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GENOMIC INSTABILITY IN CANCER — OPINION

Crosstalk between chromatin state and DNA damage response in cellular senescence and cancer

Gabriele Sulli, Raffaella Di Micco and Fabrizio d'Adda di Fagagna

Abstract | The generation of DNA lesions and the resulting activation of DNA damage response (DDR) pathways are both affected by the chromatin status at the site of damaged DNA. In turn, DDR activation affects the chromatin at both the damaged site and across the whole genome. Cellular senescence and cancer are associated with the engagement of the DDR pathways and with profound chromatin changes. In this Opinion article, we discuss the interplay between chromatin and DDR factors in the context of cellular senescence that is induced by oncogenes and in cancer.

Chromatin is comprised of nucleosomes that each consists of 146 base pairs of DNA wrapped around a core histone octamer that is comprised of two heterodimers of histone H2A and histone H2B and a tetramer of histone H3 and histone H4 (REF. 1). Histone H1 binds to the linker DNA that extends between nucleosomes. Several types of post-translational modification, which mostly occur at the fairly unstructured amino terminus of the histones (the 'histone tail'), influence chromatin condensation. The degree of compaction that is achieved varies across the genome, and densely packed chromatin (which is conventionally considered to be transcriptionally inert) is usually referred to as heterochromatin, whereas chromatin in a more open, actively transcribed conformation is known as euchromatin. Chromosome regions with structural roles, such as telomeres and centromeres, are organized into 'constitutive heterochromatin', although shorter, often non-coding and repetitive regions of constitutive heterochromatin also occur in higher eukaryotes[2]. In addition, cellular processes that require gene silencing, such as differentiation, cellular senescence and X chromosome inactivation, induce the conversion of euchromatin into heterochromatin, which is referred to as facultative heterochromatin[2].

As well as affecting transcription, chromatin can affect DNA damage response (DDR) signalling. All living organisms are constantly exposed to genotoxic stress, and DNA thus needs to be repaired to preserve the information that it encodes. DDR signalling is specific to the type of DNA damage that occurs, and the pathways that are activated are determined by the activation of the PI3K-like kinases (PIKKs) ataxia-telangiectasia mutated (ATM), ataxia-telangiectasia and Rad3-related (ATR) and DNA-dependent protein kinase catalytic subunit (DNA-PKcs), which consequently phosphorylate and

thus activate various proteins that coordinate the arrest of cell cycle progression and DNA repair pathways to preserve genome integrity (FIG. 1). The resumption of cell cycle progression occurs only when DNA damage has been removed in full. Alternatively, in the case of severe DNA damage, the DDR in some cell types can induce cell death by apoptosis. Among the various events involving DNA during the life of a cell, chromosomal DNA replication is certainly the most dangerous, and it is more dangerous in the presence of an activated oncogene[3–4].

Oncogene-induced DNA replication stress is a powerful trigger of DDR signalling pathways[5–6], in particular the ATR-dependent DDR pathways that respond to damage that is incurred during DNA replication[7].

DDR signalling can induce a permanent cell cycle arrest, termed cellular senescence, which is caused by the accumulation of unrepaired DNA lesions that fuel persistent DDR signalling[8], or the induction of apoptosis (FIG. 1). Cellular senescence is a condition that was initially described by L. Hayflick[9], in which cells, despite being alive, are unable to

progress through the cell cycle and divide. Following oncogene activation, oncogene-induced senescence (OIS) is established, which is a specific type of senescence that arrests the proliferation of checkpoint-proficient cells that have an activated oncogene[10]. It is now appreciated that OIS occurs *in vivo* and contributes to tumour suppression by preventing the expansion of oncogene-expressing cells[11,12]. The activation of DDR pathways is causally involved in the establishment of OIS[5,6,13], and activation of DDR signalling has been observed from the onset of cancer development in humans[14,15]. The observation that several DDR genes are inactivated during cancer progression[4] strengthens the idea that DDR genes have tumour suppressive roles. Although still a matter of intense investigation, both altered DNA replication[5,6] and oxidative stress[16] have been proposed to be the mechanisms that are responsible for the activation of DDR signalling following oncogene activation. Whether these two mechanisms are distinct is still unclear[17].

Oncogene activation has also been shown to have an important effect on chromatin, and cellular senescence is reportedly characterized by heterochromatin formation and chromatin condensation. Thus, oncogene activation and the process of cellular transformation are intimately linked to DDR engagement and chromatin changes. In this Opinion article, we discuss the interplay between chromatin and the DDR, with an emphasis on senescence and cancer, as well as the possible opportunities for therapeutic intervention.

The effect of chromatin on the DDR

Chromatin can affect the sensitivity of DNA to DNA-damaging agents. Evidence for this has come from studies showing that, on exposure to ionizing radiation, DNA that is depleted of histones, or of other chromatin-associated proteins, and DNA that is wrapped in chromatin with a low degree of condensation has more lesions compared with compacted chromatin[18,19]. It is now clear that in addition to physically shielding DNA from damage, the chromatin structure also affects local DDR signalling around DNA damage. For example, heterochromatin seems to be resistant to histone H2AX phosphorylation (an early event in DDR signalling) and DDR activation in general, and phosphorylated H2AX (referred to as γH2AX) is mostly detected in euchromatin or is confined to the periphery of heterochromatic domains[20,21]. Furthermore, decreasing the degree of heterochromatinization by inhibiting histone deacetylases (HDACs), which increase chromatin
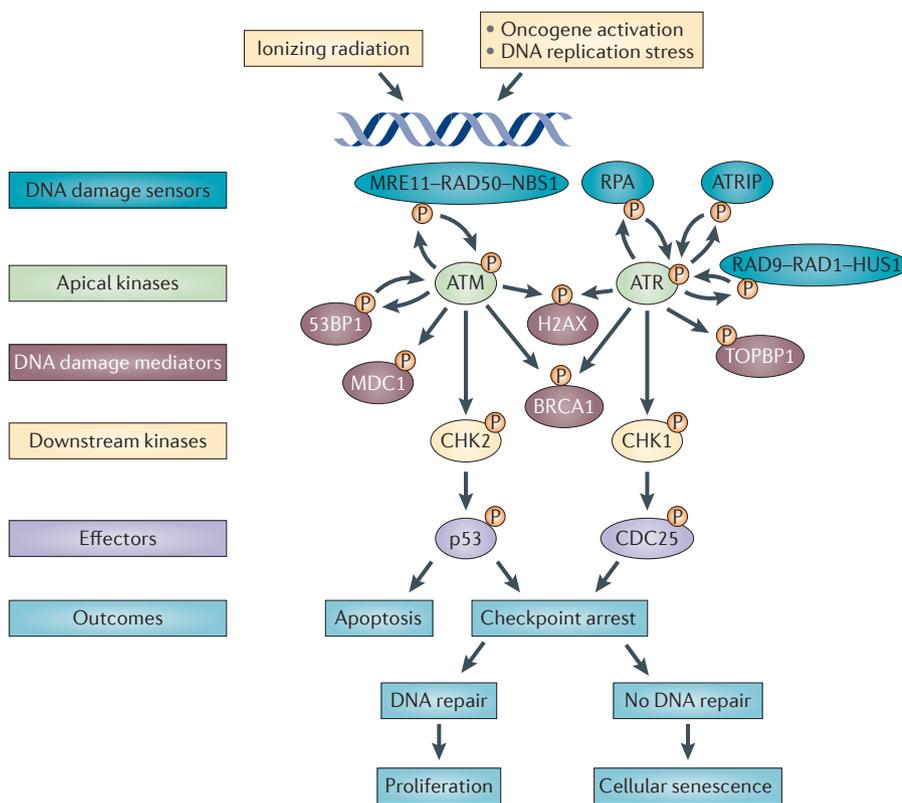

