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REVIEW

# Chromatin maintenance and dynamics in senescence: a spotlight on SAHF formation and the epigenome of senescent cells

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**Abstract** Senescence is a stable proliferation arrest characterized by profound changes in cellular morphology and metabolism as well as by extensive chromatin reorganization in the nucleus. One particular hallmark of chromatin changes during senescence is the formation of punctate DNA foci in DAPI-stained senescent cells that have been called senescence-associated heterochromatin foci (SAHF). While many advances have been made concerning our understanding of the effectors of senescence, how chromatin is reorganized and maintained in senescent cells has remained largely elusive. Because chromatin structure is inherently dynamic, senescent cells face the challenge of developing chromatin maintenance mechanisms in the absence of DNA replication in order to maintain the senescent phenotype. Here, we summarize and review recent findings shedding light on SAHF composition and formation via spatial repositioning of chromatin, with a specific focus on the role of lamin B1 for this process. In addition, we discuss the physiological implication of SAHF formation, the role of histone variants, and histone chaperones during senescence and also elaborate on the more general changes observed in the epigenome of the senescent cells.

**Keywords** Chromatin dynamics · H3.3 · SAHF · Senescence · PML-NBs

**Abbreviations**

| | |
|---|---|
| ATRX | Alpha-thalassemia/mental retardation X-linked |
| DAXX | Death-domain associated protein |
| HP1 | Heterochromatin protein 1 |
| H3K9me3 | Histone H3 trimethylated at lysine 9 |
| OIS | Oncogene-induced senescence |
| PML | Promyelocytic leukemia |
| PML-NBs | PML nuclear bodies |
| SAHF | Senescence-associated heterochromatin foci |

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## Introduction

Cellular senescence is a stable proliferation arrest that can be triggered by telomere shortening or by oxidative, genotoxic, and other cellular stresses (Rai and Adams 2011). In addition, overexpression of activated oncogenes such as H-RasV12 induces a DNA damage response that ultimately leads to oncogene-induced senescence (OIS) that can act as an anti-cancer barrier (Serrano et al. 1997; Bartkova et al. 2006; Di Micco et al. 2006; Mallette et al. 2007). Senescence has thus emerged as an important tumor-suppressing mechanism to prevent further proliferation of premalignant cells and may also be implicated in tissue and organ aging (Rodier and Campisi 2011). However, the prevailing view of senescence occurring only under pathological conditions has now been challenged by the discovery that cellular senescence has an important physiological role during development. Senescent cells were identified during mammalian embryonic development at multiple locations, and their clearance by macrophages contributes to tissue remodeling and patterning of the embryo (Muñoz-Espín et al. 2013; Storer et al. 2013). Cellular senescence may thus have originated in evolution as a crucial part of normal development and may have been further adopted as a mechanism to eliminate dangerous cells.

Despite being arrested, senescent cells are characterized by an altered physical flat and enlarged morphology and are metabolically active. A hallmark feature of senescent cells is a modified transcriptional program characterized by the prominent secretion of a complex mixture of extracellular matrix

and soluble factors. This feature—referred to as the senescence-associated secretory phenotype (SASP) (Coppe et al. 2008)—participates in the reinforcement of the arrested state (Acosta et al. 2008; Kuilman et al. 2008). Senescent cells also display senescence-associated β-galactosidase (SA-β-Gal) activity as a consequence of an increased number of lysosomes (Dimri et al. 1995; Kurz et al. 2000). In addition, one striking characteristic of senescent cells is the large-scale reorganization of their chromatin. The basic unit of chromatin, the nucleosome core particle, comprises 147 base pairs of DNA wrapped around a core histone octamer, containing two times each of the core histones (H2A, H2B, H3, and H4). The linker histone H1 and nonhistone proteins participate in the dynamic regulation of chromatin compaction within the nucleus. Upon senescence entry, chromatin structure is extensively remodeled as exemplified by the formation of senescence-associated heterochromatin foci (SAHF), visible as microscopically discernible, punctate DNA foci in DAPI-stained senescent cells (Narita et al. 2003; Zhang et al. 2005).

While the network of effector pathways contributing to the senescence phenotype, for example, the prominent roles of the pRb and p53 pathways, has been described in detail in recent years (for review, Funayama and Ishikawa 2007; Rai and Adams 2011; O'Sullivan and Karlseder 2012), little is known about the underlying mechanisms of the extensive chromatin reorganization observed in senescent cells. In addition, how senescent cells (which can persist in the human body for decades as exemplified by senescent melanocytes in benign human nevi (Michaloglou et al. 2005)) face the challenge of chromatin maintenance in the absence of proliferation has remained unclear.

In this review, we discuss recent data that have increased our knowledge about how chromatin is reorganized and maintained during senescence, which is essential for genome stability. Specifically, we focus on SAHF formation and function in senescence and highlight novel contributions of lamin B1 in chromatin reorganization. We discuss the dynamics of histone variants and histone chaperones in senescence, as well as changes in the epigenome of senescent cells.

## Chromatin reorganization and structure of SAHF

SAHF, as initially described by Narita and colleagues, are visible as punctate DNA foci upon DAPI staining of senescent human primary fibroblats (Narita et al. 2003) (Fig. 1a). These foci are enriched in heterochromatic markers such as histone H3 trimethylated at lysine 9 (H3K9me3), heterochromatin protein 1 (HP1), and histone macroH2A (Narita et al. 2003; Zhang et al. 2005), a histone variant associated with gene silencing, for example, during X inactivation (Costanzi and Pehrson 1998). Typical euchromatic histone marks such as H3K4me3 or H3K9ac, as well as RNA polymerase II, are excluded from SAHF (Narita et al. 2003; Funayama et al. 2006). As a consequence, SAHF are thought to represent transcriptionally inactive regions which is further supported by the lack of any nascent RNA signals in these structures (Narita et al. 2003; Funayama et al. 2006; Corpet et al. 2014) (Fig. 1b). In addition to H3K9me3 and HP1 enrichment in SAHF, linker histone H1, usually considered to be a facilitator of chromatin condensation, is unexpectedly downregulated upon OIS and absent from SAHF (Funayama et al. 2006). In parallel, increased levels of the high mobility group A (HMGA) proteins have been reported in SAHF (Narita et al. 2006).

Chromosome painting analyses showed that each individual chromosome condenses into a single SAHF focus (Funayama et al. 2006; Zhang et al. 2007a). Development of highly specific monoclonal antibodies against various histone marks has enabled a refined characterization of the SAHF structure. SAHF are highly organized structures composed of multiple chromatin types arranged in concentric layers. The SAHF core is enriched with the constitutive heterochromatin mark H3K9me3 and is surrounded by an outer layer of H3K27me3 (a facultative heterochromatin marker) thus allowing separation of the silent core from the transcriptionally active regions (marked with H3K36me3) (Chandra et al. 2012) (Fig. 1c). This highly structured organization supports the idea that SAHF formation may contribute to the maintenance of gene expression profile stability for both active and repressed genes. Interestingly, the H3K9me3-enriched domains of constitutive heterochromatin such as pericentromeric and telomeric heterochromatin are not a part of SAHF but localize to the periphery of SAHF (Narita et al. 2003; Narita et al. 2006; Funayama et al. 2006; Zhang et al. 2007a), thus further supporting the idea of a spatial segregation of distinct chromatin states in senescent cells.

