Mutational signatures of redox stress in yeast and men

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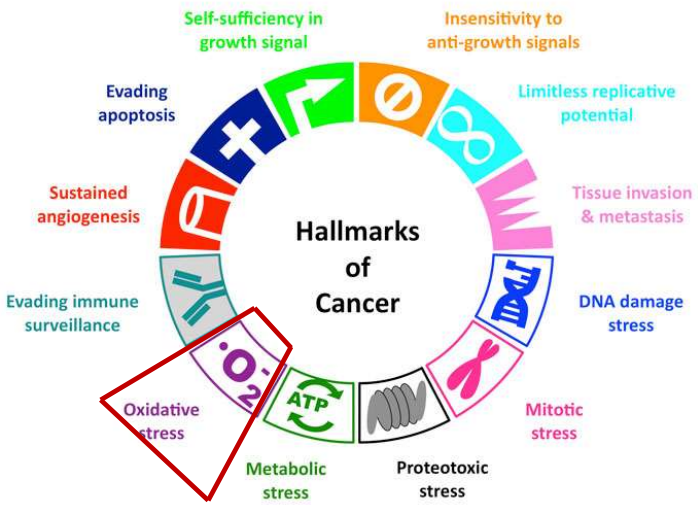
Mutagenesis & DNA Repair Regulation Group, NIEHS

DNA repair interest group videoconference
May 18, 2021

Redox stress is linked to human disease and ageing

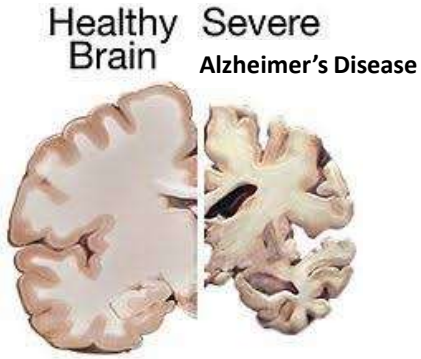
A hallmark of cancer



Luo, Solimini, Elledge, *Cell*, 2009

Neurodegenerative diseases

- Alzheimer's, Parkinson's, Huntington's, Amyotrophic lateral sclerosis (ALS)
- Associated with cell loss/degeneration in high energy consuming cells



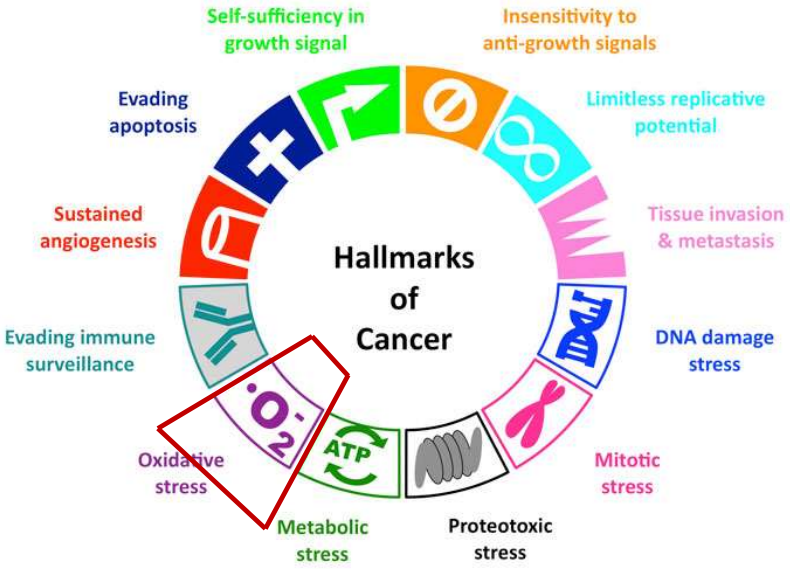
Lin and Beal, *Nature,* 2006

Ageing

- Redox theory of ageing

Go and Jones, *Clin Sci*, 2017

Redox stress is a hallmark of cancer



Redox status is deregulated in <u>majority</u> of cancers due to:

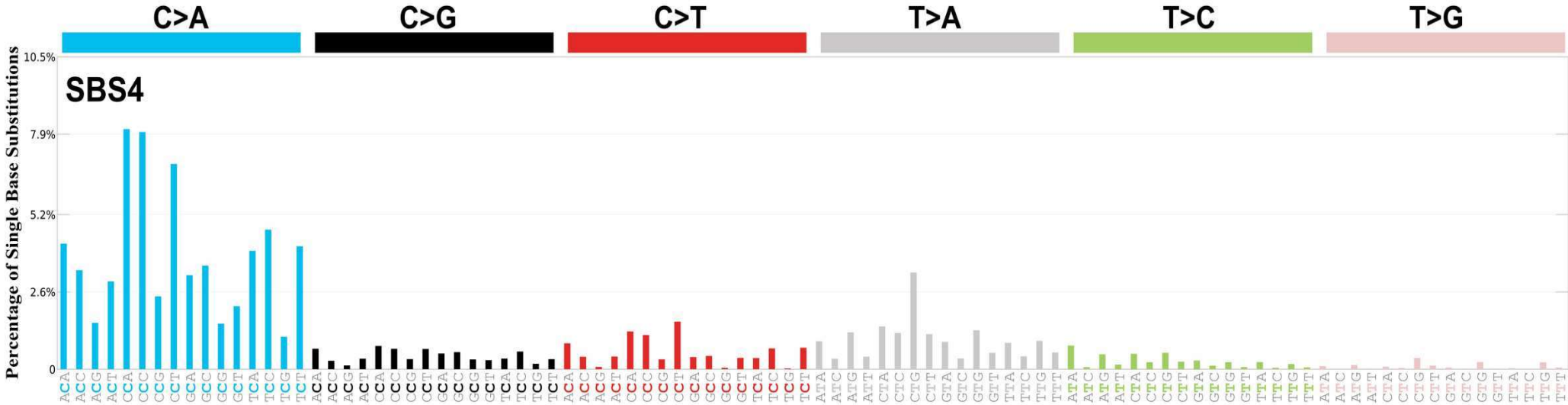
- altered metabolism;
- mitochonrdial dysfunction;
- inflammation;
- malfunctioning of peroxisomes;
- over- or under-expression of ROS-producing and ROS-scavenging enzymes.

Luo, Solimini, Elledge, *Cell*, 2009

How to assess the contribution of redox stress to disease and aging?

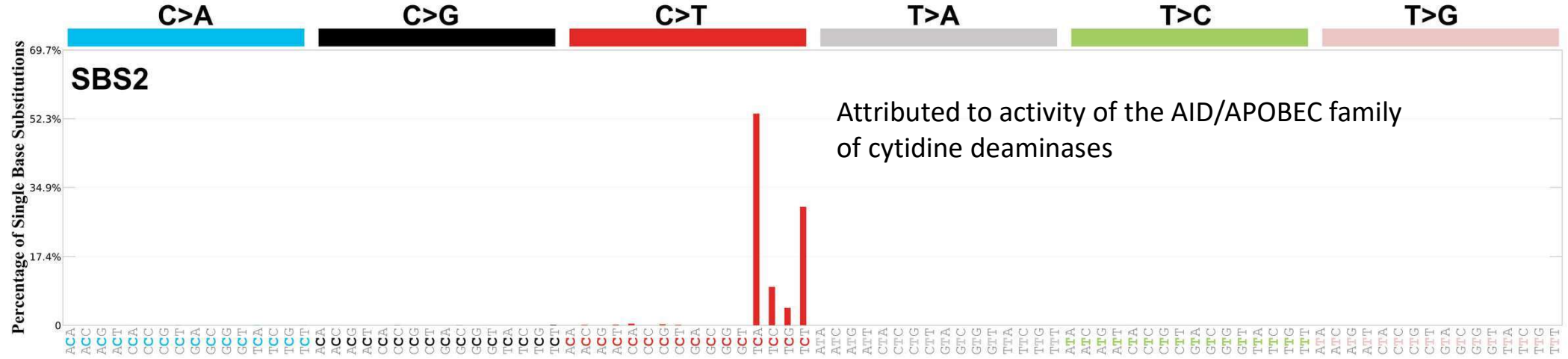
By analyzing the mutations accumulated following exposure to oxidizing agents
and in tissues affected by a disease

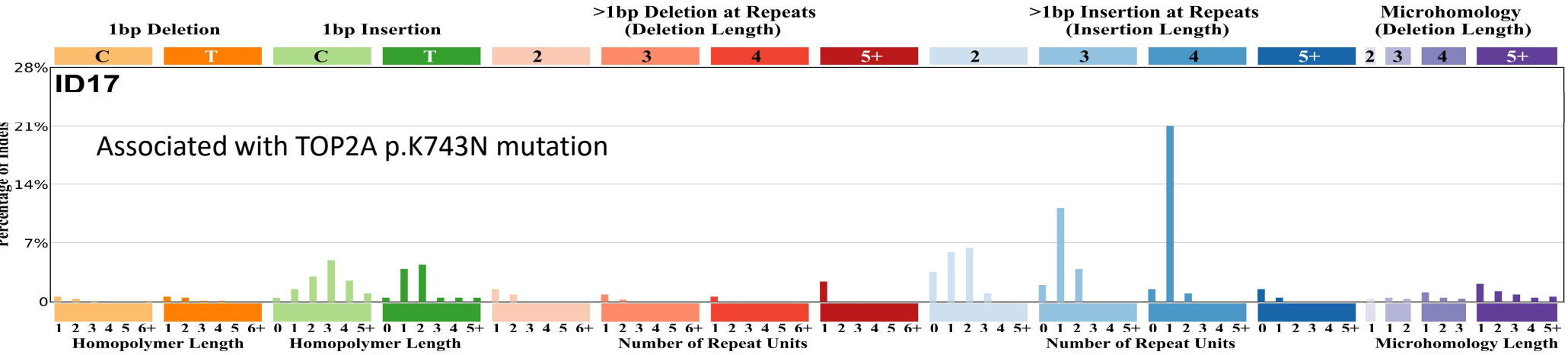
Mutational signature is a spectrum of mutations in the context of adjacent nucleotides



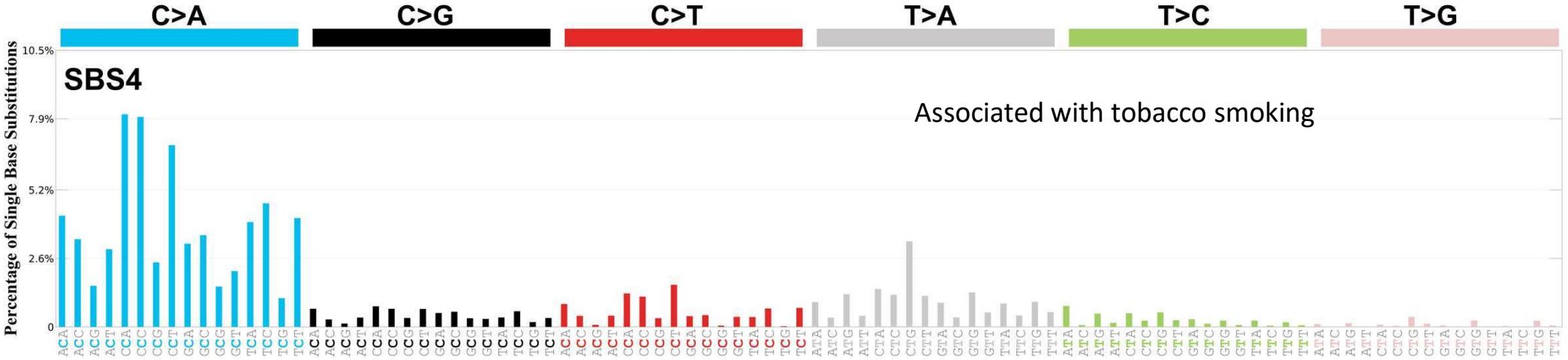
https://cancer.sanger.ac.uk/signatures/sbs/