Figure 1 | **The DNA damage response.** The DNA damage response (DDR) pathway is composed of two main DNA damage sensors: the MRE11–RAD50–NBS1 (MRN) complex that detects DNA double-strand breaks (DSBs); and replication protein A (RPA) and the RAD9–RAD1–HUS1 (9-1-1) complex that detects exposed regions of single-stranded DNA. These sensors recruit the apical kinases ataxia-telangiectasia mutated (ATM) (through the MRN complex) and ataxia telangiectasia and Rad3-related (ATR) (through RPA and the 9-1-1 complex), which is bound by ATR-interacting protein (ATRIP). These in turn phosphorylate (P) the histone variant H2AX on Ser139 (known as γH2AX) in the region proximal to the DNA lesion[65,147]. Thus, although ATM is predominantly activated by DSBs, ATR responds to the type of genotoxic stress that is caused by DNA replication stress, which is also caused by oncogenes. γH2AX is required to recruit mediator of DNA damage checkpoint 1 (MDC1) that further sustains and amplifies DDR signalling by enforcing further accumulation of the MRN complex and activation of ATM. BRCA1 is recruited at sites of DNA damage on phosphorylation by ATM and ATR. p53-binding protein 1 (53BP1) is also involved in sustaining DDR signalling by enhancing ATM activation. DDR signalling relies on additional mechanisms that are based on ubiquitylation (FIG. 3b). Eventually, DDR signalling spreads away from the damaged locus owing to the engagement of diffusible kinases CHK2 (which is mainly phosphorylated by ATM) and CHK1 (which is mainly phosphorylated by ATR) with signalling converging on downstream effectors such as p53 and the cell division cycle 25 (CDC25) phosphatases. DDR-mediated cellular outcomes may be cell death by apoptosis; transient cell cycle arrest followed by repair of DNA damage and resumption of proliferation; or cellular senescence caused by the persistence of unrepaired DNA damage. An additional layer of control of the DDR that is based on small RNAs called DDRNAs has also recently been reported[148].

compaction by removing acetyl groups from histones (the negative charges of acetyl groups repel one another and this repulsion is reduced when acetyl groups are removed), or by reducing the levels of histone H1, enhances DDR signalling and the extent of γH2AX spreading from the lesion[22,23]. However, the simple model that open chromatin favours local DDR signalling is probably an over-simplification because euchromatic regions such as gene promoters that are occupied by RNA polymerase II, for example, also constitute a barrier for γH2AX spreading[24].

In addition to DDR signalling, chromatin affects the mechanism of DNA repair. DNA double-strand breaks (DSBs) that occur near or within heterochromatin are not efficiently repaired and supplementary mechanisms are necessary in order to facilitate the processing of these lesions[25]. These supplementary mechanisms involve the reduction of the affinity of the transcriptional repressor KRAB-associated protein 1 (KAP1; also known as TIF1β and TRIM28) to heterochromatin, following its phosphorylation by ATM (FIG. 2a). This increases nucleosome flexibility, which allows the DNA repair machinery access to the lesion. A molecular mechanism for this process has recently been proposed[26]: on sumoylation of KAP1 at Lys554, Lys779 and Lys804 by ubiquitin carrier protein 9 (UBC9), KAP1 binds the nucleosome remodelling protein chromodomain helicase DNA-binding protein 3 (CHD3), which is involved in the formation of heterochromatin[27]. Although KAP1 sumoylation levels are not affected by ionizing radiation, ATM-dependent KAP1 phosphorylation — by interfering with the CHD3–KAP1 interaction — triggers hetero-chromatin decondensation and allows DNA repair (FIG. 2a).

Of particular interest, KAP1 was found to be highly expressed in gastric cancer and breast cancer metastasis[28,29]. Additionally, high KAP1 expression is a predictive marker of the occurrence and the reduced survival of patients with peritoneal carcinoma[28]. KAP1 downregulation impairs the viability of gastric cancer cell lines[28], thus it is tempting to speculate that KAP1 induction may increase chromatin compaction and in turn block tumour suppressive functions of DDR signalling.

A peculiar response to DNA damage that occurs within heterochromatin was recently reported in a study carried out in *Drosophila melanogaster*[30]. In this model, DNA damage was detectable in heterochromatin only at early time points after exposure to ionizing radiation. The authors propose that, rather than heterochromatin being refractory to
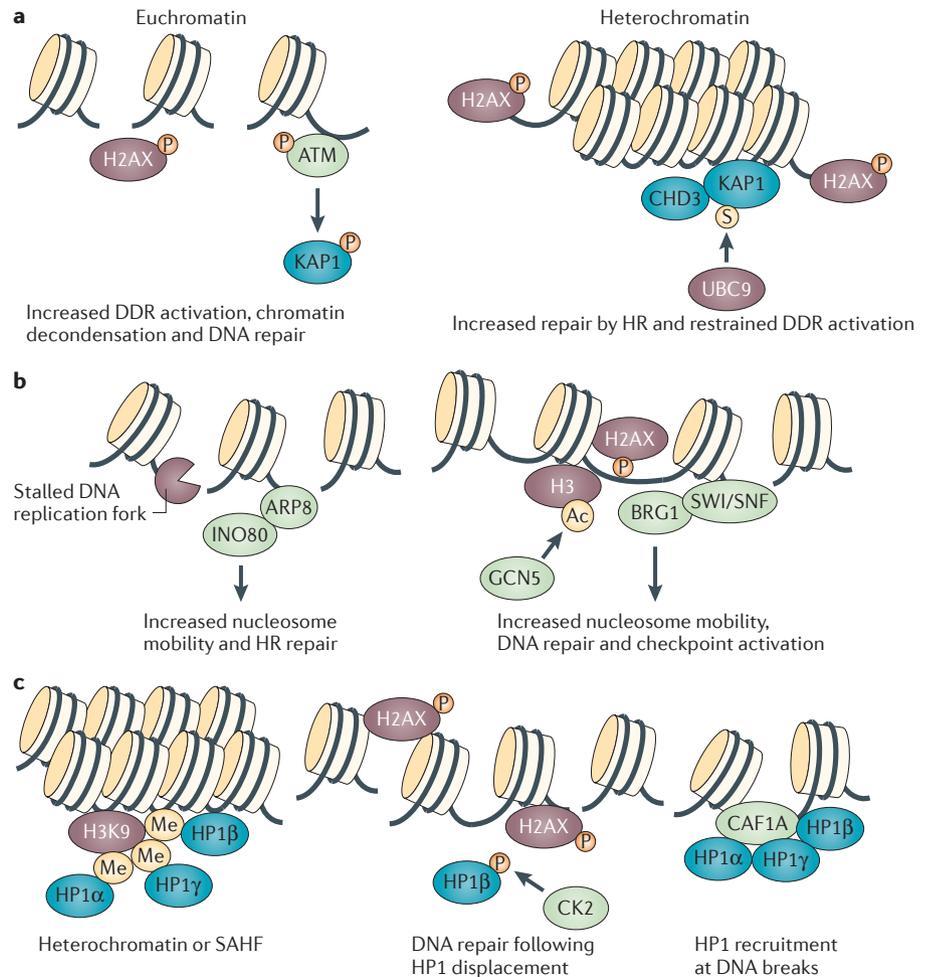
Figure 2 | **Chromatin remodellers and histone modifiers following DNA damage induction.** **a** | Chromatin conformation and DNA repair is shown. Following DNA double-strand break (DSB) generation in euchromatic regions, the phosphorylation (P) of histone H2AX (γH2AX) by ataxia telangiectasia-mutated (ATM) contributes to the repair of the DNA lesion. ATM phosphorylates KRAB-associated protein 1 (KAP1), which reduces its association with chromatin and allows the DNA repair machinery to access DNA breaks. KAP1 sumoylation (S) by ubiquitin carrier protein 9 (UBC9) is required for KAP1 binding to CHD3, which is involved in the formation of heterochromatin that restrains the activation of DNA damage response (DDR) signalling. Importantly, ATM-dependent KAP1 phosphorylation, inhibits CHD3–TRIM28 interaction and promotes heterochromatin decondensation and DNA repair. Notably, H2AX phosphorylation and DDR activation is mostly confined to the periphery of heterochromatic domains, possibly as the result of its move outside the heterochromatin regions to undergo DNA repair by homologous recombination (HR). **b** | Chromatin-remodelling complexes in DDR activation are shown. After DNA damage, the INO80-containing chromatin-remodelling complex is recruited at sites of DNA breaks through its subunit actin-related protein 8 (ARP8) independently of H2AX phosphorylation. INO80 stabilizes stalled DNA replication forks and regulates nucleosomal mobility and DNA repair by HR. The SWI/SNF complex, through its subunit BRG1, binds to both phosphorylated H2AX and acetylated (Ac) histone H3, which allows DNA repair and cell cycle checkpoint activation. GCN5 promotes histone H3 acetylation. **c** | DDR activation and chromatin conformation are shown. Heterochromatin proteins HP1α, HP1β and HP1γ associate with methylated (Me) histone H3 on nucleosomes and mediate heterochromatin formation and senescence-associated heterochromatic foci (SAHF). Following DNA damage, HP1β is phosphorylated by caseine kinase 2 (CK2) and is displaced from the chromatin, allowing γH2AX-mediated DNA repair. However, the recruitment of HP1 proteins to DNA lesions has also been reported, possibly as a later event.

ionizing radiation-induced damage, the DSB moves outside the heterochromatin regions to undergo DNA repair by homologous recombination (HR). This dynamic event seems to be distinct from the reported positional

stability of DSBs that are observed in mammals[31,32]. Notably, in OIS, focal accumulation of activated DDR factors (commonly known as DDR foci) occurs near — but outside — large heterochromatic regions[21].

