Cancer Biology I :

Topics covered

Week 1:
Lecture 1: **Hallmarks of cancer – an overview; Oncogenes and tumor suppressor genes**
(Chapters 2, 4, 7 (Weinberg book))

Week 2:
DNA repair of DNA double strand breaks; Synthetic lethality
Lecture and paper discussion

Week 3:
Lecture 3/Exercises: **Synthetic lethality continued; chromatin at double strand breaks; DNA repair: NER; the DNA damage response**

Week 4:
Lecture 4/Exercises: **p53 and apoptosis**
(Chapters 9 (Weinberg))

Links for Background Information on Techniques you Should be Familiar with from Previous Courses

(also covered in text books; e.g. Mol Biol of the Cell (Alberts et al.))

RNA interference
https://en.wikipedia.org/wiki/Small_interfering_RNA

https://horizondiscovery.com/en/applications/rnai/sirna-applications?gclid=EAIaIQobChMIkpaT4N-3-gIV6oODBx1G7Q1pEAAYAiAAEgLjiPD_BwE

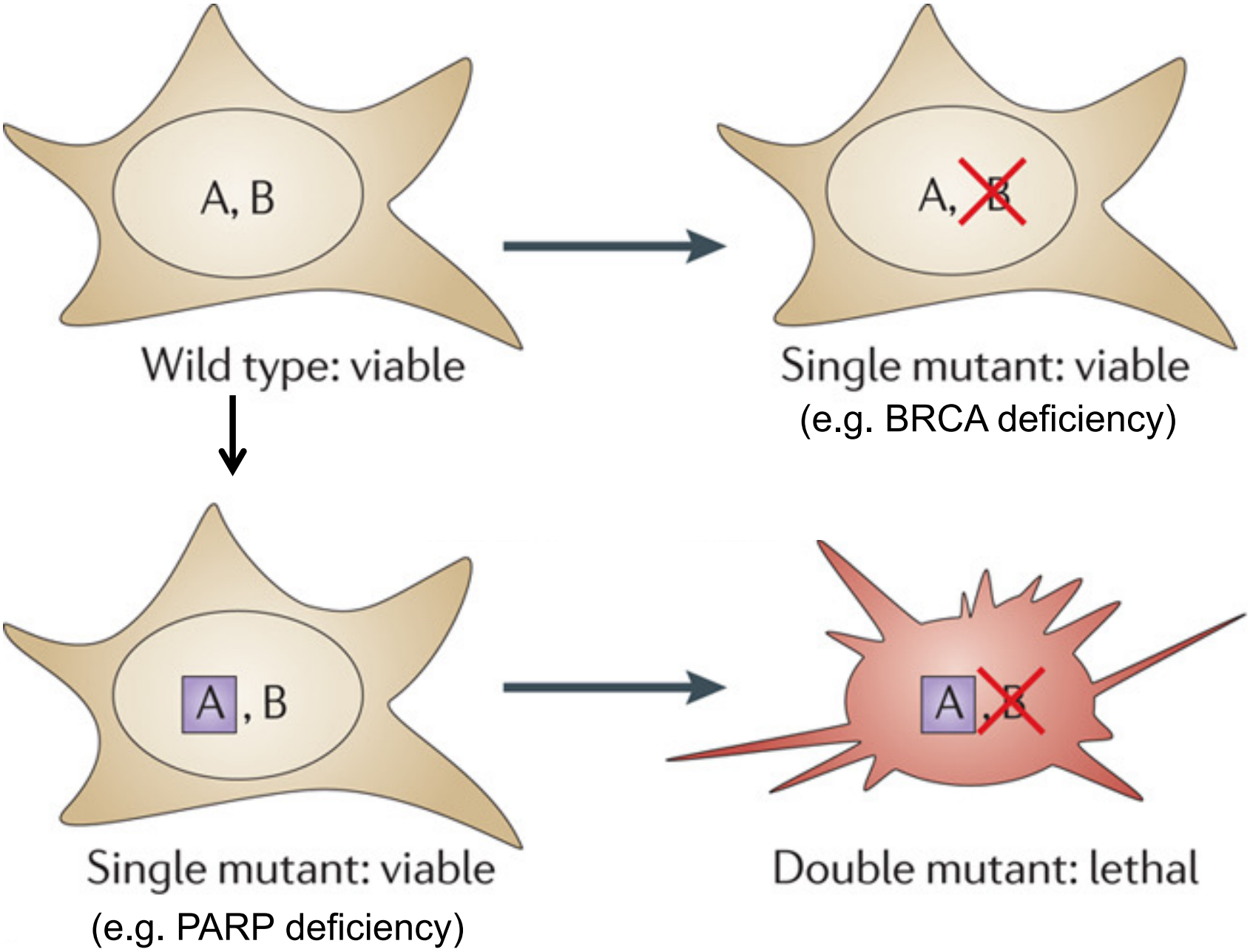
Western blot
https://en.wikipedia.org/wiki/Western_blot

Immunoprecipitation
https://en.wikipedia.org/wiki/Immunoprecipitation
https://www.youtube.com/watch?v=OrVVZ8X3n6k

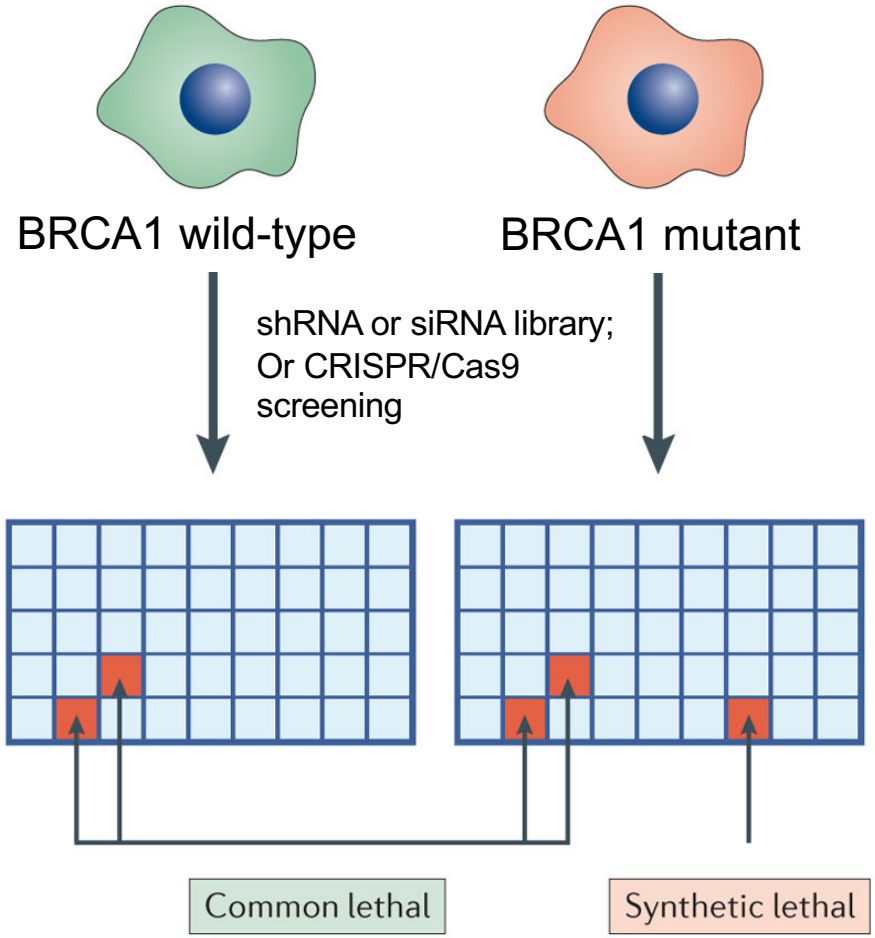
PCR
https://en.wikipedia.org/wiki/Polymerase_chain_reaction

CRISPR-Cas9 genome engineering
https://www.ibiology.org/genetics-and-gene-regulation/crispr-cas9/

From Week 2: Synthetic Lethality

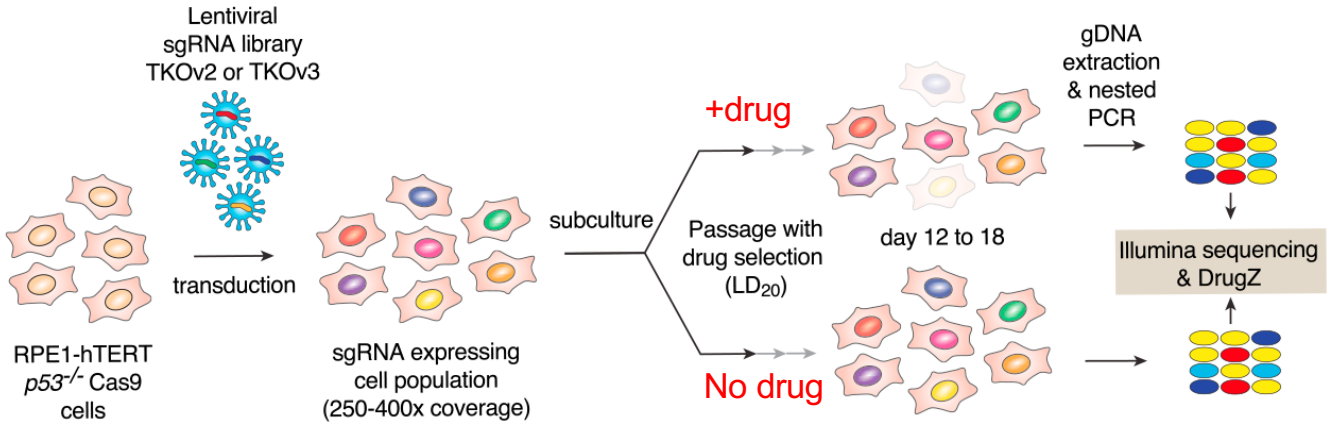


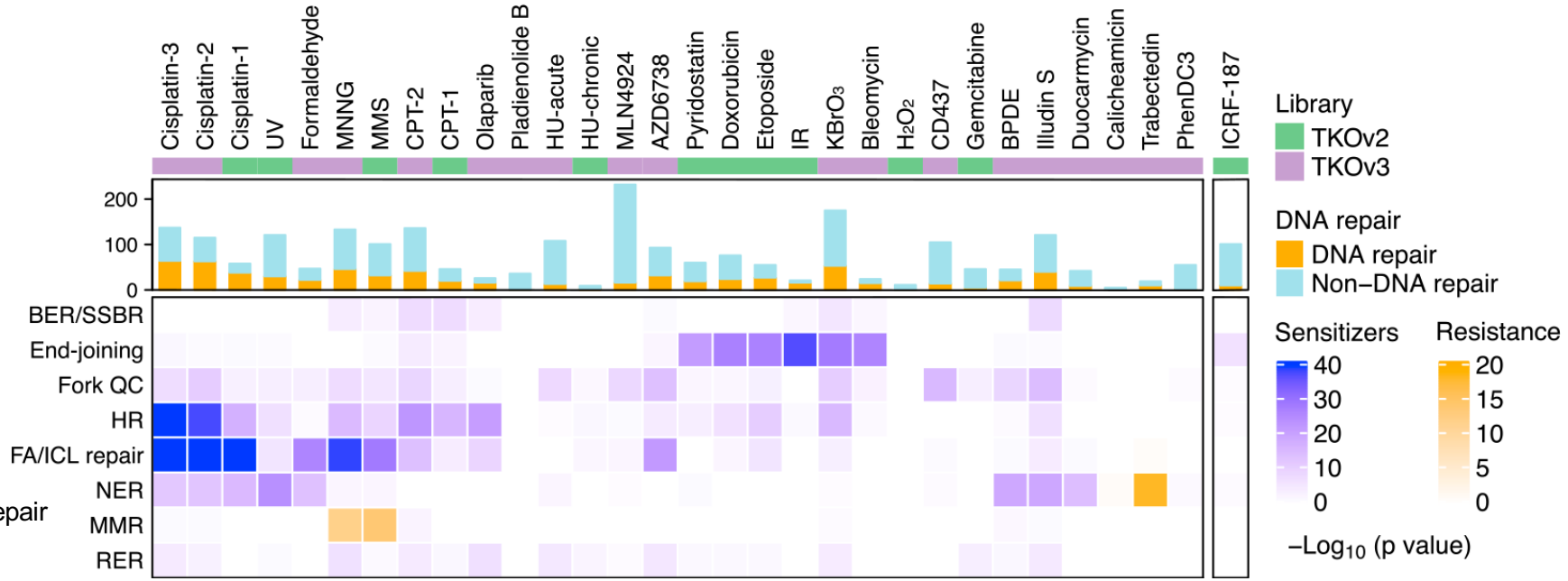
RNAi Screen for Synthetic Lethality



CRISPR-Cas9 Screens: Identification of Genes whose Loss Increases (or Decreases) Sensitivity to a Genotoxic Drug (used in cancer therapy)

RPE: retinal epithelial cells.
hTERT: cells are immortalized.
Cas9: guide RNA-directed DNA endonuclease.
p53 -/- : to avoid cell cycle arrest that may be induced by these drugs.
Essential gene products will not be identified.





BER: Base Excision Repair
HR: Homologous Recombination
NER: Nucleotide Excision Repair
MMR: Mismatch Repair

From Cell 182, 481-496 (2020)

Supplementary Table S1: Screen details including concentrations used, sources and experimenter

Name	Mechanism of Action	Dose used	library	Product supplier / machine ID	Cat#	Author	Set
Cisplatin-2	Inter- intrastrand crosslink/Helix distoring lesion	1.5 µM	TKOv3	https://www.sigmaaldrich.com/catalog/product/sigma/p4394?lang=en&region=CA	Cat# P4394	NH	1
Bleomycin	DNA strand breaks	25 nM	TKOv3	https://www.sigmaaldrich.com/catalog/product/sigma/15361?lang=en&region=CA	Cat# 15361	MZ	2
Olaparib	PARP inhibitor	5 µM	TKOv3	https://www.selleckchem.com/products/AZD2281(Olaparib).html	Cat# S1060	MZ	2
CD437	DNA replication stress	200 nM	TKOv3	https://www.sigmaaldrich.com/catalog/product/sigma/c5865?lang=en&region=CA	Cat# C5865	MO, TC	3
Illudin S	Transcription-interefering	30 nM	TKOv3	https://www.caymanchem.com/product/17451	Cat# 17451	MO, TC	3
KBrO3	Oxidative DNA damage	500 µM	TKOv3	https://www.sigmaaldrich.com/catalog/product/sigald/60085?lang=en&region=CA	Cat# 60085	MO, TC	3
MLN4924 (Pevonedistat)	NAE inhibitor	250 nM	TKOv3	https://www.activebiochem.com/product/231	Cat# A-1139	MO, TC	3
MNNG	Base alkylation	100 nM	TKOv3	https://www.trc-canada.com/product-detail/?CatNum=N493990&CAS=70-25-7&Chemical_Name=N%E2%80%99-Nitro-N-nitroso-N-methylguanidine%20(Stabilized%20with%20Water)&Mol_Formula=C%E2%82%82H%E2%82%85N%E2%82%85O%E2%82%83	Cat# N493990	MO, TC	3
Cisplatin-3	Inter- intrastrand crosslink/Helix distoring lesion	1.5 µM	TKOv3	https://www.sigmaaldrich.com/catalog/product/sigma/p4394?lang=en&region=CA	Cat# P4394	SA	4
Camptothecin (CPT)-2	DNA strand breaks	6 nM	TKOv3	https://www.sigmaaldrich.com/catalog/product/sigma/c9911?lang=en&region=CA	Cat# C9911	SA	5
Calicheamicin	DNA strand breaks	0.5 nM - 0.05nM	TKOv3	https://www.medchemexpress.com/Calicheamicin.html	Cat# HY-19609	MO, JY	6
Duocarmycin SA	Base alkylation	1 nM - 0.075 nM	TKOv3	https://www.creative-biolabs.com/adc/duocarmycin-sa-746.htm	Cat# ADC-P-043	MO, JY	6
Formaldehyde	Inter- intrastrand crosslink	63.5 µM	TKOv3	https://www.sigmaaldrich.com/catalog/product/sial/252549?lang=en&region=CA	Cat# 252549	MO, JY	6
Phen-DC3	C-quadruplex stabilizer	1 µM	TKOv3	https://www.polysciences.com/default/phen-dc3	Cat# 26000	MO, JY	6
Trabectedin	Transcription-interefering	1 nM - 0.075 nM	TKOv3	https://www.trc-canada.com/product-detail/?CatNum=T703500&CAS=114899-77-3&Chemical_Name=Trabectedin&Mol_Formula=C%E2%82%83%E2%82%89H%E2%82%84%E2%82%83N%E2%82%83O%E2%82%81%E2%82%81S	Cat# T703500	MO, JY	6
AZD6738	ATR inhibitor	0.5 µM	TKOv3	https://www.medchemexpress.com/AZD6738.html?src=google-product&gclid=CjwKCAjwxaXtBRBbEiwAPqPxcL0DcECB0Evzv-IlkhFibqfnHxGvkqQu2_gAQpqgTdDYc7jJTQOcIBoCuQ0QAvD_BwE	Cat# HY-19323	AAQ	7
Camptothecin (CPT)-1	DNA strand breaks	5 nM	TKOv2	https://www.sigmaaldrich.com/catalog/product/sigma/c9911?lang=en&region=CA	Cat# C9911	MO, TC	8
Cisplatin-1	Inter- intrastrand crosslink/Helix distoring lesion	1 mM	TKOv2	https://www.sigmaaldrich.com/catalog/product/sigma/p4394?lang=en&region=CA	Cat# P4394	MO, TC	8
Etoposide (VP-16)	DNA strand breaks	100 nM	TKOv2	https://www.sigmaaldrich.com/catalog/product/sigma/e1383?lang=en&region=CA	Cat# E1383	MO, TC	8
Hydroxyurea (HU)	DNA replication stress	100 µM (chronic)	TKOv2	https://www.sigmaaldrich.com/catalog/product/sigma/h8627?lang=en&region=CA	Cat# H8627	MO, TC	8
Ionizing radiation (IR)	DNA strand breaks	3 Gy	TKOv2	Faxitron 43855C	N/A	MO, TC	8
Doxorubicin	DNA strand breaks	5 nM	TKOv2	https://www.sigmaaldrich.com/catalog/product/sigma/d1515?lang=en&region=CA	Cat# D1515	MO, GSM	9
Hydrogen Peroxide (H2O2)	Oxidative DNA damage	15 µM	TKOv2	https://www.sigmaaldrich.com/catalog/product/sigma/h1009?lang=en&region=CA	Cat# H1009	MO, GSM	9
Methyl Metanesulfonate (MMS)	Base alkylation	25 nM	TKOv2	https://www.sigmaaldrich.com/catalog/product/aldrich/129925?lang=en&region=CA	Cat# 129925	MO, GSM	9
Pyridostatin	C-quadruplex stabilizer	0.75 µM	TKOv2	https://www.sigmaaldrich.com/catalog/product/sigma/sml0678?lang=en&region=CA	Cat# SML0678	MO, GSM	9
Ultraviolet Light (UV)	Helix distoring lesion	5 J/m2	TKOv2	UVS 254 nm lamp	N/A	MO, GSM	9
ICRF-187	TOP2 inhibitor	25 µM	TKOv2	https://www.sigmaaldrich.com/catalog/product/sigma/d1446?lang=en&region=CA	Cat# D1446	ASB, IDS	10
benzo(a)pyrene diol epoxide (BPDE)	Helix distoring lesion	200 nM	TKOv3	https://www.scbt.com/p/benzoapyrene-diol-epoxide-58917-67-2?requestFrom=search	Cat# sc-503767A	TC, SF	11
Hydroxyurea (HU)	DNA replication stress	1.5 mM (acute)	TKOv3	https://www.sigmaaldrich.com/catalog/product/sigma/h8627?lang=en&region=CA	Cat# H8627	TC, SF	11
Pladienolide B (PladB)	splicing-interefering	0.5 nM	TKOv3	https://www.tocris.com/products/pladienolide-b_6070	Cat# 6070	TC, SF	11
Gemcitabine	DNA replication stress	3 nM	TKOv2	https://www.sigmaaldrich.com/catalog/product/sigma/g6423?lang=en&region=CA	Cat# G6423	TU, MWF	12

From Cell 182, 481-496 (2020)

Example: PARP-1 inhibitors

- BRCA-silenced or mutated cells are sensitive to PARP-1 inhibitors (as discussed previously)
- Recent work (published in 2015): Pol θ (teta) which is involved in microhomology-mediated end joining is also a lethal target for the treatment of BRCA1-mutated cancers.

