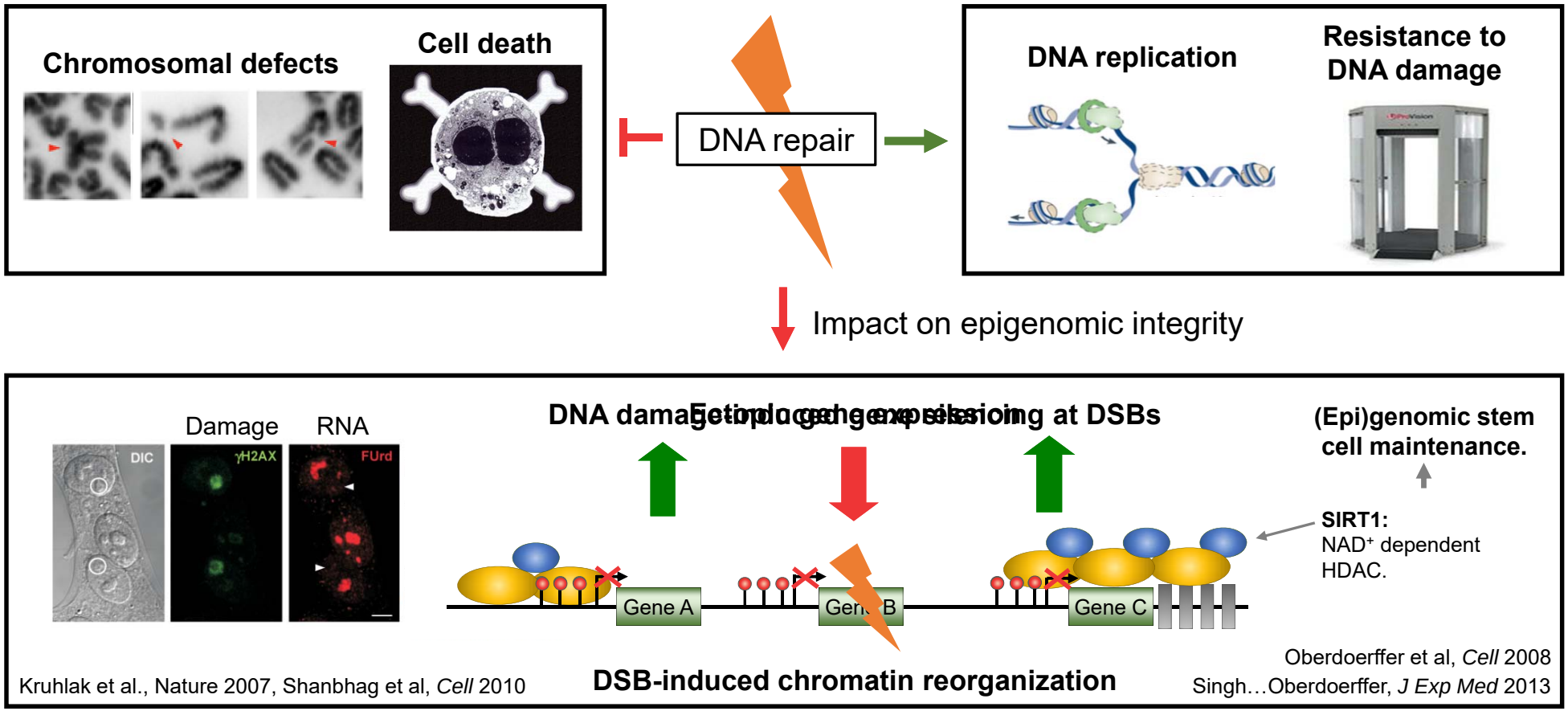
DNA damage within chromatin: *break it to shape it*

Philipp Oberdoerffer
NCI, Bethesda, MD

DNA Repair Interest Group – 2017

DNA repair: guarding genome and epigenome integrity?

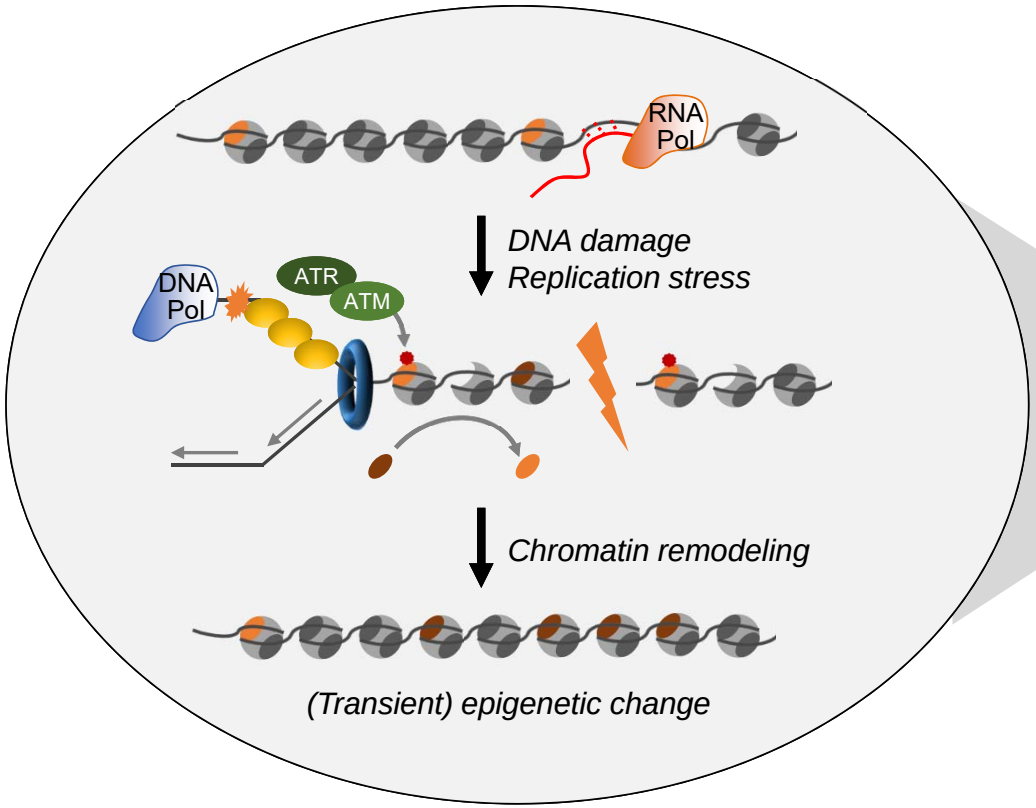


Kruhlak et al., Nature 2007, Shanbhag et al, *Cell* 2010

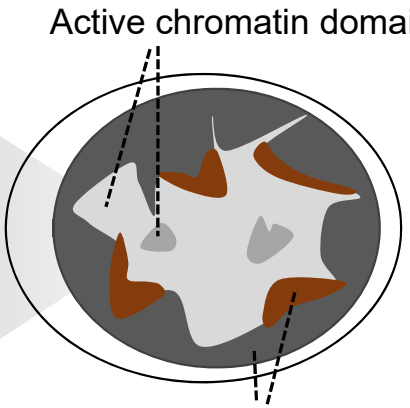
Oberdoerffer et al, *Cell* 2008

Singh…Oberdoerffer, *J Exp Med* 2013

Impact of DNA damage on chromatin organization



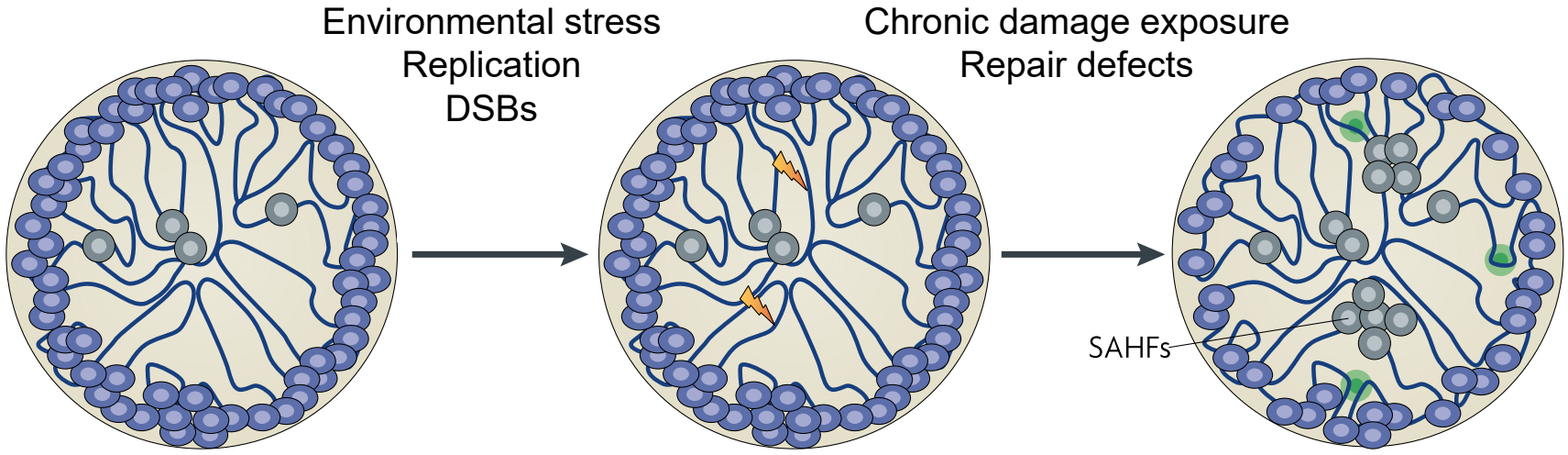
→ DNA damage as means to shape and/or modulate the epigenome?