An additional level of functional interaction between the DDR and chromatin components is the crosstalk between ATM and high mobility group protein A (HMGA), an architectural protein that can bend DNA. Both HMGA1 and HMGA2 can interact with ATM and are phosphorylated by ATM after irradiation[33,34]. This is potentially relevant to cancer given that HMGA proteins are aberrantly expressed in different types of cancer and are associated with unfavourable prognosis and resistance to chemotherapy[35].

Overall, chromatin conformation is therefore an important component of the activation of DDR signalling and DNA repair. As a common characteristic of different tumour types is to be highly heterochromatic[21], this suggests that cancer cells, through the expansion of heterochromatic regions in the genome, attenuate DDR signalling and survive in the presence of DNA lesions that occur after oncogene activation. These may be relevant considerations for the development of cancer therapeutic protocols or the implementation of existing ones, as the analysis of chromatin status may predict the efficacy of therapies that are based on inhibiting DDR signalling, or may direct the use of strategies that aim to alter chromatin compaction.

Chromatin remodellers in the DDR
In addition to histone modifications, chromatin structure is modified by chromatin-remodelling complexes. These multiprotein molecular machines contain DNA-dependent ATPases as catalytic subunits that can alter the position and density of nucleosomes. It is currently thought that chromatin remodelling facilitates the access of DDR factors by moving and ejecting nucleosomes and, conversely, by restoring the original chromatin conformation once DNA repair is completed.

The role of the INO80 chromatin-remodelling complex, which is involved in regulating the mobility and exchange of histone variants, has been extensively studied in yeast and has been shown to be involved in DNA repair[36,37] (FIG. 2b). In mammals, INO80 is an essential component of HR[38,39], and the recruitment of INO80 is mediated by the subunit actin-related protein 8 (ARP8). Unlike in yeast, mammalian INO80 localizes to DNA lesions independently of γH2AX[40]. This suggests that the INO80 complex is involved in the early phase of the DDR signalling cascade. Given the role of the INO80 complex in the stabilization of stalled replication forks[41,42], an event that is reported to be intrinsically associated with oncogene activation[5,6,43], and given

its recruitment to Holliday junctions (a DNA intermediate in HR) through the transcription factor yin and yang 1 (YY1)[38], the role of the INO80 complex in cancer deserves to be addressed in greater depth.

In mammals, the impairment of the activity of the SWI/SNF chromatin-remodelling complex results in reduced γH2AX levels, DSB repair defects and hypersensitivity to DSBs, although it does not seem to affect the recruitment of ATM and ATR[44]. Primary fibroblasts lacking SWI/SNF subunits are characterized by an enhanced sensitivity to single-strand breaks (SSBs), as well as to DSBs to a minor extent[45]. The binding of BRG1, an ATPase subunit of the SWI/SNF complex, to DNA damage sites is dependent on both γH2AX and histone H3 acetylation[46]. Histone H3 is acetylated at Lys9, Lys14, Lys18 and Lys23 on DNA damage by the histone acetyltransferase (HAT) GCN5 (also known as KAT2A)[46]. Interestingly, crosstalk between SWI/SNF and GCN5 complexes is required for efficient DSB repair (FIG. 2b).

Importantly, the processes that involve chromatin-remodelling complexes also include the restoration of the original chromatin conformation after DNA has been repaired. Thus, any alterations of these processes may in turn alter the original epigenetic architecture, thus resulting in deregulation of gene expression that may lead to malignant transformation. Indeed, several components of the SWI/SNF

chromatin-remodelling complex are mutated in cancer (TABLE 1). Interestingly, SNF5 (also known as SMARCB1, INI1 and BAF47), which is a core component of the SWI/SNF chromatin-remodelling complex that is important for the localization of this complex at specific loci and for the recruitment of HDAC1 at gene promoters[47], is inactivated in a large range of cancers[48]; it was also shown to function as a tumour suppressor in mouse models[48]. It is interesting to note that the impairment of SNF5, in addition to compromising DNA repair[45], results in the upregulation of proliferative genes, thus potentially leading to hyperproliferation in a manner that is reminiscent of oncogenic activation[48]. Moreover, another SWI/SNF subunit, BRG1, is recurrently downregulated or mutated in various cancer types[48]. In addition to its consolidated contribution to tumour suppression via transcriptional regulation of p16 (also known as INK4A)[49], more work is required to clarify the impact of the SWI/SNF chromatin-remodelling complex role on the DDR in tumorigenesis.

Finally, it is worth considering that the ATPase activities of chromatin-remodelling complexes make them potential drug targets. However, given the complexity of their roles, including their proposed tumour suppressive functions, more needs to be learned about their functions in cancer before they can be considered legitimate therapeutic targets.

Table 1 | Histone modifiers and their roles in DDR signalling and cancer

Histone modifier	Role in the DDR	Association with cancer
SWI/SNF	γH2AX levels and DSB repair	Mutated; tumour suppressor
BMI1	Ubiquitylation of Lys119 on histone H2A; blocks transcriptional elongation at the DNA damage site	Oncogene
TIP60	Acetylation of H2AX on Lys5, which is necessary for ubiquitylation of H2AX[122]; ATM acetylation	Tumour suppressor
DOT1L	53BP1 recruitment	Downregulated in pleiomorphic adenoma of the parotid gland; altered chromatin recruitment in acute lymphoblastic leukaemia, acute myeloid leukaemia, mixed lineage leukaemia and lymphoma
BBAP E3 ligase	53BP1 recruitment	Highly expressed in chemoresistant leukaemia
HDAC	γH2AX levels, HR and NHEJ	Altered expression; mutated
CBP	DNA repair and DDR signalling	Translocated in leukaemias and lymphomas
MMSET	53BP1 recruitment	Translocated in multiple myeloma

53BP1, p53-binding protein 1; ATM, ataxia-telangiectasia mutated; BBAP, B-lymphoma- and BAL-associated protein; CBP, CREB-binding protein; DDR, DNA damage response; DSB, DNA double-strand break; HDAC, histone deacetylase; HR, homologous recombination; NHEJ, non-homologous end joining; TIP60, 60kDa Tat-interactive protein.

Modulation of chromatin by the DDR

To the same extent that DDR activation is affected by chromatin state, the DDR machinery can also alter the chromatin structure. Cytological observations indicate that, on DSB generation, energy-dependent processes cause an H2AX- and ATM-independent expansion of the chromatin region surrounding the DSB[31]. This can be a consequence of remodelling and decondensation by chromatin-remodelling complexes that are recruited to DSBs. Interestingly, heterochromatic domains that are affected by DSBs are also subjected to a similar chromatin expansion in human cells[30,31], whereas in *D. melanogaster* this event is dependent on ATM and ATR[30].

An important protein family at the interface between chromatin structure and DDR signalling that has been studied in some depth is HP1 (REF. 50) (FIG. 2c). HP1 proteins (of which there are three different isoforms in mammals, HP1α, HP1β and HP1γ) were previously considered to be simply structural heterochromatin-associated proteins that associate with H3K9me2 and H3K9me3 on nucleosomes through their chromodomains[51,52]. However, they are now known to be involved in a broad range of processes, including the formation of senescence-associated heterochromatin foci (SAHF) (discussed below) and the modulation of DDR signalling[50].

