Published in final edited form as:

Gene. 2007 August 1; 397(1-2): 84–93. doi:10.1016/j.gene.2007.04.020.

Remodeling of chromatin structure in senescent cells and its potential impact on tumor suppression and aging

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Abstract

Cellular senescence is an important tumor suppression process, and a possible contributor to tissue aging. Senescence is accompanied extensive changes in chromatin structure. In particular, many senescent cells accumulate specialized domains of facultative heterochromatin, called Senescence Associated Heterochromatin Foci (SAHF), which are thought to repress expression of proliferation-promoting genes, thereby contributing to senescence-associated proliferation arrest. This article reviews our current understanding of the structure, assembly and function of these SAHF at a cellular level. The possible contribution of SAHF to tumor suppression and tissue aging is also critically discussed.

Keywords

HIRA; ASF1a; SAHF; senescence; cancer

1. An overview of cellular senescence

1.1 Senescence and its physiological impact

Cellular senescence is characterized by an irreversible arrest of cell proliferation. Senescence *in vivo* is thought to be an important tumor suppression process. Activated oncogenes trigger cell senescence, thereby blocking progression to a transformed cell phenotype, notably in primary human melanocytes, human prostatic epithelium, benign human neurofibromas, mouse lymphocytes and pre-malignant mouse lung adenomas (16,25,26,30,37,79,113). Further underscoring the importance of senescence as a tumor suppression mechanism as well as its therapeutic potential, recent studies have shown that reactivation of p53 in murine tumors causes cell senescence and associated tumor regression (130,139).

As well as activated oncogenes, senescence is caused by shortened telomeres that result from repeated rounds of cell division, and inadequate *in vitro* growth conditions and other cellular stresses (20,59,101,136). Senescence *in vivo* has also been suggested to contribute to tissue aging, through exhaustion of renewable tissue stem cell populations (20,59,63,69,82,136).

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1.2 Molecular Characteristics of Senescence

Senescence has been most widely studied in fibroblasts *in vitro*, but is also well-defined in melanocytes and epithelial cells (35,79,135). Other cell types suggested to undergo senescence include hematopoietic and neural progenitor cells (46,91). In many human cells, cellular senescence is characterized by several molecular and cytological markers, such as a large flat morphology, expression of a Senescence-Associated β -galactosidase activity (SA β -gal), formation of intracellular vacuoles, resistance of proliferation-promoting genes to mitogenic stimulation and formation of punctate highly-condensed domains of facultative heterochromatin, called Senescence-Associated Heterochromatin Foci (SAHF) (20,59,136).

However, specific senescence markers vary in their prominence depending on the species, cell type and trigger of senescence. Although some mouse cells exhibit a general increase in the amount of nuclear heterochromatin as judged by histone modifications (16), senescent mouse cells have not been shown to accumulate domains of facultative heterochromatin as pronounced as the punctate SAHF observed in human cells. In mouse cells, SAHF should not be confused with the highly-condensed domains of constitutive pericentromeric heterochromatin that are present even in growing mouse cells (53). Of human cells, WI38 and IMR90 fibroblasts and primary human melanocytes form pronounced SAHF, whereas BJ fibroblasts form less marked SAHF (35,88). In response to an activated Ras oncogene, primary human melanocytes express SA β -gal and assume the classical large, flat extensively-vacuolarized morphology. In response to an activated BRAF oncogene, the same cells express SA β -gal, but the large flat morphology and intracellular vacuoles are much less apparent (35). The physiological significance of these variations in the senescence phenotype are unknown. To understand their significance it is necessary to define the impact of the molecular phenotype, e.g. SA β -gal and SAHF formation, on potential physiological endpoints of senescence, such as tissue aging and tumor suppression. For SAHF, the major topic of this review, this is considered at the end of the article.

1.3 Senescence inducing pathways

A large body of evidence indicates that the pRB and p53 tumor suppressor pathways are master regulators of senescence. Inactivation of these two pathways typically abolishes senescence in mouse and human cells, regardless of the initial senescence trigger (20,59,136). Although human cells lacking pRB and p53 circumvent senescence, most such cells ultimately still cease proliferation through “crisis” due to erosion of telomeres to a critically short length (29). The p53 pathway is comprised of at least 3 proteins whose activity is altered in human cancer - p53, p19ARF and hdm2 (118). The p53 pathway exerts its effects through activation of downstream target genes, including the cell cycle inhibitor p21CIP1, whose expression is increased in senescent cells. The pRB pathway is generally considered to be comprised of at least 4 proteins whose activity is frequently perturbed by genetic mutations or altered level of expression in human cancers - p16INK4a, cyclin D1, cdk4 and pRB (89). By inhibiting cyclinD/cdk4 kinases, p16INK4a activates pRB. The pRB pathway inhibits cell proliferation through numerous downstream effectors. For example, pRB inhibits the E2F family of transcription factors, whose target genes are necessary for progression through S-phase (89).

The mechanisms by which activated oncogenes, short telomeres and cellular stresses drive senescence through activation of the pRB and p53 pathways has been extensively reviewed elsewhere (9,20,59), and will only be briefly summarized here to emphasize the major points, recent findings and some outstanding questions. The p53 pathway is activated by DNA damage, in response to either short telomeres or activated oncogenes. The free DNA ends of short telomeres are sensed by the cell as a form of DNA damage and activate p53 via the ATM and Chk2 DNA damage signaling cascade (58). Activated oncogenes cause a proliferative burst and rounds of error prone DNA synthesis. The DNA damage that accumulates during these rounds of unscheduled DNA synthesis also activates p53 via the ATM and ATR damage

signaling pathways (7,36,75). In mouse cells, activated oncogenes also activate p53 by upregulation of p14ARF. However, this pathway is apparently not conserved in human cells (17,42,132). Activation of the p53 pathway contributes to activation of the pRB pathway. The p53 target gene, p21CIP1, inhibits cyclin/cdk2 complexes, thereby activating pRB. The pRB pathway is also activated by upregulation of p16INK4a. The mechanism by which expression of p16INK4a is increased is not known, although it is likely to involve regulation of polycomb proteins that repress expression of p16INK4a (11,47,62).

2. Chromatin remodeling in senescent cells

2.1 Description of SAHF

The impact of chromatin structure on cell senescence has been considered previously (61, 106). SAHF were first explicitly described by Scott Lowe and coworkers (88). When stained with 4'-6-Diamidino-2-phenylindole (DAPI), normal human cells exhibit a relatively even, diffuse distribution of DNA through the cell nucleus. However, in DAPI-stained senescent human cells, SAHF appear as approximately 30–50 bright, punctate DNA foci. The chromatin in these foci appears much more compact than the chromatin in normal interphase growing cells. Indeed, chromatin from cells with SAHF is more resistant to nuclease digestion than chromatin from growing cells (88). Inclusion of proliferation-promoting genes, such as cyclin A, into these compact chromatin foci is thought to silence expression of those genes, thereby contributing to senescence-associated cell cycle arrest.

Remarkably, each SAHF focus in a senescent cell results from condensation of an individual chromosome (143). Chromosome regions normally in constitutive heterochromatin, such as pericentromeric and telomeric regions, are located at the periphery of SAHF (45,88,140,143). SAHF contain several common markers of heterochromatin, including histones that are hypoacetylated, methylation of lysine 9 of histone H3 (H3K9Me) and bound Heterochromatin Protein 1 (HP1) proteins. However, SAHF do not contain some other markers of condensed chromatin in mitotic and apoptotic cells, such as phosphoserine 10 of histone H3 (H3S10P), H2BS14P and H3S28P (45,96). SAHF are also characterized by their depletion of linker histone H1 and enrichment in at least two other proteins, namely the histone variant macroH2A and HMGA proteins (45,87,145).

MacroH2A is actually a family of three variants, macroH2A1.1, 1.2 and 2 (where 1.1 and 1.2 are splice variants). MacroH2As contain an N-terminal histone H2A-like domain and a C-terminal “macro domain” of more than 200 residues that is unrelated to other histones. MacroH2A clearly contributes to gene silencing, since it is inserted into the inactive X chromosome (23,27,28) and macroH2A-containing chromatin is resistant to ATP-dependent remodeling proteins and binding of transcription factors *in vitro* (4).

