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Genome folding and refolding in differentiation and cellular senescence

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Abstract

The spatial conformation of chromatin within the confines of eukaryotic cell nuclei is now acknowledged as a decisive epigenetic mechanism for the modulation of such cellular functions as gene expression regulation, DNA replication or DNA damage repair. Of course, these processes are tightly regulated during organismal development and markedly affected by cellular ageing. Thus, the question that arises is to what extent does folding or refolding of the genome in three-dimensional space underlie the progression of development or ageing? Herein, we discuss recent experimental and modelling evidence to address this question and revisit how these seemingly different processed might represent two sides of the same coin.

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Introduction

Over the last decade, the idea that the genome should be approached as a dynamic three-dimensional (3D) entity has started to pervade all aspects of molecular and cell biology of the nucleus. This is mostly due to the continuous development and application of genomics approaches for studying 3D genome architecture. This essentially began with the introduction of the genome-wide variant of chromosome conformation capture (3C) technology, Hi-C [1]. In Hi-C, chromatin in fixed nuclei is digested to produce free ends, which are then ligated together on the basis of their proximity. These ligation junctions are marked by the incorporation of a biotin-labelled nucleotide, which allows for the enrichment of ligated fragment pairs, and ultimately for the detection of pairwise chromatin interactions following paired-end sequencing. Nowadays, Hi-C can routinely yield sub-kbp resolution contact maps [2,3] and even reveal chromosomal interactions in single cells [4,5]. This technological advent of 3C was followed by orthogonal approaches that emerged to alleviate potential biases in Hi-C, for example, in the ligation (GAM [6] or SPRITE [7]) or fixation processes (iHi-C, TALE-iD [8,9] or DamC [10]), or that offer a single-molecule view of interaction hubs (MC-4C [11] or ChIA-Drop [12]). In parallel to these molecular approaches, super-resolution imaging of increasingly higher throughput, for example, multiplexed oligoFISH [13,14], ORCA [15] or 3D-ATAC-PALM [16], now provide additional and corroborating evidence on how chromosomes fold in 3D nuclear space.

As a result of work applying the aforementioned technologies, we have greatly advanced our initial understanding of genome folding. Thus, the original low-resolution Hi-C data revealed Mbp-sized genomic regions of similar transcriptional marking colocalising in 3D space, that is, giving rise to the mostly euchromatic A compartment, and the mostly heterochromatic B compartment [1]. With increasing resolution, we then identified such organisational features as topologically associated domains (TADs) (reviewed in the study by Beagan and Phillips-Cremins [17]). TADs are defined as self-associating chromosomal domains that exhibit strong interactions within their own domain compared with interactions with neighbouring ones. They appear generally conserved and instruct interactions between promoters and *cis*-regulatory elements in their sub-Mbp-sized insulated neighbourhoods [18]. Interestingly, disruption of TAD boundaries can explain gene misregulation in cancer, neurological and congenital disorders [19–21] with the underlying mechanism being the loss of TADs insulation and the consequent rearrangement of enhancer–promoter interactions. Boundaries between TADs are often marked by active promoters and/or CTCF-/cohesin-bound sites. CTCF (or CCCTC-binding factor) is a ubiquitously expressed protein with 11 conserved zinc-finger domains, and cohesin is a ring-shaped protein complex initially discovered to

mediate sister chromatid cohesion. The involvement of CTCF/cohesion complexes in transcriptional regulation, DNA replication and recombination is attributed to their key role in TAD insulation and, thus, in the designation of promoter-enhancer interactions [2,22,23•••]. At kbpresolution, the genome is populated by structural loops of an average size of ~250 kbp, the anchors of which are marked by convergent CTCF binding motifs [2]. These 'loop domains' further enhance or insulate regulatory interactions genome-wide and are eliminated upon removal of cohesin complex components [24–26]. This effect corroborates the key role of cohesion in loop formation as described in the prevalent 'loop extrusion' model. According to this model, structural chromatin loops form once cohesin loads onto DNA and starts extruding chromatin at a fast pace via its ATPase motor; loop elongation via this process stops once the cohesion complex meets a pair of convergently oriented and CTCF-bound motifs or once it becomes unloaded from chromatin by the protein WAPL [27,28]. Of course, in recent nucleosome-resolution interrogations of human chromosome folding, using a variant called Micro-C, interactions among promoters and enhancers arise that do not require CTCF/cohesin for their stabilisation [23••,24,29••].

