

HISTONE H2AX AND RADIOSENSITIVITY

Juan Jesús Rubio Arroyo

Radiology and Physical Medicine

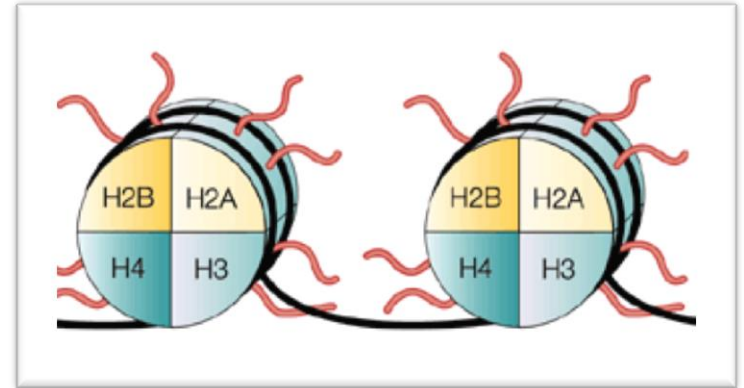
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INTRODUCTION

- ❖ One of the main objectives of the radiation use is to control tumor and normal cell DNA response to this treatment.
- ❖ Double strand breaks (DSBs) are one of the most important biological effects of radiation. If unrepaired, DSBs lead to gene mutations, chromosomal aberrations and cell transformation/death.
- ❖ In this way, the relation between DSBs detection/repair and the activity of H2AX histone becomes of great importance.

HISTONE H2AX

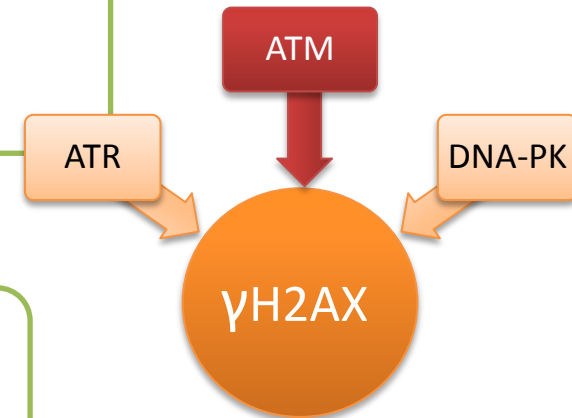
One of several variants of the histone H2A.



http://www.nature.com/nrc/journal/v1/n3/fig_tab/nrc1201-194a_F1.html

When DSBs happen, H2AX is phosphorylated via different pathways:

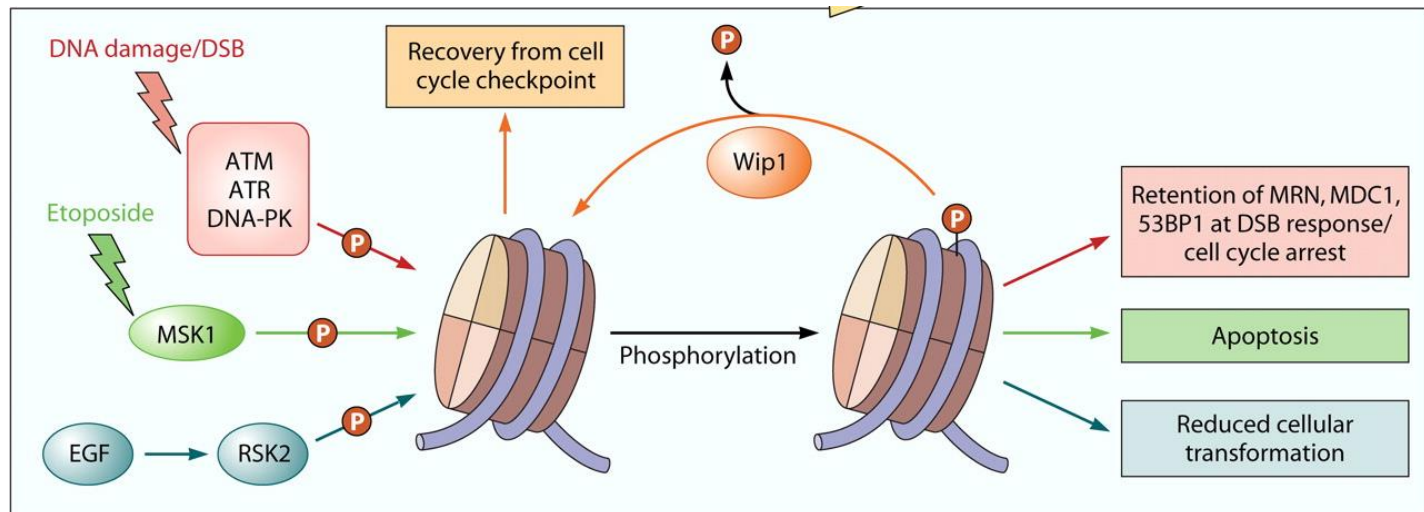
Phosphorylation occurs in Ser139, flanking the DSBs.