The HMGA1 and HMGA2 proteins (previously called HMG1Y and HMGIC, respectively) are abundant non-histone chromatin proteins (103,114). Paradoxically, the presence of HMGA proteins in chromatin is normally associated with gene activation, cell proliferation and cell transformation (103,114). Both proteins are expressed in embryos, repressed during cell differentiation and their expression is stimulated by mitogens. Mice expressing an HMGA transgene develop tumors (40,138). In human tumors, HMGA proteins are sometimes overexpressed and the genes coding for these proteins are targets of amplification and translocations (103,114). In light of these prior observations, it was a surprise when SAHF were shown to be enriched in HMGA proteins and that these proteins contribute to senescence-associated proliferation arrest and transformation suppression in fibroblasts (45,87). However, others have also confirmed that in some contexts HMGA proteins play a tumor suppression role (41). In sum, HMGA proteins contribute to senescence-associated SAHF formation and

cell cycle exit in fibroblasts, and the extent to which this role is conserved in other cell types remains to be determined.

2.2 Formation of SAHF is a multi-step process

Two lines of evidence indicate that SAHF form through a cascade of temporally and mechanistically separable events. First, a kinetic analysis of cells forming SAHF showed that condensed chromosomes, in the form of DAPI foci, are detectable before their enrichment with H3K9Me, HP1 proteins and macroH2A (145). Second, a dominant negative HP1 mutant that removes 50–80% of all three HP1 isoforms from chromatin in primary human cells has no effect on chromosome condensation or incorporation of macroH2A into condensed chromosomes (143). Together, these results indicate that formation of SAHF is a multi-step process. The earliest detectable event to date is chromosome condensation to form a SAHF focus that is detectable by DAPI staining of DNA, followed by methylation of lysine 9 of histone H3 to create H3K9Me, binding of HP1 proteins and incorporation of macroH2A.

Remarkably, these studies also indicated that loading of abundant HP1 proteins onto chromatin is not required for two additional hallmarks of the senescent phenotype, expression of SA β -gal and senescence-associated cell cycle exit (143). Conceivably, the residual chromatin-bound HP1 proteins in cells expressing the dominant negative HP1 mutant are sufficient to mediate HP1 functions that are required for these senescence phenotypes. However, these results raise the possibility that HP1 proteins do not contribute to acute onset of the senescent phenotype. Instead, HP1 proteins might be required for long-term maintenance of SAHF and the senescent state. Alternatively, HP1 proteins might secure the senescent state in the face of genetic alterations or cellular perturbations that compromise other aspects of the senescence program. These ideas remain to be tested.

2.3 Chromosome condensation is driven by histone chaperones HIRA and ASF1a

Based on the studies described above, during formation of SAHF, senescence-associated cell cycle exit appears to be linked to the process of chromosome condensation. Two chromatin regulators, HIRA and ASF1a, drive chromosome condensation during SAHF assembly in human cells (145). HIRA and ASF1a are the human orthologs of proteins known to create transcriptionally silent heterochromatin in yeast, flies and plants (64,83,97,117,121). Yeast Asf1p is required for heterochromatin-mediated silencing of telomeres and mating loci and has histone deposition activity *in vitro* (67,116,121,127). Yeast Asf1p is also required for histone eviction and subsequent replacement at transcribed genes (1,66,111), and also plays a role in DNA replication-coupled chromatin assembly (44,51,86,110,127). Yeast Hir1p and Hir2p share several biological and biochemical properties with Asf1p. Like Asf1p, they are required for heterochromatin-mediated silencing of telomeres and mating loci, and are also required for formation of proper pericentromeric chromatin (but in an Asf1p-independent manner) (64, 67,116,117). Mouse ES cells lacking HIRA have a larger pool of loosely bound histones than wild type cells, consistent with a role for HIRA in generation of compact, nucleosome-dense, transcriptionally silent heterochromatin (78). *In vitro*, HIRA has been shown to act as a histone chaperone, preferentially depositing the histone variant histone H3.3 into nucleosomes, compared to canonical histone H3.1 (49,72,100,102,125). Consistent with their partially overlapping functions, Asf1 and Hir proteins physically interact and in yeast this interaction is necessary for telomeric silencing (32,49,116).

The role of Asf1 and Hir proteins in formation of heterochromatin is conserved in human cells, through the ability of HIRA and ASF1a to drive formation of SAHF. Ectopic expression of HIRA or ASF1a in primary human cells accelerates formation of SAHF (145). This activity requires binding of HIRA and ASF1a to each other, and shRNA-mediated knock down of ASF1a blocks formation of SAHF triggered by an activated Ras-oncogene (145). Consistent

with the idea that formation of SAHF depends on histone chaperone activity of the HIRA/ASF1a complex, formation of SAHF also requires an interaction between ASF1a and histone H3 (143). Significantly, many previous reports indicate that transcriptionally active chromatin is depleted of nucleosomes. This is true, at both a genome-wide and local chromatin level (1, 2,5,6,12,24,71,80,146). Moreover, a previous study reported that the facultative heterochromatin of the inactive X chromosome has higher nucleosome density than most other regions of the nucleus (95). Together, these results suggest that chromosome condensation associated with SAHF formation may depend, in part, on increased nucleosome density due to HIRA/ASF1a-mediated nucleosome deposition.

Recently, several groups have described molecular structures of Asf1 proteins, either as free proteins or bound to histones, HIRA or fragments of either (32,39,84,126). Regardless of the species, the Asf1 protein forms an elongated immunoglobulin-like β -sandwich fold, with three α -helices in the loops between the β -strands. Together, these studies indicate that HIRA and the histone H3/H4 heterodimer bind to distinct faces of the Asf1 polypeptide (126). HIRA binds to a shallow hydrophobic groove on ASF1a, perpendicular to the strands of the β -sandwich, and is anchored at one end of the groove by a cluster of salt bridge interactions. The histone H3/H4 heterodimer binds largely to the opposite face of Asf1 (39,84). Interestingly, in the Asf1/H3/H4 trimeric complex, Asf1 binds to the C-terminus of histone H4 that normally interacts with histone H2A in the nucleosome. This suggests that release of histone H3/H4 from Asf1 will facilitate nucleosome assembly by exposing the histone H4 tail to histone H2A (39).

Questions remain regarding the specific histone substrate utilized by HIRA/ASF1a to make SAHF. Like ASF1a, HIRA also binds to histones. HIRA-containing chaperone complexes preferentially deposit the histone variant histone H3.3, over the canonical histone H3.1, in a DNA replication-independent manner (72,125). Significantly, histone H3.3 accumulates in fibroblasts approaching senescence and in non-dividing differentiated cells, in some cases to about 90% of the total histone H3, with presumably the majority being in inactive chromatin (15,18,52,68,92,98,106,128,137). Unfortunately, because human histone H3.3 and canonical H3.1 only differ by 5 amino-acids, differentiating between them immunologically is challenging, making it difficult to ask whether endogenous histone H3.3 is specifically enriched in SAHF. The idea that SAHF contains histone H3.3 may initially seem unlikely, because deposition of histone H3.3 is typically linked to transcription activation (3,77,80,112,133), whereas SAHF is a form of transcriptionally silent facultative heterochromatin (87,88,145). However, the apparent inconsistency in this idea is merely an extension of an existing paradox. Specifically, HIRA and its orthologs in other species are typically involved in gene silencing and formation of heterochromatin (13,50,64,67,97,116,117,119,123), whereas HIRA's favored deposition substrate, histone H3.3, is linked to transcriptional activation (3,77,80,112,133). However, to our knowledge, histone H3.3 *per se* has not been shown to directly cause or contribute to transcription activation, and a proportion of histone H3.3 does carry post-translational marks characteristic of transcriptionally silent chromatin (55,74,77). Therefore, histone H3.3 is unlikely to be exclusively linked to transcription activation. Instead, deposition of histone H3.3 may be associated with any major remodeling of chromatin, perhaps as a way to "re-set" histone modifications. Concordant with this proposal, after egg fertilization in flies, dHIRA activity is required for replacement of protamines by histone H3.3-containing nucleosomes in decondensing sperm chromatin (72). Also, after treatment of primary human cells with histone deacetylase inhibitors that disrupt heterochromatin structure, HIRA and histone H3.3 are required for a chromatin "repair" process that recruits HP1 proteins to pericentromeres, thereby maintaining structure and function of the adjacent chromosome kinetochores (144). Recently, van der Heijden and coworkers showed that histone H3.3 is incorporated into the X and Y chromosomes during formation of the transcriptionally silent sex body by meiotic sex chromosome inactivation (129). Replacement of canonical histone

H3.1 by variant histone H3.3 is linked to HIRA's localization to the developing sex body. Thus, the HIRA/ASF1a complex might drive formation of SAHF by deposition of histone H3.3-containing nucleosomes.

Alternatively, under some conditions, for example during SAHF formation, HIRA/ASF1a might utilize histone H3.1 as a substrate. Consistent with this idea, ASF1a interacts with histone H3.1 and histone H3.3 and inactivation of HIRA in mouse ES cells affects the nuclear mobility of both histone H3.3 and H3.1 (78,84,125). Although HIRA is thought to act in a DNA replication-independent manner and histone H3.1 is incorporated predominantly in S-phase of the cell cycle, recent studies have shown that histone H3.1 can be deposited outside of S-phase (99). In sum, the histone H3 variant utilized by HIRA/ASF1a to make SAHF and the key events in chromosome condensation are still not fully defined.

