Cancer Biology I :

Topics covered

Week 1:
Lecture 1: **Hallmarks of cancer – an overview; Mutations, oncogenes and tumor suppressor genes**
(Chapters 2, 4, 7 (Weinberg book))

Week 2:
Lecture: **DNA repair of DNA double strand breaks; Synthetic lethality**
Exercises: **Paper discussion on Wednesday**

Week 3:
Lecture 3/Exercises: **DNA repair and the DNA damage response**

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

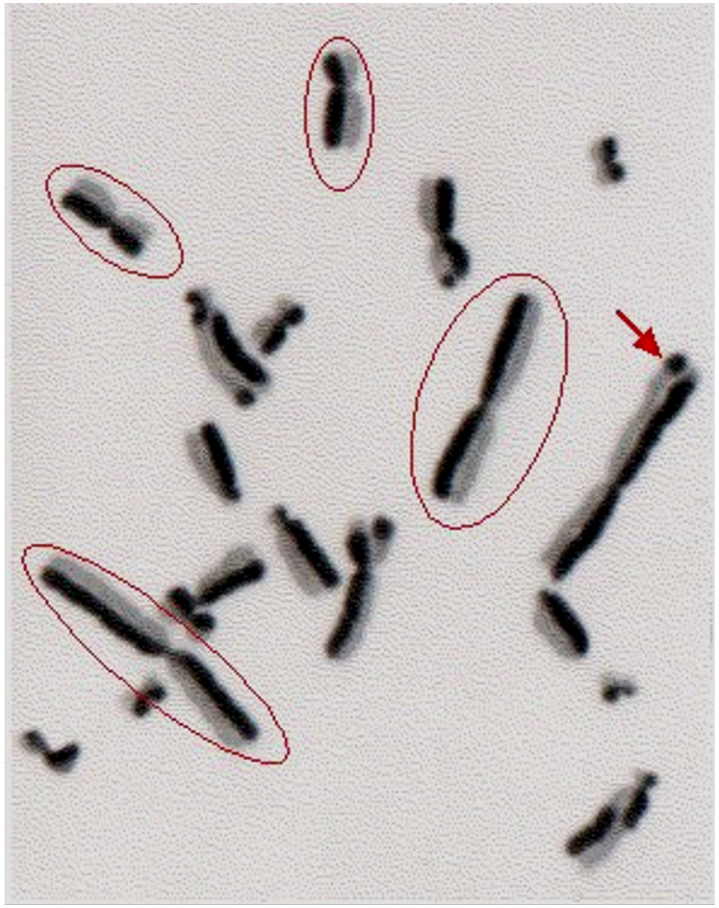
→ Double strand break repair

- **NHEJ: Non homologous end-joining**
- **MMEJ: Microhomology mediated end-joining (also called Alt-NHEJ or Theta-mediated end-joining (TMEJ))**
- **HR: Homologous recombination**

Link for an exhaustive list of proteins that are implicated in genome stability:
http://sciencepark.mdanderson.org/labs/wood/DNA_Repair_Genes.html

Sister Chromatid Exchange (SCE)

Spontaneous SCE



Induced SCE (DNA damage)

"Harlequin chromosomes"

BrDU containing DNA stains clear upon staining with <u>Giemsa</u> while the DNA with thymine stains dark

- Repair of dsDNA breaks: NHEJ, MMEJ (also called Alt-EJ), HR
- BRCA1 and BRCA2 function in HR
- PARP-1 stimulates base-excision repair and micro-homology mediated end joining
- HR-deficient cells can be killed by inhibiting PARP-1. …Concept of **synthetic lethality**

PARP Inhibitor Resistance

Strong evidence

Not discussed in this course:

Resistance mechanisms	Cause of resistance	Clinical evidence
(i) Increased drug efflux	- Upregulation of ABC transporters	- No evidence
(ii) Decreased PARP trapping	- Loss or decreased trapping of PARP1 - Loss of PARG	- Trapping-diminishing PARP1 mutation in PARPi-resistant tumour - No evidence
(iii) Restoration of HR	- Reactivation of *BRCA1/2* - Loss of 53BP1 - Loss of Shieldin factors - Loss of CTC/Polα - Loss of DYNLL1/ATMIN	- Mutations in patients and PDXs - Low expression and mutations in PDXs - Low expression and mutations in PDXs - No evidence - No evidence
(iv) Stabilization of stalled forks	- Loss of PTIP - Loss of EZH2	- No evidence - No evidence