Some of the mutational signatures reveal etiology of human cancers caused by dysregulation of specific enzymes

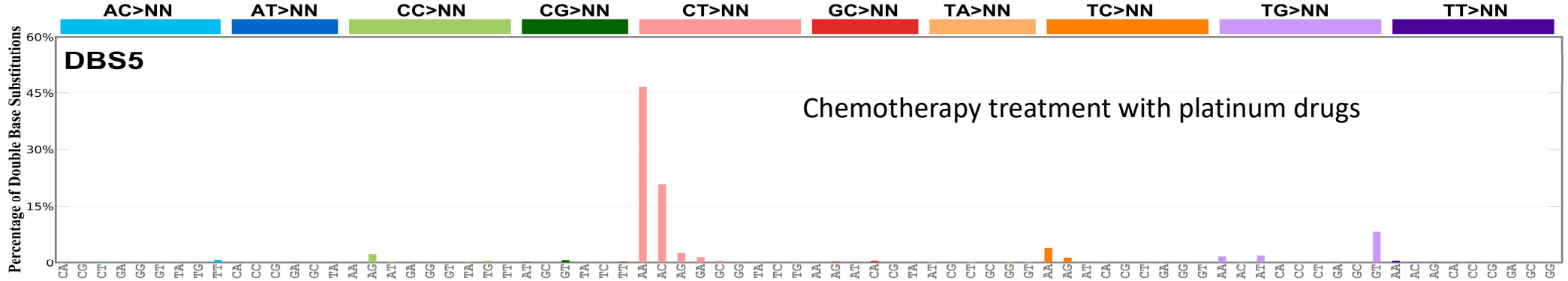




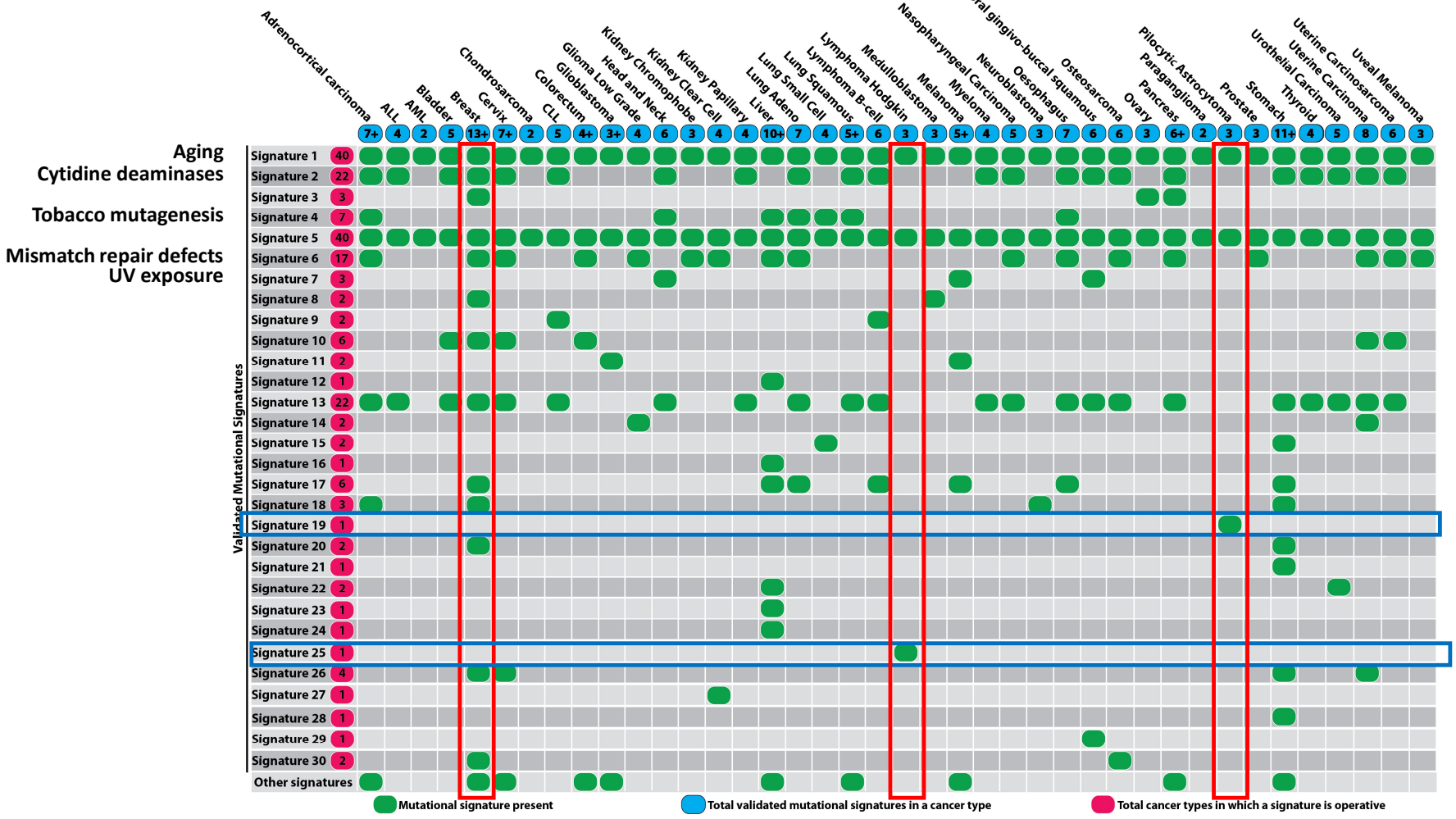
… or by environmental exposures



… or by chemotherapeutic interventions



Is there a signature of redox stress?



Alexandrov et al., Nature, 2013

Approach:

discerning the mutational signature of redox stress

-in the model organism *Saccharomyces cerevisiae*;

-in single strand DNA;

- selecting clustered (closely-spaced) mutations.

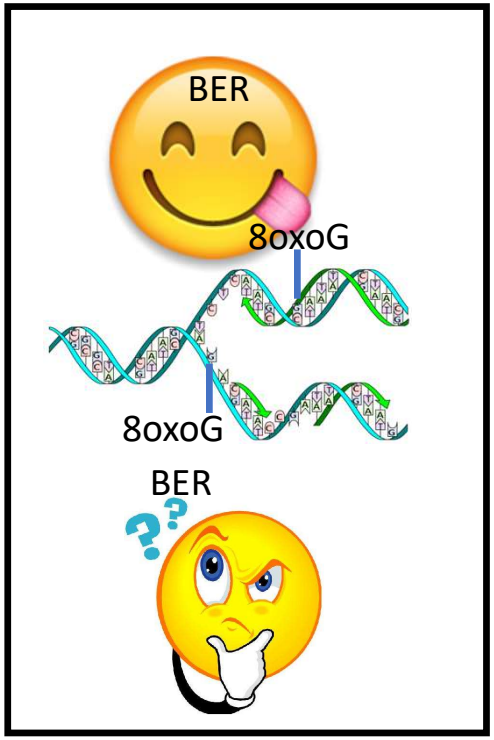
Why did we look for a signature of oxidative damage in single strand DNA?