2.4 Incorporation of other SAHF components

After HIRA/ASF1a-driven chromosome condensation, histone H3 is methylated on lysine 9 creating a binding site for HP1 proteins and the histone variant macroH2A is incorporated. In murine cells, which do not form the punctate SAHF of human cells, but do exhibit a general heterochromatinization throughout the nucleus, the increase of H3K9Me in senescent cells depends on the Suv39h1 histone methyl transferase (16).

The factors responsible for incorporation of macroH2A are unknown, but are likely to include an ATP-dependent remodeling factor as a histone H2A exchange factor (19,65,70,81,141). Recently, the yeast ATP-dependent chromatin remodeling protein, Swr1, was shown to exchange canonical H2A for another histone H2A variant, H2AZ (19,65,70,81,141). The yeast ortholog of the human NAP1 histone chaperone has also been shown to exchange canonical H2A and H2AZ *in vitro* in an ATP-independent manner (93). Human cells contain several homologs of Swr1 that might replace canonical histone H2A with macroH2A. The closest human homologs of yeast Swr1 are the INO80 family of ATP-dependent chromatin remodeling factors, namely hINO80, p400 and SRCAP (65,122). Other more distantly related enzymes include human hBrm, Brg1 and the CHD family (CHD1-7). Of note, human hBrm and Brg1 are two candidate tumor suppressor genes that are implicated in repression of E2F target genes and induction of cell differentiation and senescence, through physical and functional interactions with the pRB protein (33,38,56,76,85,104,105,115,124,134,142). Since E2F target genes are incorporated into SAHF and formation of SAHF requires an intact pRB pathway (88,140), hBrm and/or Brg1 might be involved in SAHF assembly, perhaps through deposition of macroH2A.

HMG proteins and histone H1 bind to similar sites on chromatin and can compete with each other for chromatin binding (21,22,60,148). Consequently, Ishikawa and coworkers proposed that HMGA proteins displace histone H1 from SAHF (45). The time when this occurs in relation to chromosome condensation is not clear, since a comparative kinetic analysis has not been reported. However, Lowe and coworkers showed that, in contrast to HP1 proteins, HMGA1 is required for formation of SAHF, as judged by chromosome condensation (87). This implies that displacement of histone H1 and deposition of HMGA proteins is likely to be an early event in formation of SAHF, perhaps occurring at the same time as, or prior to, chromosome condensation. If so, the mechanistic relationship between HIRA/ASF1a-mediated nucleosome deposition and HMGA recruitment will need to be defined.

2.5 Formation of SAHF depends on a dynamic cell nucleus

Formation of SAHF appears to depend on dynamic relocation of several nuclear components. In proliferating primary human cells, HIRA is evenly dispersed throughout the cell nucleus (145). However, in cells approaching senescence, regardless of whether the trigger

is an activated oncogene, short telomeres or cell stress, HIRA is translocated into a specific subnuclear organelle, the PML nuclear body. Most human cells contain 20–30 PML nuclear bodies, which are typically 0.1–1 μ M in diameter and enriched in the protein PML, as well as many other nuclear regulatory proteins (14,107). PML bodies have been previously implicated in various cellular processes, including tumor suppression and cellular senescence (34,42,94). Significantly, HIRA is translocated to PML bodies prior to formation of SAHF and prior to exit of the cells from the cell cycle (145). Two lines of evidence indicate that HIRA's translocation to PML bodies is essential for formation of SAHF. First, a dominant negative HIRA mutant, which is targeted to PML bodies but does not bind to ASF1a and which blocks localization of endogenous HIRA to PML bodies, also blocks formation of SAHF (140). Second, expression of the PML-RAR α fusion protein, that is known to inhibit function of PML bodies (107), also blocks formation of SAHF. At a molecular level, PML bodies have been proposed to serve as sites of assembly of macromolecular regulatory complexes (43,54,94). Therefore, it seems likely that PML bodies serve as a molecular "staging ground" for assembly or modification of HIRA-containing complexes, prior to export of these complexes to sites of nascent SAHF.

Additional support for this "staging ground" hypothesis comes from analysis of HP1 proteins in cells entering senescence. Like HIRA, HP1 α , β and γ isoforms are evenly distributed throughout the nucleoplasm in proliferating human cells. However, in pre-senescent cells, prior to formation of SAHF and cell cycle exit, HP1 proteins are also translocated into PML nuclear bodies (145). Enrichment of HP1 proteins in PML bodies is, however, only a transient phenomenon. Ultimately, HP1 proteins are depleted from PML bodies and accumulate in SAHF. Although based only on steady state analyses of protein distribution, these results suggest that HP1 proteins are translocated through SAHF prior to their deposition in PML bodies. Significantly, HP1 γ , but not HP1 α and β , becomes phosphorylated in cells entering senescence. Phosphorylation occurs on serine 93 and is required for accumulation of HP1 γ in SAHF, but not its recruitment to PML bodies. Thus, consistent with the "staging ground" hypothesis, HP1 γ might be phosphorylated inside PML bodies and phosphorylation might target HP1 γ to SAHF (145). Alternatively, HP1 γ might be phosphorylated after the protein exits PML bodies *en route* to SAHF. Either way, the kinase responsible for HP1 γ phosphorylation and why phosphorylation is required for accumulation in SAHF is unknown.

2.6 Relationship of SAHF assembly to the pRB and p53 tumor suppressor pathways

The pRB and p53 tumor suppressor pathways are master regulators of the senescence program. Lowe and coworkers originally showed that formation of SAHF depends on an active pRB pathway (88), and this has been confirmed by us (140). In contrast to these earlier studies, we also found that efficient formation of SAHF depends on activity of the p53 pathway (140). Our studies also showed that translocation of HIRA to PML bodies - an upstream event in the SAHF assembly process - is largely independent of pRB and p53 activity (140). Thus, pRB and p53 appear to be required at a specific downstream point in formation of SAHF. Significantly, formation of SAHF driven by ectopically expressed p16INK4a, an activator of the pRB pathway, also requires ASF1a. Together, these observations suggest that the HIRA/ASF1a and pRB pathways act in parallel to form SAHF.

Consistent with this idea, the pRB tumor suppressor protein and the HIRA and ASF1a histone chaperones are all well-established regulators of chromatin structure and function. Through an interaction with the E2F transcription factor, the pRB protein facilitates formation of heterochromatin at E2F target genes and other sites throughout the genome (48,147). For example, pRB recruits the histone methylase Suv39h1 to chromatin and, as described above, this enzyme is required for oncogene-induced formation of heterochromatin in murine T-cells (16). HIRA and ASF1a are two histone chaperones whose physical interaction is required to

form transcriptionally silent heterochromatin from yeast to humans (13,50,64,67,97,116, 145). One simple model to describe the cooperation of the pRB and HIRA/ASF1a pathways is that pRB initiates heterochromatin formation at the promoters of E2F target genes, and this heterochromatin acts as a nucleation site for HIRA/ASF1a-mediated large scale chromosome condensation. Consistent with this, E2F target genes, such as cyclin A, are incorporated into SAHF and pRB has been reported to partially colocalize with SAHF (88).

Alternatively, pRB and HIRA might both act in concert with a single protein that plays a key role in SAHF formation. One such candidate is the J domain protein, DNAJA2. DNAJA2, was independently cloned as a HIRA and pRB binding protein in two-hybrid screens performed by two different labs. Specifically, Lipinski and coworkers identified DNAJA2 as a HIRA-binding protein (73). Subsequently, Kaelin and coworkers identified DNAJA2 in a differential two-hybrid screen intended to identify proteins that contribute to pRB-mediated cell differentiation (10). J domain-containing proteins regulate the activity of 70-kDa heat-shock proteins (Hsp70s) (131). Hsp70s bind to short unfolded hydrophobic regions of substrate polypeptides, promoting assembly or disassembly of macromolecular protein-containing complexes. J domain proteins are known to regulate a wide variety of cellular processes, including protein translation, endocytosis, mRNA splicing and mitochondrial biogenesis. DNAJA2 interacts with HIRA and pRB when ectopically expressed in cells (140). Moreover, DNAJA2 accelerates formation of HP1 and macroH2A-containing SAHF when ectopically expressed in primary human fibroblasts (140). The ability of DNAJA2 to drive formation of SAHF appears to be partially independent of pRB and p53 and ASF1a, consistent with the idea that DNAJA2 is neither wholly up nor downstream of pRB, p53 and HIRA/ASF1a. Conceivably, the chaperone activity of DNAJA2 facilitates chromatin remodeling and SAHF formation by HIRA and pRB.