Epigenome roadmap

Higher level chromatin organization determines cell identity and function

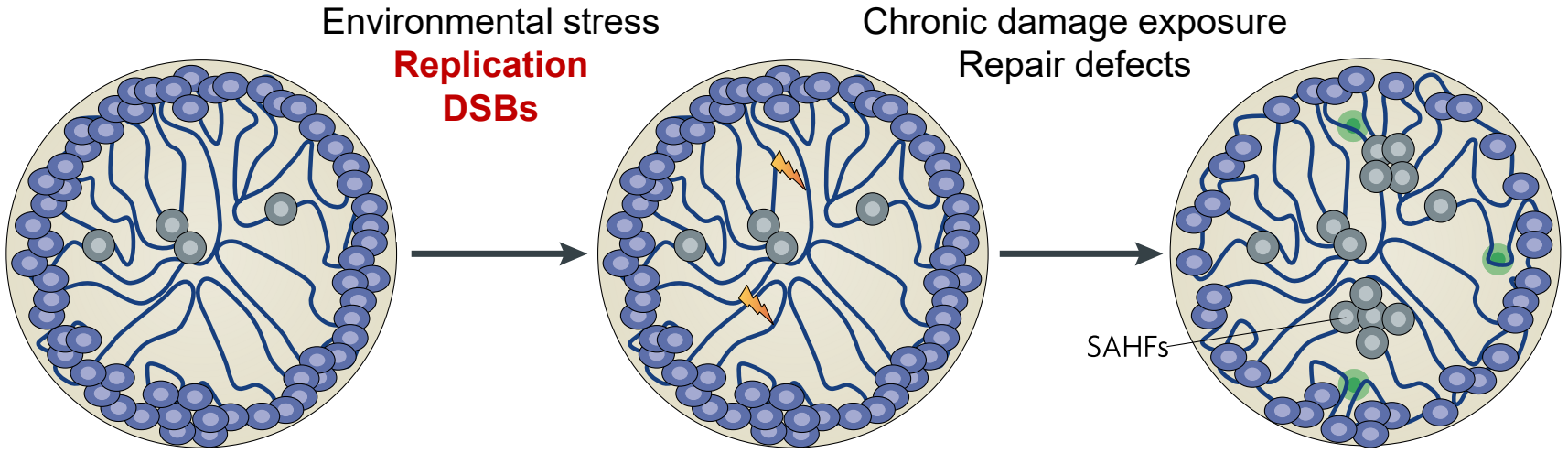
DNA damage as a driver of epigenomic change in aging and cancer?



Epigenetic changes/noise
Linked to both aging and cancer

Oberdoerffer and Sinclair, “Reorganization of Chromatin Modifiers Hypothesis” *Nature Reviews* 2007

DNA damage as a driver of epigenomic change in aging and cancer?



→ *Consequences of DSBs for transcriptional integrity in vivo?*

→ *Chronic DNA damage response as a means to shape chromatin?*

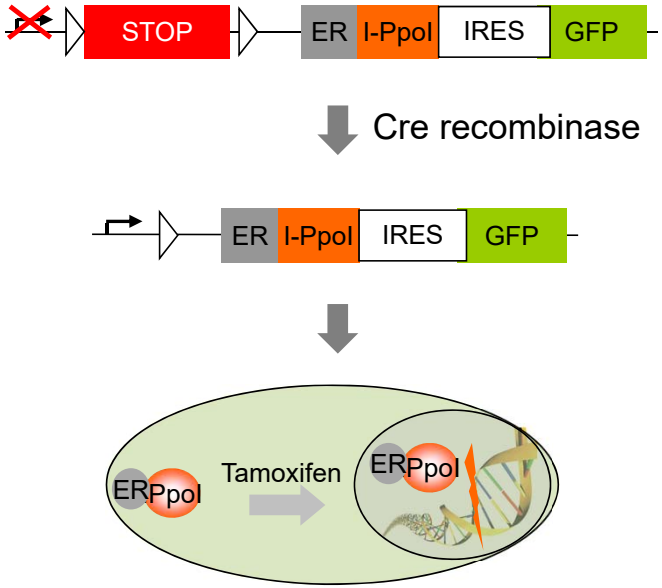
A mouse model for temporally and spatially controlled DSB induction

I-PpoI:

Intron-encoded endonuclease from ***P**hysarum **po**lycephalum*

→ *Cuts mouse genome at ~ 150 defined sites*

Inducible, tissue-specific I-PpoI mouse model



Transcriptional consequences upon T cell-specific DSB induction?

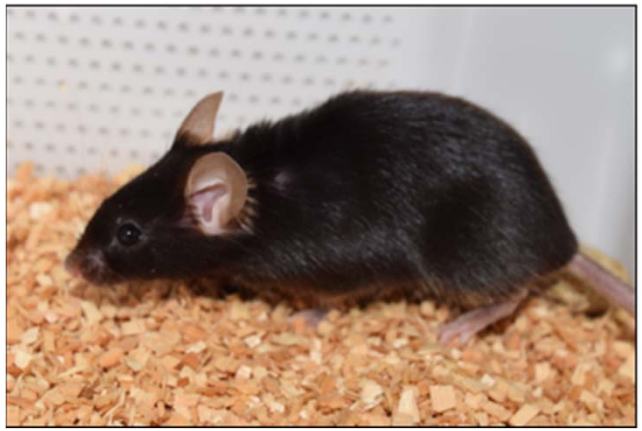
- I-PpoI causes gene repression specifically in DSB-containing genes.
- DSB-induced gene repression is transient and dependent on DNA damage signaling.

The mammalian transcriptome is sufficiently robust to accommodate short term DSB exposure.

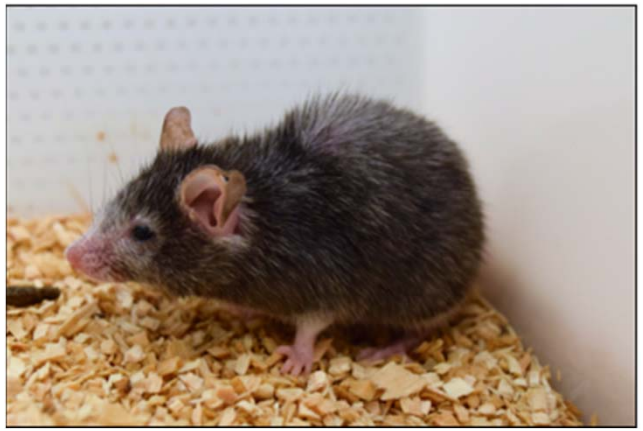
Kim et al., *Nucleic Acids Res* 2016

However…

Cre control (16 mo old)

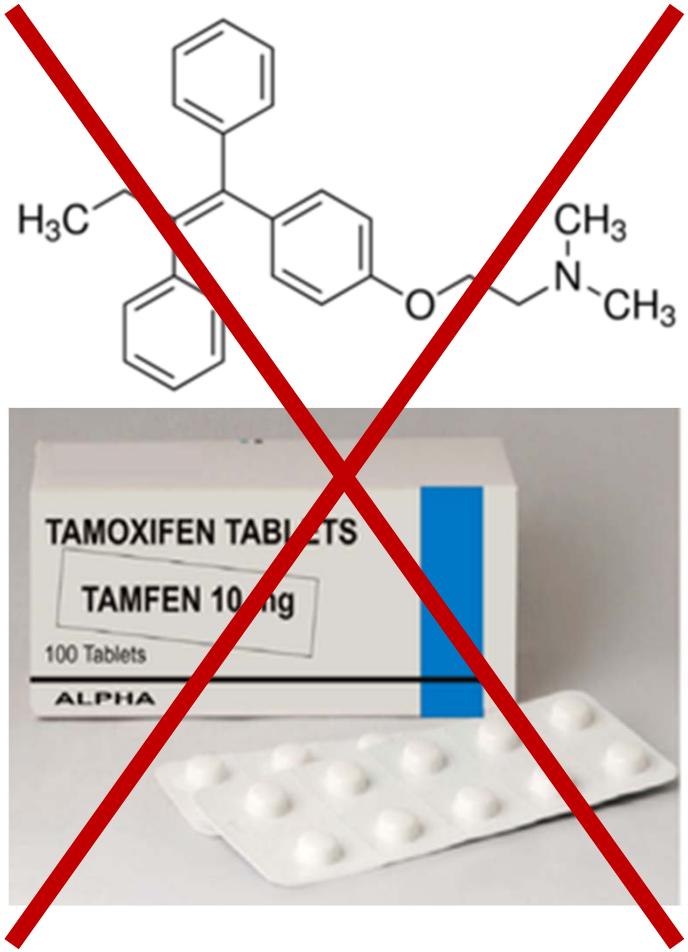


I-Ppo/Cre (16 mo old)