The incorporation of histone variants into chromatin is one way of modulating nucleosome stability and function (Talbert and Henikoff 2010; Luger et al. 2012) and is orchestrated by a family of proteins called histone chaperones (Burgess and Zhang 2013). While the histone variant H3.3 differs by only five amino acids from the canonical replicative variant H3.1, it has been technically challenging to address whether H3.3 is incorporated into SAHF and this issue has remained a matter of debate (for review, Adams 2007; Rai and Adams 2011). Because the H3.3-specific histone chaperone histone regulator A (HIRA) has been implicated in SAHF formation (see below) (Zhang et al. 2005; Zhang et al. 2007a), H3.3 was proposed to be enriched in SAHF. We have now addressed this issue by using human primary diploid fibroblasts expressing epitope-tagged forms of the H3 variants, H3.1 and H3.3, or by using an antibody highly specific for H3.3, which has recently been developed. We showed that both epitope-tagged H3.3 and endogenous H3.3 do not localize into SAHF but are actually excluded from the DAPI-dense regions. In addition,

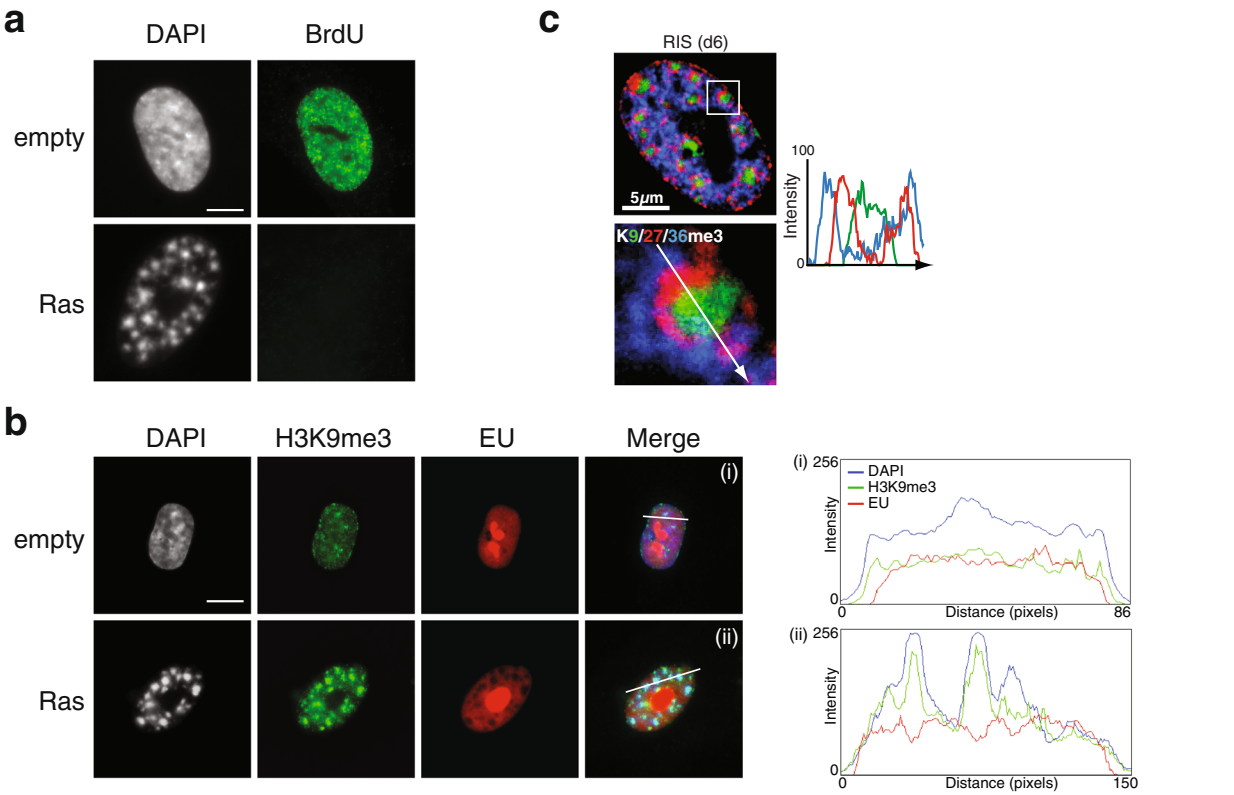


**Fig. 1** Structure and composition of SAHF. **a** Fluorescent microscopy visualization of proliferating (*empty*) or senescent human diploid fibroblasts induced into senescence with H-RasV12 overexpression (*Ras*). Only proliferating cells are labeled with BrdU (*green*). DAPI stains chromatin and is a marker of SAHF formation visible as DAPI-dense foci. *Scale bar* is 10 μm. Adapted from Corpet et al. (2014) **b** Fluorescent microscopy visualization of nascent global RNA transcription in vivo in human diploid fibroblasts treated as in **a** (*left*). Nascent RNAs were labeled with 5-ethynyl uridine (EU) and revealed by the Click-iT chemistry (*red*). SAHF are marked by the presence of H3K9me3 (*green*) and are visible as DAPI-dense foci. *Scale bar* is 10 μm. Graphs, showing fluorescent intensity profiles quantified along lines drawn through nuclei, underscores that transcriptional activity is excluded from SAHF (*right*). Adapted from Corpet et al. (2014) **c** Confocal microscopy visualization of indicated histone marks in human diploid fibroblasts induced in senescence by overexpression of H-RasV12 (RIS). The region indicated by the rectangle is magnified. The *arrow* indicates the path over which fluorescent intensity was profiled (*graphic on the right*). This image underscores the highly structured chromatin organization of SAHF in concentric layers. At the core, SAHF are enriched in H3K9me3, a mark of constitutive heterochromatin. The SAHF core is encircled by a ring of H3K27me3, a facultative heterochromatin mark. This latter ring separates the core from transcriptionally active regions marked by H3K36me3. Adapted from Chandra et al. (2012)

H3.3 localization correlates with transcriptional activity in senescent cells (Corpet et al. 2014). Interestingly, these results are in line with the "active pattern of posttranslational modifications (PTMs) found on H3.3 (Hake et al. 2006; Loyola et al. 2006) and with the fact that deposition of H3.3 is typically associated with transcriptional activation (Ahmad and Henikoff 2002; Chow et al. 2005; Wirbelauer 2005; Mito et al. 2005; Jin et al. 2009). However, this does not exclude localization of H3.3 in pericentromeric or telomeric regions, as demonstrated in proliferating cells (Wong et al. 2008; Goldberg et al. 2010; Drane et al. 2010; Corpet et al. 2014). These regions localize outside of SAHF in senescent cells. In contrast, new H3.1 was not deposited in senescent cells, but the preexisting pool of H3.1 was enriched in SAHF (Corpet et al. 2014). This is consistent with previous data showing enrichment for heterochromatin marks on arrays of nucleosomes containing the canonical H3.1 (Loyola et al. 2006). Moreover, these findings support the hypothesis that a spatial rearrangement of preexisting heterochromatin upon senescence entry is the predominant mechanism of SAHF formation (Chandra et al. 2012; Chandra and Narita 2013) (see also below).