Trends in Cell Biology

PDX: patient derived xenografts

Figure 2. Modes of Resistance to PARP Inhibitors (PARPi). An overview of the four distinct categories of PARPi resistance mechanisms. In each category (left column), all molecular mechanisms that have been identified in preclinical studies are mentioned (middle column). In addition, whether direct clinical evidence for PARPi resistance has been observed in primary tumor material or PDX-models until this date is indicated (right column). Abbreviations: HR, homologous recombination; PARP, poly(ADP-ribose) polymerase; PDX, patient-derived xenograft.

- PARG: Poly (ADP-ribose) glycohydrolase; removes poly ADP-ribose moieties
- Loss of 53BP1, shieldin, CTC1 →increased 3' overhangs at DSBs

→ reactivates resection and HR in BRCA1 cells (in BRCA1 deficiency resection is inhibited). **BRCA2 deficient cells are not acquiring PARP inhibitor resistance by loss of 53BP1!**

From: Trends Cell Biol 29, 820 (2019)

Chromatin and DNA Repair

Repair of DSB Involves Posttranslational Modifications of Nucleosomes and other Proteins

- Detect DNA damage
- Remodel local chromatin to provide access
- Reorganize nucleosome-DNA template for processing and repair
- Restore local chromatin organization after repair

See *Cell* 152, 1344 (2013) for a Review

Colocalization of BRCA1 with γ-H2AX at ds DNA Breaks

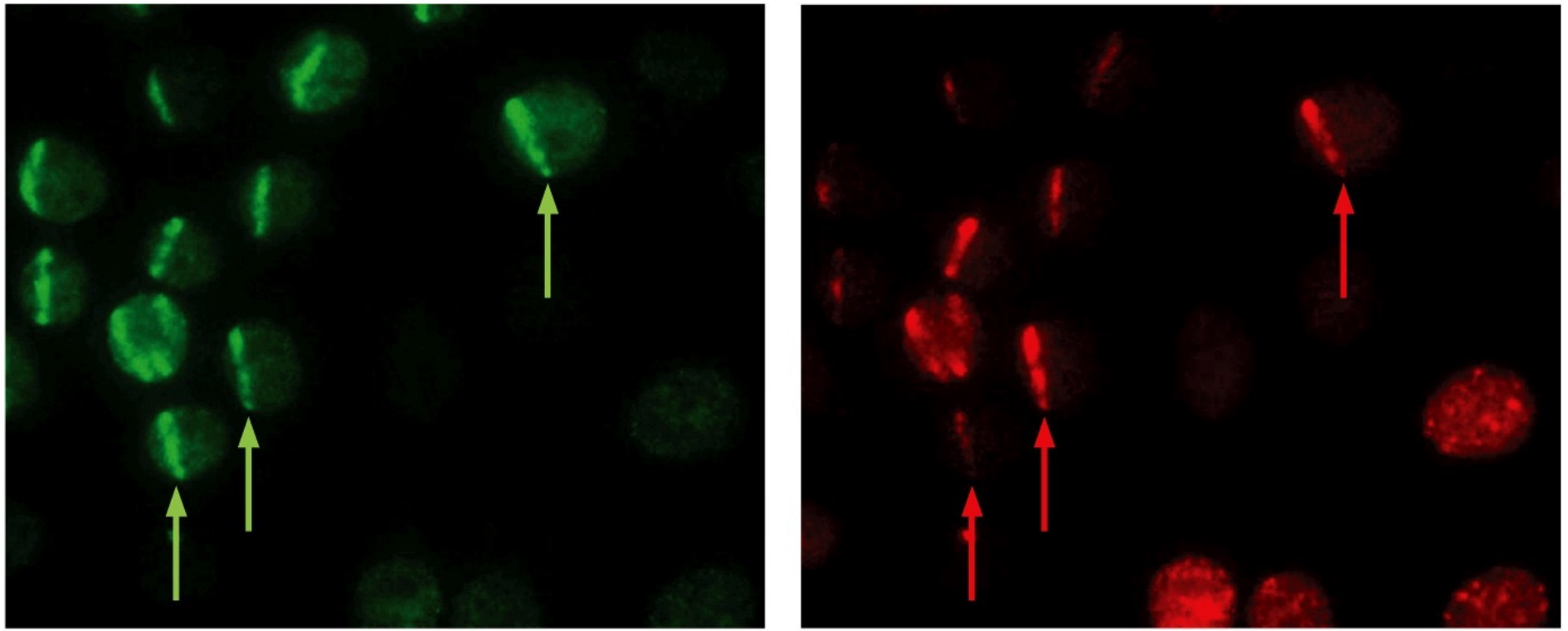
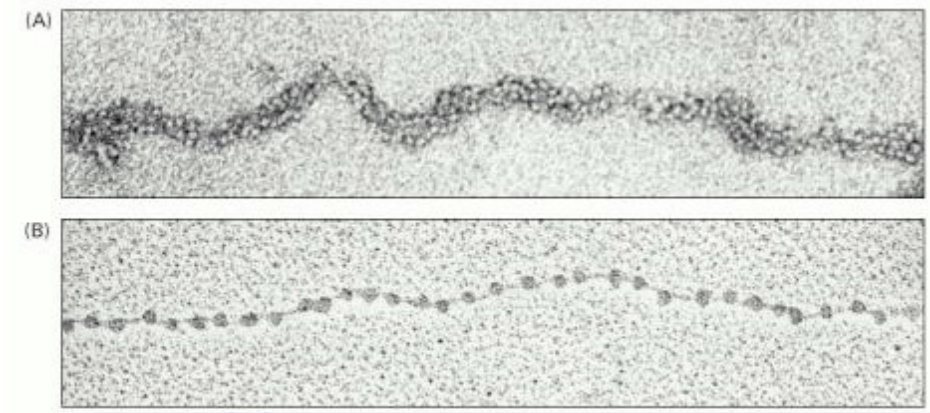


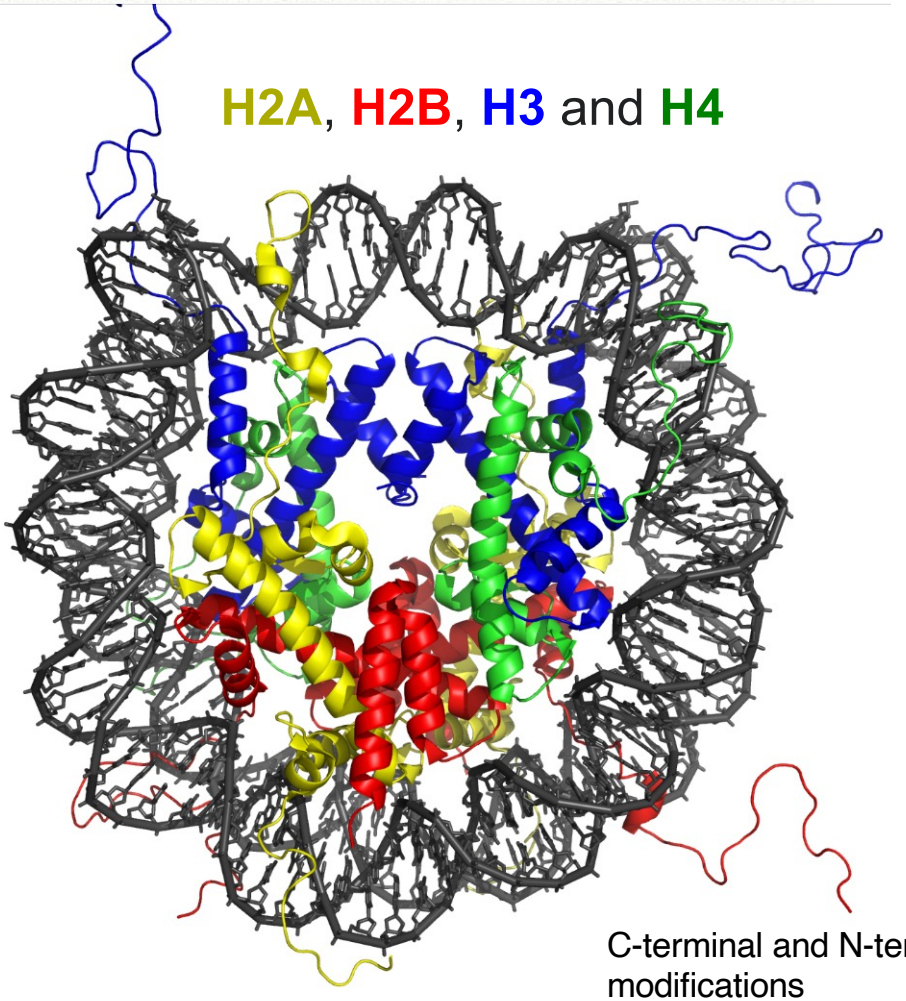
Figure 12.28. Weinberg, The Biology of Cancer

