Privileged Sites for DNA Photoproducts: genomic sensors & the control of clonal expansion

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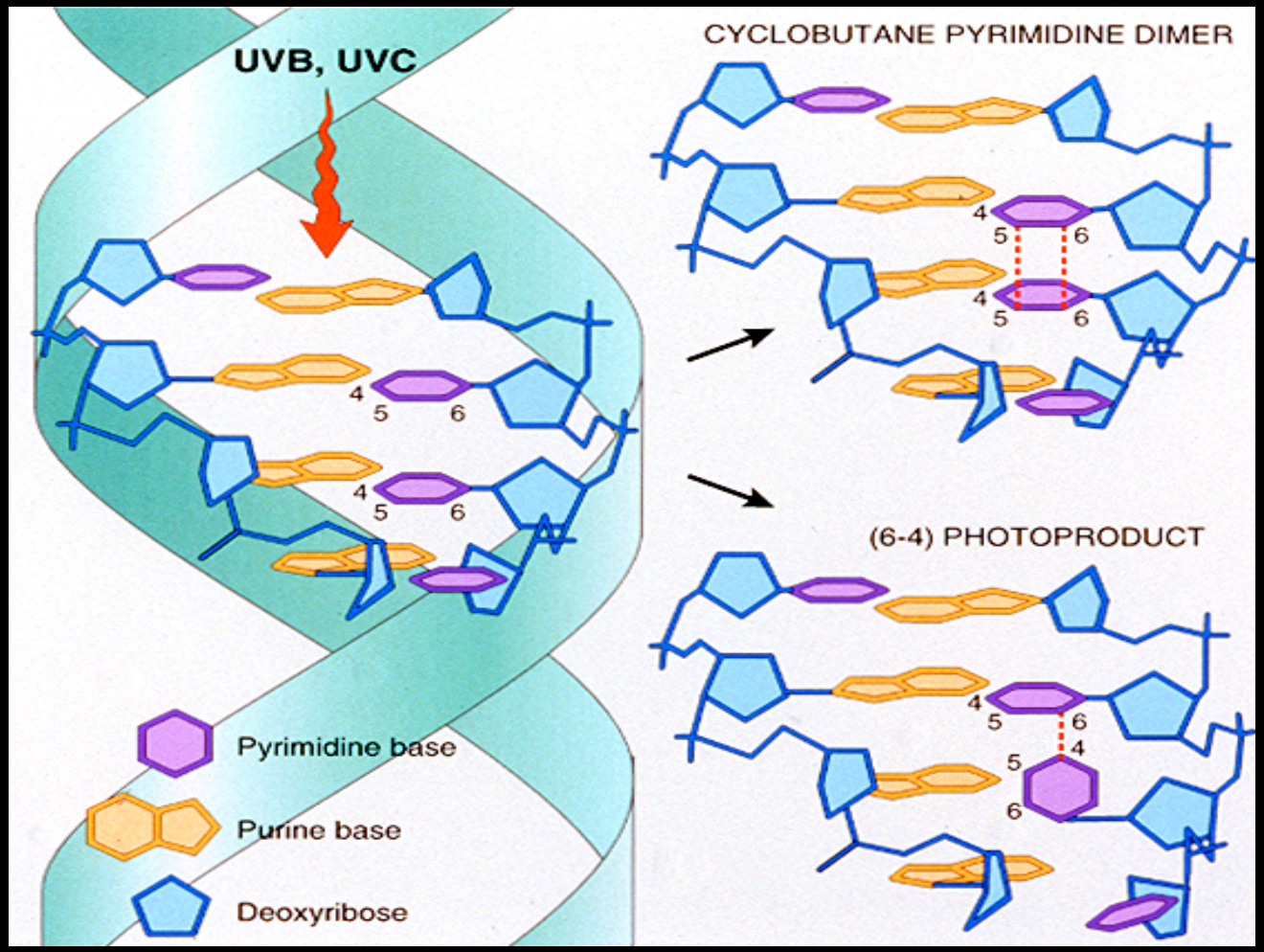
UV photoproducts in DNA



1 hr
Texas noon sun:
10,000 per cell

~ 100 UV mutations per cell

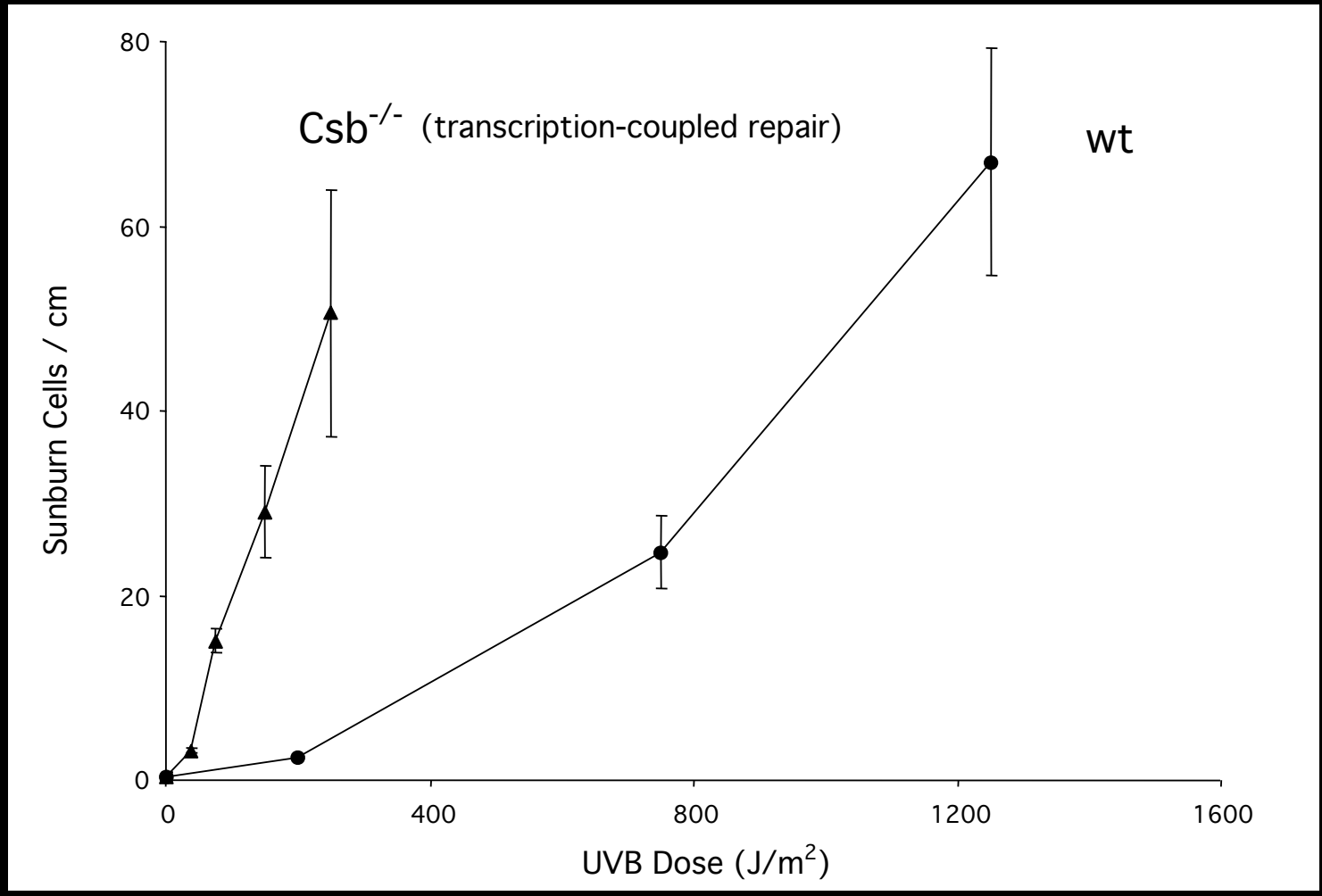
UV signature mutations

C → T at dipyrimidine sites
CC → TT are distinctive for UV

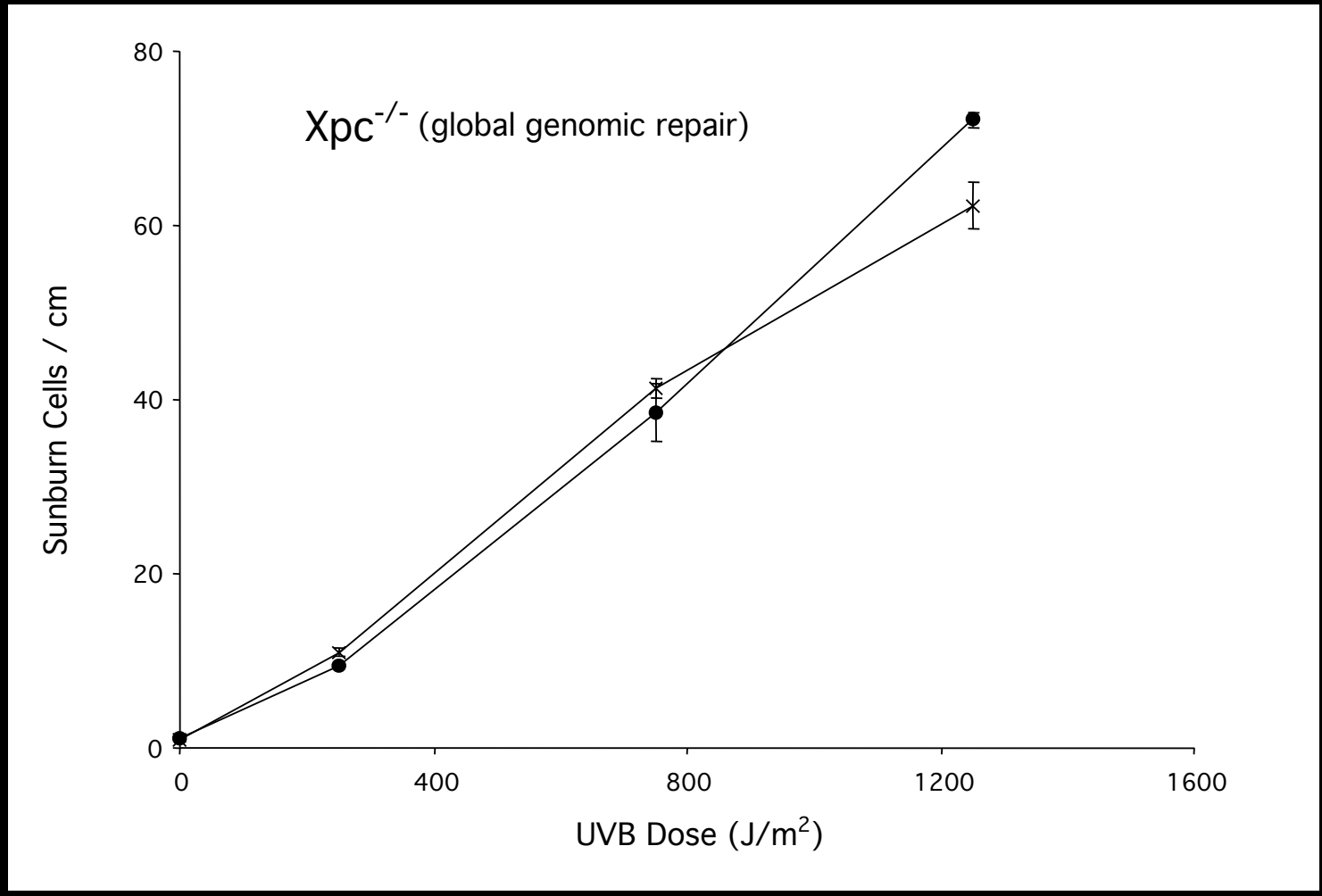
p53
PTCH
PTEN

Special Regions of the Genome

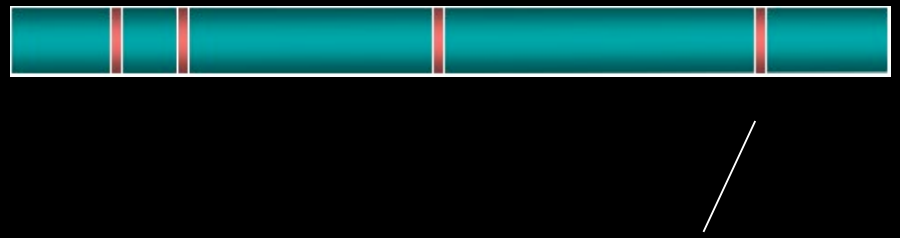
A 2nd role for sunlight: ↓ Mdm-2 → ↑ P53 → apoptosis is triggered by UV photoproducts in active genes



2nd role for sunlight: ↓ Mdm-2 → ↑ P53 → apoptosis is triggered by UV photoproducts in active genes



The apoptosis signal originates from ...



