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:
DNA repair: NER; the DNA damage response
Lecture and paper discussion

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

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.)

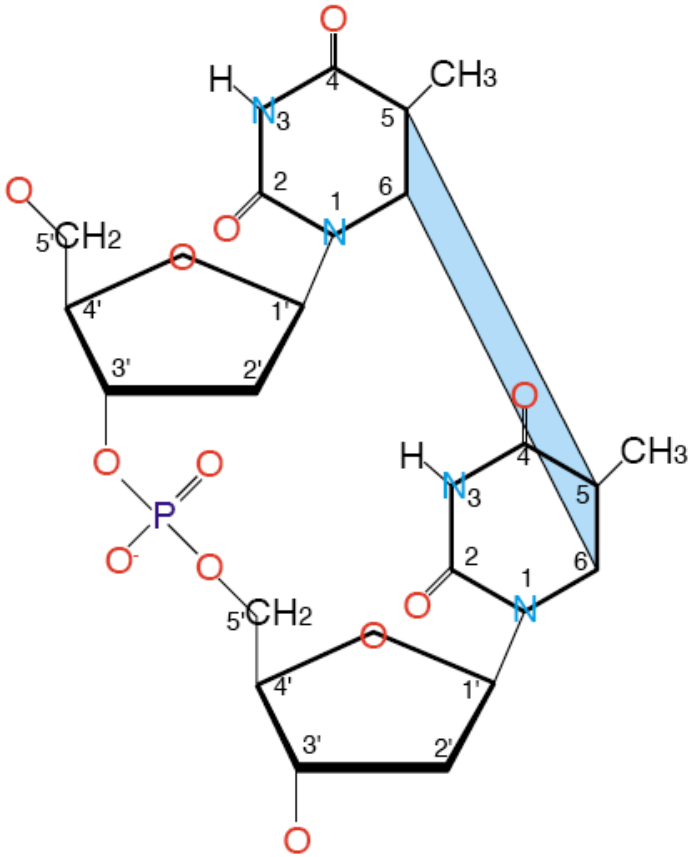
Double strand break repair. ✅

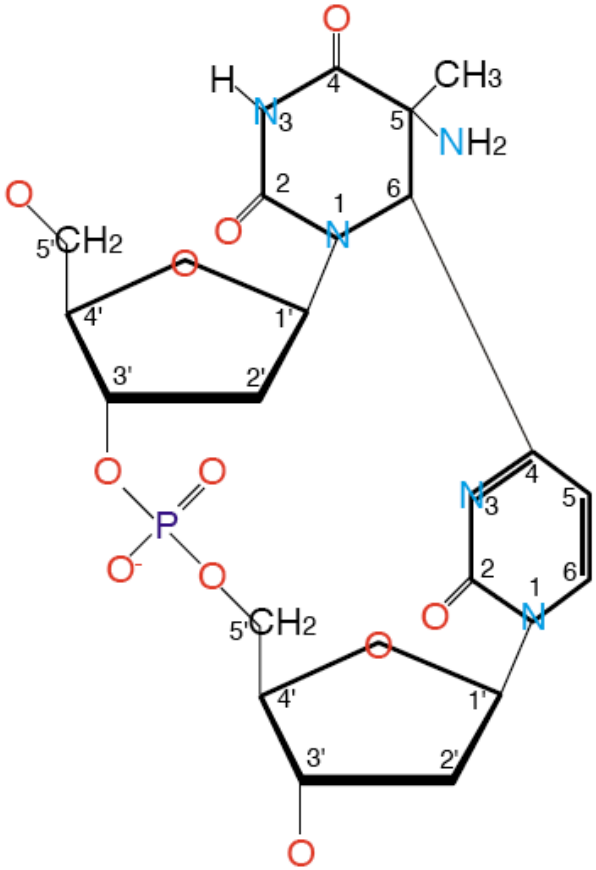
- NHEJ: Non homologous end-joining
- MMEJ: Microhomology directed end-joining (or Alt-EJ)
- HR: Homologous recombination