DNA damage induces HP1β phosphorylation on Thr51 by casein kinase 2 (CK2), although how this induction occurs remains unknown. This causes the release of HP1β from chromatin, which has been suggested to contribute to the reported local transcriptional repression that occurs surrounding DNA damage[53,54]. Importantly, HP1β displacement can be observed both in euchromatin and in heterochromatin and it promotes H2AX phosphorylation, thus sustaining the full activation of the DDR cascade.

In apparent contrast to this model, three independent reports have proposed that HP1α, HP1β and HP1γ are recruited, rather than displaced, to sites of DNA damage and that HP1α is necessary for p53-binding protein 1 (53BP1) and RAD51 foci formation[55,56]. The recruitment of the HP1 proteins is dependent on their chromoshadow domain (a chromo-like domain) and on chromatin assembly factor 1A (CAF1A); it is independent of the chromodomain in these proteins and H3K9me3. Therefore, this model presents a potentially distinct mobilization mechanism from the one proposed for the release of HP1 proteins from DNA damage sites.

A way of reconciling these observations is to consider that HP1 proteins might be immediately displaced (and this is more evident in heterochromatin than in euchromatin) and slowly recruited again to DSBs[57]. Nevertheless, despite the different experimental conditions and systems used in various studies[30,53,55–58] (which may highlight distinct aspects of a complex network of events) it is likely that the association of HP1 proteins with chromatin becomes dynamic on DNA damage and that the proteins are exchanged between the chromatin and the nucleoplasm until DDR signalling is eventually muted. In this regard, it will be interesting to study the mobility of HP1 proteins in SAHF (discussed below) and their role in DDR signalling.

Recently, HP1 proteins have been implicated in cancer in a complex manner[59,60]. Although no mutations have been reported in HP1 genes, their levels of expression are altered in several types of cancer[21]. Increased expression of HP1α, but not of HP1β or HP1γ, has been linked to proliferation and cancer progression and has been proposed to be a novel prognostic marker for breast cancer[59]. However, it has also been observed that HP1α downregulation may facilitate the invasion and metastasis of breast and colon cancer[61,62]; HP1α is also poorly expressed in some tumours such as papillary thyroid carcinomas[60]. In addition, HP1γ is abundantly expressed in a plethora of cancer types, including breast cancer, and HP1γ knockdown inhibits the proliferation of various cancer cell lines[63] and results in increased DDR signalling in oncogene-expressing cells and transformed cell lines[21]. Notably, HP1γ is downregulated during adipocyte differentiation, and its forced expression abrogates adipogenesis[63], suggesting that the upregulation of HP1γ in cancer, rather than impinging on proliferation, could also maintain cancer cells in an undifferentiated state. Finally, the role of HP1 proteins in resistance to DNA-damaging agents such as laser microirradiation and camptothecin[58] suggests that precancerous lesions would benefit from this radioprotective role. Overall, these diverse observations suggest that HP1 proteins are active participants in the regulation of DDR signalling and that they are altered on oncogene activation and in human cancer.

Histone modifications in the DDR

Many chromatin modifications involved in the modulation of the DDR have been mapped, making deciphering the DNA damage histone code an intriguing challenge. Although the role of DNA methylation in cancer and senescence has recently been discussed elsewhere[64],

we address the role of a subset of DDR histone modifications and their relevance to cellular senescence and tumorigenesis.
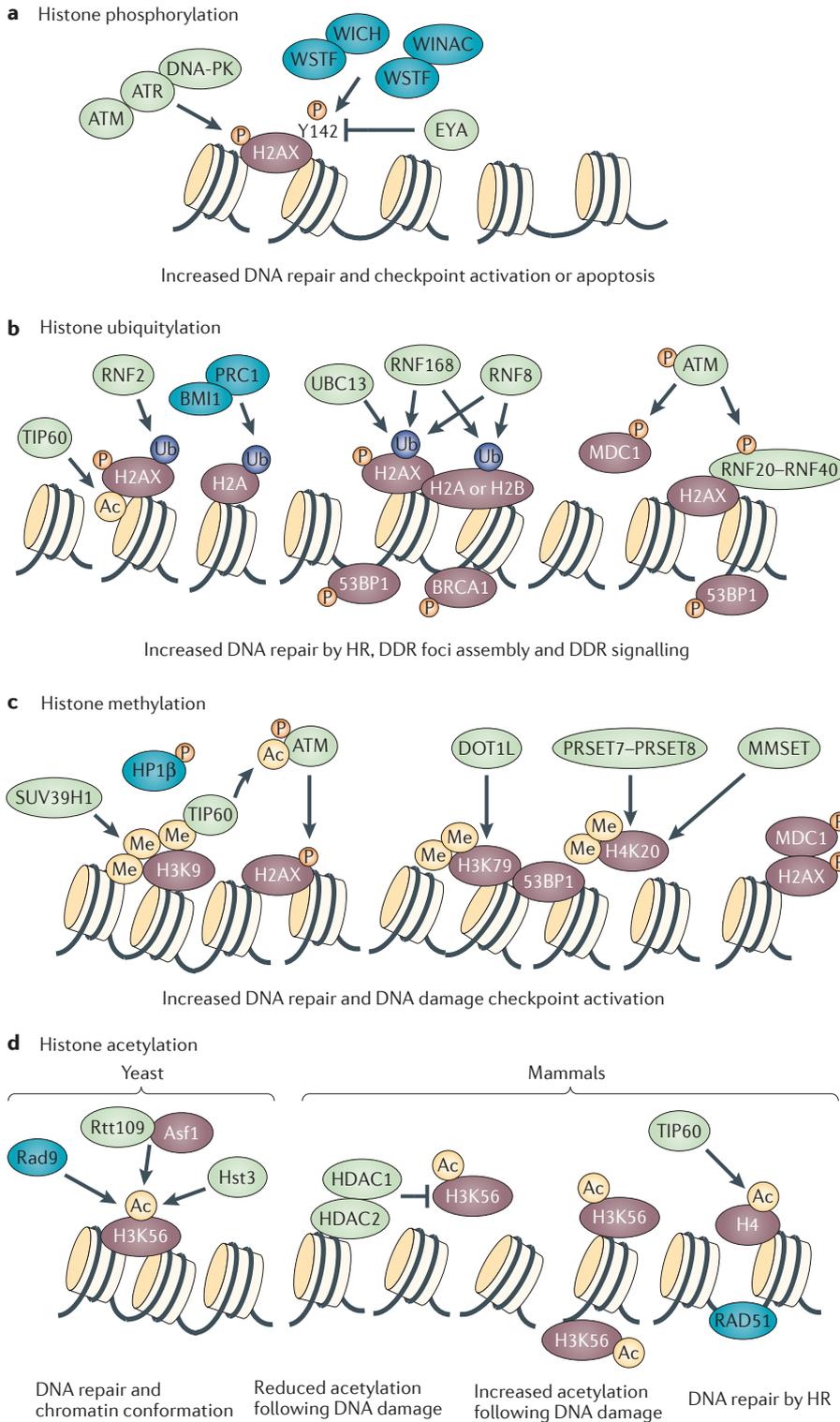
Phosphorylation. Phosphorylation is a prime example of the interplay between histone modifications and DDR signalling. H2AX phosphorylation at Ser139 is an early event that is mediated by ATM, ATR and DNA-PK[65,66] and a key step in DDR signalling (FIG. 3a). *H2ax* (also known as *H2afx*)-null mice are radiosensitive, and cells derived from these mice display DNA repair defects and chromosome instability[67]. Although H2AX is immediately phosphorylated at sites of DNA damage, this event is not strictly necessary for the initial recruitment of the DDR machinery but it is required for the amplification of the DDR signal and the formation and maintenance of DDR foci[68]. Given that mice lacking H2AX have chromosome instability and are tumour-prone in a *Trp53*-null background[67,69,70] the γH2AX status in cancer was analysed in several studies. Similar to other DDR markers, γH2AX is present in preneoplastic lesions, but in contrast to other DDR markers, it is not detectably lost during cancer progression[15,71]; this is probably due to redundant kinases that are responsible for its phosphorylation. The gene encoding H2AX is located in a region that is frequently mutated or deleted in several tumour types[72]. However, in tumours in which H2AX is not altered, the presence of γH2AX can be used as a diagnostic and prognostic marker and as a predictive biomarker of response to therapy[73,74] (reviewed in REF. 72).