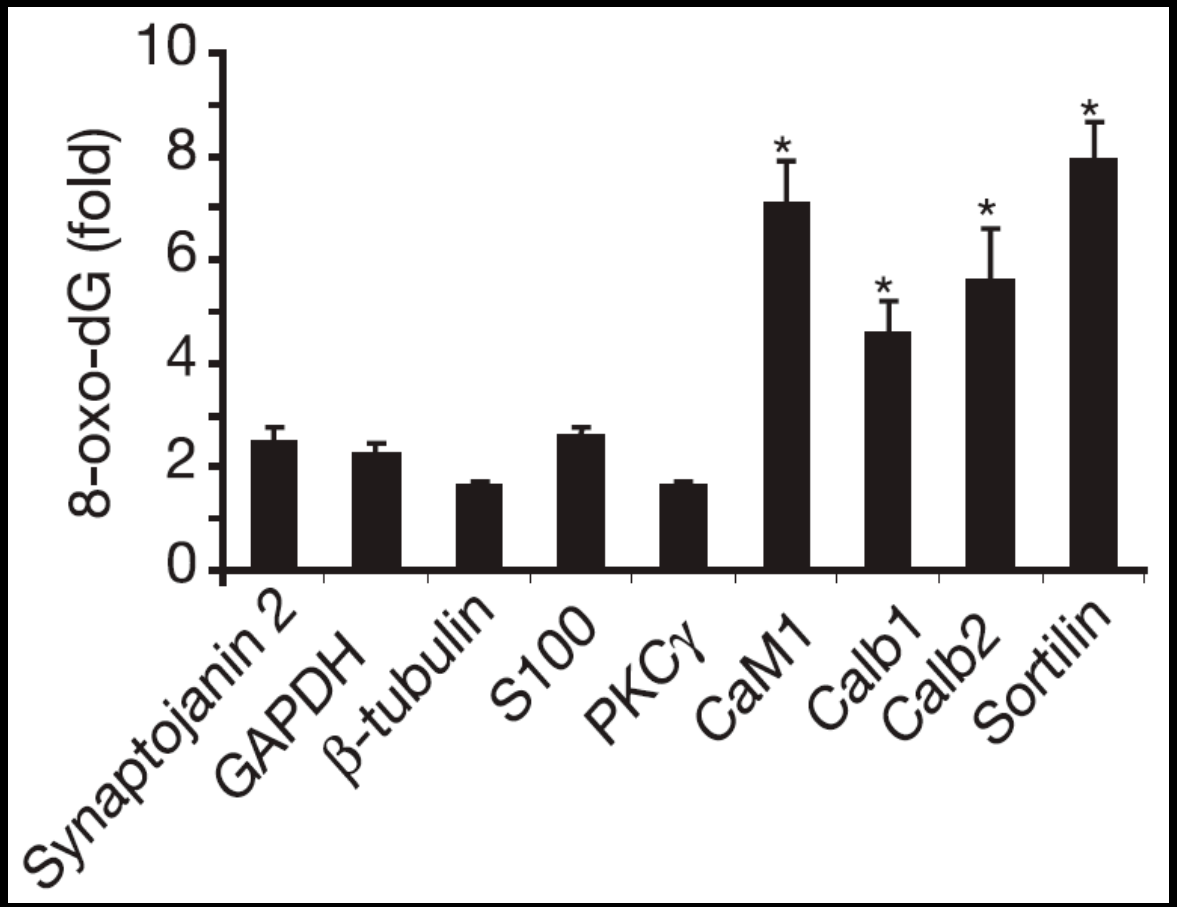
cyclobutane dimers in active genes

Domains for transcription-coupled repair

Mullenders:

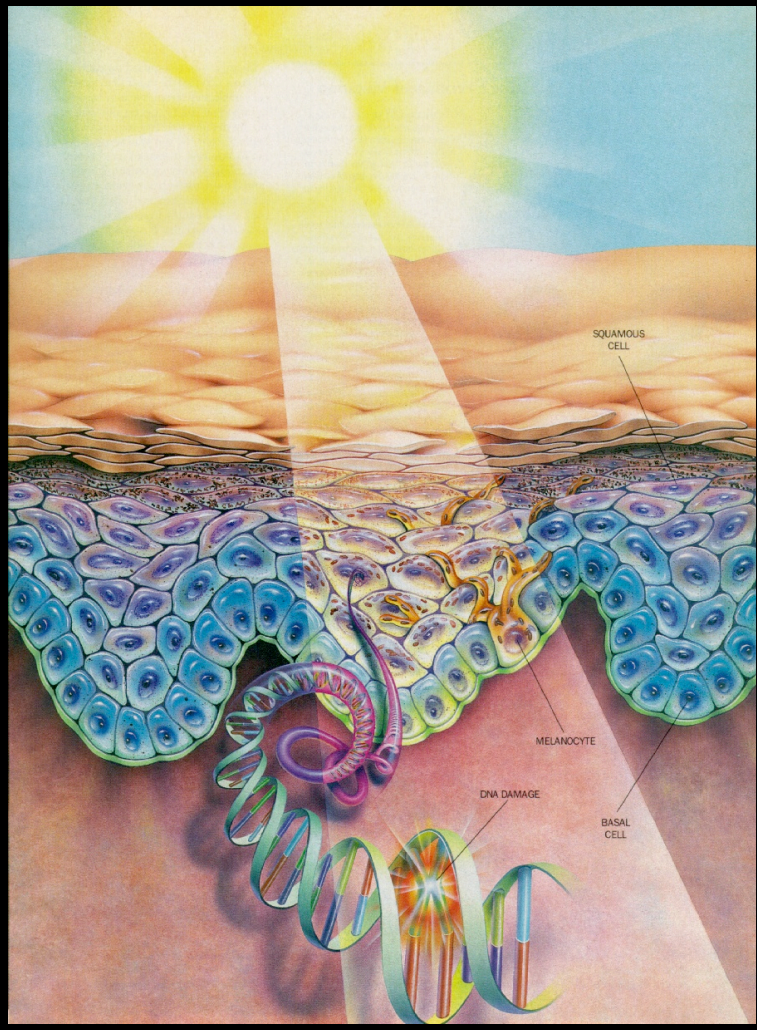
TCR sites cluster at early-replicating gene-rich bands & telomeric regions

Oxidation-sensitive promoters during brain aging



Lu et al, Nature 429:883, 2004

Genome Regions Sensitive to UV

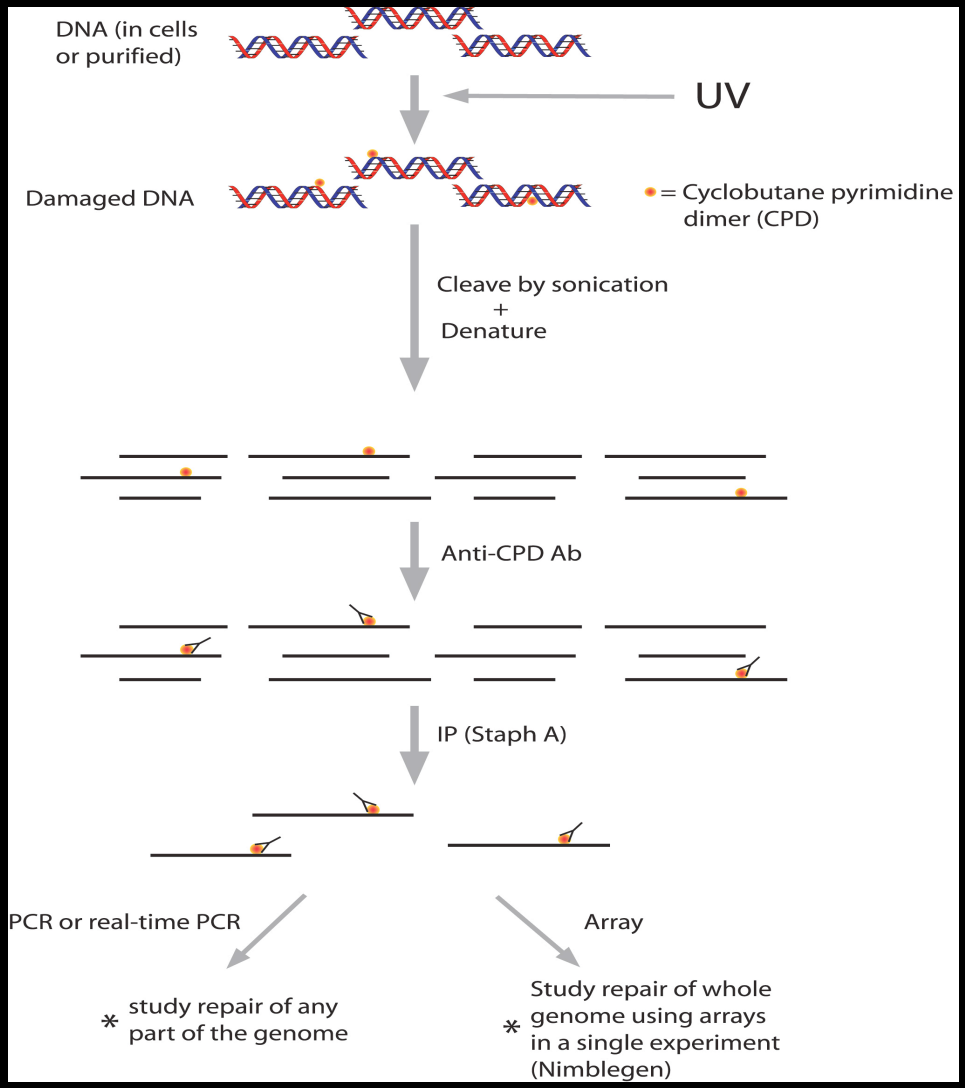


Telomeres as a trigger for UV signaling

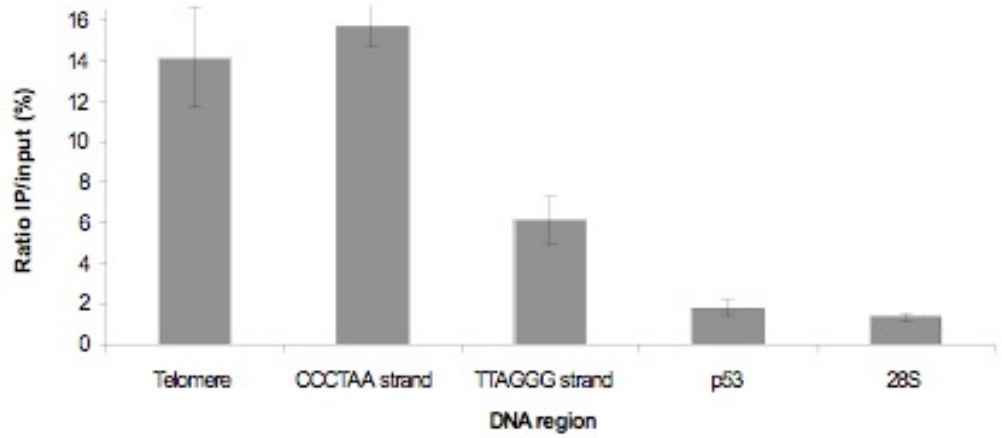
5’ … TTAGGGTTA … 3’
3’ AATCCCAAT 5’

mostly double stranded
5 to 20 kb

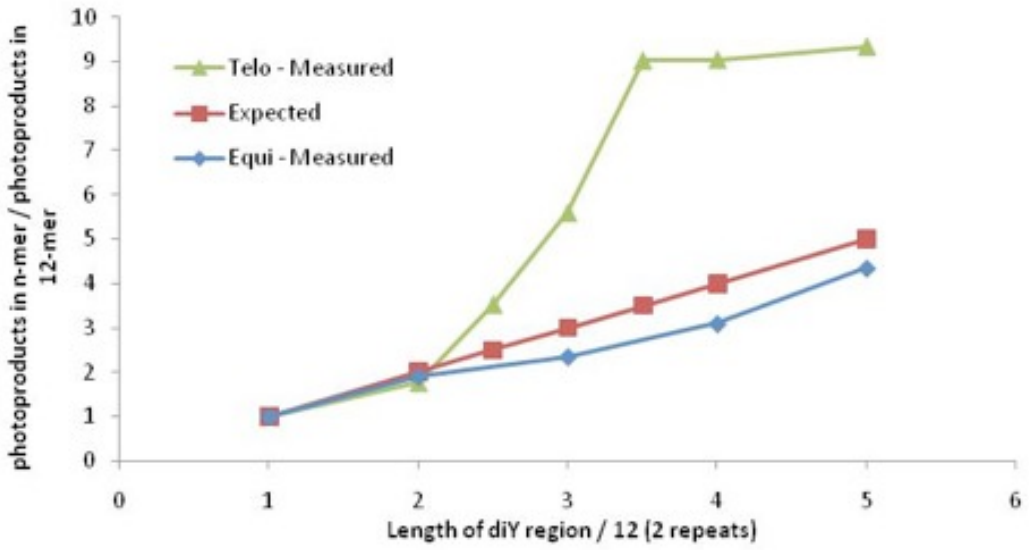
IPoD ~ immunoprecipitation of damage



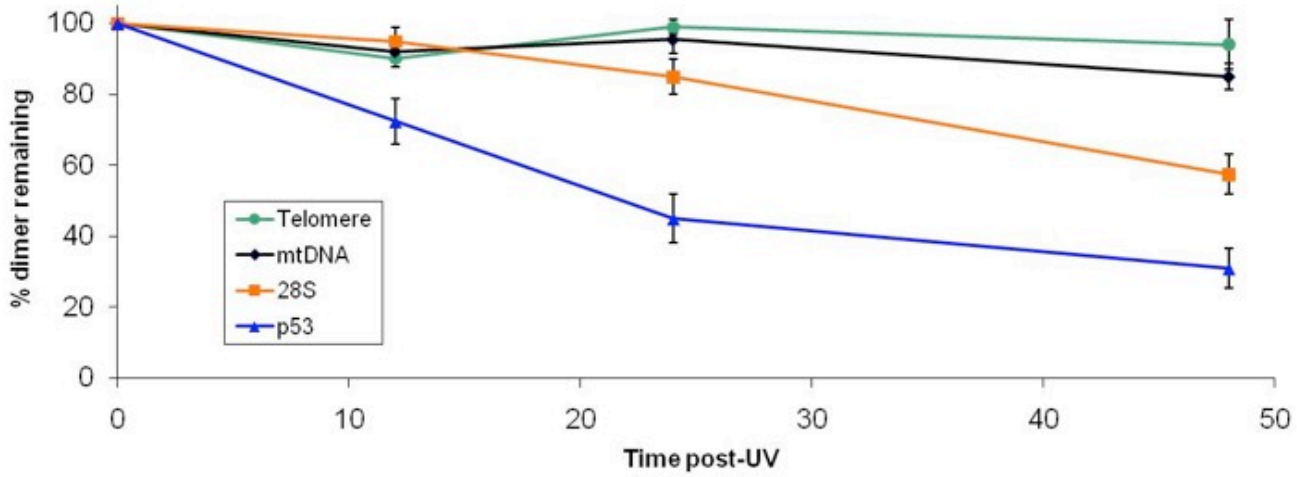
Telomeres are UV-sensitive



Telomere UV-sensitivity in oligos depends on repeat length



Telomeres don’t repair cyclobutane dimers



Why have poor repair in telomeres?

