DNA Damage Processing at Telomeres: Maintaining Youthful Chromosomes

Patty O'presko

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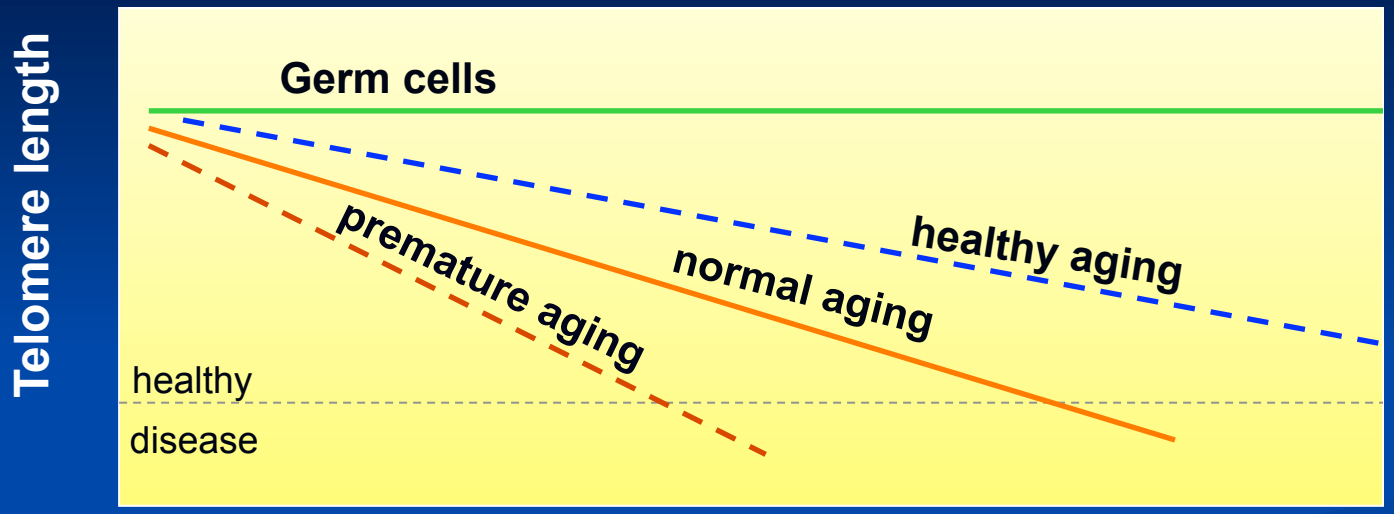
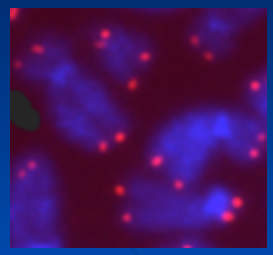
University of Pittsburgh Cancer Institute, Hillman Cancer Center

Relationship Between Telomere Length, Aging and Human Disease



$(TTAGGG)_{1500}$



Diseases
- osteoporosis
- cancer
- heart disease/ atherosclerosis
- arthritis/ ulcerative colitis
- pulmonary fibrosis/ liver cirrhosis
- diabetes
- neurodegeneration

Factors
- perceived stress
- smoking/ polycyclic aromatic HC
- obesity
- environmental metals
- lack of exercise
- diet

Genomic instability and the loss of cell viability induced by telomere dysfunction contribute to disease and tissue/organ decline with age

Mechanisms of Telomere Loss and Preservation

Significance

Understanding mechanisms of telomere loss and preservation could inform about new therapeutic strategies that:

1) Protect telomeres in healthy cells to prevent telomere dysfunction-induced pathologies

- cell senescence and degenerative disease associated with aging
- genomic instability and carcinogenesis

2) Deplete telomeres to halt cancer cell proliferation

- combine with telomerase inhibitors

Outline

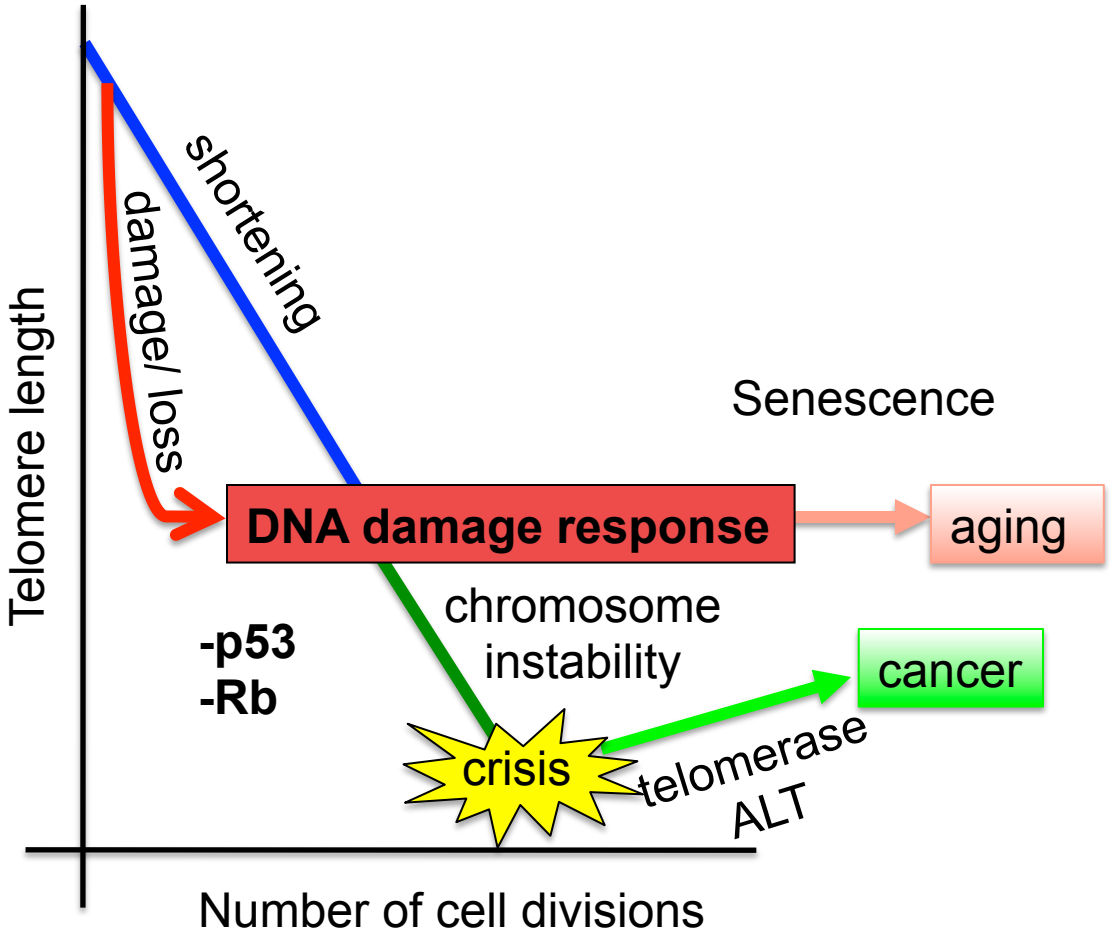
General background on telomere biology
DNA damage and repair

1. Impact of bulky DNA lesions on telomere replication

2. Repair of bulky DNA lesions at telomeres

Summary and Conclusions

Roles for Dysfunctional Telomeres in Cancer and Aging

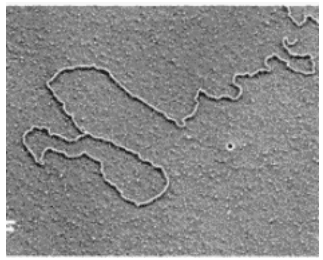


Somatic cells:
most have no telomerase activity

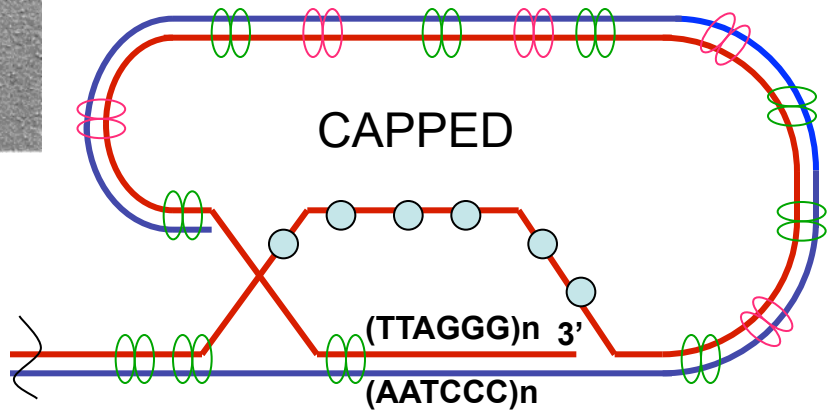
Stem cells:
variable amounts

Cancer cells:
90% express high telomerase activity

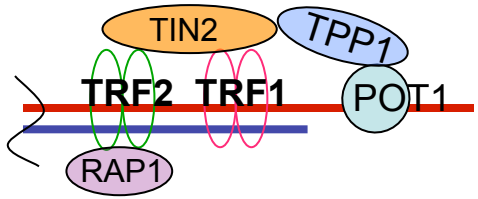
Telomere Cap is a Complex of Proteins and DNA Structure



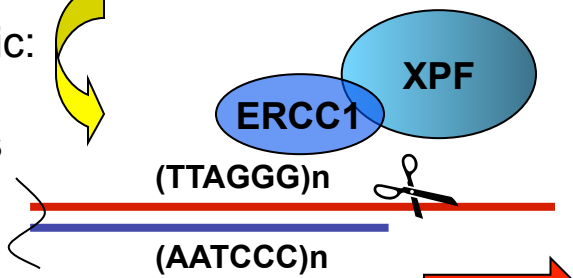
Griffith et al
1999, Cell



Shelterin



Loss of telomeric: DNA proteins



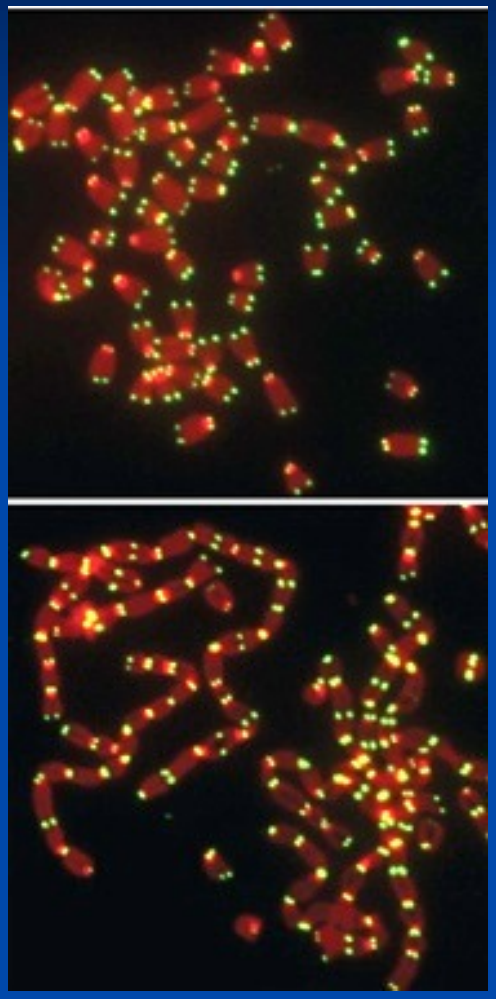
UNCAPPED

Chromosome fusions → Genomic instability
CANCER

Activate DNA damage response

Senescence/ Apoptosis
AGING

Telomere Uncapping



Conditional knock out of TRF2 in mouse embryonic fibroblasts.

p53 proficient – senescence or apoptosis

p53 deficient – chromosome fusions

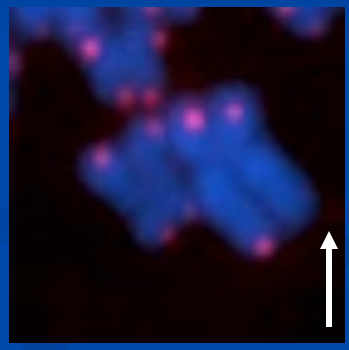
N Dimitrova et al. Nature 2008, 1-5 (2008)

Werner Syndrome

Phenotypes

- skin wrinkling
- hair loss and graying
- cataracts
- diabetes (type II)
- osteoporosis
- atherosclerosis, heart disease
- cancer; particularly sarcomas