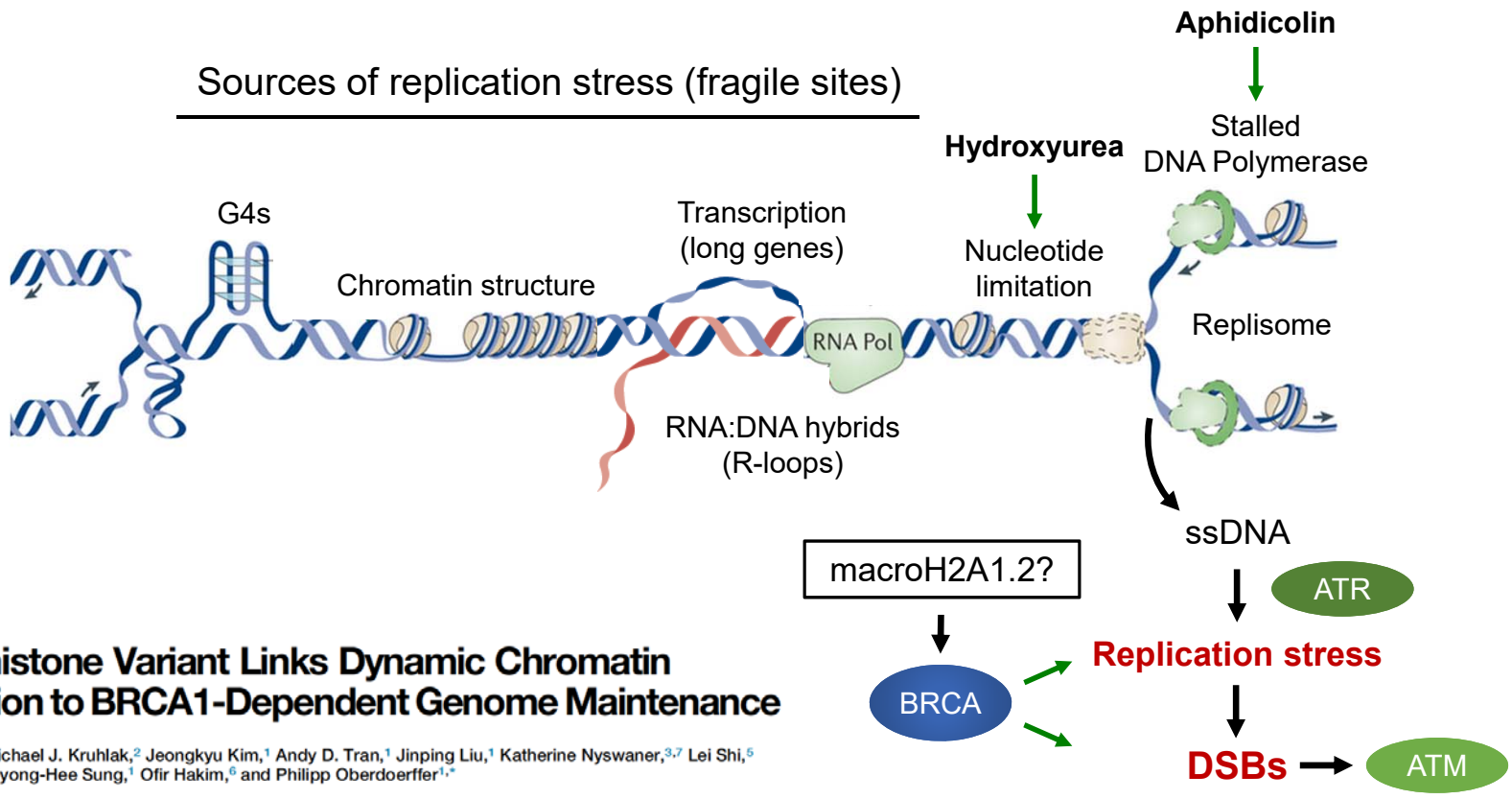
- Mice exposed to several weeks of DSB induction early in life
- Evidence for epigenetic memory of DSB induction…

Data from labs of David Sinclair, Shelley Berger and Rafa de Cabo



Can we test the impact of chronic DNA damage under physiological conditions?

Replication stress: a source of chronic, HR-associated DNA damage



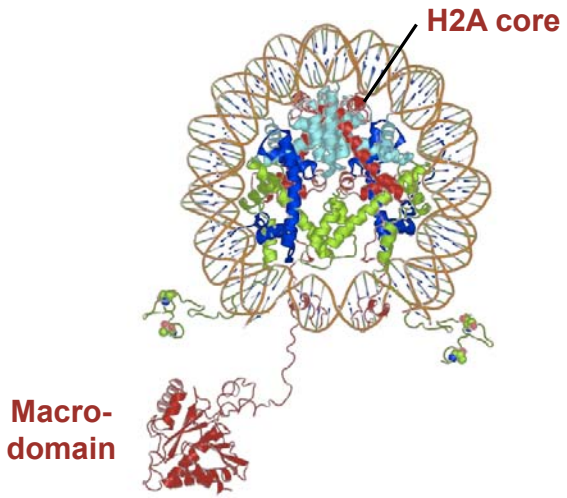
Cell Reports
Article

A Macrohistone Variant Links Dynamic Chromatin Compaction to BRCA1-Dependent Genome Maintenance

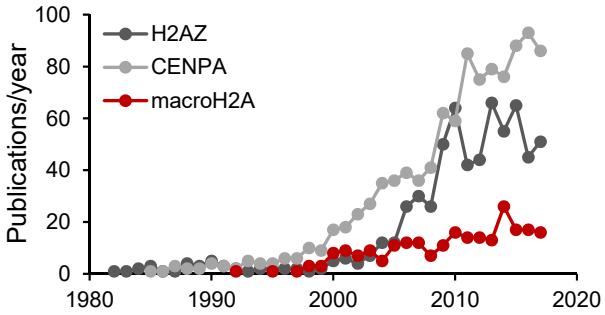
Simran Khurana,[1] Michael J. Kruhlak,[2] Jeongkyu Kim,[1] Andy D. Tran,[1] Jinping Liu,[1] Katherine Nyswaner,[3,7] Lei Shi,[5] Parthav Jailwala,[4] Myong-Hee Sung,[1] Ofir Hakim,[6] and Philipp Oberdoerffer[1,*]

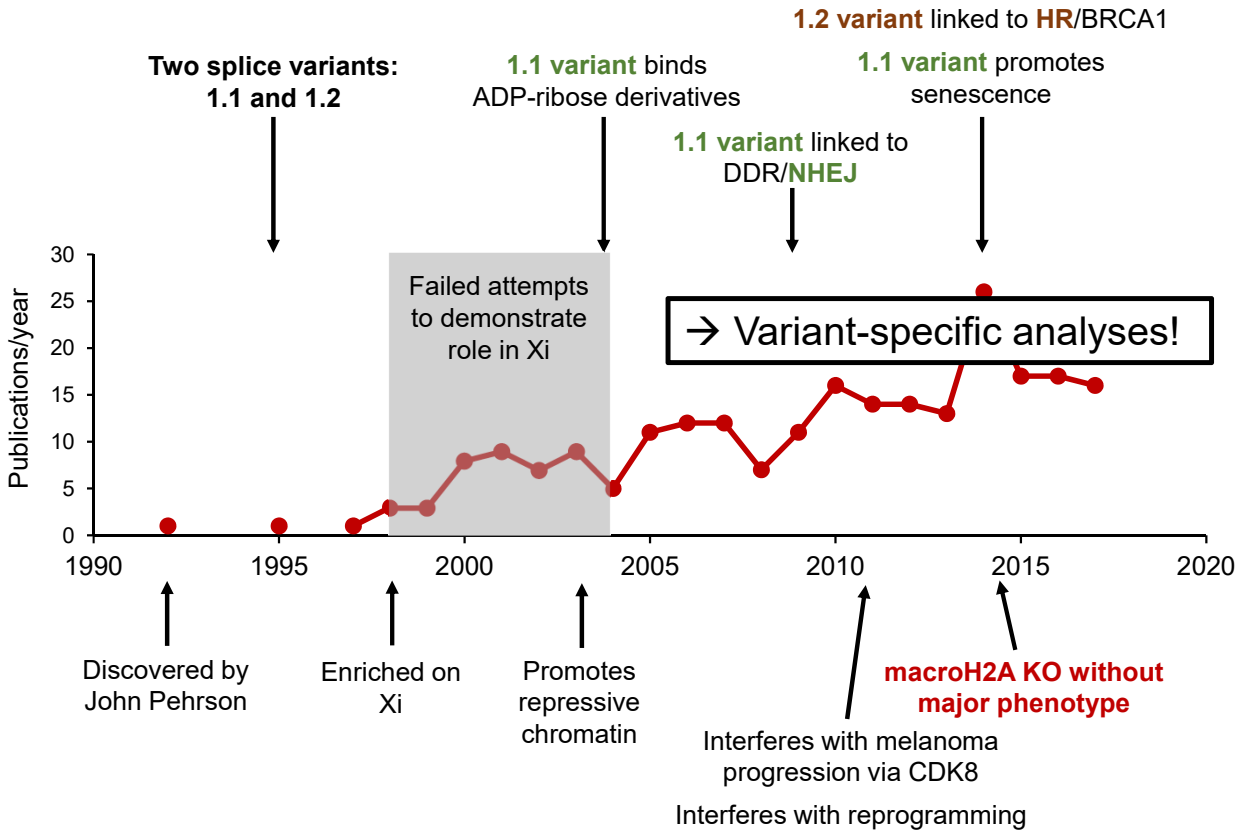
Adapted from Gaillard…Aguilera, *Nat Rev Cancer* 2015

MacroH2A1 – a unique yet underappreciated histone variant



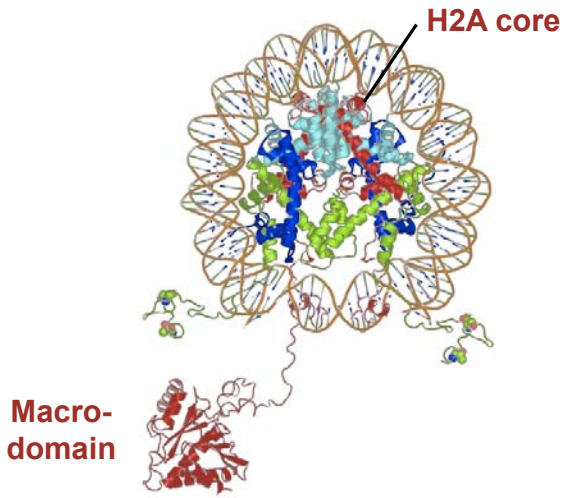
Papers on histone variants





Pehrson, Luger, Bernstein, Ladurner, Gamble, Price, Buschbeck and others…

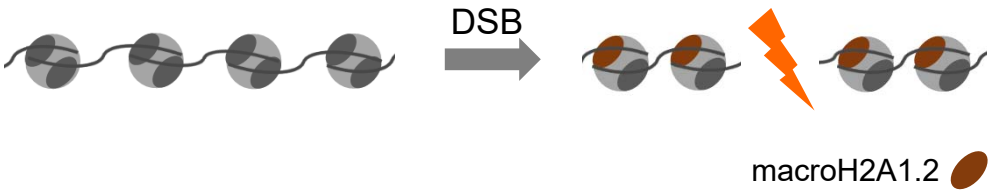
The macroH2A1.2 variant promotes BRCA1-dependent DSB repair



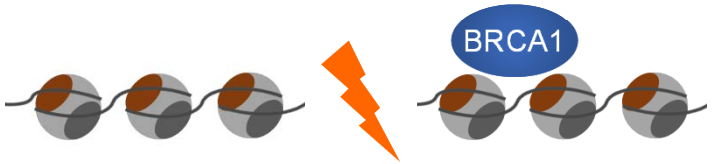
- RNAi screen identified **macroH2A1.2-specific** role in homology-directed DSB repair

Implications for (epi)genome maintenance?