Functionally, changes in both the Mbp and kbp scale of the 3D architecture of mammalian chromosomes have been linked to the modulation of cellular processes, including gene expression regulation, DNA replication, as well as induction and repair of DNA damage [18,30,31]. These processes are intricately regulated along the course of development or affected in the context of disease and cellular ageing. Here, we discuss recent evidence on how chromatin (re)folds during these transitions and how such refolding might underlie functional outcomes.

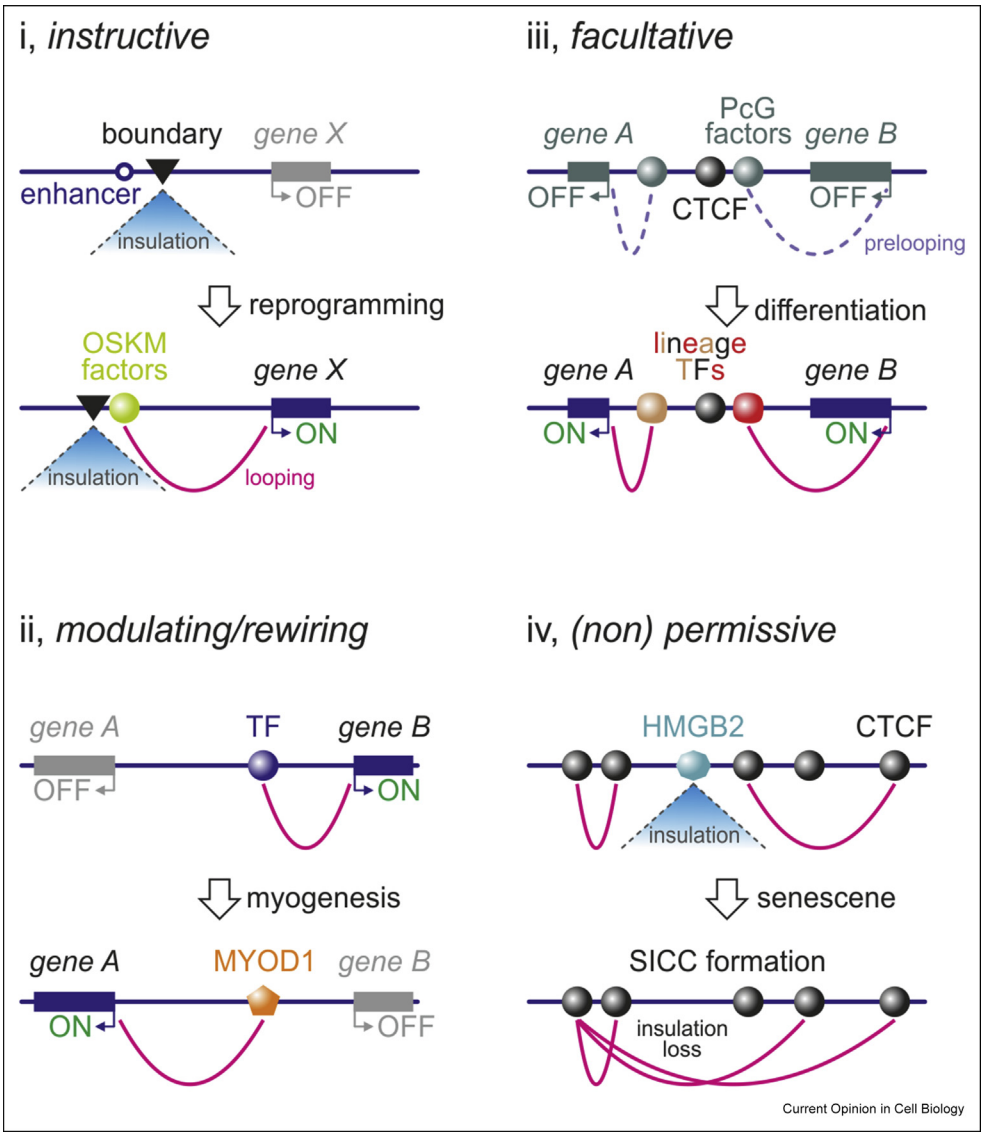
3D genome folding during cellular differentiation