The γ H2AX becomes a binding site for downloading many components of the DSB response (MDC1, MBS1...) ¹

It mediates translocation of the p53 to the radiation induced foci ²

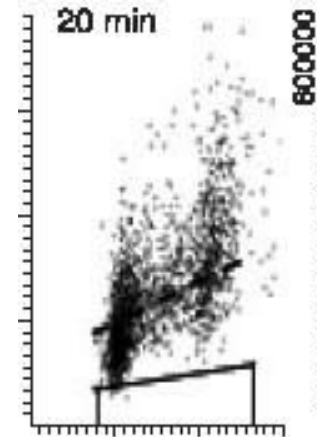
H2AX guards genomic integrity against carcinogenesis and its dephosphorylation is associated with a greater cell survival.



DETECTION METHODS

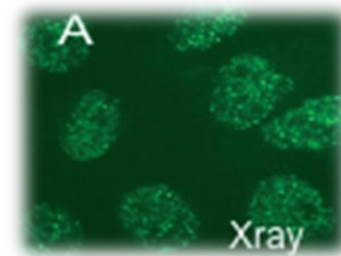
➤ Flow cytometry:

- High sensitivity
- γ H2AX-cell DNA content direct correlation
- Analyses intercellular variability

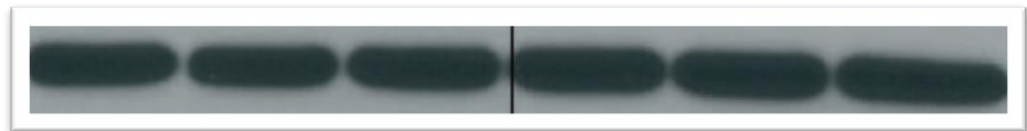


➤ Immunocytochemistry:

- Much greater sensitivity
- Easier to perform
- Each site represents a single DSB