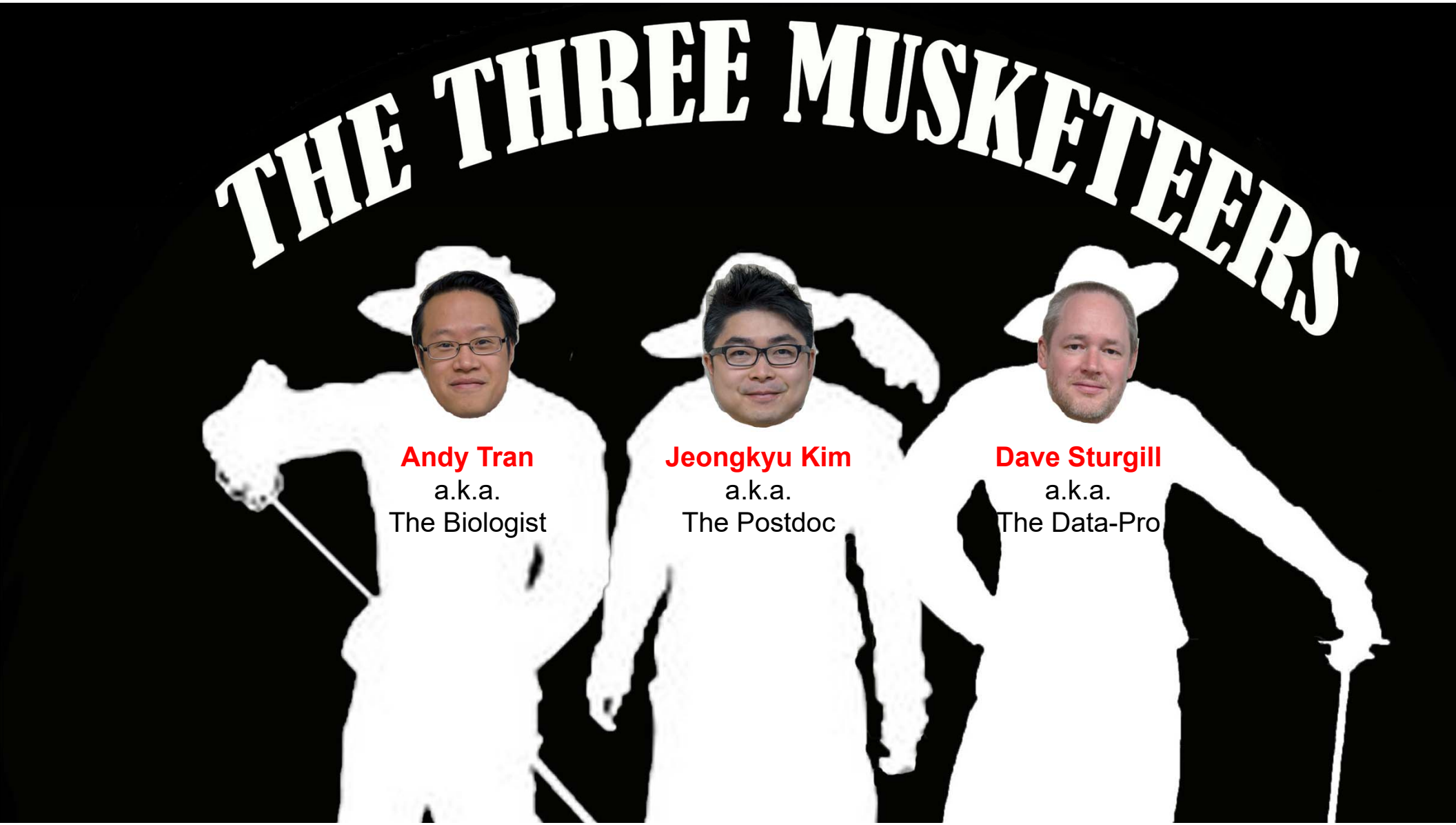
→ **MacroH2A1.2 promotes dynamic chromatin condensation at DSBs**



→ **Facilitates BRCA1 accumulation, end resection and HR**

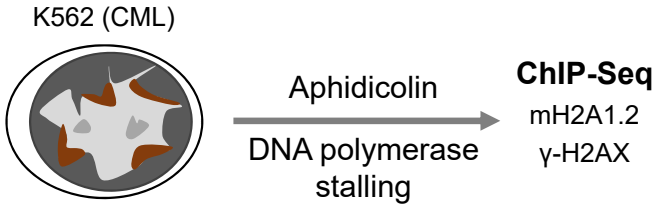


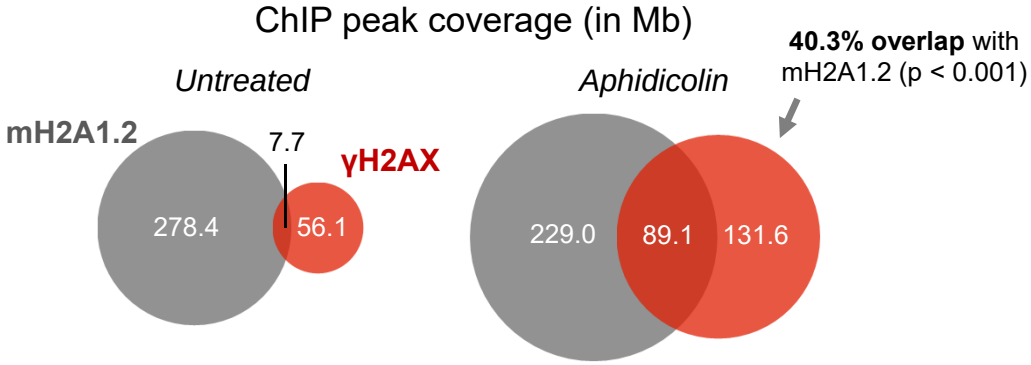
Khurana et al., *Cell Reports* 2014


THE THREE MUSKETEERS
Andy Tran
a.k.a.
The Biologist
Jeongkyu Kim
a.k.a.
The Postdoc
Dave Sturgill
a.k.a.
The Data-Pro

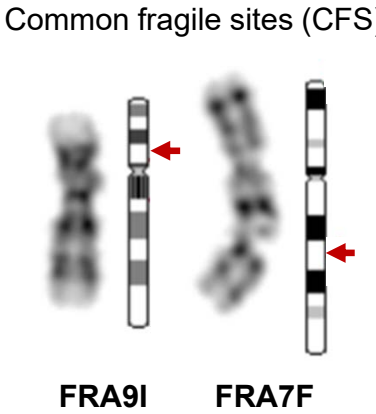
Replication stress drives macroH2A1.2 accumulation at fragile sites genome-wide

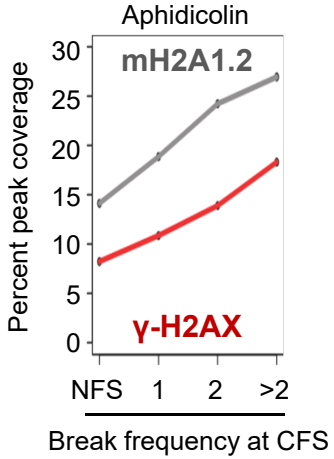
A role for macroH2A1.2 in replication stress?

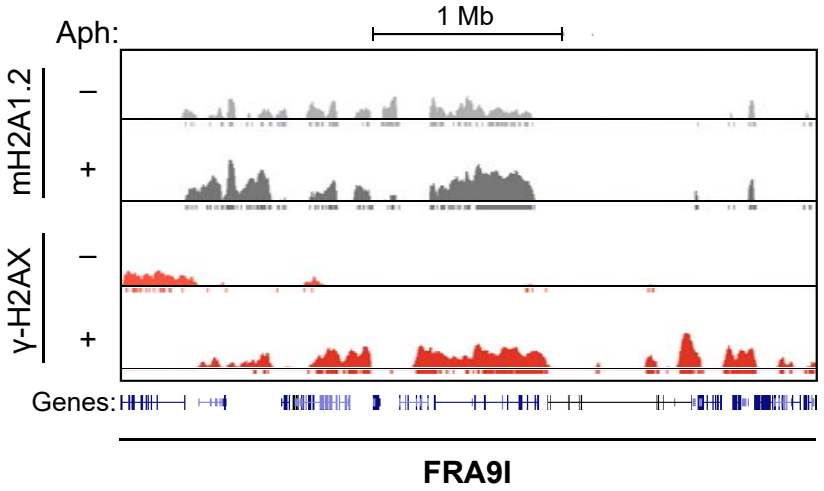




Relationship to sites of replication stress?