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

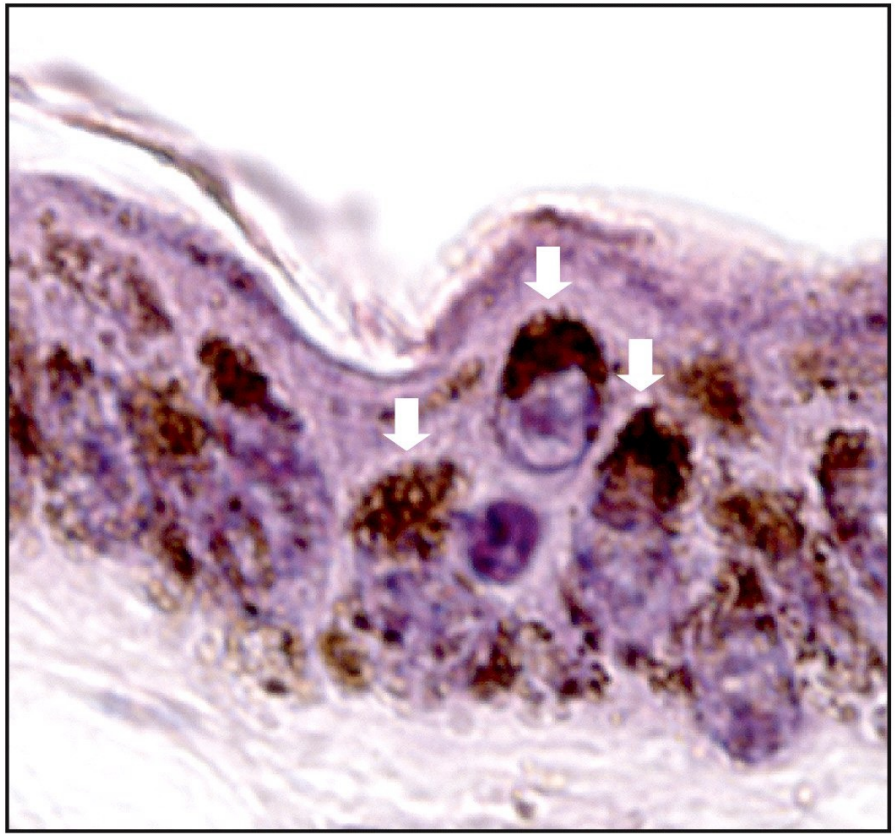


Figure 12.17 Weinberg, The Biology of Cancer

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

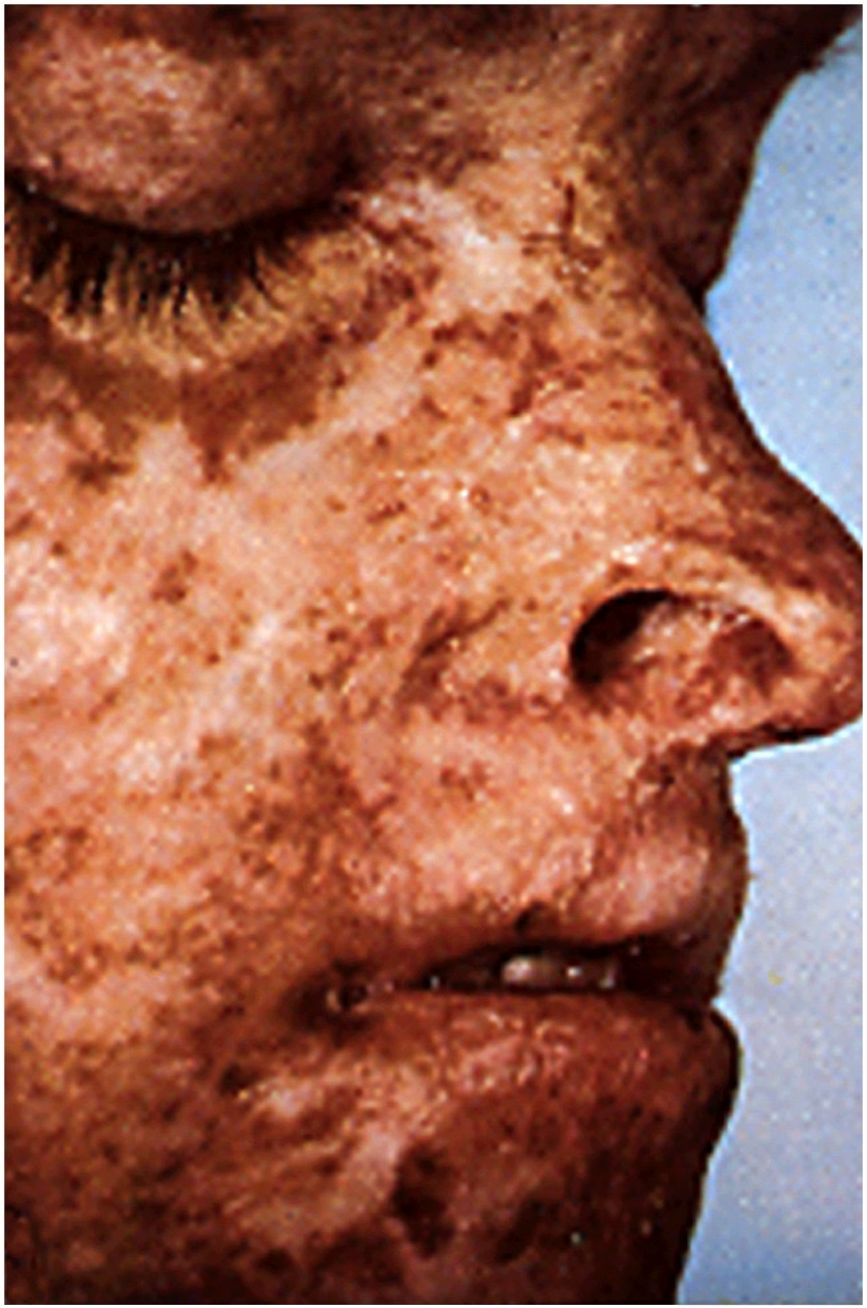


Figure 12.23 Weinberg, The Biology of Cancer