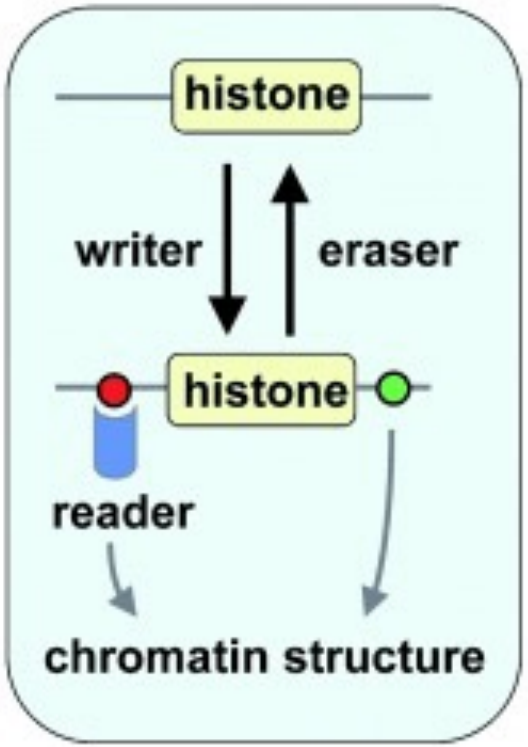
A 355-nm UV laser was used to paint stripes across nuclei



30 nm fiber (interphase chromatin analyzed by EM)

Linker histone H1 removed: “beads on a string”
147 bp are rolling 1.7 times around a histone octamer (two of histones H2A, H2B, H3 and H4).





C-terminal and N-terminal tails are subject to post-translational modifications

H2AX

10 % of the H2A pool of mammals
H2AX is the ‘normal’ histone H2A in budding yeast