The demonstration that activation of the HIRA/ASF1a SAHF assembly pathway through HIRA's localization to PML bodies is independent of pRB and p53 suggests the exciting possibility that some early events in the senescence program are independent of these two presumed master regulators of the senescence program. Interestingly, previous reports have also pointed to the existence of senescence-activating pathways that are pRB- and p53-independent (90). Defining these pRB and p53 independent events might shed light on other important unanswered questions, such as the mechanism of upregulation of the p16INK4a tumor suppressor in senescent cells.

3. SAHF, tissue aging and tumor suppression

Cellular senescence has been suggested to contribute to tissue aging (20,59,63,69,82,136). So does SAHF directly contribute to aging? In support of this idea, markers of increased heterochromatin, including activation of the HIRA/ASF1a pathway, have been reported in skin of aging primates (57). On the other hand, fibroblasts from individuals with the premature aging syndrome, Hutchinson-Gilford Progeria Syndrome (HGPS), typically exhibit decreased abundance of heterochromatic markers, such as H3K9Me and HP1 proteins (108,109,120). Moreover, after passage in culture, cells from aging normal individuals (>80 years) show the same changes in heterochromatin structure as HGPS individuals (108). At present, the heterochromatin changes associated with cell senescence, HGPS and normal human aging are all defined at a relatively crude level. To understand the impact of heterochromatin on tissue aging in humans it is necessary to better define its structure and function, and link this to the activity of the (largely unknown) genes and environmental conditions that influence the rate of normal human aging.

As discussed previously, cellular senescence is also a tumor suppression mechanism (16,25, 26,30,37,79,113). So what is the evidence that SAHF contributes to tumor suppression? Linking SAHF to tumor suppression, senescent melanocytes contain SAHF and senescence is

well-defined as a tumor suppression mechanism in these cells (35,79). Although fibroblasts are not thought to be a frequent “cell-of-origin” of human tumors, formation of SAHF in fibroblasts correlates with an irreversible senescence-associated cell cycle arrest. Specifically, SAHF form inefficiently in human BJ fibroblasts and senescence is most easily reversed in these cells (8,31). By making senescence irreversible, SAHF are likely to contribute to tumor suppression. Perhaps the best evidence linking SAHF to tumor suppression is the demonstration that shRNA-mediated knock down of HMGA2 blocks formation of SAHF and contributes to cell transformation (87). This implies that inactivation of gene products that are required for SAHF formation, such as HIRA, ASF1a and HMGA2, might contribute to development of human tumors. To date, genetic or epigenetic inactivation of these genes has not been reported in human tumors.

Abbreviations

ASF1	Anti-silencing function 1
ATM	Ataxia Telangiectasia Mutated
ATP	Adenosine 5'-TriPhosphate
Cdk4	Cyclin-dependent kinase 4
DNA	DeoxyriboseNucleic Acid
DAPI	4'-6-DiAmidino-2-PhenylIndole
ES	Embryonal Stem
H2BS14P	PhosphoSerine 14 of histone H2B
H3K9Me	Methylated Lysine 9 of histone H3
H3S10P	PhosphoSerine 10 of histone H3
H3S28P	PhosphoSerine 28 of histone H3
H4K20Me	Methylated lysine 20 of histone H4
HGPS	Hutchinson-Gilford Progeria Syndrome
HIRA	Histone Repressor A
HMGA	

	High Mobility Group A
HP1	Heterochromatin Protein 1
Hsp70	Heat Shock Protein 70
MacroH2A	macro histone H2A
mRNA	messenger RNA
p19/14ARF	p19/14 Alternative Reading Frame
p21CIP1	p21Cdk Inhibitor Protein 1
pRB	Retinoblastoma tumor suppressor protein
PML	ProMyelocytic Leukemia
RARα	Retinoic Acid Receptor α
SA β-gal	Senescence Associated β -galactosidase
SAHF	Senescence Associated Heterochromatin Foci

References

- Adkins MW, Howar SR, Tyler JK. Chromatin disassembly mediated by the histone chaperone Asf1 is essential for transcriptional activation of the yeast PHO5 and PHO8 genes. *Mol Cell* 2004;14:657–66. [PubMed: 15175160]
- Agalioti T, Lomvardas S, Parekh B, Yie J, Maniatis T, Thanos D. Ordered recruitment of chromatin modifying and general transcription factors to the IFN-beta promoter. *Cell* 2000;103:667–78. [PubMed: 11106736]
- Ahmad K, Henikoff S. The histone variant h3.3 marks active chromatin by replication-independent nucleosome assembly. *Mol Cell* 2002;9:1191–200. [PubMed: 12086617]
- Angelov D, Molla A, Perche PY, Hans F, Cote J, Khochbin S, Bouvet P, Dimitrov S. The histone variant macroH2A interferes with transcription factor binding and SWI/SNF nucleosome remodeling. *Mol Cell* 2003;11:1033–41. [PubMed: 12718888]
- Angermayr M, Bandlow W. Permanent nucleosome exclusion from the Gal4p-inducible yeast GCY1 promoter. *J Biol Chem* 2003;278:11026–31. [PubMed: 12536147]
- Angermayr M, Oechsner U, Gregor K, Schroth GP, Bandlow W. Transcription initiation in vivo without classical transactivators: DNA kinks flanking the core promoter of the housekeeping yeast adenylate kinase gene, AKY2, position nucleosomes and constitutively activate transcription. *Nucleic Acids Res* 2002;30:4199–207. [PubMed: 12364598]
- Bartkova J, Rezaei N, Lontos M, Karakaidos P, Kleitas D, Issaeva N, Vassiliou LV, Kolettas E, Niforou K, Zoumpourlis VC, Takaoka M, Nakagawa H, Tort F, Fugger K, Johansson F, Sehested M, Andersen CL, Dyrskjot L, Orntoft T, Lukas J, Kittas C, Helleday T, Halazonetis TD, Bartek J,

- Gorgoulis VG. Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints. *Nature* 2006;444:633–7. [PubMed: 17136093]
8. Beausejour CM, Krtolica A, Galimi F, Narita M, Lowe SW, Yaswen P, Campisi J. Reversal of human cellular senescence: roles of the p53 and p16 pathways. *Embo J* 2003;22:4212–22. [PubMed: 12912919]
 9. Ben-Porath I, Weinberg RA. The signals and pathways activating cellular senescence. *Int J Biochem Cell Biol* 2005;37:961–76. [PubMed: 15743671]
 10. Benevolenskaya EV, Murray HL, Branton P, Young RA, Kaelin WG Jr. Binding of pRB to the PHD protein RBP2 promotes cellular differentiation. *Mol Cell* 2005;18:623–35. [PubMed: 15949438]
 11. Bernard D, Martinez-Leal JF, Rizzo S, Martinez D, Hudson D, Visakorpi T, Peters G, Carnero A, Beach D, Gil J. CBX7 controls the growth of normal and tumor-derived prostate cells by repressing the Ink4a/Arf locus. *Oncogene* 2005;24:5543–51. [PubMed: 15897876]
 12. Bernstein BE, Liu CL, Humphrey EL, Perlstein EO, Schreiber SL. Global nucleosome occupancy in yeast. *Genome Biol* 2004;5:R62. [PubMed: 15345046]
 13. Blackwell C, Martin KA, Greenall A, Pidoux A, Allshire RC, Whitehall SK. The *Schizosaccharomyces pombe* HIRA-like protein Hip1 is required for the periodic expression of histone genes and contributes to the function of complex centromeres. *Mol Cell Biol* 2004;24:4309–20. [PubMed: 15121850]
 14. Borden KL. Pondering the promyelocytic leukemia protein (PML) puzzle: possible functions for PML nuclear bodies. *Mol Cell Biol* 2002;22:5259–69. [PubMed: 12101223]
 15. Bosch A, Suau P. Changes in core histone variant composition in differentiating neurons: the roles of differential turnover and synthesis rates. *Eur J Cell Biol* 1995;68:220–5. [PubMed: 8603674]
 16. Braig M, Lee S, Lodenkemper C, Rudolph C, Peters AH, Schlegelberger B, Stein H, Dorken B, Jenuwein T, Schmitt CA. Oncogene-induced senescence as an initial barrier in lymphoma development. *Nature* 2005;436:660–5. [PubMed: 16079837]
 17. Brookes S, Rowe J, Ruas M, Llanos S, Clark PA, Lomax M, James MC, Vatcheva R, Bates S, Vousden KH, Parry D, Gruis N, Smit N, Bergman W, Peters G. INK4a-deficient human diploid fibroblasts are resistant to RAS-induced senescence. *Embo J* 2002;21:2936–45. [PubMed: 12065407]
 18. Brown DT, Wellman SE, Sittman DB. Changes in the levels of three different classes of histone mRNA during murine erythroleukemia cell differentiation. *Mol Cell Biol* 1985;5:2879–86. [PubMed: 3018484]
 19. Bruno M, Flaus A, Stockdale C, Rencurel C, Ferreira H, Owen-Hughes T. Histone H2A/H2B dimer exchange by ATP-dependent chromatin remodeling activities. *Mol Cell* 2003;12:1599–606. [PubMed: 14690611]
 20. Campisi J. Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. *Cell* 2005;120:513–22. [PubMed: 15734683]
 21. Catez F, Brown DT, Misteli T, Bustin M. Competition between histone H1 and HMGN proteins for chromatin binding sites. *EMBO Rep* 2002;3:760–6. [PubMed: 12151335]
 22. Catez F, Yang H, Tracey KJ, Reeves R, Misteli T, Bustin M. Network of dynamic interactions between histone H1 and high-mobility-group proteins in chromatin. *Mol Cell Biol* 2004;24:4321–8. [PubMed: 15121851]
 23. Chadwick BP, Willard HF. Cell cycle-dependent localization of macroH2A in chromatin of the inactive X chromosome. *J Cell Biol* 2002;157:1113–23. [PubMed: 12082075]
 24. Chen X, Wang J, Woltring D, Gerondakis S, Shannon MF. Histone dynamics on the interleukin-2 gene in response to T-cell activation. *Mol Cell Biol* 2005;25:3209–19. [PubMed: 15798206]
 25. Chen Z, Trotman LC, Shaffer D, Lin HK, Dotan ZA, Niki M, Koutcher JA, Scher HI, Ludwig T, Gerald W, Cordon-Cardo C, Pandolfi PP. Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. *Nature* 2005;436:725–30. [PubMed: 16079851]
 26. Collado M, Gil J, Efeyan A, Guerra C, Schuhmacher AJ, Barradas M, Benguria A, Zaballos A, Flores JM, Barbacid M, Beach D, Serrano M. Tumour biology: senescence in premalignant tumours. *Nature* 2005;436:642. [PubMed: 16079833]
 27. Costanzi C, Pehrson JR. Histone macroH2A1 is concentrated in the inactive X chromosome of female mammals. *Nature* 1998;393:599–601. [PubMed: 9634239]