→ Development of PARP-inhibitors

- Olaparib: cellular model (*BRCA2*-deficient Capan-1 cells); concentration for 50% reduction in survival: IC_{50} = 259 nM ; approved by the FDA (US food and drug administration) and EMA (European medicines agency) for the treatment of BRCA-mutated advanced ovarian cancer.
- Talazoparib has also been approved. Talazoparib traps PARP on DNA and is effective at low concentrations in a cellular model (Capan-1 cells) (IC_{50} =5 nM).

Table 1 Comparison of clinical PARP inhibitors based on the individual parameters[a]

	Catalytic inhibition (IC_{50} in wild-type DT40 cells) (μM)	Cytotoxicity (IC_{90} in wild-type DT40 cells) (μM)	Cytotoxicity (IC_{90} in *Brca2*-deficient DT40 cells) (μM)	PARP trapping potency (relative to olaparib)	Anticancer clinical application (dose)
Olaparib	0.006	4.6	0.20	1	Approved as single agent for ovarian and breast cancers for maintenance and for ovarian cancers with *BRCA* mutations (300 mg × 2/day)
Niraparib	0.060	2.3	NA	2	Approved as single agent for ovarian cancer for maintenance (300 mg × 1/day)
Rucaparib	0.021	3.1	0.15	1	Approved as a single agent for ovarian cancer for maintenance and for ovarian cancers with *BRCA* mutations (600 mg × 2/day)
Talazoparib	0.004	0.5	0.006	100	Approved as a single agent for advanced metastatic HER2-negative breast cancers with deleterious or suspected deleterious germline *BRCA* mutations (1 mg × 1/day)
Veliparib	0.030	>50	15	<0.2	Combination clinical trials (400 mg × 2/day)

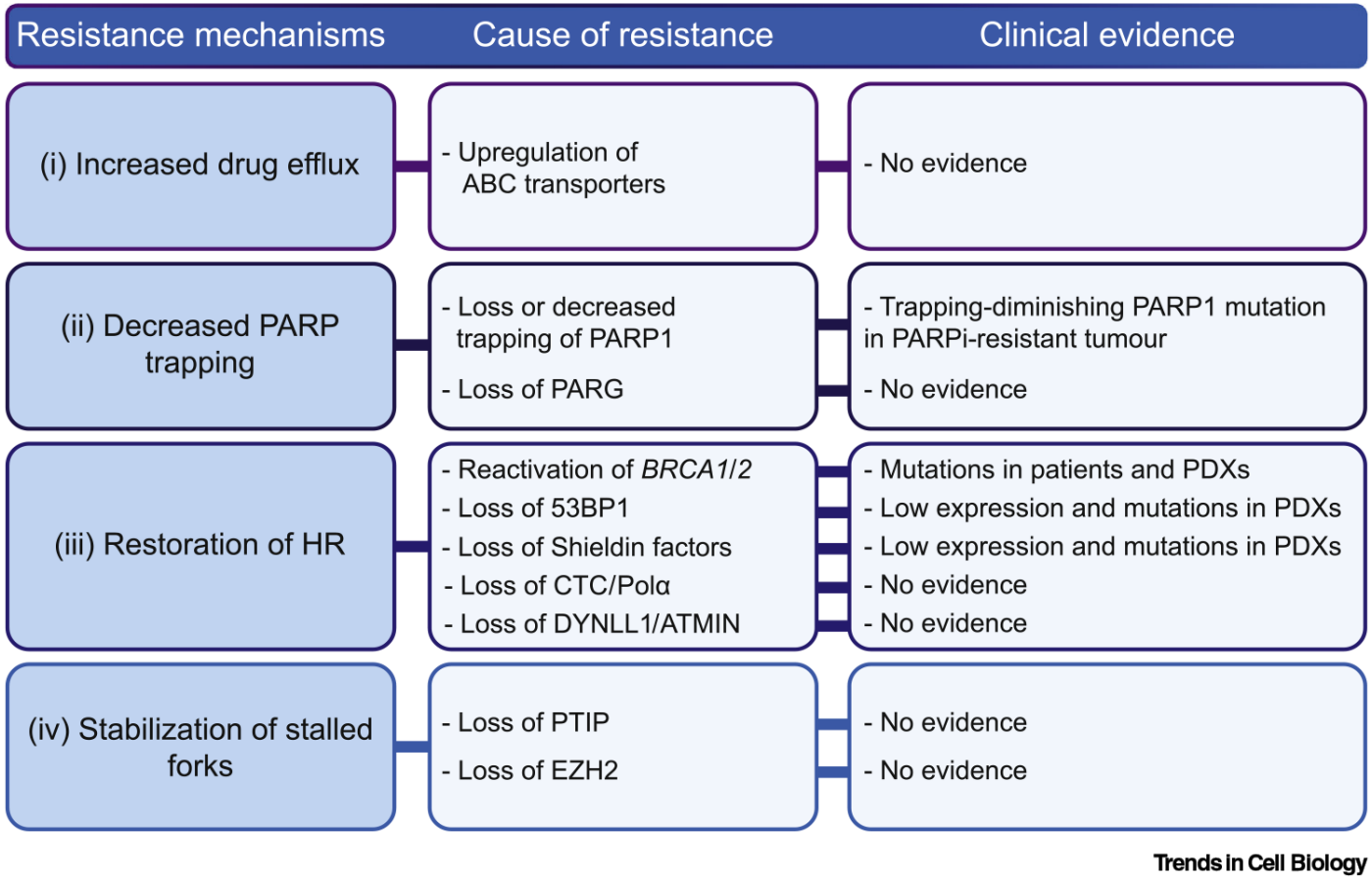
Abbreviations: IC_n, n% of maximal inhibitory concentration; NA, not available; PARP, poly(ADP-ribose) polymerase.

[a]Data from Murai et al. (2012, 2014a) and Murai & Pommier (2015).

Resistance to therapy

- Resistance to platinum-based chemotherapies is a strong predictor for PARPi resistance, indicating sharing of common mechanisms.

PARP Inhibitor Resistance



PDX: patient derived xenografts

Not discussed in this course:

Figure 2. Modes of Resistance to PARP Inhibitors (PARPi). An overview of the four distinct categories of PARPi resistance mechanisms. In each category (left column), all molecular mechanisms that have been identified in preclinical studies are mentioned (middle column). In addition, whether direct clinical evidence for PARPi resistance has been observed in primary tumor material or PDX-models until this date is indicated (right column). Abbreviations: HR, homologous recombination; PARP, poly(ADP-ribose) polymerase; PDX, patient-derived xenograft.

- PARG: Poly (ADP-ribose) glycohydrolase; removes poly ADP-ribose moieties
- Loss of 53BP1, shieldin, CTC1 →increased 3' overhangs at DSBs

→ reactivates resection and HR in BRCA1 cells (in BRCA1 deficiency resection is inhibited). BRCA2 deficient cells are not acquiring PARP inhibitor resistance by loss of 53BP1.

From: Trends Cell Biol 29, 820 (2019)

Loss of 53BP1 in *BRCA1*-mt Cells Leads to PARPi-resistance

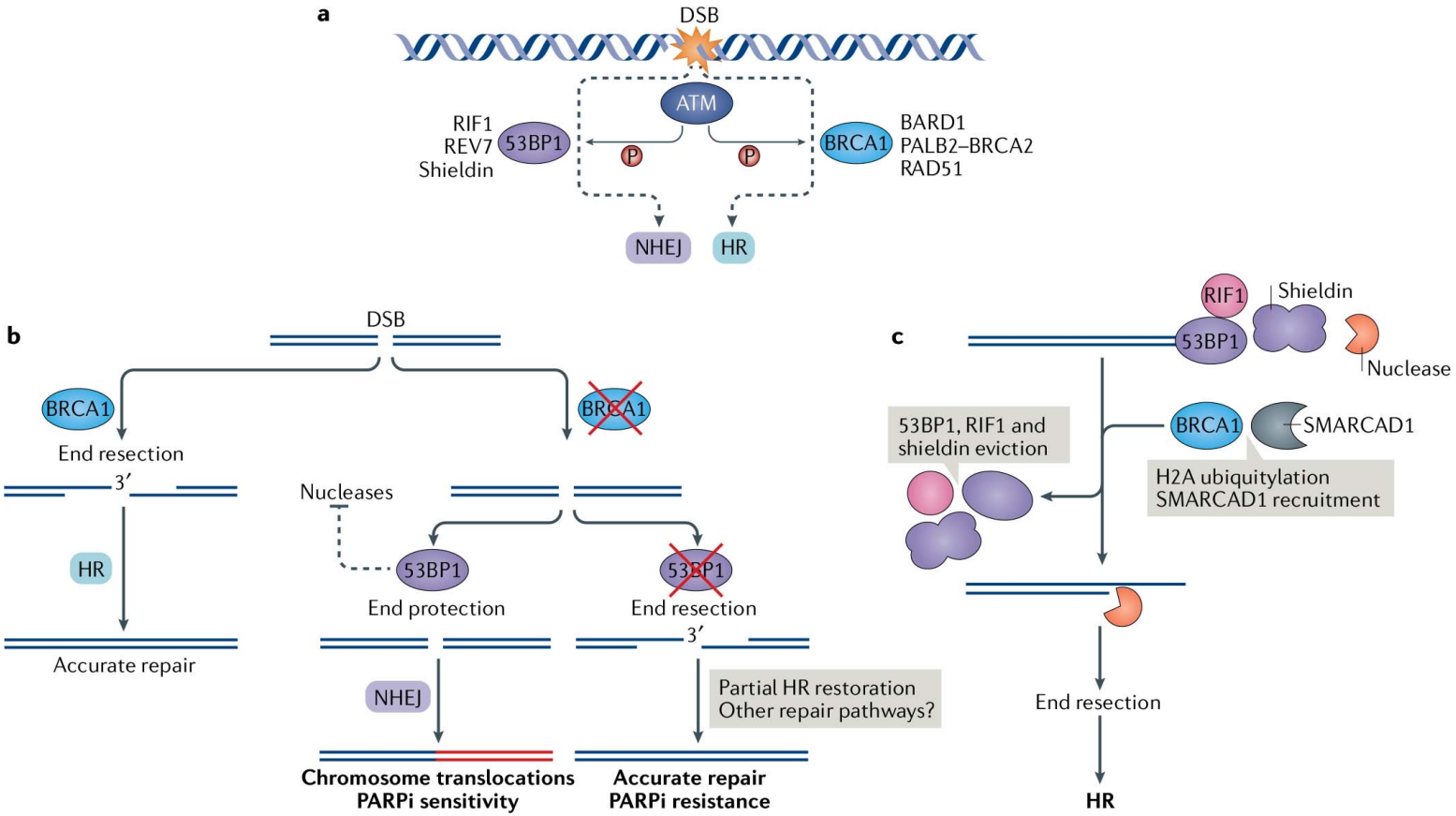


Fig. 3 | **Antagonistic roles of BRCA1–BARD1 and the 53BP1 complex. a** | A DNA double-strand break (DSB) elicits ataxia telangiectasia mutated (ATM)-dependent checkpoint responses, which activate and recruit breast cancer type 1 susceptibility protein (BRCA1)-containing and/or p53-binding protein 1 (53BP1)-containing complexes to the DNA lesion. BRCA1 and 53BP1 compete and perform opposing functions at broken DNA ends by engaging either homologous recombination (HR) or non-homologous end joining (NHEJ) for DSB repair, respectively. **b** | BRCA1 promotes DNA end resection and HR repair (left). If BRCA1 is abrogated, DSB ends are protected from resection by 53BP1-containing complexes and are channelled into NHEJ for repair (right). This process can lead to deleterious chromosome translocations, which underline the sensitivity of BRCA1-deficient cells to poly(ADP-ribose) polymerase inhibitors (PARPi). If, in addition to BRCA1, 53BP1 is concomitantly abrogated, the DNA ends are accessible to resection nucleases, leading to partial HR restoration and PARPi resistance. **c** | 53BP1-containing complexes (53BP1–RIF1–shieldin) protect DNA ends from nucleolytic digestion by nucleases. This end protection is overcome by BRCA1-mediated histone H2A ubiquitylation and recruitment of the helicase SMARCAD1. The eviction of the 53BP1-containing complexes leads to DNA end resection and HR.

From: NatRevMolCellBiol 2020: https://doi.org/10.1038/s41580-020-0218-z

Targeting Resistance

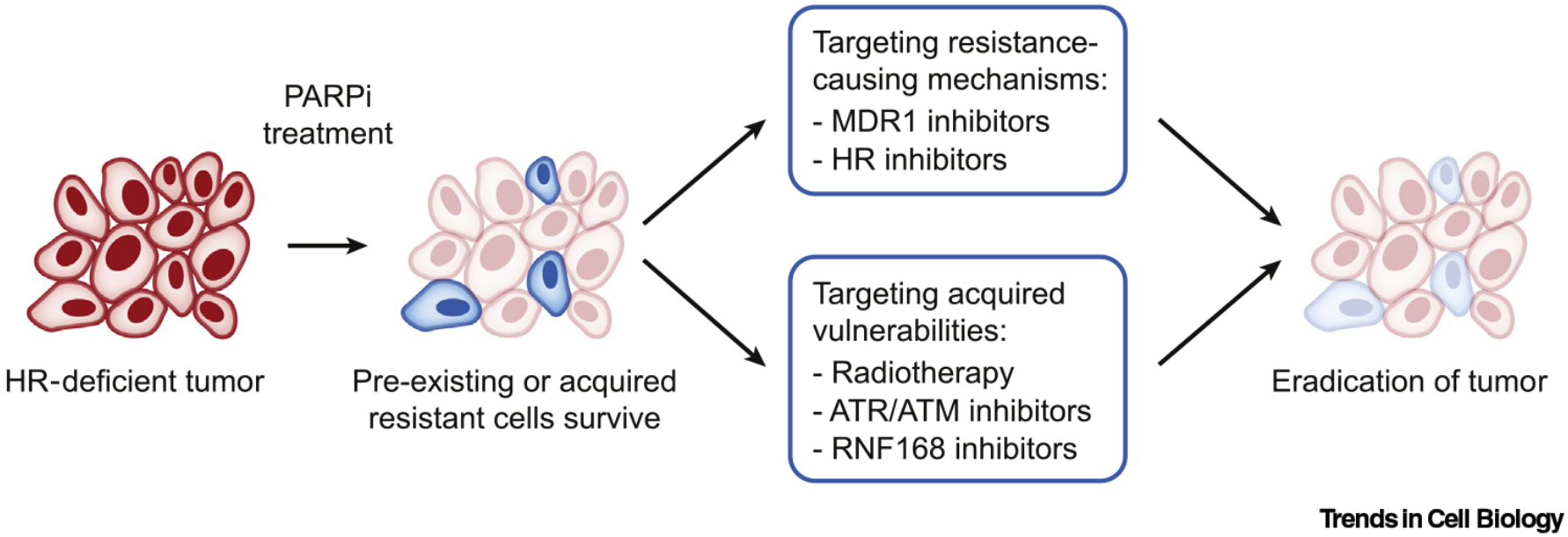


Figure 5. Novel Treatment Options for Tumors Resistant to PARP Inhibitors (PARPi). PARPi have shown great promise in the selective killing of homologous recombination (HR)-deficient tumors, although the rates of pre-existing or acquired resistance are high. Depending on the resistance mechanism, specific treatment options are available to target resistance by inhibiting increased drug efflux (MDR1 inhibitors) or by inhibiting reactivated HR (e.g., by using CDK, HDAC, or PI3K inhibitors). As a second approach, the resistant clones can potentially be killed by drugs targeting acquired vulnerabilities such as sensitivity to irradiation as well as by ATR, ATM, and RNF168 pathway inhibitors.