Beyond the typical SAHF formation, chromatin is further reorganized upon senescence. The thickness of the perinuclear H3K9me3-rich heterochromatin is significantly diminished in senescent cells (De Cecco et al. 2013; Sadaie et al. 2013). In addition, an age-related global loss of histones H3-H4 has been reported at the protein level both during aging of budding yeast and in human cells that are approaching replicative senescence (Feser et al. 2010; O'Sullivan et al. 2010). This event is thought to promote destabilization of chromatin structure together with misregulated gene expression and triggers a DNA damage response that enforces the aging phenotype. Thus, these observations suggest that chromatin alterations upon senescence might contribute to the aging process (for review, Rodier and Campisi 2011; O'Sullivan and Karlseder

2012). Interestingly, Ivanov et al. recently showed the appearance of cytoplasmic chromatin fragments (CCFs) in late senescent cells, which are processed by an autophagic/lysosomal pathway, resulting in low histone content. This would contribute to the stability and "deepness of the senescence phenotype in mature portions of human nevi (Ivanov et al. 2013). In the case of H3 variants, the centromeric CENP-A (another H3 variant) is diminished in senescent cells through transcriptional and posttranslational control mechanisms (Maehara et al. 2010). In contrast, the amount of histone H3.3 increases during replicative senescence of human fibroblasts concomitant with a decrease in the amount of canonical H3.1, suggesting an important role of H3.3 during senescence (Rogakou and Sekeri-Pataryas 1999). Intriguingly, the specific H3.3K36me3 modification that is deposited on gene bodies during transcriptional elongation was recently implicated in recruiting the tumor suppressor ZMYND11, thus linking the H3.3-mediated transcriptional elongation control with tumor suppression (Wen et al. 2014). In addition, mutations of H3.3 on G34 in pediatric brain tumors may impact on the ability of histone-modifying complexes to methylate H3.3 K36, which may be altering transcription of key target genes (for review, Liu et al. 2014). Thus, whether H3.3 and its specific modification H3.3K36me3 could contribute to senescence via maintenance of a specific transcriptional program remains to be investigated. In any case, these data emphasize the novel importance of the histone variant H3.3 in tumor suppression.

## Factors involved in SAHF formation

Since the implication of the p16INK4A-Rb and p53-Rb pathways in SAHF formation has been extensively described and reviewed (Serrano et al. 1997; Narita et al. 2003; Chan 2005; Ye et al. 2007; Chicas et al. 2010) (for review, Rodier and Campisi 2011; O'Sullivan and Karlseder 2012), we focus here on the role of chromatin-associated components in SAHF formation.

### Role of histone chaperones and how they are linked to PML nuclear bodies

Histone chaperones are factors that interact with histones throughout their cellular life and play a crucial role in nucleosome assembly (Burgess and Zhang 2013). While the canonical histone H3.1 is deposited in chromatin by chromatin assembly factor 1 (CAF-1) in a DNA synthesis-dependent manner, several specific histone chaperones exist for the histone H3 variant H3.3 and deposit this particular protein into chromatin in a DNA synthesis-independent manner. The histone chaperone HIRA along with associated factors, anti-silencing function 1a (ASF1a), ubinuclein 1 (UBN1), and Cabin1, is responsible for H3.3 deposition into active chromatin, whereas the H3.3-specific chaperone death domain-associated protein (DAXX) in cooperation with the chromatin remodeler α-thalassemia/mental retardation syndrome X-linked (ATRX) is essential for H3.3 deposition at heterochromatic loci (for review, Burgess and Zhang 2013). Since cellular senescence is associated with large-scale chromatin alterations, a long-standing question has been whether the histone chaperones have a particular role during senescence. The first clue to this question came from Adams and colleagues who reported that ectopic overexpression of histone chaperones HIRA and ASF1a leads to SAHF formation in primary human fibroblasts (Zhang et al. 2005) and requires the H3-binding region of ASF1 (Zhang et al. 2007a). In addition, it was shown that knockdown of ASF1a by RNAi impairs OIS induced by overexpression of H-RasV12 (Zhang et al. 2005). Interestingly, HIRA localizes in promyelocytic leukemia (PML) nuclear bodies (PML-NBs) as cells enter senescence, and this is required for SAHF formation. Indeed, dominant negative HIRA mutants that block HIRA's localization in PML-NBs prevent SAHF formation, as does the disruption of PML-NBs with a PML-RARα fusion protein (Zhang et al. 2005; Ye et al. 2007). PML-NBs are discrete foci, 0.2–1.0-μm wide, that are present in most mammalian cell nuclei. They stain positive for the tumor suppressor PML and have been implicated in the onset of OIS (Bernardi and Pandolfi 2007; Lallemand-Breitenbach and de The 2010). Importantly, other members of the HIRA complex, namely UBN1 and Cabin1, were also shown to localize to PML-NBs only in senescent cells, and ectopic expression of these two proteins also led to SAHF formation and senescence entry, suggesting a function of the entire HIRA histone chaperone complex in SAHF assembly (Banumathy et al. 2009; Rai et al. 2011). Thus, these data suggested that the H3.3 histone chaperones forming the HIRA complex localize to PML-NBs during senescence and that this is important for SAHF formation (Fig. 2). In addition, HP1 proteins transiently localize to PML-NBs prior to their incorporation into SAHF (Zhang et al. 2005) further highlighting a connection of these nuclear bodies with heterochromatin assembly in senescence.