28. Costanzi C, Pehrson JR. MACROH2A2, a new member of the MARCOH2A core histone family. *J Biol Chem* 2001;276:21776–84. [PubMed: 11262398]
29. Counter CM, Avilion AA, LeFeuvre CE, Stewart NG, Greider CW, Harley CB, Bacchetti S. Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. *Embo J* 1992;11:1921–9. [PubMed: 1582420]
30. Courtois-Cox S, Genther Williams SM, Reczek E, Johnson BW, McGillicuddy LT, Johannessen CM, Hollstein PE, MacCollin M, Cichowski K. A negative feedback signaling network underlies oncogene-induced senescence. *Cancer Cell* 2006;10:459–72. [PubMed: 17157787]
31. d'Adda di Fagagna F, Reaper PM, Clay-Farrace L, Fiegler H, Carr P, Von Zglinicki T, Saretzki G, Carter NP, Jackson SP. A DNA damage checkpoint response in telomere-initiated senescence. *Nature* 2003;426:194–8. [PubMed: 14608368]
32. Daganzo SM, Erzberger JP, Lam WM, Skordalakes E, Zhang R, Franco AA, Brill SJ, Adams PD, Berger JM, Kaufman PD. Structure and function of the conserved core of histone deposition protein Asf1. *Curr Biol* 2003;13:2148–2158. [PubMed: 14680630]
33. Dahiya A, Gavin MR, Luo RX, Dean DC. Role of the LXCXE binding site in Rb function. *Mol Cell Biol* 2000;20:6799–805. [PubMed: 10958676]
34. de Stanchina E, Querido E, Narita M, Davuluri RV, Pandolfi PP, Ferbeyre G, Lowe SW. PML is a direct p53 target that modulates p53 effector functions. *Mol Cell* 2004;13:523–35. [PubMed: 14992722]
35. Denoyelle C, Abou-Rjaily G, Bezrookove V, Verhaegen M, Johnson TM, Fullen DR, Pointer JN, Gruber SB, Su LD, Nikiforov MA, Kaufman RJ, Bastian BC, Soengas MS. Anti-oncogenic role of the endoplasmic reticulum differentially activated by mutations in the MAPK pathway. *Nat Cell Biol* 2006;8:1053–63. [PubMed: 16964246]
36. Di Micco R, Fumagalli M, Cicalese A, Piccinin S, Gasparini P, Luise C, Schurra C, Garre M, Nuciforo PG, Bensimon A, Maestro R, Pelicci PG, d'Adda di Fagagna F. Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication. *Nature* 2006;444:638–42. [PubMed: 17136094]
37. Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, Medrano EE, Linskens M, Rubelj I, Pereira-Smith O, et al. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proc Natl Acad Sci U S A* 1995;92:9363–7. [PubMed: 7568133]
38. Dunaief JL, Strober BE, Guha S, Khavari PA, Alin K, Luban J, Begemann M, Crabtree GR, Goff SP. The retinoblastoma protein and BRG1 form a complex and cooperate to induce cell cycle arrest. *Cell* 1994;79:119–30. [PubMed: 7923370]
39. English CM, Adkins MW, Carson JJ, Churchill ME, Tyler JK. Structural basis for the histone chaperone activity of Asf1. *Cell* 2006;127:495–508. [PubMed: 17081973]
40. Fedele M, Battista S, Kenyon L, Baldassarre G, Fidanza V, Klein-Szanto AJ, Parlow AF, Visone R, Pierantoni GM, Outwater E, Santoro M, Croce CM, Fusco A. Overexpression of the HMGA2 gene in transgenic mice leads to the onset of pituitary adenomas. *Oncogene* 2002;21:3190–8. [PubMed: 12082634]
41. Fedele M, Fidanza V, Battista S, Pentimalli F, Klein-Szanto AJ, Visone R, De Martino I, Curcio A, Morisco C, Del Vecchio L, Baldassarre G, Arra C, Viglietto G, Indolfi C, Croce CM, Fusco A. Haploinsufficiency of the Hmgal gene causes cardiac hypertrophy and myelo-lymphoproliferative disorders in mice. *Cancer Res* 2006;66:2536–43. [PubMed: 16510570]
42. Ferbeyre G, de Stanchina E, Querido E, Baptiste N, Prives C, Lowe SW. PML is induced by oncogenic ras and promotes premature senescence. *Genes Dev* 2000;14:2015–27. [PubMed: 10950866]
43. Fogal V, Gostissa M, Sandy P, Zacchi P, Sternsdorf T, Jensen K, Pandolfi PP, Will H, Schneider C, Del Sal G. Regulation of p53 activity in nuclear bodies by a specific PML isoform. *Embo J* 2000;19:6185–95. [PubMed: 11080164]
44. Franco AA, Lam WM, Burgers PM, Kaufman PD. Histone deposition protein Asf1 maintains DNA replisome integrity and interacts with replication factor C. *Genes Dev* 2005;19:1365–75. [PubMed: 15901673]
45. Funayama R, Saito M, Tanobe H, Ishikawa F. Loss of linker histone H1 in cellular senescence. *J Cell Biol* 2006;175:869–880. [PubMed: 17158953]