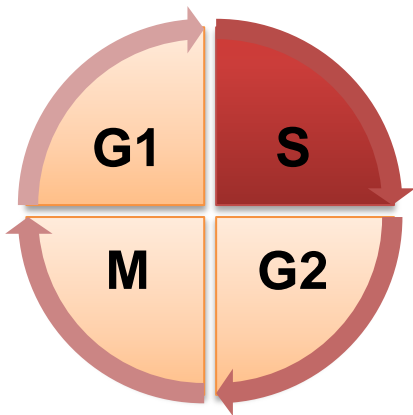


➤ Western blot

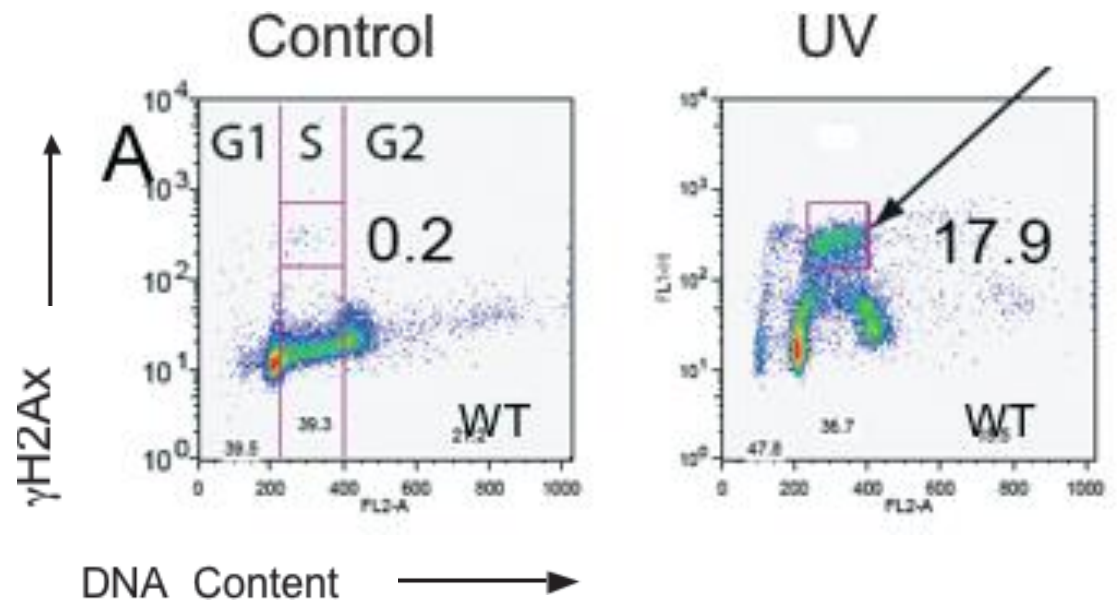


CELL RESPONSE TO RADIATION DEPENDS ON THE CELL CYCLE

Radiosensitivity
variations:
 $G2/M > G1 > S$



The highest intensity of γ H2AX
can be detected in the S phase:

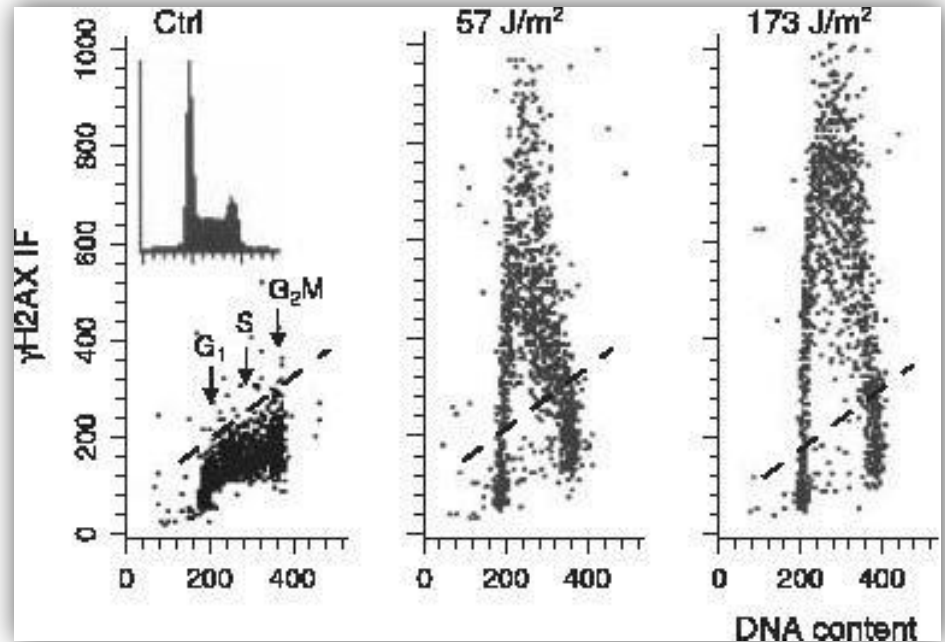


James E. Cleaver. γ H2AX: Biomarker of Damage or Functional Participant in DNA Repair "All that Glitters Is not Gold". *Photochemistry and Photobiology*, 2011,87: 1230-1239

At the same doses of exposure the induction of H2AX phosphorylation is more pronounced in S phase than in G1 and G2/M.

This has been tested using UV light and X radiation (Macphail *et al.* 2003a).

This effect occurs equally at low and high doses of radiation.



