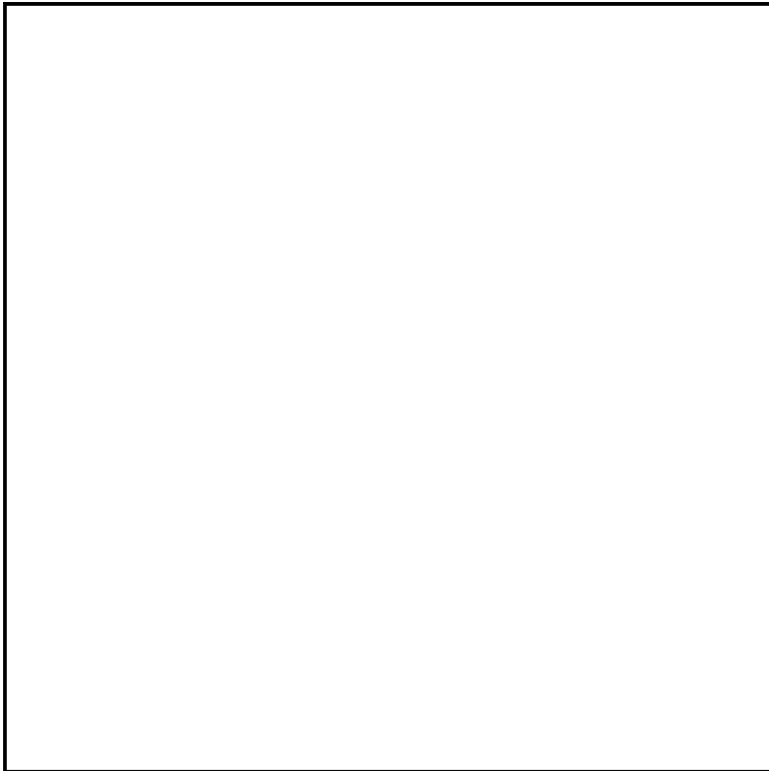Article

G R N L S C

Graphical Abstract

Authors

Tamir Chandra, Philip Andrews Ewels, ..., Peter Fraser, Wolf Reik

Correspondence

amir.chandra@babraham.ac.uk

In Brief

Chandra, Ewels, et al. map changes in genome organization in cellular senescence using Hi-C. Contrary to the believed increase in heterochromatin in senescence-associated heterochromatic foci formation, they describe a loss of local interactions in heterochromatic regions. This is in agreement with changes observed in progeria cells.

Highlights

- SAHF cells show sequence- and LAD-dependent loss of heterochromatin (HC) structure

- Senescence HC behavior is mirrored in Hutchinson-Gilford progeria

- Senescence-specific spatial clustering of HC leads to a new model for SAHF formation

- Comparing ESCs, somatic, and senescent cells links senescence to differentiation

Cell

# Global Reorganization of the Nuclear Landscape in Senescent Cells

Tamir Chandra,[1,2,7,*]

from Hutchinson-Gilford progeria syndrome (HGPS) patients show a decrease in heterochromatin and are devoid of SAHF (Scaffidi and Misteli, 2006; Shumaker et al., 2006). Cellular models of HGPS and cellular senescence of fibroblasts have proven to be relevant models for organismal aging. It is therefore important to understand the seemingly contradictory roles of heterochromatin in cellular aging and SAHF formation.