46. Geiger H, Van Zant G. The aging of lympho-hematopoietic stem cells. *Nat Immunol* 2002;3:329–33. [PubMed: 11919569]
47. Gil J, Bernard D, Martinez D, Beach D. Polycomb CBX7 has a unifying role in cellular lifespan. *Nat Cell Biol* 2004;6:67–72. [PubMed: 14647293]
48. Gonzalo S, Garcia-Cao M, Fraga MF, Schotta G, Peters AH, Cotter SE, Eguia R, Dean DC, Esteller M, Jenuwein T, Blasco MA. Role of the RB1 family in stabilizing histone methylation at constitutive heterochromatin. *Nat Cell Biol* 2005;7:420–8. [PubMed: 15750587]
49. Green EM, Antczak AJ, Bailey AO, Franco AA, Wu KJ, Yates JR 3rd, Kaufman PD. Replication-independent histone deposition by the HIR complex and Asf1. *Curr Biol* 2005;15:2044–9. [PubMed: 16303565]
50. Greenall A, Williams ES, Martin KA, Palmer JM, Gray J, Liu C, Whitehall SK. Hip3 interacts with the HIRA proteins Hip1 and Slm9 and is required for transcriptional silencing and accurate chromosome segregation. *J Biol Chem* 2006;281:8732–9. [PubMed: 16428807]
51. Groth A, Ray-Gallet D, Quivy JP, Lukas J, Bartek J, Almouzni G. Human Asf1 regulates the flow of S phase histones during replicational stress. *Mol Cell* 2005;17:301–11. [PubMed: 15664198]
52. Grove GW, Zweidler A. Regulation of nucleosomal core histone variant levels in differentiating murine erythroleukemia cells. *Biochemistry* 1984;23:4436–43. [PubMed: 6593094]
53. Guenatri M, Bailly D, Maison C, Almouzni G. Mouse centric and pericentric satellite repeats form distinct functional heterochromatin. *J Cell Biol* 2004;166:493–505. [PubMed: 15302854]
54. Guo A, Salomoni P, Luo J, Shih A, Zhong S, Gu W, Paolo Pandolfi P. The function of PML in p53-dependent apoptosis. *Nat Cell Biol* 2000;2:730–6. [PubMed: 11025664]
55. Hake SB, Garcia BA, Duncan EM, Kauer M, Dellaire G, Shabanowitz J, Bazett-Jones DP, Allis CD, Hunt DF. Expression patterns and post-translational modifications associated with mammalian histone H3 variants. *J Biol Chem* 2006;281:559–68. [PubMed: 16267050]
56. Hendricks KB, Shanahan F, Lees E. Role for BRG1 in cell cycle control and tumor suppression. *Mol Cell Biol* 2004;24:362–76. [PubMed: 14673169]
57. Herbig U, Ferreira M, Condel L, Carey D, Sedivy JM. Cellular senescence in aging primates. *Science* 2006;311:1257. [PubMed: 16456035]
58. Herbig U, Jobling WA, Chen BP, Chen DJ, Sedivy JM. Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and p21(CIP1), but not p16(INK4a). *Mol Cell* 2004;14:501–13. [PubMed: 15149599]
59. Herbig U, Sedivy JM. Regulation of growth arrest in senescence: telomere damage is not the end of the story. *Mech Ageing Dev* 2006;127:16–24. [PubMed: 16229875]
60. Hill DA, Reeves R. Competition between HMG-I(Y), HMG-1 and histone H1 on four-way junction DNA. *Nucleic Acids Res* 1997;25:3523–31. [PubMed: 9254714]
61. Howard BH. Replicative senescence: considerations relating to the stability of heterochromatin domains. *Exp Gerontol* 1996;31:281–93. [PubMed: 8706797]
62. Itahana K, Zou Y, Itahana Y, Martinez JL, Beausejour C, Jacobs JJ, Van Lohuizen M, Band V, Campisi J, Dimri GP. Control of the replicative life span of human fibroblasts by p16 and the polycomb protein Bmi-1. *Mol Cell Biol* 2003;23:389–401. [PubMed: 12482990]
63. Janzen V, Forkert R, Fleming HE, Saito Y, Waring MT, Dombkowski DM, Cheng T, Depinho RA, Sharpless NE, Scadden DT. Stem-cell ageing modified by the cyclin-dependent kinase inhibitor p16 (INK4a). *Nature* 2006;443:421–6. [PubMed: 16957735]
64. Kaufman PD, Cohen JL, Osley MA. Hir proteins are required for position-dependent gene silencing in *Saccharomyces cerevisiae* in the absence of chromatin assembly factor I. *Mol Cell Biol* 1998;18:4793–806. [PubMed: 9671489]
65. Kobor MS, Venkatasubrahmanyam S, Meneghini MD, Gin JW, Jennings JL, Link AJ, Madhani HD, Rine J. A protein complex containing the conserved Swi2/Snf2-related ATPase Swr1p deposits histone variant H2A.Z into euchromatin. *PLoS Biol* 2004;2:E131. [PubMed: 15045029]
66. Korber P, Barbaric S, Luckenbach T, Schmid A, Schermer UJ, Blaschke D, Horz W. The histone chaperone Asf1 increases the rate of histone eviction at the yeast PHO5 and PHO8 promoters. *J Biol Chem* 2006;281:5539–45. [PubMed: 16407267]

67. Krawitz DC, Kama T, Kaufman PD. Chromatin Assembly Factor I Mutants Defective for PCNA Binding Require Asf1/Hir Proteins for Silencing. *Mol Cell Biol* 2002;22:614–25. [PubMed: 11756556]
68. Krimer DB, Cheng G, Skoultschi AI. Induction of H3.3 replacement histone mRNAs during the precommitment period of murine erythroleukemia cell differentiation. *Nucleic Acids Res* 1993;21:2873–9. [PubMed: 8332496]
69. Krishnamurthy J, Ramsey MR, Ligon KL, Torrice C, Koh A, Bonner-Weir S, Sharpless NE. p16 (INK4a) induces an age-dependent decline in islet regenerative potential. *Nature* 2006;443:453–7. [PubMed: 16957737]
70. Krogan NJ, Keogh MC, Datta N, Sawa C, Ryan OW, Ding H, Haw RA, Pootoolal J, Tong A, Canadien V, Richards DP, Wu X, Emili A, Hughes TR, Buratowski S, Greenblatt JF. A Snf2 family ATPase complex required for recruitment of the histone H2A variant Htz1. *Mol Cell* 2003;12:1565–76. [PubMed: 14690608]
71. Lee CK, Shibata Y, Rao B, Strahl BD, Lieb JD. Evidence for nucleosome depletion at active regulatory regions genome-wide. *Nat Genet* 2004;36:900–5. [PubMed: 15247917]
72. Loppin B, Bonnefoy E, Anselme C, Laurencon A, Karr TL, Couple P. The histone H3.3 chaperone HIRA is essential for chromatin assembly in the male pronucleus. *Nature* 2005;437:1386–90. [PubMed: 16251970]
73. Lorain S, Quivy JP, Monier-Gavelle F, Scamps C, Lecluse Y, Almouzni G, Lipinski M. Core histones and HIRIP3, a novel histone-binding protein, directly interact with WD repeat protein HIRA. *Mol Cell Biol* 1998;18:5546–56. [PubMed: 9710638]
74. Loyola A, Bonaldi T, Roche D, Imhof A, Almouzni G. PTMs on H3 Variants before Chromatin Assembly Potentiate Their Final Epigenetic State. *Mol Cell* 2006;24:309–16. [PubMed: 17052464]
75. Mallette FA, Gaumont-Leclerc MF, Ferbeyre G. The DNA damage signaling pathway is a critical mediator of oncogene-induced senescence. *Genes Dev* 2007;21:43–8. [PubMed: 17210786]
76. Marignani PA, Kanai F, Carpenter CL. LKB1 associates with Brg1 and is necessary for Brg1-induced growth arrest. *J Biol Chem* 2001;276:32415–8. [PubMed: 11445556]
77. McKittrick E, Gafken PR, Ahmad K, Henikoff S. Histone H3.3 is enriched in covalent modifications associated with active chromatin. *Proc Natl Acad Sci U S A* 2004;101:1525–30. [PubMed: 14732680]
78. Meshorer E, Yellajoshula D, George E, Scambler PJ, Brown DT, Misteli T. Hyperdynamic plasticity of chromatin proteins in pluripotent embryonic stem cells. *Dev Cell* 2006;10:105–16. [PubMed: 16399082]
79. Michaloglou C, Vredeveld LC, Soengas MS, Denoyelle C, Kuilman T, van der Horst CM, Majoor DM, Shay JW, Mooi WJ, Peeper DS. BRAFE600-associated senescence-like cell cycle arrest of human naevi. *Nature* 2005;436:720–4. [PubMed: 16079850]
80. Mito Y, Henikoff JG, Henikoff S. Genome-scale profiling of histone H3.3 replacement patterns. *Nat Genet* 2005;37:1090–7. [PubMed: 16155569]
81. Mizuguchi G, Shen X, Landry J, Wu WH, Sen S, Wu C. ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. *Science* 2004;303:343–8. [PubMed: 14645854]
82. Molofsky AV, Slutsky SG, Joseph NM, He S, Pardal R, Krishnamurthy J, Sharpless NE, Morrison SJ. Increasing p16(INK4a) expression decreases forebrain progenitors and neurogenesis during ageing. *Nature* 2006;443:448–52. [PubMed: 16957738]
83. Moshkin YM, Armstrong JA, Maeda RK, Tamkun JW, Verrijzer P, Kennison JA, Karch F. Histone chaperone ASF1 cooperates with the Brahma chromatin-remodelling machinery. *Genes Dev* 2002;16:2621–6. [PubMed: 12381660]
84. Mousson F, Lautrette A, Thuret JY, Agez M, Courbeyrette R, Amigues B, Becker E, Neumann JM, Guerois R, Mann C, Ochsenbein F. Structural basis for the interaction of Asf1 with histone H3 and its functional implications. *Proc Natl Acad Sci U S A* 2005;102:5975–80. [PubMed: 15840725]
85. Murphy DJ, Hardy S, Engel DA. Human SWI-SNF component BRG1 represses transcription of the c-fos gene. *Mol Cell Biol* 1999;19:2724–33. [PubMed: 10082538]
86. Myung K, Pennaneach V, Kats ES, Kolodner RD. *Saccharomyces cerevisiae* chromatin-assembly factors that act during DNA replication function in the maintenance of genome stability. *Proc Natl Acad Sci U S A* 2003;100:6640–5. [PubMed: 12750463]