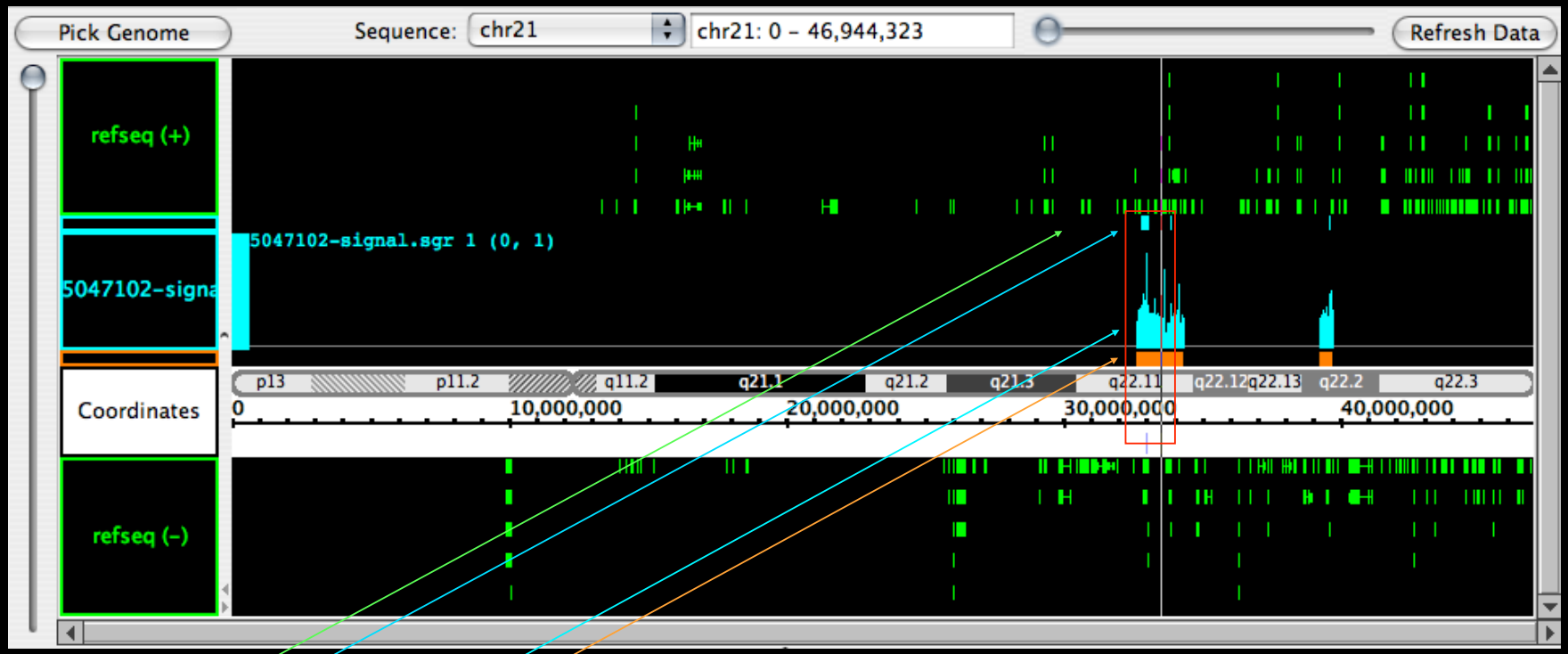
- Avoids soaking up repair proteins at long tracts of unexpressed DNA?
- Avoids overlapping repair nicks?

How can the cell prevent repair, given that repair proteins are there?

- Repair-inhibitory protein for repeated DNA?

The Whole-Genome Frontier

DNA photoproduct signals along Xsome 21



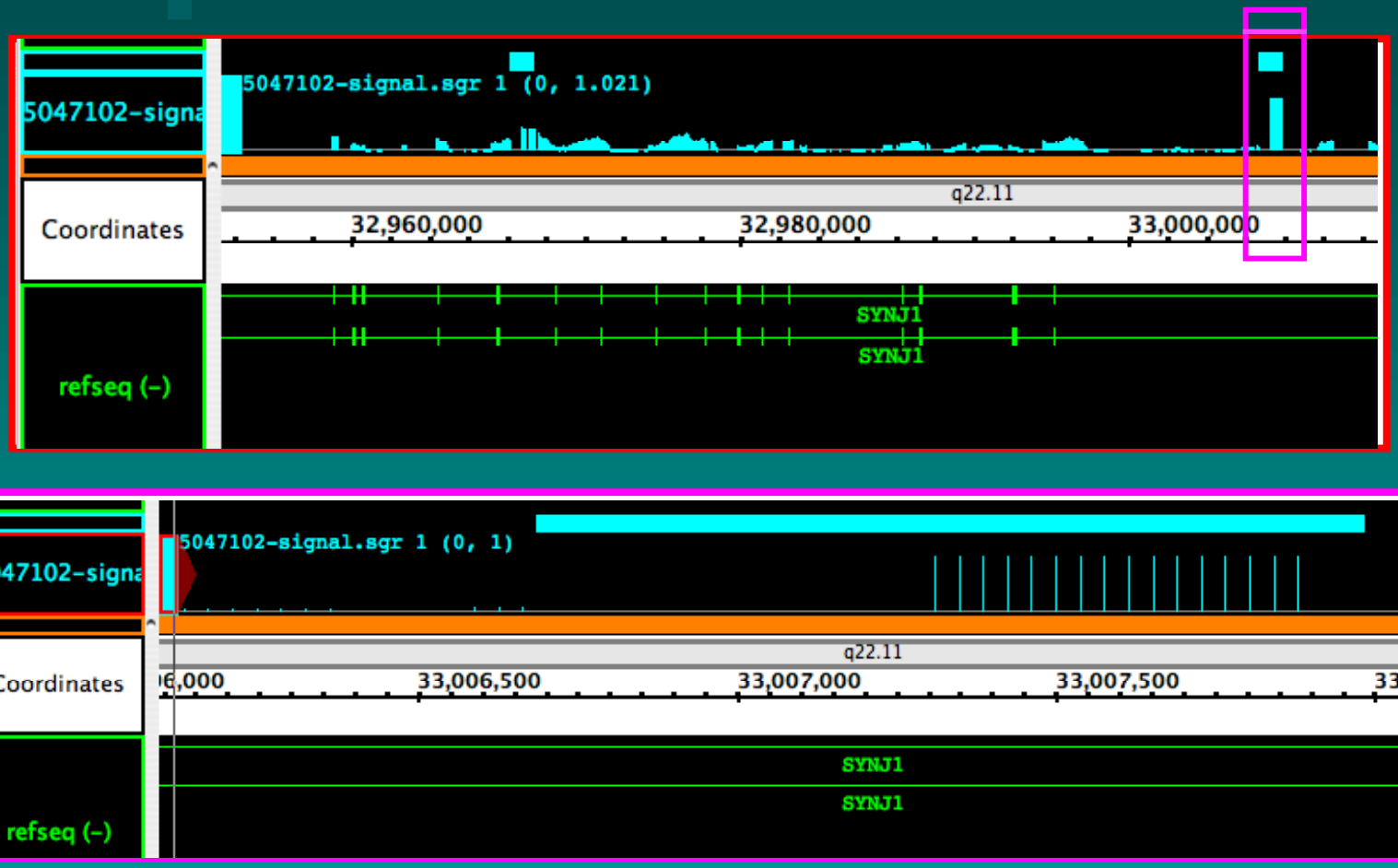
exons

ENCODE regions

CPD signal

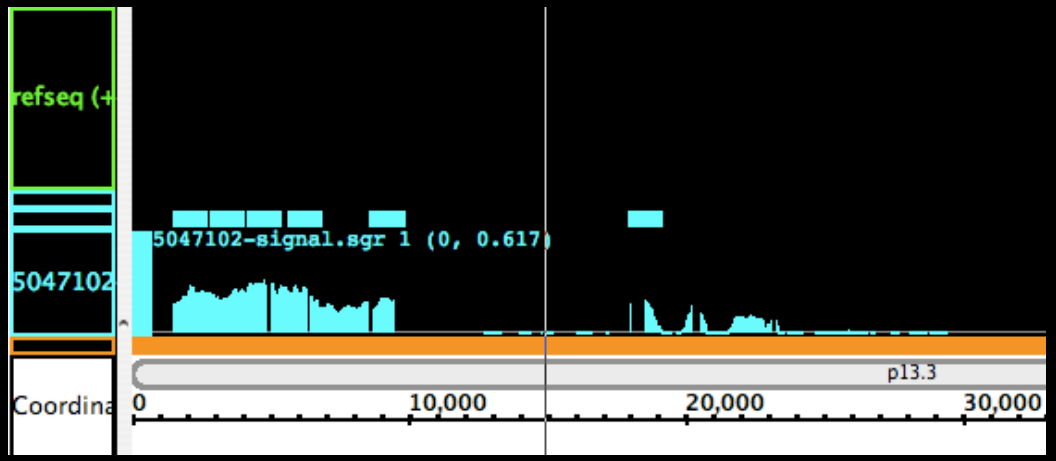
1% false-discovery peaks

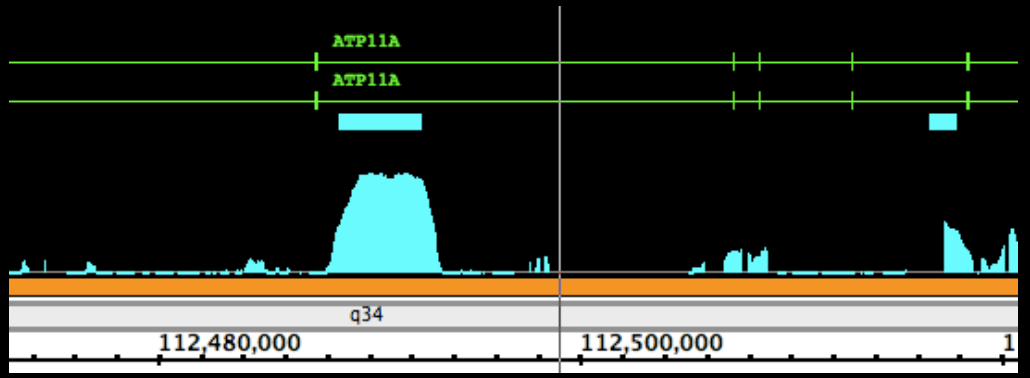
DNA photoproduct hotspots on Xsome 21



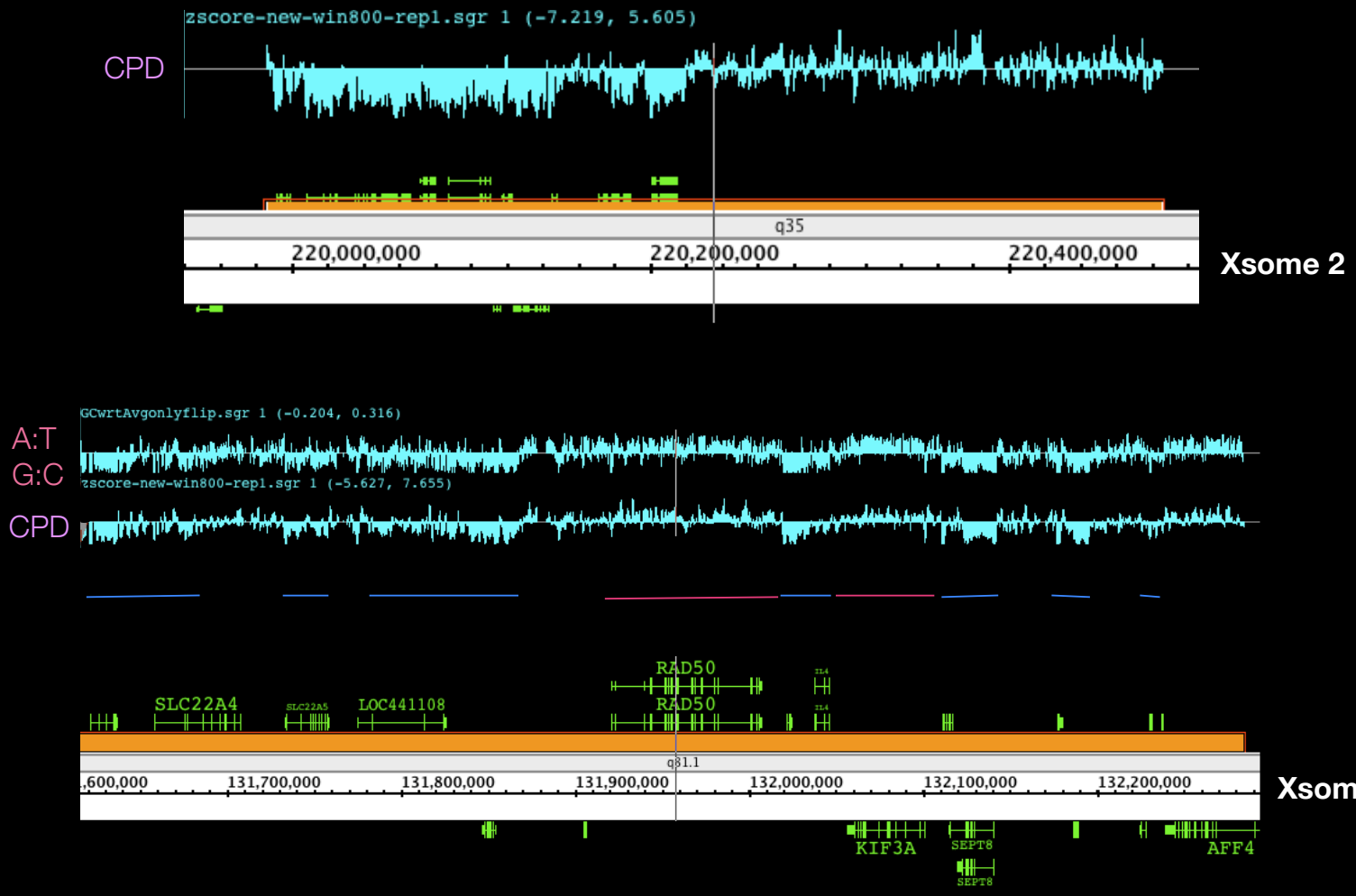
600 nt series of 28 repeats each containing 5' TTTCCC 3'