We have recently shown that SAHF chromosomes show an inversion of euchromatin, facultative heterochromatin (fHC), and constitutive heterochromatin (cHC), with cHC moving to the center of chromosomal territories (see also Figure 4C; Chandra et al., 2012). This inversion is due to a physical reorientation of the chromatin rather than a redistribution of repressive histone marks, questioning a causal role for classical heterochromatic marks, H3K9me3 (cHC) and H3K27me3 (fHC), in the formation of heterochromatin in somatic cells. A key feature of the senescent nucleus and strong correlate with SAHF formation is the loss of lamin B1 (Sadaie et al., 2013; Shah et al., 2013). Other factors involved in SAHF formation, such as the cell-cycle regulator pRb, high mobility group proteins HMGA1/HMGA2, histone chaperones HIRA and ASF1a, canonical Wnt signaling, chromatin remodelling proteins p400 and BRG1, and linker histone H1, have been identified; however, knowledge of how the chromosome structure is changed is still lacking (Chan et al., 2005; Chicas et al., 2010; Funayama et al., 2006; Narita et al., 2003, 2006; Tu et al., 2013; Ye et al., 2007a, b; Zhang et al., 2005). More importantly, the function of SAHF is controversial. Whereas the role of SAHF was initially reported as being tumor and cell-cycle suppressive (Narita et al., 2003, 2006), recent work has suggested that SAHF may in fact be proproliferative (Di Micco et al., 2011).

OCI low

Local	High		Distal	Low
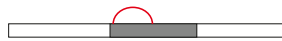
interactions in windows across the genome and normalized by subtracting the median chromosomal value from each window and then by smoothing values in a rolling 20 Mb window. The resulting OCI values give insight into the propensity of a region to form local or distal interactions. Our measurement bears some resemblance to the previously described interchromosomal contact propensity (ICP) (Kalhor et al., 2012), which itself has been found to correlate with active marks such as RNA polymerase II occupancy (Kalhor et al., 2012). When visualized over the TAD shown in Figures 1B and 1C, we find a rise in the OCI in senescence, suggesting OCI may be a suitable metric to measure a switch from local to distal interactions (Figure 1F). To explore OCI changes genome wide, we plotted OCI values in growing and senescence in a scatterplot (Figure 1G). A population of regions appears to follow the behavior of the TAD described above: an initially low OCI in growing cells with a rise in senescent cells, indicating a loss of local interactions. It has been previously suggested that high ICP values could be affected by proximity to the periphery of chromosomal territories (Kalhor et al., 2012). To rule out any such effect in our measurements, we calculated the OCI using only cis contacts, counting any interaction spanning more than 20 Mb as a distal contact (Figures S1E and S1F). We readily identified the same changing regions, confirming OCI as a suitable metric to measure changes in local architecture.

Based on the TAD shown in Figures 1B, 1C, and 1F, a low OCI in growing cells would suggest a compact structure with strong local interactions. To test this hypothesis, we correlated OCI with DNase accessibility in growing cells (Figure 1G). Corroborating our hypothesis, we found a striking overlap between the least-accessible regions in growing cells (dark blue) and regions showing the strongest rise in OCI. Next, we analyzed whether genomic regions with changing chromosomal interactions display a characteristic sequence-composition signature. We highlighted the GC content of each point within the ICP scatterplot and again identified a strong correlation, with those regions losing internal contacts strongly enriched for low GC content (Figure 1G).

Using OCI, we have identified regions of chromatin losing internal structure. These regions are the least accessible in the genome in growing cells and are rich in adenine-thymine (AT) content.

L-inter LADs showing no change in OCI. This suggests that the combination of being in an AT-rich L-isochore and a LAD predicts the dramatic OCI gain in senescence. For H2 and H3

Distance (μm)

growing/senescence-specific LMNB1 regions (Figure S3A). We find a strong enrichment for the newly forming senescence-specific LADs within TADs exhibiting greater insulation, suggesting a functional role for the senescence-specific gain of LMNB1. Our data suggest a role for the chromosomal redistribution of LMNB1 in the architectural changes we described.

