Senescence is triggered by various cellular stresses that result in genomic lesions and DNA damage response activation. However, the role of chromatin and DNA replication in senescence induction remains elusive. Here we show that downregulation of p300 histone acetyltransferase activity induces senescence by a mechanism that is independent of the activation of p53, p21<sup>CIP1</sup> and p16<sup>INK4A</sup>. This inhibition leads to a global H3, H4 hypoacetylation, initiating senescence-associated heterochromatic foci formation during S phase, together with a global decrease in replication fork velocity, and alteration of DNA replication timing. This replicative stress occurs without DNA damage and checkpoint activation, but results in a robust G2/M cell cycle arrest, within only one cell cycle. These results provide new insights into the control of S-phase progression by p300, and identify an unexpected chromatin-dependent alternative mechanism for senescence induction, which could possibly be exploited to treat cancer by senescence induction without generating further DNA damage.
Cellular senescence is characterized by a stable cell cycle arrest that is triggered by various forms of stress stimuli including oncogene activation (oncogene-induced senescence, OIS) or telomere shortening (replicative senescence), and is considered as a barrier to tumourigenesis. This arrest depends on the activation of the cyclin-dependent kinase (CDK) inhibitors (p21<sup>CDP</sup> and p16<sup>NKAA</sup>), components of the tumour-suppressor pathways that are governed by the p53 and retinoblastoma (pRB) proteins, respectively. Activation of the DNA damage response (DDR), triggered by DNA single-strand and/or double-strand breaks, has been described as a common feature of telomere-initiated or OIS. Moreover, dramatic changes in chromatin structure seem to contribute to the irreversible nature of the senescent state, especially through the formation of senescence-associated heterochromatic foci (SAHF), which are characterized by hypoacetylation of histones, tri-methylation of histone H3 on lysine 9 (H3K9me3) and the presence of facultative heterochromatin proteins. Chromatin organization is partly regulated by the balance of activity between histone acetyltransferases (HATs) and histone deacetylases. The HAT p300, which is involved in replication timing, occurring in the absence of DNA damage and checkpoint activation. Finally, we show that the downregulation of p300 HAT activity leads to p53, p21<sup>CDP</sup> and p16<sup>NKAA</sup>-independent senescence. These results reveal an unexpected and new mechanism for senescence induction involving inhibition of p300 activity. Our findings suggest that chromatin-dependent alteration can robustly induce senescence without activation of the commonly associated senescence pathways.

**Results**

**Inhibition of p300 HAT activity induces senescence.** We used two short hairpin RNAs (shp300-1 and shp300-2) to stably inhibit p300 expression in the hTERT-immortalized human diploid fibroblast (HDF) cell line TIG3(et). In response to reduced p300 expression, we observed the upregulation of the CDK inhibitors p16<sup>NKAA</sup> and p21<sup>CDP</sup>, an increase in senescence-associated β-galactosidase (SA-β-Gal) activity and SAHF formation (Fig. 1a–c). These changes are hallmarks of cellular senescence. Assuming that cellular senescence can be triggered by different stresses, including oncogene activation or telomere shortening, we wondered whether inactivation of p300 activity also occurs in these types of cellular senescence and may...

![Figure 1](image-url)