DNA photoproduct hotspot regions on Xsome 13

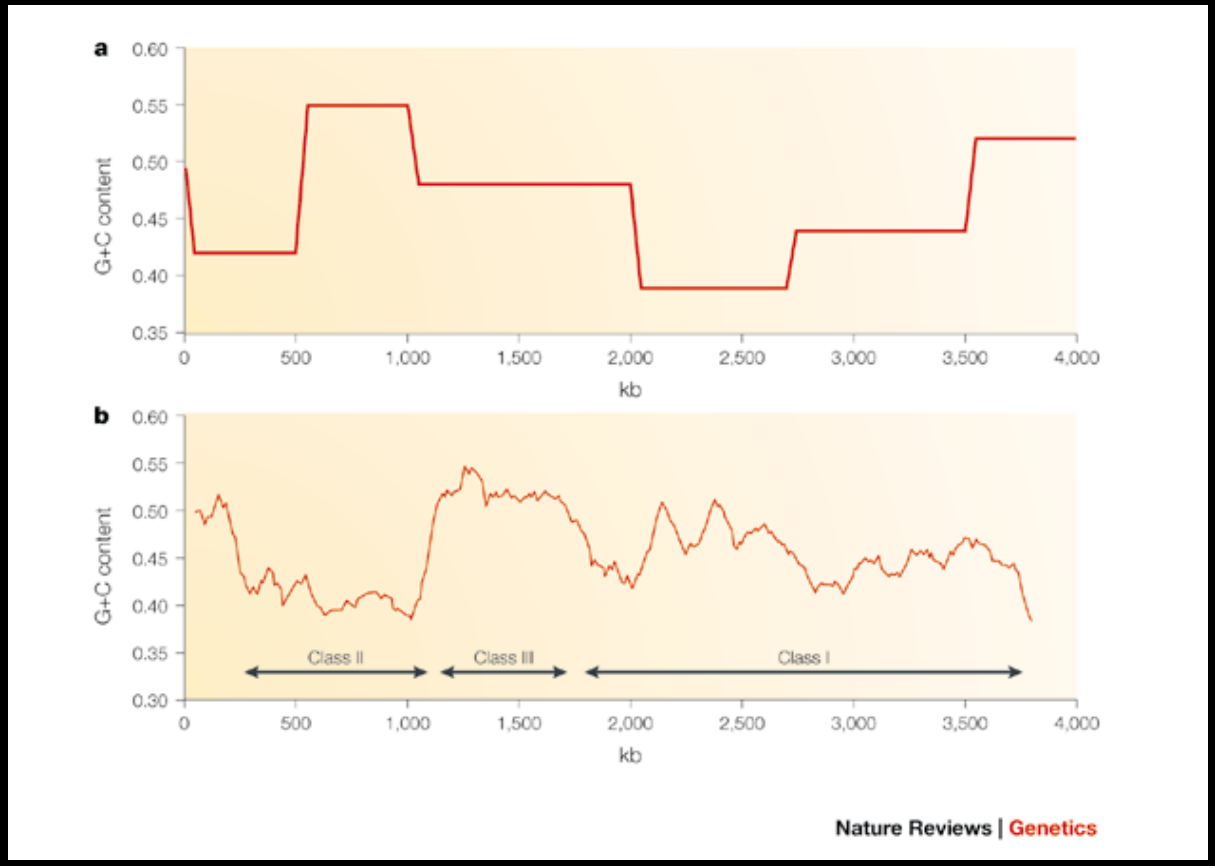




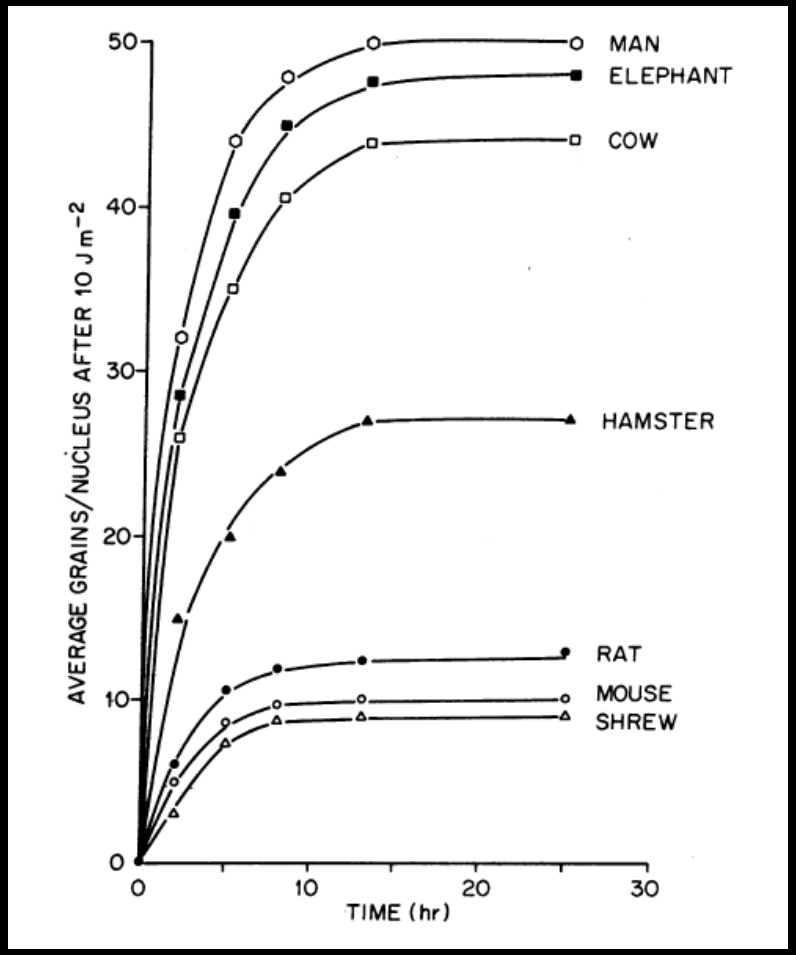
Damage frequency & G:C often follow gene boundaries



DNA isochores



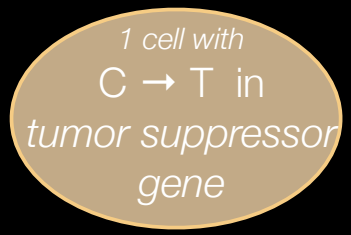
Excision Repair & Lifespan



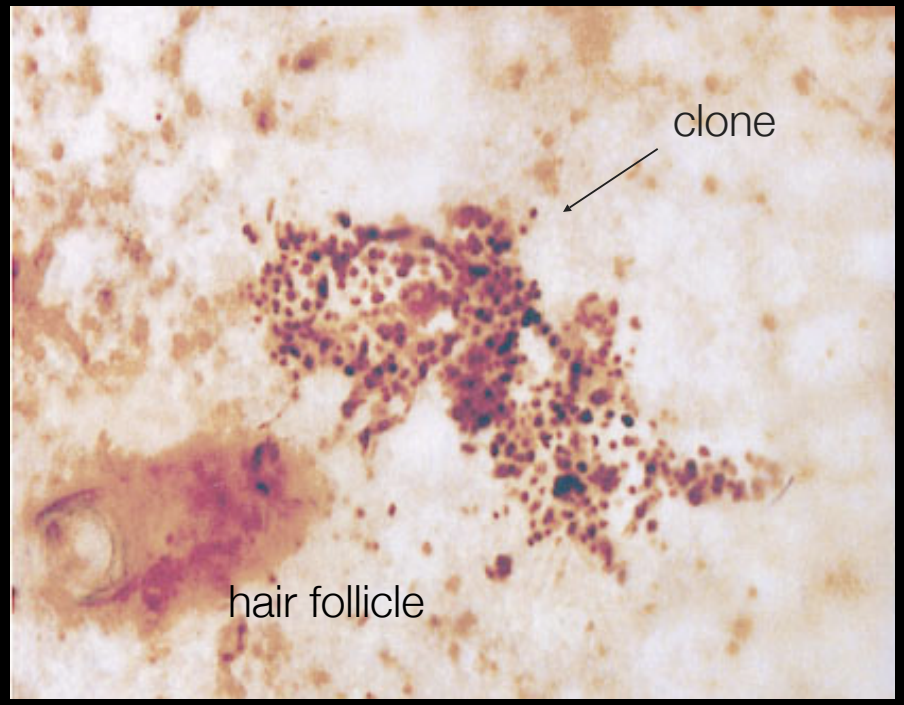
Hart & Setlow, PNAS 71:2169, 19744

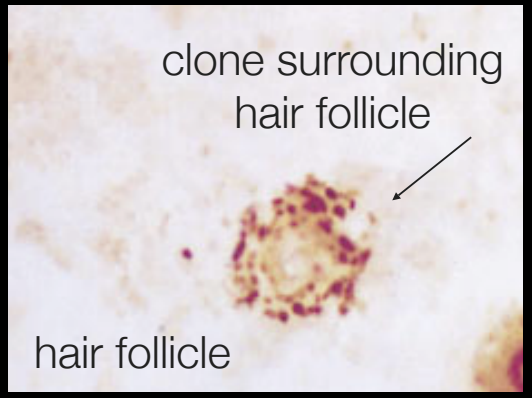
Clonal Expansion
&
DNA Photoproducts

This gets us ...

