Journal of Clinical & Experimental Pathology

Matsuno et al., J Clin Exp Pathol 2018, 8:1
DOI: 10.4172/2161-0681.1000335

Mini Review Open Access

# Establishment of Cellular Quiescence Together with *H2AX* Downregulation and Genome Stability Maintenance

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**Received date:** January 15, 2018; **Accepted date:** January 18, 2018; **Published date:** January 22, 2018



## Abstract

*H2AX* is required for genome stability. In response to DNA double-strand breaks (DSBs), *H2AX* is rapidly phosphorylated to form *γH2AX* foci, which mediate DNA repair and checkpoint signaling. This process is regulated by modifications and molecular interactions of *H2AX*. In addition, the rapid stabilization of *H2AX* in response to DSBs facilitates *γH2AX* foci formation. Although *H2AX* is markedly downregulated in many cellular states, *γH2AX* foci can still efficiently form upon DSB generation. Here, we review the regulation of *H2AX* in response to DSBs.

**Keywords:** *H2AX*; *γH2AX*; DNA double-strand breaks

**Abbreviations:** DSB: DNA Double-Strand Break; MEF: Mouse Embryonic Fibroblast

## Introduction

*H2AX* mediates repair of DNA double-strand breaks (DSBs), and hence is required for genome stability. In response to DSBs, *H2AX* is rapidly phosphorylated at Ser139 by ATM, ATR, or DNA-PK, which leads to the generation of *γH2AX* foci at DSB sites [1,2]. These foci promote DSB repair by non-homologous end joining and/or homologous recombination [3,4]. MDC1 rapidly binds to *γH2AX* and promotes recruitment of the MRN (MRE11-Rad50-NBS1) complex and ATM. This leads to enlargement of *γH2AX* foci and amplification of DNA damage signaling, which usually peaks at 30 min after damage [5]. *γH2AX* foci serve as a platform for the recruitment of DSB repair factors and chromatin-remodeling complexes [6,7]. Mono-ubiquitination at K119/K120 [8-12] and poly-ubiquitination at K13/K15 [13,14] are also involved in the effective recruitment of many DNA repair-associated factors, such as BRCA1 [15] and 53BP1 [14].

## Transient Upregulation of *H2AX* Efficiently Induces DSB Repair in Quiescent Cells

*H2AX* is a variant of histone H2A. The expression level of *H2AX* greatly differs between cell types. In particular, *H2AX* is markedly downregulated in quiescent normal cells [16]. Intriguingly, our recent studies revealed that *γH2AX* foci efficiently form in response to DSBs even in the *H2AX*-diminished quiescent state [17]. This is dependent on transient and immediate expression of *H2AX* upon DSB formation. *H2AX* is continuously transcribed and translated in non-damaged cells, but undergoes proteasomal degradation mediated by the E3 ubiquitin ligase HUWE1 (Figure 1A) [17]. This proteolytic degradation is immediately blocked after DSB formation and consequently *H2AX* rapidly accumulates and *γH2AX* foci efficiently form (Figure 1B).

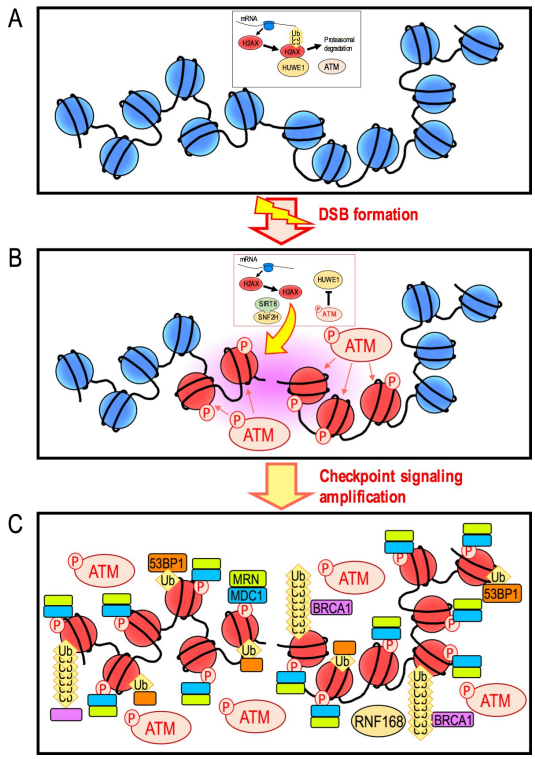


**Figure 1:** Efficient Induction of DSB Repair in Quiescent Cells. *H2AX* is markedly downregulated in quiescent cells (A). *H2AX* is immediately stabilized upon DSB formation, and this is mediated by ATM and SIRT6/SNF2H (B). Thereafter, *γH2AX* foci are enlarged, enabling efficient checkpoint signaling activation and subsequent DSB repair (C).

