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Stop relaxing: How DNA damage-induced chromatin compaction may affect epigenetic integrity and disease

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DNA damage is widely recognized for its potential to impair epigenetic integrity. Epigenetic defects are closely associated with a variety of diseases. We have recently uncovered DNA double-strand break-induced chromatin condensation as a critical modulator of repair outcome. Here, we discuss the possible implications for cell functions beyond repair.

The tightly regulated organization of genomic DNA in nuclear space is essential for the control of gene expression, DNA replication, and genome stability. To achieve this level of organization, eukaryotic DNA is packaged into chromatin, a higher order ribonucleic acid-protein complex comprised of a DNA/histone core and a plethora of accessory proteins, RNAs, and modifying enzymes. Recent comparative genome-wide analyses highlight the diverse, and often evolutionarily conserved, elements that contribute to metazoan chromatin organization.[1] It is therefore not surprising that aberrant cellular function, disease, and malignant transformation are associated with profound alterations in the epigenetic landscape.[2,3]

Over the past decade it has become apparent that DNA damage poses a significant threat to the integrity of our epigenomes. DNA damage results in a range of chromatin alterations at the sites of DNA lesions that involve changes in histone modifications, nucleosome remodeling, and histone (variant) exchange.[4] The dramatic impact of DNA damage on chromatin is perhaps best exemplified at DNA double-strand breaks (DSBs), which promote an immediate and expansive reorganization of the surrounding chromatin structure to render damaged DNA accessible for repair factors.[4] We recently showed that this process is followed by an unexpected phase of prolonged chromatin re-condensation, which is initiated well before recruitment of repair factors is completed and is in fact essential for efficient accumulation of the tumor suppressor and DSB repair mediator BRCA1.[5] DSB-induced chromatin condensation involves the coordinated, DNA damage signaling-dependent recruitment of the macro-histone variant macroH2A1 (encoded by *H2AFY*) and the histone methyltransferase PRDM2, which in turn promote dimethylation of lysine 9 on histone H3 (H3K9me2) flanking the DSB. Although the importance of this process for accurate genome maintenance has been discussed previously,[5] its implications for the epigenetic integrity of the cell remain unclear. Several intriguing possibilities are considered below.

Both macro-histones and H3K9 methylation have long been associated with cellular processes that involve the formation of repressive chromatin, such as gene silencing and X chromosome inactivation. By analogy, the macroH2A1/PRDM2-dependent modulation of DSB-proximal chromatin results in condensation of nucleosomes that can be detected up to several hundred kilobases from the DSB site.[5] Both macroH2A1 and H3K9me2 persist for hours after DNA damage, a time frame that is sufficient to cause potentially harmful changes in chromatin structure and/or gene expression. Notably, DSBs were found to promote transcriptional silencing in cis to the site of damage,[4] and it will be interesting to determine whether macroH2A1/PRDM2-dependent chromatin compaction is required for this process (**Fig. 1A**). In addition, DNA repair was found to promote the redistribution of repair-relevant chromatin modifiers from undamaged to damaged chromatin, thus accounting for epigenetic gene deregulation beyond the sites of damage.[6] Both macroH2A1 and PRDM2 were reported to act as tumor suppressors and their recruitment to DSBs may perturb these functions by depleting macroH2A1 and/or PRDM2 from the genomic loci or protein complexes they normally associate with. Consistent with this notion, a reduction in macroH2A1 was recently found to promote melanoma progression by