Base Excision Repair requires second DNA strand *

Ntg1

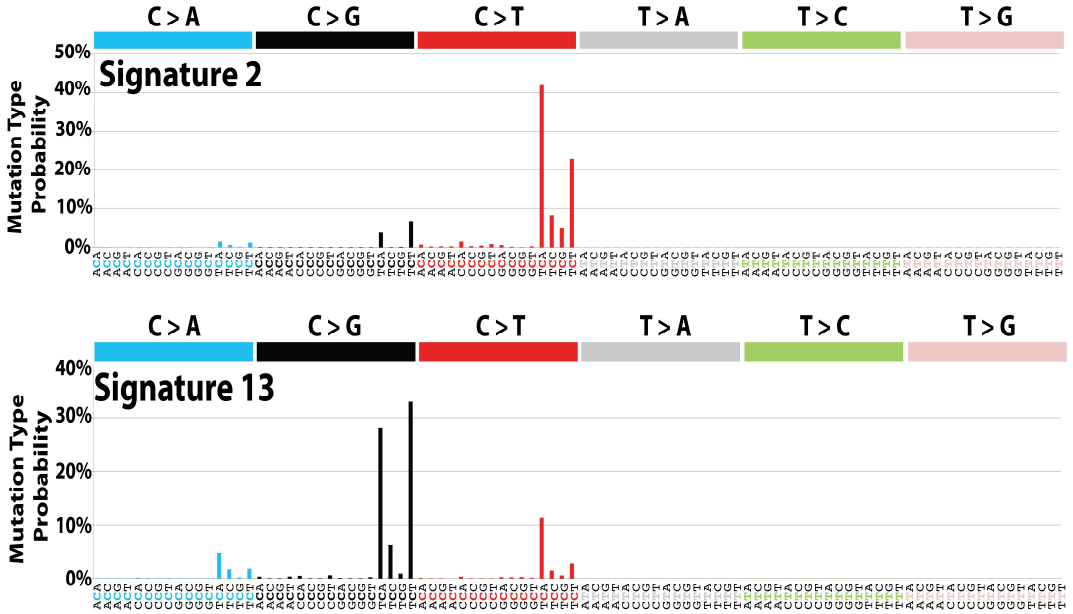
Ntg2

Ogg1



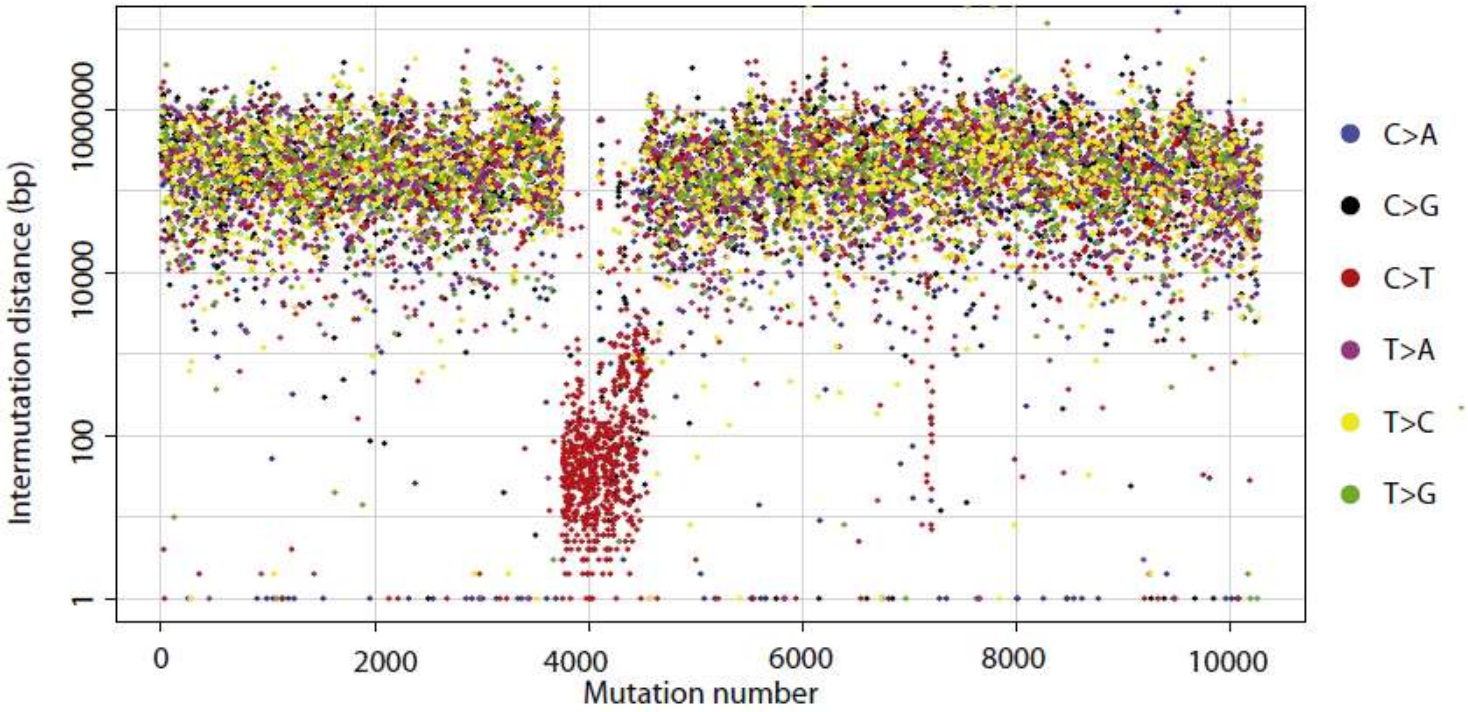
* with some exceptions

Among published cancer signatures
some are attributed to activity of enzymes that uses ssDNA as a substrate



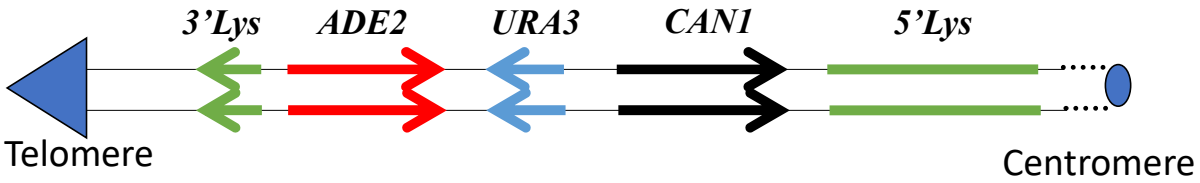
Why did we look for a signature of oxidative damage in single strand DNA?

Localized hypermutability suggests persistent presence of ssDNA in cancer genomes

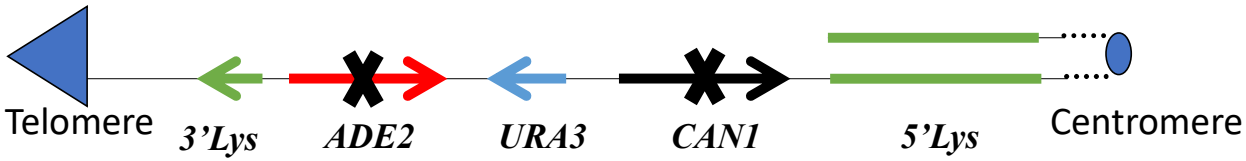


Nik-Zainal et al., 2012

The reporter system allows for the generation long stretches of ssDNA and selection for multiple mutations

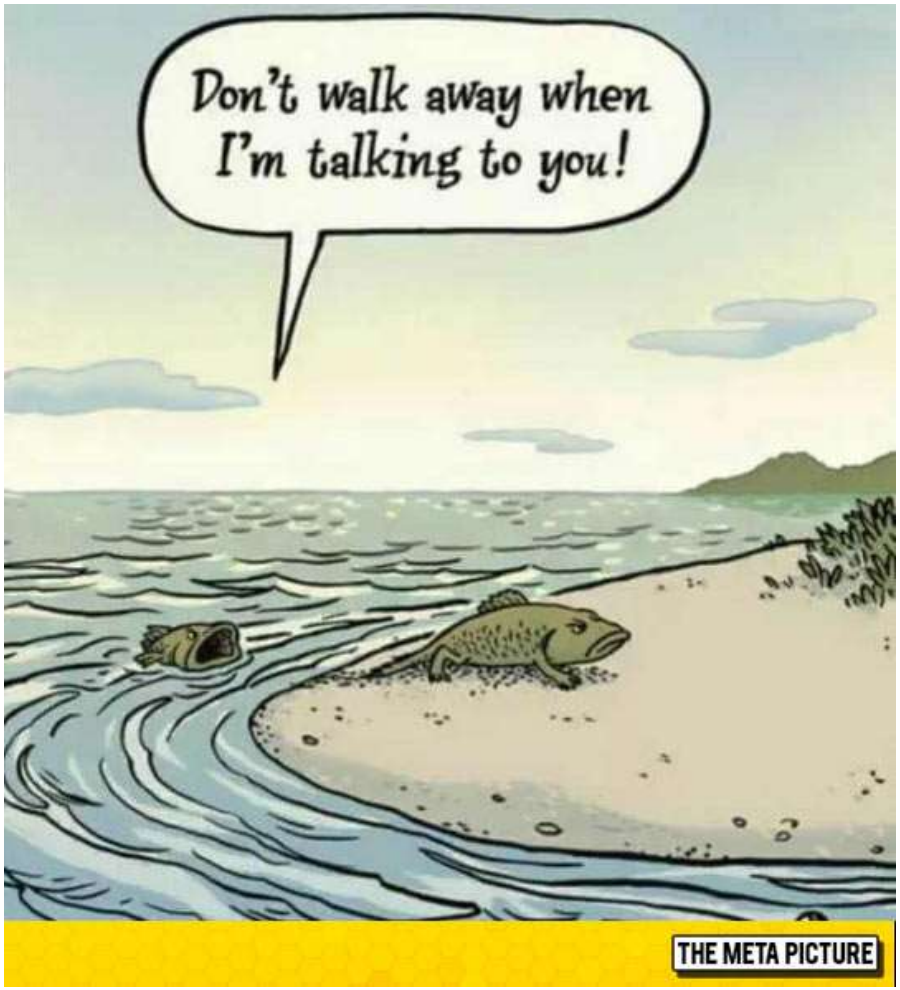


cdc13-1ts at 37^0C

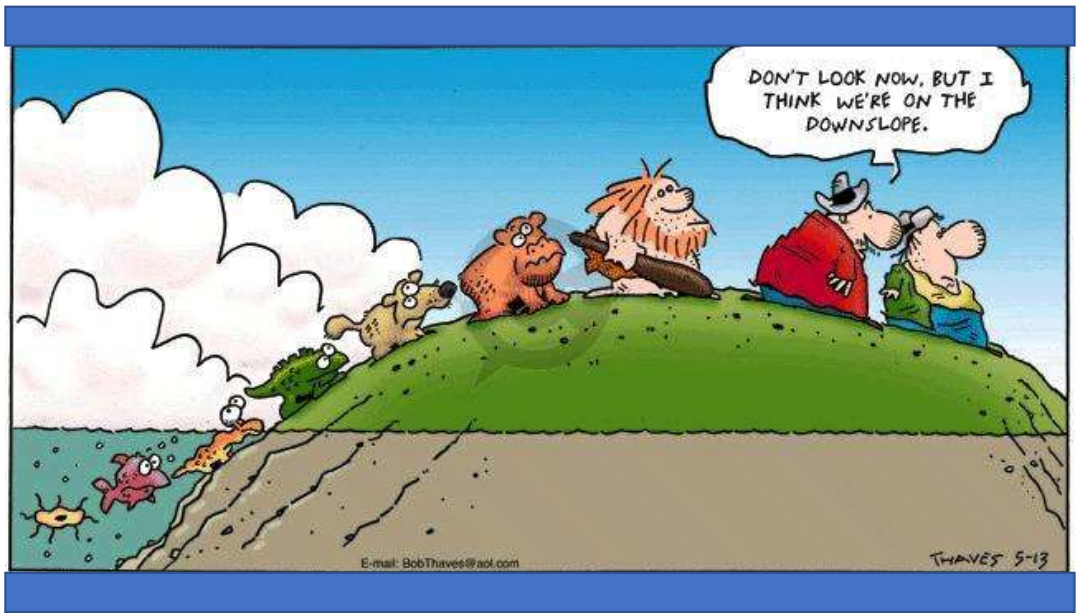


RED Can^R clones = clustered mutations

Chan et al., 2012


Don't walk away when I'm talking to you!
THE META PICTURE

O_2


DON'T LOOK NOW, BUT I THINK WE'RE ON THE DOWNSLOPE.
E-mail: BobThaves@aol.com
THAVES 5-13

A Compendium of Mutational Signatures of Environmental Agents

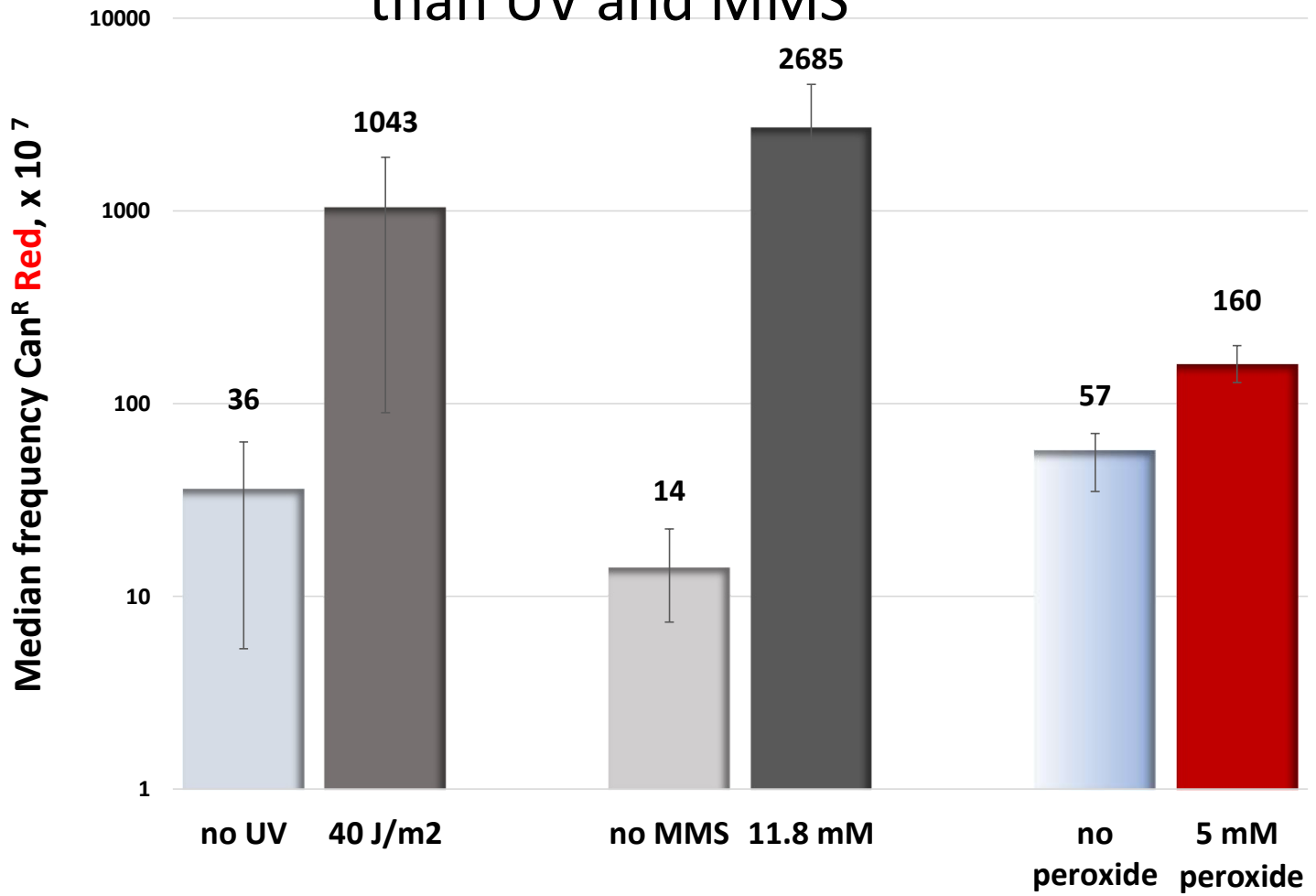
Jill E. Kucab, Xueqing Zou, Sandro Morganella, Madeleine Joel, A. Scott Nanda, Eszter Nagy, Celine Gomez, Andrea Degasperi, Rebecca Harris, Stephen P. Jackson, Volker M. Arlt, David H. Phillips, Serena Nik-Zainal
Cell, 2019