More recently, phosphorylation of H2AX on Tyr142 was discovered to be involved in cell fate decisions (FIG. 3a). Williams syndrome transcription factor (WSTF), a component shared by two chromatin-remodelling complexes — WSTF–ISWI chromatin-remodelling complex (WICH; a SWI/SNF-related complex) and WSTF including the nucleosome assembly complex (WINAC) — was identified as the tyrosine kinase responsible for this phosphorylation. H2AX-Tyr142 is constitutively phosphorylated in the absence of DNA damage, whereas Tyr142 phosphorylation is lost on DNA damage, which occurs concomitantly with an increase in γH2AX levels. WSTF at the damage site facilitates the recruitment of ATM and mediator of DNA damage checkpoint 1 (MDC1)[75]. The tyrosine phosphatases eyes absent homologue 1 (EYA1) and EYA3 can dephosphorylate H2AX at phospho-Tyr142 (REF. 76). Interestingly, on DNA damage, ATM and ATR phosphorylate EYA3, and this event is crucial for the interaction of EYA1 and EYA3 with γH2AX (FIG. 3a).

The phosphorylation of this residue has been proposed to be crucial for mediating cell fate decisions between survival and apoptosis; although γH2AX is a signal for DNA repair and DDR signalling, on induction of substantial DNA damage Tyr142 phosphorylation is preserved, and the concomitant presence of γH2AX and H2AX phospho-Tyr142 leads to the destabilization of MDC1, MRE11 and RAD50 binding to γH2AX, and allows the binding of the pro-apoptotic protein JUN N-terminal kinase 1 (JNK1; also known as MAPK8) and the promotion of the apoptotic programme. Exploring the role of EYA and WSTF in cancer may be useful for the development of novel therapeutic tools.

Ubiquitylation. H2AX phosphorylation is part of a crosstalk that also involves histone ubiquitylation, suggesting that cooperation between different histone modifications is a functional strategy during DNA damage. γH2AX is ubiquitylated by ring finger protein 2 (RNF2), which causes the recruitment of BMI1 to sites of DNA lesions. BMI1 is a

Figure 3 | **Histone modifications involved in DDR signalling. a** | Following DNA damage, γH2AX favours DNA repair and checkpoint activation. The concomitant phosphorylation (P) of H2AX at Tyr142 coordinated by the Williams syndrome transcription factor (WSTF)–ISWI chromatin-remodelling complex (WICH; a SWI/SNF-related complex), and WSTF including the nucleosome assembly complex (WINAC) and opposed by the tyrosine phosphatases eyes absent homologue 1 (EYA1) and EYA3, destabilizes the DNA repair machinery at the site of DNA damage and promotes the induction of the apoptotic programme. **b** | Ubiquitylation (Ub) of γH2AX by ring finger protein 2 (RNF2) recruits BMI1 and the polycomb repressive complex 1 (PRC1) to sites of DNA damage. The BMI1–PRC1 complex ubiquitylates histone H2A, which controls DNA repair by homologous recombination (HR) and DNA damage response (DDR) signalling. Ubiquitylation of H2AX by ubiquitin carrier protein 13 (UBC13), RNF8 and RNF168 promotes the local accumulation of p53-binding protein 1 (53BP1) and BRCA1. In addition to γH2AX, RNF8 and RNF168 also target histones H2A and H2B and enhance localization of 53BP1 and BRCA1 at DNA double-strand breaks (DSBs). Additionally, phosphorylated mediator of DNA damage checkpoint 1 (MDC1) recruits RNF8 and RNF18 proteins, which are involved in the focal assembly of DDR signalling mediators. Furthermore, ATM phosphorylates the RNF20–RNF40 heterodimer that dictates the timing of γH2AX and 53BP1 focal assembly at DSBs. **c** | On DNA damage, 60 kDa Tat-interactive protein (TIP60) binds to SUV39H1-mediated H3K9me3, which allows ataxia-telangiectasia mutated (ATM) acetylation (Ac) and enforces cell cycle checkpoint activation. TIP60 competes with HP1 proteins for the binding to H3K9me3. H3K79 methylation (Me) by DOT1L histone H3 methyltransferase and H4K20 methylation by PRSET7–PRSET8 favours 53BP1 recruitment at sites of damage. MMSET, which is recruited in a γH2AX–MDC1-dependent manner, also methylates H4K20 and is required for DNA repair. **d** | In *Saccharomyces cerevisiae*, H3K56ac is regulated by Rad9 and is involved in DNA repair and chromatin conformation. The histone acetyltransferase Rtt109, together with the histone chaperone Asf1, and the NAD+-dependent histone deacetylase (HDAC) Hst3, are important regulators of H3K56ac levels. In mammals, the role of H3K56ac in regulating DDR is still controversial. The localization of HDAC1 and HDAC2 to DSBs reduces H3K56ac levels. However, increased H3K56ac levels were also found following DNA damage[107,109].

proto-oncogene that cooperates with MYC to induce lymphomas in mice[77], and it is a component of the polycomb group chromatin-remodelling complex, polycomb repressive complex 1 (PRC1), which is recruited to DNA damage foci and ubiquitylates Lys119 of histone H2A on DNA damage[78,79] (FIG. 3b; TABLE 1). BMI1 recruitment to damaged DNA ensures the proper localization of 53BP1, BRCA1 and RAP80; furthermore, it promotes DNA repair by HR and has been reported to block transcriptional elongation at the DNA damage site[80]; therefore, ubiquitylation can be used to reach different aims. ATM can thus suppress local transcription by inducing RNA polymerase II stalling and by positively regulating the levels of histone H2A Lys119 monoubiquitylation. The link between BMI1 and the DDR suggests novel therapeutic approaches. Indeed, in an *in vivo* orthotopic mouse model of chemoresistant ovarian cancer in which cisplatin treatment alone had no effect, the depletion of BMI1 alone or together with cisplatin treatment resulted in an increase in apoptosis and a reduction in proliferation, thus suggesting that targeting BMI1 may be an effective therapeutic strategy[81].

Ubiquitylation has a major role in the recruitment of other DDR factors that are involved in enhancing DDR activation. Indeed, ubiquitylation of H2AX on Lys119 and Lys120 by UBC13, RNF8 and RNF168 favours local accumulation of 53BP1 and BRCA1 (REFS 82,83) (FIG. 3b). On DNA damage, MDC1 binds γH2AX and is phosphorylated by ATM. The phosphorylated form of MDC1 recruits RNF8 and, consequently, RNF168 to amplify the activation of DNA damage signalling[84,85] (FIG. 3b). Furthermore, ATM phosphorylates the RNF20–RNF40 heterodimer, the recruitment of which at DSBs regulates the timing of 53BP1 and γH2AX foci formation[86] (FIG. 3b). This signalling cascade represents an interesting crosstalk between various histone-modification events through which different enzymes cooperate in order to fully sustain an appropriate DDR.

As a further example of the complexity of the DNA damage histone code, acetylation of Lys5 in H2AX by 60 kDa Tat-interactive protein (TIP60; also known as KAT5) — an acetyltransferase with tumour suppressor functions — is necessary for the ubiquitylation of H2AX[82], which is important for 53BP1 and BRCA1 accumulation (FIG. 3b; TABLE 1). Besides γH2AX, RNF8 and RNF168 also ubiquitylate histones H2A and H2B, events that enhance the localization of 53BP1 and BRCA1 at DSBs[83,84,87,88]. Thus, by regulating DDR signalling and DNA repair, and by

ensuring that active transcriptional machinery is excluded from the DNA damage site, ubiquitylation seems to be a key modification of the DDR pathway, and the possibility of targeting this modification for novel cancer therapy deserves to be explored.

Methylation. Heterochromatin is often proposed to have a role in suppressing DDR signalling. Unexpectedly, however, trimethylation of Lys9 in histone H3 (H3K9me3), an event that is generally, but not exclusively, associated with the formation of heterochromatin and gene silencing, has been proposed to be a key step in a set of events that activate ATM activity and that involve HP1β and TIP60 (which can acetylate both histones and non-histone proteins, including ATM)[89] (FIG. 3c). The observation that oncogene-induced DNA replication stress induces a global increase of H3K9me3 by mechanisms that are still unclear (see below) provides a further link between this modification and DDR activation.