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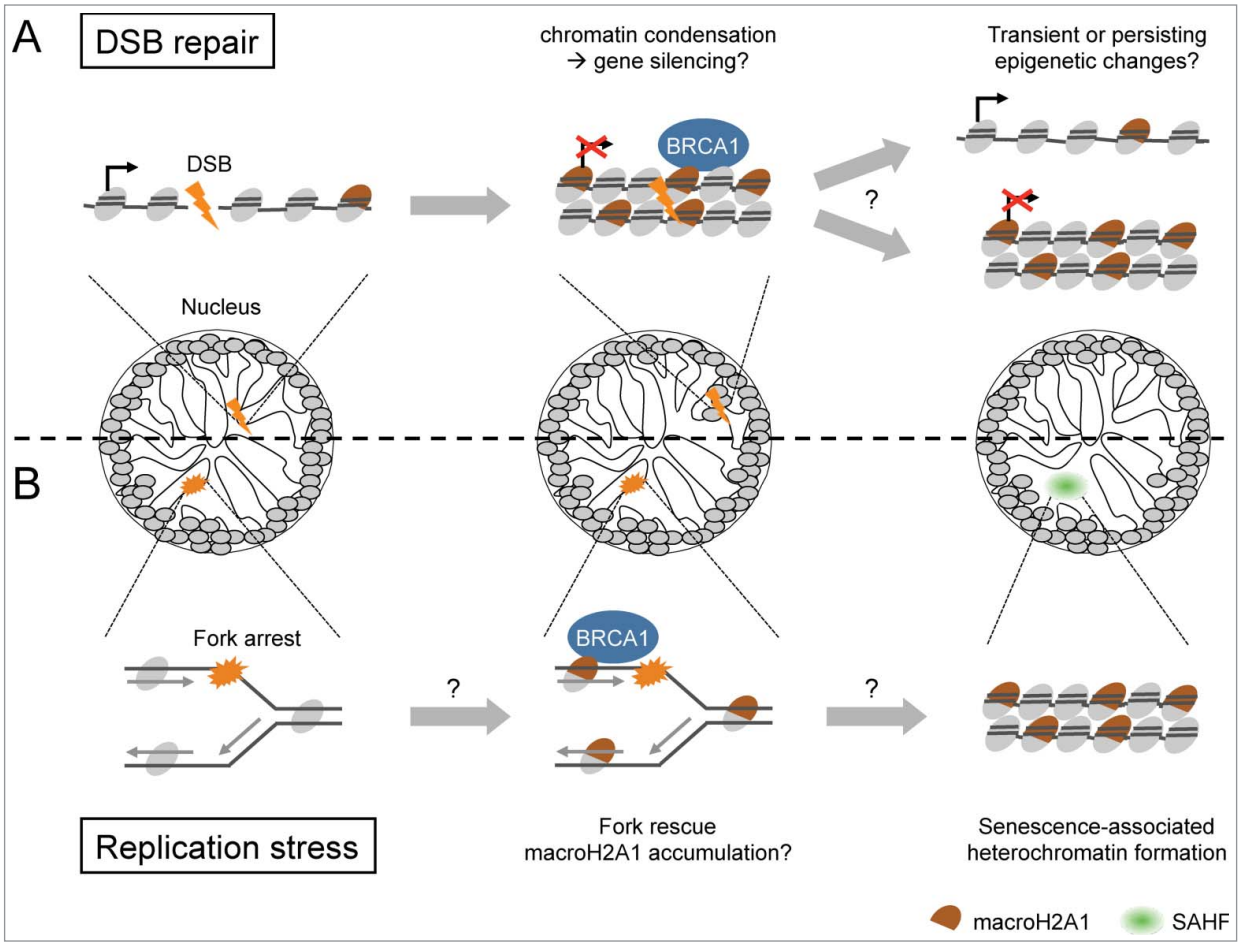

Figure 1. Epigenetic consequences of DNA double-strand break (DSB)-induced chromatin compaction. (**A**) DSBs promote concomitant macroH2A1 accumulation, chromatin condensation, and BRCA1 accumulation. DSBs may thereby cause transient or persistent changes in nuclear chromatin organization and the expression of break-proximal genes. (**B**) Stalled replication forks trigger recruitment of BRCA1 to promote fork rescue; macroH2A1 may be recruited to facilitate BRCA1 accumulation. Continued replication stress can trigger cellular senescence and the accumulation of macroH2A1-containing, senescence-associated heterochromatin foci (SAHFs). MacroH2A1 may provide a link between stalled replication forks and SAHF formation. A schematic of nuclear changes in response to DSBs (lightning bolt) or replication stress (burst) is shown. Gray ovals, repressive chromatin domains; green oval, SAHF.

causing direct transcriptional upregulation of the colorectal cancer oncogene cyclin-dependent kinase 8 (CDK8).[7] Given that cells are continuously exposed to both exogenous and endogenous sources of DSBs, it seems plausible that even transient macroH2A1/PRDM2-dependent chromatin changes may result in chronic epigenetic defects. This could be particularly relevant during aging, which displays a continuous increase in DNA damage across the genome.

Macro-histones and H3K9 methylation have also been implicated in cellular senescence, an irreversible proliferative arrest that is thought to contribute to tumor suppression and possibly aging. Specifically, macroH2A1 accumulates at so-called senescence-associated heterochromatin foci (SAHFs), which are enriched in heterochromatin protein 1 and K9-methylated histone H3.[8] SAHFs reflect an extensive transformation of transcriptionally active euchromatin into a less accessible heterochromatic state. This process has been linked to silencing of retinoblastoma (RB)/E2F transcription factor-regulated genes and may thereby contribute to senescence-associated cell cycle arrest.[9] Although the molecular basis for SAHF formation remains elusive, DNA replication stress and the concomitant activation of the DNA damage sensor ATR kinase have been positively correlated with this process. Moreover, prolonged replication stress can result in genomic aberrations including common fragile site instability and DSB formation, thereby adding to the continued activation of the DNA damage response that appears to drive senescence.[9] Notably, BRCA1 was recently reported to play an important role in the resolution of stalled replication forks, a main source of replication stress.[10] Given the newly identified role of macroH2A1 in BRCA1-dependent genome maintenance following DSB formation, it is tempting to speculate that macroH2A1 may be equally involved in preventing aberrant fork stalling. By extension, macroH2A1 may thus provide a mechanistic link between replication stress and the formation of SAHFs (**Fig. 1B**). However, more work is needed to dissect the impact

of macroH2A1 and/or PRDM2 on cellular senescence in the context of DNA damage signaling.