Caused by loss of the RecQ helicase WRN



Telomere dysfunction contributes to the disease pathology

WS cells exhibit increased:

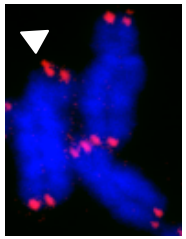
The shortest telomere, not the average telomere length, is the critical determinant of cell viability and genome stability

(Hemann Cell 2001)

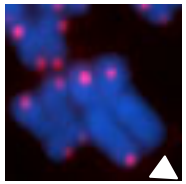
Stochastic telomere loss occurs in normal senescent cells as well

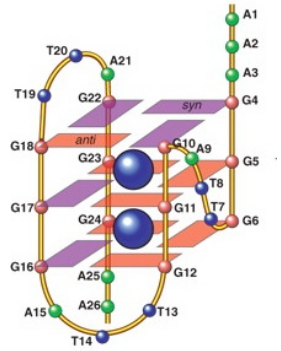
Telomeres Are Hypersensitive to Replication Stress

Fragile Telomere



Telomere loss



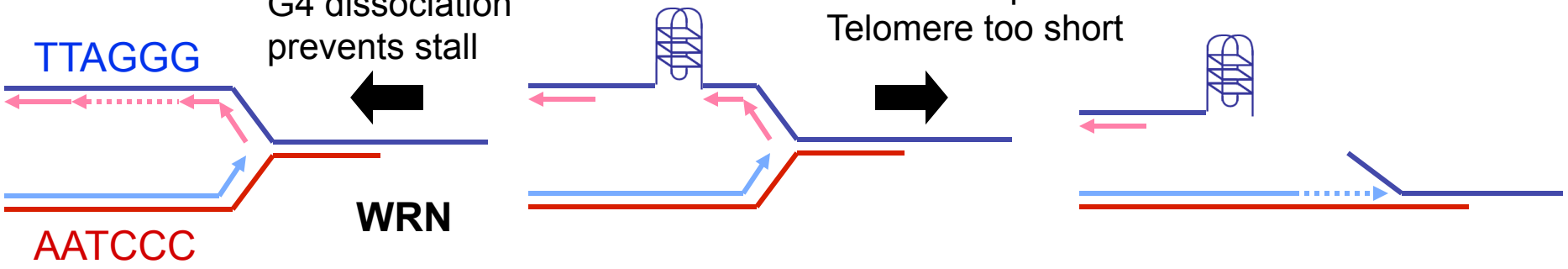


Ambrus, NAR 2006

aphidicolin (fragile only)
oncogenic signaling
G-quadruplex ligands
Loss of:
helicases WRN, BLM, RTEL1
shelterin TRF1, POT1, others
Ogg1 and Nth1 glycosylase

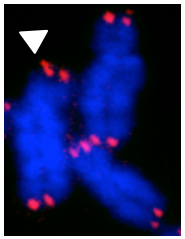
G4 dissociation
prevents stall

G4 block replication
Telomere too short

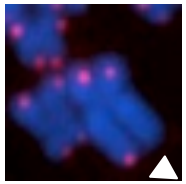


Can DNA Damage Cause Telomere Loss or Fragility?

Fragile Telomere



Telomere loss



X = blocking DNA adduct or lesion ??

aphidicolin (fragile only)
oncogenic signaling
G-quadruplex ligands
Loss of:
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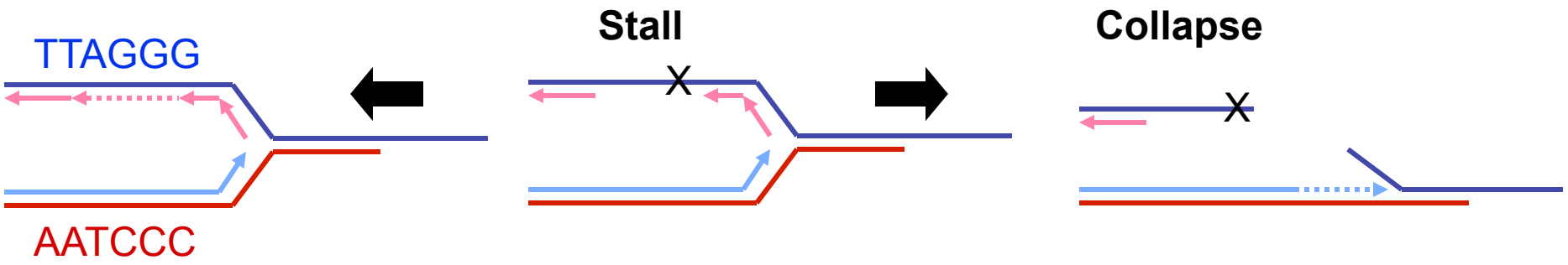
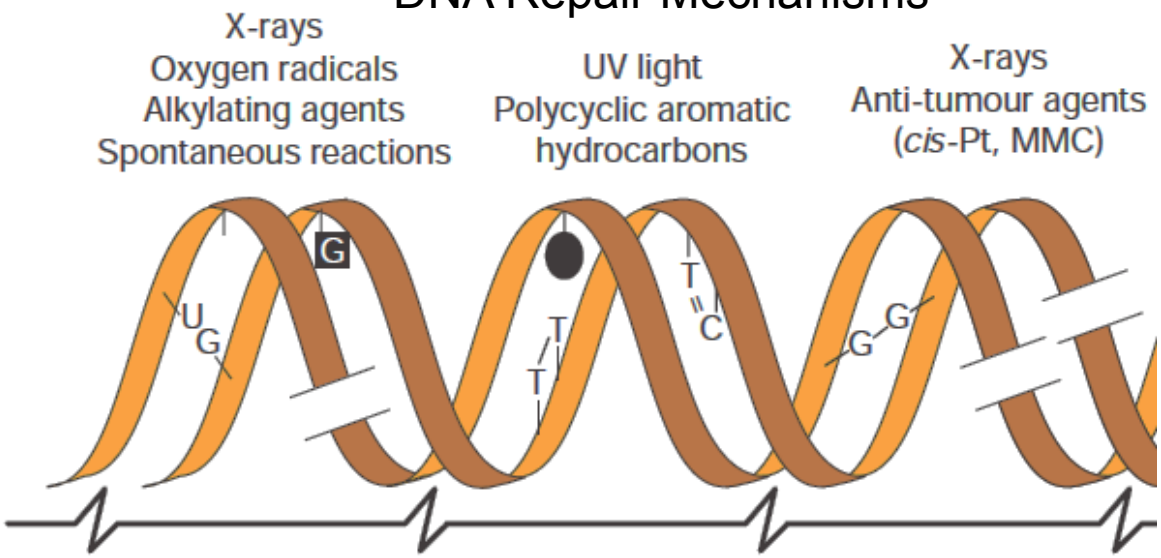
shelterin TRF1, POT1
Ogg1 and Nth1 glycosylase



How Do Telomeres Manage DNA Damage?

DNA Repair Mechanisms



Uracil
Abasic site
8-Oxoguanine
Single-strand break

(6–4)PP
Bulky adduct
CPD

Interstrand cross-link
Double-strand break

Hoeijmakers, 2001 Nature

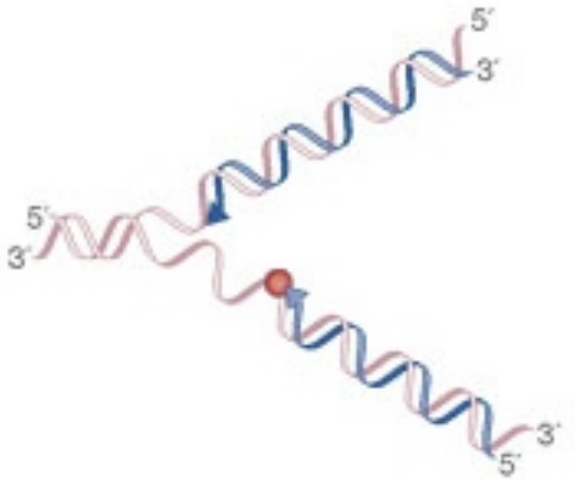
Base-excision repair (BER)

Nucleotide-excision repair (NER)

Recombinational repair (HR, EJ)

Telomere: Yes efficiency? | ? | Suppressed

DNA Damage Tolerance



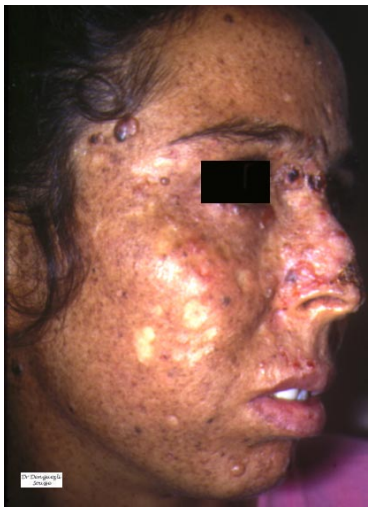
CPD, 6-4PP
bulky adducts
oxidative base damage

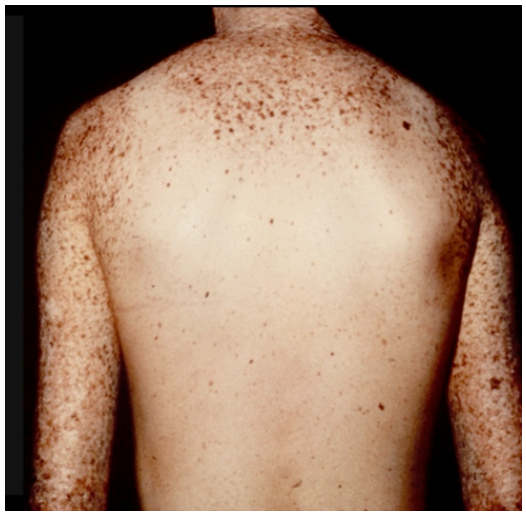
Fork remodeling
Translesion DNA synthesis

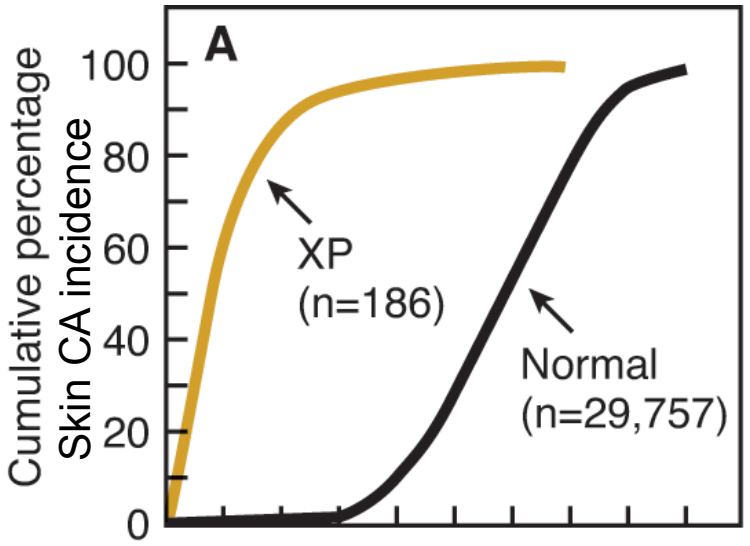
Telomere: ?