Early Onset of Skin Cancer in XP-Patients

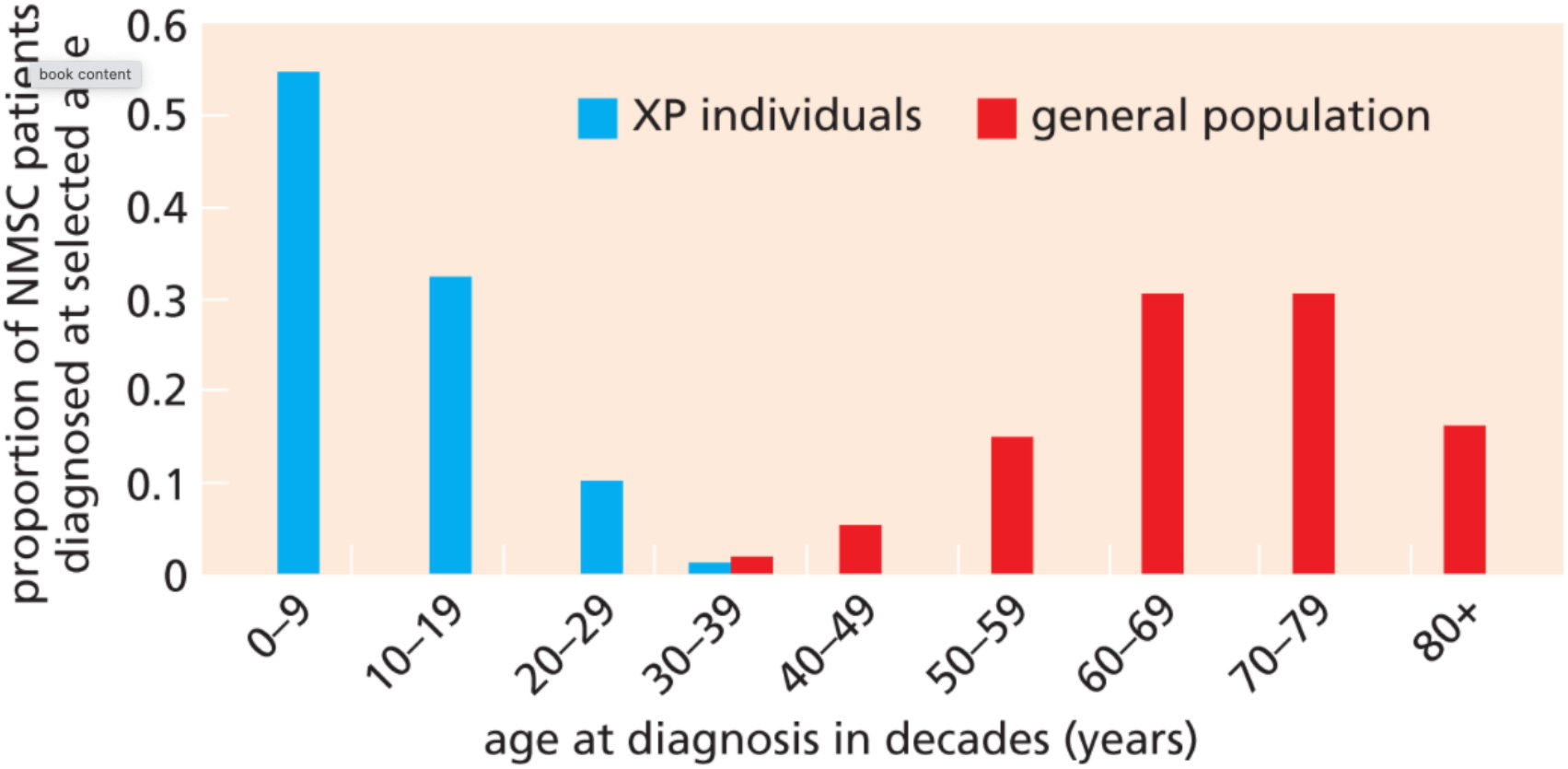


Figure 12.24. Weinberg, The Biology of Cancer

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

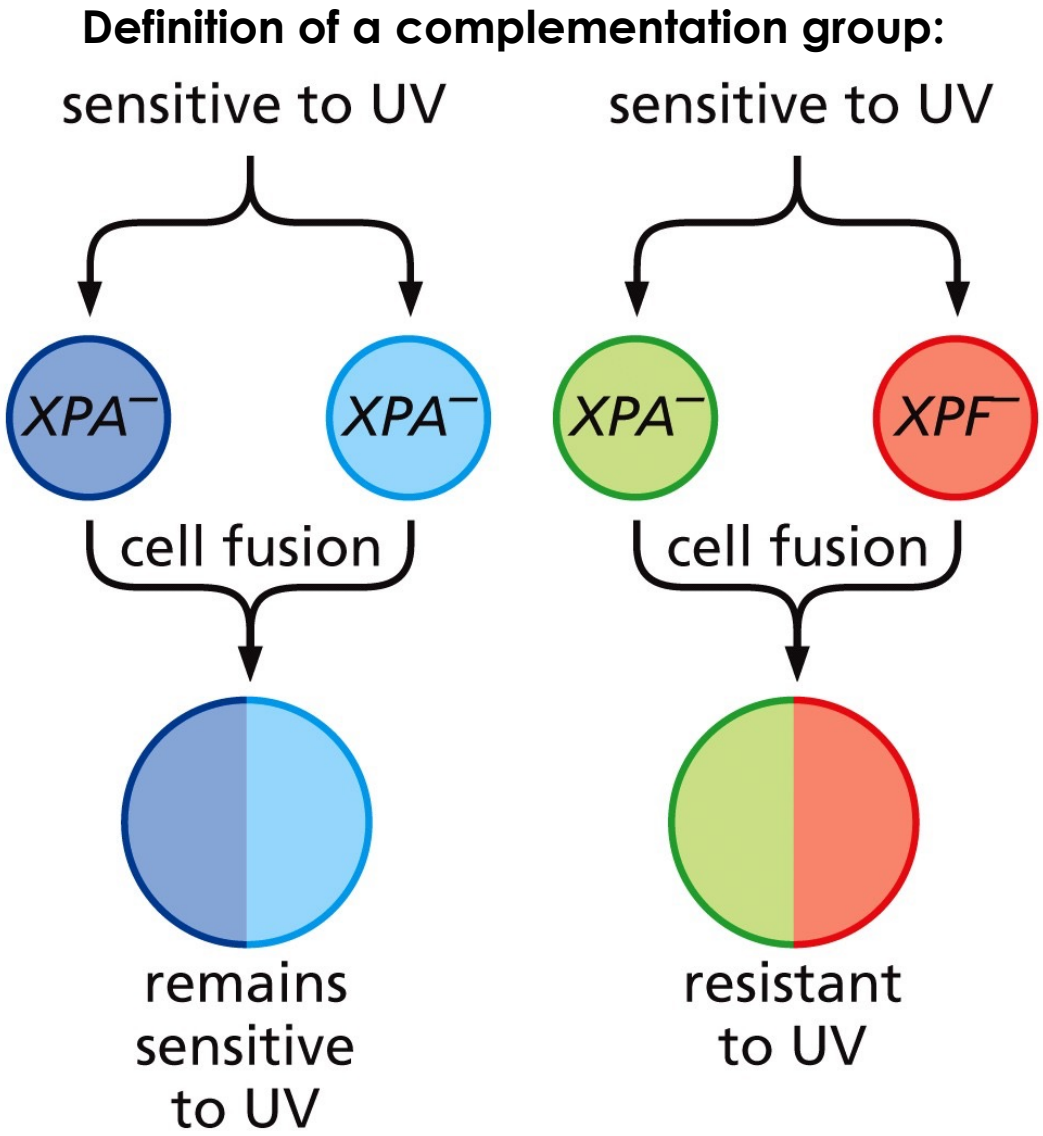
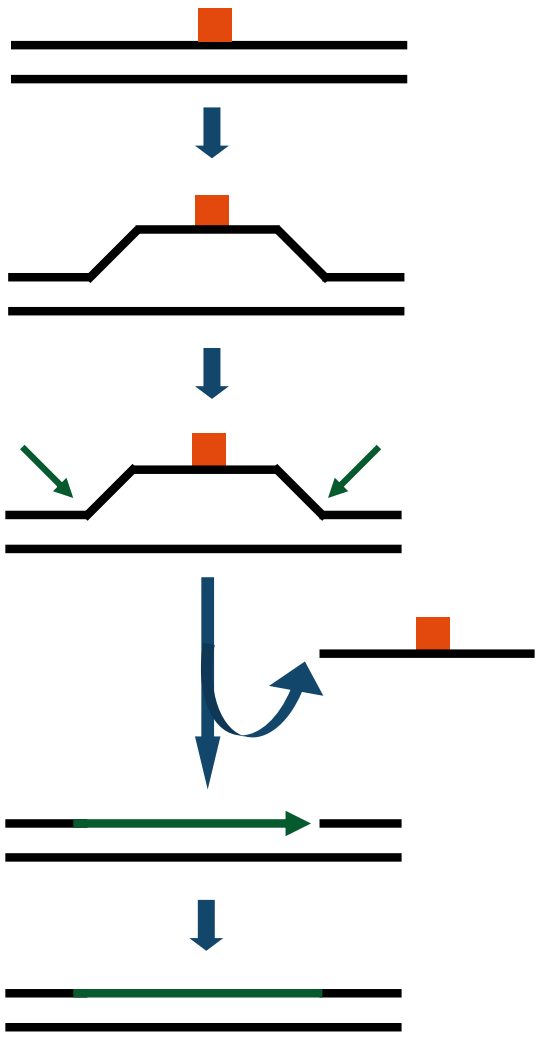