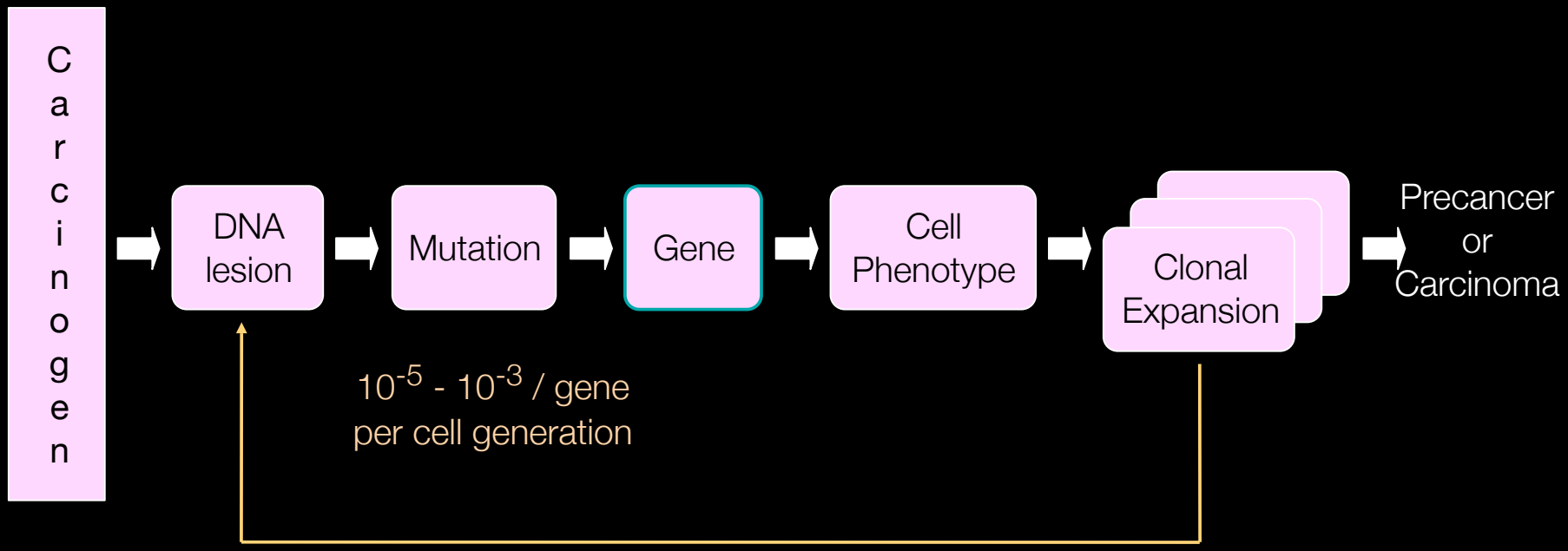


p53-mutant clones in human skin

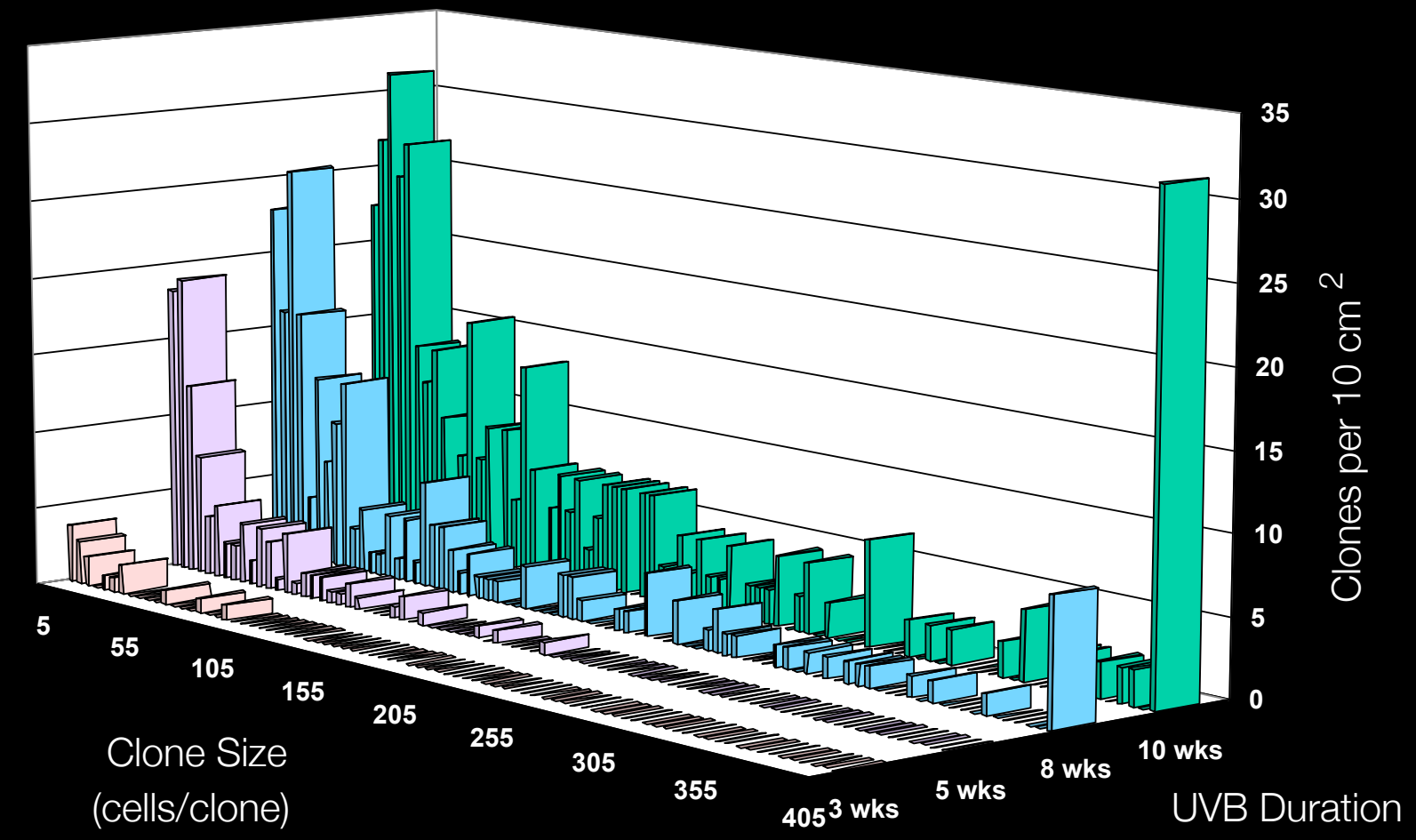




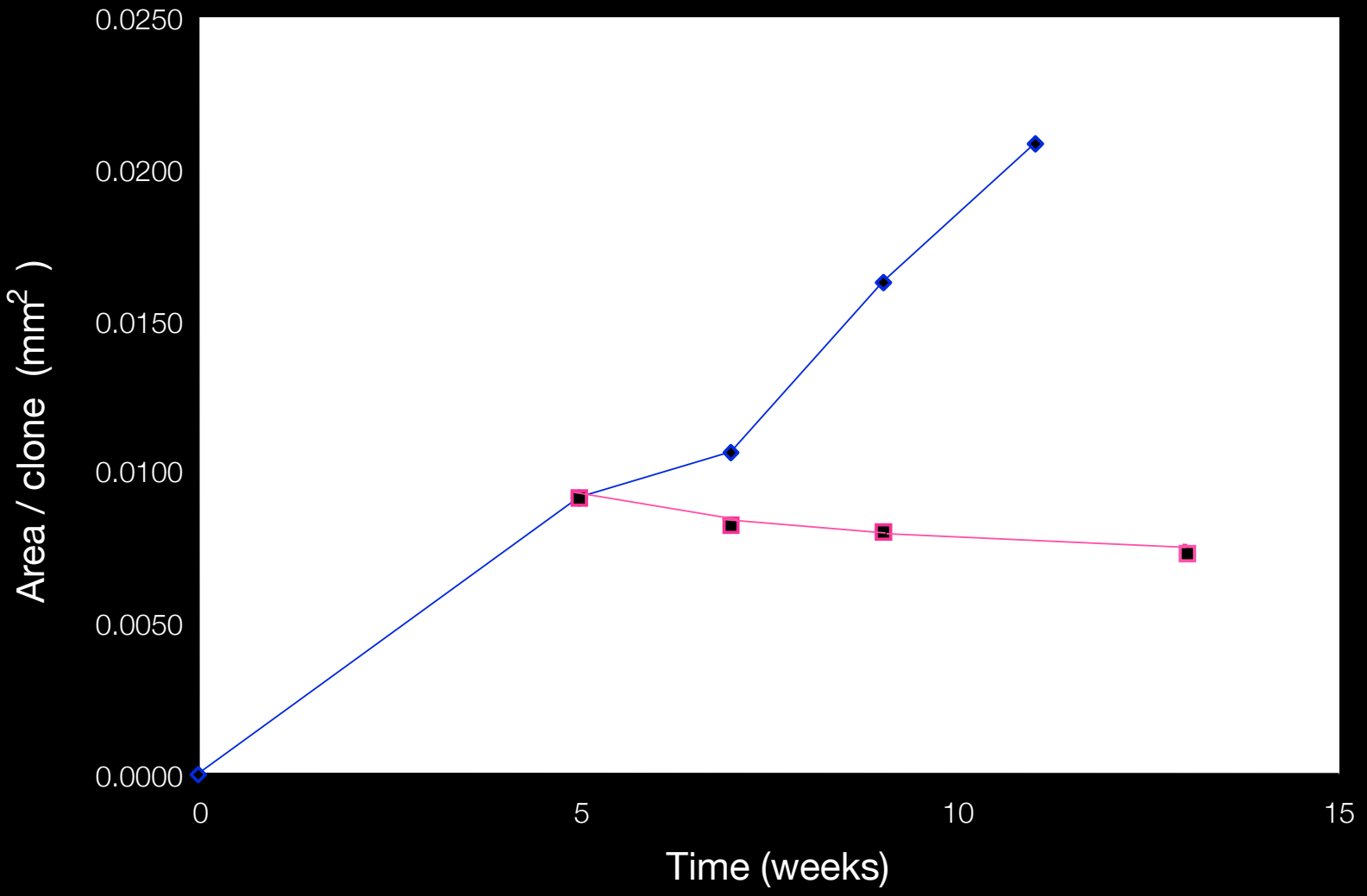
The cancer cycle



Chronic UVB drives clonal expansion of *p53*-mutant clones



Expansion of *p53*-mutant clones is not due to a mutation !



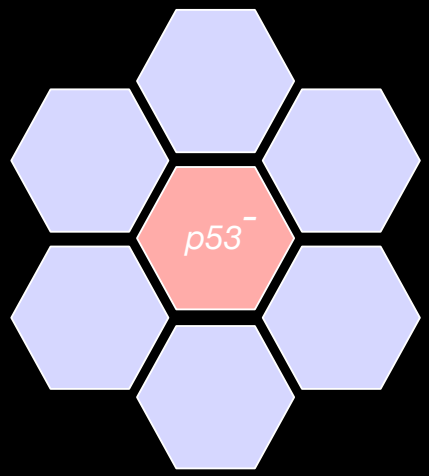
The other frontier: Physiology

Physiology is controlling mutations,
rather than the other way around.

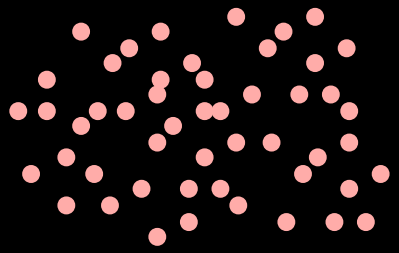
Epithelial sheet system: an in vivo petri dish

- Number of clones = initial mutations
- Clone size = clonal expansion

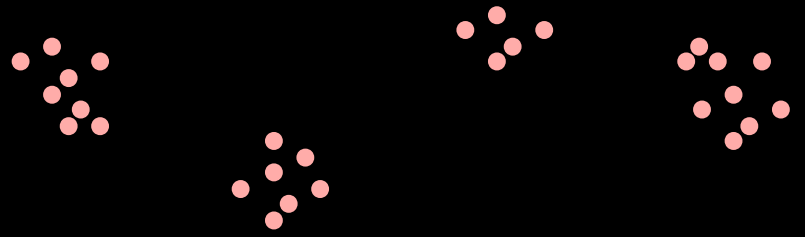
Propose: the physiological event driving clonal expansion is UV-apoptosis in adjacent stem cell compartments



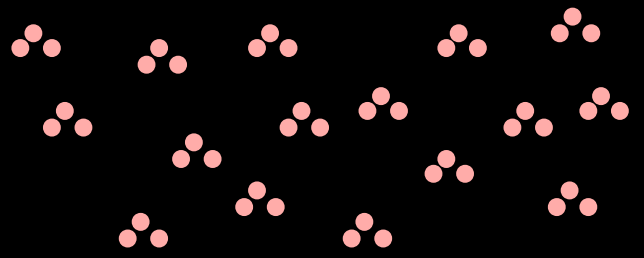
apoptosis^{S}



WT

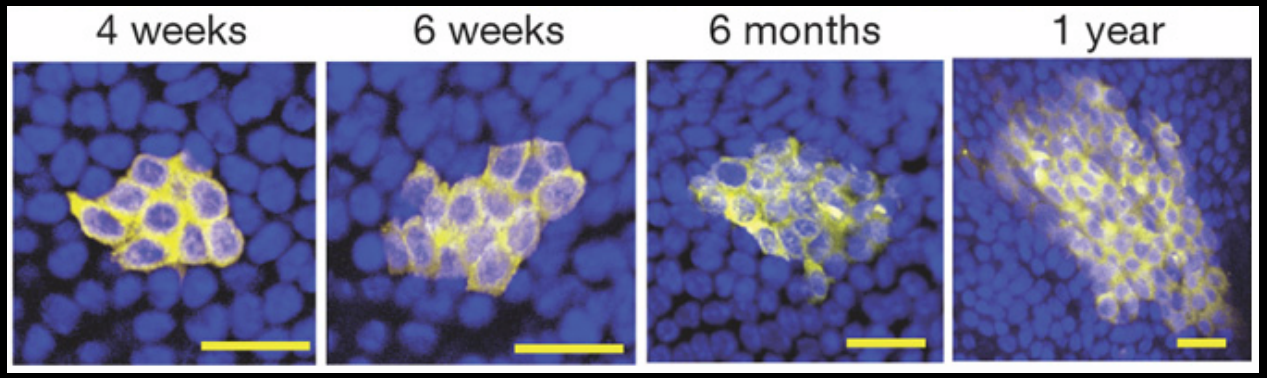


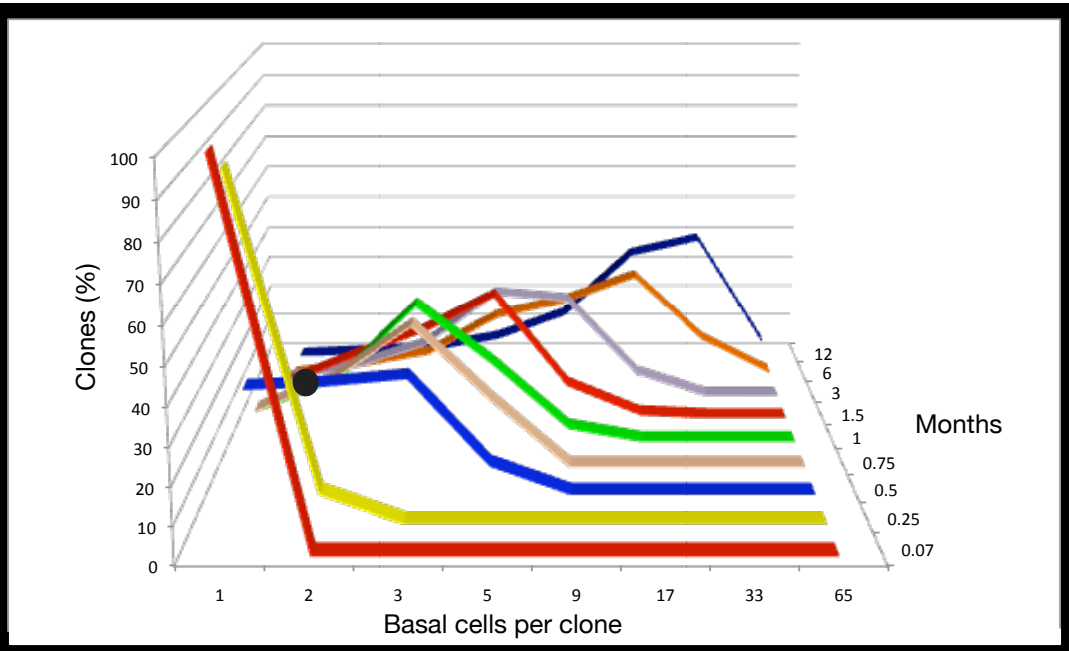
apoptosis^{R}



A 2nd route

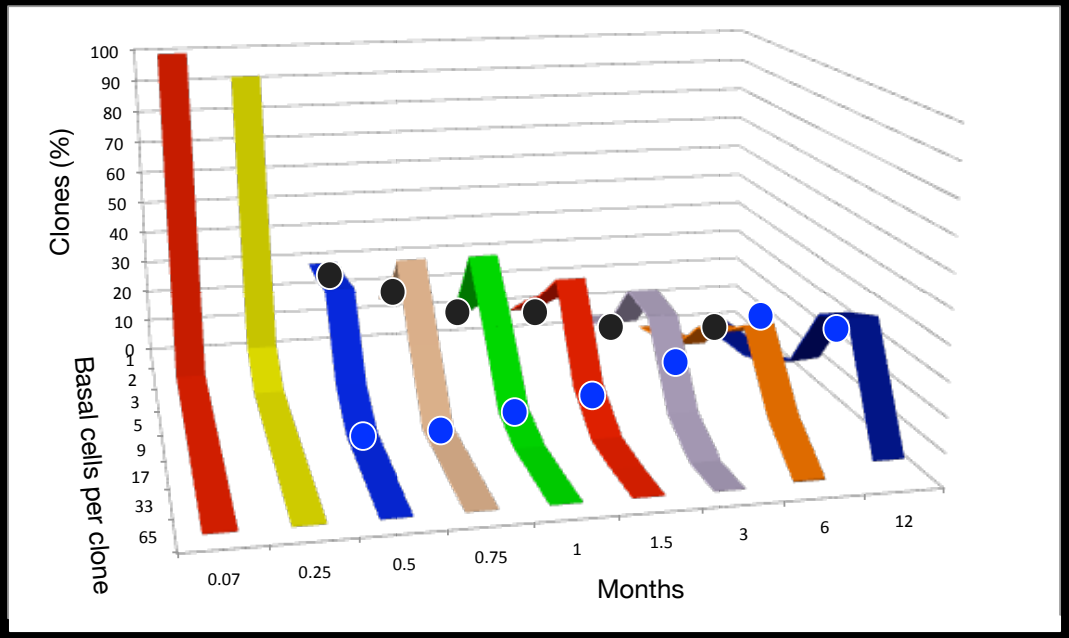
Normal clones grow larger than the putative stem cell compartment (EPU)





