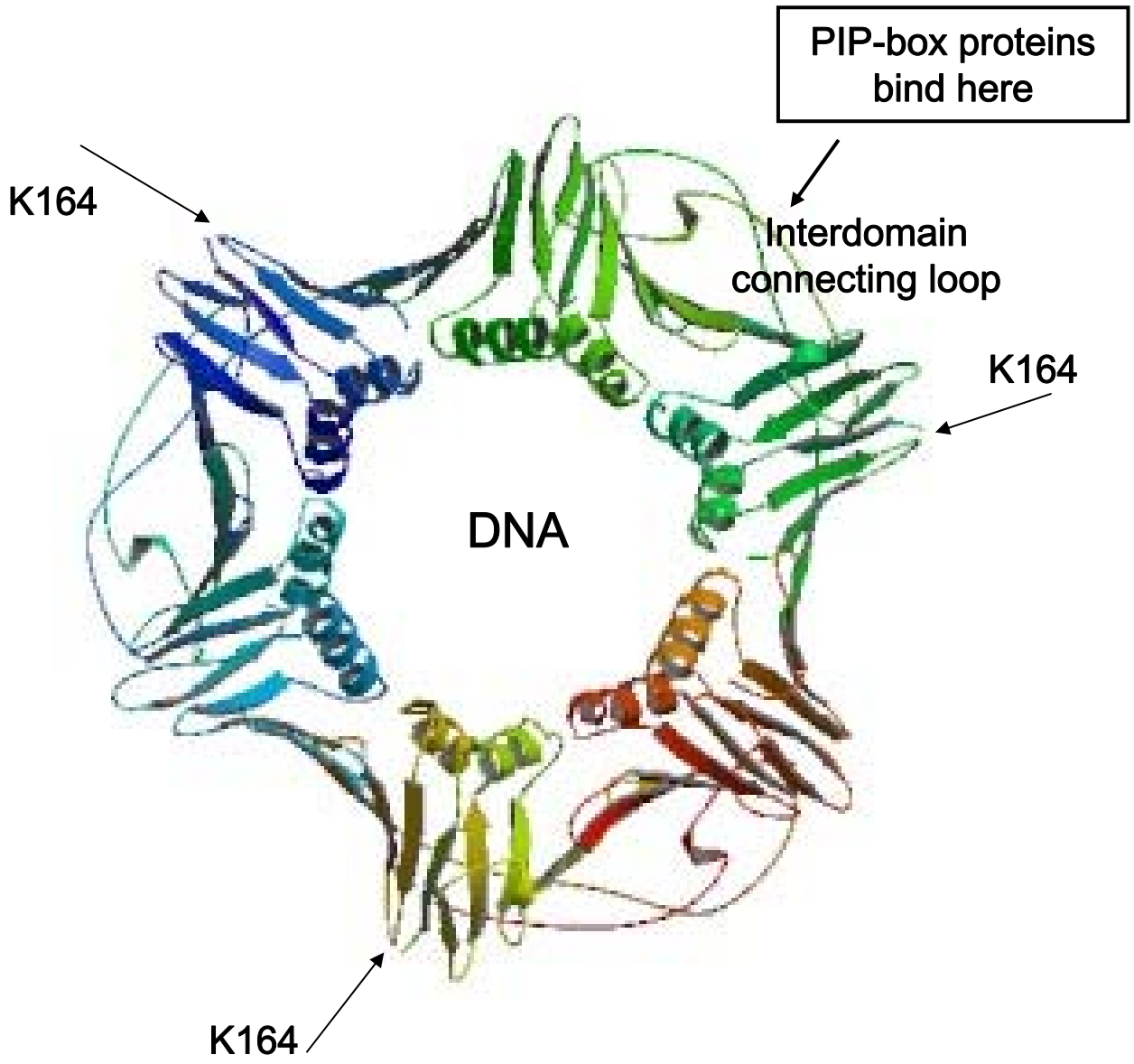
- **Rad6 and Rad18 are required for all processes of postreplication repair**
- **Mms2, Ubc13 and Rad5 are involved in an error-free branch**
- **Rad6 and Ubc13-Mms2 are E2 Ubiquitin conjugating enzymes**
- **Rad18 and Rad5 are E3 ubiquitin ligases**
- **Multiple interactions (Ulrich and Jentsch)**