Highlights

- 41 of 79 environmental agents yielded substitution signatures
- 6 agents produced double-substitution signatures and 8 produced indel signatures
- Several signatures match or exhibit similarity with signatures found in human tumors
- Topographical mutational asymmetries reveal mechanistic insights

"…hydrogen peroxide, anticipated to create ROS, and peroxynitrite, which generates reactive nitrogen (nitric oxide) species, did not yield clear mutation patterns"…

At an equitoxic dose hydrogen peroxide induces fewer mutations, than UV and MMS



Median survival 60% 70% 60%

Degtyareva et al., 2019

Even if it is possible to discern a mutational signature of oxidative stress what to expect?

8oxoG mis-pairing with A

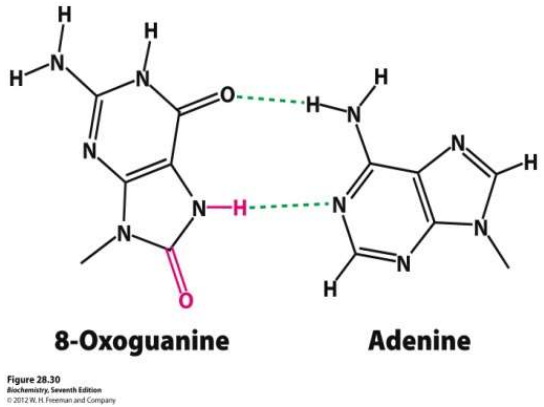


Figure 28.30
Biochemistry, Seventh Edition
© 2012 W. H. Freeman and Company

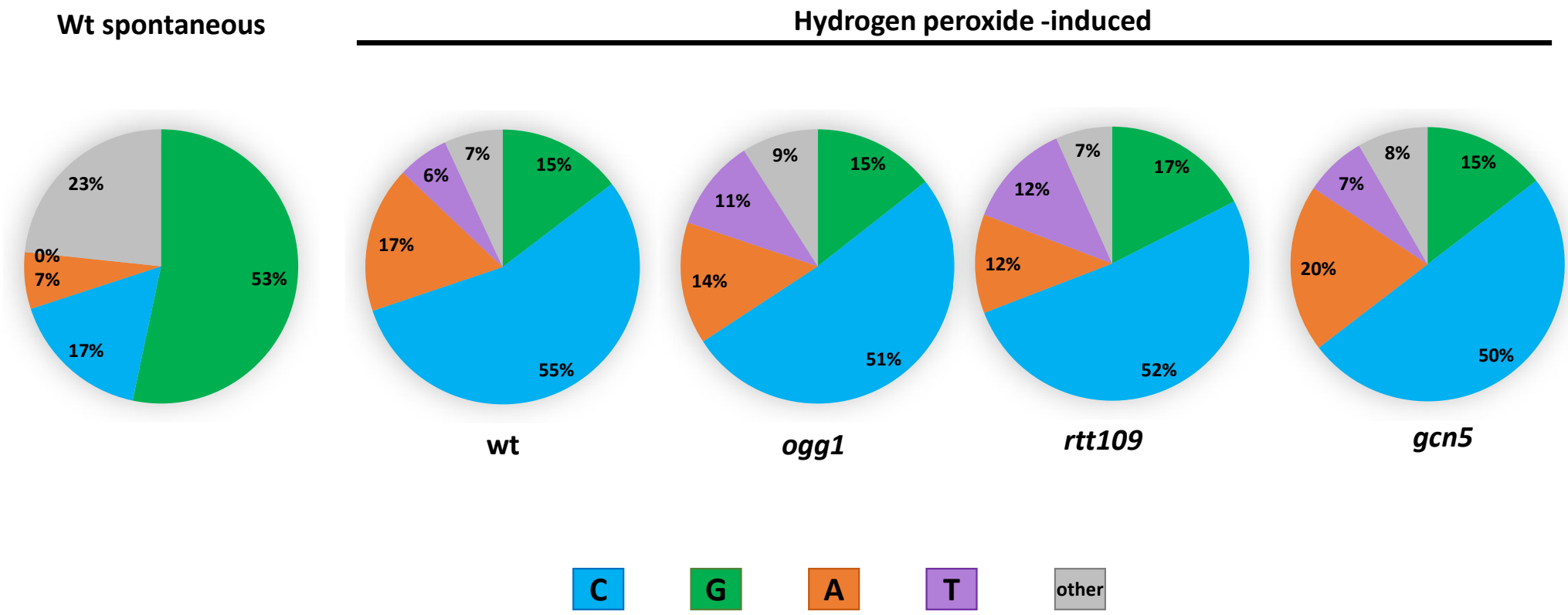
leads to

G to **T**

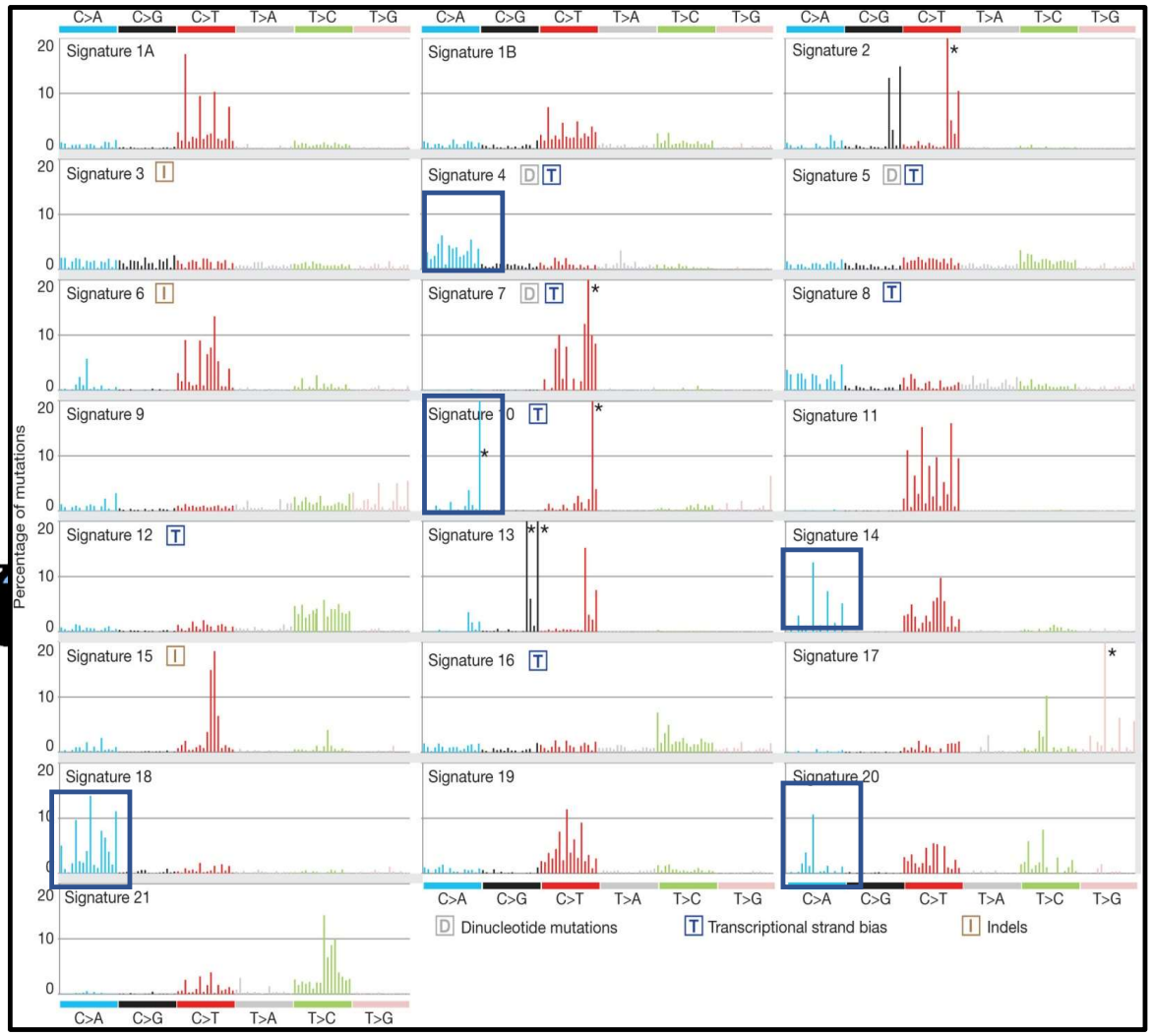
transversions

?