Figure 12.25. Weinberg, The Biology of Cancer

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

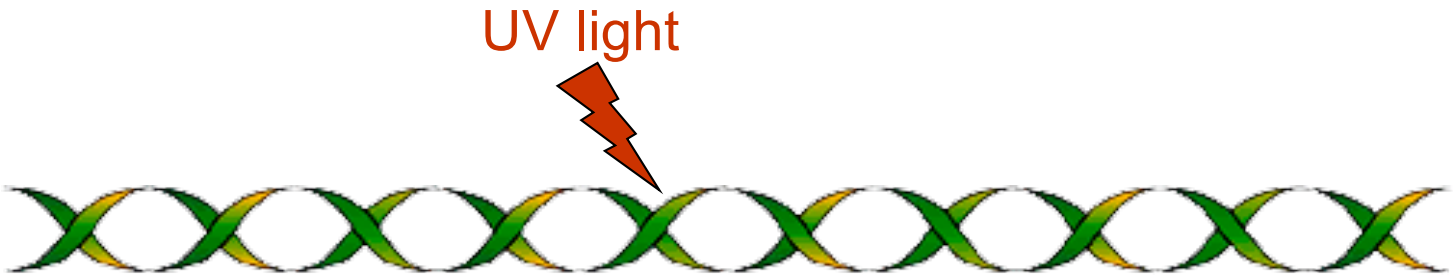
Dual incision 5’ and 3’ of the lesion

Excision of an oligonucleotide of 24-32 nt

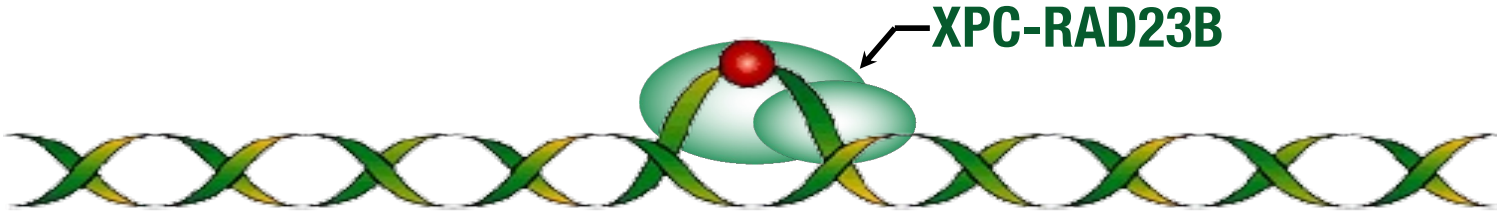
DNA repair synthesis

Ligation

Actors of NER

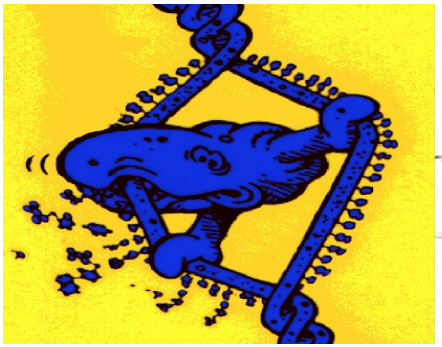


1- Recognition of lesion

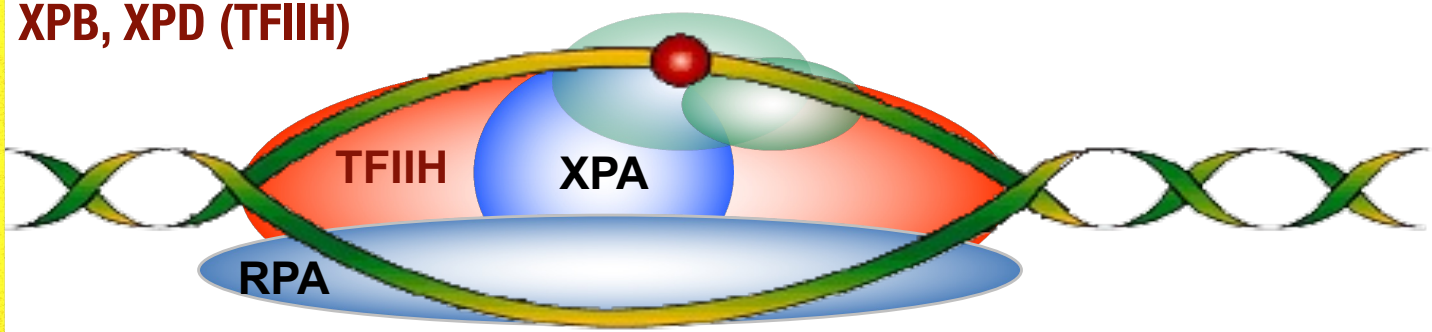


2- Formation of pre-incision complex

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

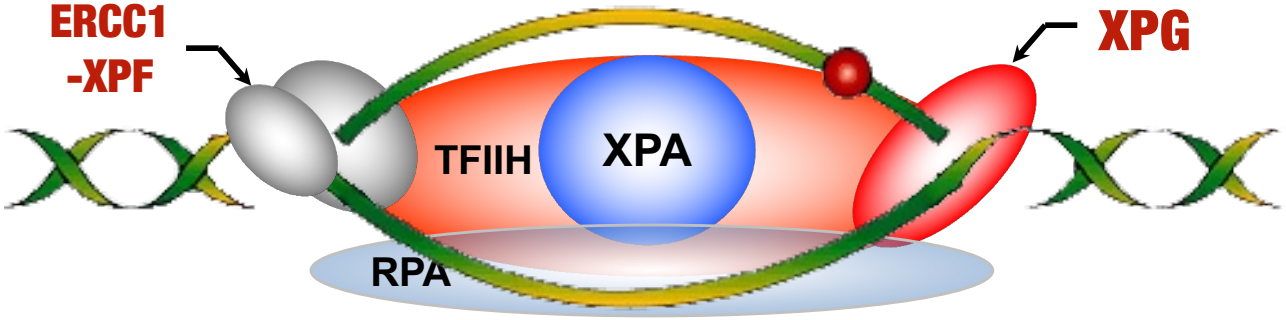


XPB, XPD (TFIIH)



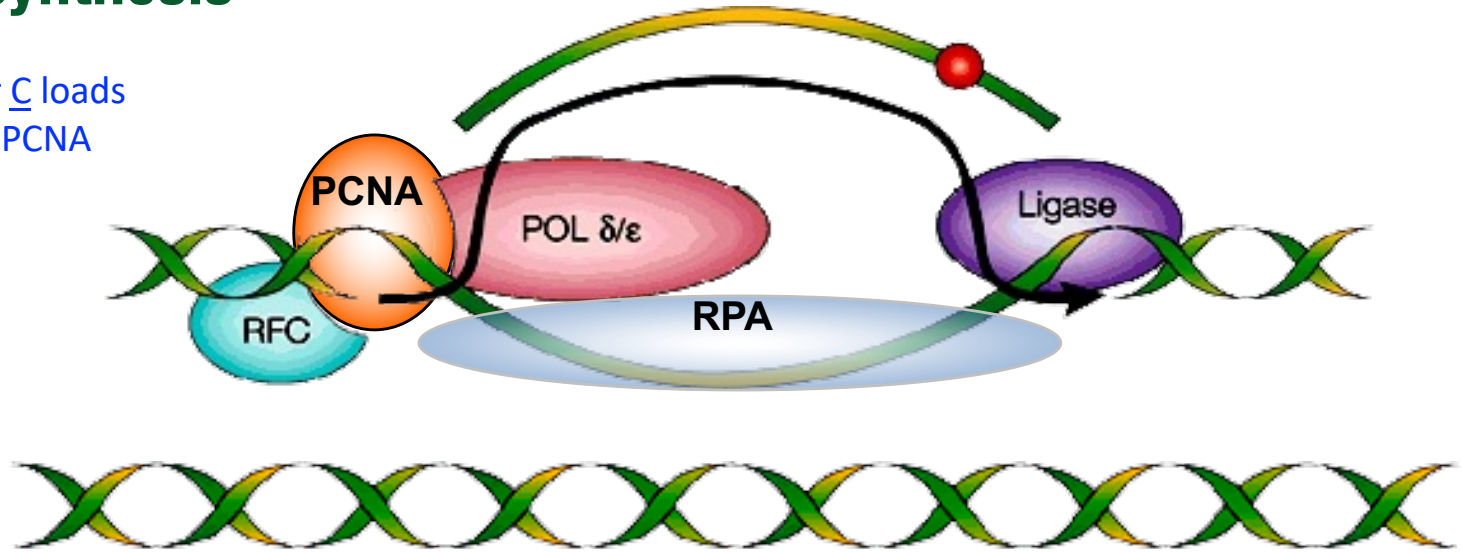
3- Double incision - excision

XPG binding precedes ERCC1-XPF1 binding but cleavage occurs first 5' to the lesion by ERCC1-XPF



4- Repair synthesis

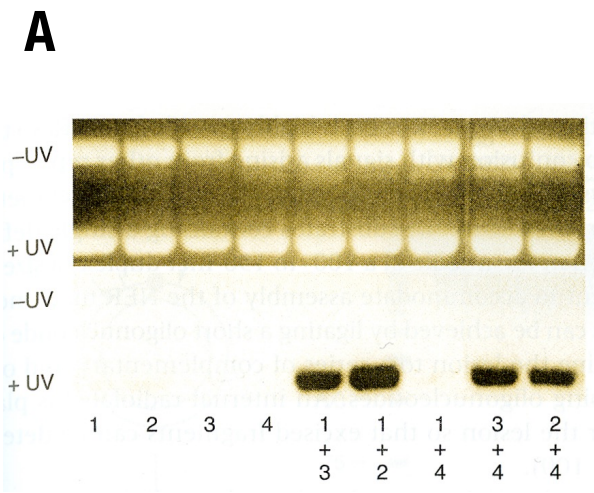
RFC: replication factor C loads trimeric sliding clamp PCNA