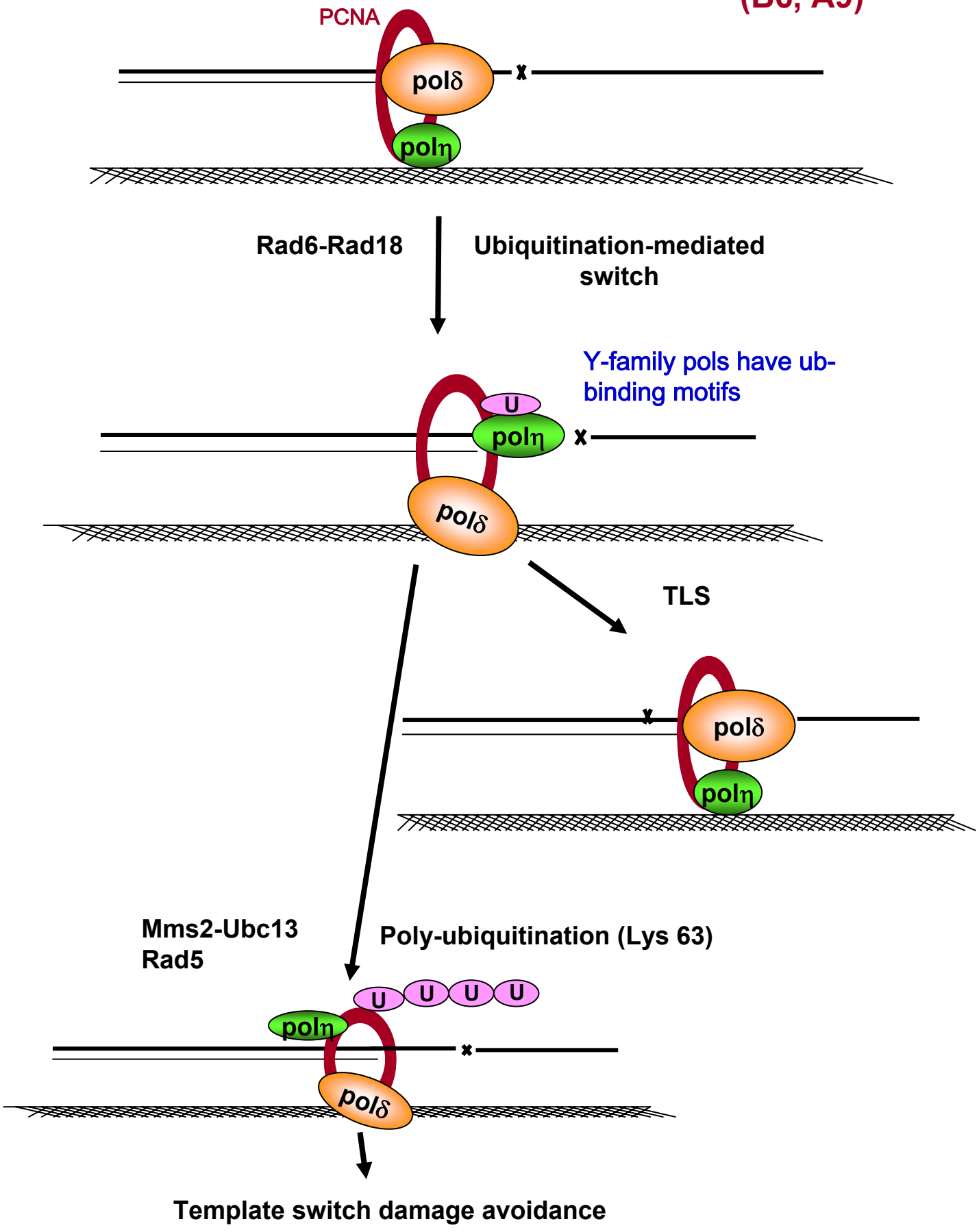
PCNA is the ubiquitination substrate

B6



Switching via ubiquitination of PCNA

(B6, A9)