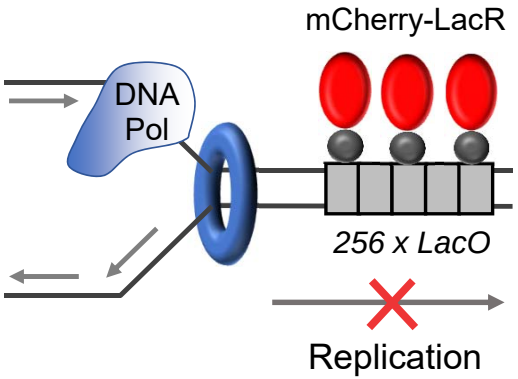


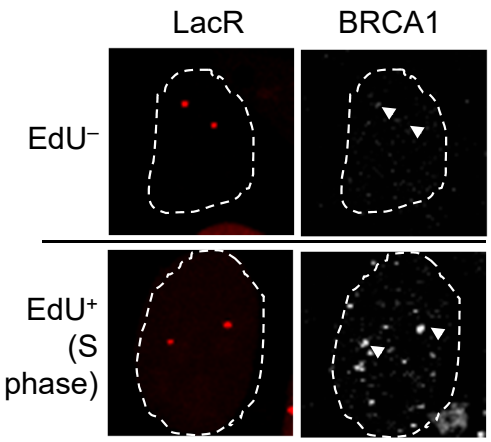




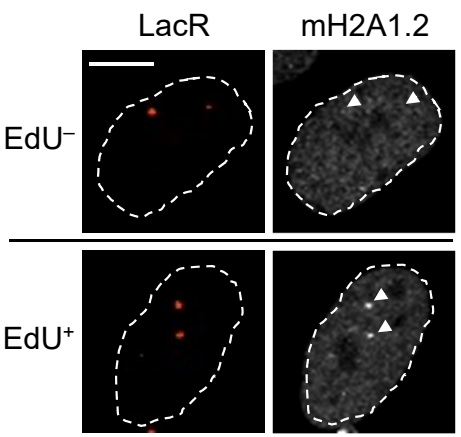
macroH2A1.2 accumulates at artificial replication blocks

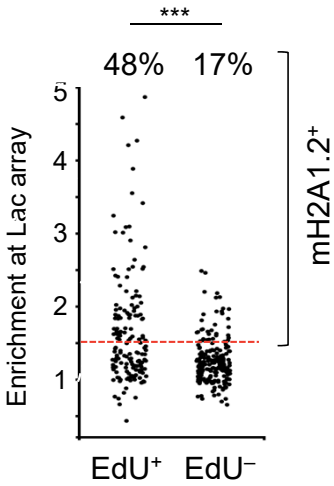
A system for locally defined replication fork arrest





macroH2A1.2 accumulates at artificial replication stops



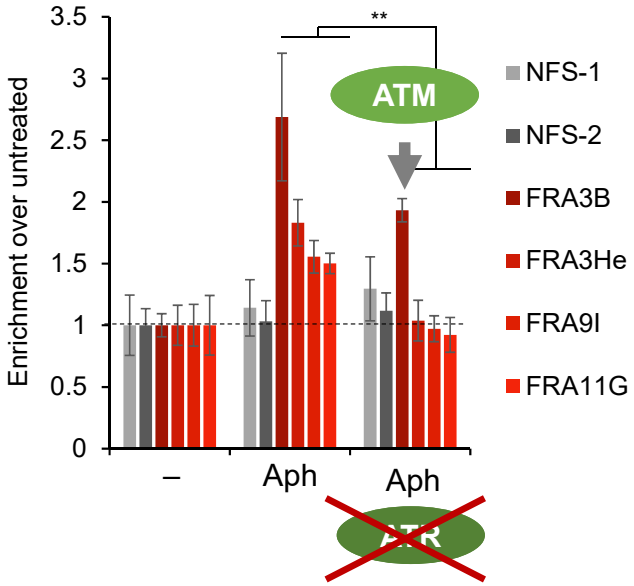


→ Link to the replication stress response?

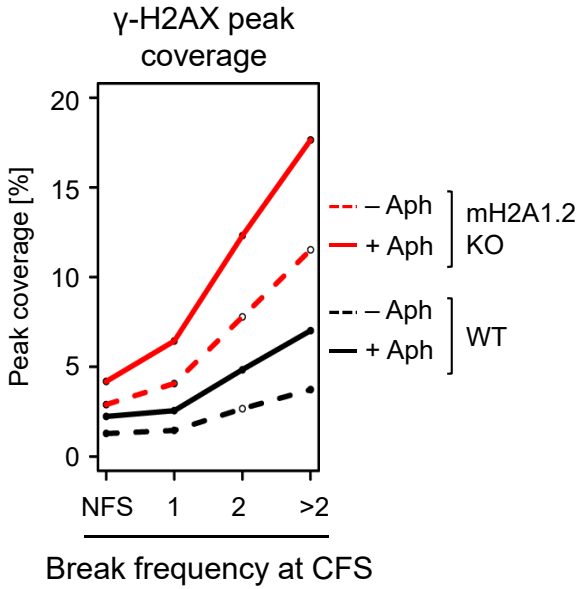
Beuzer…Almouzni, *Cell Cycle* 2014

Role of macroH2A1.2 during replication stress

RS-induced mH2A1.2 accumulation is DDR-dependent



MacroH2A1.2 loss causes increased DNA damage at CFSs

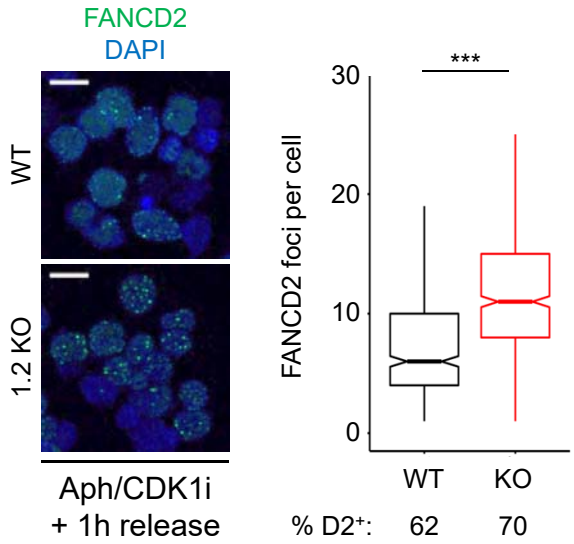


Also by comet assay…

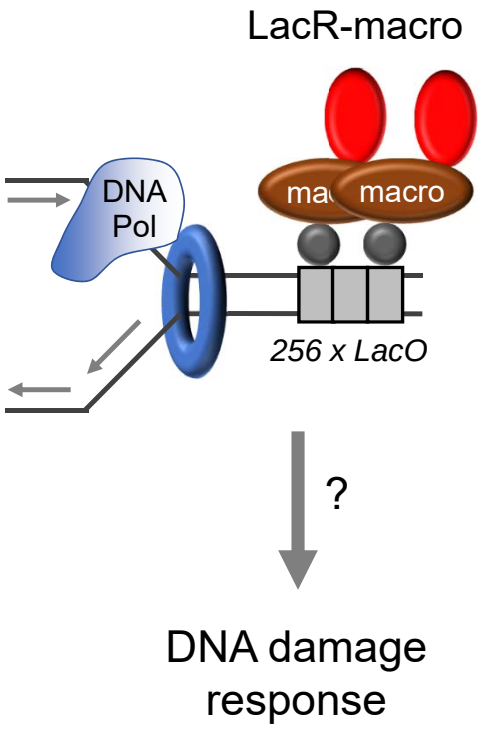
Molecular Cell
Article

FANCD2 Facilitates Replication through Common Fragile Sites

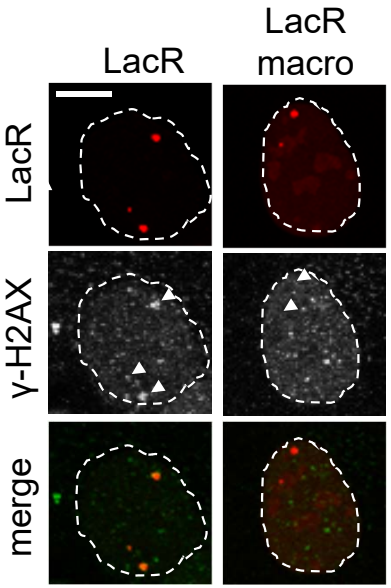
Advaitha Madireddy,[1,*] Settapong T. Kosiyatrakul,[1] Rebecca A. Boisvert,[2] Emilia Herrera-Moyano,[3] María L. García-Rubio,[3] Jeannine Gerhardt,[1] Elizabeth A. Vuono,[2] Nichole Owen,[4] Zi Yan,[1] Susan Olson,[4] Andrés Aguilera,[3] Niall G. Howlett,[2] and Carl L. Schildkraut[1,5,*]

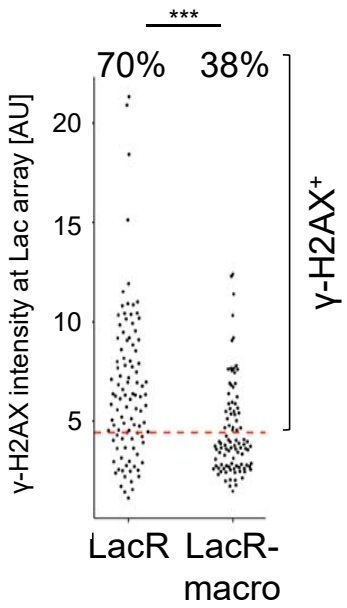


Can macroH2A1.2 protect from replication stress-induced damage?



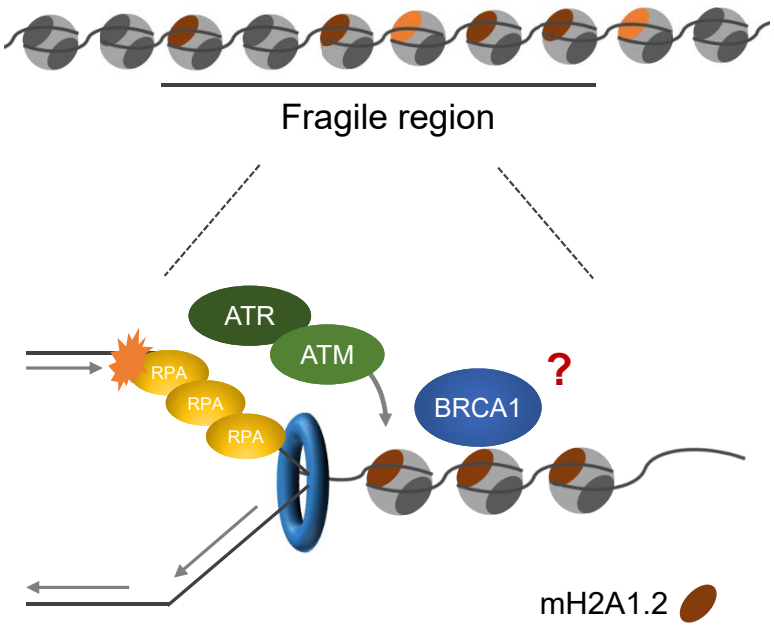
The macro domain protects from DNA damage at replication blocks