1. Nucleotide Excision Repair (NER)

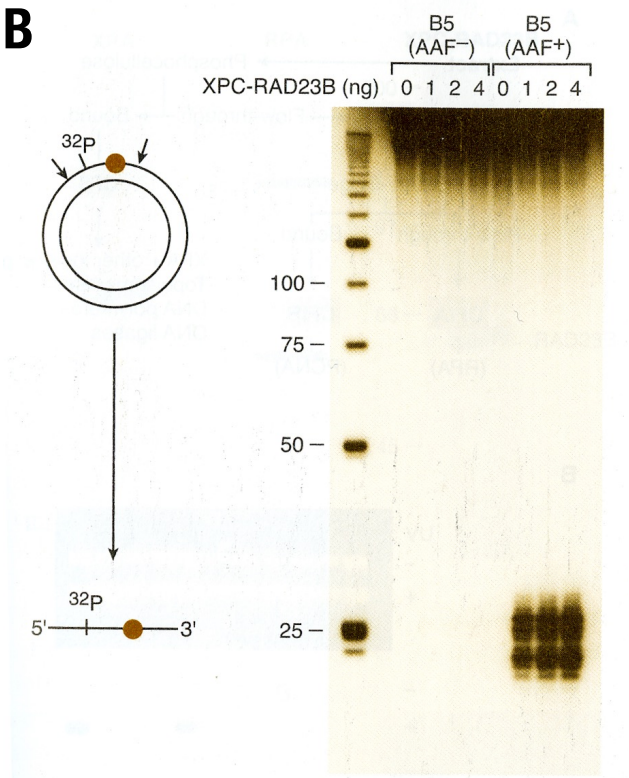
The NER system operates on a large variety of lesions with a particular affinity for **damages that induce distortions** of the double helix (alkylating agents, cis-platine, psolarenes etc). Most importantly lesions induced by UV, such as **cyclobutane pyrimdines dimers** and the **photoproducts (6-4)** pyrimidones are repaired by NER.

NER in vitro



CHO cells defective in NER complementation groups 1, 2, 3, and 4 as indicated: (defective genes: ERCC1 (cleavage 5'), XPD (TFIIH subunit; ERCC2), XPB (TFIIH subunit; ERCC3), or XPF (cleavage 5'; ERCC4).

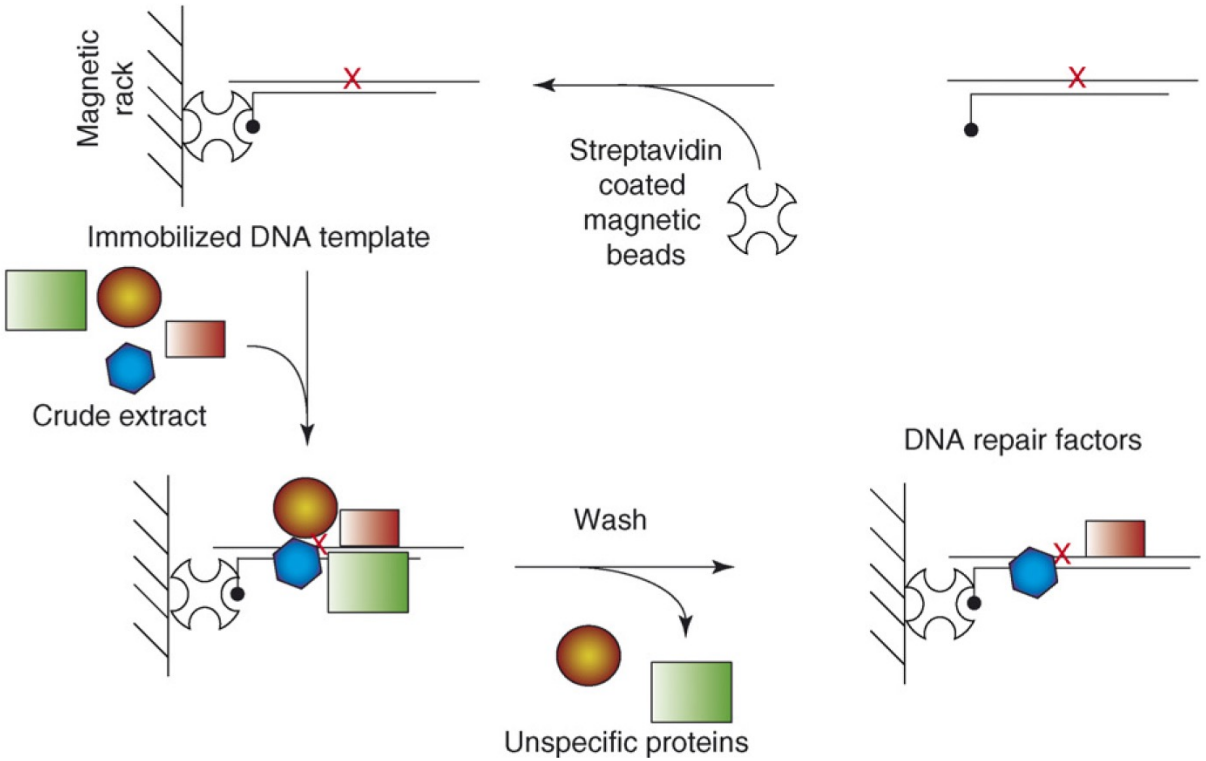
Reaction: fractionated extracts, PCNA, RPA, dNTPs, α-^{32}P-dATP, ATP, plasmids



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

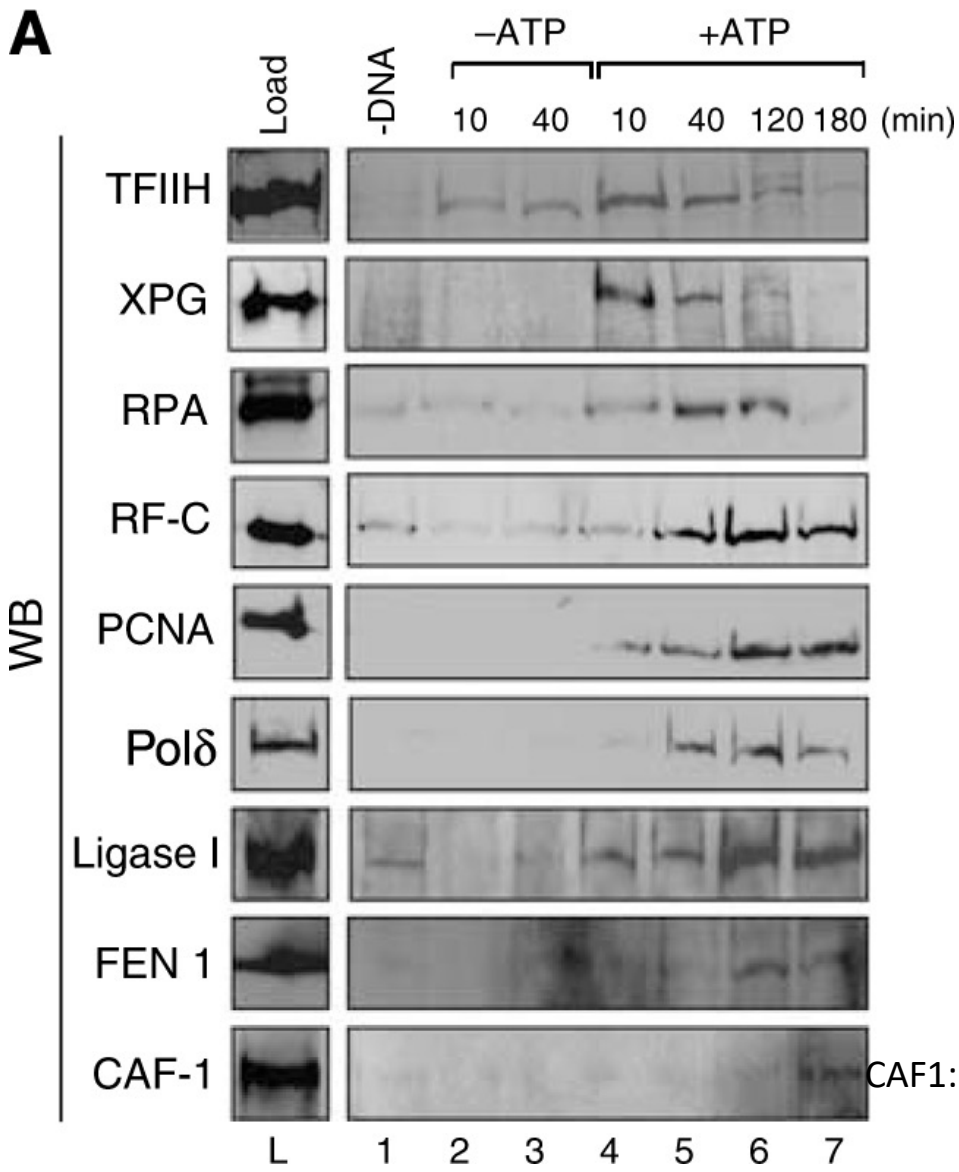
(N-Acetyl-2-aminofluorene (AAF) is a chemical carcinogen that reacts with guanines at the C8 position in DNA to form a structure that interferes with DNA replication)