However, it is still unclear if SAHF formation is mediated by the incorporation of H3.3 into chromatin. Functional implication of H3.3 in senescence as well as the role of its other specific chaperones DAXX and ATRX, which localize in PML-NBs in proliferating cells (Xue et al. 2003; Ishov 2004), was recently investigated. Remarkably, DAXX, ATRX, or H3.3 is dispensable for senescence entry, consistent with the absence of H3.3 localization at SAHF (Corpet et al. 2014). While genome-wide incorporation of H3.3 is only affected by HIRA but not by DAXX depletion (Ray-Gallet et al. 2011; Corpet et al. 2014), further studies investigating the requirement for H3.3 or HIRA in senescence maintenance should provide more insights into the role of this variant during senescence. Interestingly, H3.3 was found to be

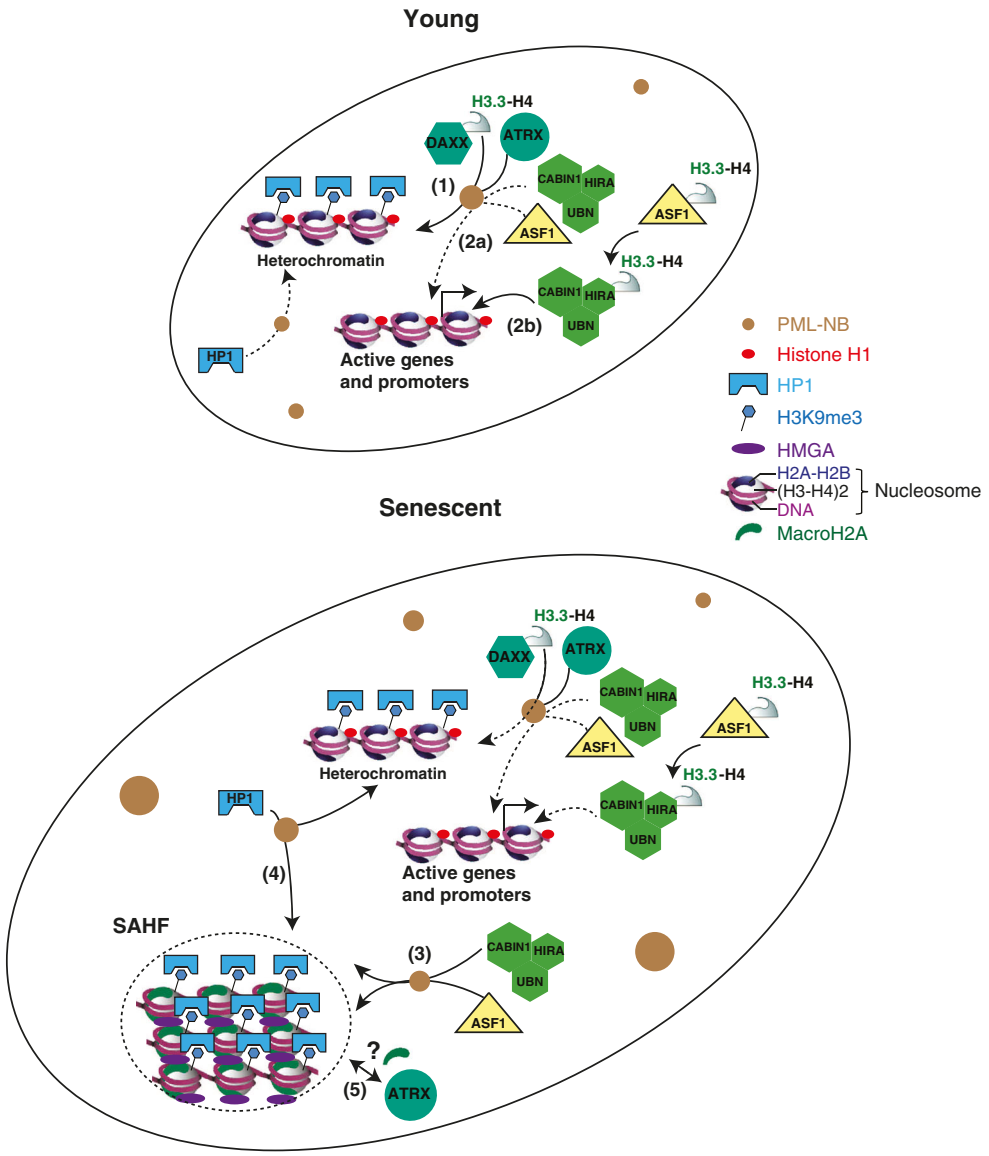


**Fig. 2** SAHF formation and PML-NBs dynamics. In proliferating cells, two pathways regulate H3.3 deposition into chromatin independently of DNA synthesis. DAXX cooperates with the chromatin remodeler ATRX for the accumulation of H3.3 at pericentric and telomeric heterochromatin (*1*). Importantly, H3.3 localizes in PML-NBs in a DAXX-dependent manner, and this is an essential process for its subsequent deposition on pericentromeric heterochromatin. In euchromatin, the HIRA complex (comprising HIRA, Cabin1, and UBN1) is responsible for H3.3 enrichment in the body of transcribed genes and at promoters of transcribed or nontranscribed genes (*2*). Whether this latter pathway requires the transient localization of the HIRA complex in PML-NBs to be loaded with H3.3 is not clear (*dashed arrow*) (*2a*). Alternatively, the histone chaperone ASF1 acts directly as a histone donor for the HIRA complex (*2b*). In senescent cells, PML-NBs increase in size and number. As an early event during senescence, localization of the HIRA complex as well as ASF1 in PML-NBs is required for SAHF formation (*3*). HP1 proteins also transiently localize in PML-NBs before their incorporation into SAHF, but their presence is actually not a prerequisite for SAHF formation (*4*). As in proliferating cells, DAXX is required for H3.3 localization in PML-NBs. However, whether this is important for H3.3 incorporation at pericentromeric heterochromatin and whether the HIRA complex regulates H3.3 deposition at active genes in senescence has not been investigated yet. In addition, it remains unknown if ATRX, which localizes at SAHF, can regulate incorporation of macroH2A in SAHF (*5*). PML-NBs thus appear as important regulatory structures involved in chromatin dynamics in senescent cells, but, of note, this figure does not depict the well-established role of PML-NBs in the regulation of p53 and Rb/E2F dynamics in senescence

targeted to PML-NBs in a DAXX-dependent manner both in proliferating and senescent cells (Delbarre et al. 2013; Corpet et al. 2014) (Fig. 2). Thus, while PML-NBs play important functions during senescence through regulation of the p53 and Rb/E2F pathways (Pearson et al. 2000; Ferbeyre et al. 2000; de Stanchina et al. 2004; Vernier et al. 2011; Martin et al. 2011), they may also represent novel interstages in the replication-independent chromatin assembly pathway (Fig. 2). However, the exact functional role of PML-NBs in chromatin dynamics in senescence remains to be elucidated.

Concerning the loading of the macroH2A variant into SAHF, it is interesting to note that ATRX has also been

identified as a macroH2A chaperone (Ratnakumar et al. 2012). MacroH2A is incorporated into chromatin after the appearance of SAHF suggesting that this variant may play a role in the maintenance of SAHF rather than in their induction (Zhang et al. 2005; Funayama and Ishikawa 2007). Thus, it should be further investigated if ATRX, which localizes at SAHF in senescent cells but is not essential for SAHF formation (Corpet et al. 2014), could contribute to SAHF maintenance in senescent cells via the regulation of macroH2A dynamics (Ratnakumar et al. 2012).

## Dynamics of architectural components upon SAHF formation

Since SAHF are characterized by the presence of heterochromatin marks, as well as by the presence of chromatin-associated HMGA proteins, we will discuss how these architectural components affect SAHF formation.