From: Trends Cell Biol 29, 820 (2019)

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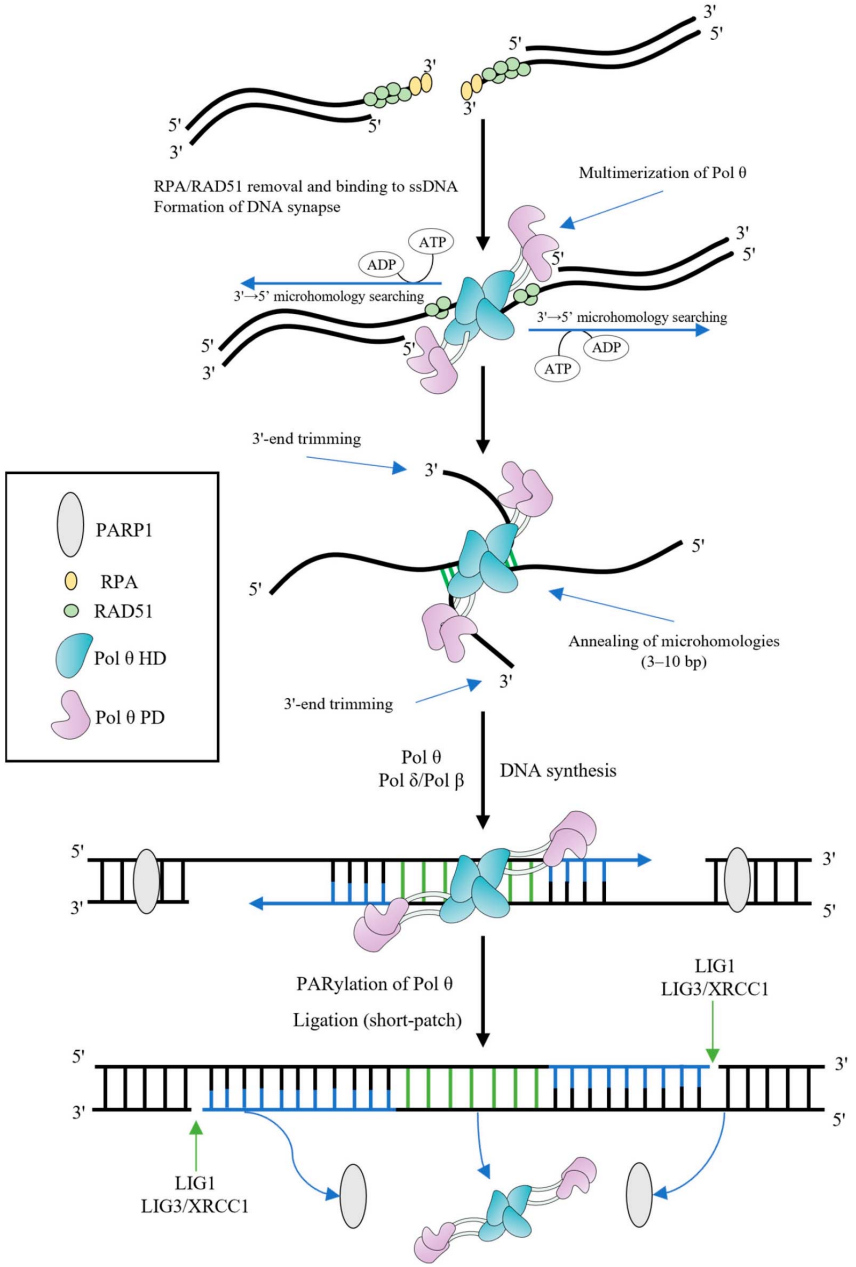
Pol θ (teta) and MMEJ

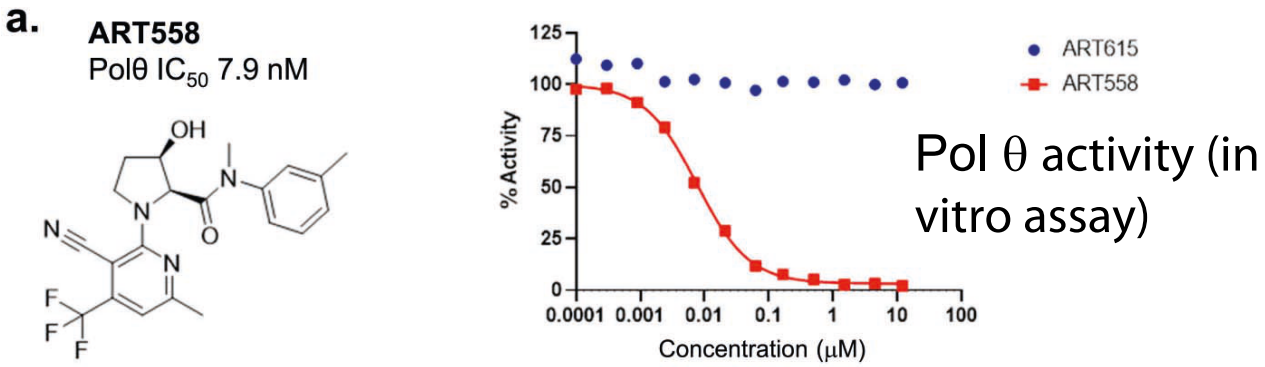


Model of MMEJ. . The resected 3′-overhangs are coated by RPA and RAD51 filaments. The HD of Pol θ removes both of them in ATP-dependent manner, contributing to TMEJ. Multimerization of Pol θ promotes the DNA synapse formation and initiates the 3′→5′ bidirectional searching for microhomologies, using the energy of ATP hydrolysis. Aligned 3′-ends are annealed in microhomology-rich regions. Unannealed 3′-overhangs are trimmed before DNA extension. Pol θ (or Pol β) fills gaps from annealed microhomologies, using them as primers. PARP1 PARylates Pol θ in order to remove the polymerase from DNA and complete the synthesis step. LIG1 or LIG3/XRCC1 complex finish the end joining (the short-patch resolution is shown).

From https://doi.org/10.3390/ijms24043619

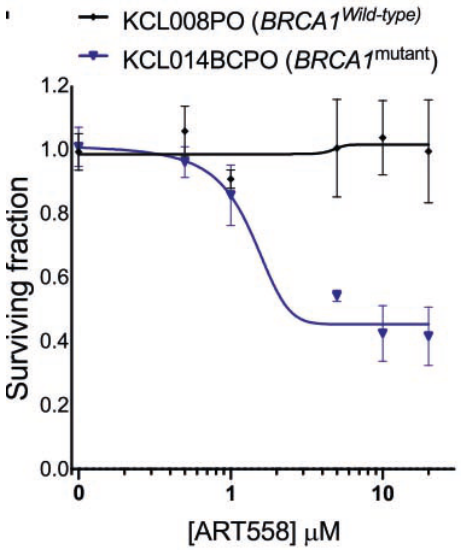
Development of Pol θ (teta) Inhibitors

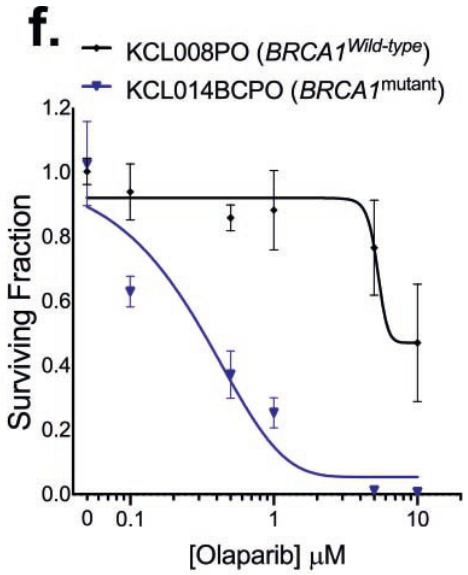
(e.g. NatComm 12: 3636 (2021); NatCancer 2, 598 (2021))



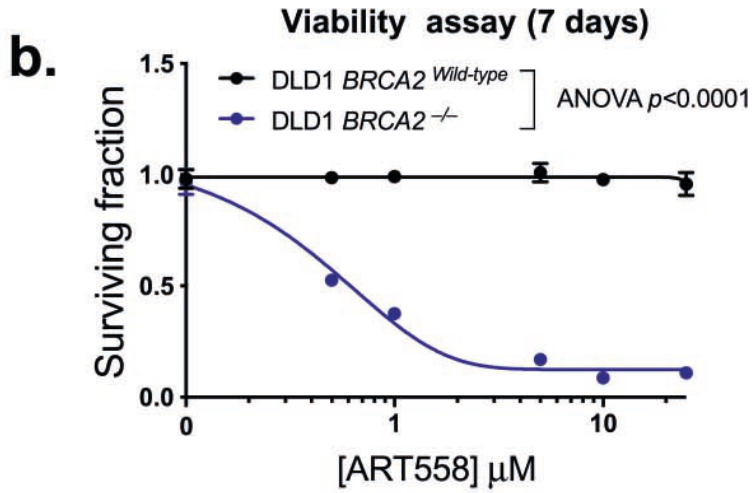
Pol θ activity (in vitro assay)

Breast cancer cells:





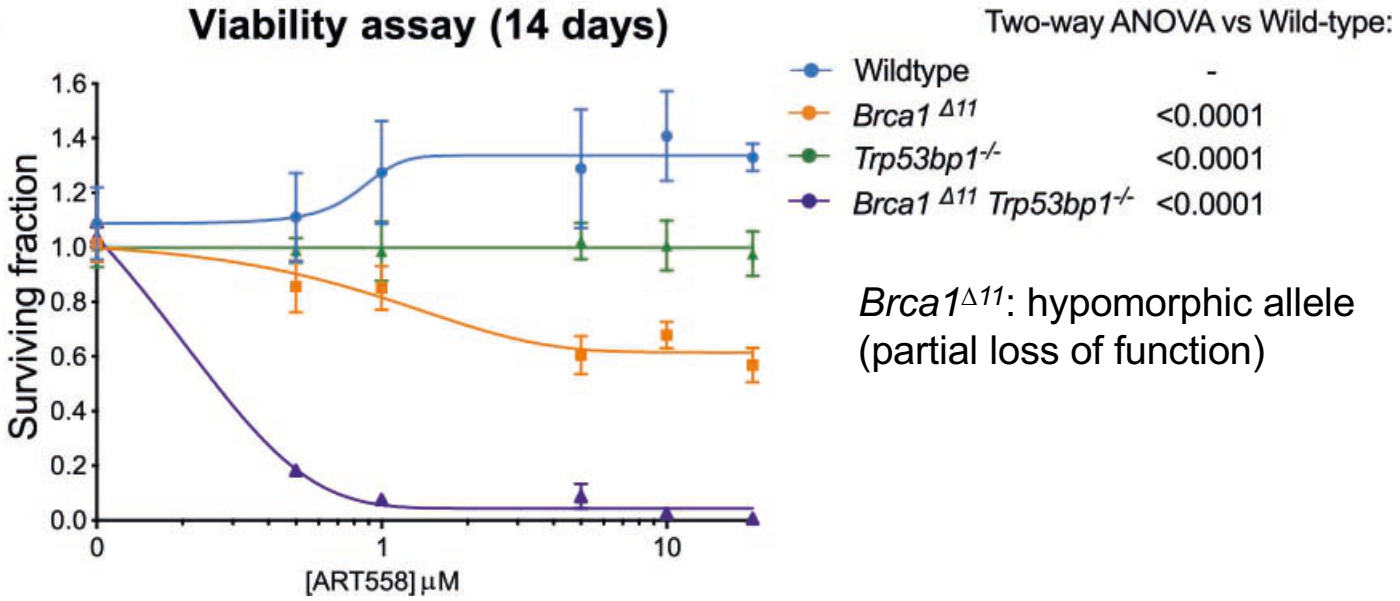
Colorectal adenocarcinoma cells:



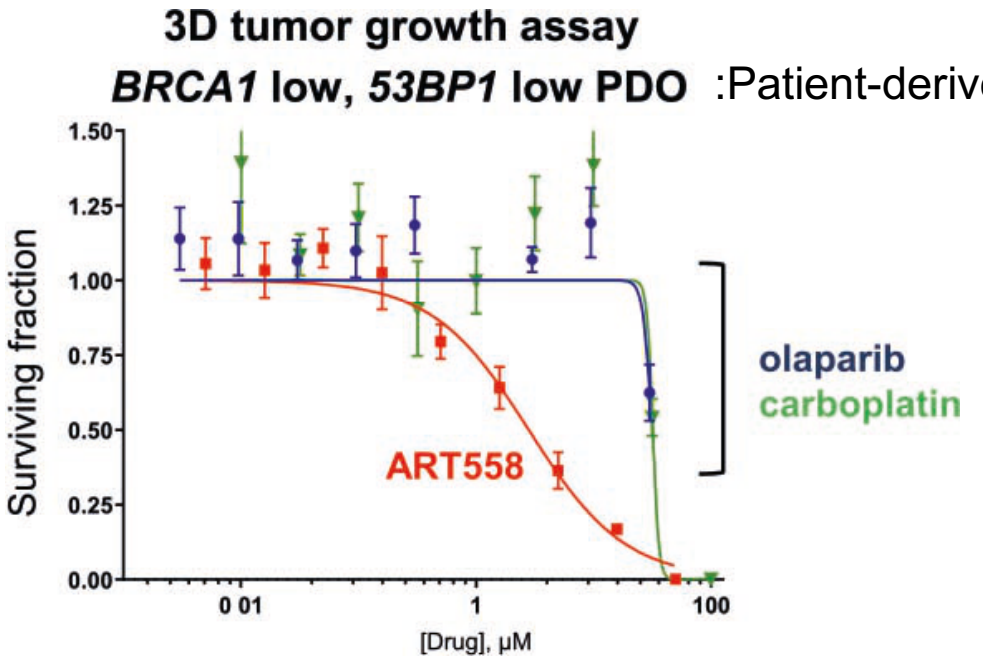
…the Pol θ inhibitor targets BRCA1 and BRCA2-deficient cells

Pol θ (teta) Inhibitor Targets even a PARPi Resistant *BRCA1-mt* Cells

(NatComm 12: 3636 (2021))



Brca1$^{\Delta 11}$: hypomorphic allele (partial loss of function)

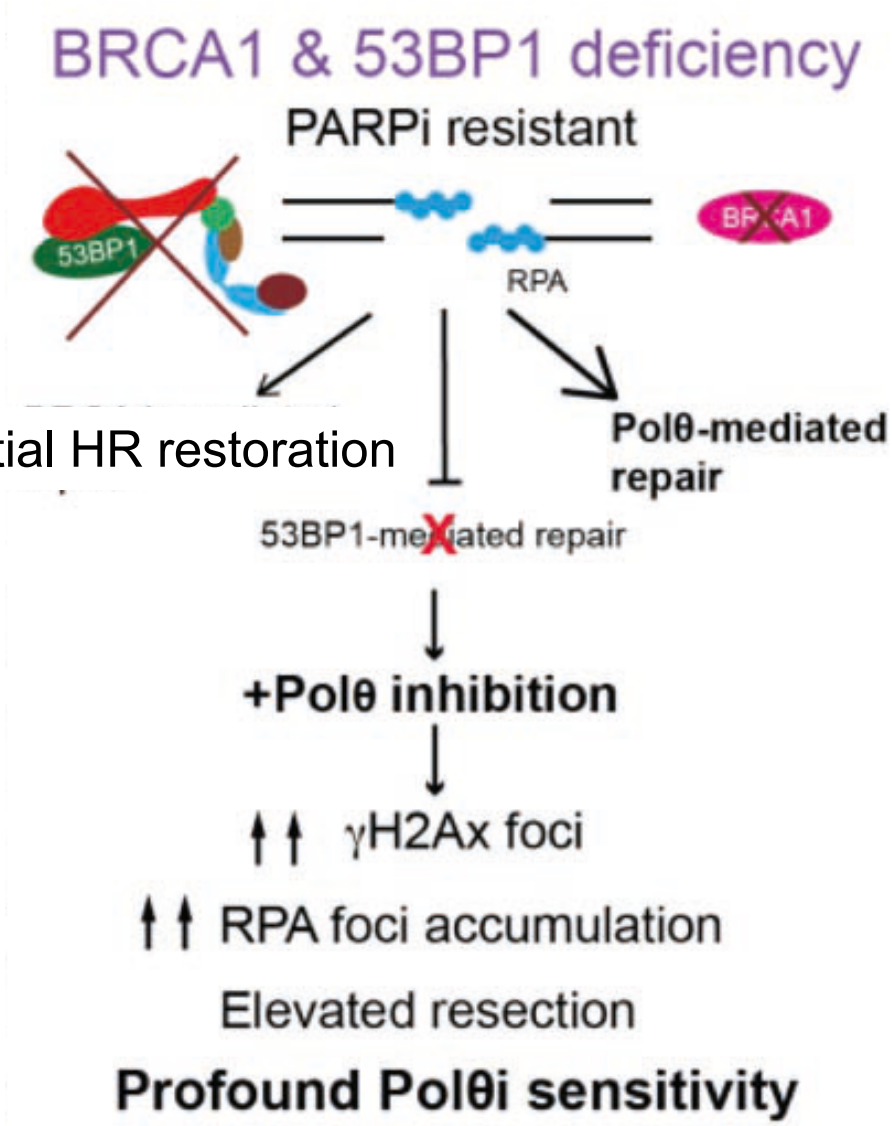


:Patient-derived Organoid

Pol θ (teta) Inhibitor Targets PARPi Resistant *BRCA1-mt* Cells

(NatComm 12: 3636 (2021))

Model: the authors observed elevated DNA end resection upon Pol θ inhibition.
Therefore, they speculate that when both BRCA1 and 53BP1 function become impaired, Pol θ becomes essential for repairing resected ssDNA caused by the exposure of DSB ends due to 53BP1-loss.
I.e.This would correspond to a MMEJ-independent function of pol θ.
…to be investigated further.



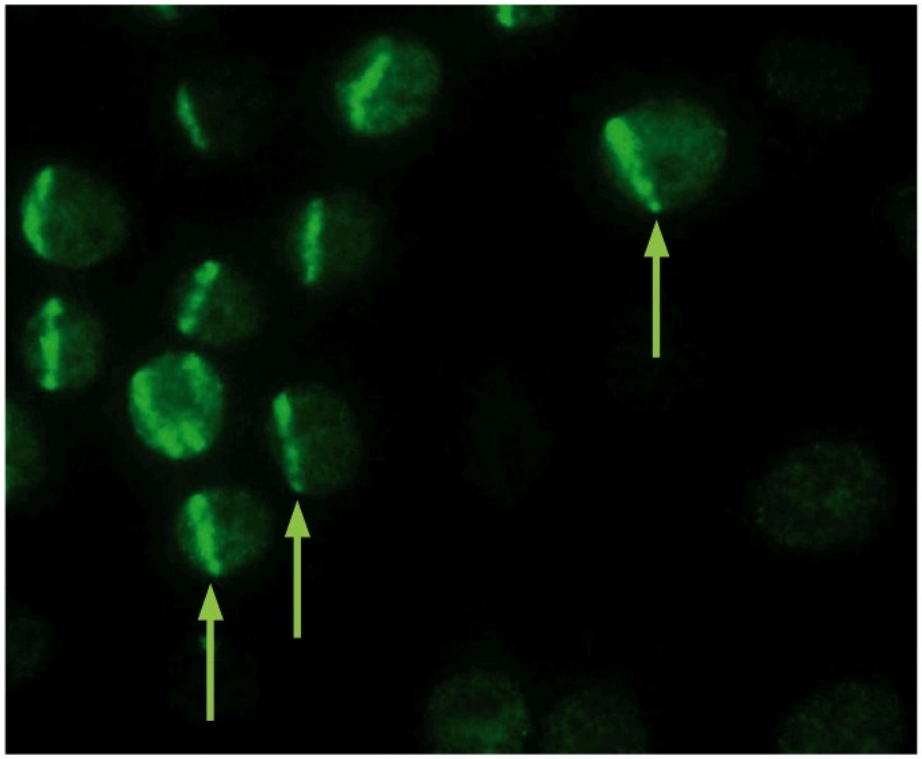
Chromatin and DNA Repair

Repair of DSB Involves Posttranslational Modifications of Nucleosomes and other Proteins

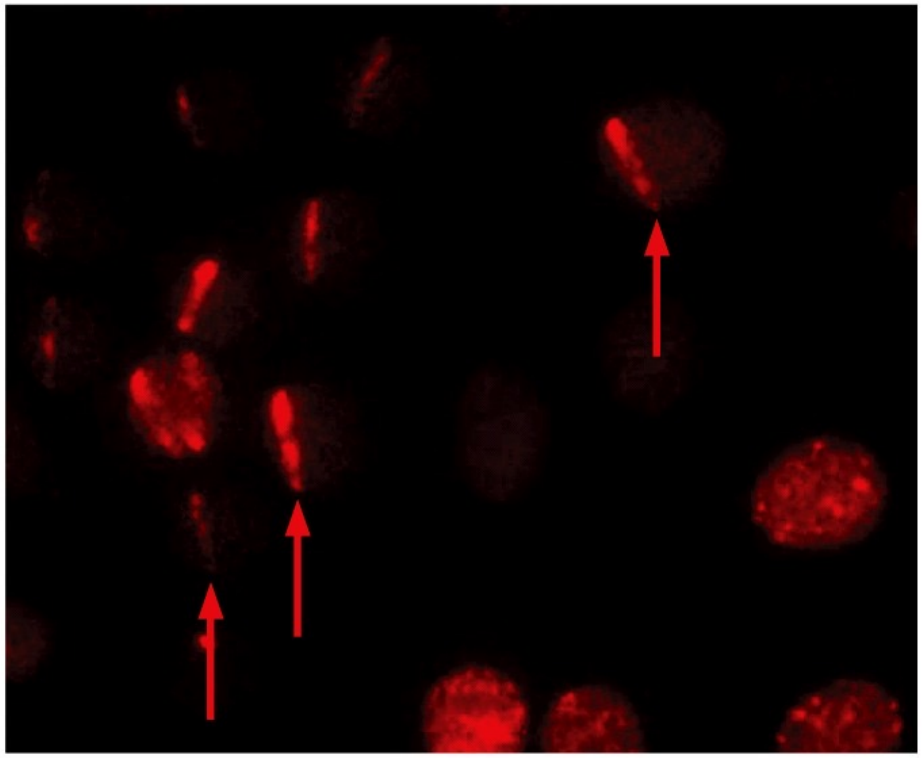
- Detect DNA damage
- Remodel local chromatin to provide access
- Reorganize nucleosome-DNA template for processing and repair
- Restore local chromatin organization after repair