Although H3K9me3 remains globally unchanged in exogenously damaged cells[53,89], the localization of TIP60 to DNA damage sites has been proposed to be dependent on this modification[53,89]. TIP60 can interact with the available H3K9me3 moiety and can activate ATM through acetylation[89]. However, at DNA damage lesions, TIP60 must compete with HP1 proteins for binding to H3K9me3 (REF. 89). Different mechanisms have been proposed to regulate HP1 binding to chromatin and competition with TIP60: phosphorylation of HP1β by CK2 (REFS 53,89) and phosphorylation of Ser10 in histone H3 by aurora kinase B[90,91]. TIP60 restrains MYC-driven lymphomagenesis by allowing full DDR activation[92]. H3K9me3 and HP1 levels increase in various cancer types[21], and H3K9me3 seems to be a predictor of poor prognosis in different types of cancer[93,94]. Although H3K9me3 may be necessary to trigger TIP60 activity, and thus ATM activation, and this could explain the retention of this chromatin modification, it is important to note that the activated form of ATM is excluded from SAHF, which are enriched for H3K9me3. Indeed, the inhibition or depletion of SUV39H1, a histone methyltransferase that is responsible for the generation of H3K9me3, enhances rather than suppresses ATM signalling in cancer cells[21]. Overall, these results suggest that H3K9me3 regulates the DDR and, although generally repressive, it can also specifically promote DDR activation through TIP60.

Similar to ubiquitylation, methylation is also involved in the recruitment of DDR factors. An essential docking site for 53BP1

binding to a DNA lesion, which is mediated by the tandem tudor domain in 53BP1, is thought to be dimethyl Lys79 of histone H3 (H3K79me2) — a modification that is uniquely mediated by the methyltransferase DOT1L[95] (FIG. 3c). Because the global levels of H3K79 are not modified during DNA damage, Huyen *et al.*[95] proposed that this residue becomes exposed when a DSB occurs owing to changes in chromatin conformation and that the resulting H3K79me2 can interact with 53BP1. Conversely, Botuyan *et al.*[96] proposed that dimethyl Lys20 of histone H4 (H4K20me2) is responsible for the recruitment of 53BP1 to DSBs given that 53BP1 binds more strongly to this modification than to H3K79me2. The suppression of DOT1L expression does not affect 53BP1 localization; whereas, depletion of the histone methyltransferase (HMT) PRSET7 (also known as SETD8), which is responsible for H4K20me1, does affect 53BP1 localization. Similarly, it has been shown that PRSET7 is recruited to DNA lesions through proliferating cell nuclear antigen (PCNA), that it is necessary for 53BP1 recruitment, and that 53BP1 and the yeast orthologue Crb2 can also bind to H4K20me1 (REFS 97,98).

H4K20 monomethylation, dimethylation and trimethylation were shown to increase in regions adjacent to DNA lesions[99], and the HMT that is responsible for the increase in dimethylation and trimethylation is MMSET (also known as NSD2), which is recruited in a γH2AX–MDC1-dependent manner[99] and is involved in 53BP1 localization. Thus, it can be speculated that coordination between PRSET7 and MMSET may be established to ensure proper localization of 53BP1.

An additional layer of regulation is represented by the crosstalk between monoubiquitylation of Lys91 in histone H4 and monomethylation and dimethylation of H4K20. Indeed B-lymphoma and BAL-associated protein (BBAP; also known as DTX3L), an E3 ubiquitin ligase that is responsible for monoubiquitylation of histone H4 at Lys91, is required for H4K20me1 and H4K20me2 — probably because BBAP regulates the chromatin association of PRSET7 (REF. 100). Therefore, different types of histone modifications (H4K91 ubiquitylation and H4K20 methylation) are required in a stepwise cascade to control 53BP1 recruitment.

Interestingly, BBAP is highly expressed in chemotherapy-resistant lymphomas[100] (TABLE 1). The observation that resistance to chemotherapy is dependent on the role of BBAP in recruiting 53BP1 suggests that the expression of BBAP may be relevant for the choice of the best therapeutic protocols[100].

The assessment of H4K20 methylation levels has also been proposed as a potential predictive biomarker in bladder cancer[101].

Acetylation. In addition to its role in transcriptional regulation, histone acetylation has been extensively studied in the context of DDR modulation. Histone acetylation functions both in DDR signalling and in DNA repair.

Acetylation of Lys56 in histone H3 (H3K56ac) was initially characterized in yeast, where it is regulated by Rad9 in *Saccharomyces cerevisiae*[102], and is proposed to favour nucleosome reassembly after DNA damage, thus facilitating full completion of DNA repair[102,103]. More recently, two reports[104,105] have identified the HAT Rtt109, together with the histone chaperone Asf1, and the Sir2-related HDAC Hst3, as important regulators of H3K56ac levels. Conflicting results have been reported regarding the regulation of this modification in higher eukaryotes[106,107]. In a screen for histone modifications following DNA damage, it was observed that H3K56ac levels decrease[106], and it was proposed that the reduction of H3K56ac is caused by the localization of histone deacetylases HDAC1 and HDAC2 to DNA breaks (FIG. 3d). This is consistent with the observation that HDAC1 and HDAC2 depletion or inhibition results in increased DDR signalling[21,108]. Conversely, two other reports showed that H3K56ac increases on DNA damage[107,109]. Despite this controversy, the role of H3K56ac in DDR regulation is worth further investigation given that the levels of this modification are reduced in senescent and differentiated cells and are increased in cancer cell lines and human tumours — possibly owing to high expression levels of ASF1A[108,109].

Other important acetyltransferases that are involved in the DDR are TIP60 and the cofactor TRRAP, which localize to regions surrounding DNA lesions[110]. TRRAP regulates local histone H4 acetylation that occurs on DNA damage, RAD51 foci formation and HR repair, probably by enhancing the accessibility to damaged chromatin[110]. Furthermore, TIP60 seems to be crucial for DNA repair and ionizing radiation-induced apoptosis[111] and can confer resistance to DNA-damaging therapeutic agents, such as cisplatin, probably through transcriptional activation of DNA repair genes[112].

DDR and chromatin in cellular senescence
One of the outcomes of activating the DDR is the induction of cellular senescence. We have recently proposed that the choice between transient DNA damage checkpoint-induced cell cycle arrest and the persistent cell cycle arrest that is typical of cellular senescence is the generation of irreparable telomeric DNA damage, which leads to persistent DDR activation and thus to a permanent cell cycle arrest[8,113]. Dramatic chromatin alterations occur during the establishment of senescence, and DDR factors have been proposed to both affect them and be affected by them.

Cellular senescence and SAHF. The establishment of cellular senescence can be associated with dramatic chromatin changes, the most striking of which is the formation of SAHF, which was first described by Lowe and collaborators[114]. SAHF are DNase-resistant, DAPI-dense, subnuclear cytological structures that are enriched for heterochromatin proteins (such as HP1) and histone modifications associated with transcriptional repression (such as H3K9me)[114,115] (BOX 1). Notably, each SAHF originates from the compaction of an individual chromosome[99]. The model proposed by Lowe and colleagues is that SAHF formation leads to the transcriptional repression of E2F target genes (which are usually involved in promoting cell cycle progression) and that the permanent cell cycle arrest that is typical of cellular senescence is enforced through the deposition of heterochromatin marks at the promoters of these genes[114]. The appearance and functions of SAHF require an efficient INK4A–RB pathway, as INK4A inactivation prevents SAHF formation and RB is recruited to genes that are associated with proliferation in order to repress them. Indeed, RNA polymerase II is excluded from SAHF. The observation that many genes remain active to preserve cell viability and that some are actually induced on senescence entry indicates that regions of active transcription must nevertheless persist in the cell.

SAHF formation is undoubtedly a key event in cellular senescence and fully deserves the attention of all those interested in understanding the mechanisms and consequences of senescence establishment. However, recent findings have challenged some of the early interpretations and suggest a more complex picture both in regard to the mechanisms of SAHF formation and in regard to their roles. First, it has now become clearer that cellular senescence can be robustly established in the absence of SAHF. Indeed, telomere shortening, ionizing radiation and prolonged exposure to hydroxyurea (HU) or DNA-damaging chemotherapeutic agents (such as etoposide) can trigger senescence without overt SAHF formation or a robust induction of heterochromatin markers that are typical of SAHF[21,114]. When, under similar conditions but using different cell lines, SAHF formation was observed, this was associated with INK4A induction[116]. Currently, the mechanism that links DNA damage generation and INK4A induction is still unclear. In tissue sections of normal respiratory epithelium no SAHF or heterochromatin induction was detected despite persistent radiotherapy-induced DDR signalling[21]. Conversely, when the same cells that do not have SAHF on treatment with DNA-damaging agents (ionizing radiation, HU or DNA-damaging chemotherapeutic agents) were challenged with an activated oncogene, SAHF were robustly detected, and DDR and INK4A induction occurred in parallel[21]. A conservative interpretation of these results is that cellular senescence can be established in the absence of SAHF formation. A more provocative intepretation is that SAHF do not have a causative role in the establishment of senescence.