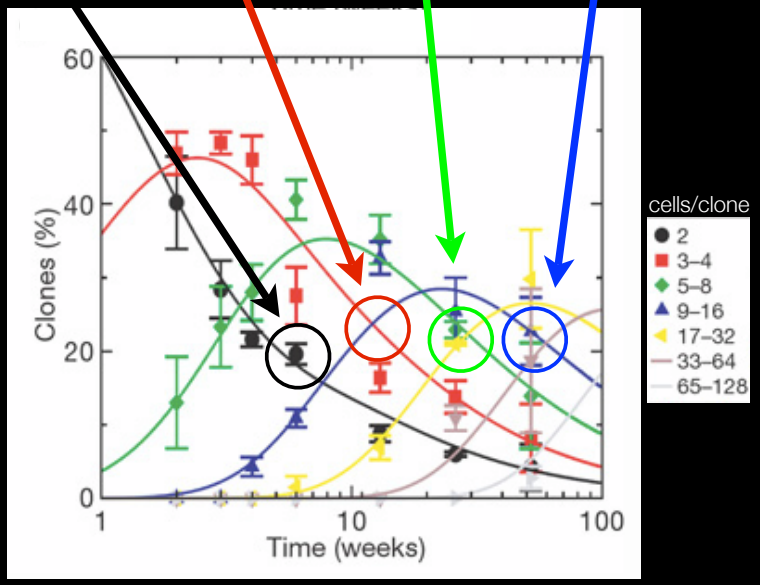
Clayton et al, *Nature* 446:185, 2007

Normal clone growth



Clone growth follows scaling behavior

2 @ 6 wks 4 @ 12 wks 8 @ 24 wks 16 @ 48 wks



The % of clones of size n at time t is the same as the
% of clones of size 2n at time 2t

Properties of Scaling Processes
(Galton-Watson branching stochastic process)

the events are stochastic

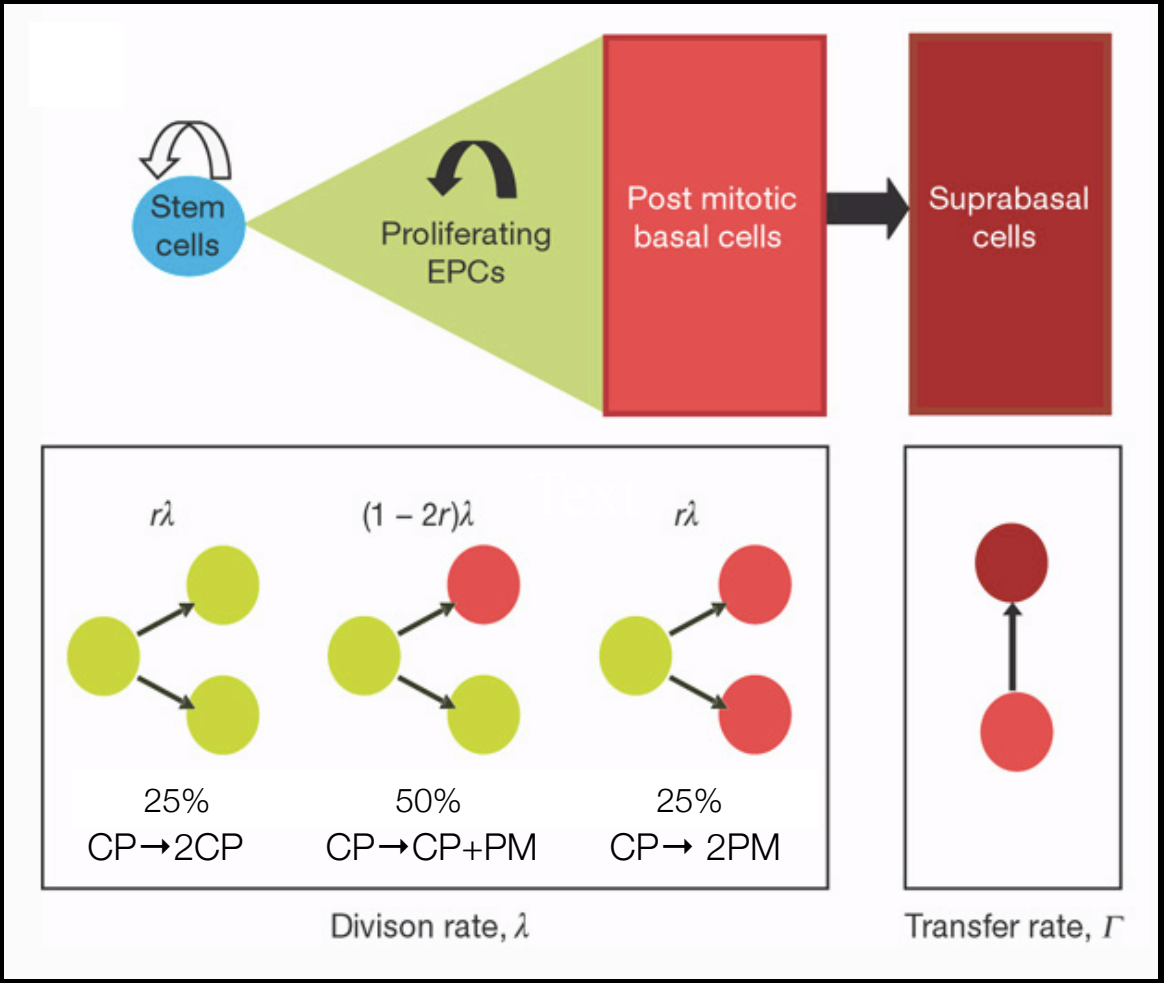
one time scale controls all events

Clayton et al, *Nature* 446:185, 2007

Stochastic progenitor cells

EPU Model

Stochastic Model
Phil Jones
Ben Simons



No long-lived, identifiable stem cells but, rather, progenitors with random cell fate.
Fits CP Leblond's measurements in the 1960's.

Clayton et al, *Nature* 446:185, 2007

Non-intuitive properties of stochastic progenitor cells

- Number of cells in a clone $= 1 + (r\,\lambda)t$

  So "lucky" clones grow linearly with time.

- Probability that a clone persists until time t $= \dfrac{1}{1 + (r\,\lambda)t}$

  So clones go extinct.

  Only "lucky" clones that undergo several CP→2CP divisions in succession will survive very long.

  Several weeks after the start of the experiment, a labeled clone may not be continguously connected to its cell of origin.

- The total number of cells = constant

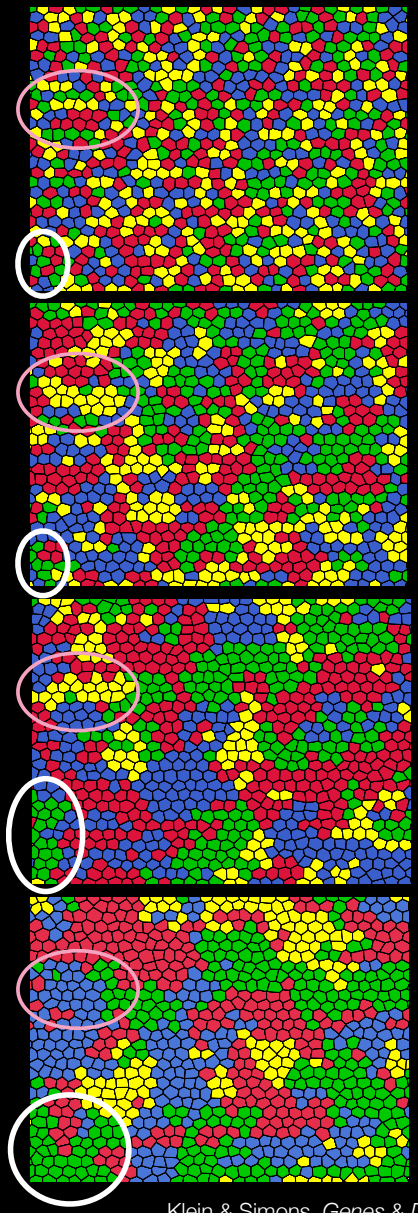
  So a diverse collection of independent clones trends toward a few very large clones.

- Cell cycle time is also stochastic, days to weeks between divisions.

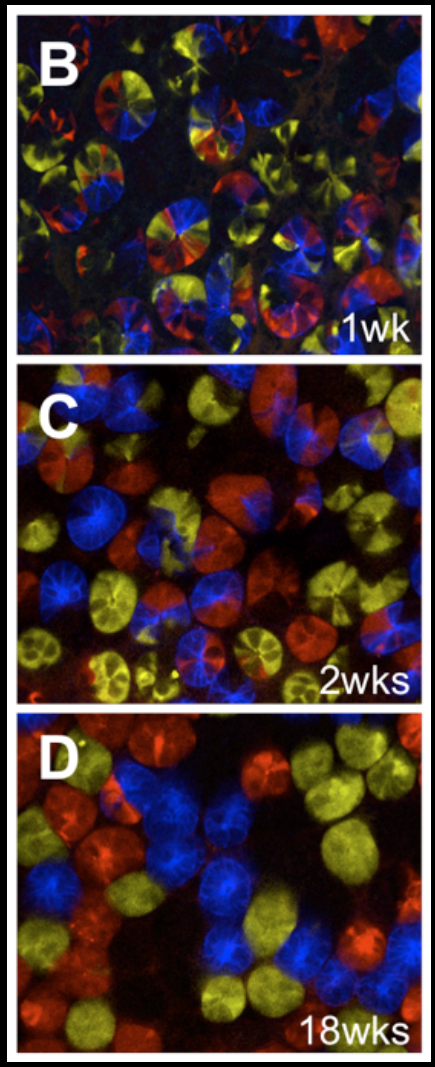
  (measured average CP division rate $\lambda = \sim 1$ per week)

This could not have been discovered by observing single cells. A coup of computational biology!

Ebb and flow of stem cell clones

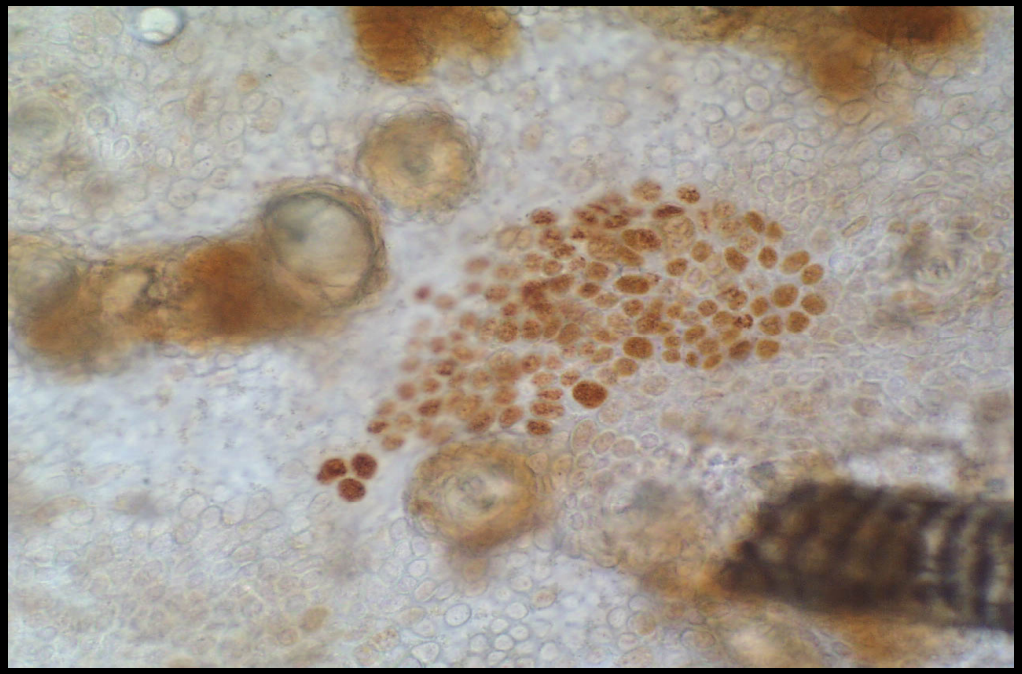


Klein & Simons, *Genes* & *Development,* in press

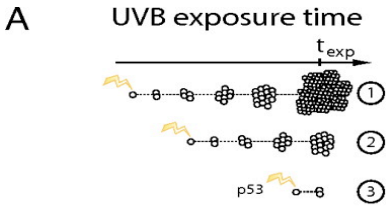


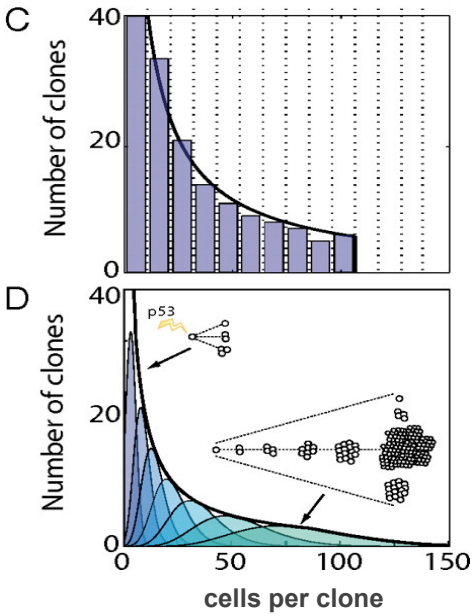
Snippert et al, *Cell* 143:134, 2010

Are *p53*-mutant clones growing via stochastic stem cells?