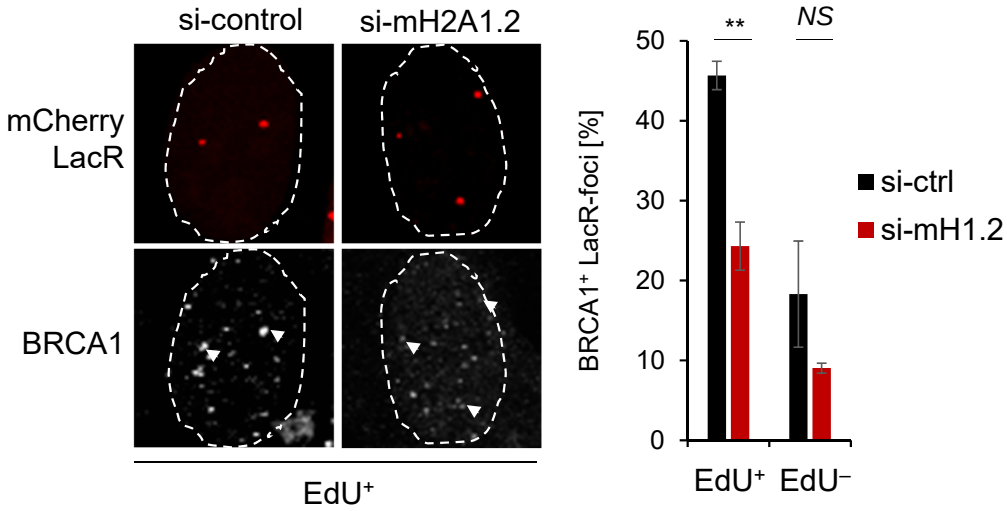


→ Molecular basis for macroH2A1.2-dependent protection from RS?

macroH2A1.2 facilitates BRCA1 accumulation upon replication stress

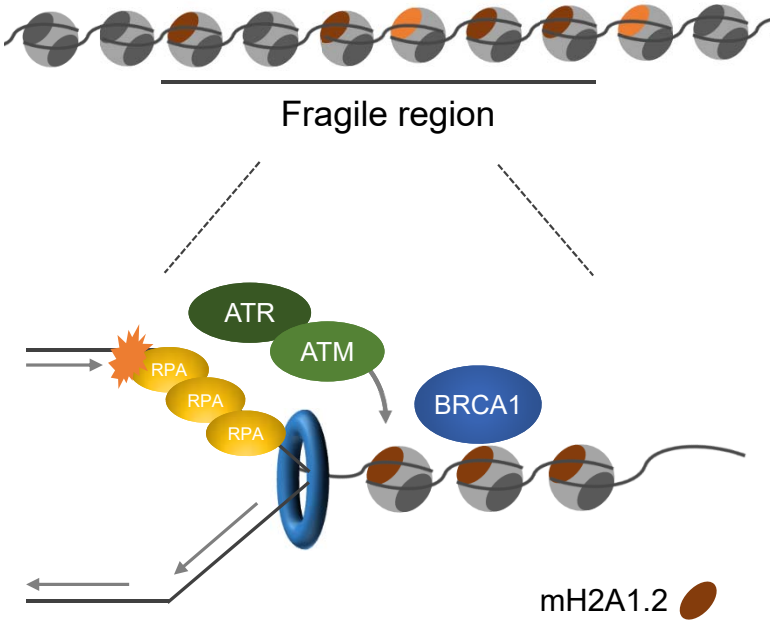


macroH2A1.2 promotes BRCA1 accumulation at replication blocks



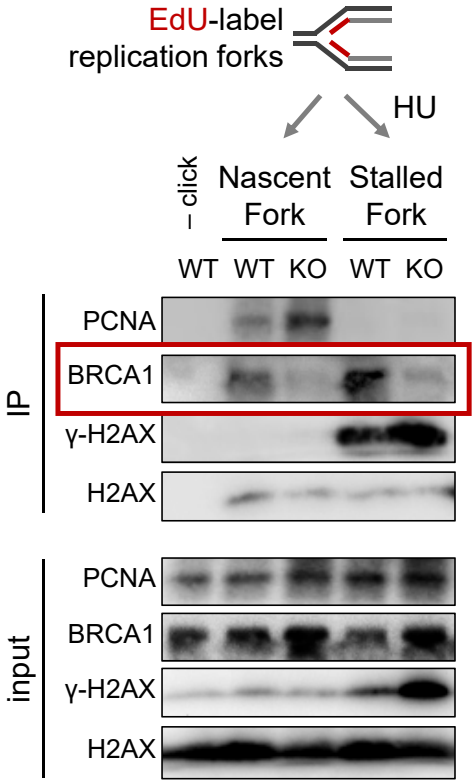
→ **Fork specific effect?**

macroH2A1.2 promotes BRCA1 recruitment to replication forks

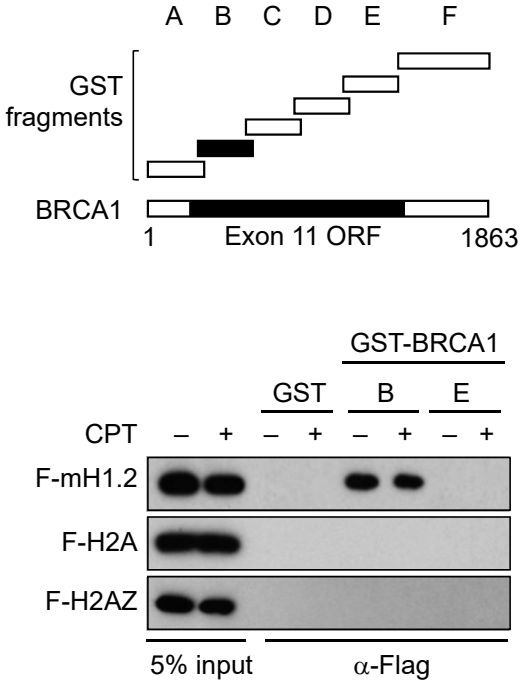


→ **What mediates macroH2A1.2 accumulation upon replication stress?**

Isolation of proteins on nascent DNA (iPOND)



BRCA1 interacts with macroH2A1.2

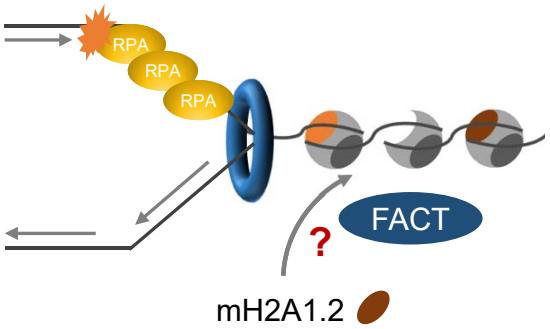


No known macroH2A1.2 chaperones!

Histone or variant	Tissues (location within the genome)	Chaperones and remodellers	Function
MacroH2A1.1	All (facultative and constitutive heterochromatin)	Not known	Not known
MacroH2A1.2	All (facultative and constitutive heterochromatin)	ATRX (remodeller)	Negative regulation, possibly indirect
MacroH2A2	All (facultative and constitutive heterochromatin)	Not known	Not known

Buschbeck & Hake, Nat. Reviews, **2017**

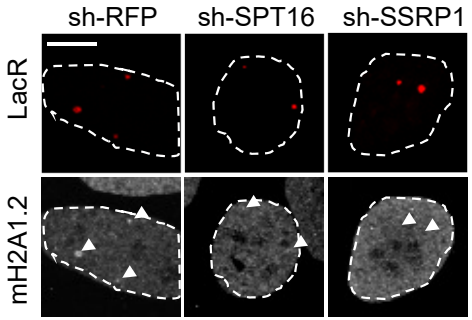
FACT remodels H2A/H2B dimers at replication forks

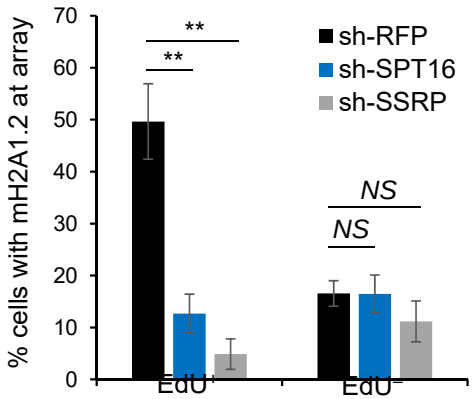


- FACT (SUPT16H/SSRP1) complex is the main H2A/H2B chaperone at replication forks.
- FACT loss causes HR and replication defects.

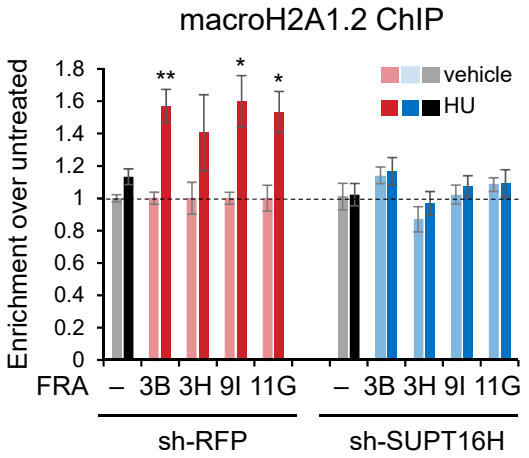
→ **FACT mediates macroH2A1.2 accumulation in a *replication stress-dependent* manner.**

FACT subunits promote RS-associated mH2A1.2 accumulation at Lac arrays…





… and common fragile sites.

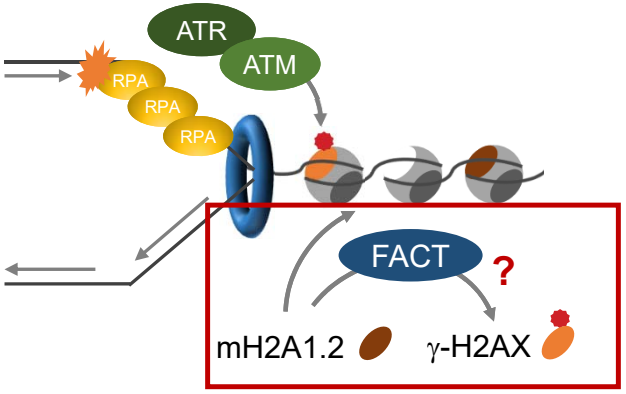


→ FACT interacts with macroH2A1.2/H2B dimer in vitro and in vivo.