1. Kruk et al. 1995, *PNAS*
2. Rochette & Brash 2010, *PLoS Genetics*

Xeroderma Pigmentosum



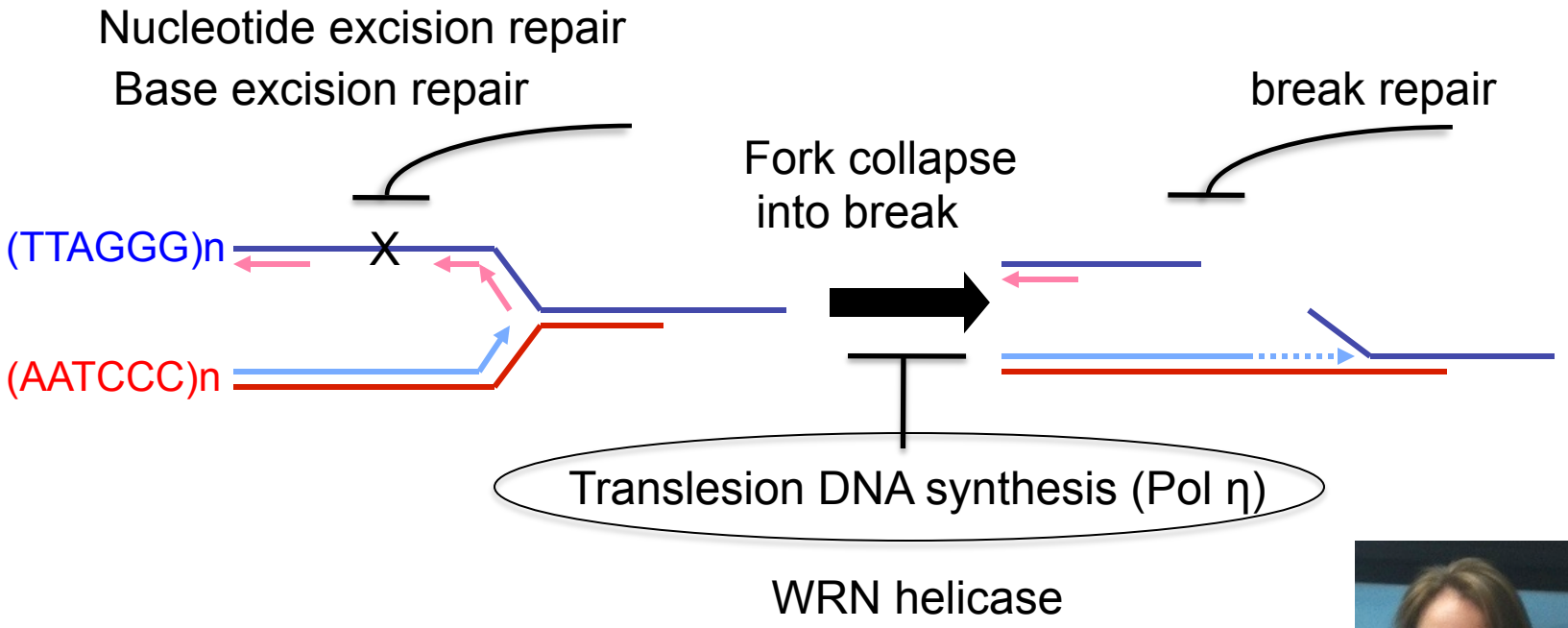




- Caused by mutations in genes that code for NER proteins (XP-A to XP-G); or polymerase η (XPV)
- Photosensitivity
- Pigmentation abnormality and atrophic skin
- >1000x increase in skin cancer and 10x increase in internal tumors
- neurological degeneration in 30% of the cases
- Shortened telomeres were observed in sunlight or UVB exposed skin tissue from humans and mice

(Ikeda et al. *Human Pathology*. 2014; Stout & Blasco *Cancer Research*. 2013)

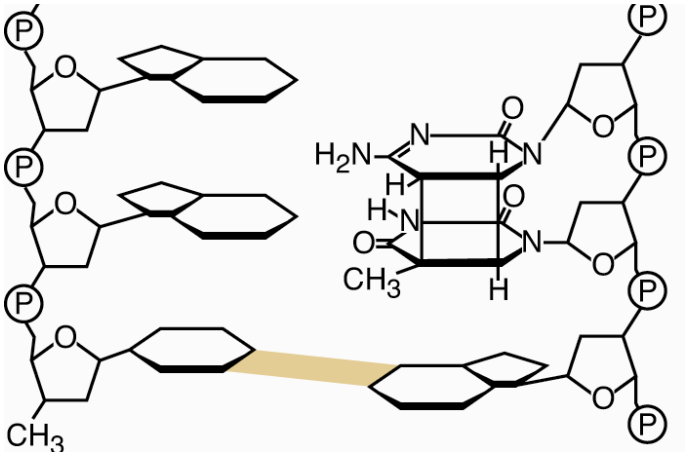
Do DNA lesions cause telomere aberrations ?
Are they repaired at telomeres?



X = UV photoproducts (acute physical exposure)
Cr-DNA adducts (chronic chemical exposure)

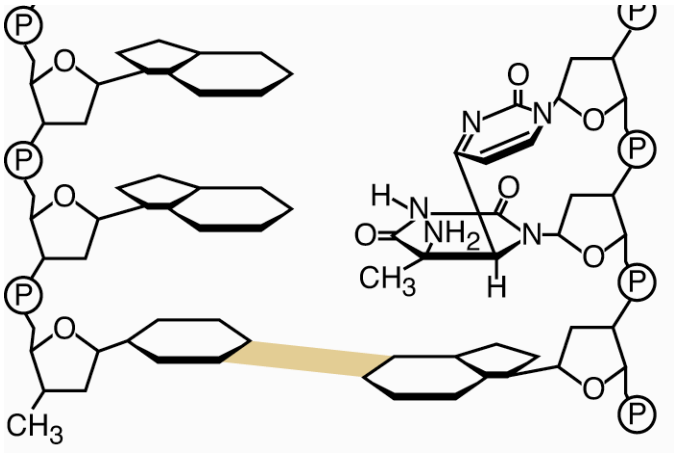
Hannah Varsalona-Pope

UV Light and Cr(VI) Exposures Induce Bulky Lesions



- removed by NER
- block replicative polymerases
- cause replication dependent breaks
- bypassed by polymerase η
  - in yeast for Cr(VI)

5'**TTAGGG**
3'**AATCCC**



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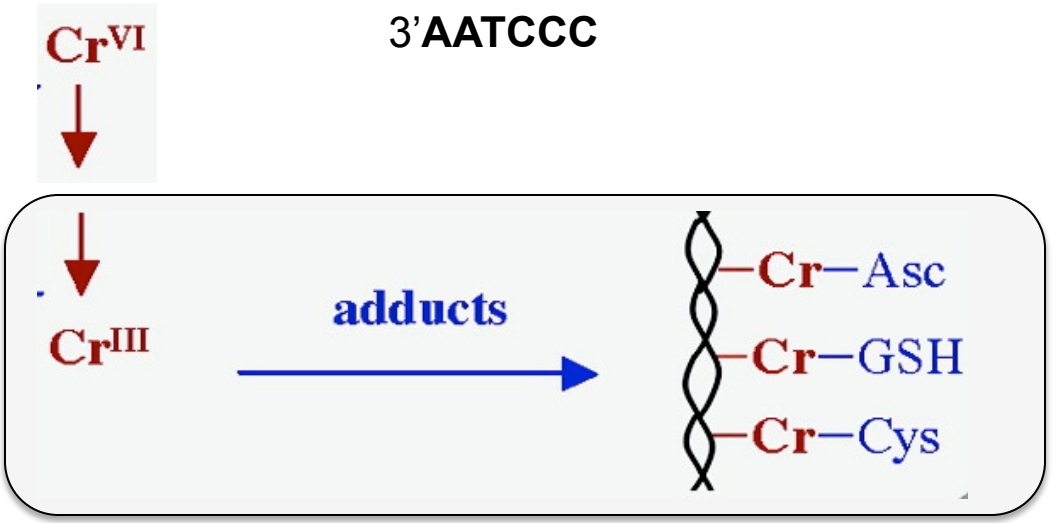
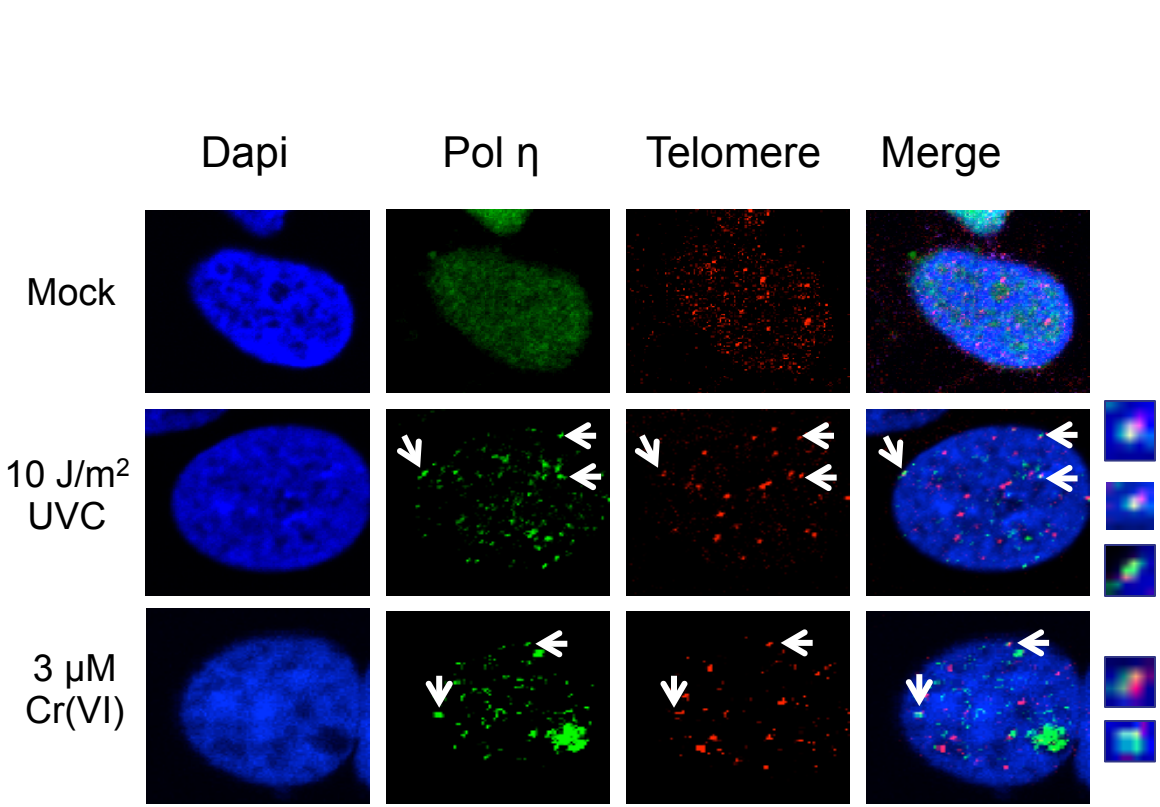
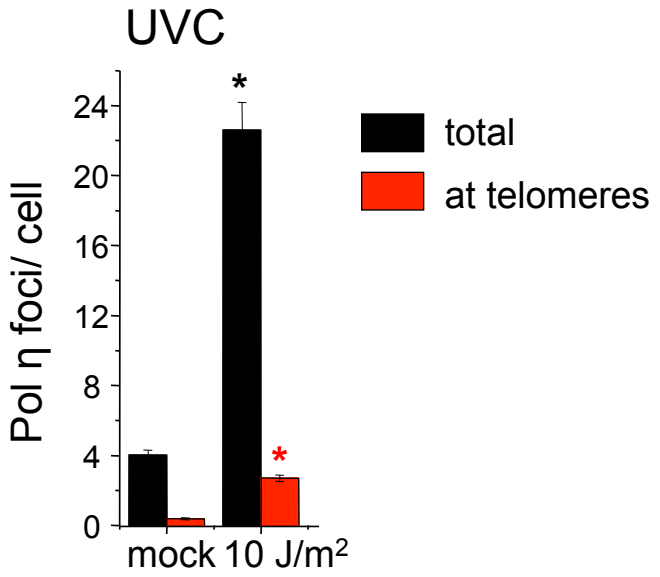


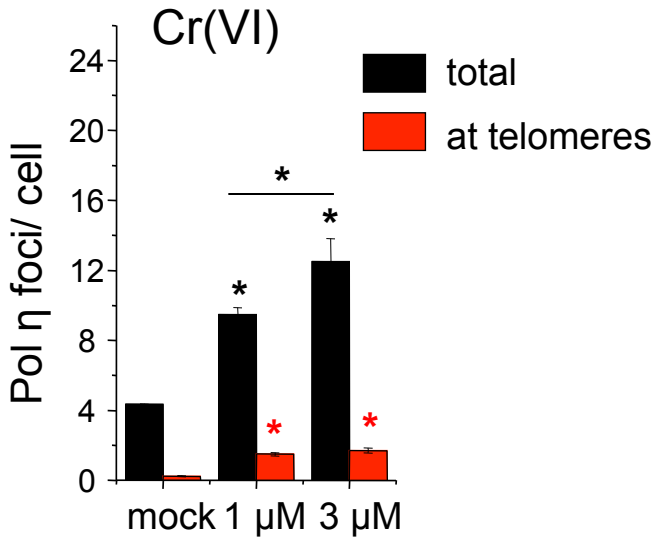
DNA Repair & Mutagenesis,
2nd Ed, ASM Press