Figure 1 | Inhibition of p300 HAT activity induces senescence. (a–c) Stable downregulation of p300 by expression of two distinct shRNAs #1, #2 in TIG3(et) cells. Cells were collected and stained 12 days after selection. (a) Immunoblot analysis with β-actin as a loading control and Q for quiescent cells. (b) Images of SA-β-gal-stained (upper panel) and Hoechst-stained (lower panel) cells to detect SAHF. Scale bars are equal to 5 μm. (c) Images of large field with H3K9me3 and Hoechst staining to detect SAHF. Scale bars are equal to 10 μm. (d–g) TIG3(et) and BJ(et) cells treated with different doses of curcumin or vehicle (O) as control. (d) Analysis by proliferation curve. Means of three experiments with standard deviation. (e) Immunoblot analysis with β-actin as a loading control for TIG3(et) cells. (f) SA-β-gal activity. Scale bars are equal to 40 μm. (g) Immunofluorescence analysis with specific antibodies for co-localization with SAHF for TIG3(et) cells. Scale bars are equal to 5 μm. (b,f) Percentage of SA-β-gal-positive cells is indicated in insert.
p300 HAT activity is required for senescence induction. Moreover, formation (Supplementary Fig. S2), confirming that inhibition of by the rescue of their proliferation rate and the decrease in SAHF vented curcumin-induced senescence in HDF cells, as observed of p300 HAT activity is involved in senescence induction, we used min induces premature senescence in HDF, thus recapitulating the cell cycle arrest associated with the upregulation of CDK inhibitors, p16INK4A, and p21CIP1, an increase in SA-Bgal activity and formation of SAHF, which co-localized with HP1γ, H3K9me3 and HMG1A, as previously described (Fig. 1e,f,g). We confirmed these results in another hTERT-immortalized HDF cell line, B(etc)(Fig. 1d and f), demonstrating that inhibition of p300 HAT activity by curcin induces premature senescence in HDF, thus recapitulating the effects of p300 downregulation. To further confirm that inhibition of p300 HAT activity is involved in senescence induction, we used CTPB, a specific activator of p300 activity. CTPB treatment prevented curcumin-induced senescence in HDF cells, as observed by the rescue of their proliferation rate and the decrease in SAHF formation (Supplementary Fig. S2), confirming that inhibition of p300 HAT activity is required for senescence induction. Moreover, the use of C646, another specific inhibitor of p300 HAT activity, confirmed that inhibition of p300 HAT activity induces SAHF formation and senescence-like growth arrest, when compared with the inactive form C37 (Supplementary Fig. S3). Together these results demonstrate that the specific loss of p300 HAT activity can induce cellular senescence.

SAHF-dependent G2/M arrest within one cell cycle. To further elucidate the mechanism of this senescence-associated growth inhibition, we analysed the cell cycle profile of HDF cells in the presence of curcin by FACS. Surprisingly, a 72-h curcin treatment gave rise to an accumulation of cells in G2/M phase, when compared with mock-treated cells (6.5% versus 26.6%, respectively) whereas no change in G1 phase proportion was observed (63.4% versus 65.4%, respectively) (Fig. 2a). This contrasts with the common cell cycle arrest of senescent fibroblasts that is usually observed in G1 phase. Assuming that the senescence phenotype generally accumulates progressively over multiple cell cycles, we tested the effect of p300 HAT inhibition on the cell cycle over time. We synchronized cells in G0/G1 by serum starvation and released them in the presence of shp300-1 (hereafter TIG3(etc)p300kd HDF cells). Interestingly, 72 h after the release, we also observed an accumulation of cells in G2/M phase, demonstrating that the inhibition of p300 triggers this cell cycle arrest (Fig. 2b). Similarly, synchronized cells, released in the presence of curcin, accumulate at the G2/M transition in a dose-dependent manner (Fig. 2c). Finally, to formally show that the cells enter senescence in one cell cycle, we pulse-labelled HDF cells with a nucleotide analogue EdU at the beginning of S phase (16 h after the serum release). Remarkably, two weeks after the EdU pulse labelling, curcumin-treated cells retained a staining pattern identical to that observed in the 1 h post-labelling control, whereas control-treated cells were no longer labelled. This indicates that the treated cells were stably arrested in their first cell cycle (Fig. 2d). Together, these results show that p300 HAT inhibition induces a G2/M block in HDF cells that leads to a senescence-like growth arrest within one cell cycle.

Because the G2/M senescence-like growth arrest occurs within one cell cycle, we also hypothesized that S-phase progression might also be required for senescence induction by p300 HAT inhibition. To test this possibility, we co-treated curcumin-treated cells with mimosine, an inhibitor of the G1/S transition, and found that ongoing replication was necessary for SAHF formation in this context (Fig. 2e).

Finally, we wondered whether p300 inhibition could promote a SAHF-dependent induction of senescence. We inhibited the expression of HMG1A, which is known to block SAHF formation and senescence induction, with a specific shRNA. We observed that SAHF formation was required for the G2/M senescence-like growth arrest induced by inhibition of p300 HAT activity (Fig. 2f-h), establishing a direct link between SAHF formation and cell cycle arrest. Alteration of S-phase progression by p300 HAT inhibition. Because SAHF formation is described as a multistep process involving acetylation changes of chromatin, we investigated the effect of p300 inhibition on histone acetylation. Interestingly, we observed an immediate and global hypoacetylation of histones H3 and H4 in curcumin-treated and p300kd HDF cells (Fig. 3a), which was also prevented by CTPB, the specific activator of p300 HAT activity (Fig. 3b). Moreover, the chromatin loading of the minichromosome maintenance protein 7 (MCM7), a member of the pre-replicative complex, and the sliding clamp protein PCNA, were impaired, revealing that histone hypoacetylation might interfere with the DNA replication process (Fig. 3a). This hypoacetylation was followed by a rapid accumulation of SAHF after release in the presence of curcin in more than 50% of cells 48 h after treatment. This indicates that SAHF initiates during the S phase preceding the G2/M block, as confirmed by the presence of SAHF during ongoing DNA synthesis (Fig. 3c).