Double strand breaks → Phosphorylation of Ser 139 (Ser c-4)
→γH2AX

a

Species	Core (C-terminal portion)	Linker	Tail
Human	NDEELNKLLGGVTIAQGGVLPNIQAVLLPKK	TSATVGPKAPSGGKKATQA	SQEY
Chimpanzee	NDEELNKLLGGVTIAQGGVLPNIQAVLLPKK	TSATVGPKAPSGGKKATQA	SQEY
Mouse	NDEELNKLLGGVTIAQGGVLPNIQAVLLPKK	SSATVGPKAPAVGKKASQA	SQEY
Chicken	NDEELNKLLGGVTIAQGGVLPNIQAVLLPKK	TGGGAASPAKAGKKGSGQQ	SQEY
Zebrafish 1	NDEELNKLLGGVTIAQGGVLPNIQAVLLPKK	TGQAAASSGKSGKKGSSQ	SQEY
Xenopus laevis	NDEELNKLLGGVTIAQGGVLPNIQAVLLPKK	SSGGVSTSGKKSSQQ	SQEY
Anopheles gambiae	NDEELSKLLQGTTISQGGVMPNIHSVLLPRK	TGAKAAGNSSSQEKQ	SQEY
Zebrafish 2	NDEELNTLLGGVTISEGGVLPNIQAVLLPKK	TKAAREPNAGTEAQ	SQDF
Chickpea	NDEELSKLLGSVTIANGGVLPNIHQTLLPKK	VGKGKGEIGSA	SQEF
Tetrahymena pyriformis	NDEELNKLMANTTIADGGVLPNINPMLLPSK	SKKTESRGQA	SQDI
Budding yeast	NDDELNKLLGNVTIAQGGVLPNIHQNLLPKK	SAKATKA	SQEL
Fission yeast	NDEELNKLLGHVTIAQGGVVPNINAHLLPKT	SGRTGKP	SQEL
Giardia intestinalis	KDKELATIFANVTIREGGVARSAKEGREGKG	SHR	SQDL

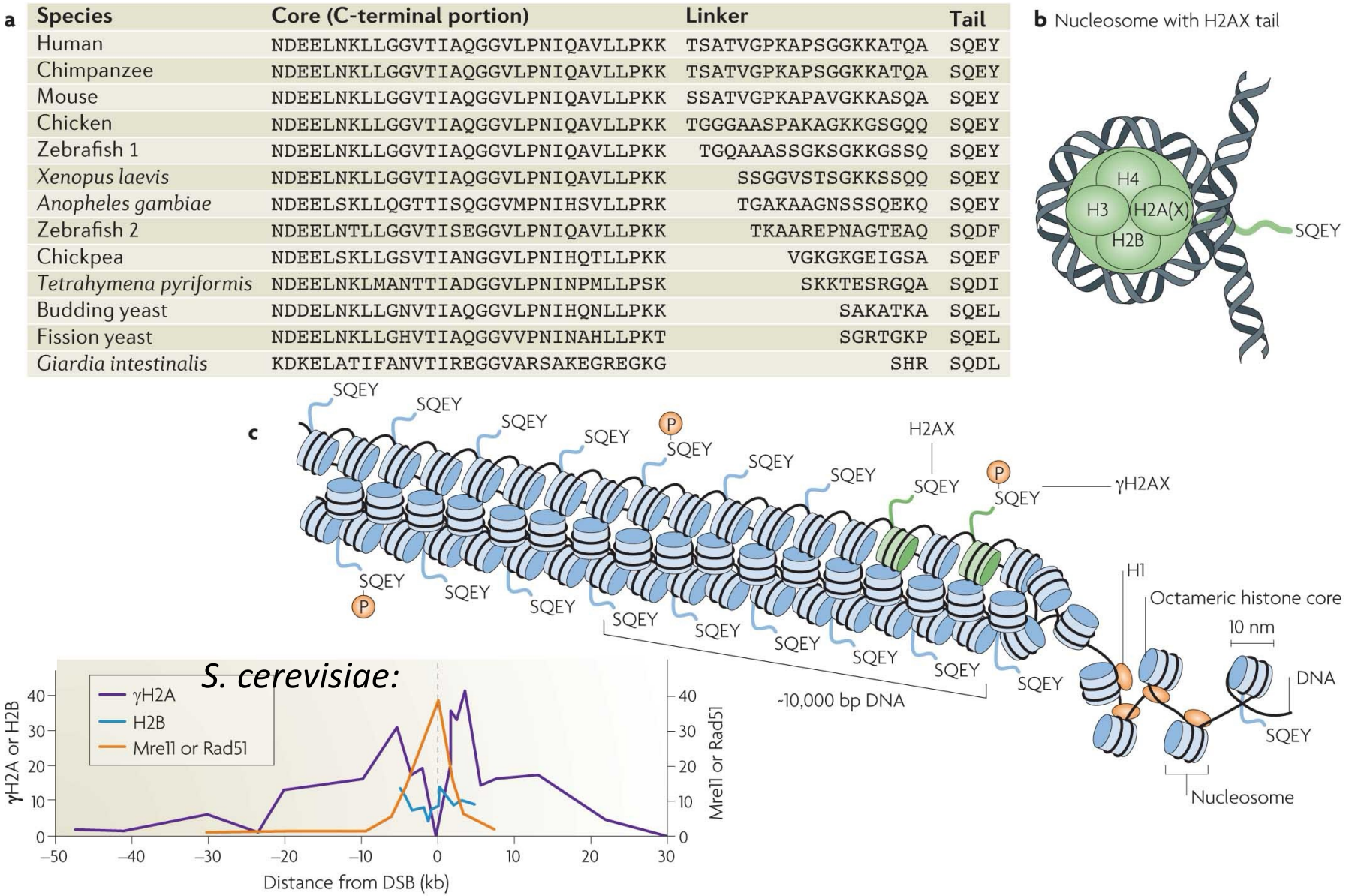
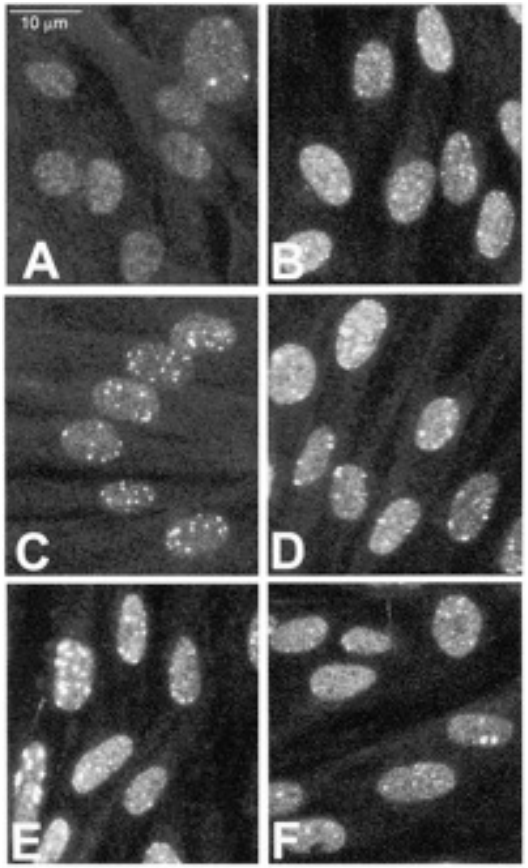