Salnikow & Zhitkovich; *Chem. Res. Toxicol.* 2008

Polη Foci Formation and Localization to Telomeres



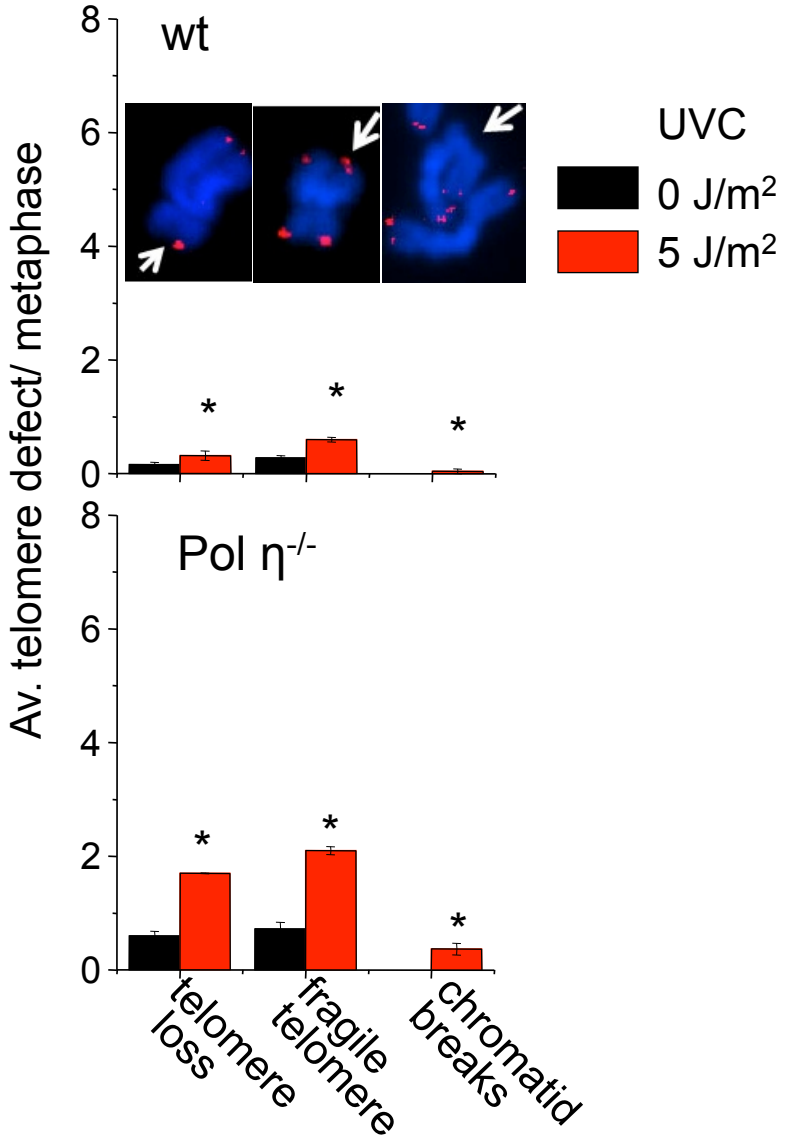


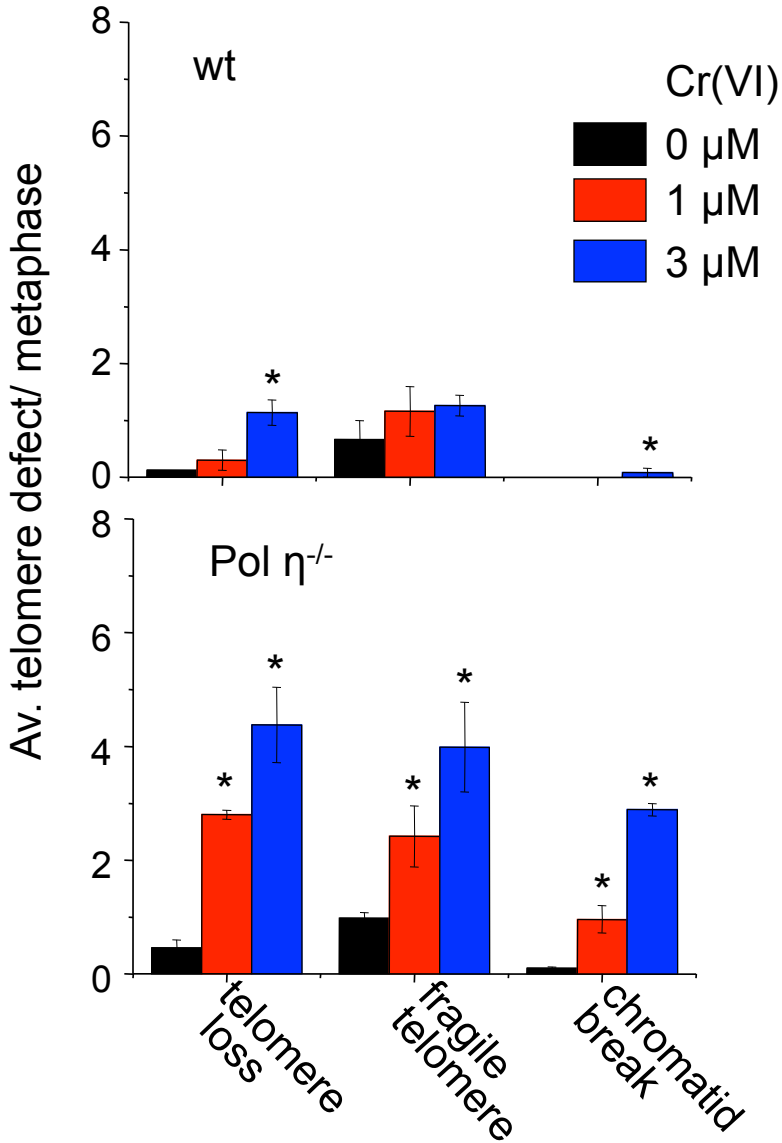


SV40 Transformed GFP-Polη XPV cells (Alan Lehmann)

Pope-Varsalona, Liu, Guzik, Opresko. *NAR*, 2014

Translesion Synthesis is Required for Telomere Preservation After DNA Damage





Pope-Varsalona, Liu, Guzik, Opresko. *NAR*, 2014

Summary and Conclusions

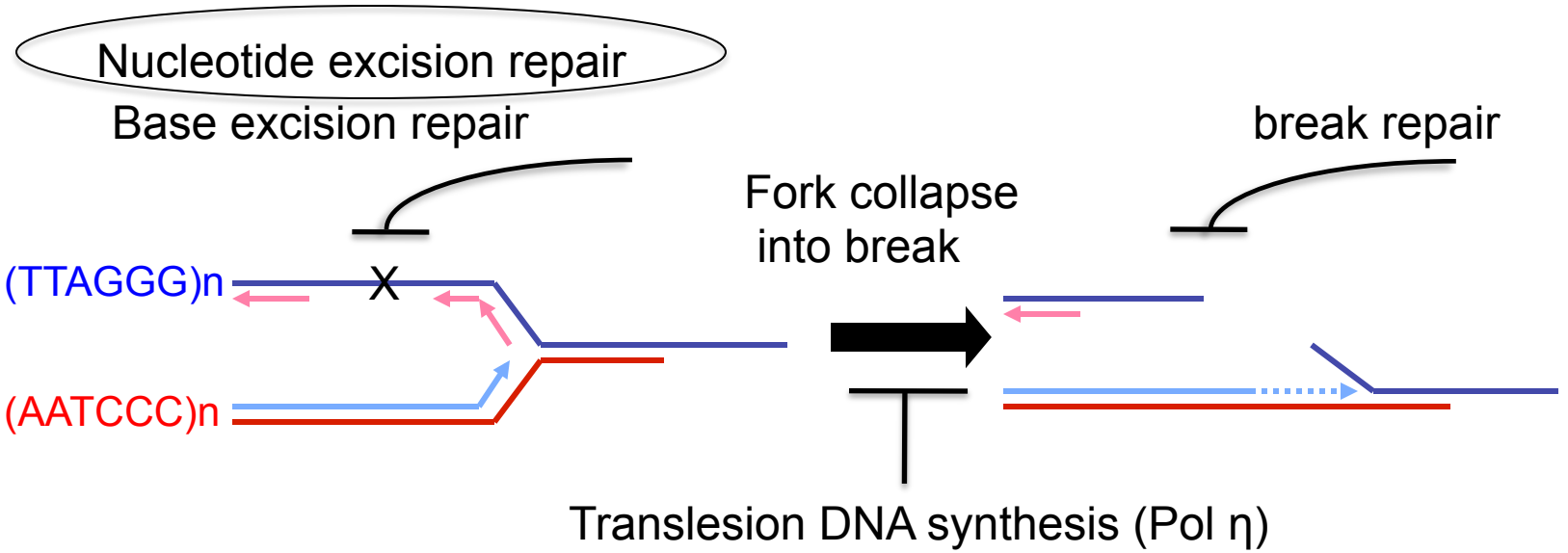
- Uncovered new evidence that Pol η protects against Cr(VI)-induced cytotoxicity and replication stress.

- The induction of bulky DNA lesions generates telomere defects consistent with failures in telomere replication – loss and fragility
  - UVC-induced DNA photoproducts
  - Cr(VI)-induced DNA adducts

- Pol η deficient cells show significantly higher levels of UVC- and Cr(VI)-induced telomere defects.
  - Translesion DNA synthesis required for replication of damaged telomeres

Telomeres may be highly dependent on damage bypass mechanisms due to deficiencies in break repair and possible lack of damage removal.

DNA lesions can cause telomere aberrations
Are they repaired at telomeres?

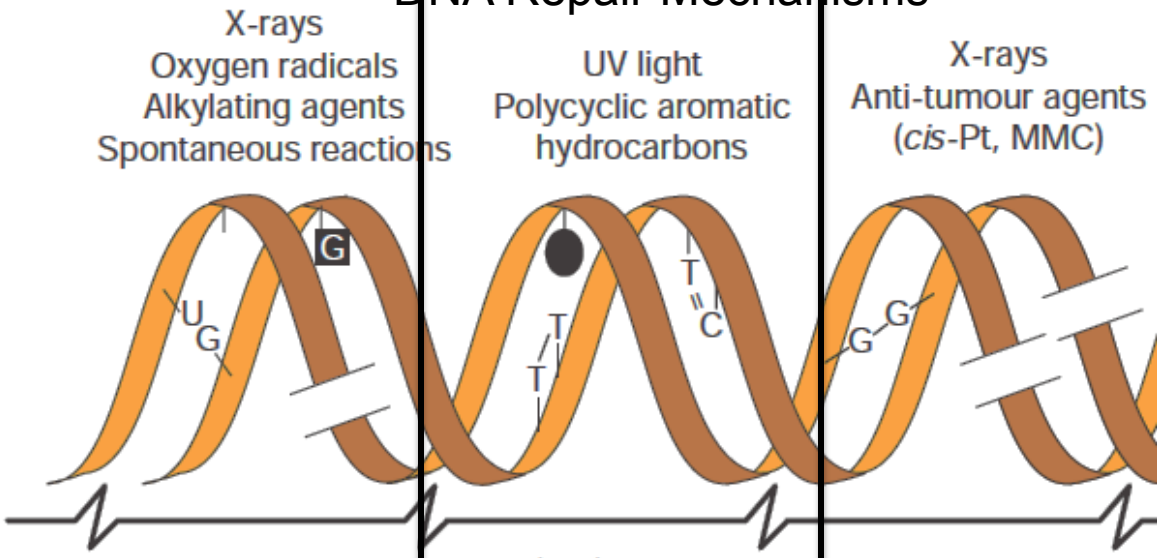


X = UV photoproducts
Cr-DNA adducts

Dhvani Parikh

How Do Telomeres Manage DNA Damage?

DNA Repair Mechanisms



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Hoeijmakers, 2001 Nature

Base-excision repair (BER)

Nucleotide-excision repair (NER)

Recombinational repair (HR, EJ)

Telomere

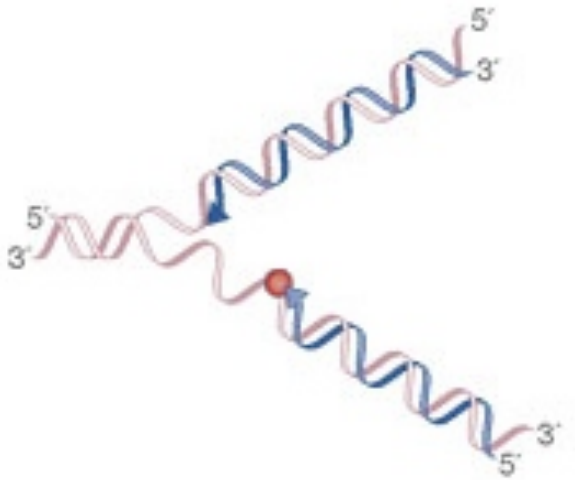
Yes
efficiency?