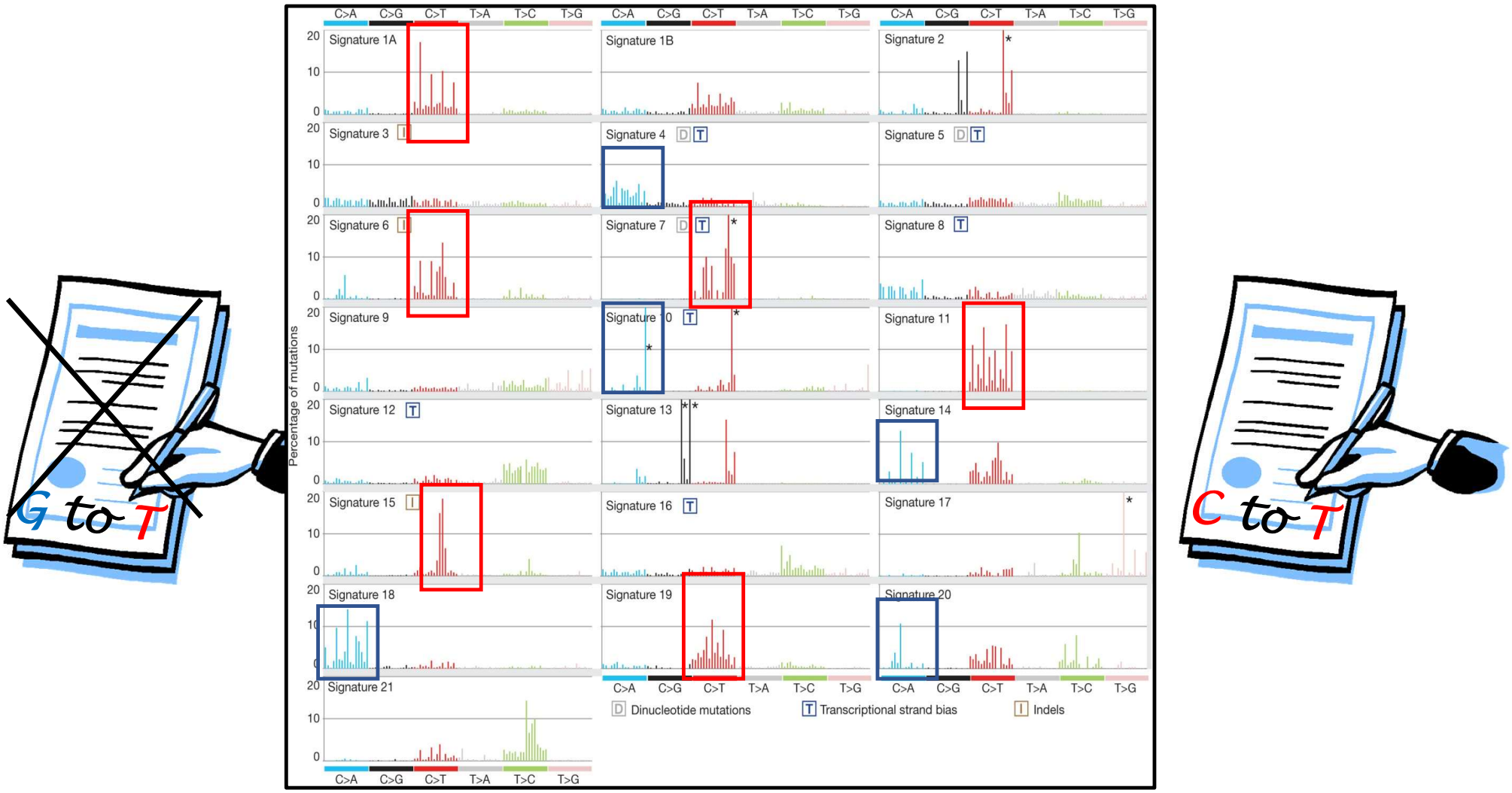
Oxidative stress-induced mutagenesis in ssDNA occurs primarily at C



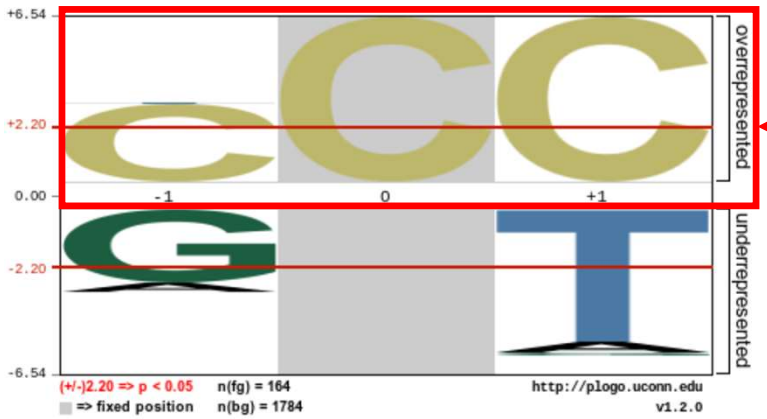
Expected: G to T enrichment in oxidative stress signature

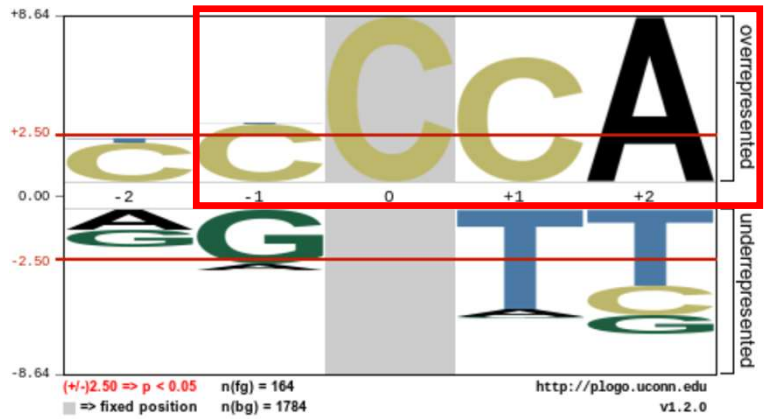


C to T enrichment in oxidative stress signature?



Signature of hydrogen peroxide - induced oxidative stress





https://plogo.uconn.edu/

Significant enrichment in C at position +1 and -1

Significant enrichment in A at position +2

Confirmed signature of hydrogen peroxide - induced oxidative stress

$$(E)nrichment = \frac{Mutations_{(cCc \rightarrow cTc)} \times Context_{(c)}}{Mutations_{(C \rightarrow T)} \times Context_{(ccc)}}$$

- Produces sample-specific P-values
- Not affected by “topography” preferences

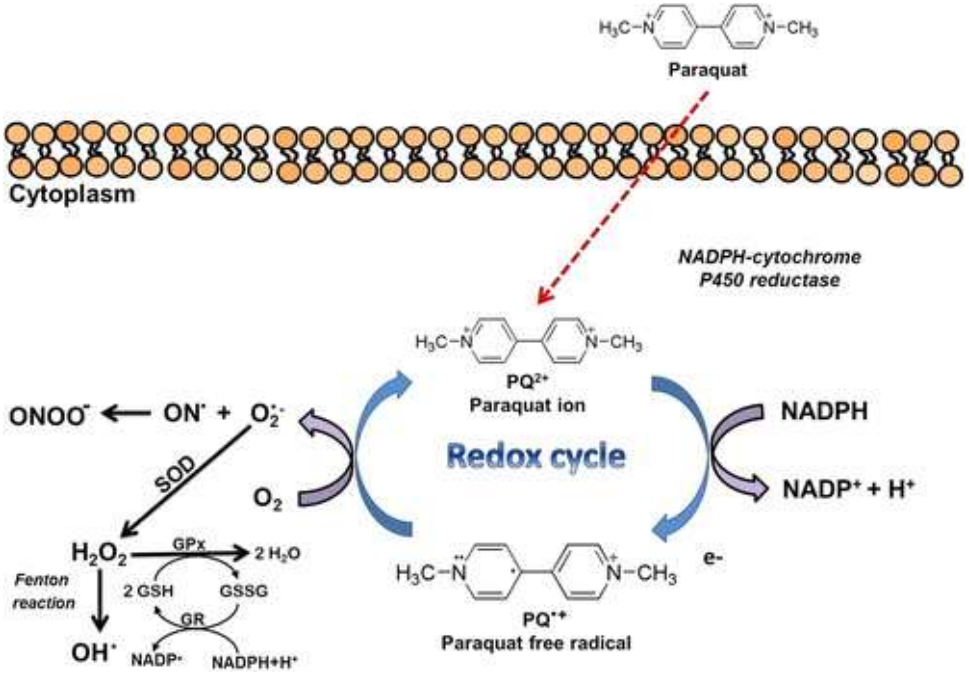
Roberts et al., 2012

Motif	Fold enrichment	Mutational load	Bonferroni-corrected Fisher P value
Cca to Tca	**3.00**	**21**	**1.95E-08**
cCca to cTca	**3.40**	**4**	**1.04E-02**
Cca toTna	**1.49**	**19**	**2.43E-04**

Is this signature hydrogen peroxide-specific?

Paraquat (PQ)

- Widely used herbicide
- Toxic to humans and animals
- Exposure has been linked to Parkinson's Disease