Rapid, robust and coordinated changes in gene expression levels are essential in the process of differentiation. At the same time, transcriptional activation or repression have been shown to be interdependent to changes in the spatial organisation of chromatin across multiple scales. The data from recent studies that are discussed in the following passages, with a focus on mammals, enhance the amount of mechanistic detail on the interplay between genomic conformation changes and transcriptional regulation in differentiation. For example, TAD formation is crucial for proper gene regulation during X chromosome inactivation in mammals. In mouse ES cells, the X-inactivation center (Xic) comprises two consecutive TADs such that each of the essential noncoding loci, *Xist* and *Tsix*, are insulated from one another in *cis*. Using various CRISPR/Cas9-induced inversions, it was shown that this bipartite organisation is needed for *Xist* activation and proper spreading of inactivation in female X chromosomes [32]. Moreover, at the single-cell level, X-chromosome TADs are lost only in the inactivated allele as genes become silenced but are still detected in loci harbouring 'escapee' genes and generally arise in a non–parental-specific manner via a 'second wave' of genome folding [33••].

Similarly, Hi-C and super-resolution imaging concurred that exit of mouse Embryonic Stem Cells (ESCs) from naïve pluripotency coincides with a gain of 'strong' TAD boundaries and of multiple new loop domains that favour specific enhancer–promoter associations, while insulating others. Interestingly, upon neural stem cell reprogramming back to the naïve state, TAD topology also reverts back to its ground state [34]. Naïve stem cells display a correlation of H3K4me1-marked 'poised' enhancers (i.e. bivalent *cis*-regulatory elements functionally important for pluripotency and differentiation on which activating and repressive histone marks coexist) and TADs that exhibit most spatial remodelling during differentiation [35]. This often entails preloop-ing of such 'poised' enhancers to cognate gene promoters, which was shown to be mediated by proteins of the polycomb repressive complex 2 (PRC2). Intriguingly, loss of PRC2 and H3K27me demarcation does not lead to the derepression of these 'poised' loci but rather compromises their ability to robustly activate in later stages of differentiation [36••]. Such a 'facilitator' role taken up by H3K27me-mediated 3D interactions was also documented using single-cell Hi-C during early mouse preimplantation stages. Chromatin folding after fertilisation closely matches allele-specific enrichment of H3K27me3 at early-emerging parental-specific domains that impose gene repression and allow for parentally biased gene activation [33••]. Moreover, low input Hi-C applied during mouse oocyte development revealed that late-stage cells form multiple self-interacting, cohesin-independent H3K27me3-marked domains (PADs). PAD regions show strong inter-domain interactions and segregate from interPADs much like A compartments segregated from B compartments. PADs disappear upon meiotic resumption but are again briefly detectable on maternal alleles after fertilisation [37].

On the other hand, during spermatogenesis in both Rhesus monkeys and mice, extensive loss of TADs is observed in the meiotic prophase, despite spermatocytes being highly transcriptionally active at this stage [38,39]. This then implies that transcription occurs and is regulated independently of a need for TAD insulation. Despite no TADs detected, A and B compartments are well maintained in these pachytene spermatocytes. In Rhesus monkeys, they appear well refined, except for the X chromosome during meiotic sex chromosome inactivation. In mice, A compartments were also found