Box 1 | **Events and key proteins involved in SAHF formation**

Seminal work mainly pioneered by P. Adams's laboratory[115,116,129] described the events that lead to senescence-associated heterochromatin foci (SAHF) formation. Two chromatin regulators and histone chaperones, HIRA and ASF1A, are required for initiating chromatin condensation[115]. HIRA phosphorylation following glycogen synthase kinase 3β (GSK3β) activation and its translocation to promyelocytic leukaemia (PML) bodies (which are nuclear foci that contain PML) precede SAHF formation[130]. HIRA functions in the deposition of the histone variant H3.3 into nucleosomes. The chromatin at SAHF is enriched with specific histone modifications such as histone H3 Lys9 dimethylation (H3K9me2) and trimethylation (H3K9me3), and consequent accumulation of HP1 proteins. When cells approach cellular senescence, the linker histone H1 is lost[131]; more recently, the entire histone pool has been reported to be reduced in senescent cells[132], although its relation to SAHF formation remains unclear. Concomitantly, high mobility group A (HMGA) proteins accumulate, potentially taking the place of histone H1 on chromatin. HMGA proteins have been proposed to be components of SAHF that are required for the maintenance of senescence[133]. As a late step and only after SAHF appearance by DAPI staining, the histone variant macroH2A, which is known to be resistant to chromatin remodelling, is incorporated into SAHF[115,134]. The contribution of macroH2A to cellular senescence might be specific for macroH2A1.1 and macroH2A2 isoforms, as the macroH2A1.2 variant was recently shown not to correlate with cell cycle arrest in premalignant lesions undergoing senescence[135].

SAHF and DDR. What is the mechanism of SAHF formation that seems to be preferentially associated with oncogene activation? As oncogene activation in the absence of proliferation does not lead to the formation of SAHF, it seems that they are formed in a DNA replication-dependent manner[21] (FIG. 4a). We also know that the induction of heterochromatic markers such as H3K9me3 and the formation of SAHF in OIS is dependent on ATR, as downregulation of ATR prevents the induction of heterochromatin[21]. Conversely, ATR activation in the absence of DNA damage has been shown to be sufficient to induce SAHF formation[117]. This suggests that robust ATR activation correlates with SAHF formation. However, HU — which specifically activates ATR-mediated DDR signalling — is not sufficient to induce SAHF formation. Although this would seem to rule out ATR as a key component in their formation, it is worth noting that although HU does not induce SAHF, it can nevertheless reproducibly induce an increase of heterochromatin marks, such as H3K9me3 (REF. 21). At first glance the picture looks complicated because ATR seems to control H3K9me3 levels[21], and this modification has been implicated in triggering ATM activation via TIP60 (REF. 89): this suggests that replicative stress activates ATR, which in turn leads to ATM activation. However, we also know that H3K9me3 seems to repress, rather than activate, ATM. This may be due to the sequestration of H3K9me3, the product of SUV39H1 by HP1 proteins (instead of TIP60), which thus impairs ATM activation. Therefore, the heterochromatinization that is triggered by oncogenes results in the attenuation of ATM signalling that can be obtained both by the sequestration of H3K9me3 and by limiting DDR signalling through the formation of heterochromatin.

The histone chaperone ASF1B was recently identified as a core component of nuclear and cytosolic complexes that mediate histone deposition during DNA replication[118]. Following treatment with HU, ASF1B temporarily binds the evicted histones from stalled replication forks and releases them once replication is restored[118]. Interestingly, after replication stress, ejected histone H3 that is bound by ASF1B–CAF1 (a factor that is involved in chromatin reorganization following DNA synthesis occurring during replication[119] and repair[120]) are enriched for K9me1 (REF. 118), a modification that is also predominantly found on newly synthesized histone H3, and as such may be a precursor for heterochromatin formation. Thus, it is possible that ASF1B is involved in seeding heterochromatin formation following replicative stress.
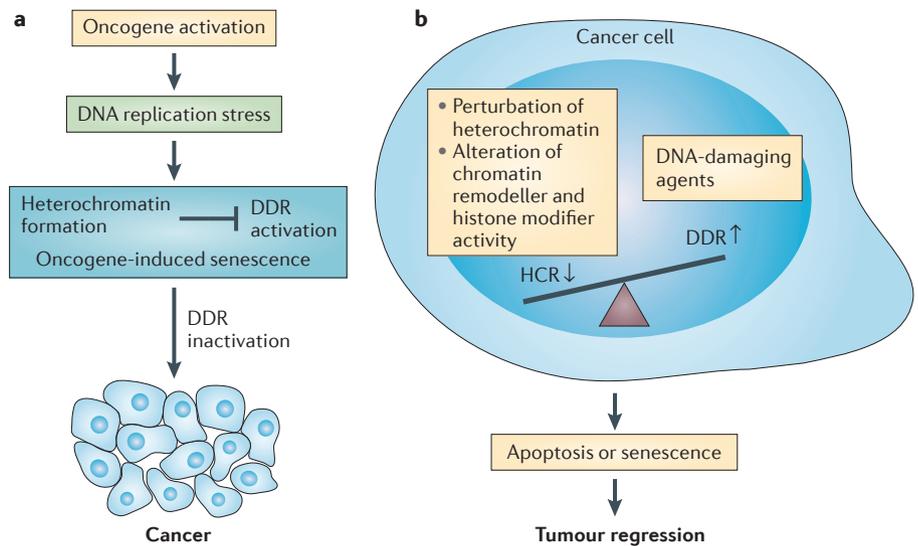
Figure 4 | **Proposed model of the connection between chromatin alterations, the DDR and OIS.** **a** | On oncogene activation, cells undergo an initial hyperproliferative phase that is associated with DNA replication stress that culminates with the generation of DNA damage signals that trigger the activation of DNA damage response (DDR) signalling and the establishment of senescence. This is associated with a widespread increase of heterochromatin (HCR) that limits DDR signalling. Inactivation of DDR functions by means of mutation of DDR genes allows cells to escape senescence and undergo full transformation. **b** | Interventions that aim to perturb or reduce the formation of heterochromatin or to impair its DDR-repressive functions may boost both endogenous (oncogene-induced) DDR signalling and its activation following therapeutic treatments based on DNA-damaging agents. Enhanced DDR signalling can result in apoptosis in competent cells and thus a more efficacious removal of cancer cells than DNA-damaging treatments alone.

Whether ATR regulates the balance between nuclear and cytosolic ASF1B complexes that are involved in histone recycling remains to be elucidated. It is worth noting that in *S. cerevisiae* the absence of Asf1 leads to the activation of the ATR homologue, Mec1 (REF. 121). Similarly, in human cells, the loss of CAF1 causes ATR activation and S phase arrest[122]. Therefore, these mechanisms seem to be evolutionary conserved, as suggested by the independently reported induction of H3K9me3 on depletion of deoxyribonucleotides (which induces replication fork stalling) in yeast[123]. This, together with the observation that SAHF formation is dependent on DNA replication and ATR, supports the idea that oncogene-induced DNA replication stress may directly affect the epigenome (FIG. 4a).

Furthermore, the overexpression of the histone chaperone HIRA, which is involved in deposition of variant histone H3.3 in nucleosomes and in SAHF formation (BOX 1), results in activation of ATR and ATM and S phase arrest[122]. Thus, multiple lines of evidence suggest that ATR, more so than ATM, may be a central component in a novel checkpoint that responds to replication stress and consequent chromatin alterations, although a more direct investigative approach is required to fully sustain this hypothesis.