?

Suppressed

Kruk et al. *PNAS*. 1995
Rochette & Brash, *PLoS Genetics*. 2010

DNA Damage Tolerance

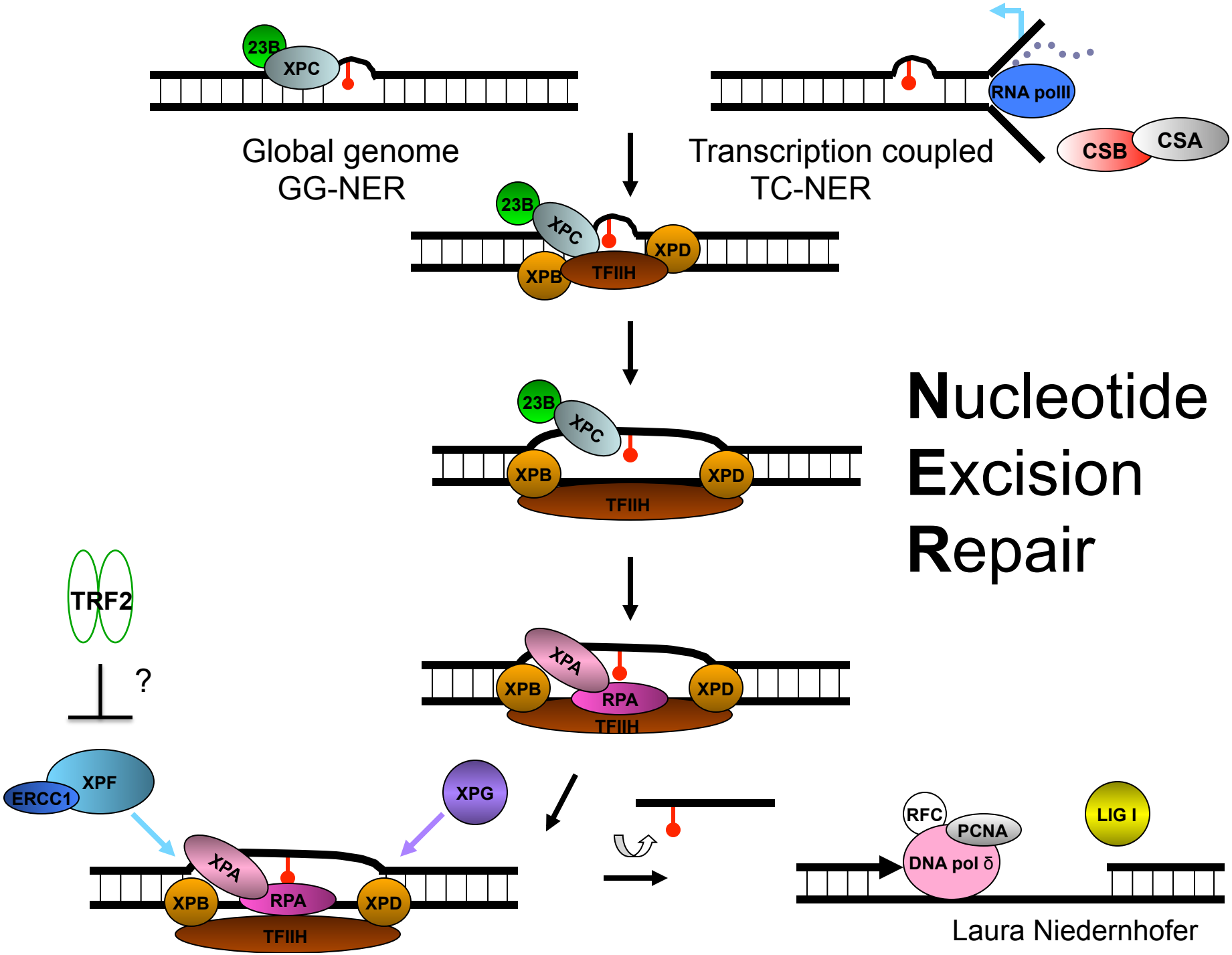


CPD, 6-4PP
bulky adducts
oxidative base damage

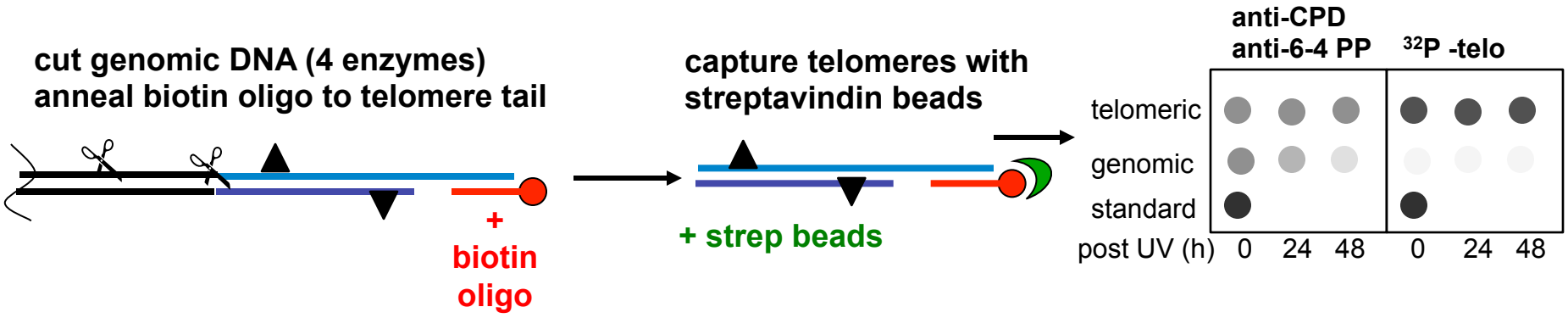
Translesion DNA synthesis
Polymerase η

Yes; required for replication after UV

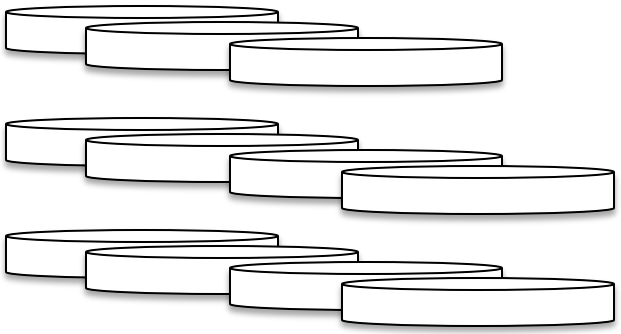
Pope-Varsalona, Liu, Guzik, Opresko, *NAR*. 2014


23B
XPC
Global genome
GG-NER
RNA polII
CSB
CSA
Transcription coupled
TC-NER
XPD
XPB
TFIIH
Nucleotide
Excision
Repair
TRF2
?
XPA
RPA
XPF
ERCC1
XPG
RFC
PCNA
DNA pol δ
LIG I
Laura Niedernhofer

Telomere Capture Assay



▲ = pyridimine dimer



11x 100 mm dishes

Telomere < 0.025% of the genome !

20×10^6 cells

↓

100 µg genomic DNA

↓

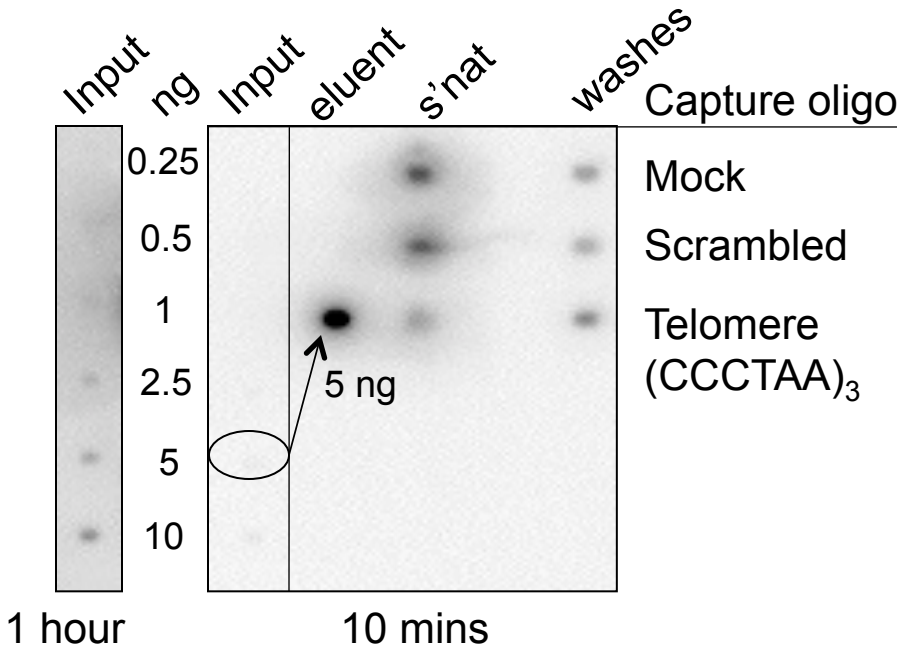
25 ng telomeric DNA

Dhvani Parikh

Wright et al. *Genes & Dev*. 1997

Efficiency of Telomere Isolation

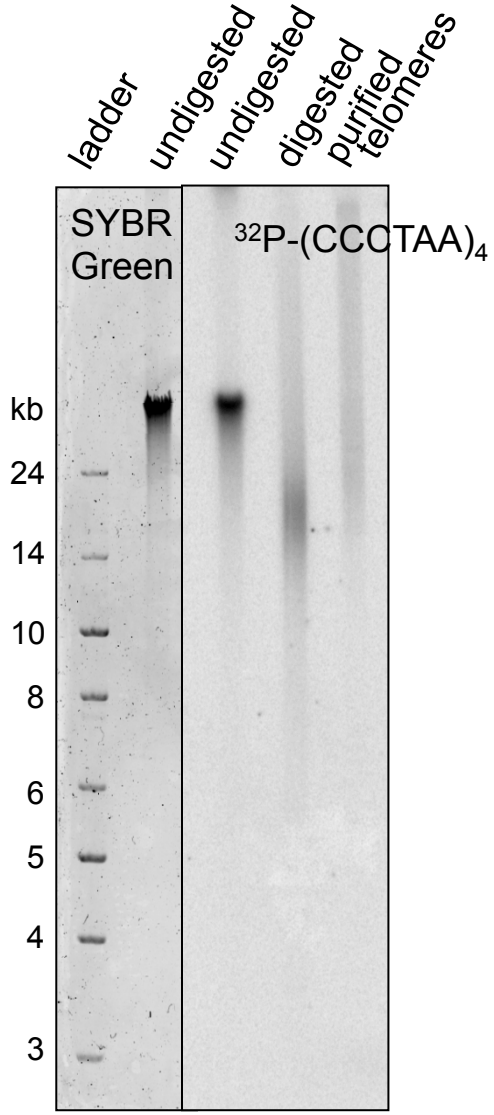
Telomere Blot - ^{32}P-(CCCTAA)$_4$



- Eluent yields: Mock = 0 ng, Scrambled = 2.6 ng, and Telomere = 10 ng

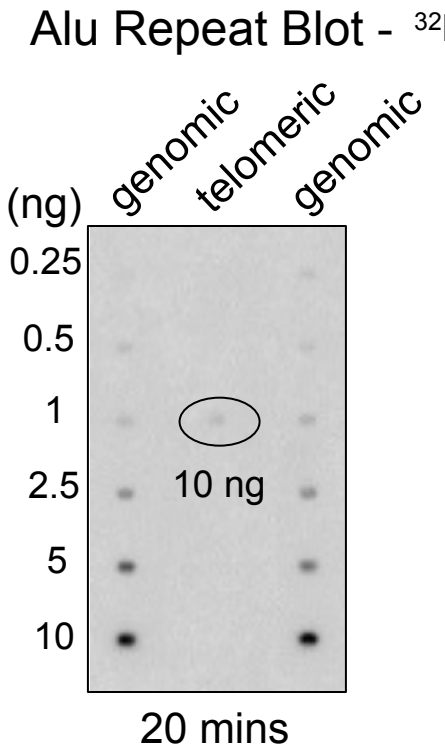
- Estimated telomere recovery:
  bound/ bound + unbound = 33% ± 0.6%