Figure 3 | **H2AX and γH2AX foci. a** | H2AX is an H2A histone with a core sequence conserved with other H2A species and a tail conserved through evolution connected by a linker of variable length. **b** | The SQEY tail extends from the core nucleosome near the entry and exit point of the DNA. **c** | The nucleosomes form a 30 nm fibre with H2AX molecules in every fifth nucleosome on average in mammals and every nucleosome in yeast. Approximately 10% of the H2AX molecules are phosphorylated at any one time in a focus.

From NatureRevCancer 8, 957 (2008)

Nuclear γ-H2AX foci are a Direct Consequence of DNA Double Strand Breaks

Irradiation: 0.6 Gy



Control

3 min.

15 min.

30 min.

60 min.

180 min.

Rogakou et al. (1999). JCB (146) pp. 905-916

Ionizing radiation
Replicative stress

↓

ATM/ATR

↓

γH2AX

Spreading of γ-H2AX at DSB

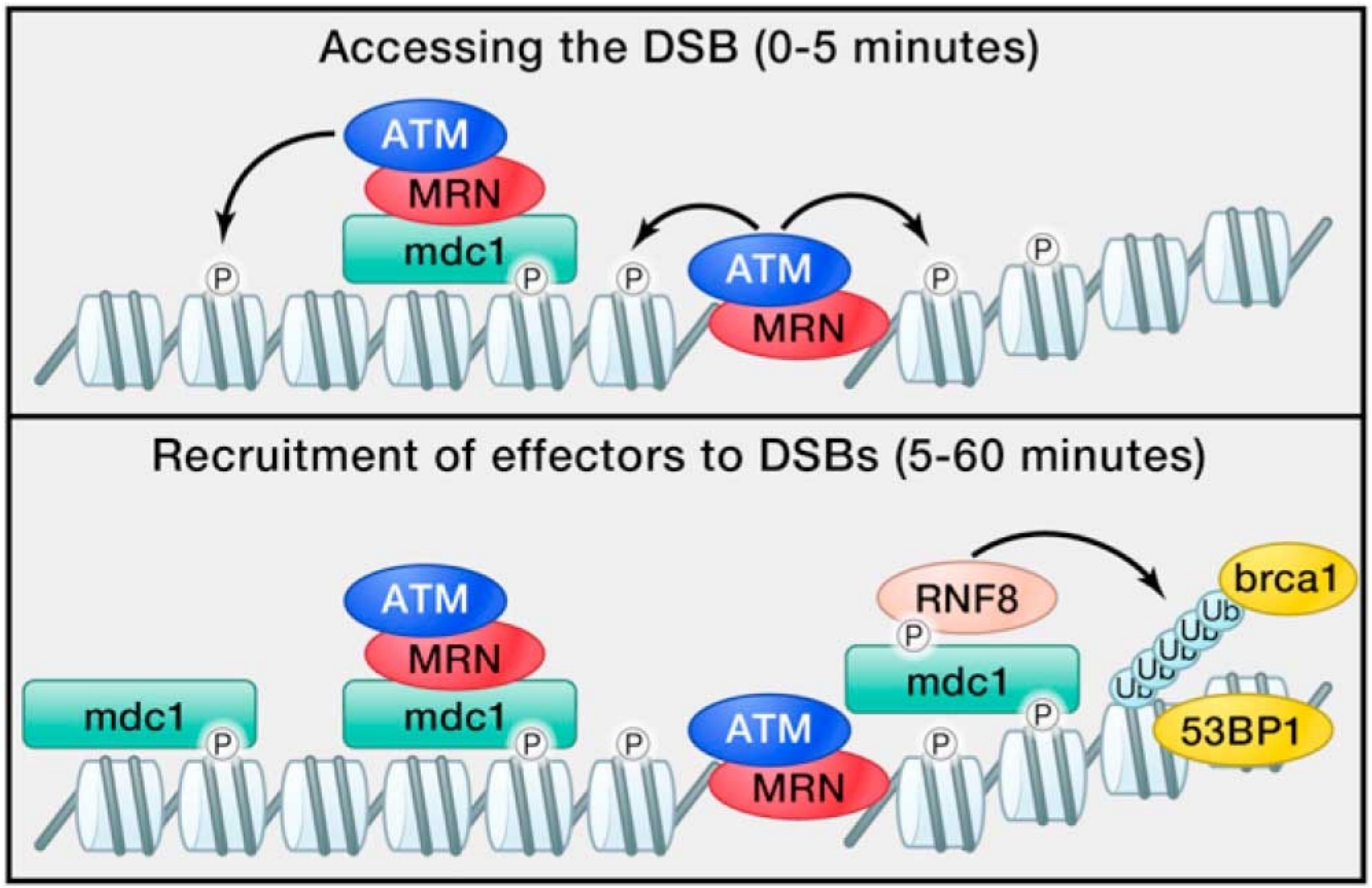


Figure 1. The Mechanism of DSB Repair

Top: ATM phosphorylates H2AX at DSBs, creating a binding site for the mdc1 protein. ATM-MRN complexes then associate with mdc1, promoting the spreading of γH2AX along the chromatin for hundreds of kilobases.

Bottom: mdc1 recruits multiple DSB-repair proteins, including the RNF8/RNF168 ubiquitin ligases, to sites of damage. Chromatin ubiquitination then facilitates loading of the brca1 complex and 53BP1 DSB-repair proteins.

P = phosphorylation, Ub = ubiquitination, MRN = mre11-rad50-nbs1 complex.

Role of H2AX

- **Repair of double-strand breaks: NHEJ & HR**

(impaired recruitment of 53BP1 and BRCA1 in absence of H2AX)

- **Mouse H2AX -/- :**

Chromosomal breaks and translocations
Small size
Lymphomas & solid tumors