Taken together, these findings suggest that DNA damage-associated formation of repressive chromatin is likely to have significant implications for the epigenetic integrity of eukaryotic cells. Its functional consequences may range from the formation of senescence-associated heterochromatin to the aberrant activation of oncogenes, depending on the genomic context of the DNA lesion as well as the cell or tissue type. Irrespective of the specific molecular outcome, both macroH2A1 and PRDM2 can be expected to impinge on cellular senescence, tumor suppression, and possibly aging through their roles in DSB repair.

References

1. Ho JW, Jung YL, Liu T, Alver BH, Lee S, Ikegami K, Sohn KA, Minoda A, Tolstorukov MY, Appert A, et al. Comparative analysis of metazoan chromatin organization. Nature 2014; 512:449-52; PMID:25164756; http://dx.doi.org/10.1038/nature13415
2. Cruickshanks HA, McBryan T, Nelson DM, Vanderkraats ND, Shah PP, van Tuyn J, Singh Rai T, Brock C, Donahue G, Dunican DS, et al. Senescent cells harbour features of the cancer epigenome. Nat Cell Biol 2013; 15:1495-506; PMID:24270890; http://dx.doi.org/10.1038/ncb2879
3. Shah PP, Donahue G, Otte GL, Capell BC, Nelson DM, Cao K, Aggarwala V, Cruickshanks HA, Rai TS, McBryan T, et al. Lamin B1 depletion in senescent cells triggers large-scale changes in gene expression and the chromatin landscape. Genes Dev 2013; 27:1787-99; PMID:23934658; http://dx.doi.org/10.1101/gad.223834.113
4. Lukas J, Lukas C, Bartek J. More than just a focus: The chromatin response to DNA damage and its role in genome integrity maintenance. Nat Cell Biol 2011; 13:1161-9; PMID:21968989; http://dx.doi.org/10.1038/ncb2344
5. Khurana S, Kruhlak MJ, Kim J, Tran AD, Liu J, Nyswaner K, Shi L, Jailwala P, Sung MH, Hakim O, et al. A Macrohistone Variant Links Dynamic Chromatin Compaction to BRCA1-Dependent Genome Maintenance. Cell Rep 2014; 8:1049-62; PMID:25131201; http://dx.doi.org/10.1016/j.celrep.2014.07.024
6. Oberdoerffer P, Michan S, McVay M, Mostoslavsky R, Vann J, Park SK, Hartlerode A, Stegmuller J, Hafner A, Loerch P, et al. SIRT1 redistribution on chromatin promotes genomic stability but alters gene expression during aging. Cell 2008; 135:907-18; PMID:19041753; http://dx.doi.org/10.1016/j.cell.2008.10.025
7. Kapoor A, Goldberg MS, Cumberland LK, Ratnakumar K, Segura MF, Emanuel PO, Menendez S, Vardabasso C, Leroy G, Vidal CI, et al. The histone variant macroH2A suppresses melanoma progression through regulation of CDK8. Nature 2010; 468:1105-9; PMID:21179167; http://dx.doi.org/10.1038/nature09590
8. Zhang R, Poustovoitov MV, Ye X, Santos HA, Chen W, Daganzo SM, Erzberger JP, Serebriiskii IG, Canutescu AA, Dunbrack RL, et al. Formation of MacroH2A-containing senescence-associated heterochromatin foci and senescence driven by ASF1a and HIRA. Dev Cell 2005; 8:19-30; PMID:15621527; http://dx.doi.org/10.1016/j.devcel.2004.10.019
9. Sulli G, Di Micco R, d'Adda di Fagagna F. Crosstalk between chromatin state and DNA damage response in cellular senescence and cancer. Nat Rev Cancer 2012; 12:709-20; PMID:22952011; http://dx.doi.org/10.1038/nrc3344
10. Willis NA, Chandramouly G, Huang B, Kwok A, Follonier C, Deng C, Scully R. BRCA1 controls homologous recombination at Tus/Ter-stalled mammalian replication forks. Nature 2014; 510:556-9; PMID:24776801; http://dx.doi.org/10.1038/nature13295

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No potential conflicts of interest were disclosed.

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