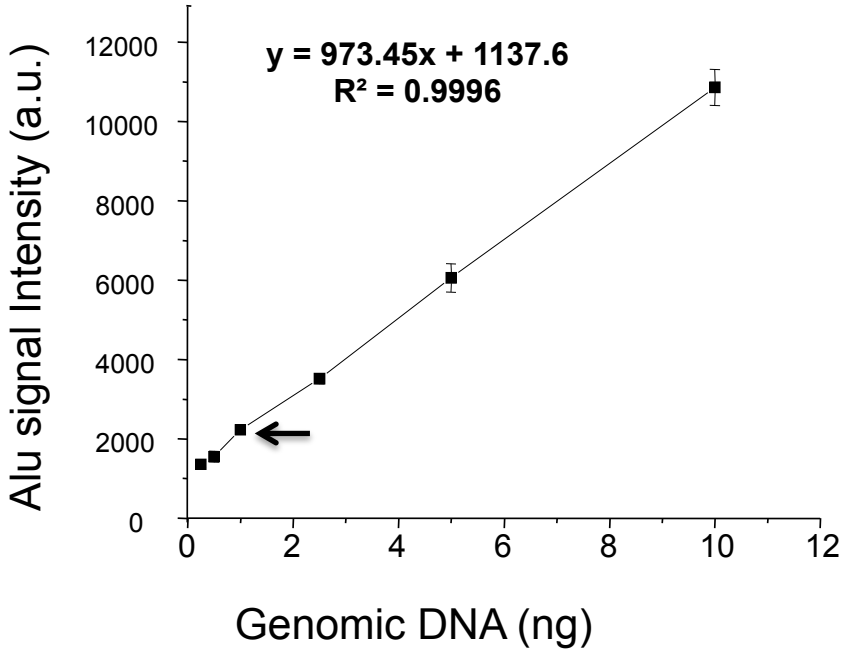
Telomere Southern Blot



Connor Murphy

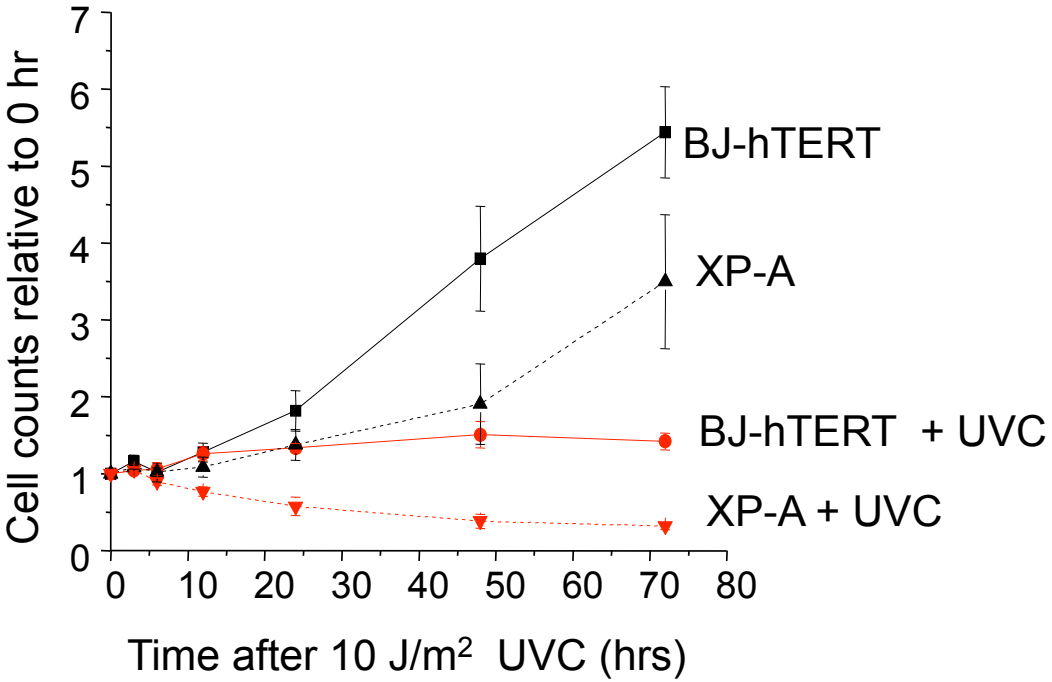
Purity of Isolated Telomeres

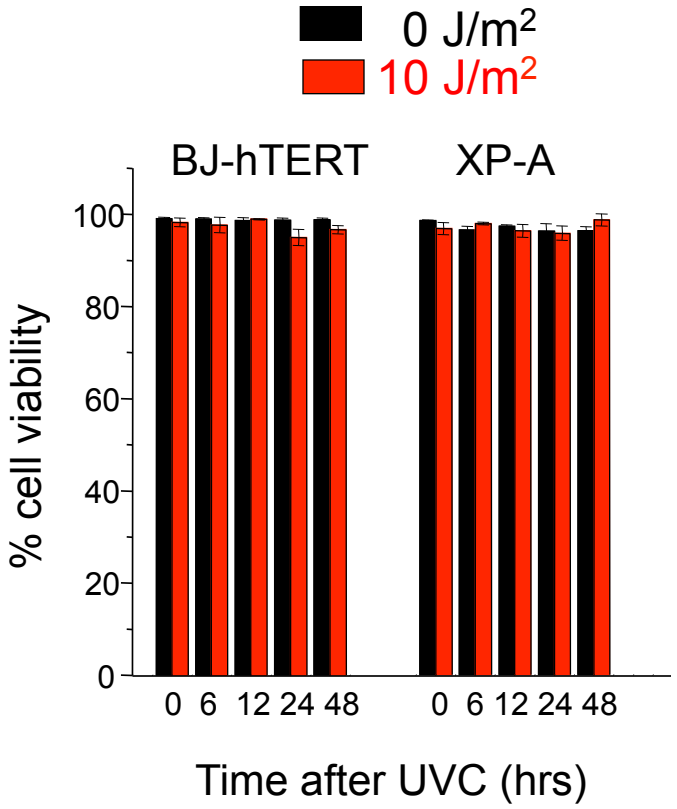




Estimated purity: 1.2 ± 0.2 ng non-telomeric DNA/ 10 ng eluent ➔ ~90% pure

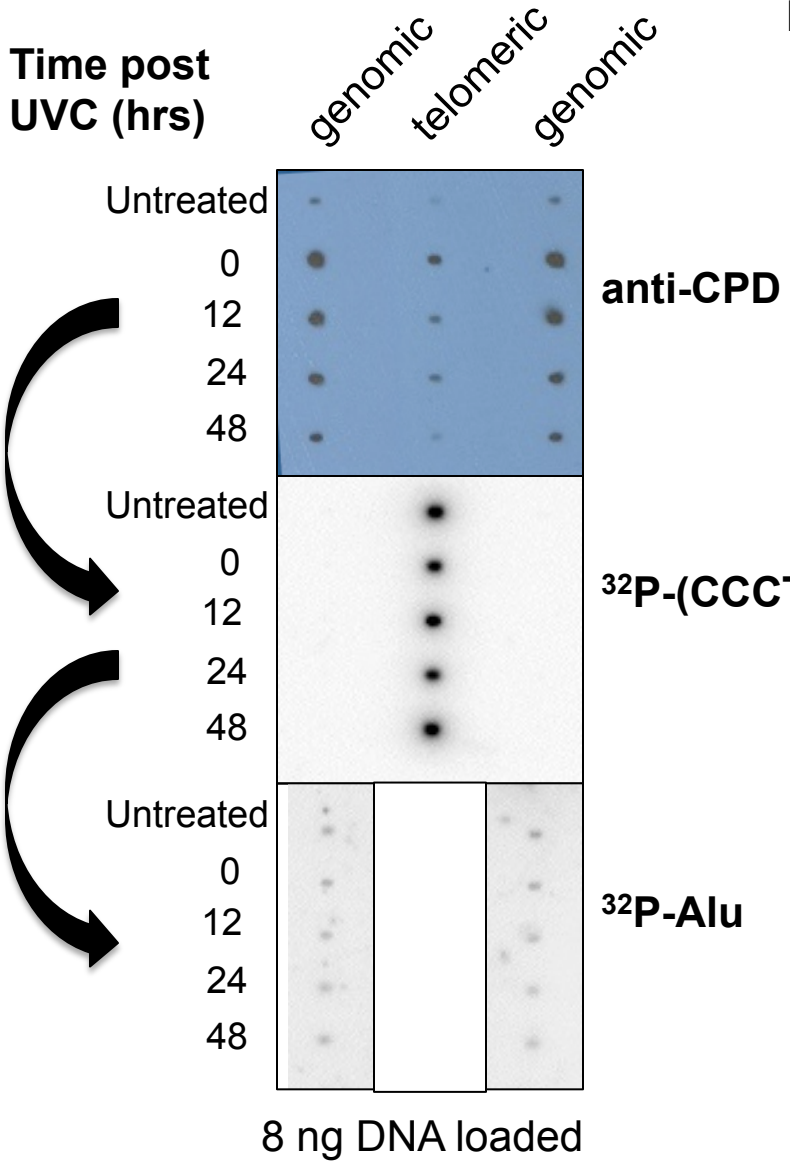
UVC Exposure Delays Cell Proliferation





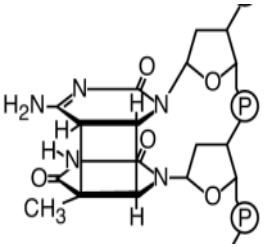
- Following UVC exposure cells do not double even after 72 hrs
- We do not expect photoproduct dilution by cell division
- The majority of cells are viable (trypan blue assay)

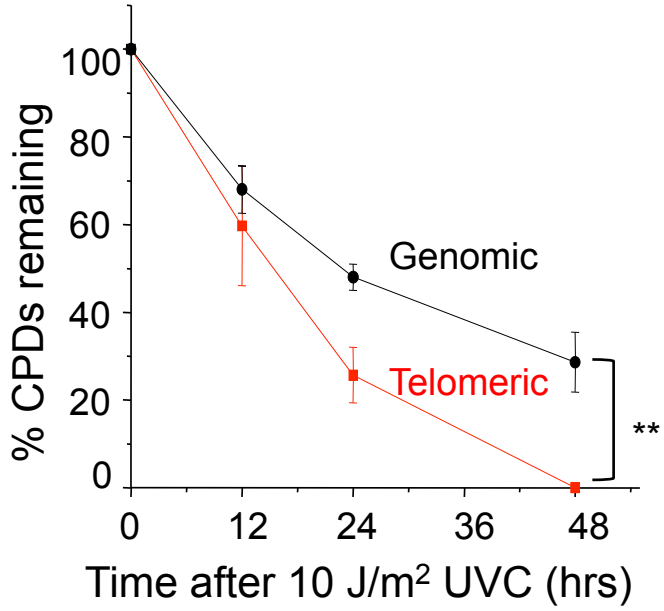
CPD Repair in Genomic and Telomeric DNA



BJ-hTERT cells

5'**TTAGGG**
3'**AATCCC**





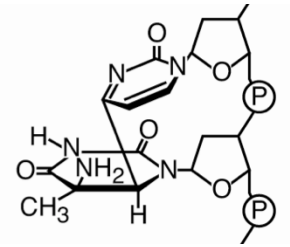
- CPD signal ~2.5-fold lower in purified telomeres