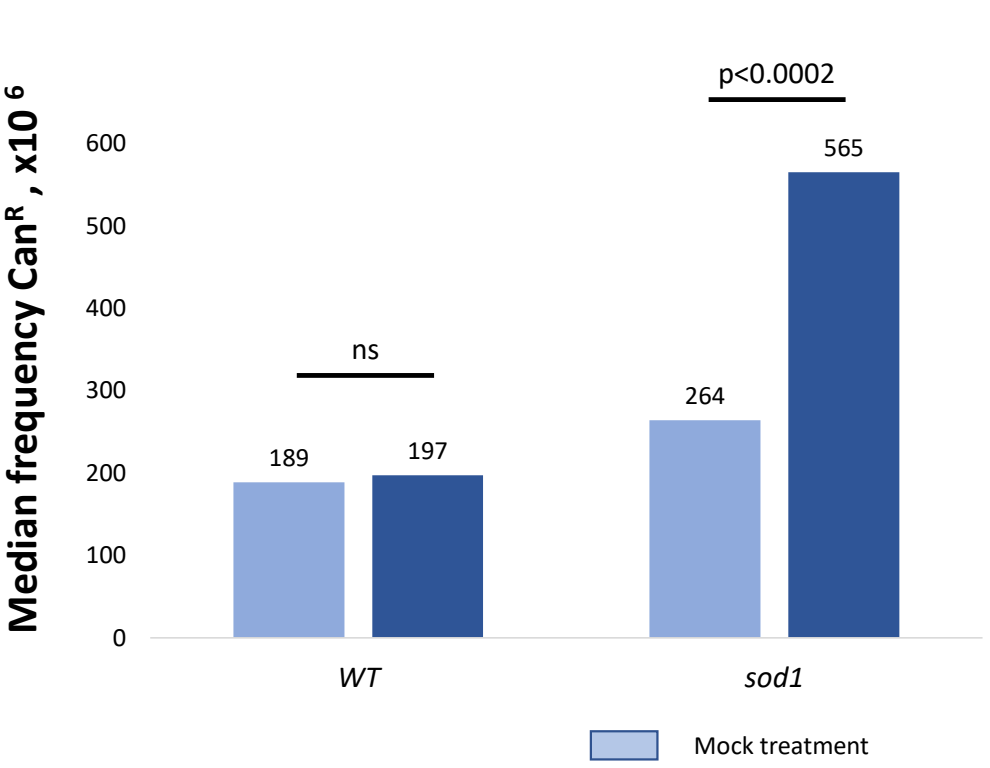


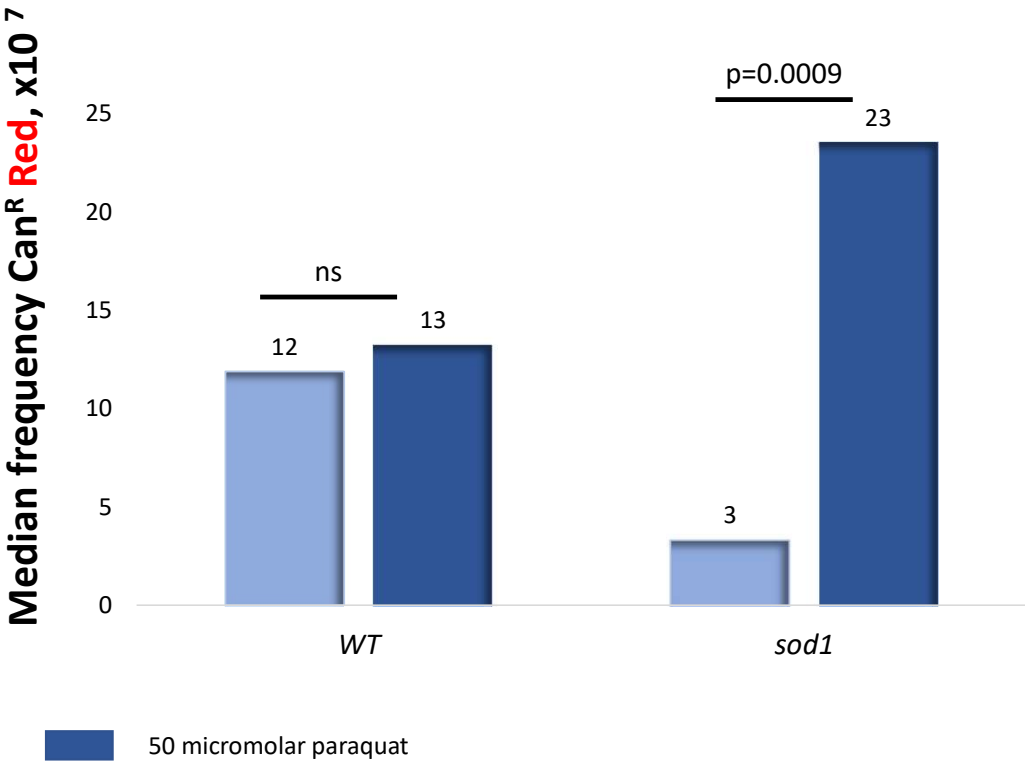
https://emergency.cdc.gov/agent/paraquat/basics/facts.asp

Blanco-Ayala et. al., Free Radical Research, 2014

Is there a signature of paraquat – induced mutagenesis?

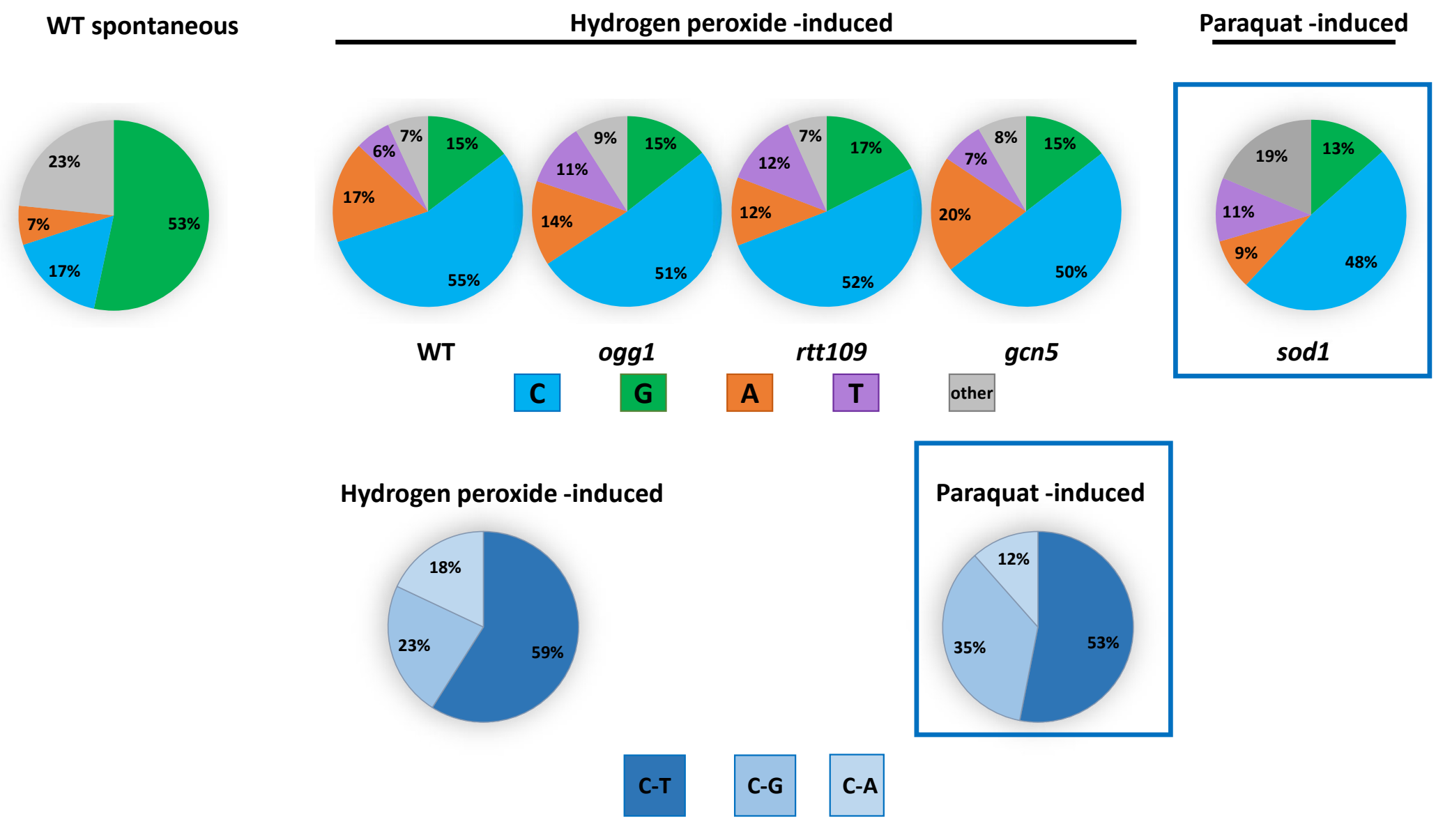
Exposure to paraquat increases mutation frequencies in *sod1* mutants





Mock treatment

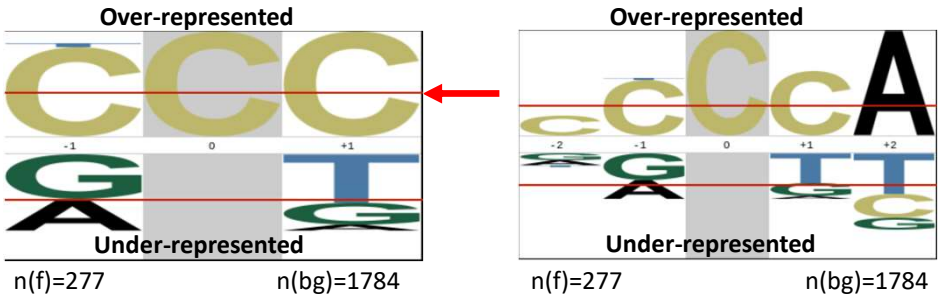
50 micromolar paraquat


WT spontaneous
Hydrogen peroxide -induced
Paraquat -induced
23%
7%
17%
53%
7%
6%
17%
15%
55%
9%
11%
14%
15%
51%
7%
12%
12%
17%
52%
8%
7%
20%
15%
50%
19%
11%
9%
13%
48%
WT
ogg1
rtt109
gcn5
sod1
C
G
A
T
other
Hydrogen peroxide -induced
Paraquat -induced
18%
23%
59%
12%
35%
53%
C-T
C-G
C-A

Mutational signatures of H_2O_2 and paraquat in ssDNA are similar

Hydrogen peroxide

WT

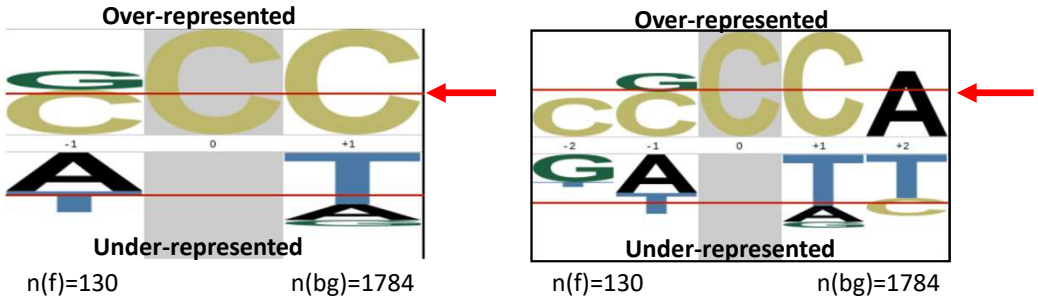


H_2O_2

cCca to cTca

Paraquat

sod1



$O_2^{\cdot-}$

Cca to Tca

Two different oxidizing agents, hydrogen peroxide and paraquat, generate similar mutational signatures in ssDNA in yeast

From yeast to men:

Is oxidative stress a major contributor to aging-related somatic mutations ?

Where to look for redox stress – related signatures in human DNA?

Mitochondria accumulate somatic mutations with age, but they are not oxidative stress-induced (?)

OPEN ACCESS Freely available online

PLOS | GENETICS

Ultra-Sensitive Sequencing Reveals an Age-Related Increase in Somatic Mitochondrial Mutations That Are Inconsistent with Oxidative Damage

Scott R. Kennedy[1], Jesse J. Salk[1,2], Michael W. Schmitt[1,2], Lawrence A. Loeb[1,3]*