As S phase is tightly regulated to ensure total genomic duplication and stability, we hypothesized that p300 HAT inhibition could trigger an alteration of S-phase progression by modifying chromatin status during ongoing S phase, leading to SAHF formation and G2/M arrest. To address this question, we first estimated the length of S phase in synchronized cells released in the presence of curcin, and found a twofold increase in S-phase length when compared with untreated cells (24 h versus 12 h, respectively) (Supplementary Fig. S4), suggesting a possible alteration of replication fork progression.

To gain further insight into the alteration of the replication process in p300 inhibited cells, we performed in vivo DNA labelling and molecular DNA combing analysis. Interestingly, we observed a marked slowdown of the median replication fork velocity in curcumin-treated synchronized cells (Fig. 3d), and we obtained the same results when we analysed the replication fork velocity in non-synchronized cells, showing that p300 HAT inhibition interferes with the progression of DNA replication forks. Whereas modifications of fork progression are generally associated with activation of dormant origins, we did not detect significant changes in the median inter-origin distances (Fig. 3e). Furthermore, we did not observe modifications in the distribution of the classified replication figures in curcumin-treated cells (Supplementary Fig. S5), suggesting that the increased S-phase length might not be due to replisomal stress, even though we measured a doubling of S-phase length. However, we calculated that this increase in S-phase length could not be explained only by the 30% decrease in replication fork velocity (1.3 kb min−1 versus 0.9 kb min−1, for untreated and 9 μM curcumin-treated cells, respectively), and might be partly because of an extended period of the time for replication origin cluster activation.

The entire genome is replicated in a programmed manner, with specific regions undergoing DNA synthesis at different times during S phase. The histone acetylation level is one of the marks that distinguish early- and late-replicating regions. For instance, in early S phase, histones H3 and H4 embedded in chromatin are generally
Figure 2 | Inhibition of p300 HAT induces a SAHF-dependent senescence G2/M cell cycle arrest within one cell cycle. (a) Cell cycle distribution of curcumin-treated or vehicle (0) as control non-synchronized TIG3(et) cell population analysed by FACS at different time points of treatment. (b) Synchronized TIG3(et) cells in GO by serum starvation during 5 days with medium containing 0.5% FBS, then released in 10% FBS medium in presence of shp300-1 or empty vector (–) as control and analysed by FACS 72 h after serum release for cell cycle distribution. (c) Synchronized TIG3(et) cells in GO by serum starvation, released in presence of 6 and 9 µM curcumin (Curc.) or vehicle (0) as control and, analysed by FACS at different time points after release, for cell cycle distribution. (d) Immunofluorescence of EdU pulse-labelled TIG3(et) cells for 1 hour, 16 hours after serum release in presence of curcumin or vehicle (0) as control and analysed at the indicated times. (e) TIG3(et) cells were synchronized by serum starvation, released in serum in presence or absence of 9 µM curcumin (Curc.) and in presence or absence of 0.5 mM mimosine (Mimo.). Cells were collected 72 h after release and assessed by FACS (upper panel) for cell cycle distribution and by immunofluorescence (lower panel) to detect SAHF. (f) Immunoblot analysis with β-actin as a loading control. (g) Immunofluorescence analysis with specific antibodies for co-localization with SAHF. (h) FACS analysis for cell cycle distribution. (b,c,e,h) Shown are the results of two independent experiments performed in duplicate. Error bars show the range. (d,e,g) Scale bars are equal to 5 µm.