SAHF and cancer. The DNA replication-licensing factor cell division control protein 6 (CDC6) provides another link between replicative stress and SAHF formation. Expression of oncogenic RAS (HRAS-G12V) is associated with the induction of CDC6 (REF. 5), which positively correlates with heterochromatin marks in human tumours[21]. The overexpression of CDC6 has been shown to be sufficient to induce DNA replication and cellular senescence *in vitro* and, interestingly, this is associated with the induction of H3K9me3 and SAHF formation[6]. Additionally, CDC6 expression positively correlates with heterochromatin marks in colon and lung tumours[21].

DNA lesions generated by the augmented DNA replication origin firing that is associated with oncogene activation and/or with CDC6 overexpression, together with consequent robust ATR activation, might be the seed of SAHF formation. This indicates that genomes which experience widespread replicative stress must be converted into a more compact chromatin structure, possibly to ensure genome integrity and cell viability. However, by doing so, the viability of oncogene-expressing cells may be preserved, thus fostering tumorigenesis. Therefore, if oncogenic stimuli lead to increased heterochromatin, and this

attenuates ATM signalling, the possible conclusion is that heterochromatin has an oncogenic role, rather than a tumour suppressive role (FIG. 4a).

Consistent with this, inactivating mutations of chromatin remodellers or of chromatin-binding proteins involved in SAHF formation are not currently known to be widespread in cancer. Furthermore, although inactivation of SUV39H1 was shown to bypass senescence and cause cancer progression in mouse haematopoietic cells, this does not seem to occur in human and mouse fibroblasts[21,124].

The induction of heterochromatin markers, such as H3K9me3 and HP1γ, was observed in benign human adenomas from colon and bladder tissues, and this upregulation paralleled the establishment of OIS[14,125]. However, the analysis of cancer specimens from lung, colon, and head and neck tissues revealed sustained expression of heterochromatin markers, organized in subnuclear structures that resembled SAHF, which indicates that the induction of heterochromatin is associated with early oncogenic events and is not limited to OIS. In agreement with this, data mining of published gene expression profiles revealed induction, not suppression, of many components of SAHF in various cancer types[21]; SUV39H1 and the histone chaperone ASF1B[59,126,127] are also aberrantly upregulated in several types of cancer.

Together, these observations indicate a lack of strong pressure for their loss, thus not supporting a tumour suppressive role for SAHF. Consistent with these *in vivo* observations, bypass of OIS *in vitro* by DDR inactivation allows the proliferation of cells with heterochromatin and structures that are morphologically reminiscent of SAHF[21]. Surprisingly, in the same *in vitro* experiment, the E2F target genes tested were free from heterochromatin marks at their promoters. This highlights how heterochromatin triggered by oncogene-induced DNA replication stress and the transcriptional repression of proliferative genes can be uncoupled in the same cells.

In keeping with the previously reported observations that heterochromatin represents a barrier to DDR signalling[22,23] it has recently been shown that SAHF formation restrains DDR activation in oncogene-expressing cells[21] (FIG. 4a). Interestingly, the perturbation of heterochromatin by depletion or inactivation of SAHF components or by using HDAC inhibitors increased DDR signalling, independently of the proliferative state of the cells[21] (BOX 2; FIG. 4b). Importantly, heightened DDR signalling caused cells to undergo

senescence or apoptosis. Furthermore, downregulation of a histone acetyl transferase complex, p300-CREB-binding protein (CBP), promotes heterochromatin formation, inhibits histone acetylation and ultimately induces cellular senescence[128]. Therefore, the study of both DDR factors and SAHF components may predict the response to cancer therapy based on DNA-damaging agents (FIG. 4b). Indeed, in cancer cells that exhibit increased levels of heterochromatin, treatments that aim to relax chromatin through the inhibition of HDACs or specific histone methyltransferases (such as SUV39H1) might lead to cell death by boosting ATM-dependent DDR signalling (BOX 2; FIG. 4b). By the same reasoning, the inhibition of DDR pathways might allow cancer cells to survive and escape cell death.

Conclusions

One of the most important points that has come to be appreciated in the past two decades of research on the DDR is the identification of chromatin components and chromatin modifications as new, active components in the regulation of the mechanisms that

preserve genome integrity. Similar mechanisms are engaged in the processes of cellular senescence induction and tumorigenesis.

Some important consequences can be drawn. The response to DNA damage and exogenous DNA-damaging agents is not uniform among cells or within a cell. During cancer initiation, oncogene activation induces a global heterochromatinization that could confer a different sensitivity to genotoxic insults compared with other cells present in the same tissues that do not express an oncogene. This different sensitivity may have an impact on therapeutic efforts that are based on DNA-damaging treatments. Conversely, this diversity could also be exploited and may open avenues to more specialized treatments that take advantage of the observed different chromatin arrangements of oncogene-expressing cells.

Finally, the identification of specific alterations in chromatin modulators in cancer cells offers the opportunity to develop targeted therapies that are based on the specific inhibition or alteration of the functions of chromatin-modifying enzymes or other non-enzymatic, but crucial, components.

Box 2 | **Old and new chromatin-oriented therapeutic approaches**

Histone deacetylases (HDACs) are promising targets for cancer therapy because HDAC inhibition is preferentially deleterious to cancer cells[136,137] and has been shown to remove cancer cells that acquire drug resistance through activation of DNA damage response (DDR) signalling and to impair the generation of drug-resistant cancer cells[138]. Intriguingly, the development of drug tolerance is linked to global chromatin alterations[138] and, similarly, the increase of DDR signalling observed on HDAC inhibitor treatment occurs specifically in cells that are affected by global heterochromatinization[21]. The effects of HDAC inhibitors on DDR signalling are observed in cancer or oncogene-expressing cells but normal cells are only mildly affected[21]. It is becoming increasingly evident that the combination of HDAC inhibitors with DNA damage-inducing agents is a powerful tool, and this is probably due to the enhancement of DDR signalling that is obtained by suppressing HDAC activity[139,140]. However, to improve the efficacy of their combined use in the clinical setting, analysis of both the chromatin and DDR status may be an important parameter to consider[21,138].

DOT1L and H3K79 methylation seem to be involved in tumorigenesis processes and are thus interesting therapeutic targets. DOT1L expression is downregulated in pleomorphic adenoma of the parotid gland[141]. Unfortunately, in other cancer types the scenario is more complex. In leukaemias that are driven by different mixed lineage leukaemia (MLL) translocations, DOT1L proteins can interact with MLL fusion proteins, and the loss of DOT1L reduces the survival of MLL fusion protein-immortalized cells[142]. It has recently been shown that H3K79me2 is abundantly present in MLL-fusion target genes[143,144] and that DOT1L positively controls an MLL-fusion dependent transcription profile, the oncogenic potential of cells harbouring MLL fusion protein and leukaemia development *in vivo*[144].

Phosphatidylinositol-binding clathrin assembly protein (CALM; also known as PICALM)–AF10 — another fusion protein that occurs in acute lymphoblastic leukaemia, acute myeloid leukemia (AML) and lymphoma — also interacts with DOT1L and thus has a role in leukaemogenesis through H3K79 hypermethylation and the upregulation of HOXA5 expression (REF. 145). By contrast, the global levels of H3K79 methylation are reduced in human and mouse leukaemic cells bearing the CALM–AF10 fusion protein through the impairment of DOT1L recruitment to chromatin. Strikingly, the reduction of H3K79 methylation by CALM–AF10 expression or DOT1L downregulation induces enhanced sensitivity to ionizing radiation and an increase in chromosome aberrations[145]. Indeed, a recently developed specific inhibitor of DOT1L was shown to be a powerful agent that selectively caused the death of cells with MLL translocations, thus indicating a bright future for the treatment of MLL-driven leukaemias[146].

Gabriele Sulli, Raffaella Di Micco and Fabrizio d'Adda di Fagagna are at IFOM Foundation - FIRC Institute of Molecular Oncology Foundation, Milan 20139, Italy.

Gabriele Sulli's present address is at The Salk Institute for Biological Studies, California 92037, USA.

Raffaella Di Micco's present address is at The New York University School of Medicine, Department of Pathology, New York 10016, USA.

Fabrizio d'Adda di Fagagna is at Istituto di Genetica Molecolare – Consiglio Nazionale delle Ricerche, Via Abbiategrasso 207, 27100 Pavia, Italy.

Correspondence to F.d'A.d.F.
e-mail: fabrizio.dadda@ifom.eu

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