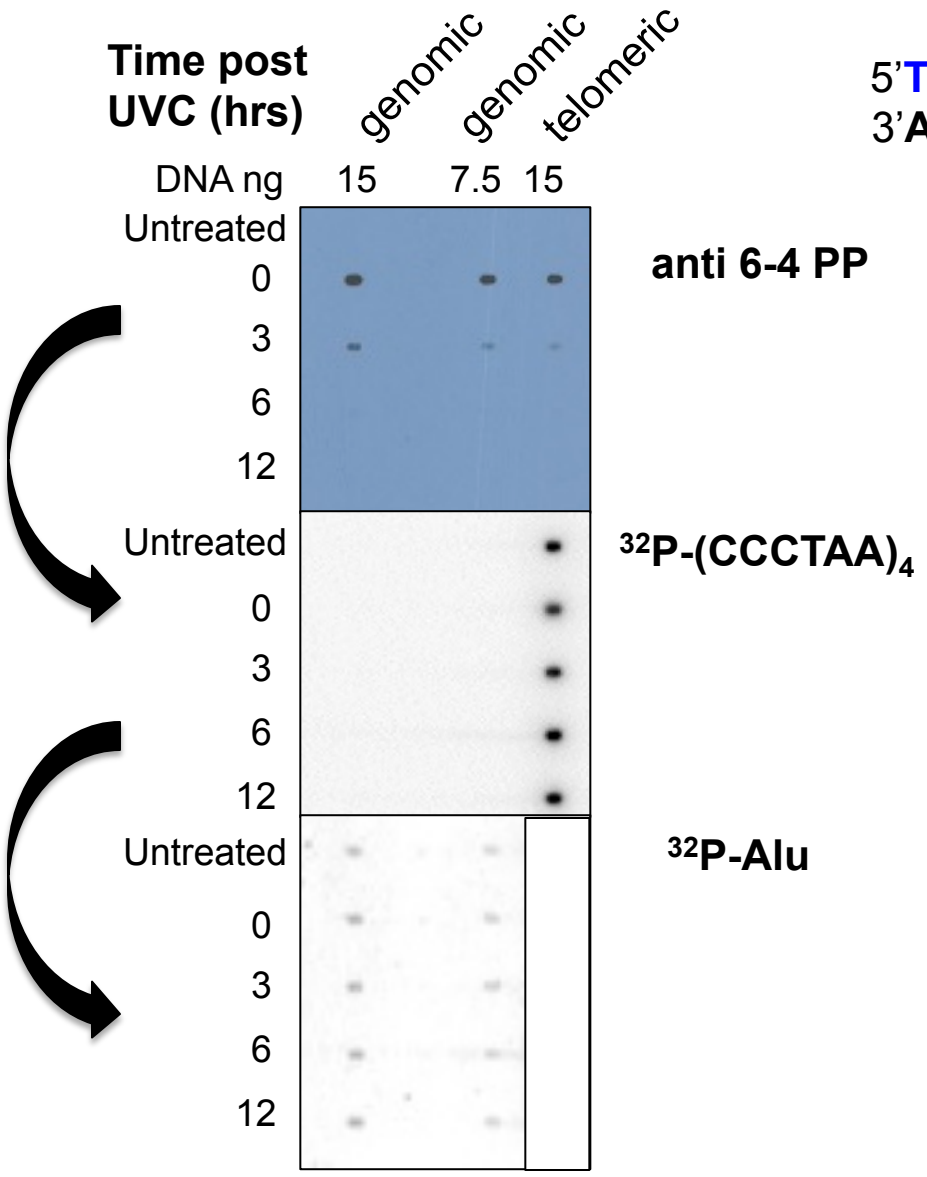
- CPD removal is 1.5 fold faster in telomeres compared to the bulk genome

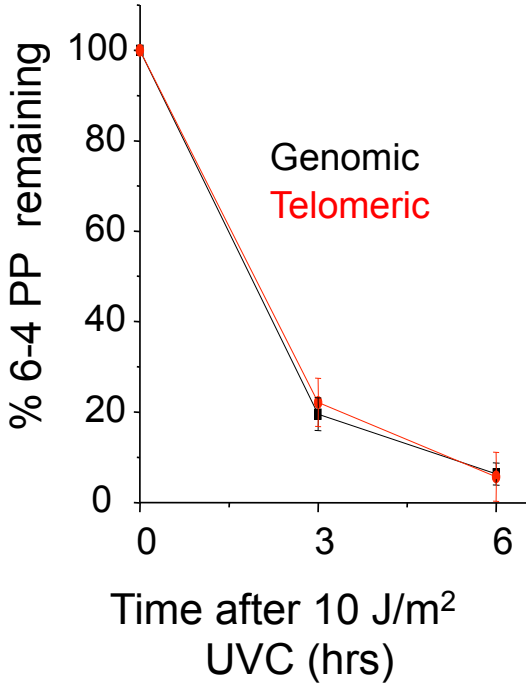
6-4 PP Repair in Genomic and Telomeric DNA

BJ-hTERT cells

5'**TTAGGG**
3'**AATCCC**

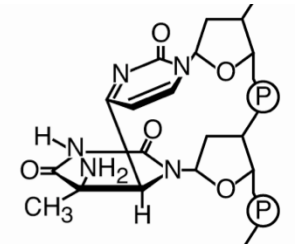






- is telomerase required?

Telomerase is Not Required for Telomere Repair

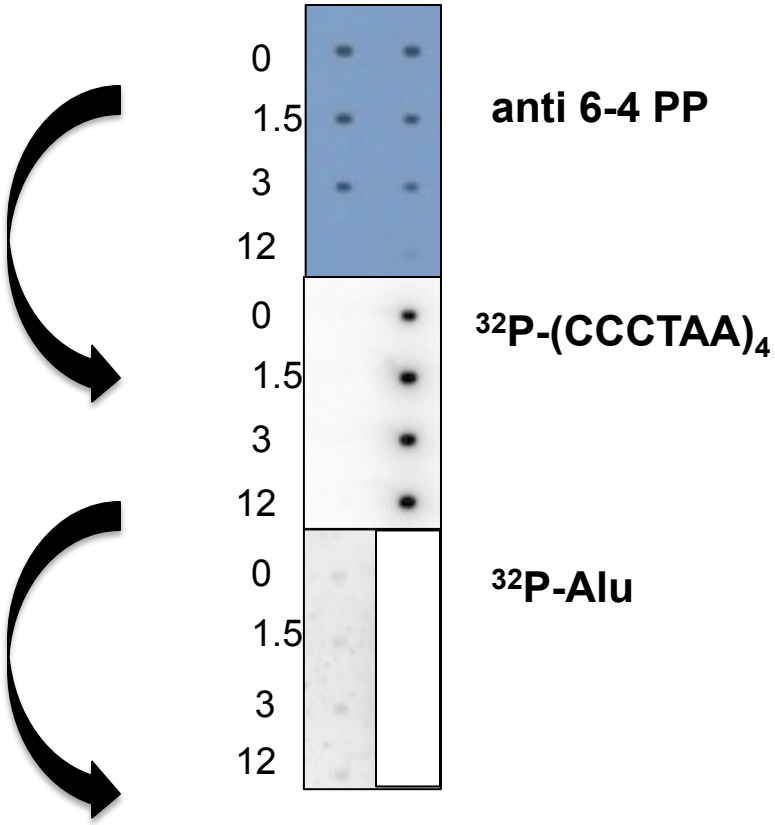


Time post UVC (hrs)

genomic telomeric

DNA ng 7.5 15

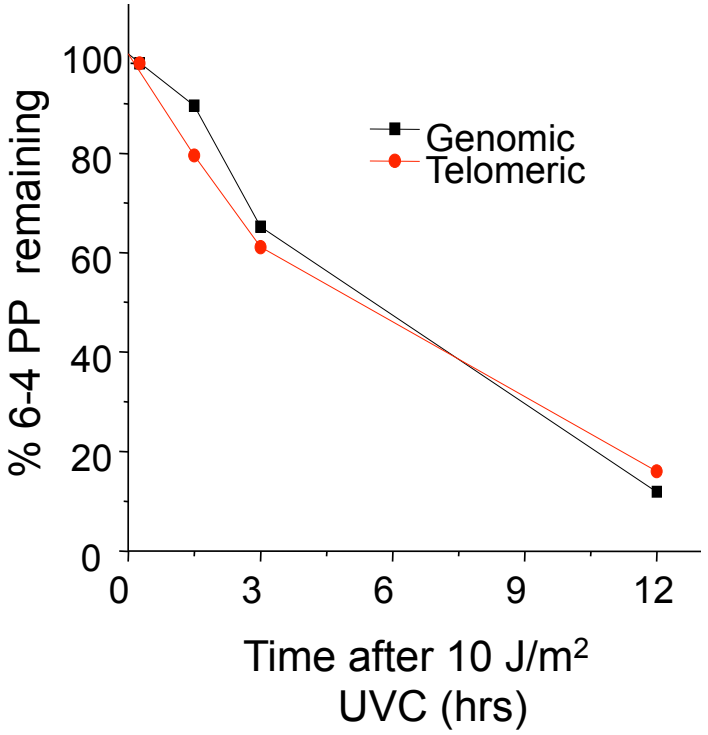
U2OS cells – telomerase negative



anti 6-4 PP

^{32}P-(CCCTAA)$_4$

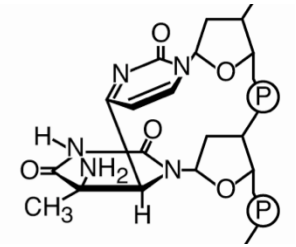
^{32}P-Alu

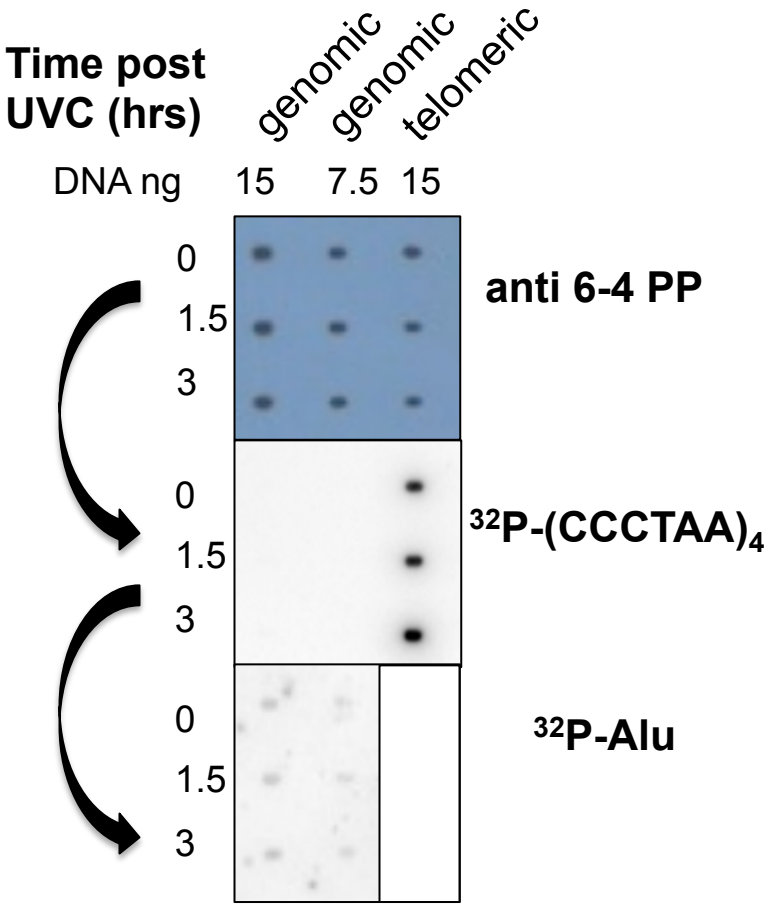


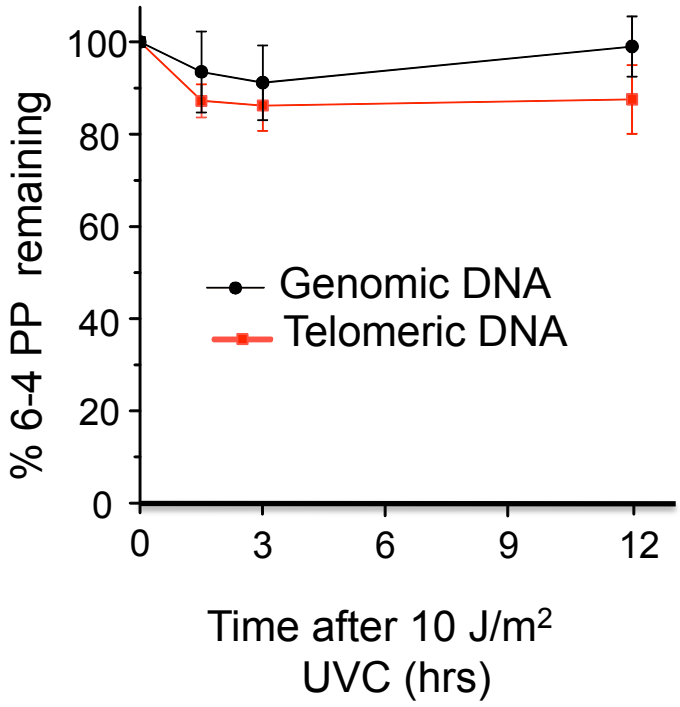
• are photoproducts removed by NER?