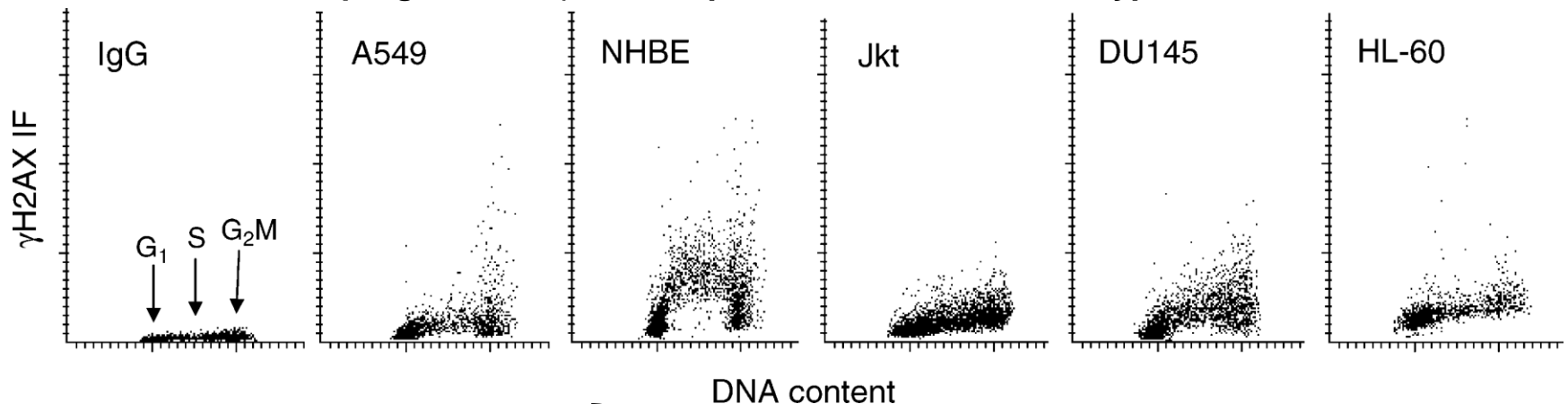
Xuan Huang *et al.* Cytometric assessment of DNA damage in relation to cell cycle phase and apoptosis. *Cell Prolif.* 2005 August; 38(4): 223-243. Available in: PMC 2006 February 3

PROGRAMMED OR INTRINSIC γ H2AX

H2AX is phosphorylated not only in response to DNA damage caused by environmental genotoxic factors, but there is also an “intrinsic” phosphorylation that depends on ²:

- Cell cycle: S phase
- Cell type
- Apoptosis induction

Variable level of ‘programmed’ γ H2AX expression in different cell types.



Xuan Huang et al. Cytometric assessment of DNA damage in relation to cell cycle phase and apoptosis. *Cell Prolif.* 2005 August; 38(4): 223-243. Available in: PMC 2006 February 3

γ H2AX USES

BIOMARKER

- ✓ Histone H2AX is phosphorylated when DSBs occur, but the presence of γ H2AX does not necessarily mean existence of DSBs.
- ✓ Its detection using different techniques makes it possible to:
 - Identify nuclear foci and determine their number and frequency.
 - Detect genomic damage and repair.
 - Determine cell radiosensitivity.
- ✓ The induction of γ H2AX provides a sensitive means to measure the extent of DNA damage following exposure to any genotoxic factor (radiation, chemotherapy...)².

FUNCTIONAL ACTIVITY

- ✓ H2AX phosphorylation might not be an important component of the DNA damage response.
- ✓ Only when the damage is known to be predominantly DSBs can γ H2AX be assumed to be functionally important.¹
- ✓ The fact that γ H2AX dephosphorylation kinetics are slower than those of DSBs rejoining suggests that other mechanisms are implicated.

BIBLIOGRAPHY

- 1. James E. Cleaver. γ H2AX: Biomarker of Damage or Functional Participant in DNA Repair “All that Glitters Is not Gold”. *Photochemistry and Photobiology*, 2011,87: 1230-1239
- 2. Xuan Huang *et al.* Cytometric assessment of DNA damage in relation to cell cycle phase and apoptosis. *Cell Prolif.* 2005 August; 38(4): 223-243. Available in: PMC 2006 February 3
- 3. Peggy L. Olive *et al.* Phosphorylation of histone H2AX as a Measure of Radiosensitivity. No 2, pp 331-335, 2004. Elsevier Inc.