See *Cell* 152, 1344 (2013) for a Review

Colocalization of BRCA1 with γ-H2AX at ds DNA Breaks



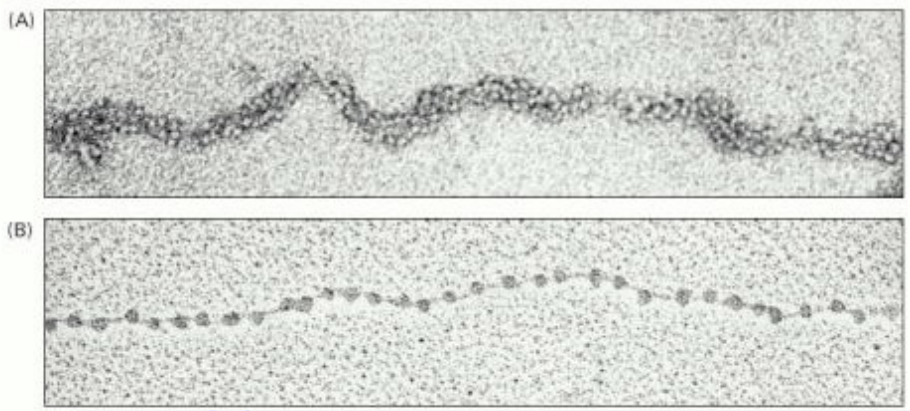
γ-H2AX



BRCA1

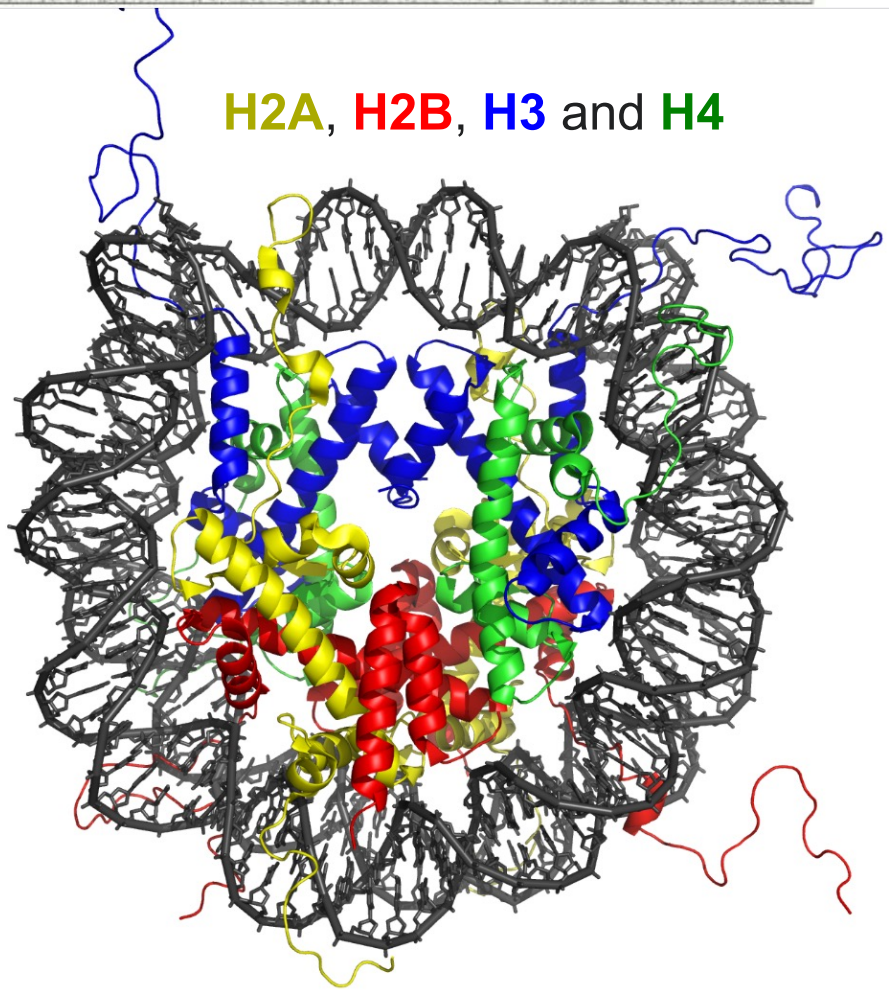
Figure 12.28. Weinberg, The Biology of Cancer

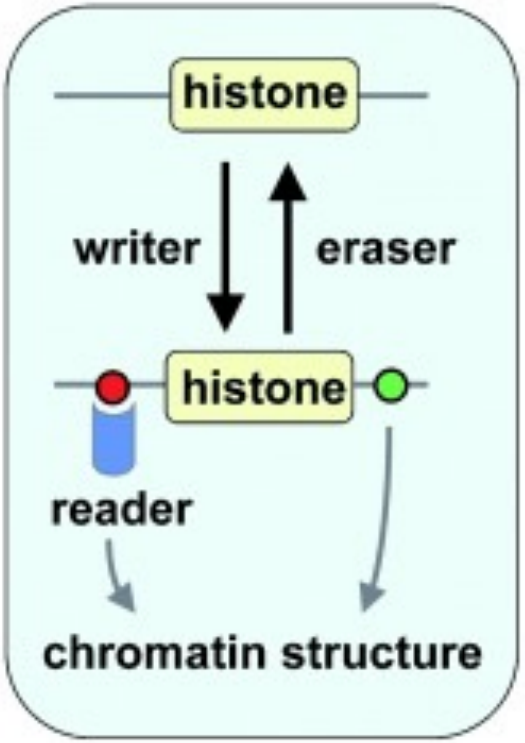
A 355-nm UV laser was used to paint stripes across nuclei



30 nm fiber

Linker histone H1 removed: “beads on a string”
147 bp are rolling 1.7 times around a histone octamer (two of histones H2A, H2B, H3 and H4).





H2AX

10 % of the H2A pool of mammals
H2AX is the 'normal' histone H2A in budding yeast

Posttranslational modifications

Ser 1	Phosphorylated
Lys 5	Acetylated
Lys 119	Ubiquitylated

Double strand breaks → Phosphorylation of Ser 139 (Ser c-4)
→ γH2AX

a

Species	Core (C-terminal portion)	Linker	Tail
Human	NDEELNKLLGGVTIAQGGVLPNIQAVLLPKK	TSATVGPKAPSGGKKATQA	SQEY
Chimpanzee	NDEELNKLLGGVTIAQGGVLPNIQAVLLPKK	TSATVGPKAPSGGKKATQA	SQEY
Mouse	NDEELNKLLGGVTIAQGGVLPNIQAVLLPKK	SSATVGPKAPAVGKKASQA	SQEY
Chicken	NDEELNKLLGGVTIAQGGVLPNIQAVLLPKK	TGGGAASPAKAGKKGSGQQ	SQEY
Zebrafish 1	NDEELNKLLGGVTIAQGGVLPNIQAVLLPKK	TGQAAASSGKSGKKGSSQ	SQEY
Xenopus laevis	NDEELNKLLGGVTIAQGGVLPNIQAVLLPKK	SSGGVSTSGKKSSQQ	SQEY
Anopheles gambiae	NDEELSKLLQGTTISQGGVMPNIHSVLLPRK	TGAKAAGNSSSQEKQ	SQEY
Zebrafish 2	NDEELNTLLGGVTISEGGVLPNIQAVLLPKK	TKAAREPNAGTEAQ	SQDF
Chickpea	NDEELSKLLGSVTIANGGVLPNIHQTLLPKK	VGKGKGEIGSA	SQEF
Tetrahymena pyriformis	NDEELNKLMANTTIADGGVLPNINPMLLPSK	SKKTESRGQA	SQDI
Budding yeast	NDDELNKLLGNVTIAQGGVLPNIHQNLLPKK	SAKATKA	SQEL
Fission yeast	NDEELNKLLGHVTIAQGGVVPNINAHLLPKT	SGRTGKP	SQEL
Giardia intestinalis	KDKELATIFANVTIREGGVARSAKEGREGKG	SHR	SQDL

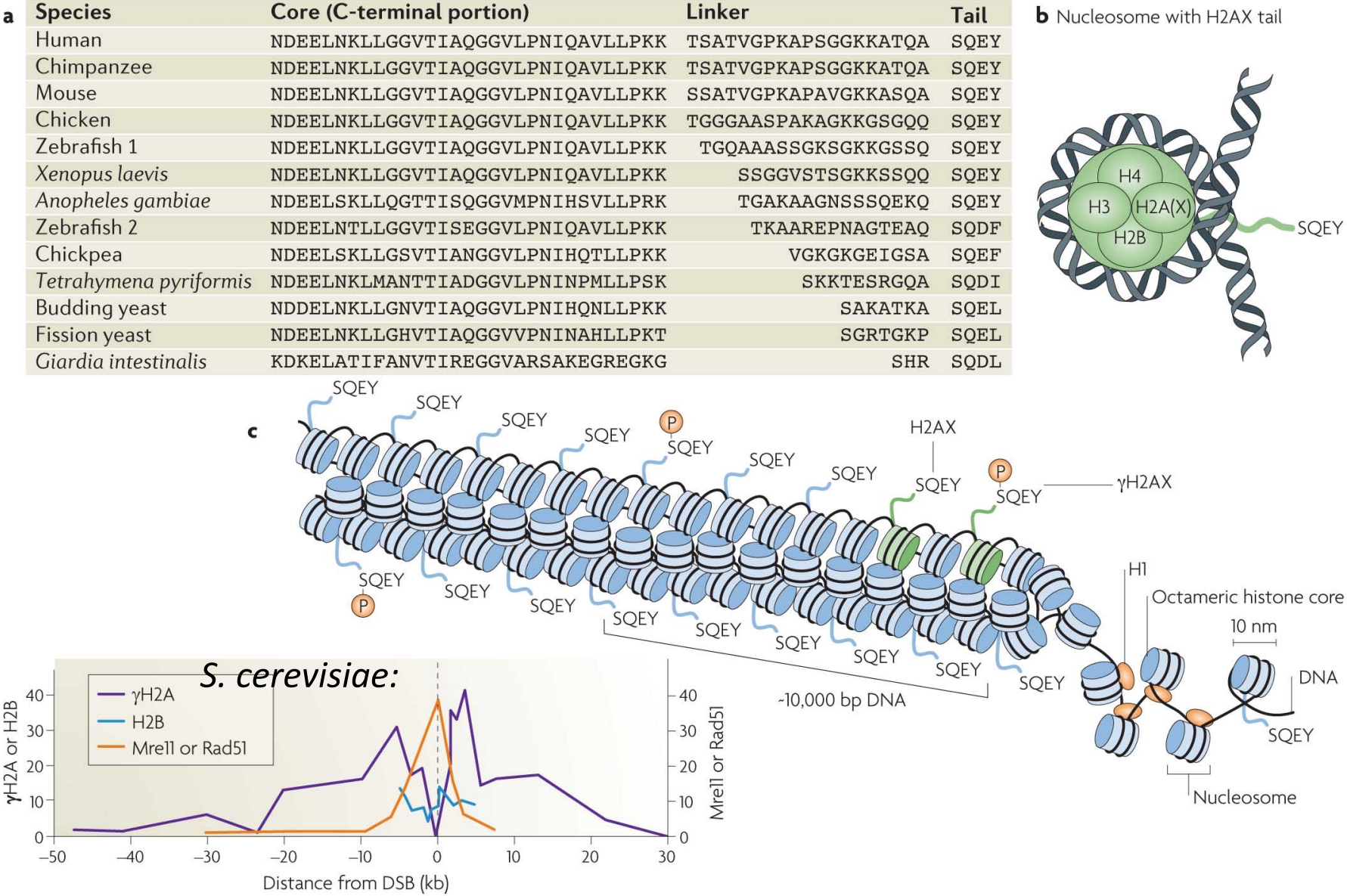
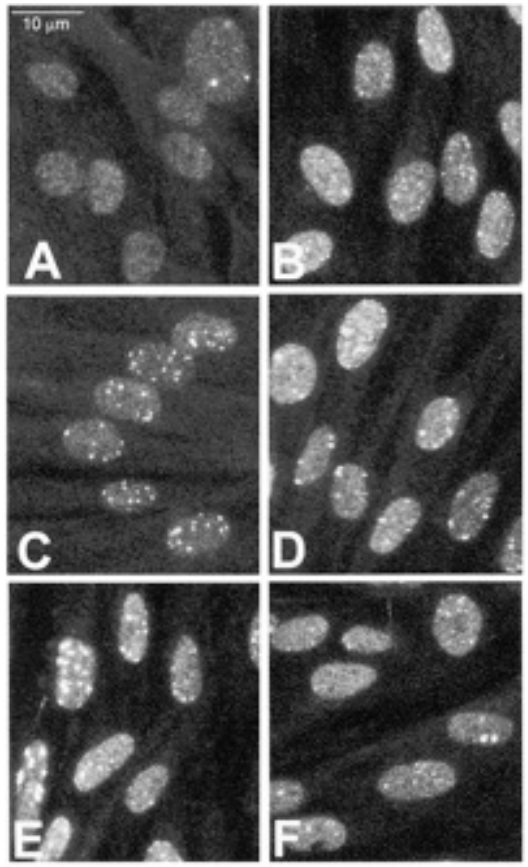


Figure 3 | **H2AX and γH2AX foci. a** | H2AX is an H2A histone with a core sequence conserved with other H2A species and a tail conserved through evolution connected by a linker of variable length. **b** | The SQEY tail extends from the core nucleosome near the entry and exit point of the DNA. **c** | The nucleosomes form a 30 nm fibre with H2AX molecules in every fifth nucleosome on average in mammals and every nucleosome in yeast. Approximately 10% of the H2AX molecules are phosphorylated at any one time in a focus.

From NatureRevCancer 8, 957 (2008)

Nuclear γ-H2AX foci are a Direct Consequence of DNA Double Strand Breaks

Irradiation: 0.6 Gy



Control

3 min.

15 min.

30 min.

60 min.

180 min.

Rogakou et al. (1999). JCB (146) pp. 905-916

Ionizing radiation
Replicative stress

↓

ATM/ATR

↓

γH2AX

Spreading of γ-H2AX at DSB

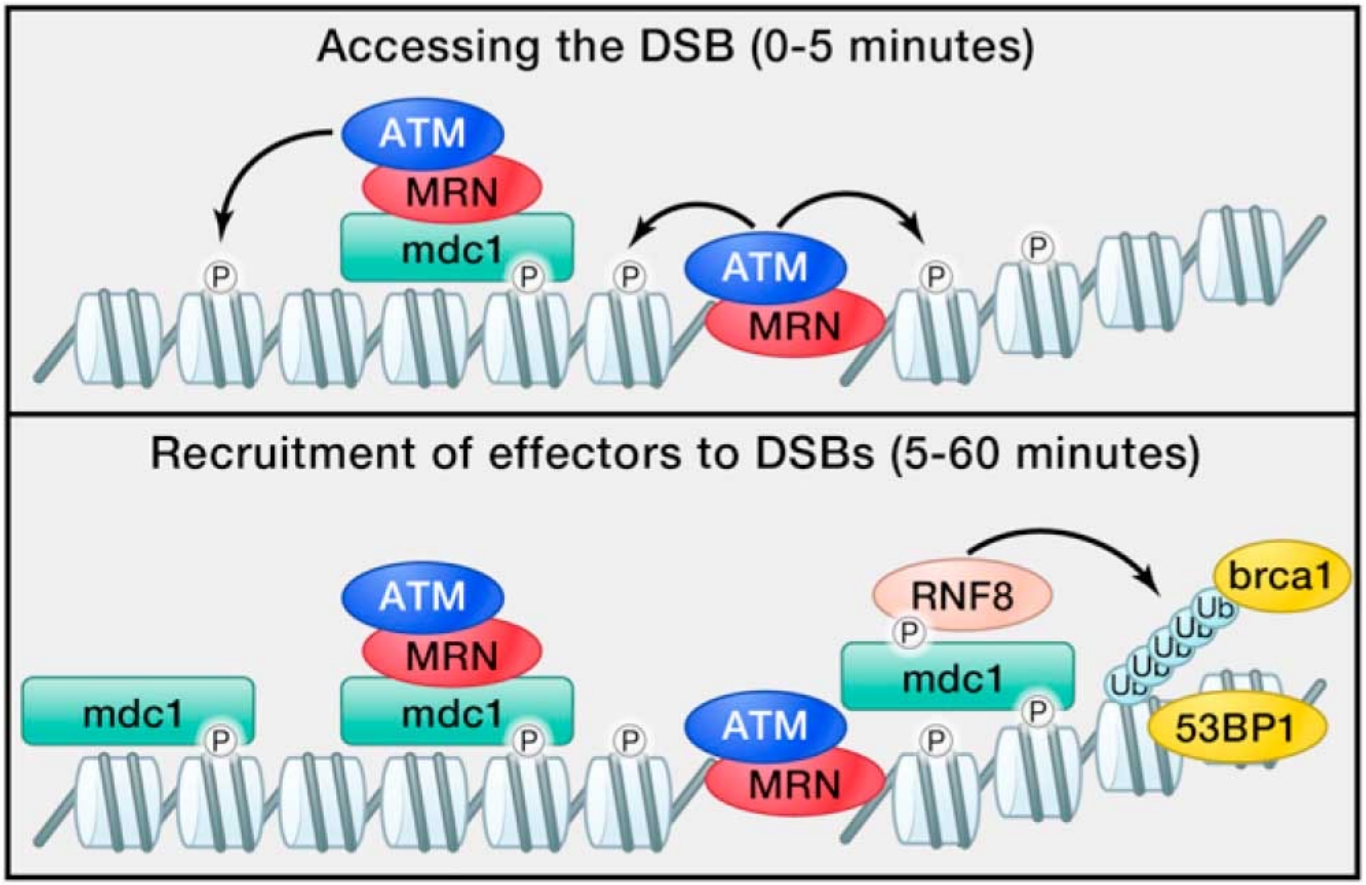


Figure 1. The Mechanism of DSB Repair

Top: ATM phosphorylates H2AX at DSBs, creating a binding site for the mdc1 protein. ATM-MRN complexes then associate with mdc1, promoting the spreading of γH2AX along the chromatin for hundreds of kilobases.