87. Narita M, Narita M, Krizhanovsky V, Nunez S, Chicas A, Hearn SA, Myers MP, Lowe SW. A novel role for high-mobility group a proteins in cellular senescence and heterochromatin formation. *Cell* 2006;126:503–14. [PubMed: 16901784]
88. Narita M, Nunez S, Heard E, Lin AW, Hearn SA, Spector DL, Hannon GJ, Lowe SW. Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. *Cell* 2003;113:703–16. [PubMed: 12809602]
89. Nevins JR. The Rb/E2F pathway and cancer. *Hum Mol Genet* 2001;10:699–703. [PubMed: 11257102]
90. Olsen CL, Gardie B, Yaswen P, Stampfer MR. Raf-1-induced growth arrest in human mammary epithelial cells is p16-independent and is overcome in immortal cells during conversion. *Oncogene* 2002;21:6328–39. [PubMed: 12214273]
91. Palmer TD, Schwartz PH, Taupin P, Kaspar B, Stein SA, Gage FH. Cell culture. Progenitor cells from human brain after death. *Nature* 2001;411:42–3. [PubMed: 11333968]
92. Pantazis P, Bonner WM. Specific alterations in the pattern of histone-3 synthesis during conversion of human leukemic cells to terminally differentiated cells in culture. *Differentiation* 1984;28:186–90. [PubMed: 6597116]
93. Park YJ, Chodaparambil JV, Bao Y, McBryant SJ, Luger K. Nucleosome assembly protein 1 exchanges histone H2A-H2B dimers and assists nucleosome sliding. *J Biol Chem* 2005;280:1817–25. [PubMed: 15516689]
94. Pearson M, Carbone R, Sebastiani C, Cioce M, Fagioli M, Saito S, Higashimoto Y, Appella E, Minucci S, Pandolfi PP, Pelicci PG. PML regulates p53 acetylation and premature senescence induced by oncogenic Ras. *Nature* 2000;406:207–10. [PubMed: 10910364]
95. Perche PY, Vourc'h C, Konecny L, Souchier C, Robert-Nicoud M, Dimitrov S, Khochbin S. Higher concentrations of histone macroH2A in the Barr body are correlated with higher nucleosome density. *Curr Biol* 2000;10:1531–4. [PubMed: 11114523]
96. Peterson CL, Laniel MA. Histones and histone modifications. *Curr Biol* 2004;14:R546–51. [PubMed: 15268870]
97. Phelps-Durr TL, Thomas J, Vahab P, Timmermans MC. Maize rough sheath2 and its Arabidopsis orthologue ASYMMETRIC LEAVES1 interact with HIRA, a predicted histone chaperone, to maintain knox gene silencing and determinacy during organogenesis. *Plant Cell* 2005;17:2886–98. [PubMed: 16243907]
98. Pina B, Suau P. Changes in histones H2A and H3 variant composition in differentiating and mature rat brain cortical neurons. *Dev Biol* 1987;123:51–8. [PubMed: 3622934]
99. Polo SE, Roche D, Almouzni G. New histone incorporation marks sites of UV repair in human cells. *Cell* 2006;127:481–93. [PubMed: 17081972]
100. Prochasson P, Florens L, Swanson SK, Washburn MP, Workman JL. The HIR corepressor complex binds to nucleosomes generating a distinct protein/DNA complex resistant to remodeling by SWI/SNF. *Genes Dev* 2005;19:2534–9. [PubMed: 16264190]
101. Ramirez RD, Morales CP, Herbert BS, Rohde JM, Passons C, Shay JW, Wright WE. Putative telomere-independent mechanisms of replicative aging reflect inadequate growth conditions. *Genes Dev* 2001;15:398–403. [PubMed: 11230148]
102. Ray-Gallet D, Quivy JP, Scamps C, Martini EM, Lipinski M, Almouzni G. HIRA is critical for a nucleosome assembly pathway independent of DNA synthesis. *Mol Cell* 2002;9:1091–100. [PubMed: 12049744]
103. Reeves R. Molecular biology of HMGA proteins: hubs of nuclear function. *Gene* 2001;277:63–81. [PubMed: 11602345]
104. Reisman DN, Sciarrotta J, Wang W, Funkhouser WK, Weissman BE. Loss of BRG1/BRM in human lung cancer cell lines and primary lung cancers: correlation with poor prognosis. *Cancer Res* 2003;63:560–6. [PubMed: 12566296]
105. Reisman DN, Strobeck MW, Betz BL, Sciarrotta J, Funkhouser W Jr, Murchardt C, Yaniv M, Sherman LS, Knudsen ES, Weissman BE. Concomitant down-regulation of BRM and BRG1 in human tumor cell lines: differential effects on RB-mediated growth arrest vs CD44 expression. *Oncogene* 2002;21:1196–207. [PubMed: 11850839]

106. Rogakou EP, Sekeri-Pataryas KE. Histone variants of H2A and H3 families are regulated during in vitro aging in the same manner as during differentiation. *Exp Gerontol* 1999;34:741–54. [PubMed: 10579635]
107. Salomoni P, Pandolfi PP. The role of PML in tumor suppression. *Cell* 2002;108:165–70. [PubMed: 11832207]
108. Scaffidi P, Misteli T. Lamin A-dependent nuclear defects in human aging. *Science* 2006;312:1059–63. [PubMed: 16645051]
109. Scaffidi P, Misteli T. Reversal of the cellular phenotype in the premature aging disease Hutchinson-Gilford progeria syndrome. *Nat Med* 2005;11:440–5. [PubMed: 15750600]
110. Schulz LL, Tyler JK. The histone chaperone ASF1 localizes to active DNA replication forks to mediate efficient DNA replication. *Faseb J* 2006;20:488–90. [PubMed: 16396992]
111. Schwabish MA, Struhl K. Asf1 Mediates Histone Eviction and Deposition during Elongation by RNA Polymerase II. *Mol Cell* 2006;22:415–22. [PubMed: 16678113]
112. Schwartz BE, Ahmad K. Transcriptional activation triggers deposition and removal of the histone variant H3.3. *Genes Dev* 2005;19:804–14. [PubMed: 15774717]
113. Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell* 1997;88:593–602. [PubMed: 9054499]
114. Sgarra R, Rustighi A, Tessari MA, Di Bernardo J, Altamura S, Fusco A, Manfioletti G, Giancotti V. Nuclear phosphoproteins HMGA and their relationship with chromatin structure and cancer. *FEBS Lett* 2004;574:1–8. [PubMed: 15358530]
115. Shanahan F, Seghezzi W, Parry D, Mahony D, Lees E. Cyclin E associates with BAF155 and BRG1, components of the mammalian SWI-SNF complex, and alters the ability of BRG1 to induce growth arrest. *Mol Cell Biol* 1999;19:1460–9. [PubMed: 9891079]
116. Sharp JA, Fouts ET, Krawitz DC, Kaufman PD. Yeast histone deposition protein Asf1p requires Hir proteins and PCNA for heterochromatic silencing. *Curr Biol* 2001;11:463–73. [PubMed: 11412995]
117. Sharp JA, Franco AA, Osley MA, Kaufman PD, Krawitz DC, Kama T, Fouts ET, Cohen JL. Chromatin assembly factor I and Hir proteins contribute to building functional kinetochores in *S. cerevisiae*. *Genes Dev* 2002;16:85–100. [PubMed: 11782447]
118. Sherr CJ, McCormick F. The RB and p53 pathways in cancer. *Cancer Cell* 2002;2:103–12. [PubMed: 12204530]
119. Sherwood PW, Tsang SV, Osley MA. Characterization of HIR1 and HIR2, two genes required for regulation of histone gene transcription in *Saccharomyces cerevisiae*. *Mol Cell Biol* 1993;13:28–38. [PubMed: 8417331]
120. Shumaker DK, Dechat T, Kohlmaier A, Adam SA, Bozovsky MR, Erdos MR, Eriksson M, Goldman AE, Khuon S, Collins FS, Jenuwein T, Goldman RD. Mutant nuclear lamin A leads to progressive alterations of epigenetic control in premature aging. *Proc Natl Acad Sci U S A* 2006;103:8703–8. [PubMed: 16738054]
121. Singer MS, Kahana A, Wolf AJ, Meisinger LL, Peterson SE, Goggin C, Mahowald M, Gottschling DE. Identification of high-copy disruptors of telomeric silencing in *Saccharomyces cerevisiae*. *Genetics* 1998;150:613–32. [PubMed: 9755194]
122. Smith CL, Peterson CL. ATP-dependent chromatin remodeling. *Curr Top Dev Biol* 2005;65:115–48. [PubMed: 15642381]
123. Spector MS, Raff A, DeSilva H, Lee K, Osley MA. Hir1p and Hir2p function as transcriptional corepressors to regulate histone gene transcription in the *Saccharomyces cerevisiae* cell cycle. *Mol Cell Biol* 1997;17:545–52. [PubMed: 9001207]
124. Strobeck MW, Knudsen KE, Fribourg AF, DeCristofaro MF, Weissman BE, Imbalzano AN, Knudsen ES. BRG-1 is required for RB-mediated cell cycle arrest. *Proc Natl Acad Sci U S A* 2000;97:7748–53. [PubMed: 10884406]
125. Tagami H, Ray-Gallet D, Almouzni G, Nakatani Y. Histone H3.1 and H3.3 complexes mediate nucleosome assembly pathways dependent or independent of DNA synthesis. *Cell* 2004;116:51–61. [PubMed: 14718166]