hyperacetylated, whereas in late S phase they are hypoacetylated\[14,15\], regulating the time of replication origin firing\[15\]. We thus examined whether the observed hypoacetylation of histones induced by the curcumin-mediated inhibition of the p300 HAT activity, might be responsible for an alteration of the replication timing. To answer this question, we used our synchronized cell system and pulse-labelled the early S phase replicating DNA in the absence of curcumin and then, the early, middle or late S phase replicating DNA of the following cell cycle, in the presence of curcumin. We first confirmed the timing specificity of each S-phase population by FACS analysis (Fig. 3f). Then, we analysed whether the early S-phase-labelled DNA from the first cell cycle might be replicated in early, middle or late S phase in the second cell cycle after inhibition of p300 HAT by curcumin, using molecular DNA combing. Interestingly, we observed an important and similar overlap between the early-replicating DNA regions of the first S phase and each of the early-, middle- or late-replicating DNA of the second S phase (Fig. 3g). This indicates that regions usually defined as early replicating in normal conditions have an altered replication time following p300 inhibition. This result demonstrates that downregulation of p300 HAT activity leads to a global desynchronization of origin activation timing in S phase, as previously suggested by the progressive increase of SAHF formation preceding the G2/M block (Fig. 3c). Altogether, these results indicate that hypoacetylation of chromatin triggered by p300 inhibition initiates both SAHF formation and a desynchronization of the replication timing, leading to a doubling of S-phase length.

p300 HAT inhibition induces senescence without DNA damage. DNA damage, the primary inducer of replication fork stalling, activates the intra-S-phase checkpoint\[16\]. Activation of the DDR, that is triggered by DNA single-strand and/or double-strand breaks, is a common feature associated with senescence\[16\]. Key DDR-signalling components are the protein kinases Ataxia-telangiectasia-mutated (ATM) and Ataxia-telangiectasia and Rad3-related (ATR), which activate the downstream kinases CHK2 and CHK1, respectively, leading to a cell cycle arrest\[17\]. Furthermore, acetylation of histone
was also dependent on the activation of DDR. We used aphidicolin to investigate whether senescence induced by p300 HAT inhibition was due to the DDR response. Furthermore, we observed a specific decrease of Cdc25B expression in curcumin-treated cells in G2/M phase (Fig. 4e), confirming that curcumin-induced DNA damage and DDR activation in curcumin-treated and p300kd cells. Finally, we showed that p300 HAT inhibition does not prevent DNA damage and DDR activation in curcumin-treated and p300kd cells. These findings suggest that DDR activation in p300 HAT inhibition is due to the DDR response. Furthermore, this could explain the G2/M phase cell cycle arrest.

H3 on lysine 56 (H3K56) has recently been shown to be mediated by p300 HAT activity and to have a critical role in packaging DNA into chromatin for DNA replication and repair. We therefore investigated whether senescence induced by p300 HAT inhibition was due to the DDR response. Furthermore, we observed a specific decrease of Cdc25B expression in curcumin-treated cells in G2/M phase (Fig. 4e), confirming that curcumin-induced DNA damage and DDR activation in curcumin-treated and p300kd cells. These findings suggest that DDR activation in p300 HAT inhibition is due to the DDR response. Furthermore, this could explain the G2/M phase cell cycle arrest.