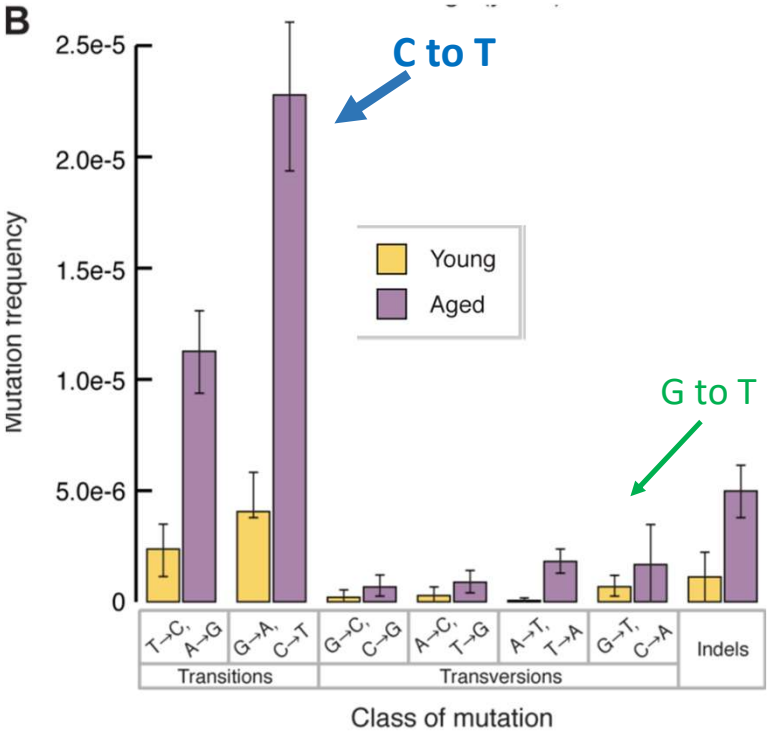
OPEN ACCESS Freely available online

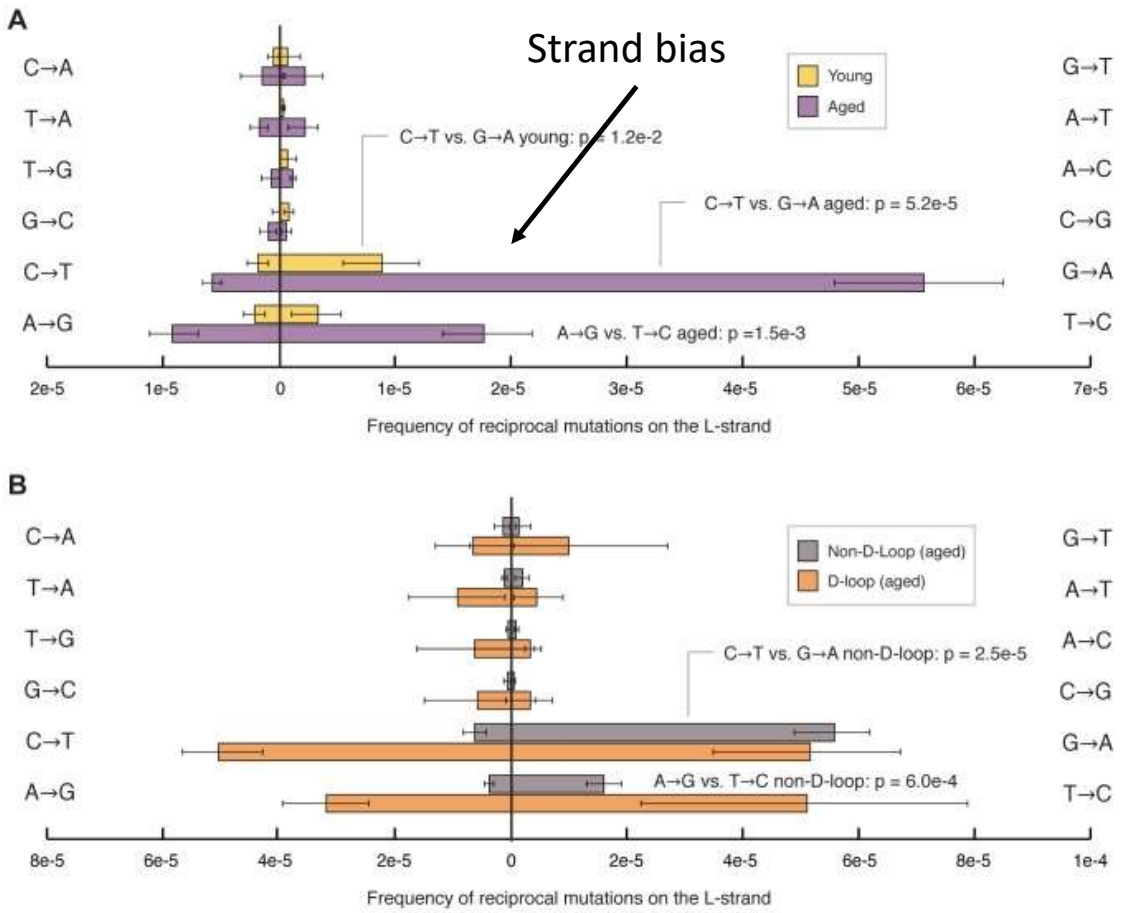
PLOS | GENETICS

Oxidative Stress Is Not a Major Contributor to Somatic Mitochondrial DNA Mutations

Leslie S. Itsara[1,2], Scott R. Kennedy[3], Edward J. Fox[3], Selina Yu[1], Joshua J. Hewitt[1,4], Monica Sanchez-Contreras[5], Fernando Cardozo-Pelaez[6], Leo J. Pallanck[1]*

The major type of somatic mutations in aging mitochondria is C to T changes





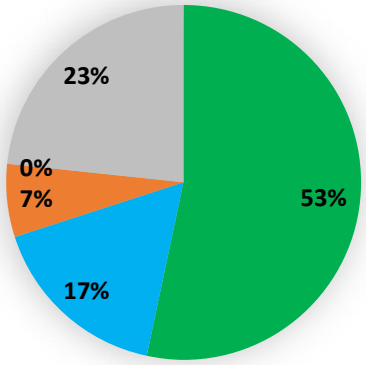
"Surprisingly, comparison of the mutation spectra of the young and old samples reveals a **notable absence of the mutational signature of oxidative damage**"... "We failed to find either a preponderance of **GtoT/CtoA** substitutions or a proportionally greater increase with age in this type of mutation relative to other types, despite a span of 80 years between our sequenced sample groups"

Kennedy et al., 2013

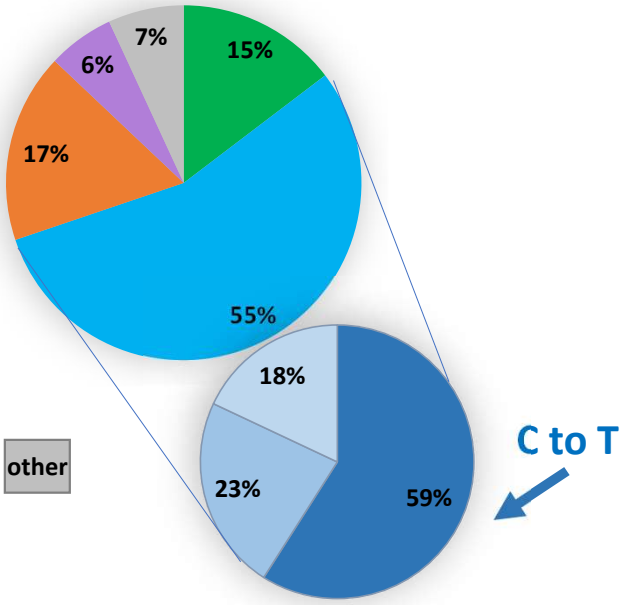
Mutational spectra of redox stress in yeast ssDNA and of aging in human mitochondrial DNA share a common feature