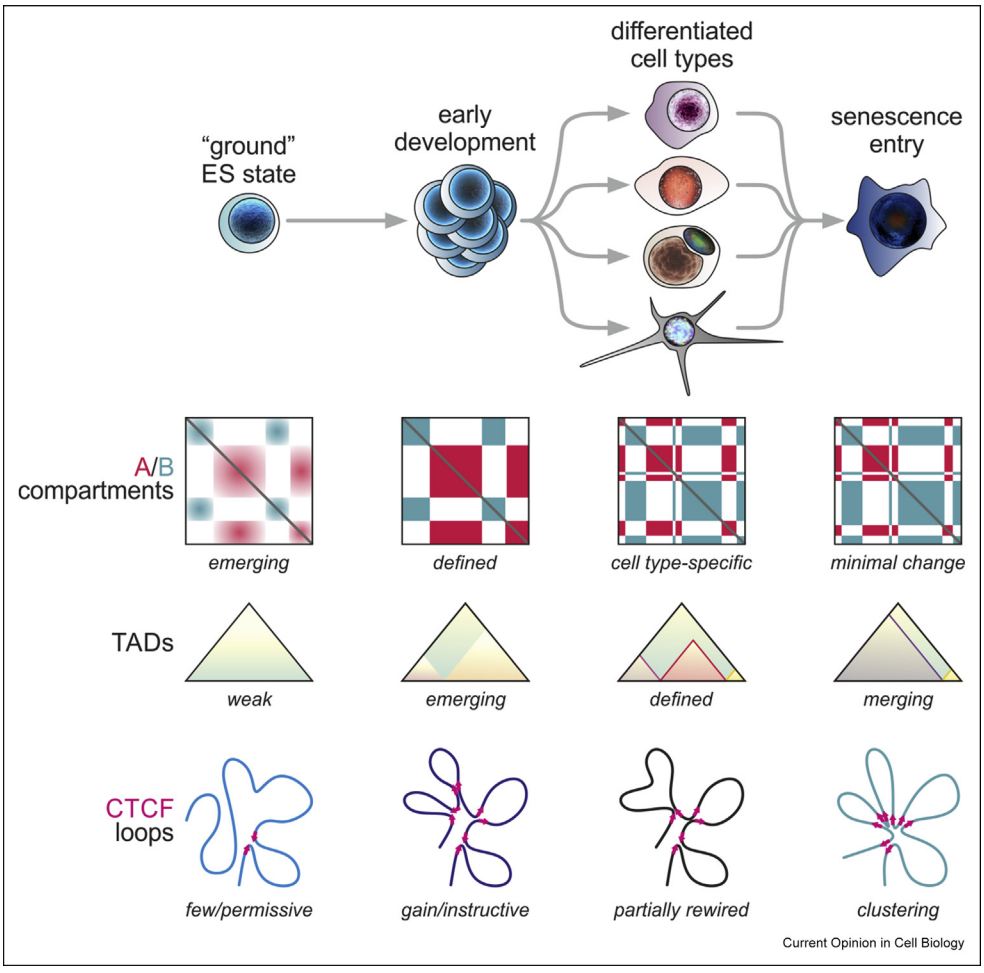
Figure 1



Some scenarios describing the interplay between transcription factor binding and chromatin reorganisation. Transcription factors (TFs) have evolved to exploit different modes of action to regulate gene expression. They can bind chromatin at an enhancer before gene activation and, only in a second step, induce looping and transcription (scenario *i*, 'instructive' TFs such as in the study by Stadhouders et al. [42]). They may modulate enhancer–promoter loops and rewire them to confer both gene repression and activation (scenario *ii*, 'modulating' TFs such as MYOD1 in the study by Dall'Agnese et al. [41]). They can facilitate later activating enhancer–promoter loops by ensuring 'prelooping' at an earlier developmental stage (scenario *ii*, 'facultative' polycomb-complex factors, PcGs, such as for 'poised' enhancers in the study by Cruz-Molina et al. [36]) or hinder long–range interactions by conferring local insulation (scenario *iv*, 'nonpermissive' TFs such as HMGB2 before senescence entry and to the formation of senescence-induced CTCF clusters, SICCS, in the study by Zirkel et al. [55]).

to be more prone to DNA breaks and recombination events, thus rendering 3D conformation of pachytene chromatin a guiding platform for homologous recombination [38,39]. Compared with events in early developmental states such as preimplantation or the naïve pluripotency exit, processes linked to adult stem cell lineage commitment, transdifferentiation or reprogramming show considerable reliance on transcription factor–driven rewiring of chromatin contacts, typically within the bounds of TADs. For example, the transcription factor BCL11B allows increased intra-TAD interactions upon differentiation of haematopoietic stem and progenitor cells into mature CD4+CD8+ T-cells. This increased connectivity coincides with abrupt changes at both the TAD and A/B compartment levels, while *Bcl11b* deletion abrogated cell fate commitment because of the inability to form the required spatial regulatory interactions [40]. Similarly, the