Reinberg, Diffley, Aguilera, Formosa, Stillman, others…

With help from Luger lab

MacroH2A1.2 accumulation at fragile sites requires H2AX phosphorylation



- FACT (SUPT16H/SSRP1) complex is the main H2A/H2B chaperone at replication forks.
- FACT loss causes HR and replication defects.

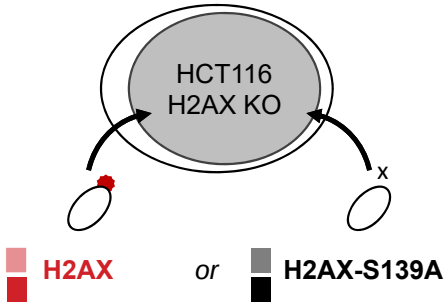
→ **FACT mediates macroH2A1.2 accumulation in a *replication stress-dependent* manner.**

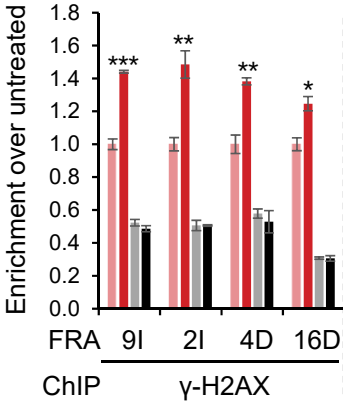
→ **MacroH2A1.2 incorporation <u>also</u> depends on DNA damage signaling.**

Molecular Cell
Article

FACT-Mediated Exchange of Histone Variant H2AX Regulated by Phosphorylation of H2AX and ADP-Ribosylation of Spt16

Kyu Heo,[1] Hyunjung Kim,[1] Si Ho Choi,[2] Jongkyu Choi,[1] Kyunghwan Kim,[1] Jiafeng Gu,[1,3,4,5] Michael R. Lieber,[1,3,4,5] Allen S. Yang,[2] and Woojin An[1,*]

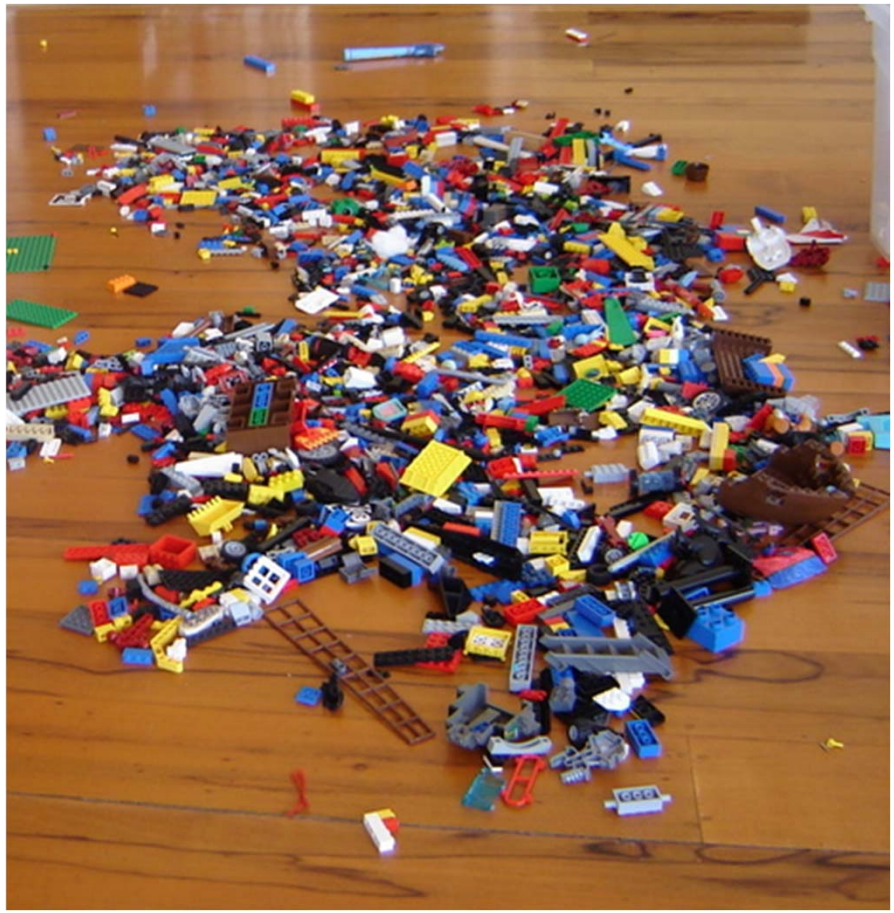




→ MacroH2A1.2 incorporation requires H2AX phosphorylation.

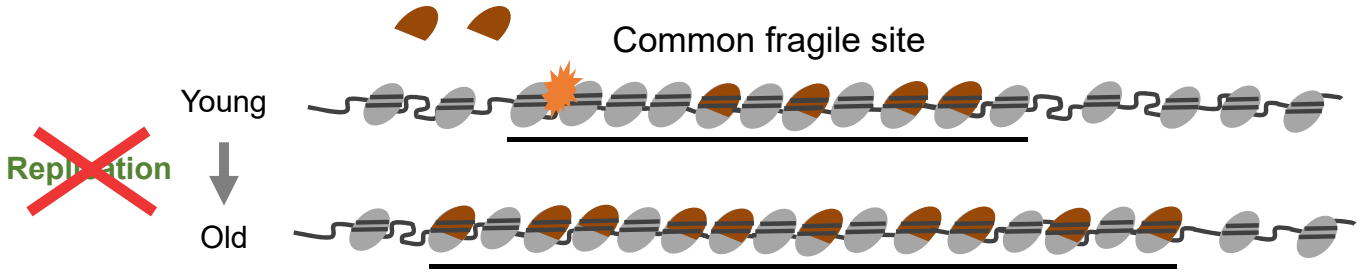
Urbain Weyemi / Bill Bonner

Potential to shape chromatin during continued replication stress?

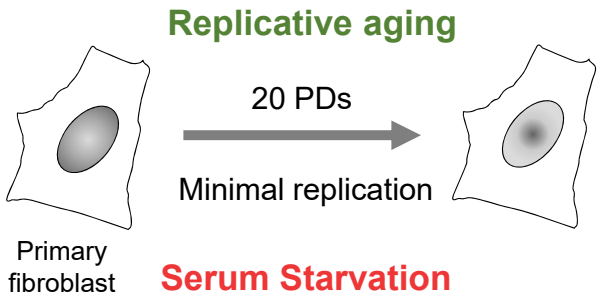


The Lego problem…

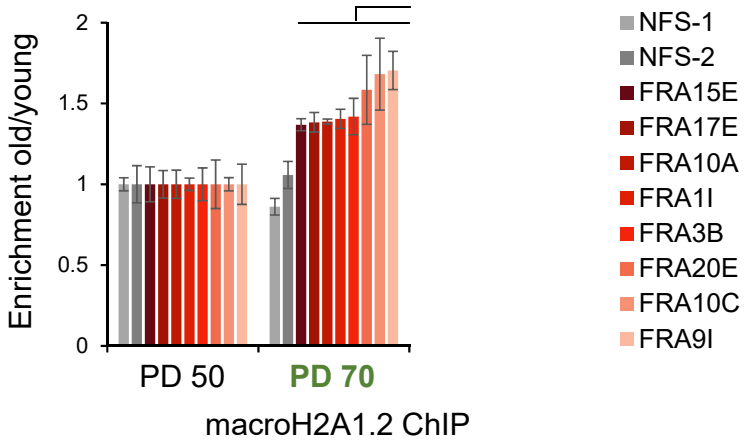
Continuous replication drives persistent macroH2A1.2 accumulation



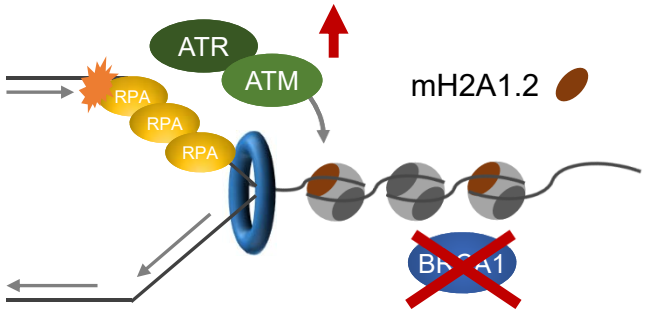
→ **Dependence on replication?** ✓



Replicative age causes mH2A1.2 accumulation at CFSs



Consequences of macroH2A1.2 loss in primary cells

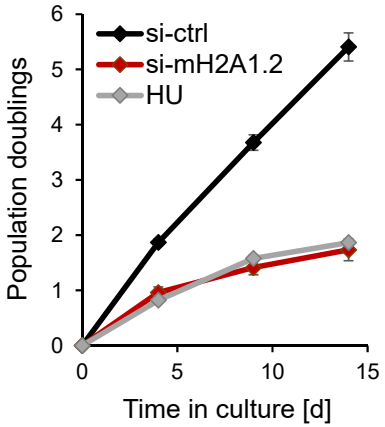


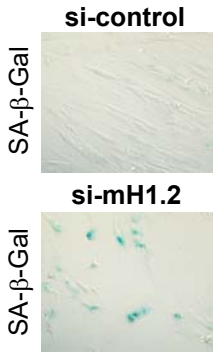
→ Barrier to malignant transformation

Replication stress → DDR → **Senescence**

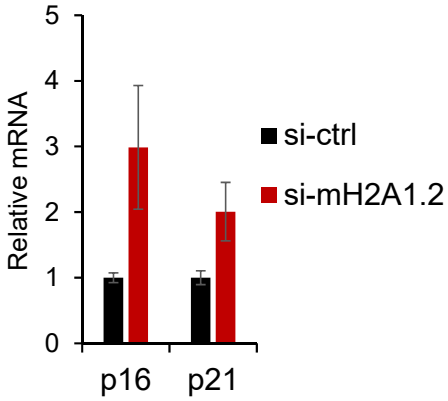
macroH2A loss?