In conclusion, our results show that SAHF formation in the H3K56 acetylation chromatin mark, as expected for a mechanism unrelated to DNA damage induction and DDR activation (Fig. 4d). Moreover, the inhibition of checkpoint activation with caffeine (1 mM) did not prevent the accumulation of curcumin-treated cells in G2/M phase (Fig. 4e), confirming that curcumin-induced senescence is independent of ATM and ATR activation. Interestingly, it was recently described that the Cdc25B dual specificity phosphatase, which controls the G2/M phase checkpoint by activating CDK1-Cyclin B activity, was specifically degraded in cells treated with drugs causing non-genotoxic stress, in the absence of DDR activation, leading to a G2/M cell cycle arrest. As shown in Figure 4c, we observed a decrease of Cdc25B expression in curcumin-treated and p300kd cells. This result is consistent with the absence of DNA damage and DDR activation in curcumin-treated and p300kd cells. Furthermore, this could explain the G2/M phase cell cycle arrest. Finally, we showed that p300 HAT inhibition does not prevent DNA damage, when the DNA damaging agent aphidicolin is added, as observed by the presence of γH2AX foci in aphidicolin-curcumin-treated cells (Fig. 4f,g). Our results show that SAHF formation in the H3K56 acetylation chromatin mark, as expected for a mechanism unrelated to DNA damage induction and DDR activation (Fig. 4d). Moreover, the inhibition of checkpoint activation with caffeine (1 mM) did not prevent the accumulation of curcumin-treated cells in G2/M phase (Fig. 4e), confirming that curcumin-induced senescence is independent of ATM and ATR activation. Interestingly, it was recently described that the Cdc25B dual specificity phosphatase, which controls the G2/M phase checkpoint by activating CDK1-Cyclin B activity, was specifically degraded in cells treated with drugs causing non-genotoxic stress, in the absence of DDR activation, leading to a G2/M cell cycle arrest. As shown in Figure 4c, we observed a decrease of Cdc25B expression in curcumin-treated and p300kd cells. This result is consistent with the absence of DNA damage and DDR activation in curcumin-treated and p300kd cells. Furthermore, this could explain the G2/M phase cell cycle arrest. Finally, we showed that p300 HAT inhibition does not prevent DNA damage, when the DNA damaging agent aphidicolin is added, as observed by the presence of γH2AX foci in aphidicolin-curcumin-treated cells (Fig. 4f,g).
Senescence growth arrest usually depends on the activation of the p53/p21CIP1 and p16INK4A pathways, respectively, and is generally associated with a G1 senescence cell cycle arrest. However, the inhibition of p300 HAT activity led to G2/M senescence arrest within one cell cycle, which requires SAHF formation and S-phase progression, suggesting that increased expression of both proteins p21^{CIP1} and p16^{INK4A} might not be directly involved in this senescence induction. To investigate whether this senescence growth arrest might be independent of the activation of p53/p21^{CIP1} and p16^{INK4A} pathways, we treated TIG3(et) cells with stable knockdown of p16^{INK4A}, p21^{CIP1}, p53 or in combination with curcumin. After 8 days of curcumin treatment, we observed, as previously shown, an induction of p21^{CIP1} and p16^{INK4A} expression in the parental cells, but only the induction of p21^{CIP1} in p16kd cell lines and only the induction of p16^{INK4A} in p21^{CIP1} kd and p53kd cell lines (Fig. 5a). Strikingly, single or double knockdown of these genes did not prevent senescence induction by inhibition of p300 HAT activity, as observed by reduced proliferation, an increase in SA-β-Gal activity and SAHF formation (Figure 5a,b; Supplementary Fig. S6). Finally, TIG3(et) cells with simultaneously stable knockdown of p53, p16^{INK4A} and p21^{CIP1} produced a similar result. Furthermore, we still observed a G2/M cell cycle arrest in these different TIG3(et) knocked-down cell lines (Fig. 5c), indicating that this specific cell cycle arrest is not triggered by upregulation of these CDK inhibitors. Because the accumulation of p21^{CIP1} and p16^{INK4A} in
curcumin-treated cells does not seem to be required for SAHF formation and senescence arrest, we hypothesized that these proteins might be inactivated or mislocalized in cells where p300 activity is inhibited. We performed nuclear and cytoplasmic cell fractions and showed that p21<sup>CIP1</sup> and p16<sup>INK4A</sup> accumulate primarily in the cytoplasm of curcumin-treated cells (Fig. 5d), suggesting that the induction of these proteins does not regulate the cell cycle but might have other functions<sup>24,25</sup>. Although we cannot exclude that the induction of p21<sup>CIP1</sup> and p16<sup>INK4A</sup> might participate in an extra step for stabilizing the cell cycle arrest later in the senescence process, we can conclude that senescence induction by inhibition of p300 HAT activity is independent of the p53/p21<sup>CIP1</sup> and p16<sup>INK4A</sup> pathways. To further demonstrate the efficiency of this senescence-inducing mechanism, we used the osteosarcoma SAOS-2 cancer cell line, that is p53- and pRB-deficient<sup>26</sup>. We were also able to induce premature senescence and specific G2/M cell cycle arrest in SAOS-2 curcumin-treated cell line in the absence of p53 and pRB pathway activation (Fig. 5e–g). Altogether, these results show that inhibition of p300 HAT is able to induce a p53/p21<sup>CIP1</sup>- and p16<sup>INK4A</sup>-independent senescence-like growth arrest in normal and cancer cell lines.