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)



CAF1: reassembly of chromatin after repair

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

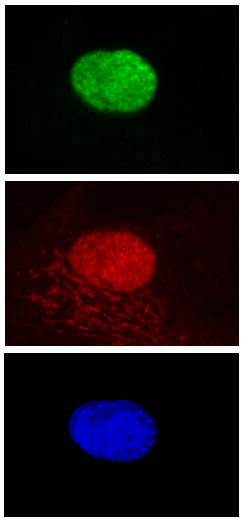
Irradiation of cells through a filter with a pore diameter= 8 μm

- UV

XPG

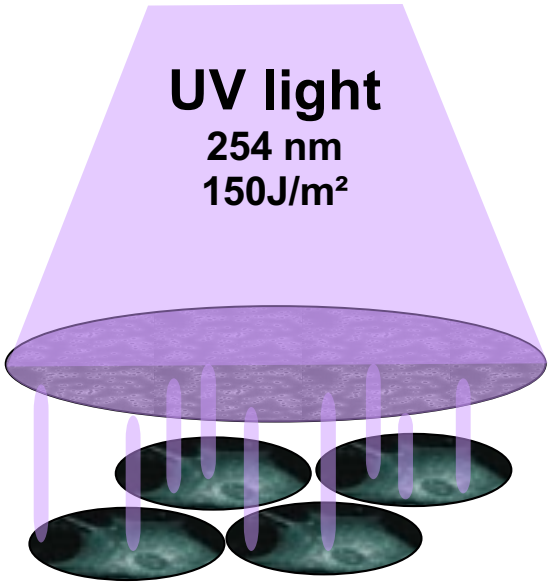
XPB

DAPI

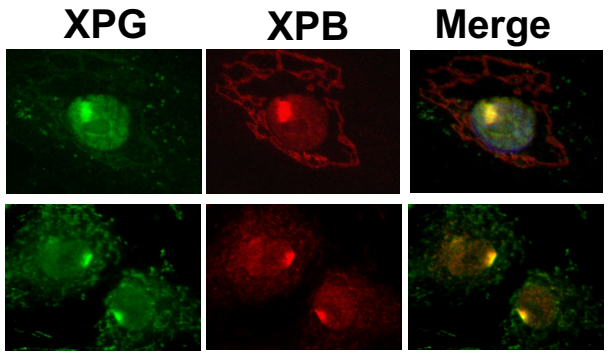


Diffuse

NER proteins are distributed throughout the nucleus



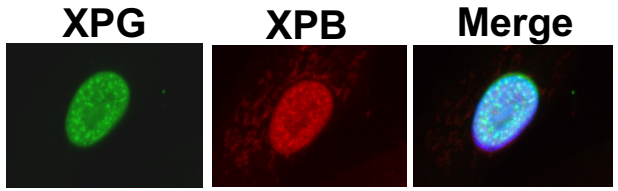
30 minutes post-UV



Formation nuclear foci

= Recruitement of NER proteins to irradiated regions

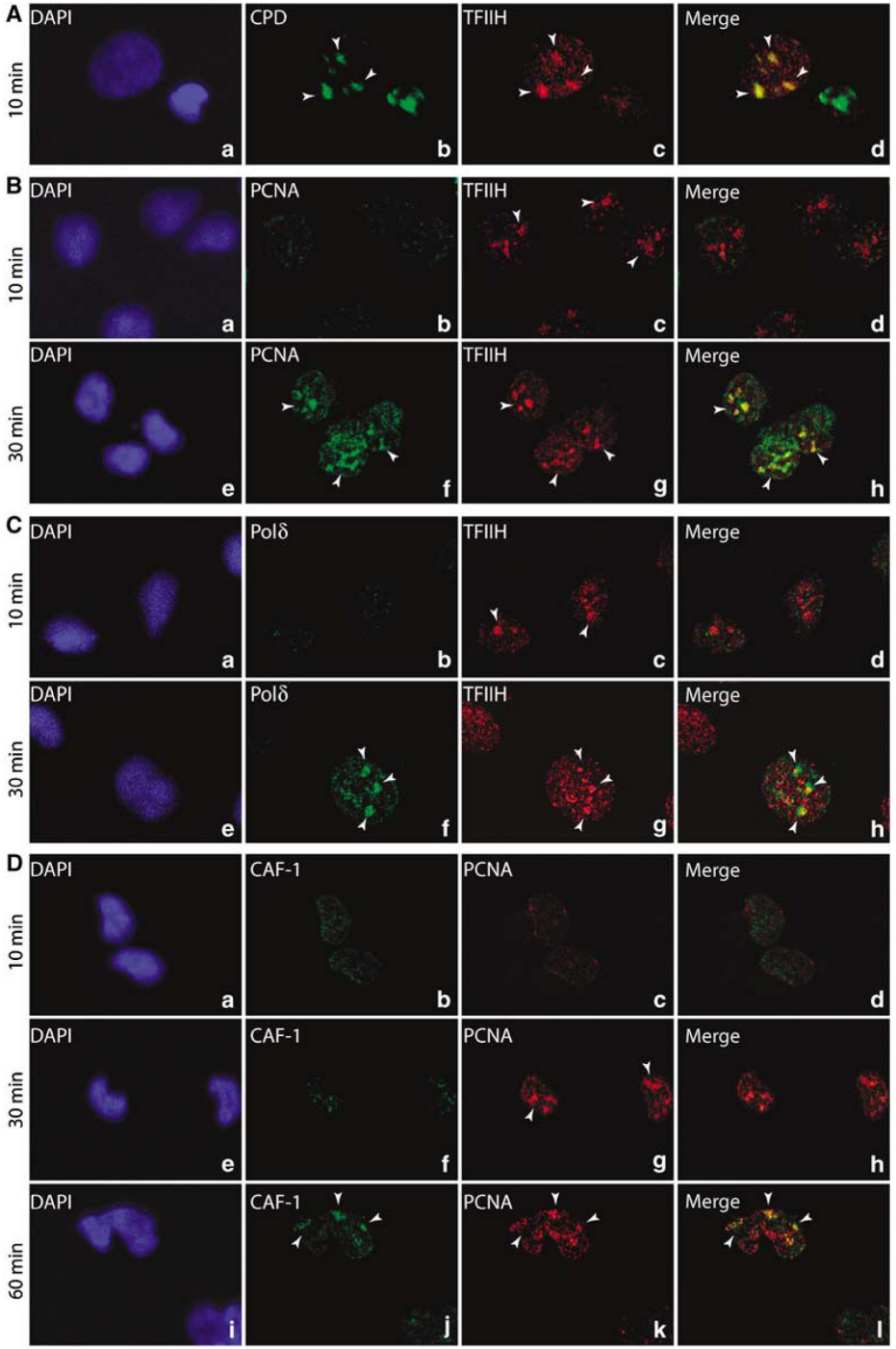
24 hours post-UV



Diffuse

= Delocalization upon achieved repair

CPD: Cyclobutane Pyrimidine Dimer



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.