macroH2A loss promotes senescence

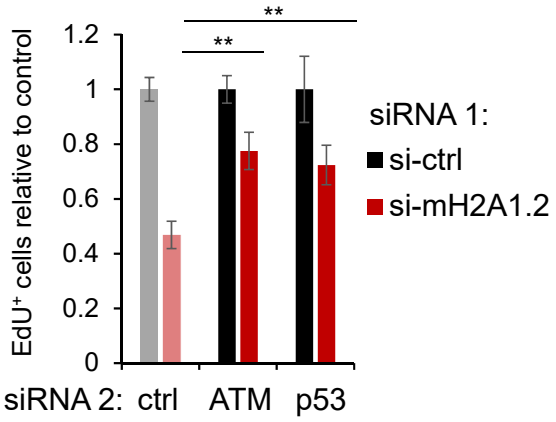




DDR is activated

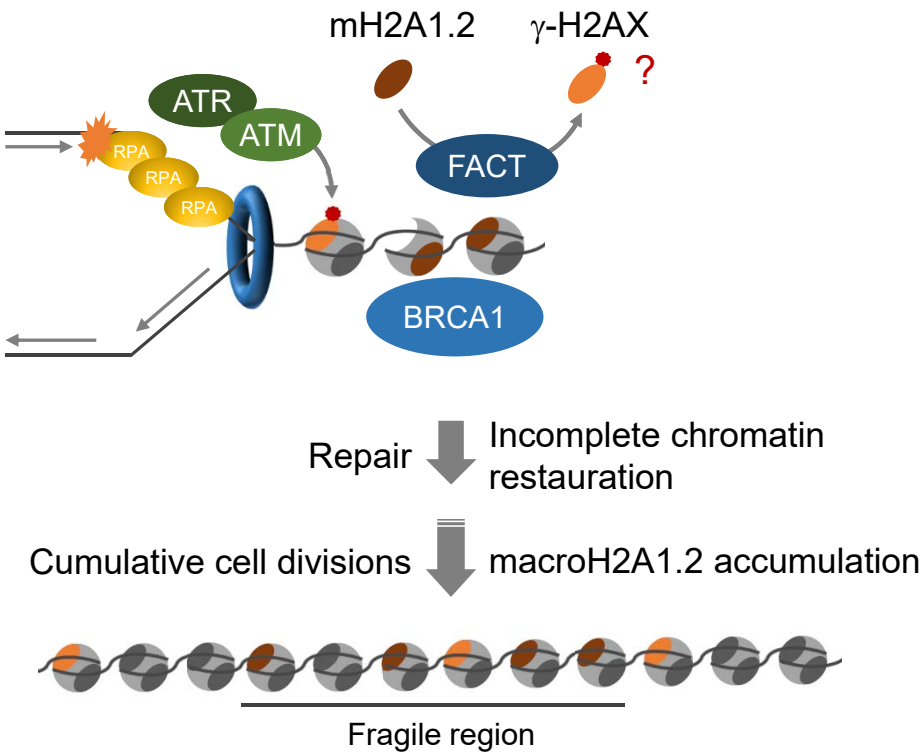


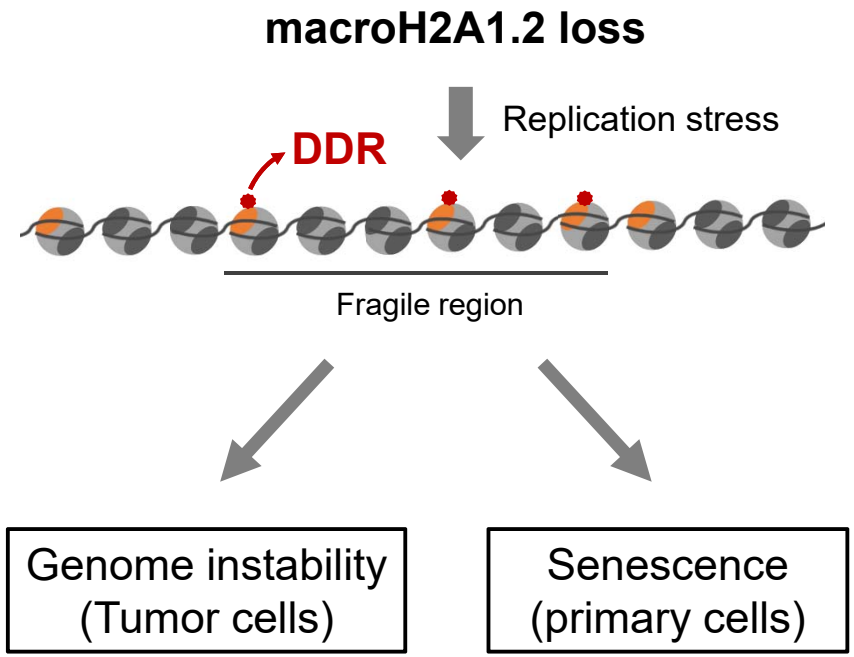
Cell cycle arrest is DDR-dependent



Bartek, d'Adda di Fagagna and others

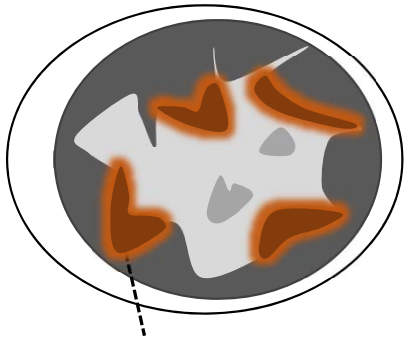
Replication stress shapes a protective chromatin environment at fragile regions





Kim et al, *Mol Cell, in press*

Implications for the epigenome

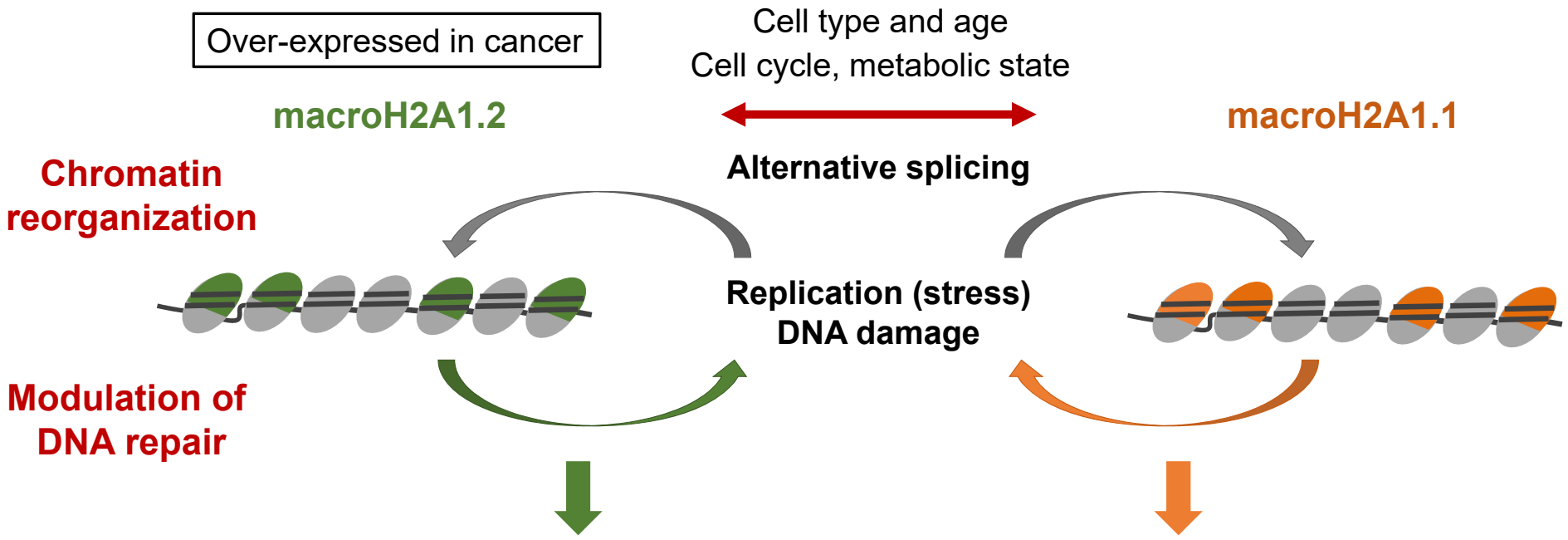


Fragile regions / macroH2A1.2 domains

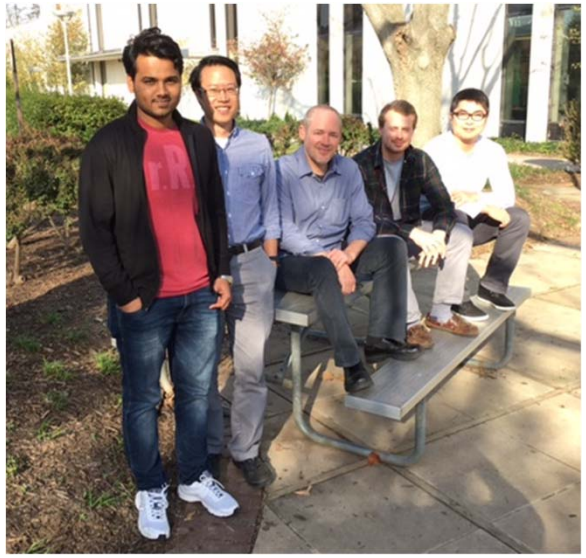
- **A subset of chromatin domains are the result of chronic DNA damage exposure.**
- **Implications for age-associated chromatin reorganization and epigenomic dysfunction.**

A more general role for macroH2A1.2 in HR and/or replication stress-associated genome maintenance?

Feedback between DNA repair and chromatin modulates cell function



Acknowledgements



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