Figure 2



Overview of spatial chromatin organization changes occurring from early development to senescence. The transition of cells from the 'ground' embryonic stem cell state into early development and all the way to the eventual senescence entry of essentially all differentiated cells in an organism is characterised by coordinated changes in multi-Mbp A- (euchromatic; *red rectangles*) and B compartments (heterochromatic; *blue rectangles*), in sub-Mbp topologically associating domains (TADs) (*coloured triangles*), as well as in hundred-kbp-sized loops anchored at convergent CTCF-bound sites (*magenta arrows*).

transdifferentiation of fibroblasts into skeletal muscle cells cannot occur without the action of the transcription factor MYOD1. It does so by coordinating transcriptional repression of cell-of-origin genes and transcriptional activation of muscle lineage–specific genes. Dall'Agnese et al. [41•] find that MYOD1 rewires chromatin interactions before the changes in gene expression observed and argue that such transcription factor–driven rewiring organises a set of multiloop hubs that set the stage for acquisition of muscle identity.

Along these lines, Stadhouders et al. [42••] reprogrammed B cells into pluripotent stem cells *in vivo* and followed this transition at high temporal resolution. They found TFs driving few but crucial changes in chromatin topology before subsequent changes in gene expression. More recently, Di Giammartino et al. [43] described how the transcription factor KLF4 is needed for rewiring both standalone and clusters of cell type–specific enhancers for mouse embryonic fibroblasts to be reprogrammed back to a 'ground' ESC state. Here, HiChIP and Hi-C data rather point to changes concomitant between topology and gene expression.

Finally, heterochromatic domains may also undergo remodelling during development. For example, H3K9me2-marked heterochromatin embedded at the nuclear lamina (i.e. at the inner part of the nuclear envelope, where fibrous lamin proteins form a complex with nuclear membrane components to create a largely transcriptionally-inert environment for any associated chromatin) of multipotent P19 cells undergoes remodelling during differentiation into the neural or the

cardiac lineage. This remodelling underlies fate commitment by repositioning gene away from the 'silencing environment' of the nuclear periphery and into A compartment domains. Although not all repositioned genes are activated, lineage-specific gene expression changes did occur, for example, *Mef2c* or *Myocd* for neurogenic and cardiogenic commitment, respectively [44].

In summary, multiple aspects of cellular differentiation rely on the spatiotemporal regulation conferred by TADs and/or A/B compartments. Changes at the boundaries of these domains can be either CTCF/cohesin or transcription factor—driven and mainly act to rewire enhancer—promoter interactions for both gene activation and repression. As timing and robustness are key in development, different classes of transcription factors and epigenetic regulators have evolved to exploit different modes of action (Figure 1), although we still lack in understanding these processes fully.

3D genome refolding in cellular senescence

The functional decline that organs and cells exhibit along the course of their lifespan is the major effect of ageing, and as such it is accompanied by increased risk for a number of diseases, including cancer. One of the hallmarks of ageing is cellular senescence: the irreversible growth arrest induced by a variety of stress factors, such as telomere shortening, DNA damage, chemotherapeutic drugs or oncogene activation [45,46]. Senescence entry has a cell type—specific character, but it is universally characterised by multiscale epigenetic changes that affect nucleosome positioning and chromatin accessibility, histone marks, DNA methylation (reviewed in the study by Rai and Adams [47]) but not nucleolar organisation [48].

An intriguing early observation was that oncogene-induced senescence (OIS) leads to acute reorganisation of heterochromatin into prominent senescence-associated heterochromatin foci (SAHF). SAHF form through the spatial repositioning of pre-existing repressively marked chromatin domains [49]. This implied the apparent spatial reorganisation of chromatin, and Hi-C in OIS fibroblasts later revealed pronounced loss of sequence- and lamin-dependent interactions in heterochromatin but negligible changes at the TAD level [50]. Thus, much like in the aforementioned lineage commitment [44], it is also H3K9me2/3-marked chromatin remodelling that facilitates a cellular transition observed here. In addition to heterochromatin, it was shown that members of the 'architectural' high mobility group A (HMGA) protein family are essential SAHF components [51]. A recent study combining high-resolution Hi-C with the dissection of the DNMT1-HMGA2 axis identified the SAHF-contributing domains in OIS as indeed heterochromatic and lamin-embedded. Interestingly, DNMT1 depletion deters SAHF formation, and at this point OIS genome organisation resembles more that of replicative senescence (RS) cells[52•]. Similarly, nuclear pore density was shown to control SAHF formation, and SAHF loss did not at all affect cell cycle arrest [53]. These changes in spatial genome reorganisation were confirmed by assumption-free *in silico* simulations that also included changes in heterochromatin observed in cells from patients with progeria[50,54]. This modelling approach suggests that phase transitions explain heterochromatin clustering and that, once established, senescent genome architecture would be 'metastable' even if interactions with the lamina were reinstated; such metastability would guarantee the permanent cell cycle arrest that defines the senescent stare[54•].