**Discussion**

p300 HAT was originally identified as a binding partner of the adenovirus early region 1A (E1A)<sup>27</sup>. p300 catalyzes the acetylation of all four core histones, and has been reported to acetylate several other proteins and itself<sup>28</sup>. p300 has been implicated in many different cellular functions, and gene mutations have been detected in human tumours, with loss-of-function point mutations found in colorectal, breast, ovarian, lung, gastric and pancreatic carcinomas, leading to the hypothesis that p300 might be a tumour suppressor gene<sup>29–30</sup>. Senescence can be triggered through either the activation of oncogenes or the loss of tumour suppressor gene, such as PTEN, RB1 or NF1 (refs 31–33). Our results revealed that loss of p300 HAT activity or p300 expression had the same effect on cellular senescence induction.

The hypoacetylation of the histone H3/H4 and the increase of nucleosome density due to the histone chaperone complex HIRA/ASF1a-mediated nucleosome deposition are among the earliest step of SAHF formation<sup>7</sup>. Interestingly, the inhibition of p300 HAT activity by specific inhibitors or shRNA induces the immediate and global hypoacetylation of histones H3 and H4, followed by a subsequent S-Phase-initiated SAHF formation (Figs 1 and 3), suggesting that p300 HAT inactivation could be implicated in the first step of SAHF formation. This hypothesis is consistent with the decrease in SAHF, observed when HMGAA1 (one of the first proteins involved in SAHF formation), was downregulated in addition to p300 inhibition (Fig. 2f,g). This is further corroborated by the strong decrease in p300 expression in oncogene-induced replicative senescence
DNA replication is a tightly regulated process that was previously described to be altered in senescence. In particular, expressing an activated oncogene in normal human cells leads to a first hyper-proliferation phase. However, our results showed that p300 HAT inhibition-induced senescence is associated rather with a decrease in cell cycle progression. Moreover OIS is related to an over-replicative phase with an increased number of stress-induced active replicons leading to alterations in DNA replication fork progression and a subsequent robust DDR. Strikingly, although we showed that p300 HAT inhibition-induced senescence leads to a marked slowdown of the global replication fork velocity, we did not detect any significant changes in the mean number of active replicons related to replication stress (Fig. 3d,e; Supplementary Fig. S5). Furthermore, we did not observe any activation of DDR or induction of DNA damage (Fig. 4). The absence of DDR was also described in another type of senescence induction, Pten-loss-induced cellular senescence, but in this case, cellular senescence occurred in the absence of DNA replication12, which is in contrast to the senescence induction by p300 HAT inactivation, in which DNA replication is required for SAHF formation and cell cycle arrest (Fig. 2e). These results highlight the difference between previously described mechanisms for senescence induction and this new type of senescence regarding the presence of DNA damage and activation of supplementary replicons.

In this report, we described, for the first time, the link between senescence induction and the alteration of replication timing (Fig. 3g). A correlation between replication time, chromatin structure and transcriptional activity has long been observed, but recent genome-wide studies revealed that a large fraction of the genome is subjected to dynamic changes during development, allowing the establishment of cell-type specific replication timing profiles35–37. Replication timing is therefore thought to represent a mitotically stable cell-type specific feature of chromosomes. Many studies suggest that replication timing can affect chromosome condensation, sister chromatid cohesion, and genome stability48, promoting abnormal replication timing control as clinical marker in cancers.

The level of histone acetylation is a mark that distinguishes early- and late-replicating regions. For instance, in early S phase, histones H3 and H4 embedded in chromatin are generally hyperacetylated, whereas in late S phase they are hypoacetylated, regulating the time of replication origin firing14,15. Because the replication time is coordinate regulated at the level of large megabase-sized domains, a change in replication timing would rapidly transmit a change in chromatin state to entire chromosomal domains, that could be responsible for the formation of SAHF and then to the senescence-induced growth arrest. The immediate and global histone hypo-acetylation triggered by p300 HAT inactivation, promoting the formation of heterochromatin alters the replication timing marks, leading to a doubling of S-phase length. Although not related to DDR activation, these alterations changing chromosome condensation are likely to be sensed as a sudden onset of stress, such as is observed in osmotic shock or hypothermia46. A rapid response system, that does not require transcription or translation could then quickly trigger a block of the cell cycle in G2/M, involving Cdc25B46, Indeed, G1 cell cycle arrest is a common feature of cellular senescence and occurs progressively over multiple cell cycles1, related to accumulation of genomic alterations that threaten genome stability. Interestingly, the inhibition of p300 HAT activity leads to an atypical G2/M cell cycle arrest that occurs within a single cell cycle and requires SAHF formation during the preceding S-phase (Fig. 2). Furthermore, we observed a decrease in Cdc25B, a dual specificity phosphatase, (Fig. 4c) that controls the G2/M phase checkpoint by activating CDK1-Cyclin B activity and was shown to be a specific target of a nongenotoxic stress checkpoint23. Interestingly, Cdc25B is degraded by the ubiquitin-proteasome pathway and depends on the F-box protein β-TrCP. Thomas and colleagues established a stabilized mutant of Cdc25B, which can not interact with βTrCP, increasing the half-life of the protein49. It might be interesting to overexpress this stabilized mutant to see whether it could impair the G2/M cell cycle arrest induced by p300 inhibition. But, assuming that different types of chromatin, assembled at the replication fork at different times during S-phase, can also influence gene expression48,49, we cannot exclude that SAHF formation due to changes in chromatin status might also change gene expression, which may also participate to the establishment of the senescent phenotype.