Mutant clone sizes during ongoing UVB radiation



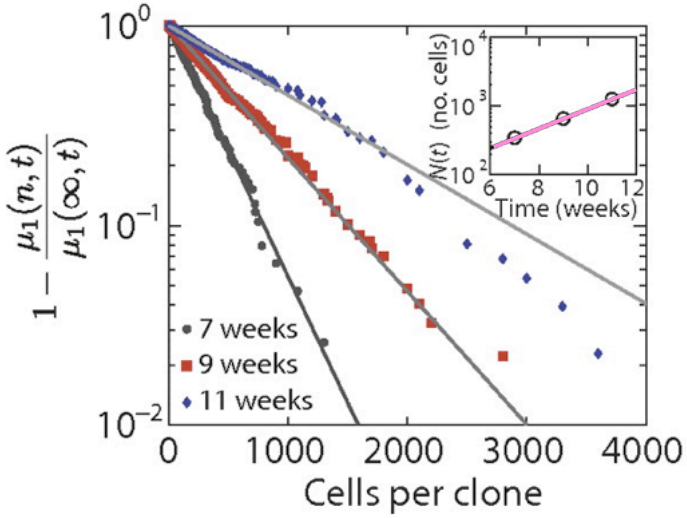


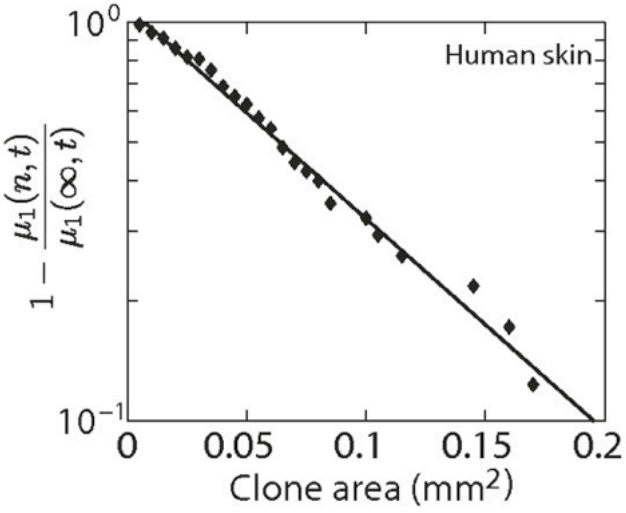
deterministic

stochastic

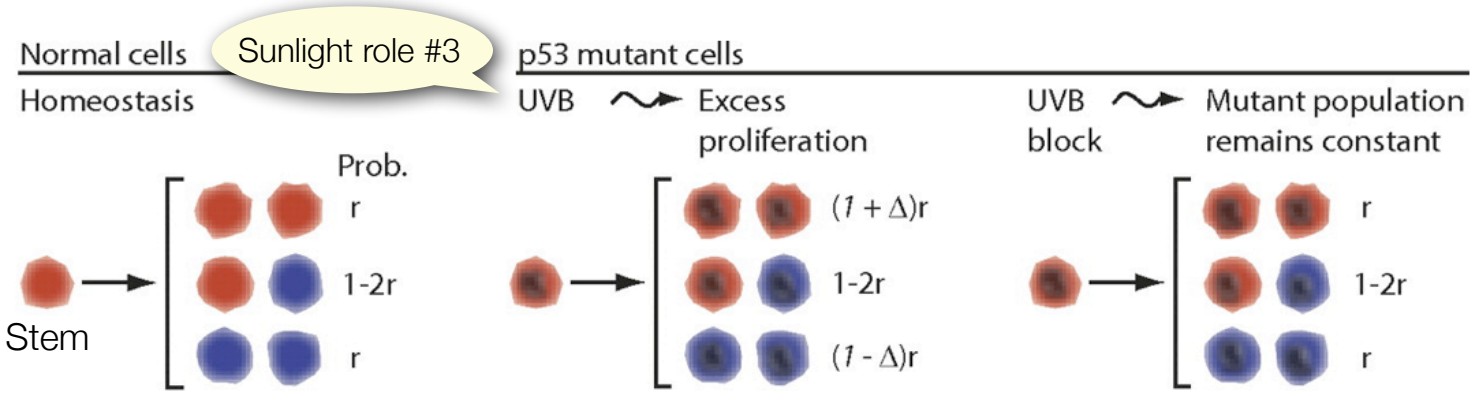
Klein A M et al. PNAS 107:270, 2010

p53-mutant clones grow stochastically – and exponentially !





So *p53*-mutant clones are also growing, shrinking, going extinct – as long as UV is present.

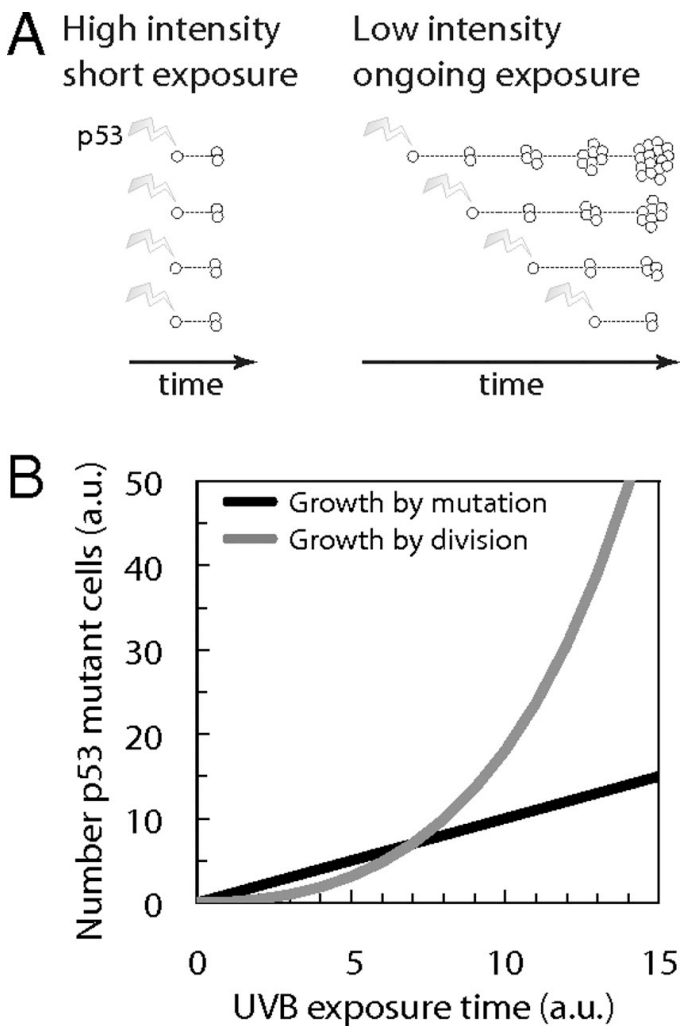


But only the *p53*-mutant cells are growing exponentially – or UV'd skin would get very thick.
Increasing the rate of proliferation and cell loss would just cause faster *linear* growth.

Klein A M et al. PNAS 107:270, 2010

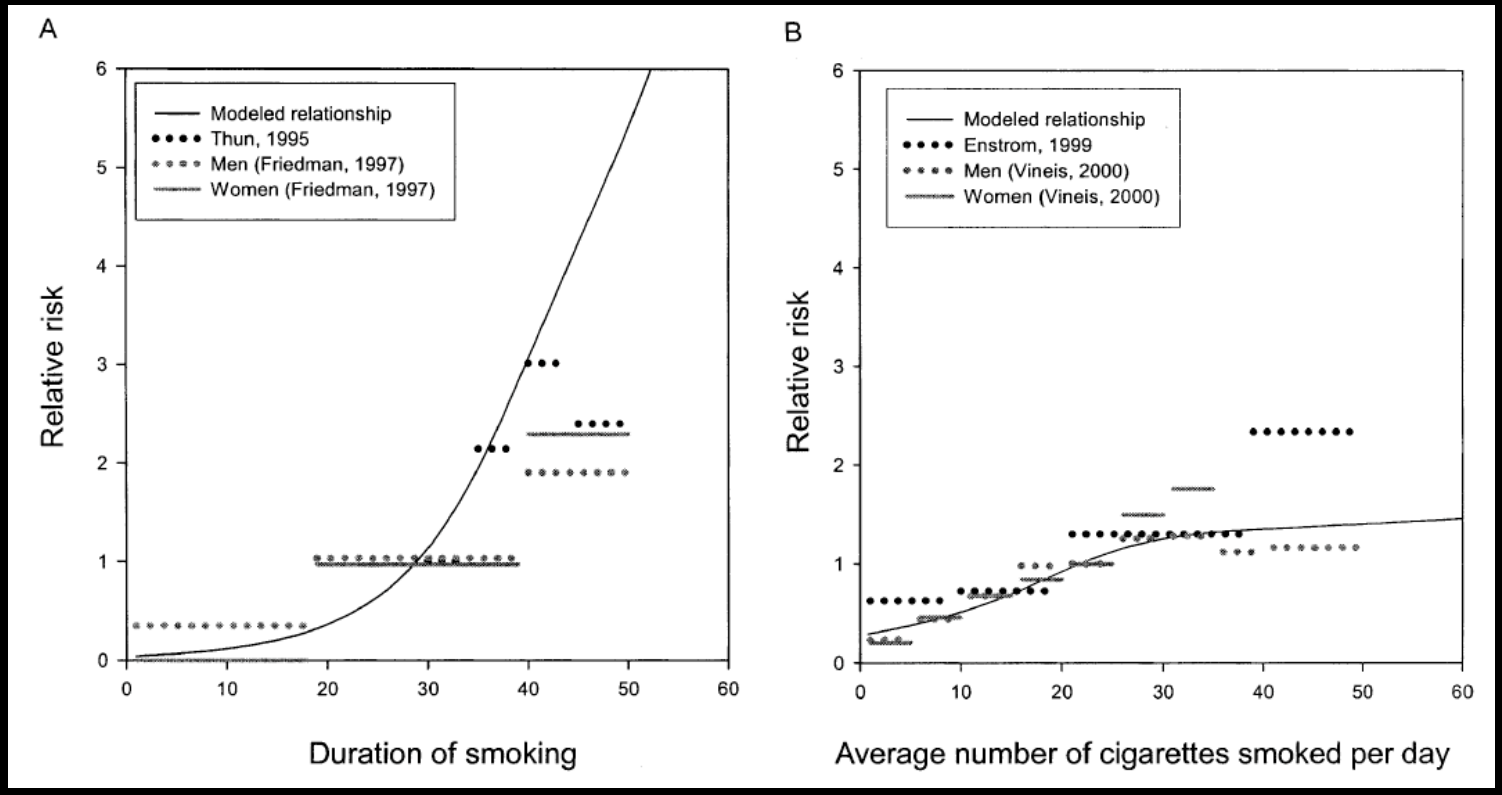
PNAS

Exponential clone growth means that skin cancer risk is highest for ongoing low exposures



In fact, most human and mouse cancers depend weakly on dose and strongly on duration of exposure – a mystery, if the role of a carcinogen is to mutate N genes !

Most human and mouse cancers depend weakly on dose & strongly on duration of exposure

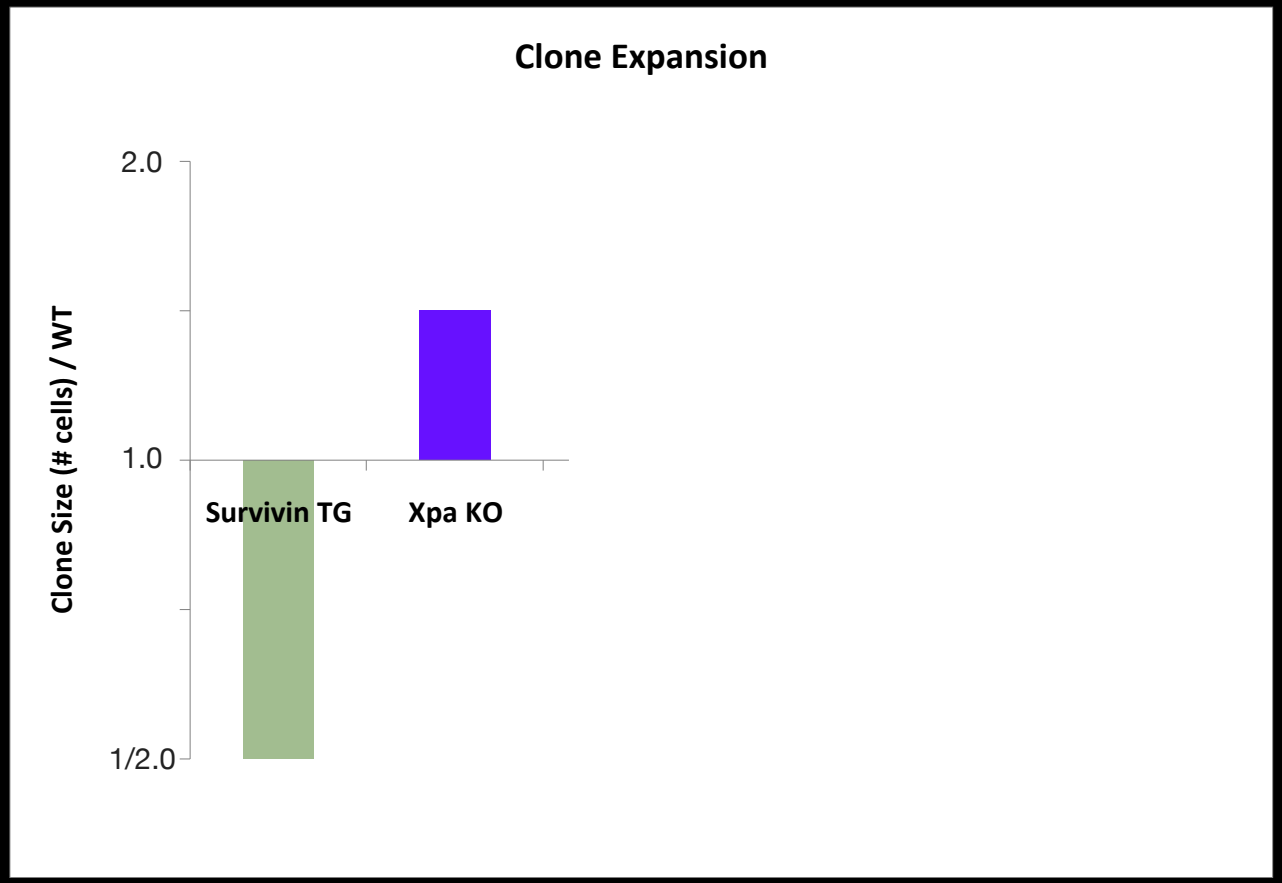


This is a mystery if the role of a carcinogen is to mutate N genes !