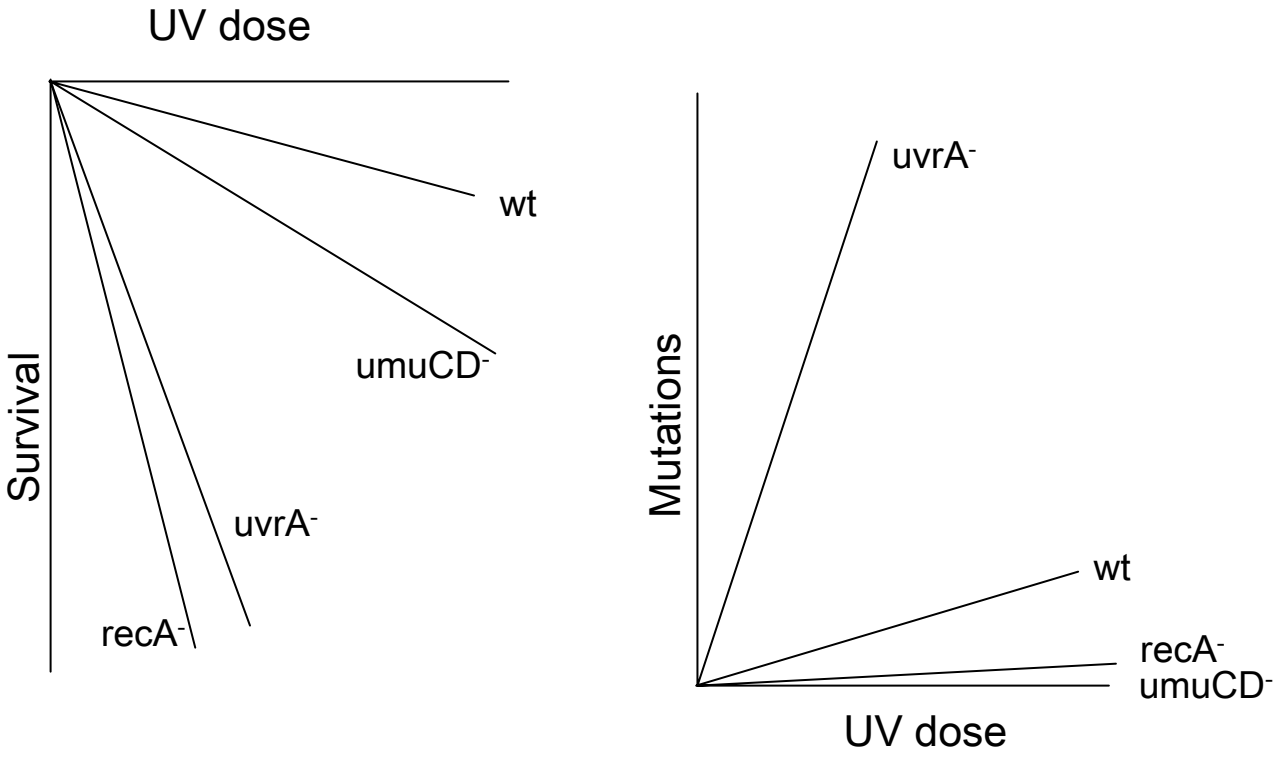


Replication of damage and errors

- **All Y-family polymerases have ubiquitin-binding domains**
- **So they can all bind to Ubiquitinated PCNA**
- **With UV-irradiated DNA, polη makes few errors**
- **In its absence, others can substitute. They make more errors**