The specific role of H3K9me3 and HP1 in SAHF formation is still unclear. Senescent cells display an increase in the total amount of chromatin-bound HP1 (β or γ), which binds to H3K9me3 (Narita et al. 2003; Di Micco et al. 2011). In addition, relative to proliferating cells, a general increase in the resistance of nuclear chromatin to digestion by nucleases can be observed (Narita et al. 2003). However, the total amount of H3K9me3 does not substantially change in senescent cells (Funayama et al. 2006; Chandra et al. 2012). Thus, it is possible that the nonstoichiometric increase in the total amount of HP1 proteins compared to H3K9me3 in senescent cells could be explained by the association of HP1 with other histone marks (Dawson et al. 2009) or to a change in affinity of HP1 for H3K9me3. Interestingly, expression of a dominant negative form of HP1β (HP1βΔN) that reduces the amount of all three chromatin-bound isoforms of HP1 by about 80 % does not affect SAHF formation and senescence entry (Zhang et al. 2007b). In addition, profiling of the repressive histone mark H3K9me3 in senescent human primary fibroblasts surprisingly showed that the landscape of H3K9me3 remains remarkably unchanged upon OIS despite normal SAHF formation (Chandra et al. 2012) (see further in Fig. 4a). Thus, although this repressive mark is locally enriched at specific genes (Narita et al. 2003), its highly static pattern strongly suggests a 3D repositioning of preexisting marks to form SAHF, rather than formation and spreading of new heterochromatin in SAHF (Chandra and Narita 2013). Of note, H3K9me3 is also dispensable for SAHF formation in cells where the H3K9me3 demethylase JMJD2D is overexpressed (Chandra et al. 2012). Thus, these data suggest that HP1 and H3K9me3, hallmarks of constitutive heterochromatin, are dispensable for the formation of the highly compacted SAHF core. While other repressive histone marks might be involved in this process, chromatin proteins that do not require binding to histones may also contribute to SAHF formation.

In particular, one structural component of SAHF, the architectural protein HMGA1, an "AT-hook containing protein that binds the minor groove of AT-rich DNA, is required for both the establishment and maintenance of SAHFs as shown by ectopic overexpression and knockdown experiments (Narita et al. 2006). This was unexpected because HMGA proteins are known to favor transcriptionally active chromatin and promote tumor progression (Reeves 2001; Hock et al. 2007). Interestingly, another AT-hook DNA-binding protein has been associated with repressive chromatin independently of heterochromatic histone marks in Drosophila (Filion et al. 2010). Thus, HMGA proteins may contribute to SAHF formation by bending linker DNA and thus promote chromatin condensation (Funayama and Ishikawa 2007). In parallel, it was shown that histone H1 is absent from chromatin and from the entire cell upon senescence entry and that its loss is correlated with frequency of SAHF formation (Funayama et al. 2006; Funayama and Ishikawa 2007). Since histone H1 and HMGA proteins compete for the same binding site on DNA, this suggests that histone H1 could be substituted with HMGA proteins on chromatin in senescent cells (Catez et al. 2004). In summary, while the role of HMGA proteins seems to be essential in SAHF formation, the role of HP1 and H3K9me3 remains to be further analyzed. Investigating the role of histone H3K9me3 methylases/demethylases should help to determine the importance of this modification in senescence.

## The role of the nuclear lamina in histone PTM dynamics and SAHF formation

The nuclear lamina (NL) forms a scaffold of lamin proteins underneath the inner nuclear membrane. Beyond this structural role, interaction of the NL with the genome suggests that the lamina plays a role in organizing chromatin into domains marked by distinct epigenetic modifications that are widely involved in the control of gene expression programs during lineage commitment and terminal differentiation (Peric-Hupkes et al. 2010) (for review, Burke and Stewart 2013; Bickmore and van Steensel 2013). In particular, genome-wide mapping of lamin B1 (LMNB1) identified large lamina-associated domains (LADs), forming a repressive environment in which the majority of genes are transcriptionally inactive and enriched with repressive histone marks (Guelen et al. 2008; Bickmore and van Steensel 2013) (Fig. 3). While it was previously known that global LMNB1 levels are down-regulated during senescence (Shimi et al. 2011; Freund et al. 2012), LMNB1 alterations have now been mechanistically linked to the large-scale changes in chromatin organization and gene expression (Sadaie et al. 2013; Shah et al. 2013).

Using ChIP-Seq to map the genomic binding profiles of LMNB1 in primary human fibroblasts, an uneven redistribution of LMNB1 genomic binding upon senescence, with a