Bottom: mdc1 recruits multiple DSB-repair proteins, including the RNF8/RNF168 ubiquitin ligases, to sites of damage. Chromatin ubiquitination then facilitates loading of the brca1 complex and 53BP1 DSB-repair proteins.

P = phosphorylation, Ub = ubiquitination, MRN = mre11-rad50-nbs1 complex.

Role of H2AX

- **Repair of double-strand breaks: NHEJ & HR**

(impaired recruitment of 53BP1 and BRCA1)

- **Mouse H2AX -/- :**

Chromosomal breaks and translocations
Small size
Lymphomas & solid tumors

Ionizing Radiation (IR)

•Deposits energy to yield ionized molecules or reactive oxygen species (ROS) that then attack the DNA.
•Causes base damage, DNA-protein cross-links, phosphate backbone damage, single-strand breaks, and double-strand breaks (DSBs).
•Cell killing by IR generally reflects the generation of DSBs, as these are difficult to repair.
•Does not occur “often” but note that 200,000,000 gamma rays pass through your body every hour (these come from decay of naturally-occurring radioactive isotopes).

•Used in cancer therapy.

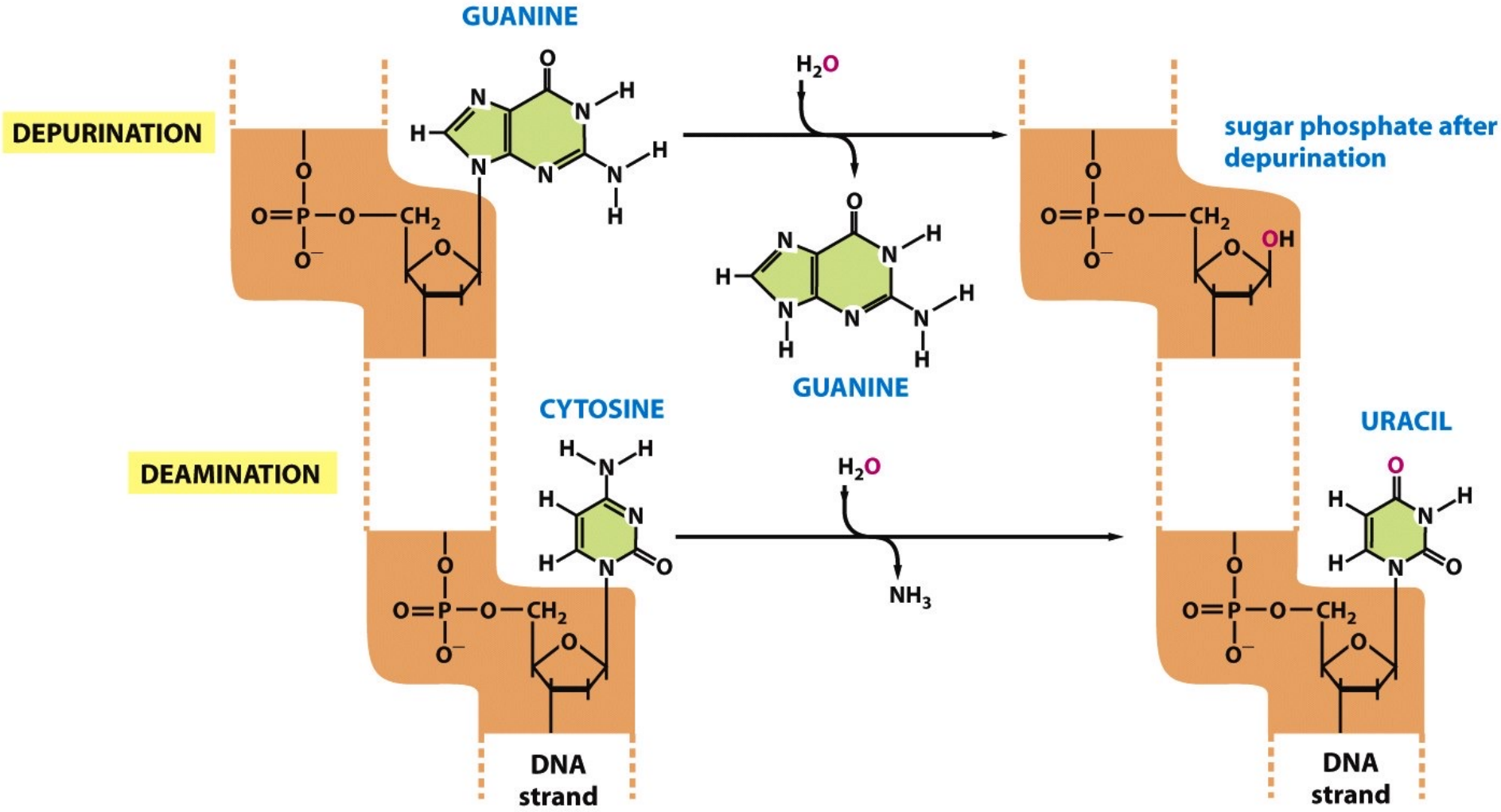
DNA Lesions Generated by Endogenous and Exogenous Sources

Endogenous DNA Damage	DNA Lesions Generated	Number Lesions/Cell/Day	
Depurination	AP site	10000[a]	
Cytosine deamination	Base transition	100–500[a]	
SAM-induced methylation	3meA	600[a]	
	7meG	4000[a]	
	O^6meG	10–30[b]	
Oxidation	8oxoG	400–1500[c]	
Exogenous DNA Damage	**Dose Exposure (mSv)**	**DNA Lesions Generated**	**Estimated Number Lesions/Cell**
Peak hr sunlight	—	Pyrimidine dimers, (6–4) photoproducts	100,000/day[d]
Cigarette smoke	—	aromatic DNA adducts	45–1029[e]
Chest X-rays	0.02[f,g,h]	DSBs	0.0008[i]
Dental X-rays	0.005[f,g,h]	DSBs	0.0002[i]
Mammography	0.4[f,g,h]	DSBs	0.016[i]
Body CT	7[f]	DSBs	0.28[i]
Head CT	2[f,g]	DSBs	0.08[i]
Coronary angioplasty	22[h]	DSBs	0.88[i]
Tumor PET scan (^{18}F)	10[h]	DSBs	0.4[i]
^{131}I treatment	70–150[h]	DSBs	2.8–6[i]
External beam therapy	1800–2000[j]	DSBs	72–80
Airline travel	0.005/hr[f]	DSBs	0.0002/hr[i]
Space mission (60 days)	50[k]	DSBs	2[i]
Chernobyl accident	300[l]	DSBs	12[i]
Hiroshima and Nagasaki atomic bombs	5–4000[k]	DSBs	0.2–160[i]

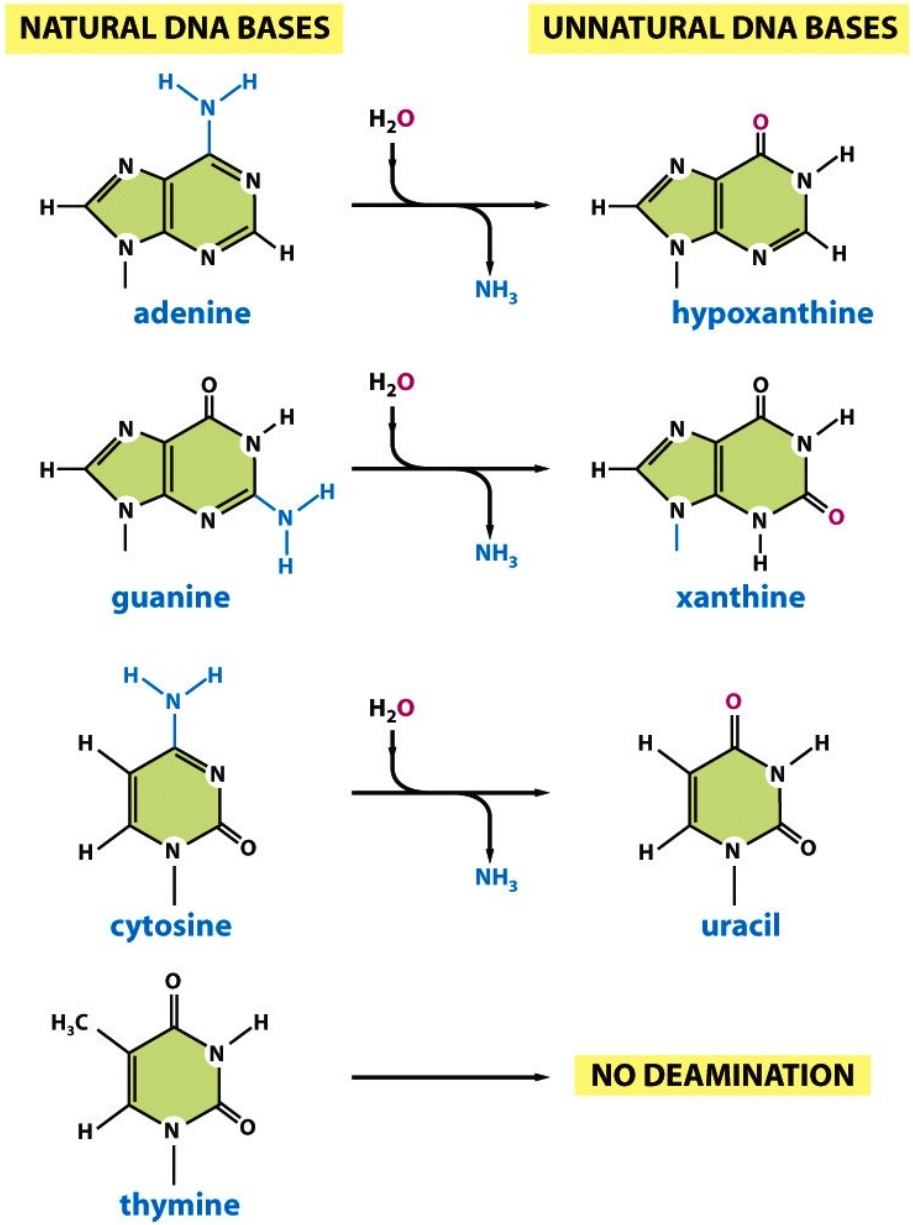
Type and number of DNA lesions are indicated. The number of lesions/cell has been estimated as described.

Ciccia and Elledge, (2010) Mol Cell, Volume 40, 179

Depurination and Deamination



Deamination Yields Unnatural Bases



APOBEC3 proteins catalyze deamination reactions in ssDNA to target viruses and retroelements as part of the innate immune response. However, **APOBEC3-mediated mutagenesis has also been observed in 70% of all cancer types and 50% of all cancer genomes!** (*Nature* **607**, pages 799–807 (2022)**.**

Figure 5-50a *Molecular Biology of the Cell* (© Garland Science 2008)

Deamination of 5-Methyl Cytosine Yields Thymine!

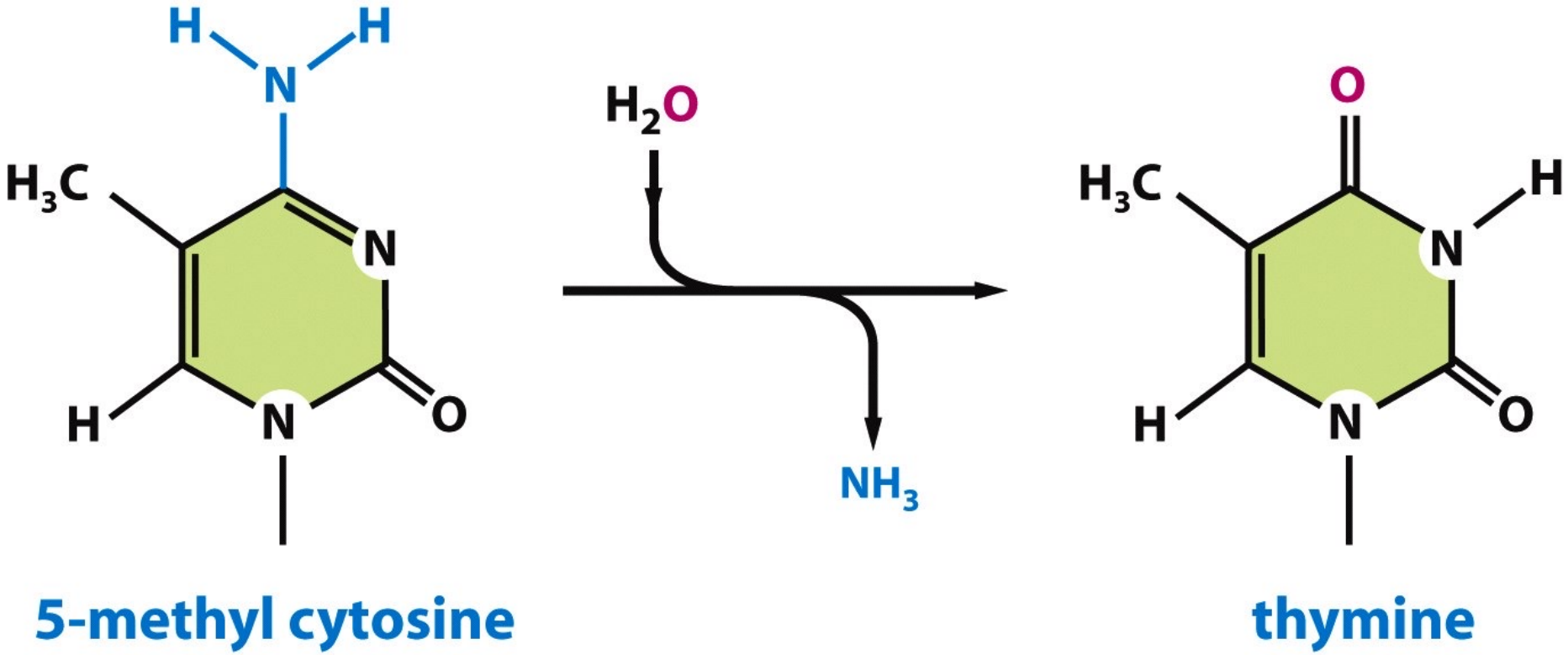


Figure 5-50b *Molecular Biology of the Cell* (© Garland Science 2008)

Thymine-Dimer: Caused by UV-light in the Skin

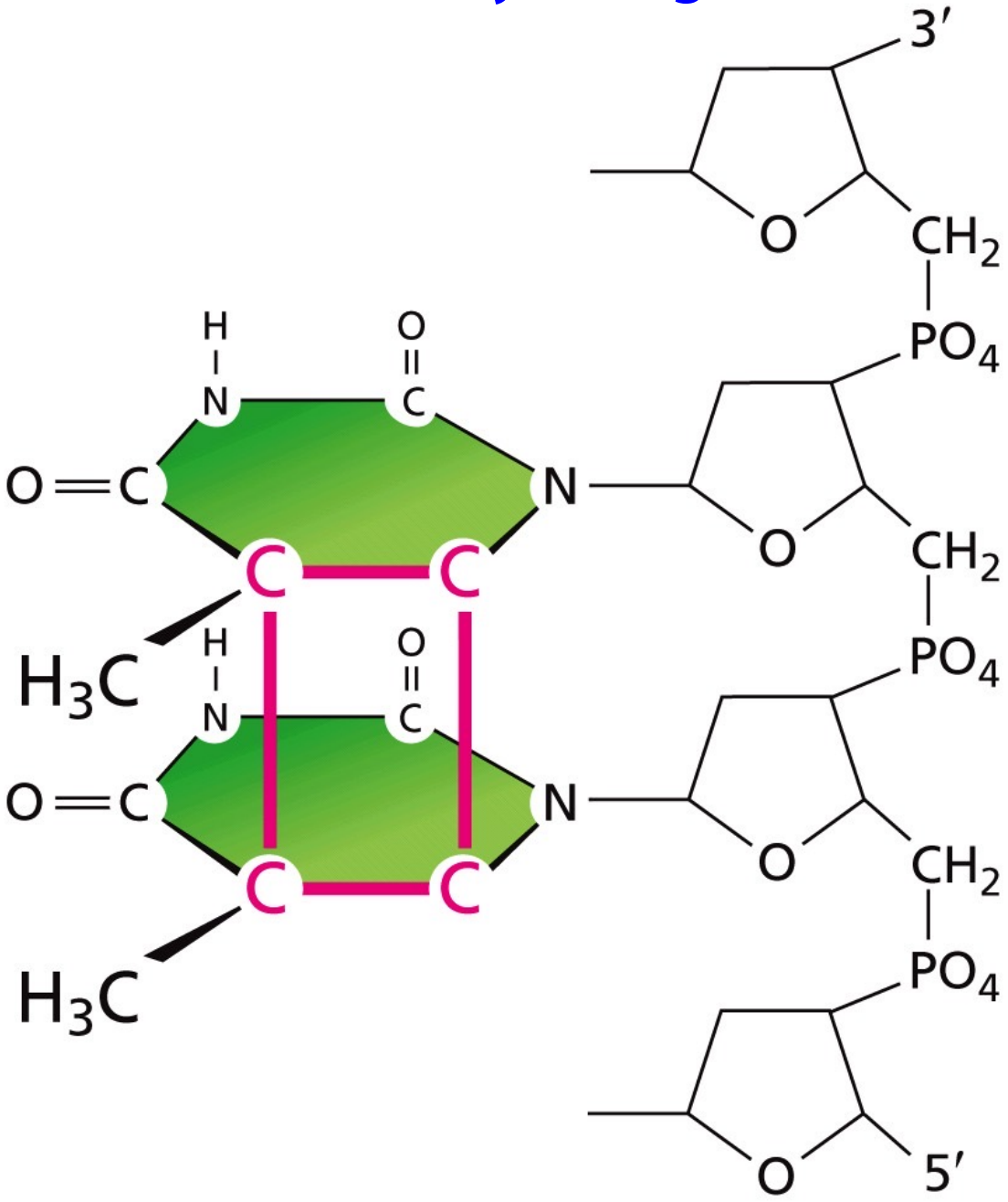


Figure 12.12a The Biology of Cancer (© Garland Science 2014)