Senescence growth arrest usually relies on the activation of the CDK inhibitors (p21CIP and p16INK4A), components of the tumour-suppressor pathways governed by the pRB proteins, respectively. Interestingly, although we observed increased expression of these proteins, we demonstrated that p300 HAT inhibition-induced senescence is independent of p53, p21CIP, and p16INK4A (Fig. 5). Moreover, we showed that p21CIP and p16INK4A accumulate primarily in the cytoplasm of p300 inhibited cells, suggesting that the induction of these proteins does not regulate the cell cycle but might have other cellular functions such as previously identified in apoptosis resistance or metastatic inhibition48,49. Furthermore, the lack of a requirement of p53, which is usually linked with DDR activation, reinforces our observation that p300 HAT inhibition-induced senescence occurs in the absence of DNA damage.

Senescence has been shown to inhibit tumour progression in vivo50, thus the induction of senescence may represent a new alternative strategy to eradicate cancer41. Unfortunately, senescence usually relies on the activation of p53 and pRB tumour suppressor pathways that are often mutated or lost in tumours44, reducing the number of cancer types treatable by senescence induction. Furthermore, senescence induction by drugs or oncogenes often triggers a DDR that might challenge the genomic integrity of cells. Interestingly, we were able to induce senescence in the osteosarcoma SAOS-2 cancer cell line, that is p53- and pRB-deficient, by treating them with a low dose of curcumin that inhibits p300, and does not trigger the DDR, potentially opening a new window for the treatment of tumours that are resistant to classical chemotherapy and that carry mutations in the p53 and/or pRB pathways (Fig. 5e–g; Supplementary Fig. S6). Curcumin, that is found in the diet, has already proven to be useful in chemoprevention for colorectal40 and pancreatic cancer46.

Overall, our results reveal a new and unexpected mechanism for senescence induction, by a chromatin-dependent alteration of S-phase progression, unrelated to genomic alterations. It may occur within only one cell cycle, independently of the activation of p53/ p21CIP and p16INK4A/pRB pathways, but depends on SAHF formation and progression through S phase, providing new insights into the control of S-phase progression by p300. Interestingly, this new senescence-induced state, initiated by chromatin deacetylation, is also recapitulated by curcumin, a drug commonly found in the diet. These results illustrate a possible epigenetic control of senescence in aging, and also reveal unanticipated targets and mechanisms controlling the chromatin state that might be specifically triggered as an alternative strategy to treat proliferation disorders without generating further DNA damage.

**Methods**

**Reagents**. pBABE-puro-RAS12 and pRetroSuper-p21CIP1 were a kind gift from Dr. D. Peeper (The Netherlands Cancer Institute, Amsterdam). pBABE-puro-miR30-HMG1 was a kind gift from Dr. M. Narita (Cancer Research UK, Cambridge).