NER is Required for 6-4 PP Removal at Telomeres

XP-A skin fibroblasts – NER deficient



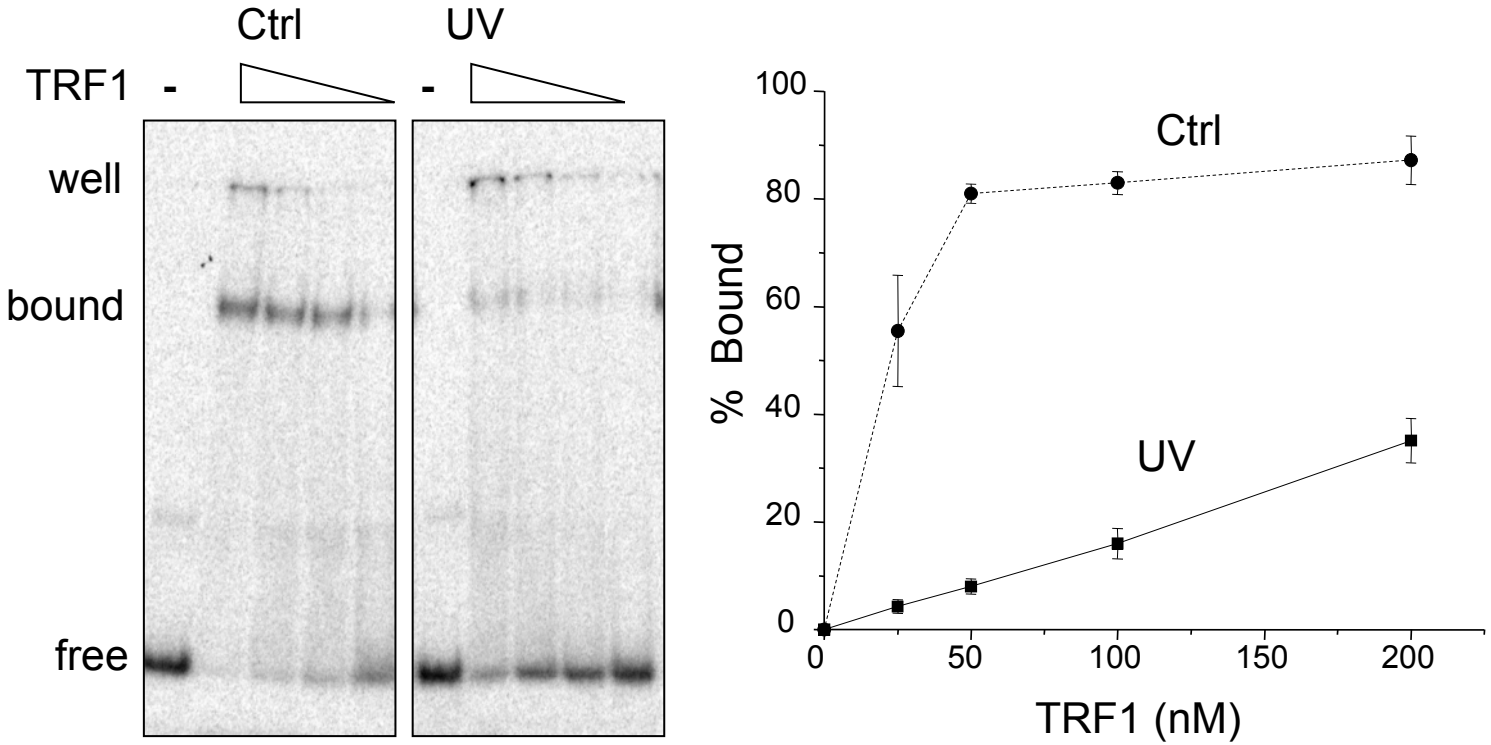




UV Photoproduct Inhibits TRF1 Binding

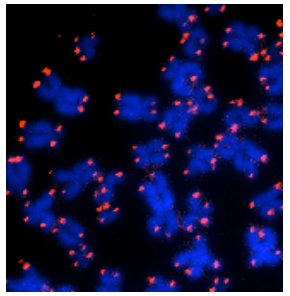
UV substrate

TTAGGGT T AGGGTTA
AATCCCA A TCCCAAT



CPD causes a 13-fold inhibition in TRF1 binding

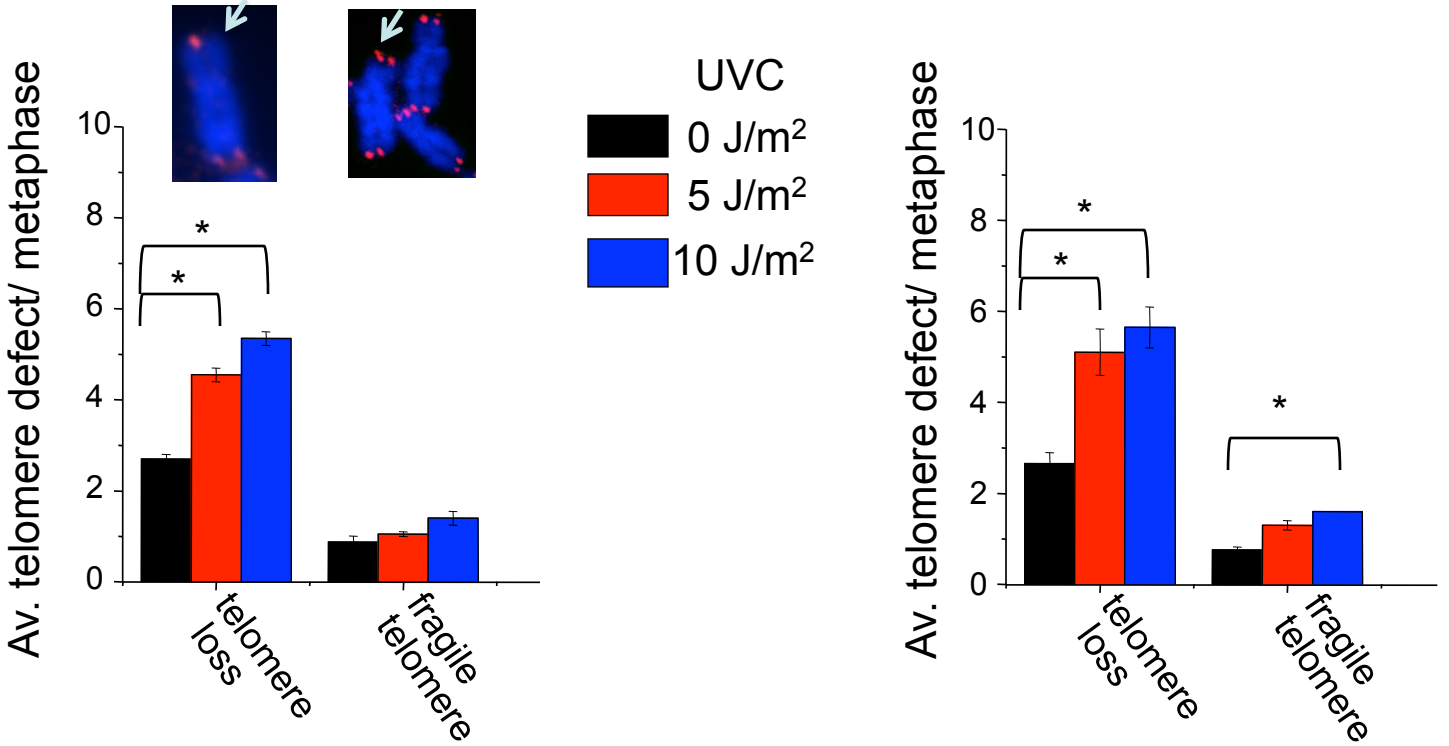
Role for Nucleotide Excision Repair At Telomeres After Damage



Normal fibroblasts
(XPA complemented)

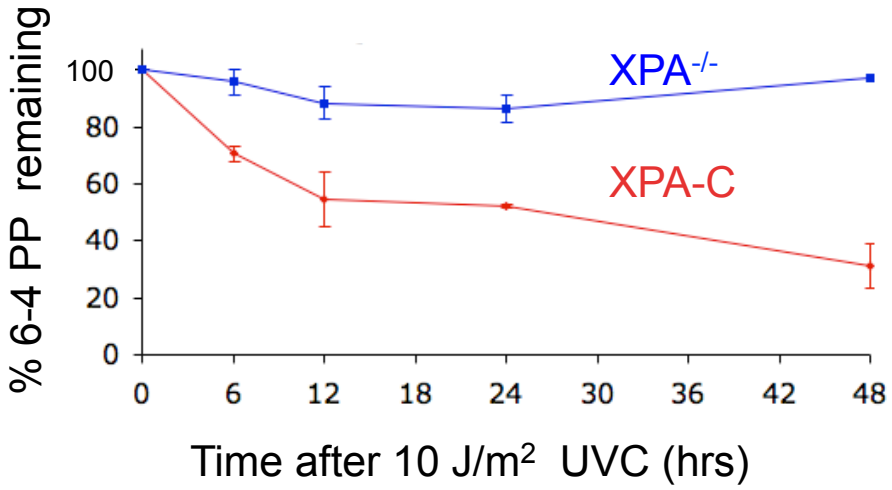
XP20S-derived cells
SV40 transformed

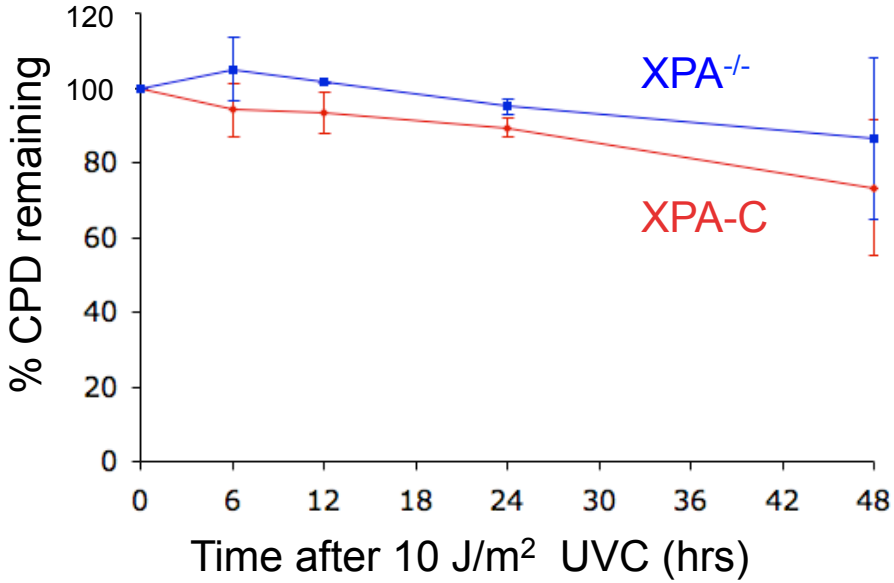
XP-A fibroblasts
($XPA^{-/-}$)

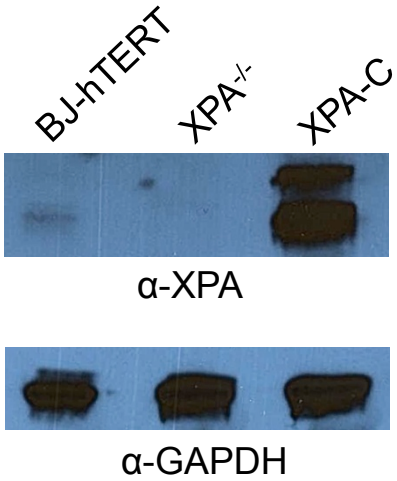


- UVC irradiation induces a near 2-fold increase in telomere loss and doublets
- Similar results in NER proficient and deficient cells?

XPA Complemented Cells Repair Slowly







Elise Fouquerel

Summary and Future Directions

1. UVC induces CPD and 6-4 PP formation at telomeres
- frequency is about 2-fold less than the bulk genome

2. CPDs are repaired 1.5-fold faster at telomeres compared to the bulk genome. Transcription coupled repair?

3. 6-4 PPs persist at telomeres and the bulk genome in XPA deficient cells
- Indicates that NER is active at telomeres

4. 6-4 PPs removal rates at telomeres are similar to the bulk genome in telomerase positive (BJ-hTERT) and telomerase negative (U2OS) cells

5. UVC generates telomere defects consistent with failures in telomere replication
- Aberrations are increased in translesion synthesis deficient cells
- NER deficient cells?

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CNAST (CMU)

Postdoctoral positions available