6-4 Photoproducts of Pyrimidines

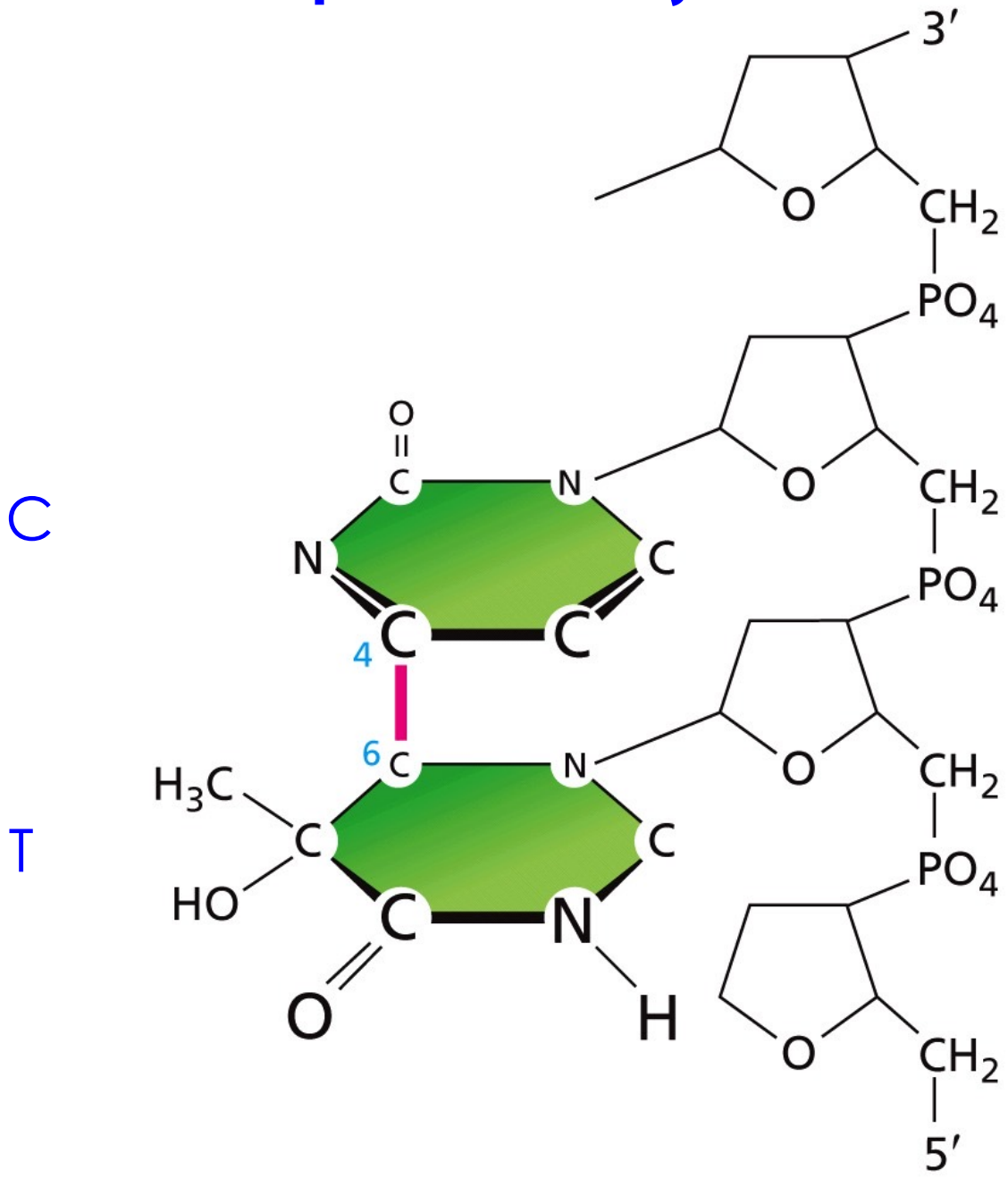
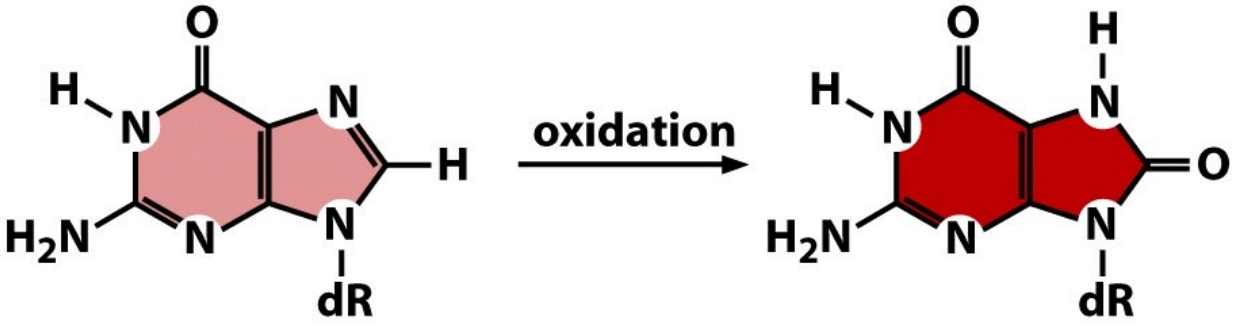


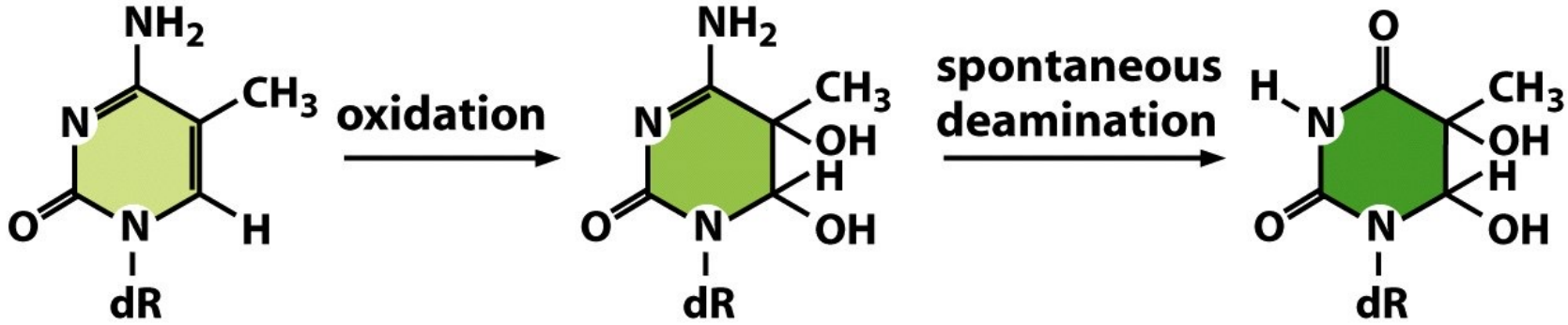
Figure 12.12b The Biology of Cancer (© Garland Science 2014)

Oxidation of DNA Bases



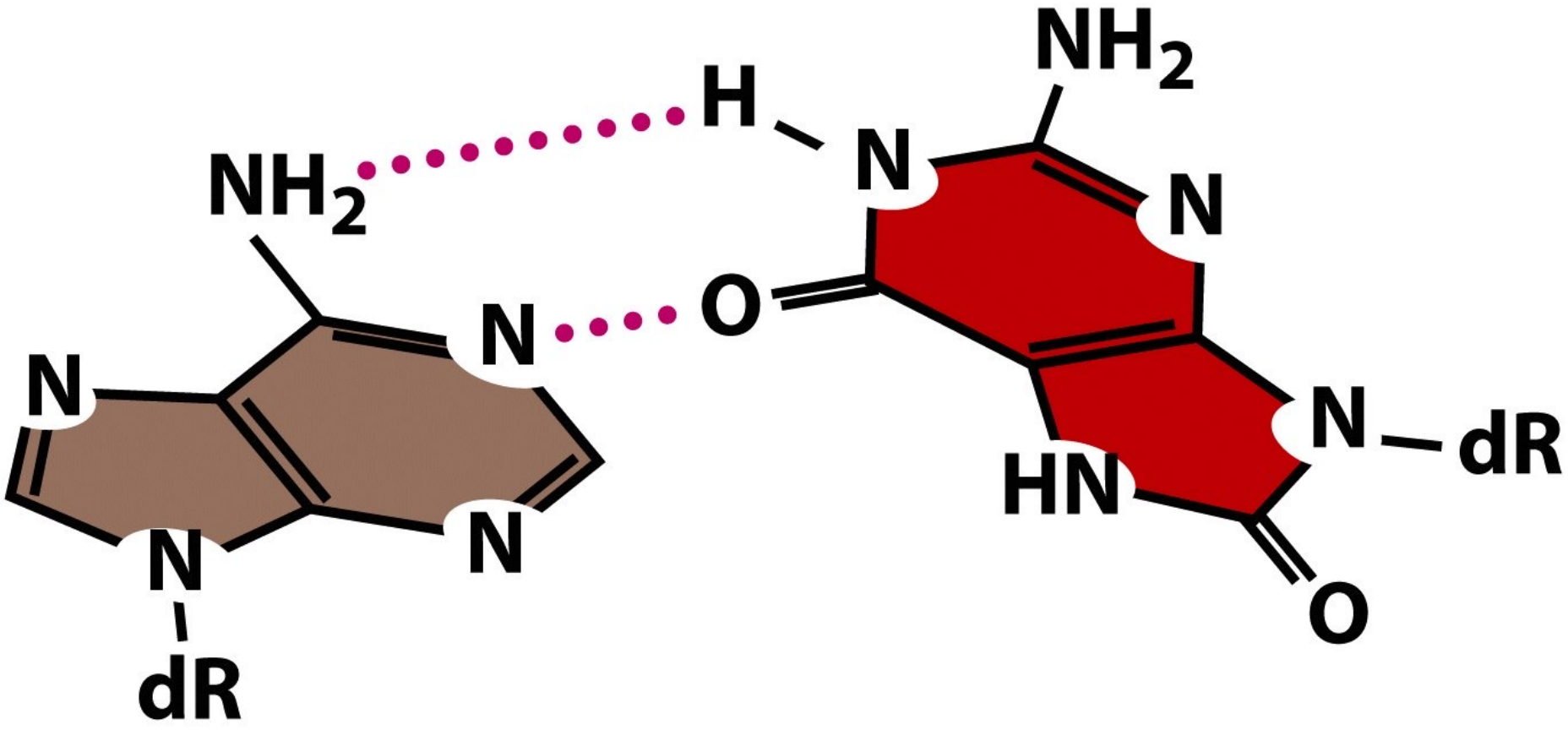
deoxyguanosine (dG)

8-oxo-deoxyguanosine
(8-oxo-dG)



deoxy 5-methyl-cytosine
(d 5'mC)

deoxythymidine glycol
(dTg)



mispairing of 8-oxo-dG with deoxyadenosine (dA)

DNA Repair Mechanisms

Repair by excision

- **BER: Base excision repair**
- MMR: Mismatch repair
- **NER: Nucleotide excision repair**
- Ribonucleotide excision repair

Low fidelity DNA polymerases-Translesion polymerases

(Some play a role,in excision repair while others are able to replicate through a lesion which would normally block progression of polymerase during replication.)

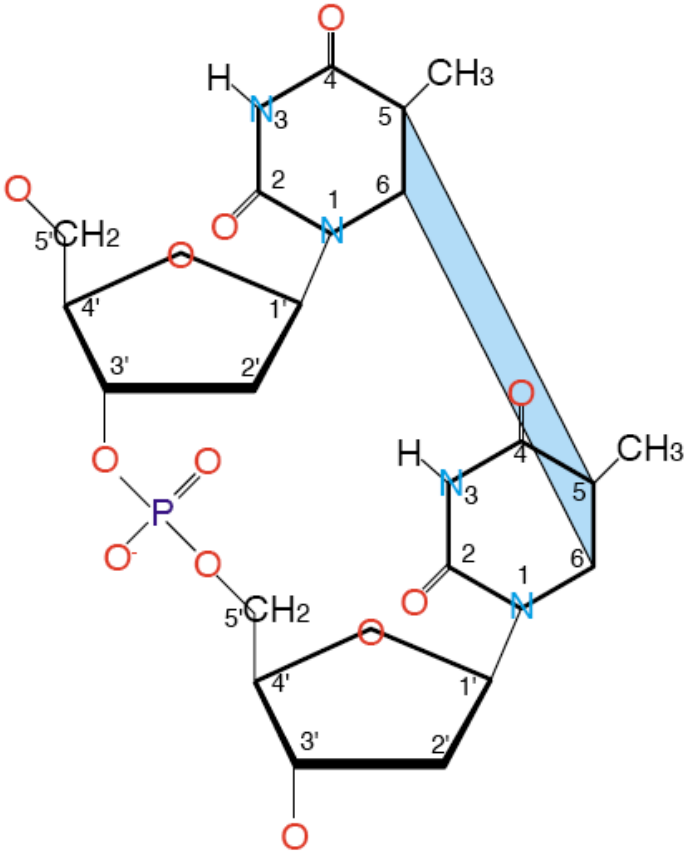
Double strand break repair. ✅

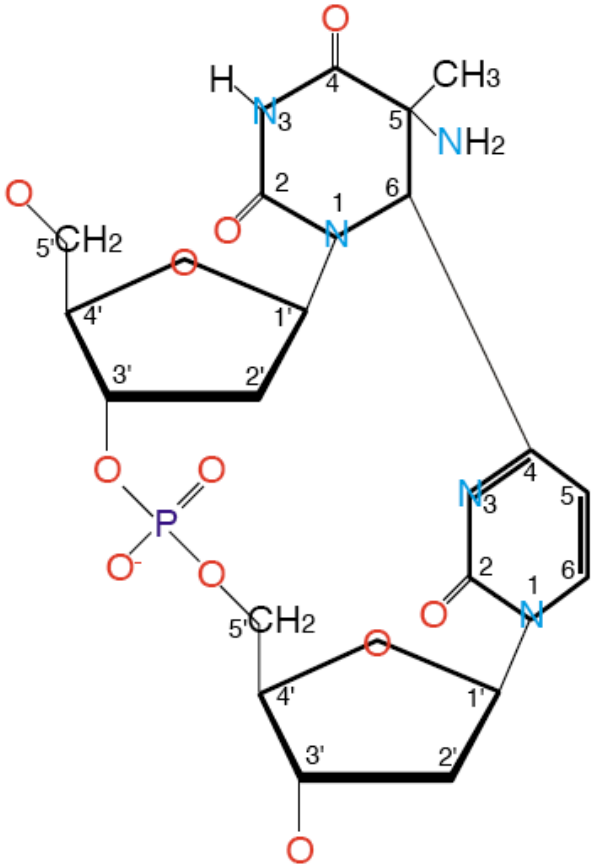
- NHEJ: Non homologous end-joining
- MMEJ: Microhomology directed end-joining (or Alt-EJ)
- HR: Homologous recombination

For an exhaustive list of proteins that are implicated in genome stability.
Wood, R. D., Mitchell, M., Sgouros, J., and Lindahl, T. (2001). Human DNA repair genes. Science *291*, 1284-1289.
http://sciencepark.mdanderson.org/labs/wood/DNA_Repair_Genes.html

1. Nucleotide Excision Repair (NER)

UV-induced Lesions

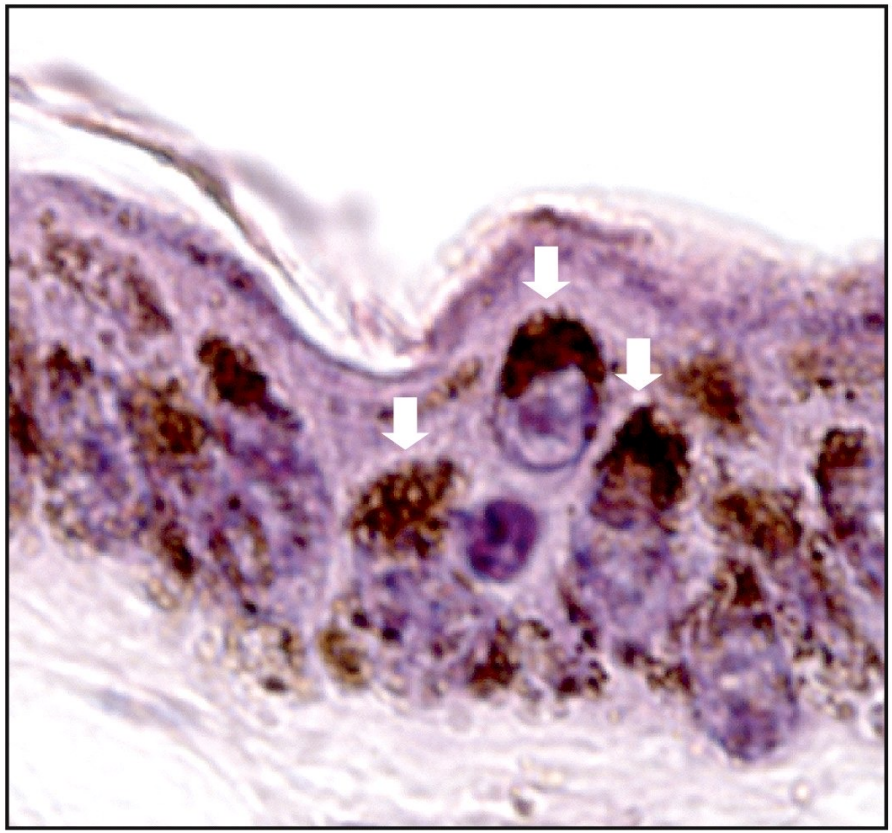




Cyclobutane pyrimidine dimers:
T-T > T-C, C-T > C-C
Helix curvature 7-9º

6-4 photoproducts:
T-C > > C-C > T-T > C-T
Helix curvature 44º

Physical Shielding of Keratinocytes Nuclei from UV



Melanosomes that sit above keratinocyte nuclei (arrows) shield these nuclei from UVB radiation.