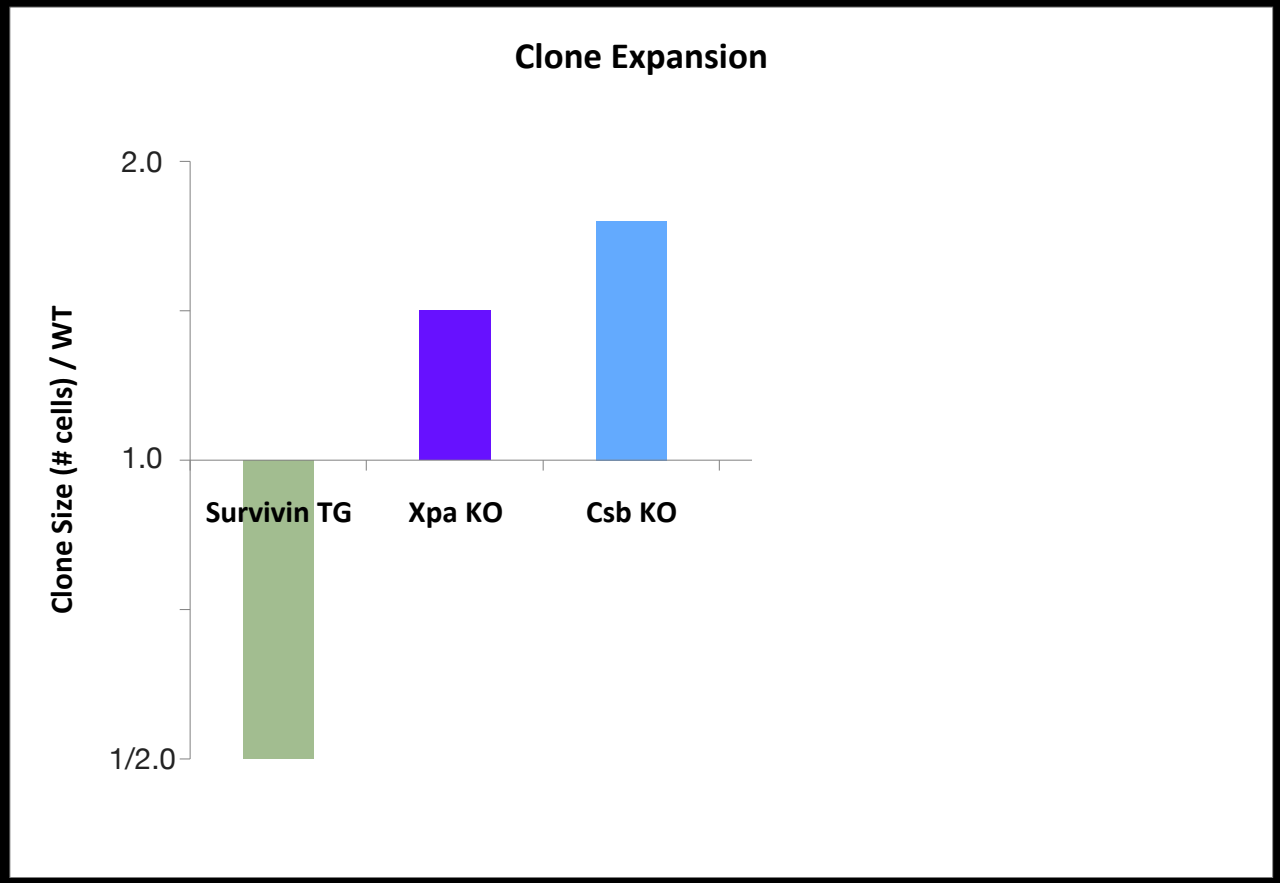
Bach et al. *JNCI* 95:470, 2003

How does UV tip the CP/PM fate-decision toward CP ?

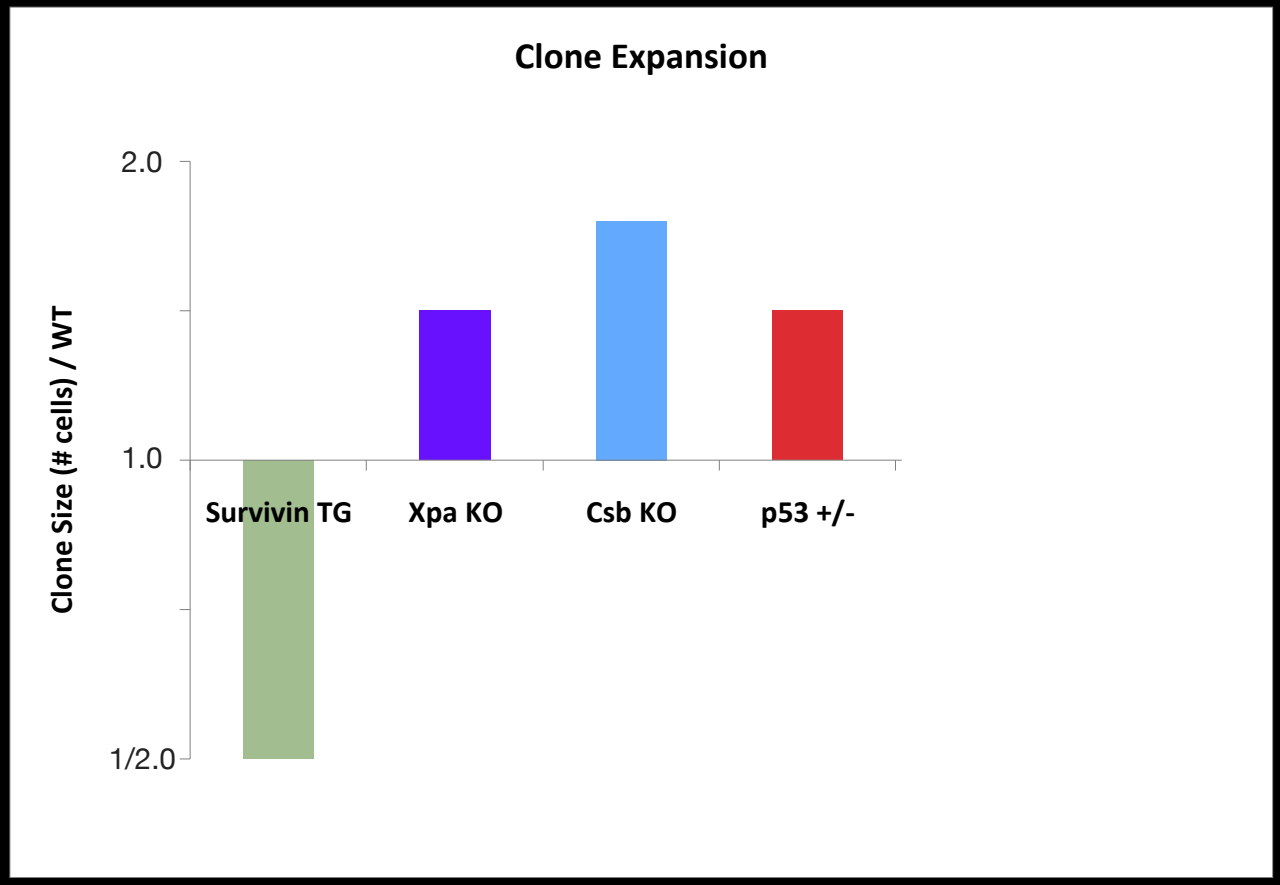
The clone expansion signal comes from DNA photoproducts



The clone expansion signal comes from DNA photoproducts in active genes



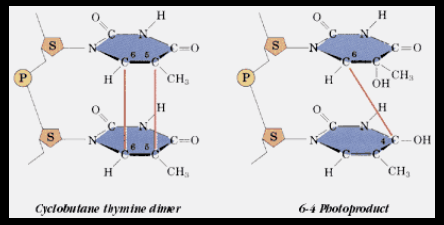
P53 *suppresses* clonal expansion



Normal cells: UV signal from active genes i) upregulates CP self-renewal, favoring clone growth but ii) inhibits CP self-renewal via P53 (↑ P21, ↓ Nanog, ↓ Notch?).
p53-mutant cells: Don't get the 'no' signal.

How Sunlight Works

Mutates a Gene

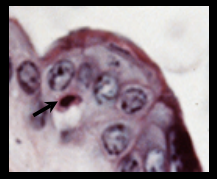


1 cell with
C → T in
p53
PTCH

Drives Clonal Expansion

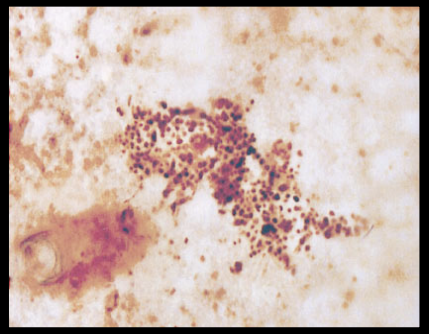
DNA photoproducts
in active genes

repeated UV

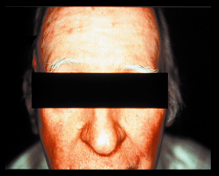


apoptosis
but not in p53⁻ cells

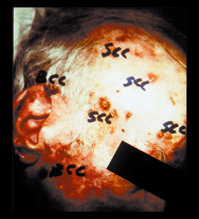
stem cell renewal *vs* differentiation
tilts to renewal
But only in p53⁻ cells



p53-mutant clones
30/cm^2
in normal skin

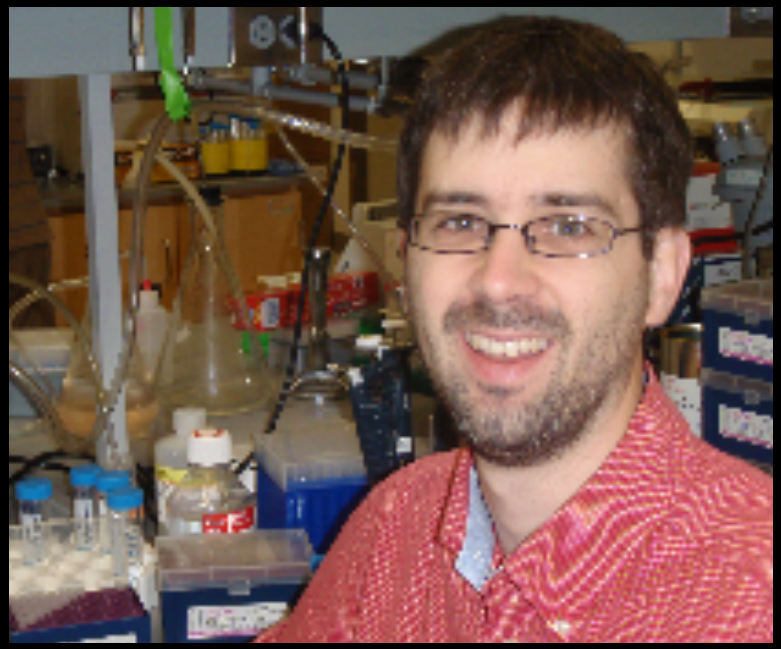


actinic keratosis



squamous
cell carcinoma

Wengeng Zhang & Dejan Knezevic



Pat Rochette

Colleagues – clonal expansion, melanin, IPoD

Brash lab

Clonal expansion
Alan Jonason
Norbert Wikonkal
Eva Remenyik
Wengeng Zhang

Melanin
Seiji Takeuchi

IPoD
Pat Rochette

Yale

Clonal expansion
David Leffell
Gary Price
John Persing
Richard Restifo
Henry Spinelli
Daniel Zelterman
Dario Altieri

Imaging
Rob Roorda
Len Milstone
Bob Tigelaar
Rick Edelson

Melanoma
Ruth Halaban
Marcus Bosenberg

IPoD Bioinformatics
Chao Cheng
Mark Gerstein

Elsewhere

Clonal expansion
Doug Grossman
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Clone computational biology
Allon Klein
(Harvard)
Ben Simons
(U Cambridge)

Melanin
Ken Kraemer
Vince Hearing
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(Fujita Univ.)

Thank you

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