Recapitulation

- **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
- Biochemical and cell biological assays reveal function

Paper Discussion

Sensing DNA Damage Through ATRIP Recognition of RPA-ssDNA Complexes

Lee Zou[1,2] and Stephen J. Elledge[1,2,3]*

The function of the ATR (ataxia-telangiectasia mutated– and Rad3-related)–ATRIP (ATR-interacting protein) protein kinase complex is crucial for the cellular response to replication stress and DNA damage. Here, we show that replication protein A (RPA), a protein complex that associates with single-stranded DNA (ssDNA), is required for the recruitment of ATR to sites of DNA damage and for ATR-mediated Chk1 activation in human cells. In vitro, RPA stimulates the binding of ATRIP to ssDNA. The binding of ATRIP to RPA-coated ssDNA enables the ATR-ATRIP complex to associate with DNA and stimulates phosphorylation of the Rad17 protein that is bound to DNA. Furthermore, Ddc2, the budding yeast homolog of ATRIP, is specifically recruited to double-strand DNA breaks in an RPA-dependent manner. A checkpoint-deficient mutant of RPA, *rfa1-t11*, is defective for recruiting Ddc2 to ssDNA both in vivo and in vitro. Our data suggest that RPA-coated ssDNA is the critical structure at sites of DNA damage that recruits the ATR-ATRIP complex and facilitates its recognition of substrates for phosphorylation and the initiation of checkpoint signaling.

The ATR (ATM- and Rad3-related) protein kinase plays a central role in the cellular response to replication stress and DNA damage such as double-strand breaks (DSBs) (*1*, *2*). In response to these events, ATR phosphorylates substrates such as p53, Brca1, Chk1, and Rad17. The phosphorylation of ATR substrates collectively inhibits DNA replication and mitosis and promotes DNA repair, recombination, or apoptosis. Despite the identification of many effectors of ATR, the mechanism by which ATR is activated by replication stress or DNA damage remains unsolved.

In human cells, ATR exists in a stable complex with ATRIP (ATR-interacting protein), a potential regulatory partner (*3*). Mec1 and Rad3, the budding and fission yeast homologs of ATR, respectively, also form similar complexes with Ddc2 (also called Lcd1 or Pie1) and Rad26, respectively (*4*–*7*). In budding yeast, the Mec1-Ddc2 complex is recruited to DSBs induced by the HO endonuclease (HO) or single-stranded DNA (ssDNA) at telomeres caused by a mutation in Cdc13. The recruitment of Mec1-Ddc2 to sites of DNA damage is independent of the replication factor C (RFC)–like protein Rad24 and the proliferating cell nuclear antigen (PCNA)–like proteins Ddc1, Mec3, and Rad17 (*8*, *9*). Likewise, ATR localization to DNA damage–induced foci does not require human Rad17, the homolog of yeast Rad24 (*10*). Thus, the ATR-ATRIP and Mec1-Ddc2 complexes might recognize certain DNA or DNA-protein structures at damage sites in the absence of the RFC-like and PCNA-like checkpoint complexes.

Many distinct DNA damaging agents can elicit the DNA damage or stress response that is mediated by ATR. Thus, a central question in checkpoint signaling is whether there is a sensor for each type of damage or whether all of these are converted to a common intermediate that is detected by a single sensor. A plausible candidate for a common intermediate is ssDNA. In yeast, ssDNA is present at telomeres because of the loss of function of Cdc13 and at the sites of damage caused by HO-induced breaks (*11*, *12*). It has been proposed as a requirement for strong and sustained activation of the checkpoint (*13*).

[1]Verna & Marrs McLean Department of Biochemistry and Molecular Biology, [2]Howard Hughes Medical Institute, [3]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, USA.

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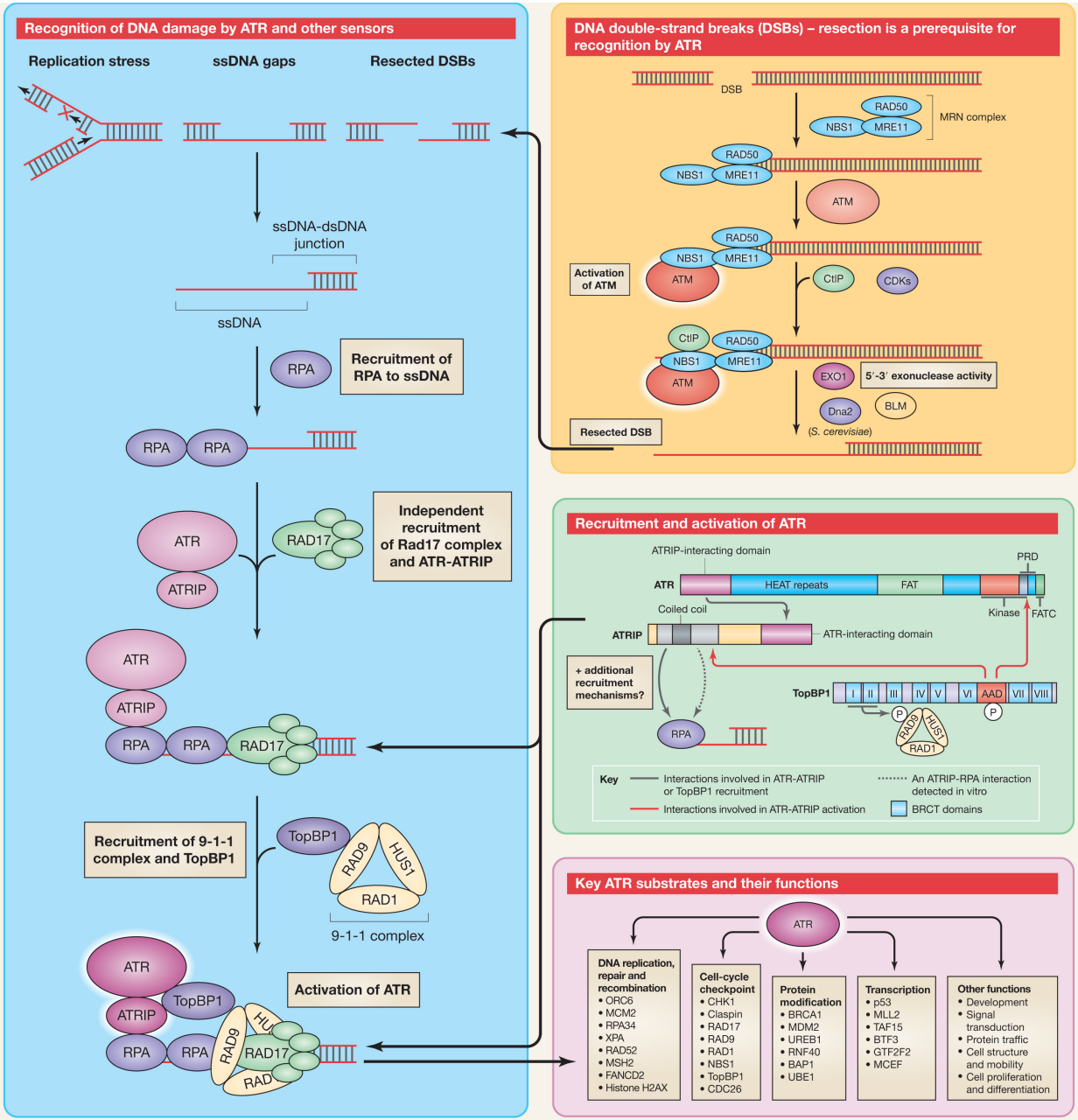
- **Cell cycle checkpoints** exist at specific points in the cell cycle to prevent them from progressing to the next phase of the cell cycle in the event of DNA damage or another condition which would make cell division dangerous for the cell.