pLKO-3p00 with the following shRNA sequences No 1 CCTCAGCTTATGGAA GAGTT sequences, No. 2 GCCCTCAACATCCGAGACAT are from Openbiosyst em. pMKO-1-puro-p53 (plasmid 10672) was purchased from Addgene. Anti-p21 (C-19), anti-p16 (C-20), anti p53 (DO1), anti-miinchromosome maintenance protein 7 (H-300), anti-PCNA (FL-26), anti-Cdc25B (C-20) and anti-CHIK1 (G-4) were purchased from Santa-Cruz, Anti- histone H3K9me3 (Ab8898), anti-H3K56ac (ab76307), anti-H4 (ab52178) and anti-H3 (Ab1791)
from Abcam; anti-HPYt (2-MOD-166 as) were purchased from Euromedex; and anti-β-actin (H9397) and anti-H4ac (60-806) from Millipore; anti-p300 (05-257) were purchased from Upstate. Anti-Ras (610002) was purchased from BD laboratories and anti-CHK2 (2628), Phospho-CHK2 (thr68) (2661), Phospho-p53 (ser15) (2141) and cathepsin D (C9016) were purchased from Cell Signaling. Finally, anti-γH2AX (07-219) and anti-tubulin (T6199) were purchased from Sigma. HMGAG1 antibody was kindly provided by Dr. M. Narita (Cancer Research UK, Cambridge). All the antibodies were used at 1/1,000 dilution except anti-H3 and anti-β-actin that were used at 1/10,000 dilution, Curcumin (81025.1) was purchased from Cayman Chemical; aphidicolin (A0718), mimouse (M2053) and cathepsin D (C9016) were purchased from Sigma; GTPase (ALX-420-593-M005) from Cogex, C646 and C37 were a kind gift from Dr. P Cole (Johns Hopkins University School of Medicine, Baltimore).

Cell culture. TIG3 and BJ human diploid fibroblasts expressing the ecotropic receptor and hTERT TIG3(et), B(et), TIG3(et)p16kd, TIG3(et)p53kd and Phoenix cells were a kind gift from Dr. P. Cole (Johns Hopkins University School of Medicine). The osteosarcoma SAOS-2 cell line was a kind gift from Dr. O. Delattre (University of Amsterdam). For the whole cell extract, cells were synchronized in G0 phase by serum starvation during 5 days with medium containing 0.5% FBS, then cells were re-set up on glass slides. Then, arrested cells were released in 10% FBS in presence of 0.5% curcumin and pulse labelled for 3 h with CiDu in early, middle or late S phase, according to the results of S-phase length obtained in Supplementary Figure S4. Cells were collected and treated for DNA combing. For specific measurement of replication fork velocity and inter-origin distances, cells were successively labelled for 15 min with 20 µM IdU and for 30 min with 100 µM CiDu and collected for DNA combing.

DNA combing. For replication timing studies, TIG3(et) cells were synchronized in G0 phase by serum starvation for 5 days with medium containing 0.5% FBS, then re-set up at 10^6 cells per 10-cm dish. The day after, cells were released in 10% FBS medium and pulse labelled for 3h with 25µM IdU followed by a 3h chase with thymidine to label DNA at the early S phase. Cells were resynchronized by serum starvation for 5 days and re-set up on glass slides. Then, arrested cells were released in 10% FBS in presence of 0.5% curcumin and pulse labelled for 3 h with CiDu in early, middle, or late S phase, according to the results of S-phase length obtained in Supplementary Figure S4. Cells were collected and treated for DNA combing.

For specific measurement of replication fork velocity and inter-origin distances, cells were successively labelled for 15 min with 20 µM IdU and for 30 min with 100 µM CiDu and collected for DNA combing. DNA combing was performed as followed. Briefly, cells were collected after the CiDu labelling and embedded in agarose plugs (about 1.5×10^6 cells/plug). DNA fibres were stretched on silanized coverslips. Combed DNA fibres were denatured for 30 min with 0.5N NaOH. IdU was detected with a mouse monoclonal antibody (BD4 Becton Dickinson; 1:20 dilution) and a secondary antibody coupled to Alexa 488 (A11006, Invitrogen; 1:50 dilution). CiDu was detected with a rat monoclonal antibody (BU1/75, AbCys; 1:20 dilution) and a secondary antibody coupled to Alexa 488 (A11006, Invitrogen; 1:50 dilution). DNA was detected with an anti-siDNA antibody (MAB3034, Eurodixona; 1:100 dilution) and an anti-mouse IgG2a coupled to Alexa 647 (A21411, Invitrogen; 1:50 dilution). Then immunodetection of DNA fibres were analysed on a Leica DM600B microscope equipped with a CoolSnap HQ CCD camera (Roper Scientifics). Data acquisition was performed with MetaMorph (Universal Imaging). Box-and-whiskers graphs were plotted with Prism v5.0 (GraphPad Software). For all graphs, whiskers correspond to 5–95 percentile and the line in the middle of the box marks the median (50th percentile). Data not included between the whiskers are plotted as outliers (dots). Statistical analysis was performed in Prism v5.0 (GraphPad) using the unpaired t test.

References.