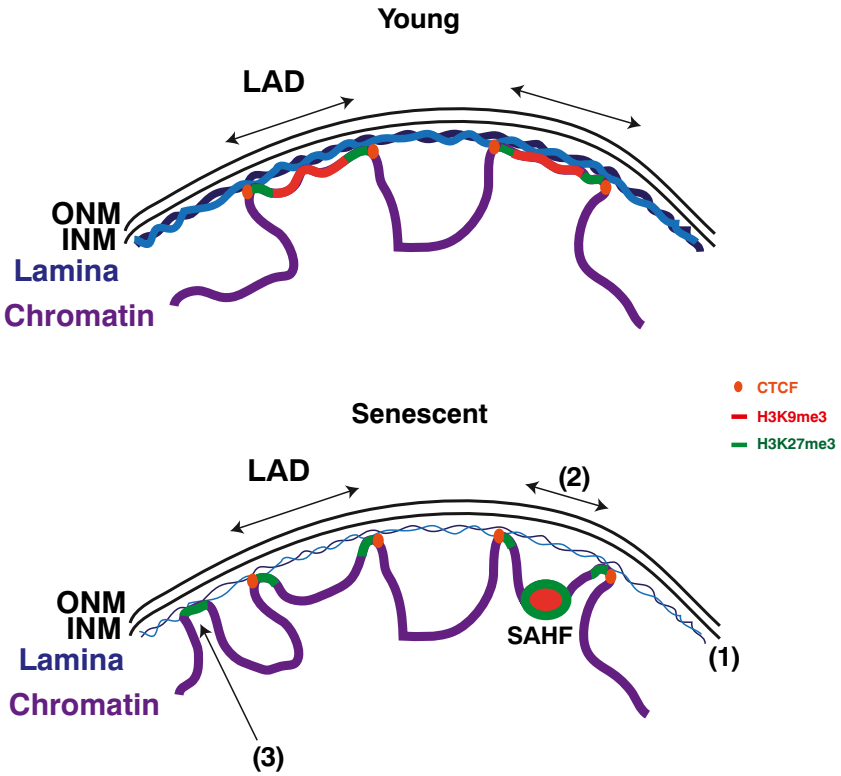


**Fig. 3** Model for the role of lamin B1 (LMNB1) in SAHF formation. In proliferating cells, LMNB1 forms a filamentous structure beneath the inner nuclear membrane (INM), which is implicated in the integrity of nuclear structure and regulation of gene expression. In particular, lamina-associated domains (LADs) form a repressive environment in which the majority of genes are transcriptionally inactive and enriched with repressive histone marks such as H3K9me2/3 and H3K27me3. In senescent cells, three mechanisms affecting LMNB1 could potentially contribute to the senescent phenotype: (*1*) LMNB1 levels are globally downregulated in senescence, and this is associated with reduced cell proliferation (Shimi et al. 2011; Barascu et al. 2012). (*2*) Loss of LMNB1 binding preferentially from H3K9me3 rich-regions at the center of LADs participates in the spatial repositioning of heterochromatin (H3K9me3-enriched), and this would help SAHF formation (Sadaie et al. 2013). In addition, LMNB1 loss would also contribute to establishment of the changes observed in histone PTMs upon senescence (Shah et al. 2013). (*3*) In about 2 % of the genome, LMNB1 shows an increased binding, together with increased H3K27me3 across the gene bodies. Genes within these regions tend to be repressed and include cell cycle genes. Thus, LMNB1 could participate in gene regulation, and this could help to reinforce the proliferation arrest

more pronounced reduction of LMNB1 in H3K9me3 regions, at the center of LADs, was observed (Sadaie et al. 2013) (Fig. 3). Interestingly, small hairpin RNA (shRNA)-mediated LMNB1 reduction induced a mild senescence phenotype (Sadaie et al. 2013; Shah et al. 2013) associated with moderate SAHF formation, which was increased by combined overexpression of HMGA proteins (Sadaie et al. 2013). This suggests that LMNB1 reduction during senescence facilitates repositioning of H3K9me3-rich heterochromatin toward the nuclear center, and this could help SAHF formation (Fig. 3).

In addition, changes in the nuclear lamin interactions with the genome have now been linked to histone PTM changes upon senescence. Shah et al. mapped the histone H3K4me3 and H3K27me3 modifications in replicative senescent human fibroblasts and found that the global levels of these marks do not change upon senescence (Shah et al. 2013). However, genome-wide altered patterns of these modifications were observed. Specifically, H3K4me3 was enriched in large domains of a few hundreds of kilobases (called "mesas ), overlapping with large domains of enrichment of H3K27me3 and with LADs (Shah et al. 2013). In addition, large domains of reduced H3K27me3 modification were also observed (called "canyons ), corresponding to transcriptional enhancer regions (Fig. 4a). Interestingly, shRNA-mediated LMNB1 depletion triggered these pattern alterations of H3K4me3 and H3K27me3 during senescence (Shah et al. 2013). This suggests that loss of LMNB1 could facilitate the genome-wide local changes of H3K4me3 and H3K27me3 with an enrichment of these modifications at the center of LADs.

Importantly, genome-wide studies revealed that alterations in LMNB1 and histone PTMs patterns are also linked to the regulation of gene expression thus suggesting that these may be important effectors of senescence. Indeed, H3K4me3 depletion is correlated with downregulation of cell cycle and proliferating genes (Shah et al. 2013). In addition, H3K27me3 loss (a repressive mark) is strongly correlating with upregulation of a number of genes involved in senescence entry and