- **May need two pols to get past some types of damage, for insertion and extension**
- **TLS can be error-free, but is usually error-prone**
- **The template – switch mechanism is error-free**

Genetics of UV mutagenesis

A. *E. coli*



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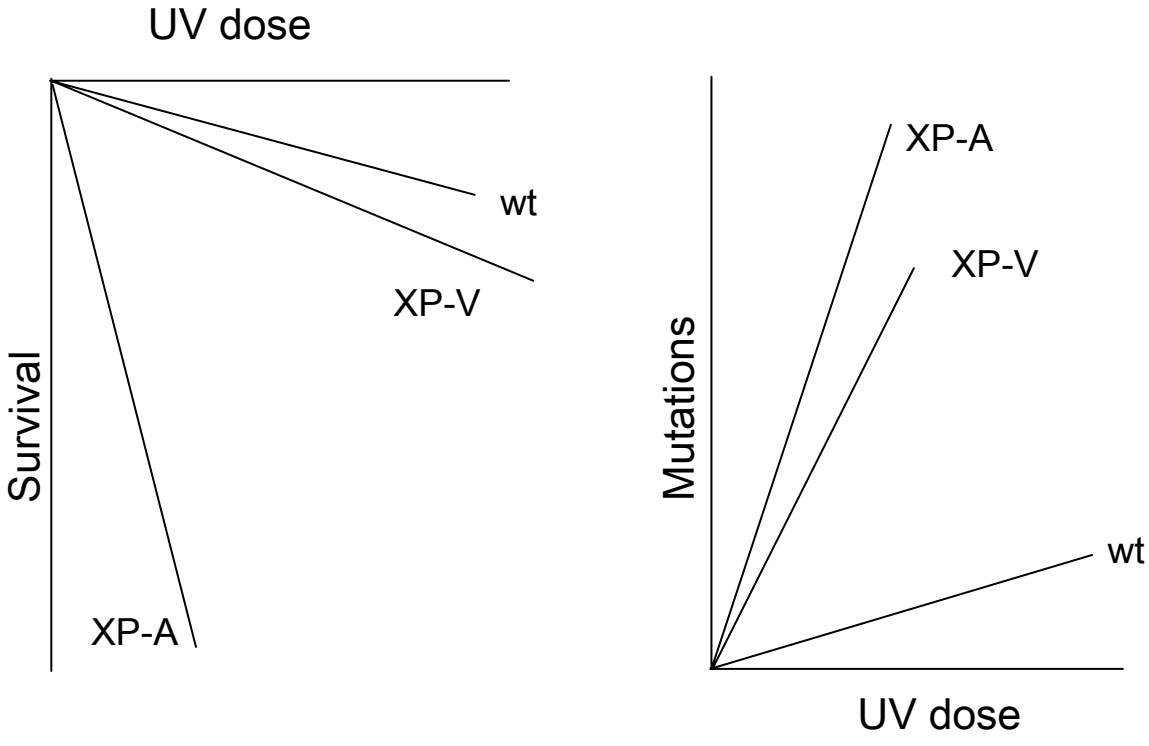
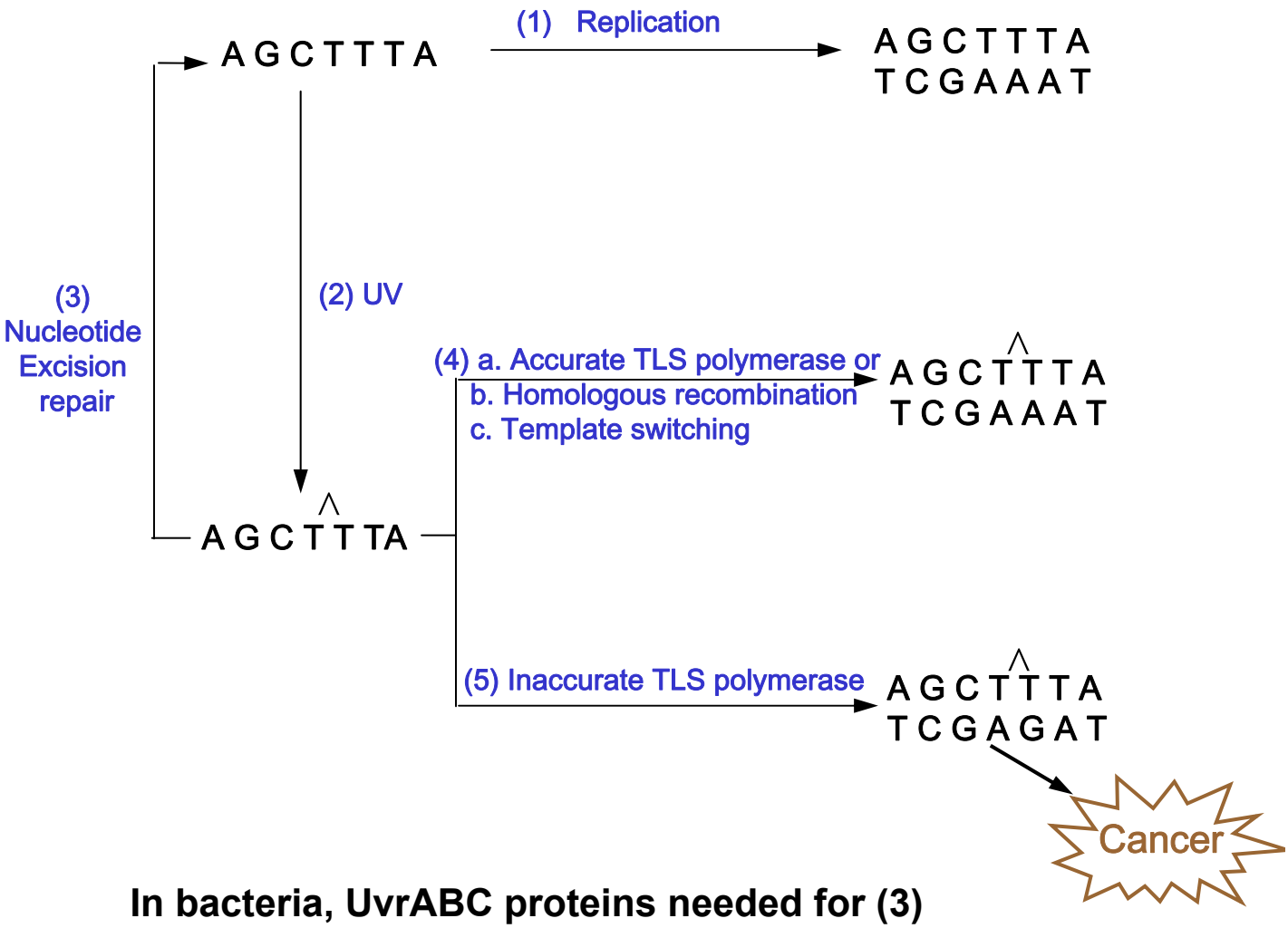