Cells of different developmental origin enter RS without forming SAHF, yet they still exhibit obvious DAPI-dense foci because of increased local levels of the heterochromatin-associated factor HP1α[55••]. Hi-C, applied to fibroblasts kept in 'deep' senescence for months revealed a general compaction of chromosomal arms together with decompaction of pericentromeric regions, which was later linked to the aberrant activation of LINE-1 repeats [56,57]. However, higher-resolution Hi-C interrogation of endothelial, lung fibroblast and mesenchymal stromal cells entering RS unveiled earlier reorganisation events. According to this, senescence entry is marked by a considerable fraction of TAD boundaries being remodelled and by the widespread and pronounced spatial clustering of CTCF-bound chromatin [55••]. The former can, at least in part, be attributed to the demarcation of hundreds of these TADs by the high mobility group proteins B1 and B2 (HMGB1/B2). HMGBs are strongly depleted from cell nuclei upon RS entry and the corresponding TAD boundaries are remodelled [55••,58]. At the same time, a combination of HiChIP and functional assays showed that the reororganisation of the CTCF-bound chromatin into senescence-induced CTCF clusters (see Figure 1) occurs despite no change in the binding repertoire of CTCF on linear chromatin and is directly due to the senescence-induced loss of HMGB2 from cell nuclei. Thus, *de novo* longer-range CTCF loops form across senescent chromsomes and gene expression changes close to these new loop anchors were seen [55]. HMGB1 and HMGB2 are the most abundant nonhistone proteins in mammalian nuclei and are known for their inherent ability to bend, kink or loop DNA [59]. Thus, HMG-family proteins seem to be central for senescence-induced refolding of chromatin.

In addition to HMG proteins, SMC complexes have now also been implicated in 3D genome organisation changes seen upon senescence. For example, the SMC2 condensin complex component is strongly suppressed in senescence, but residual SMC2 is enriched in A

compartments, mostly those arising via B-to-A transitions. In addition, SMC2 is needed for the activation of paracrine senescence genes [60]. Cohesin complex components, such as SMC1A and Rad21, are also downregulated upon senescence entry. By using both Hi-C and capture Hi-C in OIS cells, Olan et al. [61] find extensive promoter–enhancer rewiring that closely follows changes in cohesin chromatin occupancy. They also map senescence-specific cohesin peaks at the 3' ends of activated genes, including those involved in paracrine senescence. Thus, condensin and cohesin redistribution in cells that have irreversibly exited the cell cycle can dictate compartmental and looping transitions that facilitate senescent transcriptional programs.

Summary and outlook

Reprogramming of differentiated cells into pluripotent cells by enforced expression of the 'Yamanaka' factors (OCT4, SOX2, cMYC, and KLF4) offers an unprecedented window of opportunity in regenerative medicine efforts to counteract ageing-associated disease and increase healthspan. However, such reprogramming paths were proven to enhance senescence phenotypes in cells, including formation of SAHF-like structures [62]. In addition, senescence is selectively induced to prevent aberrant cell growth and thus counter malignancy and to shape tissues throughout development [46]. Thus, one could view differentiation and senescence as opposing yet coupled processes that impinge on common mechanisms and genome-organising complexes (Figure 2). In fact, lineage commitment appears to rely on the active rewiring of interactions, starting out with the presumed multiconnectivity of pluripotent genomes and gradually transforming into precise mixtures or reconnected or decommissioned enhancer–promoter interactions in differentiated cell types. Then, once senescence inevitably kicks in, a loss of longer-range connectivity is observed, followed by general chromosomal compaction, and even spatial CTCF clustering. It is therefore important to study those mechanisms that differentiation, senescence and escape into malignancy share and to obtain *in vivo* and single-cell understanding of how genomes are folded and refolded to accommodate their respective programs.

Conflicts of interest statement

Nothing declared.

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- •• of outstanding interest

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