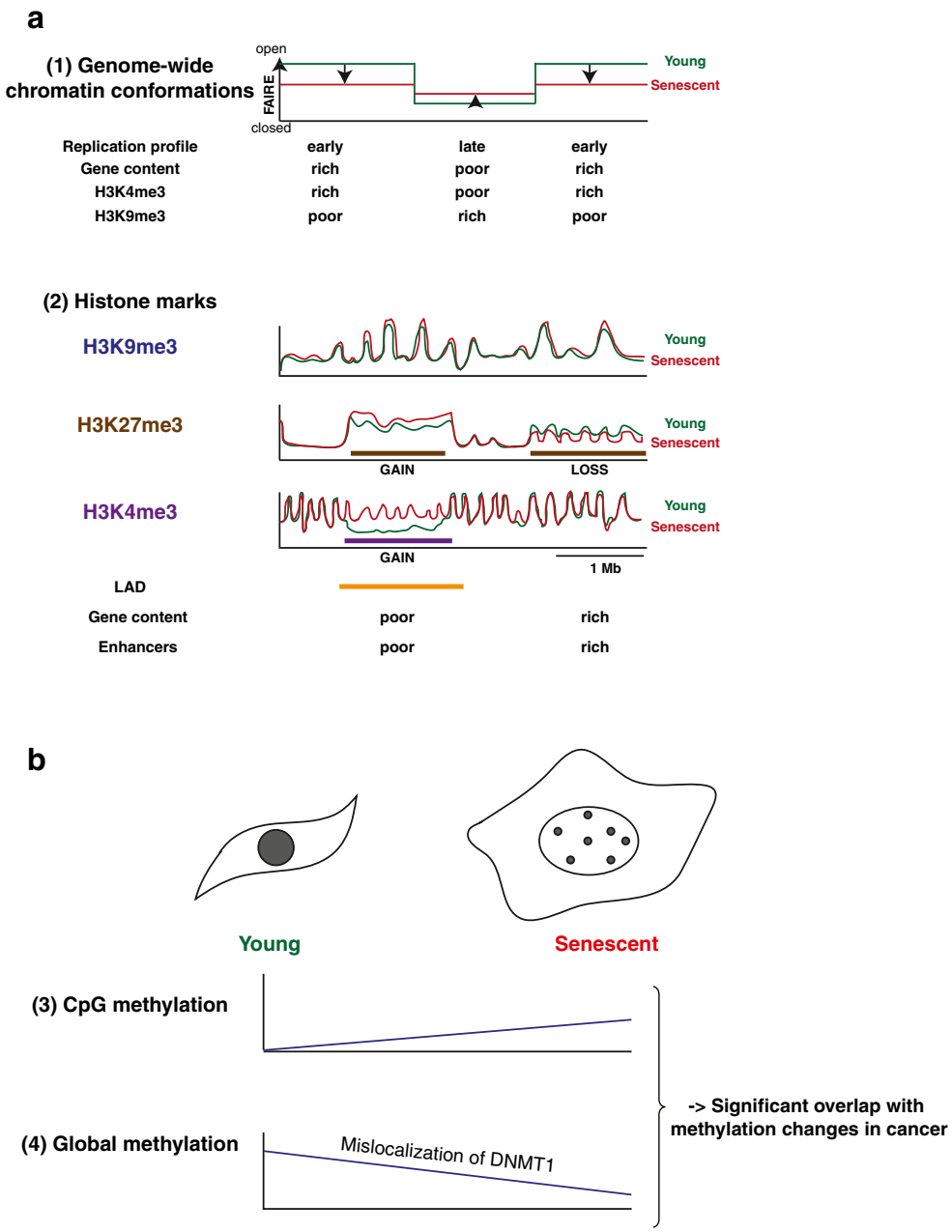


**Fig. 4** Altered epigenome in senescent cells. This figure presents a model for how the altered epigenome in senescent cells may contribute to the senescent phenotype. **a** Altered chromatin and histone PTMs in senescence. (*1*) Genome-wide mapping of open chromatin regions by the formaldehyde-assisted isolation of regulatory elements (FAIRE) method identified two processes: a "closing of gene-rich, H3K4me3-rich, early-replicating regions, and a paradoxical "opening of heterochromatin regions, which is associated with expression of transposable elements and satellite DNA as described in *4*. (*2*) Schematic view of the genome-wide mapping of specific histone PTMs via ChIP-Seq. Several independent processes were identified: for H3K9me3, the landscape remains globally unchanged and this underscores the spatial repositioning of heterochromatin upon senescence. For H3K27me3, another repressive mark, the situation is less clear (Sadaie et al. 2013; Shah et al. 2013). Nevertheless, Shah et al. identified some large regions of gains and losses that correlate with the repression of cell cycle genes or the upregulation of key senescence genes such as SASP genes, respectively. In addition, regions of H3K27me3 gains also correlate with increased H3K4me3 and LAD regions. Thus, large-scale changes in histone PTMs might contribute to the senescent phenotype by regulating gene expression. **b** Altered methylation in senescent cells. (*3*) Local hypermethylation at CpG islands can contribute to the repression of cell cycle genes, and this would reinforce the senescence-associated proliferation arrest. (*4*) On the other hand, global hypomethylation at gene-poor, late-replicating, or LAD regions would increase transcription of satellite DNA and transposable elements. The physiological relevance of this process remains to be fully understood, but the increased RNA transcription of satellite DNA could hypothetically contribute to the senescent phenotype by reinforcing heterochromatinization of these regions. In any case, the significant overlap of methylation changes with those observed in cancer cells suggests that some features of the cancer methylome could originate from a premalignant senescent state that has been overcome

maintenance such as the key SASP genes (Shah et al. 2013). In contrast, local increase of the H3K27me3 modification associated with LMNB1 binding in a subset of gene-rich regions facilitates the repression of genes belonging to the cell cycle Gene Ontology category during senescence (Sadaie et al. 2013) (Fig. 4a). In conclusion, these studies have helped to further our understanding of the dynamic changes in chromatin structure upon senescence. They shed new light on the functional role of lamins as effectors of senescence through their ability to regulate spatial chromatin reorganization and hence, gene expression. However, some conflicting observations upon overexpression of LMNB1 still need to be resolved. Two studies reported increased senescence upon overexpression of LMNB1 (Barascu et al. 2012; Dreesen et al. 2013), while others found delayed senescence (Shimi et al. 2011; Sadaie et al. 2013; Shah et al. 2013) (for review, Burke and Stewart 2013).

These studies revealed the importance of changes of histone PTMs in senescence and the role of the nuclear lamina in this process, but it is important to note that changes in histone-modifying enzymes have also been implicated in senescence (for review, David 2012). For example, knockdown of the H3K27me3 methylase EZH2 leads to rapid senescence in primary human cells, consistent with the dispensable role of H3K27me3 for SAHF formation (Chandra et al. 2012). This process could be mediated in part by upregulation of p16INK4A (Bracken et al. 2007). It remains to be determined to which extent these histone modifiers affect senescence at the level of transcriptional regulation of specific genes or genome-wide in the context of chromatin reorganization.

## Global epigenetic changes in senescent cells resemble epigenetic alterations in cancer

While we have discussed chromatin changes occurring in senescence at the levels of histone variants, histone modifications, and heterochromatin formation, it has become apparent that global epigenetic changes such as changes in chromatin accessibility and DNA methylation also occur in senescent cells. Previous studies had shown that DNA methylation of constitutive heterochromatin decreases in senescence and aging which is probably caused by downregulation of the DNA methyltransferase DNMT1 (Wilson and Jones 1983; Young 2003; Zhang et al. 2008). In addition, potent inhibitors of DNA methyltransferase such as 5-azacytidine (aza-C) led to a reduction of DNA methylation in normal human fibroblasts followed by premature senescence (Young 2001). However, the genome-wide distribution of these changes remained unknown. Using whole genome analyses, two recent papers now demonstrate that large epigenetic changes occur in senescent cells. De Cecco et al. used the formaldehyde-assisted isolation of regulatory elements (FAIRE) method to map open chromatin regions across the whole genome in replicative senescent human diploid fibroblasts (De Cecco et al. 2013). Interestingly, the authors observe an overall condensation of chromatin in euchromatin gene-rich regions and a relaxation of the late-replicating heterochromatin regions in senescent cells (Fig. 4a). This latter event is associated with increased transcription of retrotransposons as well as pericentromeric satellite repeats, consistent with previous studies showing transcription of satellites 2 and 3 as a result of decondensation and demethylation of the satellite repeats in senescent cells (Suzuki et al. 2002; Enukashvily et al. 2007). Remarkably, hypomethylated satellite DNA present in immunodeficiency, centromeric instability, and facial dismorphy (ICF) cells localized in large PML-NBs (Luciani 2006). Maybe satellite repeats could also localize in the larger PML-NBs that have been observed upon senescence (Pearson et al. 2000; Ferbeyre et al. 2000), and this may explain their increased transcription through an enhanced targeting of H3.3 to these regions. Of note, increased transcription of retrotransposons and satellite repeats occurred at late time points after senescence entry, thus suggesting that these changes are likely a consequence rather than a cause of epigenetic changes observed upon senescence (De Cecco et al. 2013).

To what extent changes in DNA methylation are implicated in the senescence process has also been largely unexplored until recently. Cruickshanks et al. now provide the first comprehensive analysis of DNA methylation in senescent cells by performing a whole-genome single-nucleotide bisulfite sequencing. They observe extensive changes in DNA methylation in senescent cells as compared to proliferating cells. Senescent cells harbor widespread hypomethylation in late-replicating, gene-poor regions, including pericentromeric satellites and LADs (Cruickshanks et al. 2013), consistent with the "open conformation of these regions observed by FAIRE-chip (De Cecco et al. 2013) and the enrichment in H3K4me3 in these large domains (Shah et al. 2013). This hypomethylation resulted in increased transcription of these regions, correlating with the decreased expression of DNMT1 (De Cecco et al. 2013; Cruickshanks et al. 2013). On the other hand, local hypermethylation was observed at promoter-proximal regions, flanking cytosine-phosphate-guanine (CpG) islands, and this was associated with repression of cell cycle genes in senescence (Cruickshanks et al. 2013) (Fig. 4b). Thus, these results suggest that global changes in DNA methylation might be effectors in the senescence process, although they might also be redundant with other senescence triggers and effectors (for review, Kuilman et al. 2010).