UV mutagenesis



In bacteria, UvrABC proteins needed for (3)

Therefore in *uvr ABC*$^{-}$ cells, more mutations via step (5)

RecA needed for (4) and (5). So no mutations in *recA*$^{-}$ cells

UmuCD needed for (5). So no mutations in *umuCD*$^{-}$ cells

In humans, no excision-repair in excision-defective XPs, so more mutations via step (5)

In XP variants, step (4) a. is deficient, so more mutations via step (5)

Ubiquitination of PCNA modulates channelling into different pathways

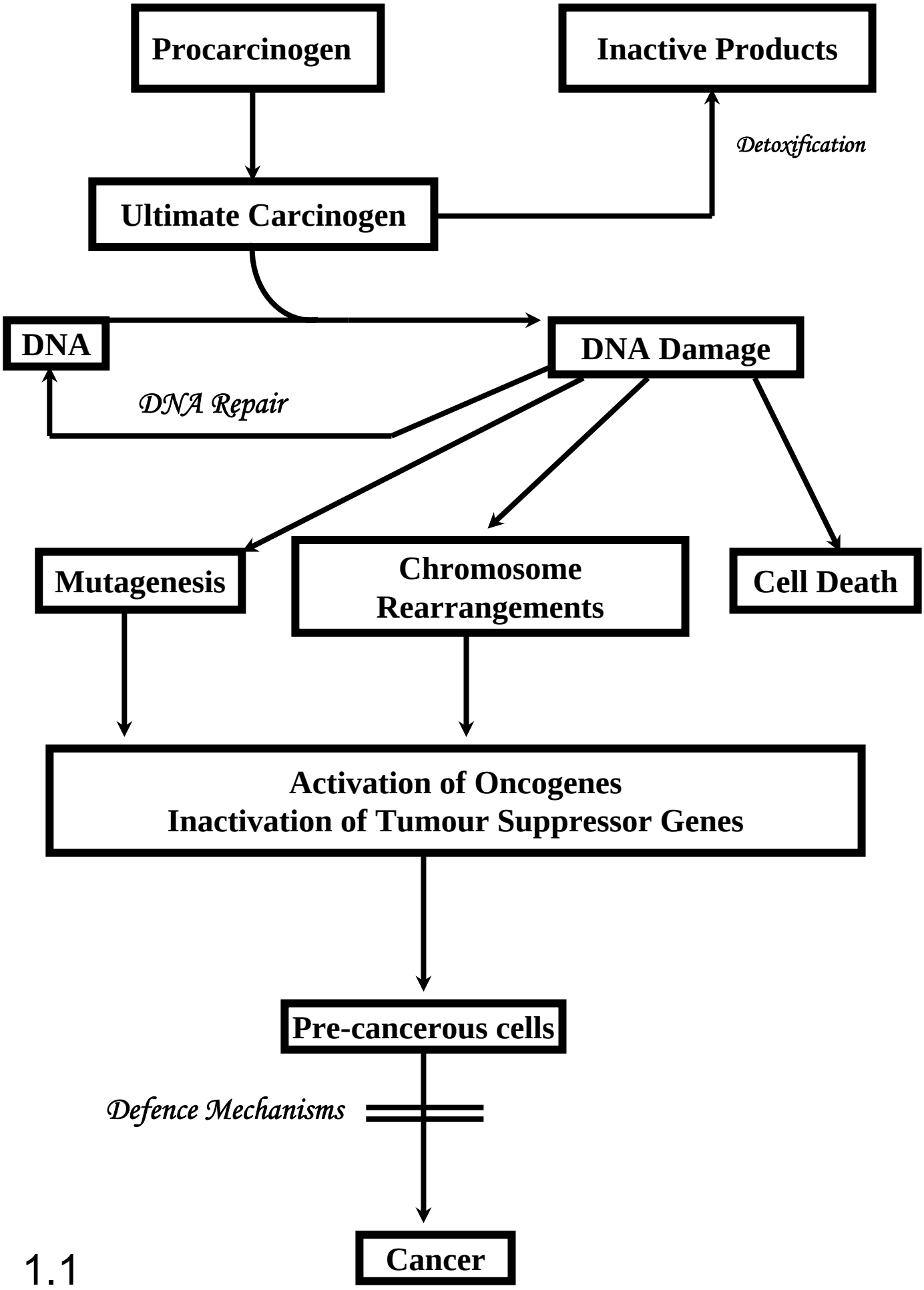
Summary (Lecture 3a)

- ***In E. coli*** **avoidance of damage by recombination is the major pathway**
- **Mutations are generated by translesion synthesis (TLS) using PolV**
- **TLS is carried out by the specialised Y-family of DNA polymerases**
- **XP variants are defective in polη**
- **Polymerase switching is mediated by the ubiquitination of PCNA**

Learning outcomes (Lecture 3b)

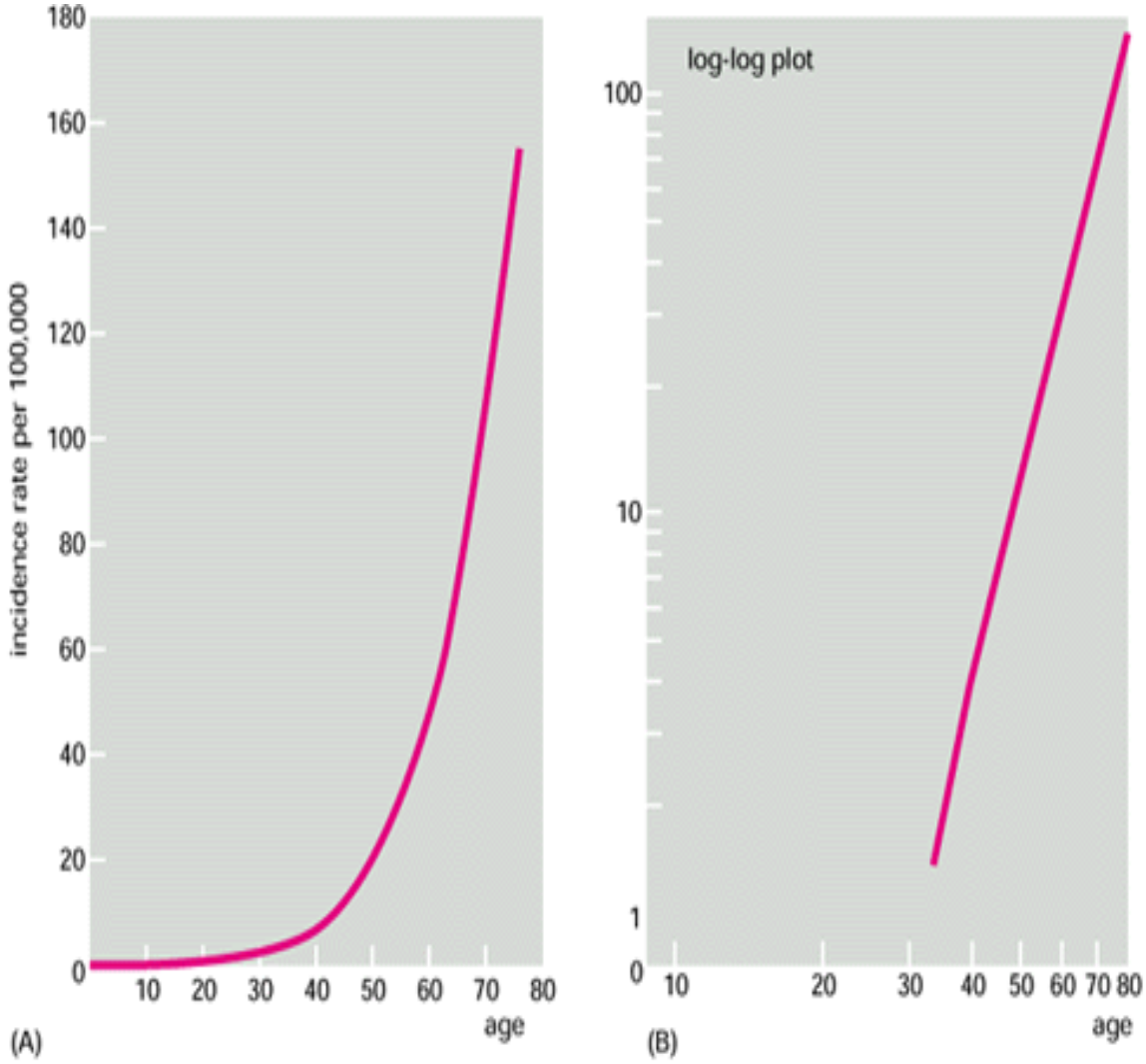
Understanding:

- Age-related incidence of cancer
- Interpretation of mutation signatures in tumours
- Links between DNA damage and ageing


Procarcinogen
Inactive Products
Detoxification
Ultimate Carcinogen
DNA
DNA Damage
DNA Repair
Mutagenesis
Chromosome Rearrangements
Cell Death
Activation of Oncogenes
Inactivation of Tumour Suppressor Genes
Pre-cancerous cells
Defence Mechanisms
Cancer

1.1

Age-related cancer incidence



Cancer incidence proportional to $(Age)^6$

Interpreted to indicate need for 6 events (mutations, chromosome rearrangements)

Mutations in skin cancer (A11)

- Skin cancers, Basal Cell Carcinoma (BCC)
  Squamous cell carcinoma (SCC)
  Malignant Melanoma (MM)

- Cell culture: UV mutations are mainly C →T; CC→TT **at dipyrimidine sites**.

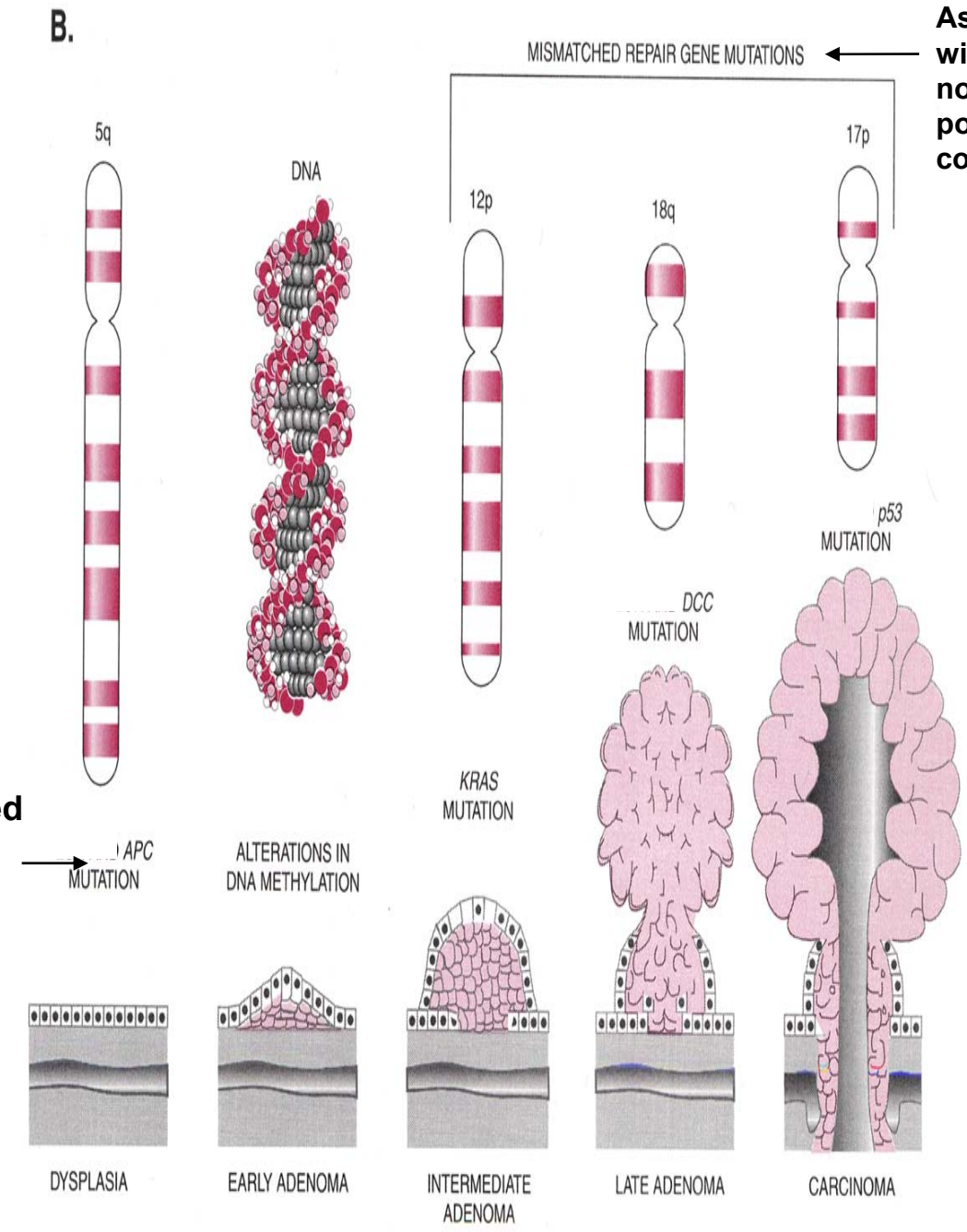
P53

- Database of mutations in **p53 gene**, at sites of dipyrimidines
- 60% skin cancers have p53 mutations. All at dipyrimidines, 65% C→T
- BCC 12% CC → TT, SCC 15%, very characteristic of UV mutations, very different from internal tumours.
- More striking in XP tumours as well. 90% C →T; 60%CC → TT.
- Strong evidence that sunlight induced damage results in p53 mutations.

HPTC

- **Gorlin's syndrome** – high frequency of BCC.
- Gene cloned and found to be *HPTC*, human homologue of Drosophila *patched.*
- Protein is a transmembrane glycoprotein receptor for Hedgehog signalling. Involved in control of differentiation and proliferation. **Not** a DNA repair gene
- Mutations in *HPTC* gene in BCCs in XPs.
- Found in 73% XP BCCs, half are CC to TT. Implies important step in BCC development.

Colon cancer



Associated with familial non-polyposis coli (HNPCC)

Associated with familial polyposis coli (FPC)