126. Tang Y, Poustovoitov MV, Zhao K, Garfinkel M, Canutescu A, Dunbrack R, Adams PD, Marmorstein R. Structure of a human ASF1a-HIRA complex and insights into specificity of histone chaperone complex assembly. *Nat Struct Mol Biol* 2006;13:921–9. [PubMed: 16980972]
127. Tyler JK, Adams CR, Chen SR, Kobayashi R, Kamakaka RT, Kadonaga JT. The RCAF complex mediates chromatin assembly during DNA replication and repair. *Nature* 1999;402:555–60. [PubMed: 10591219]
128. Urban MK, Zweidler A. Changes in nucleosomal core histone variants during chicken development and maturation. *Dev Biol* 1983;95:421–8. [PubMed: 6825941]
129. van der Heijden GW, Derijck AA, Posfai E, Giele M, Pelczar P, Ramos L, Wansink DG, van der Vlag J, Peters AH, de Boer P. Chromosome-wide nucleosome replacement and H3.3 incorporation during mammalian meiotic sex chromosome inactivation. *Nat Genet* 2007;39:251–8. [PubMed: 17237782]
130. Ventura A, Kirsch DG, McLaughlin ME, Tuveson DA, Grimm J, Lintault L, Newman J, Reczek EE, Weissleder R, Jacks T. Restoration of p53 function leads to tumour regression in vivo. *Nature* 2007;445:661–5. [PubMed: 17251932]
131. Walsh P, Bursac D, Law YC, Cyr D, Lithgow T. The J-protein family: modulating protein assembly, disassembly and translocation. *EMBO Rep* 2004;5:567–71. [PubMed: 15170475]
132. Wei W, Hemmer RM, Sedivy JM. Role of p14(ARF) in replicative and induced senescence of human fibroblasts. *Mol Cell Biol* 2001;21:6748–57. [PubMed: 11564860]
133. Wirbelauer C, Bell O, Schubeler D. Variant histone H3.3 is deposited at sites of nucleosomal displacement throughout transcribed genes while active histone modifications show a promoter-proximal bias. *Genes Dev* 2005;19:1761–6. [PubMed: 16077006]
134. Wong AK, Shanahan F, Chen Y, Lian L, Ha P, Hendricks K, Ghaffari S, Iliev D, Penn B, Woodland AM, Smith R, Salada G, Carillo A, Laity K, Gupte J, Swedlund B, Tavtigian SV, Teng DH, Lees E. BRG1, a component of the SWI-SNF complex, is mutated in multiple human tumor cell lines. *Cancer Res* 2000;60:6171–7. [PubMed: 11085541]
135. Wright WE, Shay JW. Cellular senescence as a tumor-protection mechanism: the essential role of counting. *Curr Opin Genet Dev* 2001;11:98–103. [PubMed: 11163158]
136. Wright WE, Shay JW. Historical claims and current interpretations of replicative aging. *Nat Biotechnol* 2002;20:682–8. [PubMed: 12089552]
137. Wunsch AM, Lough J. Modulation of histone H3 variant synthesis during the myoblast-myotube transition of chicken myogenesis. *Dev Biol* 1987;119:94–9. [PubMed: 3792638]
138. Xu Y, Sumter TF, Bhattacharya R, Tesfaye A, Fuchs EJ, Wood LJ, Huso DL, Resar LM. The HMG-I oncogene causes highly penetrant, aggressive lymphoid malignancy in transgenic mice and is overexpressed in human leukemia. *Cancer Res* 2004;64:3371–5. [PubMed: 15150086]
139. Xue W, Zender L, Miething C, Dickins RA, Hernando E, Krizhanovsky V, Cordon-Cardo C, Lowe SW. Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. *Nature* 2007;445:656–60. [PubMed: 17251933]
140. Ye X, Zerlanko B, Zhang R, Somaiah N, Lipinski M, Adams PD. Definition of pRB- and p53-dependent and independent steps in HIRA/ASF1a-mediated formation of Senescence-Associated Heterochromatin Foci (SAHF). *Mol Cell Biol*. 2007In press
141. Zhang H, Richardson DO, Roberts DN, Utley R, Erdjument-Bromage H, Tempst P, Cote J, Cairns BR. The Yaf9 component of the SWR1 and NuA4 complexes is required for proper gene expression, histone H4 acetylation, and Htz1 replacement near telomeres. *Mol Cell Biol* 2004;24:9424–36. [PubMed: 15485911]
142. Zhang HS, Gavin M, Dahiya A, Postigo AA, Ma D, Luo RX, Harbour JW, Dean DC. Exit from G1 and S phase of the cell cycle is regulated by repressor complexes containing HDAC-Rb-hSWI/SNF and Rb-hSWI/SNF. *Cell* 2000;101:79–89. [PubMed: 10778858]
143. Zhang R, Chen W, Adams PD. Molecular Dissection of Formation of Senescent Associated Heterochromatin Foci. *Mol Cell Biol*. 2007
144. Zhang R, Liu ST, Chen W, Bonner B, Pehrson J, Yen TJ, Adams PD. HP1 proteins are essential for a dynamic nuclear response that rescues the function of perturbed heterochromatin in primary human cells. *Mol Cell Biol* 2007;27:949–62. [PubMed: 17101789]

145. Zhang R, Poustovoitov MV, Ye X, Santos HA, Chen W, Daganzo SM, Erzberger JP, Serebriiskii IG, Canutescu AA, Dunbrack RL, Pehrson JR, Berger JM, Kaufman PD, Adams PD. Formation of MacroH2A-containing senescence-associated heterochromatin foci and senescence driven by ASF1a and HIRA. *Dev Cell* 2005;8:19–30. [PubMed: 15621527]
146. Zhao J, Herrera-Diaz J, Gross DS. Domain-wide displacement of histones by activated heat shock factor occurs independently of Swi/Snf and is not correlated with RNA polymerase II density. *Mol Cell Biol* 2005;25:8985–99. [PubMed: 16199876]
147. Zhu L. Tumour suppressor retinoblastoma protein Rb: a transcriptional regulator. *Eur J Cancer* 2005;41:2415–27. [PubMed: 16209920]
148. Zlatanova J, van Holde K. Linker histones versus HMG1/2: a struggle for dominance? *Bioessays* 1998;20:584–8. [PubMed: 9723008]

Figure 1. Formation of SAHF in senescent human cells is linked to HIRA's localization to PML nuclear bodies

Senescent human WI38 cells were stained with antibodies to HIRA (red), PML (green) and with DAPI to visualize DNA (blue). Yellow foci result from colocalization of HIRA and PML in PML nuclear bodies. SAHF is evident in the uneven distribution of DNA through the nucleus.

Figure 2. A model for formation of SAHF in senescent human cells

Senescence is triggered by short telomeres, activated oncogenes and other cell stresses. The HIRA/ASF1a pathway cooperates with the p16INK4a/pRB pathway to drive chromosome condensation. The mode of activation of HIRA/ASF1a and p16INK4a are largely unknown. After chromosome condensation, HP1 proteins and histone variant macroH2A are incorporated into SAHF. Recruitment of HP1 γ to SAHF depends on HP1 γ phosphorylation. Dashed lines indicate steps that are poorly defined at present. See text and references for further details.