Importantly, the global epigenetic events described above, including derepression of retrotransposons, increased satellite transcription, global DNA hypomethylation, and local hypermethylation at CpG islands, have been previously documented in cancer cells (Belancio et al. 2010; Hansen et al. 2011;

Berman et al. 2011; Jones and Jones 2012; Tilman et al. 2012). Thus, it was hypothesized that these changes could prefigure epigenetic cancer changes (Cruickshanks et al. 2013). First, Cruickshanks et al. show that altered methylation states in senescence (differentially methylation regions, DMRs) overlap with those described previously in cancer cells. While replicative and OIS are barriers to cell transformation, these data suggest that the premalignant senescent cells present features of the cancer methylome. Second, this global epigenetic landscape is retained in senescent cells that were forced to bypass senescence by inactivation of p53 and pRb (Cruickshanks et al. 2013). This suggests that if senescent cells can escape the proliferative barrier, they may already harbor an epigenetic landscape likely to promote genome instability and cancer.

## Potential functional roles of chromatin alterations in senescent cells

### Potential functions associated with chromatin reorganization upon senescence

The stability of the growth arrest of senescent cells contributes to the tumor suppressor function of cellular senescence, and it is therefore crucial that senescent cells actively maintain a nondividing state. Two mechanisms potentially ensure the irreversibility of the senescent state: first, the existence of mechanisms that prevent cell cycle reentry in S-phase and in M-phase via p53 and pRb pathways (for review, Funayama and Ishikawa 2007). In addition, it has been hypothesized that chromatin reorganization contributes to the stability of the cell cycle arrest. Indeed, SAHF contribute to the senescence-associated cell cycle arrest in part by silencing proliferation-promoting genes, such as cyclin A, through heterochromatinization (Narita et al. 2003; Zhang et al. 2007a). In addition, an alternative function for SAHF was recently put forward: D'Adda di Fagagna and colleagues showed that oncogene-induced SAHF formation may protect premalignant cells from undergoing apoptosis by limiting extensive DNA damage caused by replication stress to sublethal levels (Di Micco et al. 2011). These findings led them to suggest that there are two types of heterochromatin in senescent cells: one that suppresses expression of cell cycle-related genes and one that suppresses the DNA damage response. Inactivation of ATM or p53 is sufficient to release proliferation-promoting genes from heterochromatin-mediated repression even though SAHF persisted (Di Micco et al. 2011). Thus, SAHF may not be sufficient to maintain the proliferation arrest (Kosar et al. 2011).

It is still unclear to what extent the chromatin reorganization plays a role in the tumor suppression function of senescence in vivo. While it is well established that senescent cells are present in vivo in several types of benign or premalignant tumors but not in malignant tumors of mouse and human tissues (Denchi et al. 2005; Braig et al. 2005; Chen et al. 2005; Collado et al. 2005; Michaloglou et al. 2005), one study failed to detect SAHF in premalignant human tissues (Kosar et al. 2011). However, other studies identified SAHF-like alterations in the early stages of in vivo OIS (Denchi et al. 2005; Collado et al. 2005; Di Micco et al. 2011). In addition, disruption of the Suv39h1 histone methyltransferase, that is responsible for methylation of the H3K9me3 present in SAHF, dramatically accelerates Ras-induced T cell lymphomagenesis in a mouse model (Braig et al. 2005), thus implicating these structures in the tumor-suppressing role of senescence. Taken together, while it is clear that chromatin reorganization plays a crucial role in the senescence state both in vitro and in vivo, the specific contribution of SAHF in this process still needs to be better defined.

### Dynamics of histone incorporation in senescent cells

While senescent cells do not divide by definition and because chromatin structure is inherently dynamic (Deal et al. 2010), the question of how chromatin is maintained in the absence of DNA replication has remained elusive. Using the SNAP tag technique that allows dynamic labeling of preexisting or new histone pools (Ray-Gallet et al. 2011; Bodor et al. 2012), we addressed the question of the dynamic incorporation of histone H3 variants in oncogene-induced senescent cells. We showed that there is a de novo incorporation of new H3.3 histones in chromatin in senescent cells, in regions correlating with the transcriptional activity of senescent cells (Corpet et al. 2014). This suggests that replication-independent chromatin assembly is an important mode of histone replacement in nondividing senescent cells, consistent with the accumulation of H3.3 in aging human fibroblasts (Rogakou and Sekeri-Pataryas 1999). Recent studies identifying driver H3.3 mutations in pediatric glioblastomas (Schwartzentruber et al. 2012; Wu et al. 2012; Khuong-Quang et al. 2012; Sturm et al. 2012) or linking H3.3 incorporation with cellular memory (Ng and Gurdon 2007) further underscore the physiological importance of H3.3 deposition for the maintenance of chromatin structure and cellular phenotype. However, it remains to be established to what extent H3.3 deposition is important for the stability of the senescent phenotype. Since HIRA has been implicated in transcription-coupled deposition of H3.3 (Ray-Gallet et al. 2011), knockdown experiments of this particular chaperone should address this question.

In contrast, new H3.1 is not incorporated in senescent cells, consistent with its replication-coupled assembly mode. However, H3.1 is accumulated in SAHF upon induction of senescence (Corpet et al. 2014), possibly as a result of the high condensation of chromatin in these regions. The mechanism of H3.1 maintenance in SAHF, possibly over decades of senescence, is unknown. Its specific chaperone CAF-1 is

downregulated in senescence indicating other modes of maintenance (Corpet et al. 2010). The recently demonstrated longevity of histone H3.1 (Toyama et al. 2013) might provide a potential explanation. Thus, the senescent state requires chromatin maintenance mechanisms that are independent of DNA replication. It remains to be determined if senescence-associated H3.1 and H3.3 dynamics is similar to that in terminally differentiated postmitotic cells.

## Conclusion

In this review, we summarized recent progress in understanding the dynamics of chromatin conformation and epigenetic marks in cellular senescence and discussed how they may act as potential effectors of senescence. In particular, development of techniques that allow genome-wide studies has been instrumental for the analysis of the molecular alterations in the epigenome of the senescent cells (Chandra et al. 2012; De Cecco et al. 2013; Sadaie et al. 2013; Shah et al. 2013; Cruickshanks et al. 2013). While the relative contribution of local versus global chromatin changes in the senescence process remains to be further deciphered, these recent studies revealed major chromatin rearrangements induced by LMNB1 loss and these have been shown to impact on the gene expression profiles of senescent cells.

Besides, alterations in chromatin states changes in cell metabolism also regulate senescence entry (Young et al. 2009; Weyemi et al. 2011; Jiang et al. 2013; Aird et al. 2013; Kaplon et al. 2013) (for review, Salama et al. 2014). Because strong evidence now supports the idea of a physiological role of senescence both in development (Muñoz-Espín et al. 2013; Storer et al. 2013) and as an anticancer barrier (for review, Collado and Serrano 2010) understanding the molecular mechanisms of chromatin changes upon senescence might open up new avenues to modulate this complex phenotype in cancer therapy. In summary, all the findings that we reviewed here indicate that dynamic alterations of chromatin states are important for the senescent phenotype on many different levels and are part of a network of multiple effector pathways that contribute to this heterogeneous phenotype.

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