- Two kinases– ataxia telangiectasia mutated (ATM), and ATM- and Rad3-related (ATR) – are master regulators of two major checkpoint pathways.
- ATM is primarily activated by DNA double-strand breaks (DSBs), whereas ATR responds to a much broader spectrum of DNA damage, including DSBs and many types of DNA damage that interfere with DNA replication.

→ **ATR** senses single stranded DNA via ATR-interacting protein (ATRIP), which binds directly to RPA- coated ssDNA.



Abbreviations: AAD, ATR-activating domain; ATM, ataxia telangiectasia mutated; ATR, ATM- and RAD3-related; ATRIP, ATR-interacting protein; BAP1, BRCA1-associated protein 1; BLM, Bloom syndrome protein; BRCA1, breast cancer type 1 susceptibility protein; BRCT, BRCA1 carboxy-terminal domain; BTF3, RNA polymerase B transcription factor 3; CDC26, cell-division cycle protein 26; CDK, cyclin-dependent kinase; CHK1, checkpoint kinase 1; CtIP, CtBP-interacting protein; Dna2, DNA replication mutant 2; DSB, double-strand break; dsDNA, double-stranded DNA; EXO1, exonuclease 1; FANCD2, Fanconi anemia group D2 protein; FAT, FRAP-ATM-TRRAP domain; FATC, FAT carboxy-terminal domain; GTF2F2, general transcription IIF subunit 2; HEAT repeat, Huntington-elongation-factor-3–PP2A–TOR repeat; HUS1, hydroxyurea-sensitive mutant 1; MCEF, major CDK9 elongation-factor-associated protein; MCM2, minichromosome maintenance protein 2; MLL2, mixed-lineage leukemia protein 2; MRE11, meiotic recombination protein 11; MSH2, MutS protein homolog 2; NBS1, Nijmegen breakage syndrome protein 1; ORC6, origin recognition complex subunit 6; PRD, PIKK regulatory domain; RAD, radiation-sensitive mutant; RNF40, RING finger protein 40; RPA, replication protein A; RPA34, DNA-directed RNA polymerase subunit RPA34; TAF15, TATA-box-binding-protein-associated factor; ssDNA, single-stranded DNA; TopBP1, topoisomerase II binding protein 1; UBE1, ubiquitin-activating enzyme E1; UREB1, upstream regulatory element binding protein 1; XPA, xeroderma pigmentosum A.

Bunsyo Shiotani,Lee Zou, ATR signaling at a glance, J Cell Sci, 2009

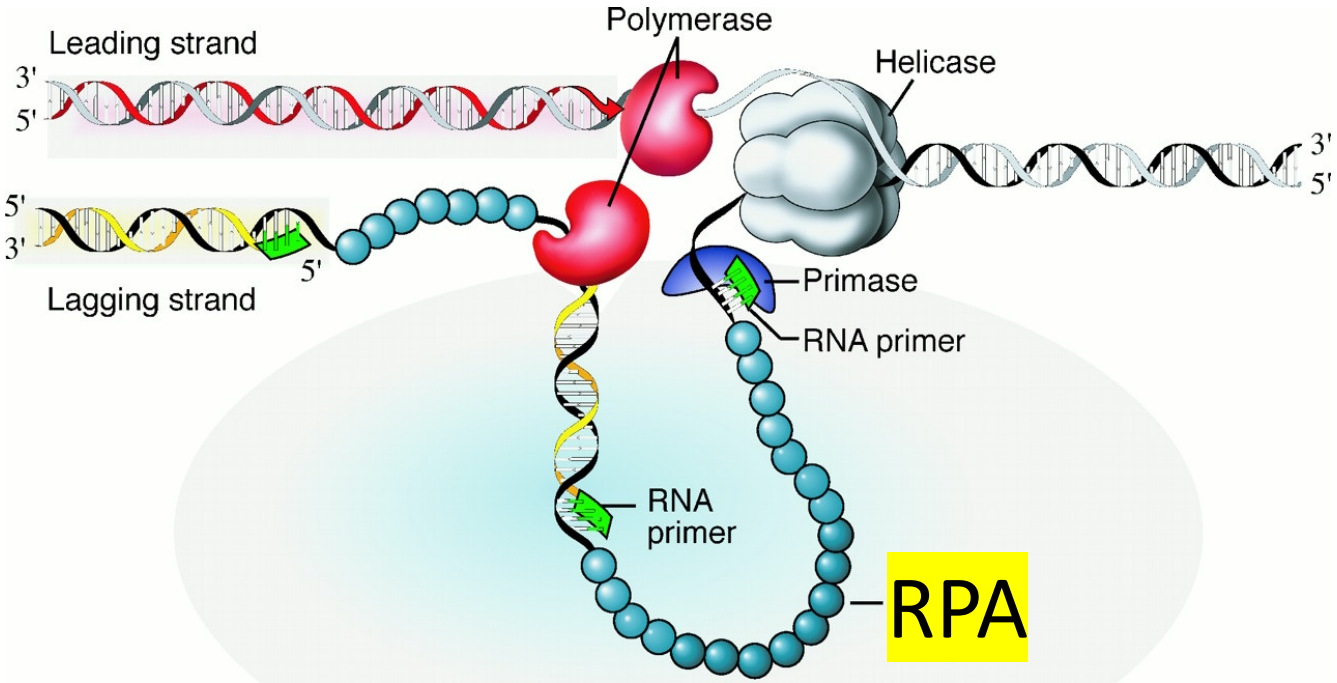
ATR is an essential gene

Hypomorphic alleles

Clinical symptoms (Seckel syndrome):

Microcephaly (small circumference of the head)
Dwarfism
Large eyes, low ears, small chin
Severe mental retardation

Hematological abnormalities and chromosome breaks



Replication protein A (RPA):

heterotrimer, composed RPA1 (RPA70) (70kDa subunit), RPA2 (RPA32) (32kDa subunit) and RPA3 (RPA14) (14kDa subunit). The three RPA subunits contain six OB-folds (oligonucleotide/oligosaccharide binding), with which RPA binds to single-stranded DNA

Function of DNA Replication Fork Proteins:

Proteins	Functions
RPA	Heterotrimer; single-stranded DNA binding; stimulates DNA polymerases; facilitates helicase loading; checkpoint signaling
RFC	5 subunits; DNA-dependent ATPase; primer template binding; displaces pol α; loads PCNA onto 3' primer/template junction and then dissociates
PCNA	Homo-trimer forms a ring; stimulates processivity of DNA pol δ;
Polα/primase	4 subunits; p48 synthesizes ~12-nucleotide RNA primer followed by ~20 nt DNA which is synthesized by p180
Polδ	4 subunits; replicative DNA polymerase; lagging strand synthesis.
Pol ε	4 subunits; replicative DNA polymerase; leading strand synthesis.
MCM	MCM2-7; 3'-5' replicative helicase
FEN1	Nuclease for removal of RNA primers
RNase HI nuclease	Removal of RNA primers
DNA ligase I	Ligation of DNA
Topo 1/2	Release of tortional stress during pol movement and for decatenation of replicated sister molecules.