NER deficiency syndrome: Xeroderma Pigmentosum

Dry, parchment-like skin (xeroderma)
Many freckles (pigmentosum)

Autosomal recessive disorder

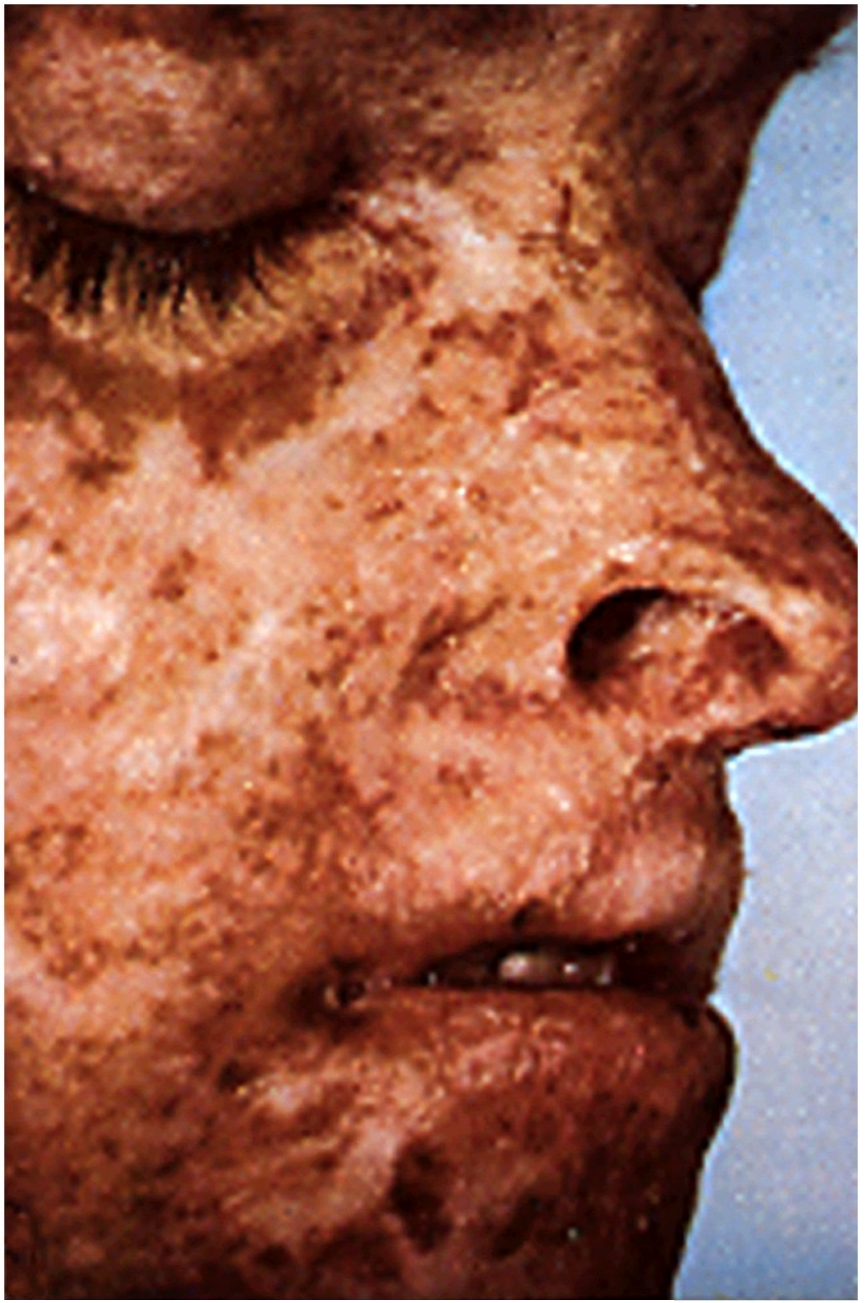


Figure 12.23. Weinberg, The Biology of Cancer

Early Onset of Skin Cancer in XP-Patients

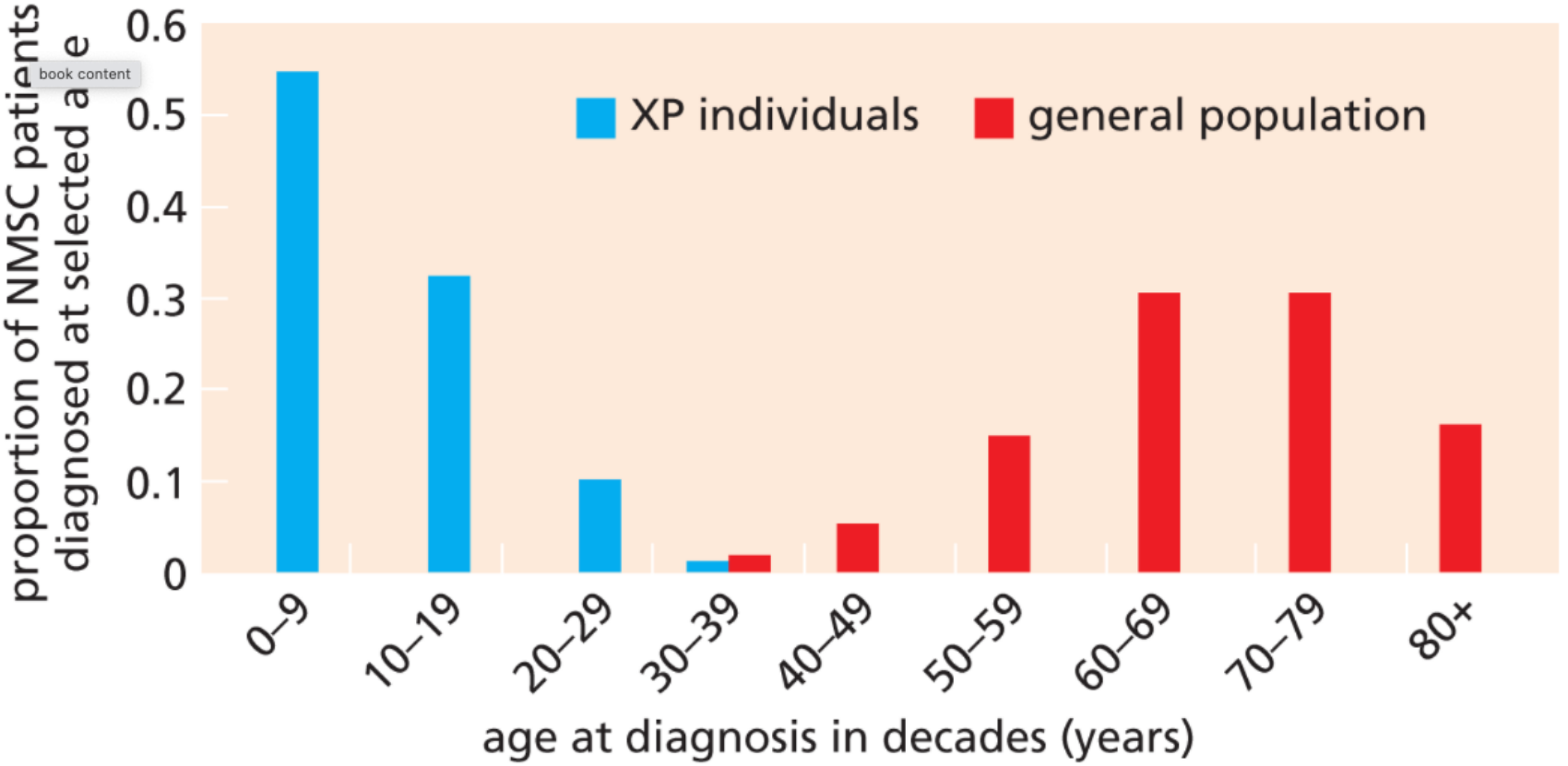


Figure 12.24. Weinberg, The Biology of Cancer

The graph shows individuals which were diagnosed with non-melanoma skin cancer (NMSC). The median age of diagnosis was 9 years for XP individuals and 67 years for the general population.

Complementation Groups of XP

8 genes: XP-A, XP-B, XP-C, XP-D, XP-E, XP-F, XP-G, XP-V

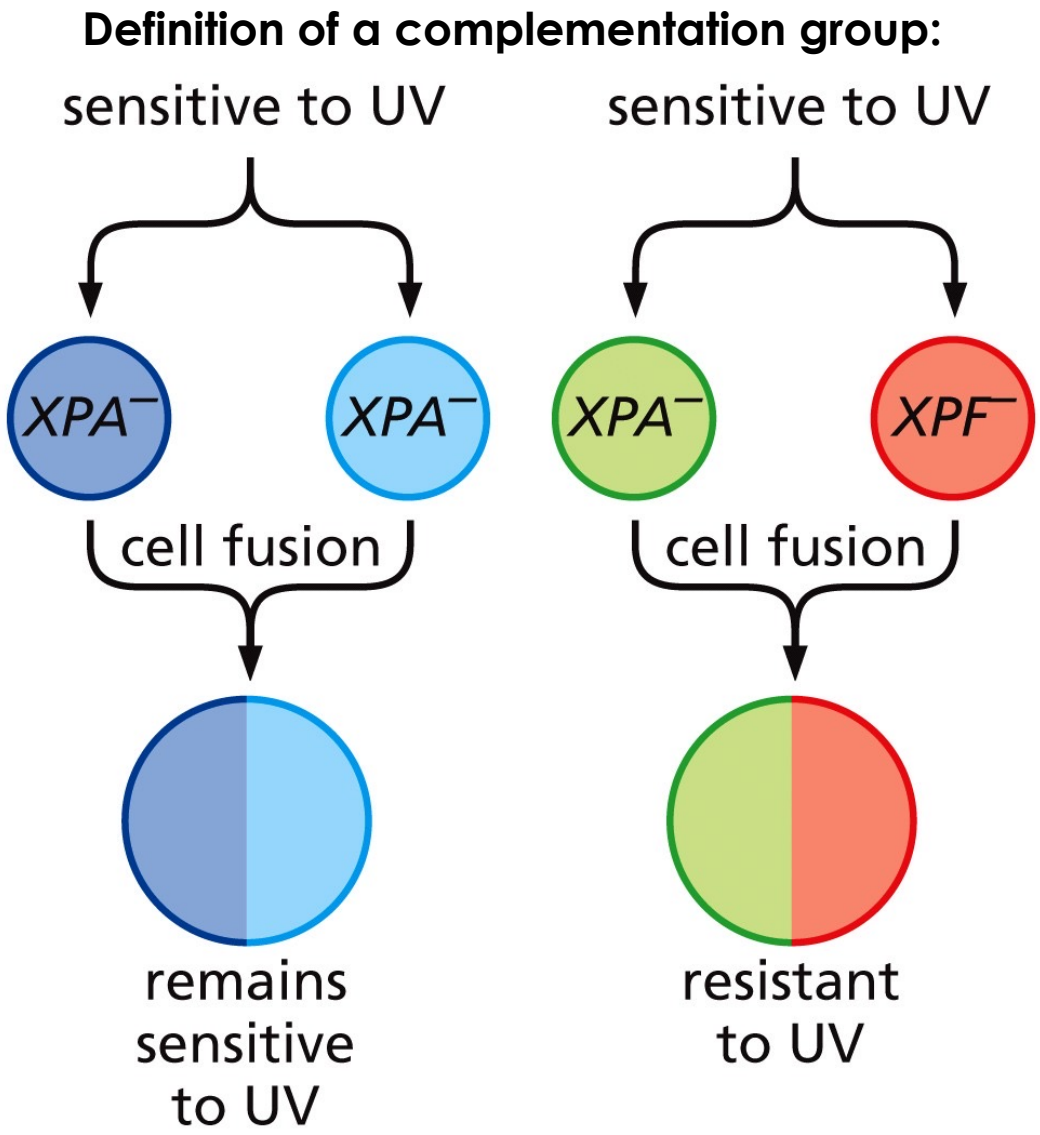
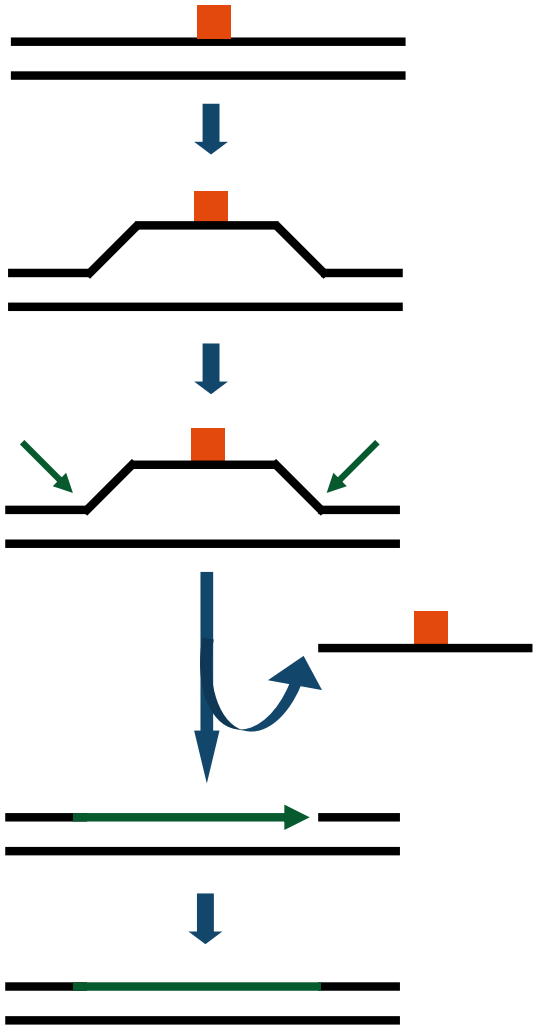


Figure 12.25. Weinberg, The Biology of Cancer

Thought Questions

- Are XP mutations dominant or recessive?
- How would you identify the mutated genes?
- How would you identify proteins that interact with your new protein?
- How would you test if the mutant gene product is responsible for recognition of damaged DNA ((6-4) Photo Products and Cyclobutane pyrimidine dimers) ?

NER Pathway



Distortion recognition

Formation of open structure and damage location

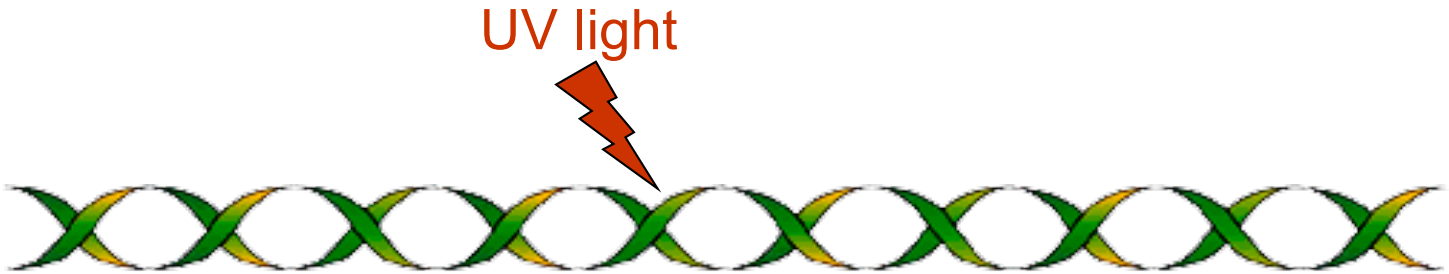
Dual incision 5’ and 3’ of the lesion

Excision of an oligonucleotide of 24-32 nt

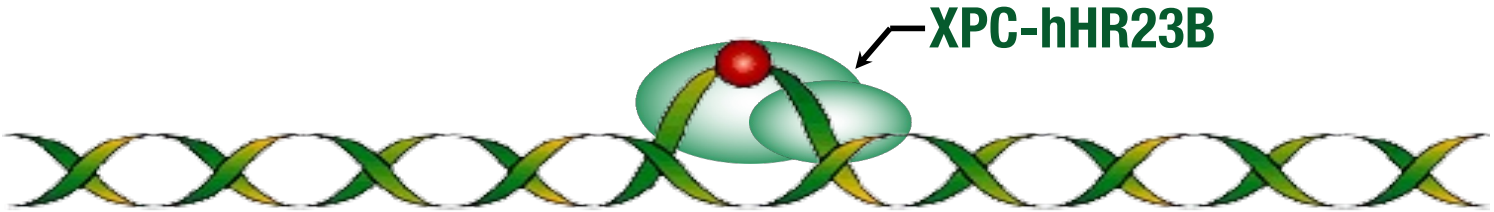
DNA repair synthesis

Ligation

Actors of NER

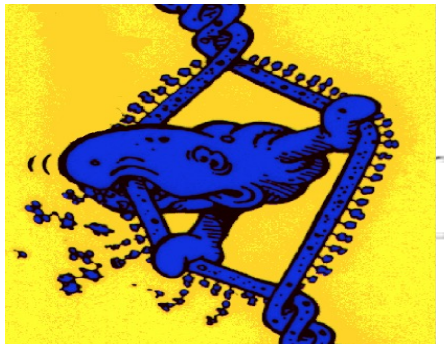


1- Recognition of lesion

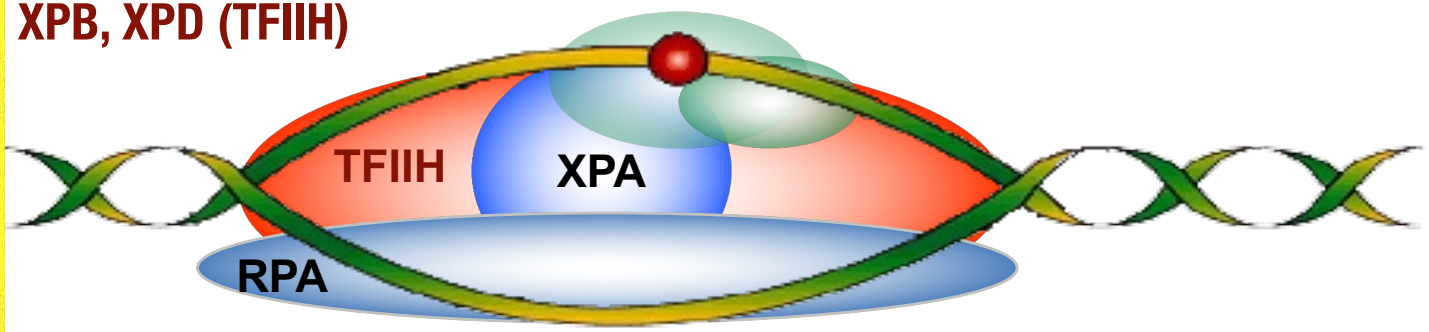


2- Formation of pre-incision complex

TFIIH: ten subunit complex involved in DNA unwinding for transcription initiation; but also required for NER.

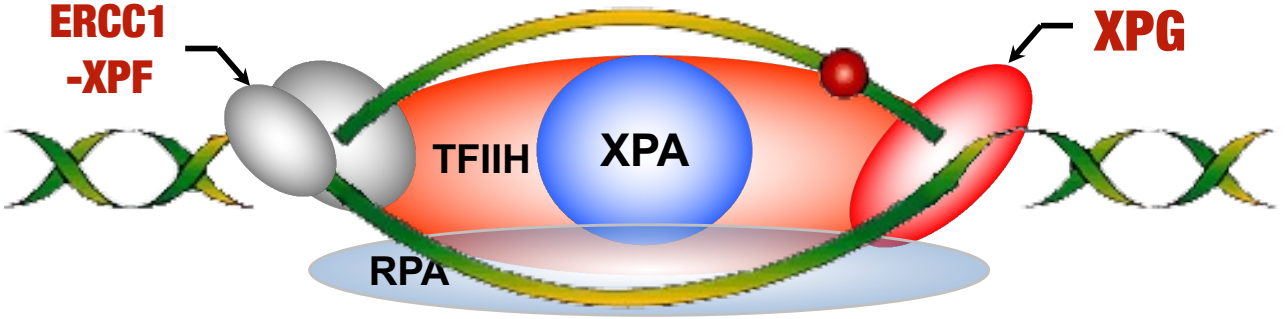


XPB, XPD (TFIIH)



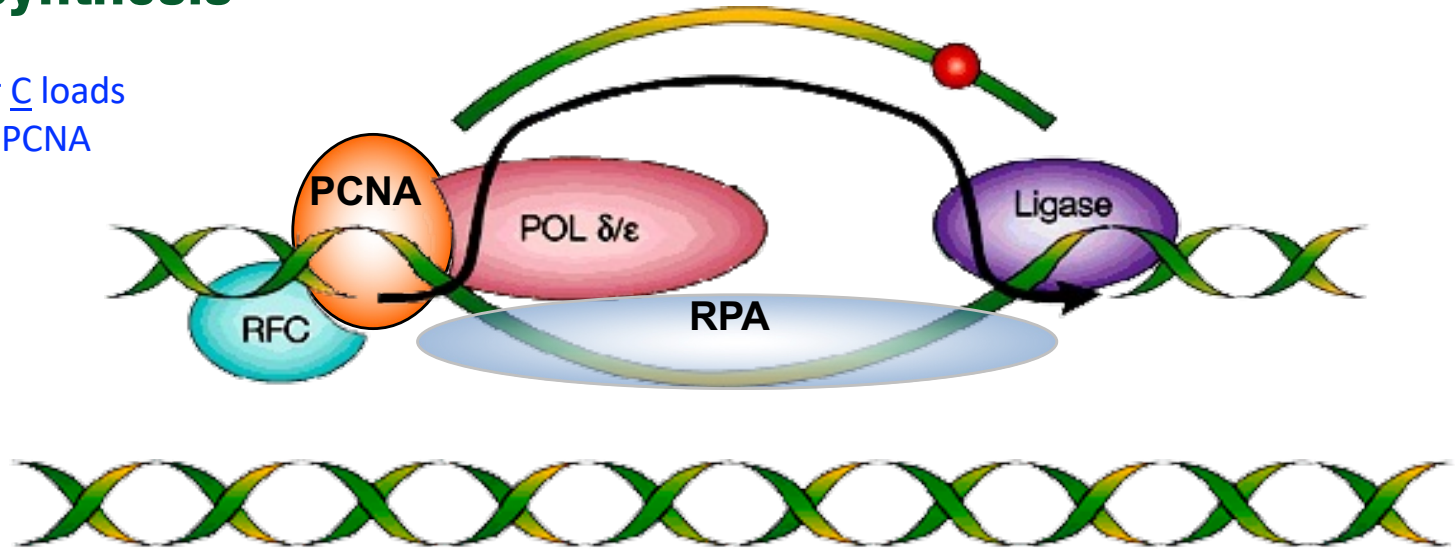
3- Double incision - excision

Incision 3’ of the lesion precedes incision 5’