Although the mechanism that regulates this process has not been fully elucidated, it involves SIRT6 and SNF2H for chromatin remodeling and ATM to halt proteasomal degradation [17]. This leads to the recruitment of repair factors in association with additional modifications, including ubiquitination of H2A/*H2AX* (Figure 1C).

After DSB repair is complete, the cellular *H2AX* level generally decreases and returns to that observed in the initial quiescent state [17]. Thus, *H2AX* is markedly downregulated in quiescent cells, but these cells still express *H2AX* in response to DSBs. However, *H2AX* is only transiently expressed to efficiently induce DSB repair, and *H2AX* expression decreases once this repair is complete.

Unlike normal cells, many cancer cells constantly express *H2AX*, and the *H2AX* level in these cells is generally 0.1-10% of the total H2A level [2]. However, such cells still demonstrate upregulation of *H2AX* in response to DSBs in an ATM and a SIRT6/SNF2H-dependent manner [17]. In addition, transient *H2AX* upregulation is required for efficient DSB repair in *H2AX*-expressing cancer cells. Thus, transient upregulation of *H2AX* is a general requirement for the efficient induction of DSB repair [17].

## Establishment of Cellular Quiescence with Downregulated H2AX

*H2AX* is highly expressed in actively growing cells [16], but is usually downregulated in normal cells after serial proliferation. In fact, normal cells generally enter a growth-arrested state with marked downregulation of *H2AX in vivo* and *in vitro* [16]. There are several growth-arrested cellular states, including senescence and quiescence, which can be clearly discriminated. The quiescent state is widely established with marked downregulation of *H2AX*; *H2AX* downregulation may directly lead to the acquisition of quiescence because cells enter an identical state upon knockdown of *H2AX* [16]. In addition, quiescence is associated with organ homeostasis, as demonstrated in normal cells in the liver, spleen and pancreas *in vivo* [16]. By contrast, senescent cells express some *H2AX* [16] and contain *γH2AX* foci, which are usually seen in cells in aging organs and those in a precancerous state [18]. Consistent with these observations *in vivo*, similar findings were made in mouse embryonic fibroblasts (MEFs) *in vitro*. Whereas *H2AX* is largely downregulated in quiescent MEFs, *γH2AX* foci form when MEFs become senescent and are subjected to genomic destabilization [16]. Thus, *H2AX*/*γH2AX* expression is strongly associated with the establishment of cellular states, i.e., quiescence is established in cells with marked downregulation of *H2AX* and senescence is established in cells with *γH2AX* foci.

Importantly, ARF and p53 regulate establishment of the *H2AX*-downregulated quiescent state [19]. Consequently, this state is abrogated in cells with mutations in ARF or p53, such as cancer cells and immortalized MEFs, in which *H2AX* expression and growth activity are recovered [19]. Notably, many quiescent cells with downregulated *H2AX* are protected against transformation. These observations illustrate the importance of the *H2AX*-diminished cellular state for the protection of cells from the transformation. However, it remains unclear how ARF and p53 regulate the establishment of this state.

## Quiescent Cells are Vulnerable to Replication Stress-Associated DSBs

Quiescent cells can still repair DSBs directly caused by γ-rays *via* upregulation of *H2AX* [17], but are vulnerable to replication stress-associated DSBs [20,21]. Replication stress-associated DSBs generally accumulate in quiescent cells exposed to exogenous growth stimuli, and these cells become senescent and often display genomic instability. In addition, senescent MEFs displaying genomic instability further lead to the generation of immortalized MEFs that are mutated in the ARF/p53 module [16,20]. These findings are analogous to cancer development, as cancer development is associated with aging and genomic instability. These results indicate that responses to replication stress-associated DSBs and DSBs directly caused by γ-rays clearly differ; however, the cause of this difference is unknown.

## Conclusion

*H2AX* downregulation is associated with establishment of cellular quiescence, which contributes to homeostasis in many organs. This state is regulated by ARF and p53, and is abrogated by mutation of the ARF/p53 module. Accumulating knowledge illustrates the importance of establishment of the *H2AX*-downregulated state and maintenance of genome stability in this state. Cells with downregulated *H2AX* can still repair DSBs directly caused by γ-rays, but are vulnerable to replication stress-associated DSBs caused by continuous exposure to growth stimuli. However, these findings raise a number of further questions. First, what underlies the difference in repair efficiency between DSBs directly caused by γ-rays and DSBs caused by replication stress? Second, how do ARF and p53 regulate establishment of the *H2AX*-downregulated state? Given that cancers widely develop together with genomic destabilization and mutations in the ARF/p53 module, investigation of these issues may help to prevent cancer.

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