Yeast

Wt spontaneous

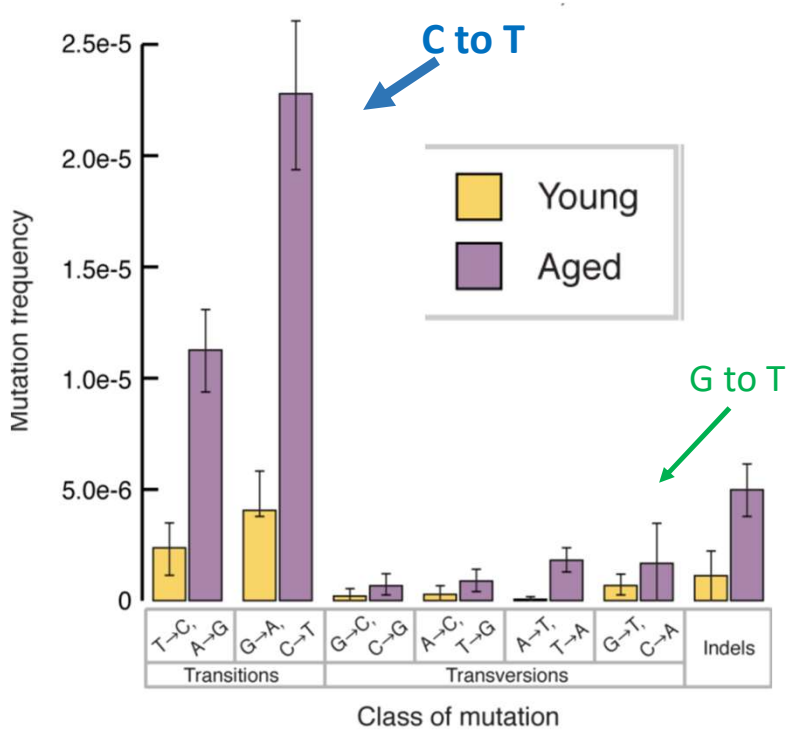


Hydrogen peroxide -induced



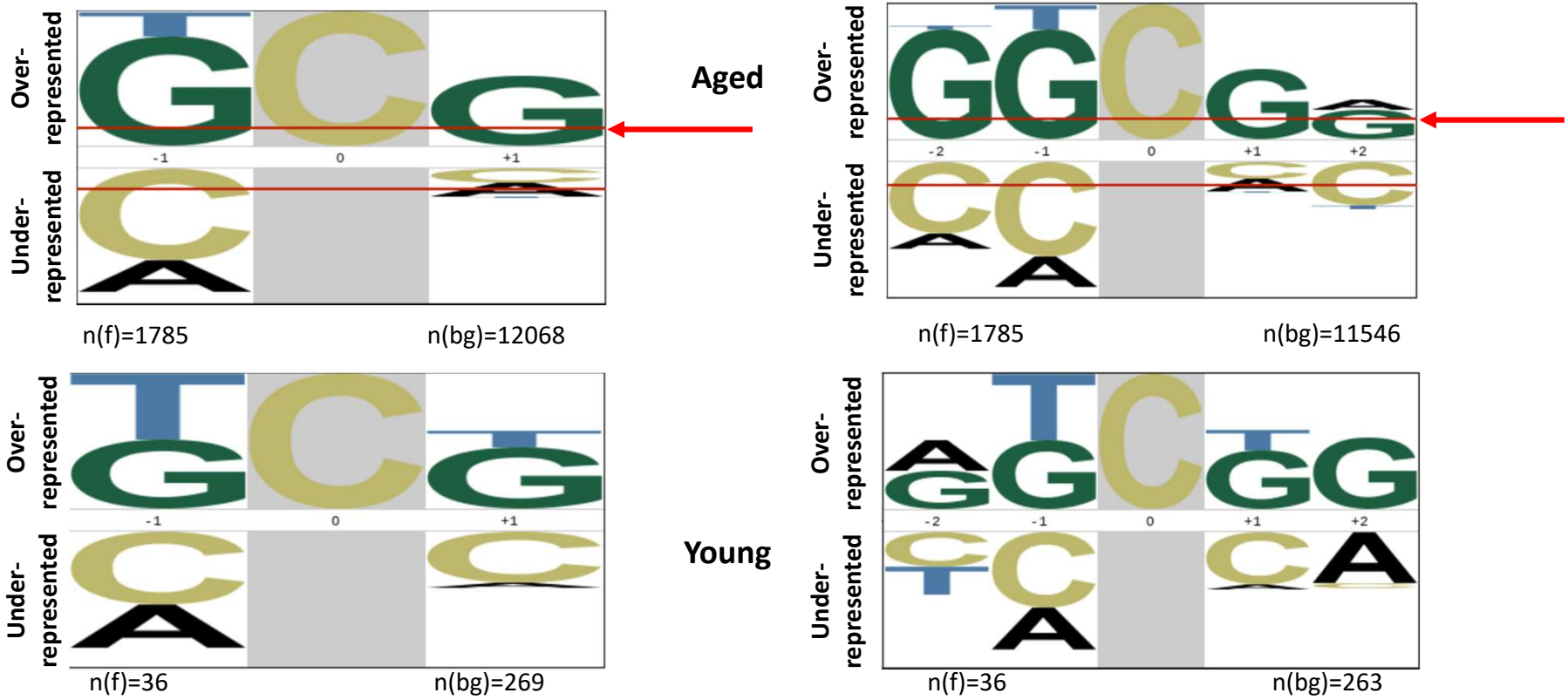
C G A T other

Men



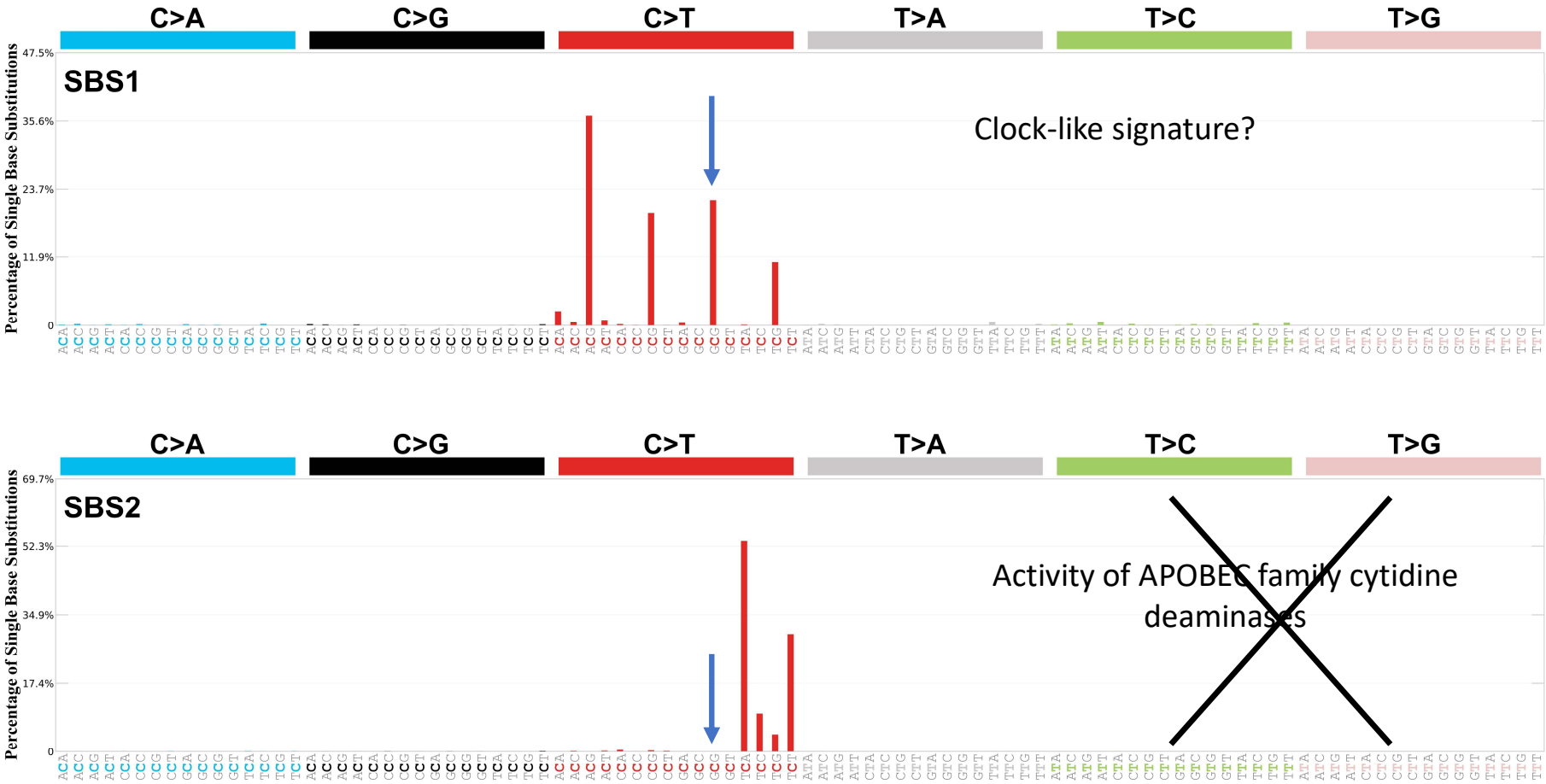
Kennedy et al., 2013

Is there a distinct signature of aging in mtDNA?

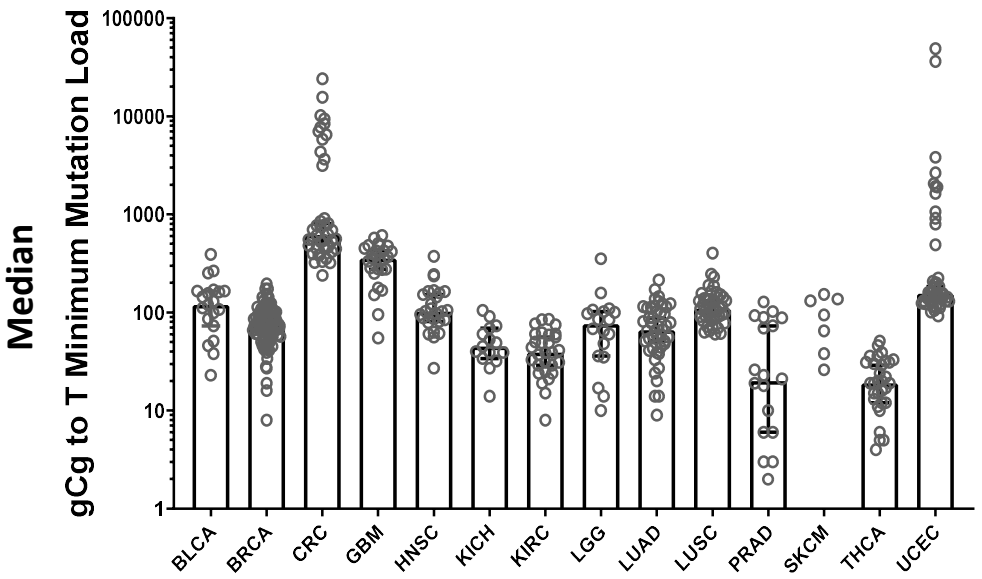


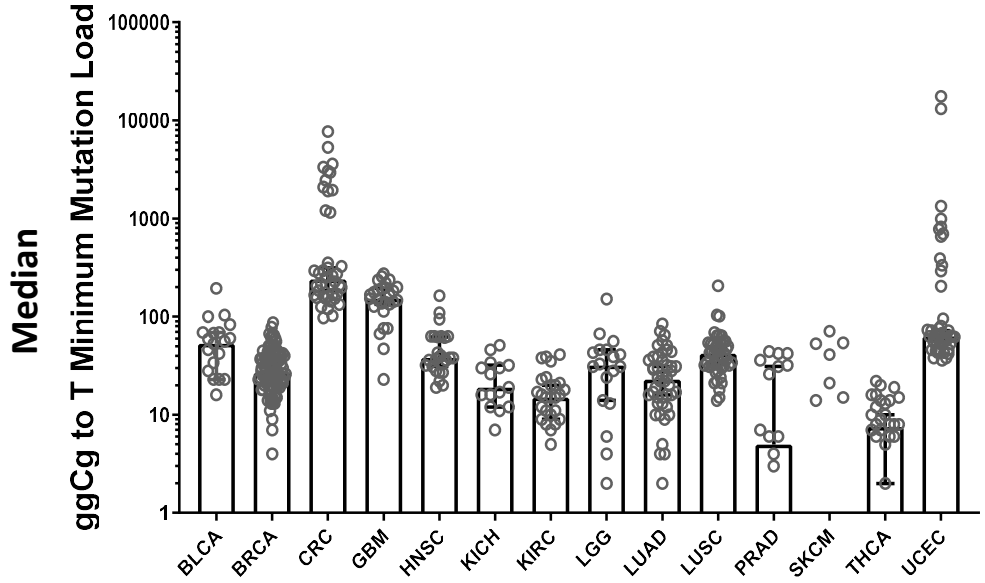
Motif	Fold enrichment	Mutational load	Bonferroni-corrected Fisher P value
gCg to gTg	2.44	25	3.26E-06
ggC to ggT	2.04	36	2.89E-07
ggCg to ggTg	3.10	15	6.50E-05

ggCg to ggTg signature of mitochondria aging: (methyl)cytosine deamination?



Evidence for redox-stress related signature in human cancers





Conclusions

- The majority of hydrogen peroxide- and paraquat-induced mutations in ssDNA occurs at C;
- C to T is the main type of redox stress-induced substitutions in ssDNA in yeast;
- 8oxoG is not the major redox stress-induced mutagenic lesion in ssDNA;
- The mutational signatures of hydrogen peroxide and paraquat in ssDNA in yeast are similar;
- Mutational signatures of redox stress in yeast ssDNA and of aging in human mitochondrial DNA share a common feature
- Many cancer genomes are enriched for redox stress-related signatures.

Questions

- What is the underlying DNA lesion at C?
- What are the molecular mechanisms of protection of ssDNA from oxidative damage?
- Do antioxidants prevent oxidative damage in ssDNA?
- Is it possible to find mutational signatures of oxidative stress in cancers by utilizing the new, broadened databases?
- Is there a mutational signature of redox stress in cells exposed to chronic inflammation?
- Are there other mutational signatures attributable to oxidative stress?

Acknowledgements

NIEHS

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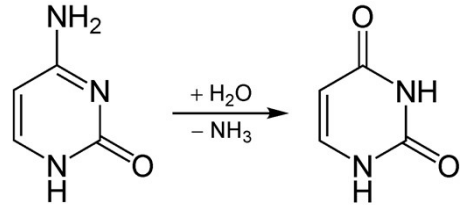
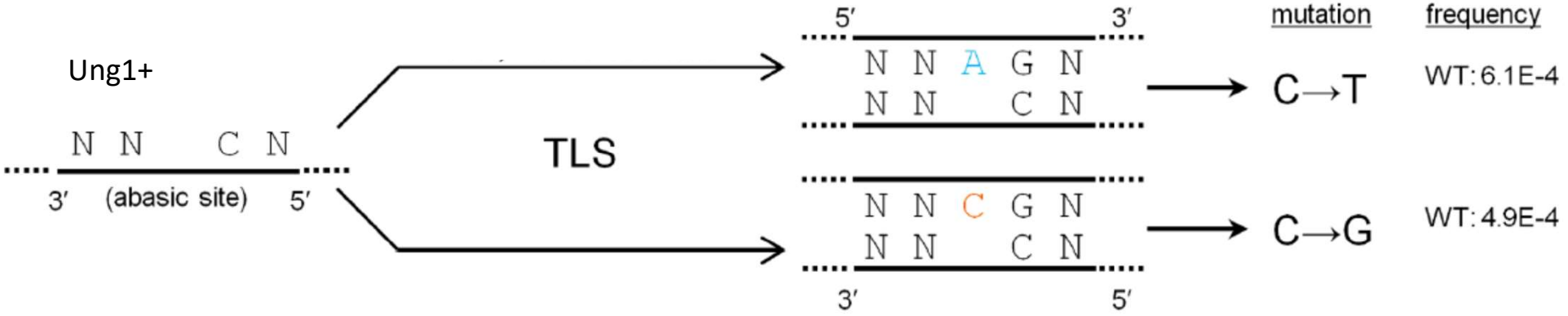
Scott Kennedy (University of Washington)

Cytosine deamination?





Frequency C to T = Frequency C to G

Chen et al., 2013