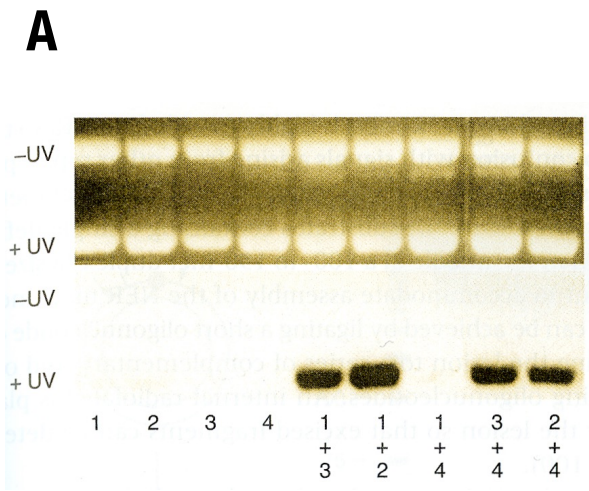


4- Repair synthesis

RFC: replication factor C loads trimeric sliding clamp PCNA

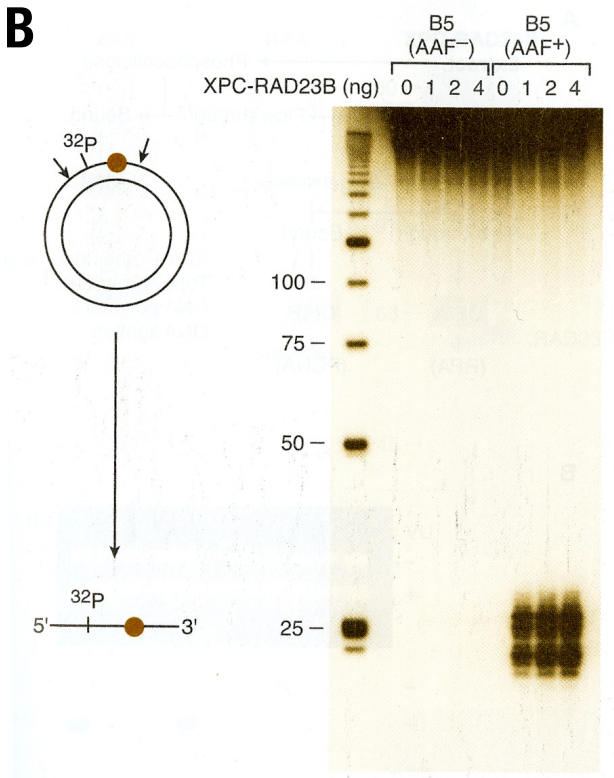


NER in vitro



CHO cells defective in NER complementation groups 1, 2, 3, and 4 as indicated: (defective genes: ERCC1, XPD (ERCC2), XPB (ERCC3), or XPF (ERCC4).

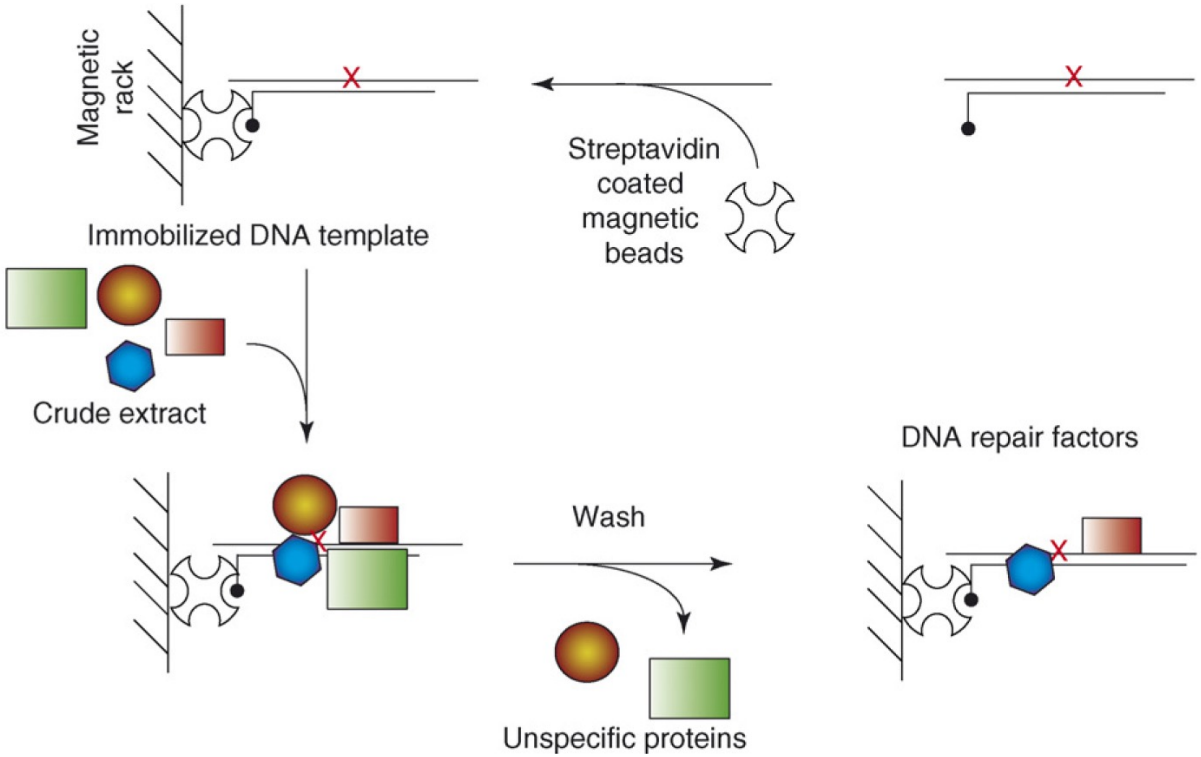
Reaction: fractionated extracts, PCNA, RPA, dNTPs, α-^{32}P-dATP, plasmids



The DNA samples were incubated with whole-cell extract from XPC-defective cells, supplemented with the indicated amounts of purified XPC-RAD23B protein

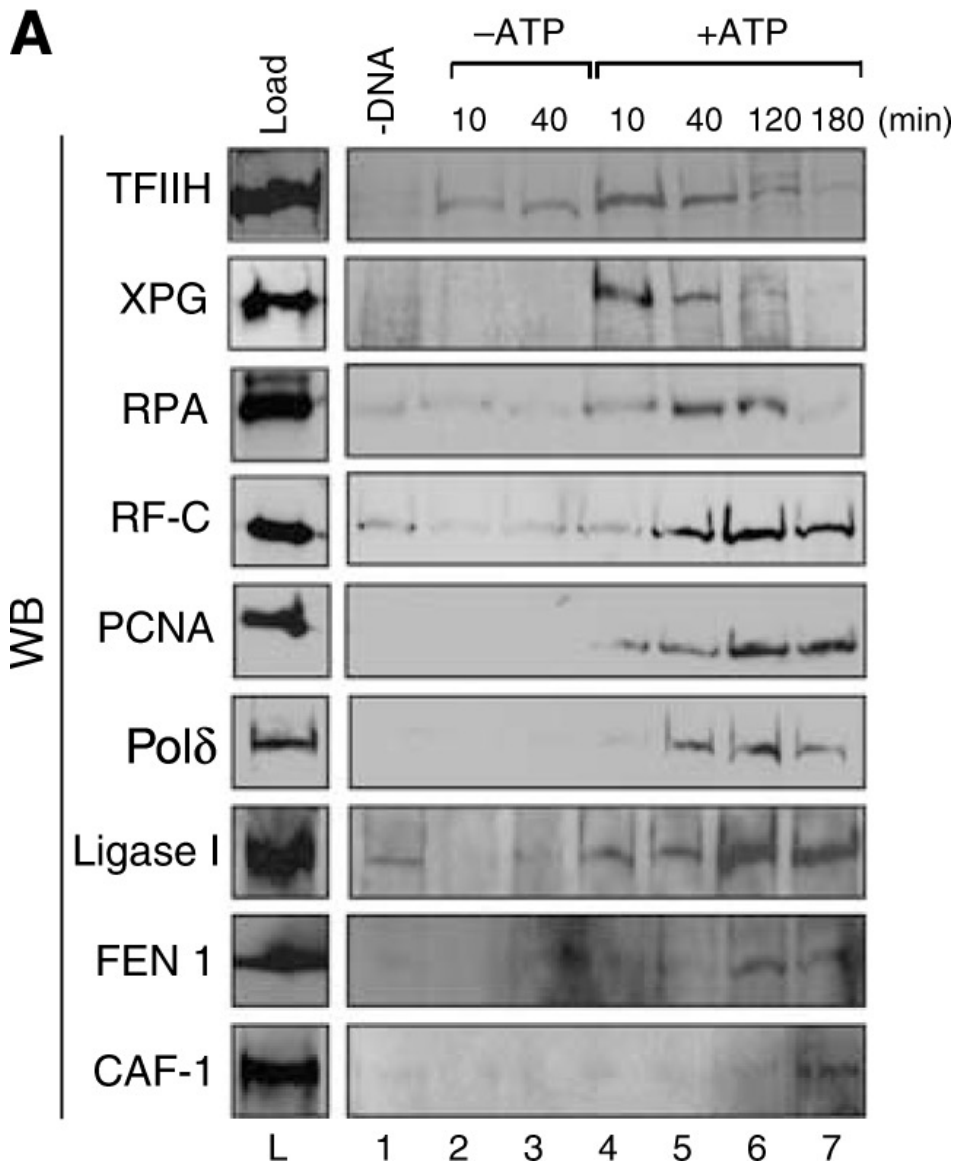
(N-Acetyl-2-aminofluorene (AAF) is a chemical carcinogen that reacts with guanines at the C8 position in DNA to form a structure that interferes with DNA replication)

Immobilized Repair Template



Generation and use of an immobilized DNA template containing a single, site directed lesion. The immobilized repair template can be incubated with crude extract or purified factors. …Identification of intermediate complexes.

Feuerhahn and Egly, TIG 24, 467 (2008)



In vitro sequential recruitment of the NER factors. The immobilized damaged DNA fragment was incubated with nuclear extract. At different time points the immobilized DNA was washed with 0.05 M KCl and the remaining bound factors were further analyzed by Western blot.

EMBO J., **27**, 155 (2008)

Local Damage Induction Method: Visualisation of Recruitment of NER Proteins upon UV Exposure

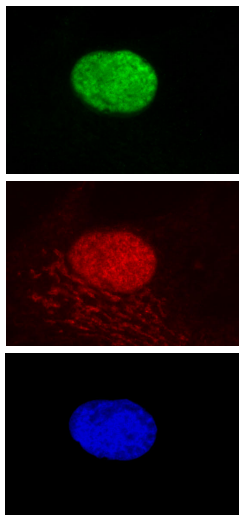
Irradiation of cells through a filter with a pore diameter= 8 μm

- UV

XPG

XPB

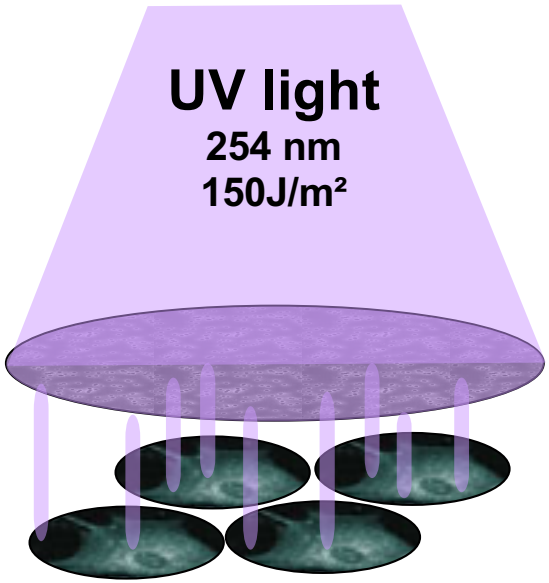
DAPI



Diffuse

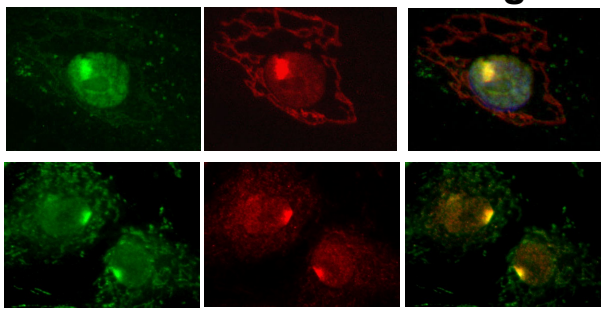
NER proteins are distributed throughout the nucleus

UV light
254 nm
150J/m²



30 minutes post-UV

XPG **XPB** **Merge**



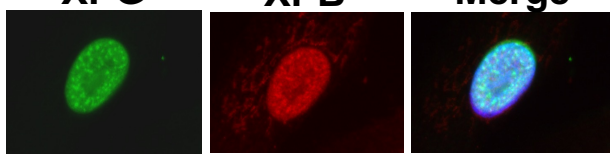
6-4 PPs **XPB** **Merge**

Formation nuclear foci

= Recruitement of NER proteins to irradiated regions

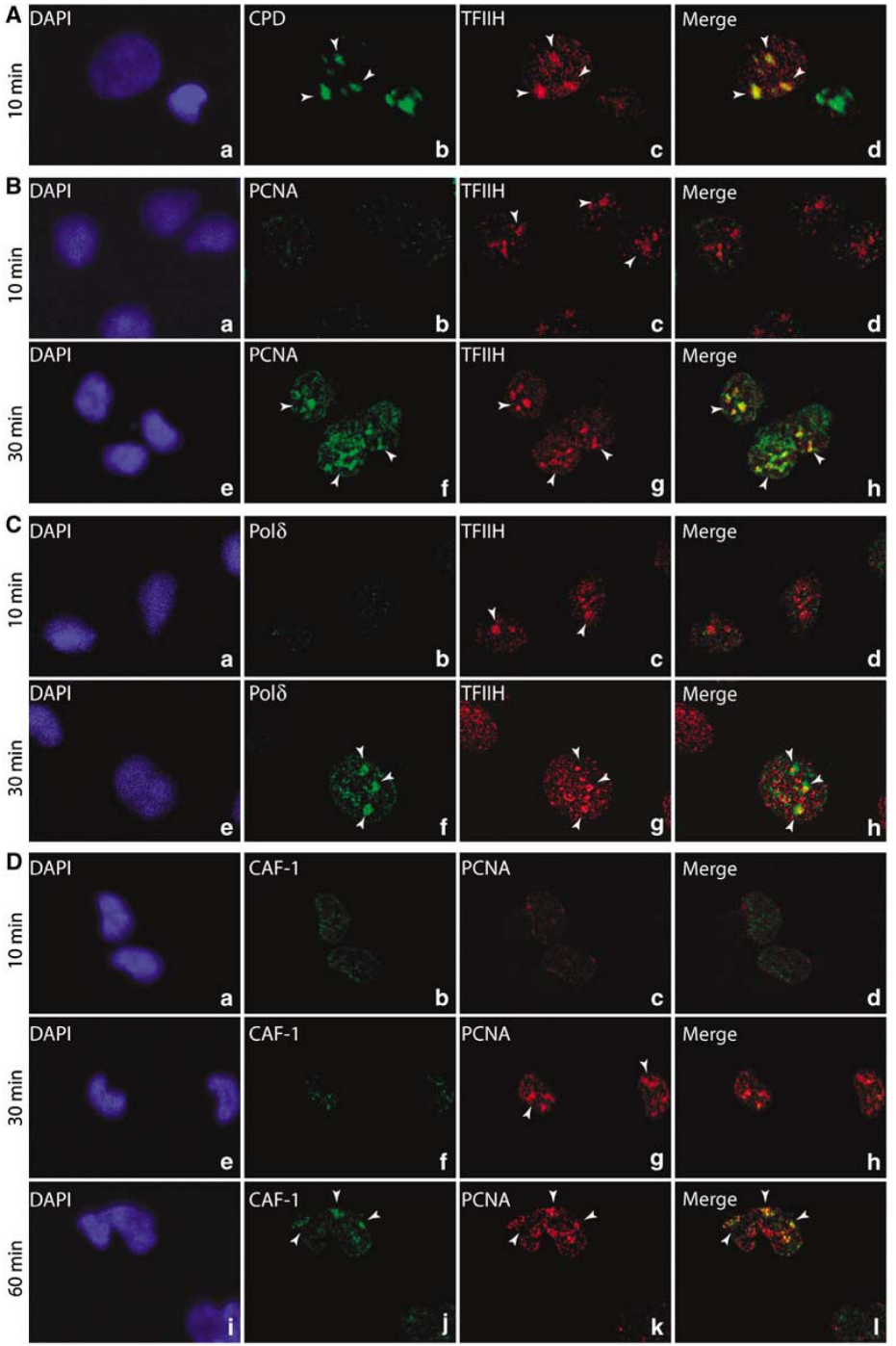
24 hours post-UV

XPG **XPB** **Merge**



Diffuse

= Delocalization upon achieved repair



EMBO J., **27**, 155 (2008)

Figure 1 *In vivo* sequential recruitment of NER factors. Rescued XPCS2BA human fibroblasts were locally UV irradiated and labelled at 10, 30 and 60 min after UV irradiation with the indicated MAbs or PAbs. Colocalization of (**A**) CPD and TFIIH (XPB) (panels a–d), (**B**) TFIIH and PCNA (panels a–h), (**C**) TFIIH and Polδ (panels a–h) and (**D**) PCNA and CAF1 (panels a–l). Nuclei were counterstained with DAPI, and pictures were merged.

Key Concepts

- Synthetic lethality:
→Screening strategies
→resistance to PARP-inhibitors
→Pol θ (teta)

- Mentioned chromatin and DNA repair: γ-H2AX

- Generation of DNA lesions by endogenous and exogenous sources

- **NER**:~30 factors involved. Repair of lesions that induce **helix distortion** (e.g. induced by UV). Repair involves DNA unwinding by TFIIH, dual incisions that flank the damaged location (XPF-ERCC1, XPG), excision and DNA repair synthesis

-->Xeroderma Pigmentosum

- Complementation