Loss of a TSG

Change in Gene expression

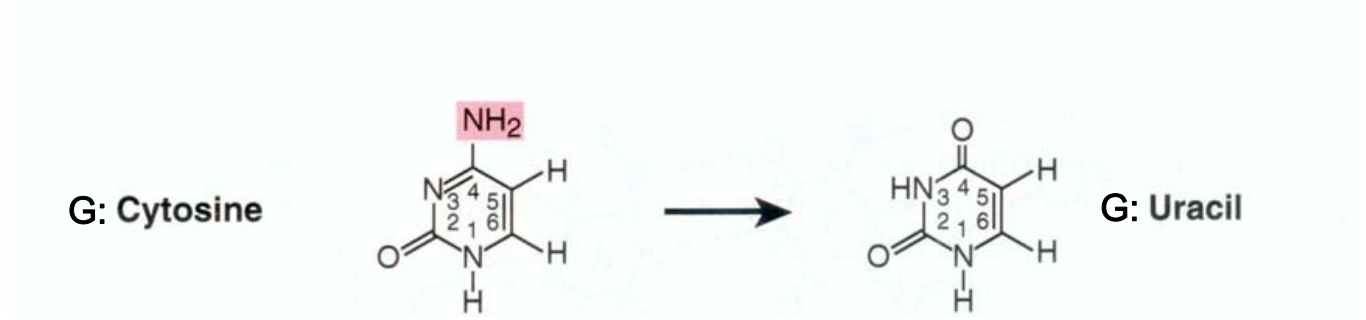
Activation of oncogene

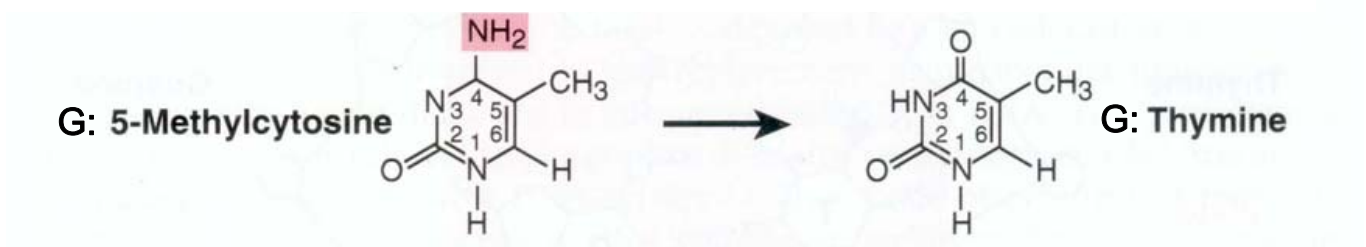
Loss of a TSG

Loss of a TSG

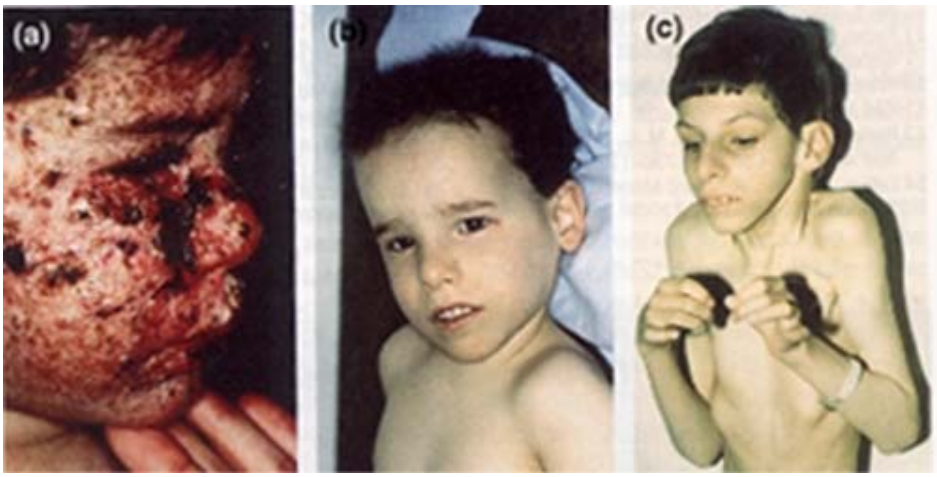
p53 mutations in HNPCC

- Mismatch repair deficiency results in general increase in mutation frequency
- 65% of HNPCC tumours have p53 mutations
- Mutations mainly C →T, but not at dipyrimidine sites, at CpG sites
- Cytosine spontaneously hydrolyses to uracil, which is removed by BER
- Cytosines are methylated at 5 position at many CpG sites
- 5MeC hydrolyses to thymine, resulting in a G:T mismatch, repaired by MMR not BER
- In HNPCC, G:T mismatches repaired poorly.
- This is the major source of p53 mutations in HNPCC





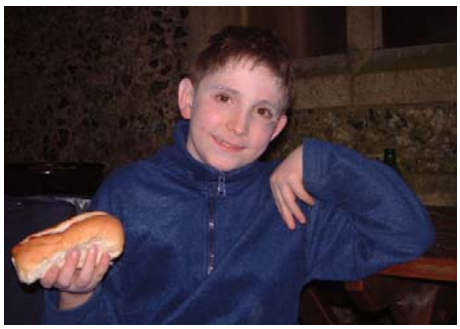
Unanswered questions in XP, CS and TTD



XP TTD CS

Cancer in XP, TTD, CS

- Why no cancer in TTD and CS despite NER defects?
- TTD? Transcription defect interferes with cancer progression?
- What about CS, not essential genes? Most mutations nulls.
- How can we explain the complex combined features of XP and CS, in some XP-B, XP-D, XP-G patients.



Neurological abnormalities

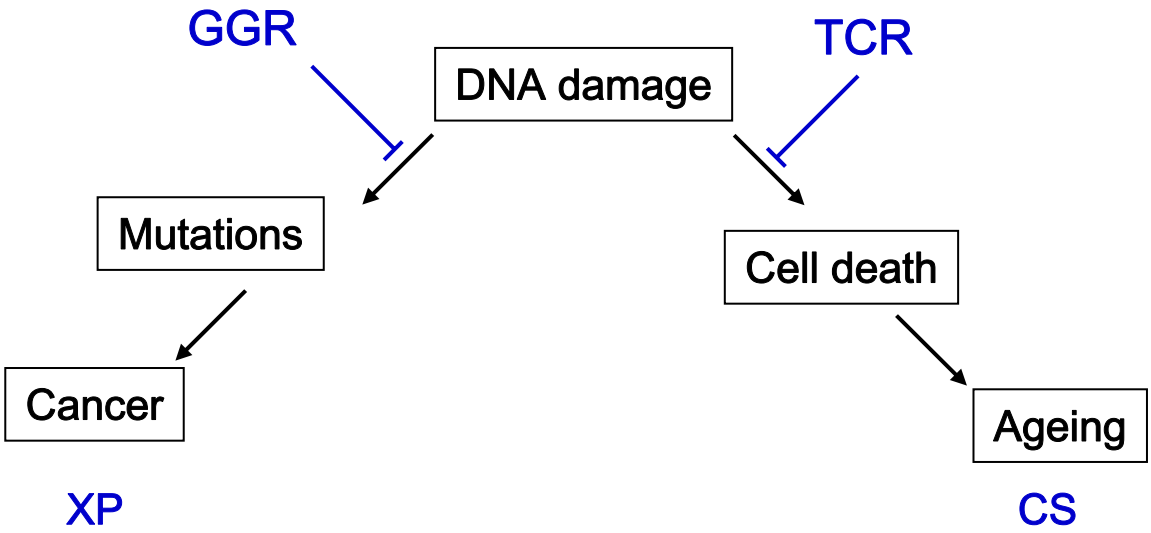
XP-A, D, G progressive neurological degeneration
CS, TTD dysmyelination, mental retardation
?oxidative damage in brain?

Ageing (A12, B7)

- Long-standing hypothesis that decreased repair is a cause of ageing.
- Aspects of premature ageing in CS.
- TTD mouse: after 1 year looks very old.
- XP-A/TTD double even more extreme, implies DNA damage and transcriptional defect result in premature ageing. What is damage?

TTD mouse

Hoeijmakers hypothesis of ageing and cancer



Summary (Lecture 3b)

- **Cancer results from about 6 genetic changes**
- **Mutation signatures in skin cancers show importance of UV damage in p53 and PTCH genes**
- **p53 mutations at CpG sites are important in HNPCC**
- **Unrepaired DNA damage plays a role in ageing**