To investigate whether the changes in LAD OCI are accompanied by changes in positioning in the nucleus, we used DNA-FISH microscopy to measure the distance of specific LADs to the nuclear periphery. We designed probes within two adjacent L1- and H2-LADs (Figure 3C); the L1-LAD showed a significant increase in the mean distance to the periphery (Figures 3D and

B
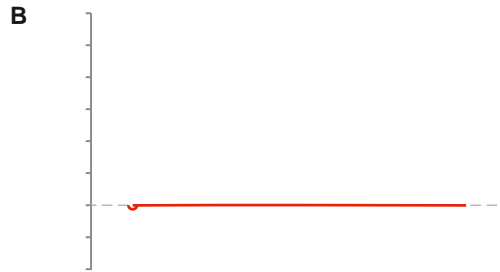
have distinct chromatin features; for example, SAHF formation is exclusive to OIS. Whereas OIS is accompanied by a major loss in LMNB1, premature aging in HPGS is due to the accumulation of progerin, a mutated version of LMNA/LMNC (Eriksson et al., 2003). As such, a commonality found within both models is the destabilization of the nuclear lamina. To investigate the breadth of our findings regarding local chromatin changes in senescence and to examine similarities between OIS and HGPS fibroblasts, we compared our data to recently published Hi-C data sets in HGPS fibroblasts (McCord et al., 2013).

Unsupervised hierarchical clustering of OCI in growing, senescent, and progeroid fibroblasts split the data into six classes,

which show strong correlation with GC content (Figure 6A). Classes 1 and 5 show similar trends for senescence and progeria and represent 58% of the genome (Figure S4). Class 1 is GC poor and highly enriched in L1-LADs (Figure 6B), suggesting a common loss of internal structure for these regions in both senescence and progeria. Cluster 5 shows the strongest enrichment of all clusters for regions gaining LMNB1 in senescence, suggesting that a common mechanism may lead to the compaction of these regions.

Whereas the decompaction of heterochromatin for HGPS cells is consistent with previous observations (Scaffidi and Misteli, 2006; Shumaker et al., 2006), the congruency in changing OCI

seems to contradict the fact that HGPS cells do not form SAHF, suggesting that changes in OCI are not solely responsible for the formation of SAHF. To understand these differences, we mapped the difference in distal interactions between growing cells and HGPS cells (Figure 6C). Interestingly, we do not observe a spatial clustering of constitutive heterochromatin in HGPS cells, suggesting that SAHF formation is a two-step process, with the initial L1-LAD decompaction shared between cellular senescence and HGPS.

The similar trends in OCI between senescence and progeria may suggest that the perturbation of the lamina has comparable consequences, independent of its cause, and that these consequences connect the nuclear changes seen in both cell types.

A role for changes in nuclear lamina interactions and RT dynamics has been described in differentiation (Hiratani et al., 2004; Peric-Hupkes et al., 2010). Although senescence and differentiation are both being studied thoroughly, there are few studies focusing on the crosstalk between the two. Senescence has been identified as a barrier to dedifferentiation with induced pluripotent stem cell (iPSC) reprogramming (Banito et al., 2009; Li et al., 2009), and a role for senescence in embryonic development has been highlighted in two recent studies (Muñoz-Espín et al., 2013; Storer et al., 2013). The emerging concept of epigenetic rejuvenation aims to reverse the aging process without dedifferentiating target cells and depends on our ability to discriminate these two processes (Manukyan and Singh, 2012; Rando and Chang, 2012). To position the higher-order chromatin structure dynamics described above within the wider context of differentiation, we compared our Hi-C data to an ESC Hi-C data set (Dixon et al., 2012). We plotted the changes in OCI for ESCs, growing, and senescent cells over LADs, grouped by LAD overlap and isochore (Figure 6D). Remarkably, we find LAD OCI behavior in senescence to be inversed with respect to the ESC configuration (Figure 6D). L1-LADs show a similar profile in both ESCs and growing cells with an inverted profile in senescence, again highlighting the unique behavior of the L1-LADs for the senescence phenotype. The growing OCI shows an intermediate behavior between ESCs and senescent cells, suggesting a potentially continuous process of higher-order -186.3(thel43.1(.9(std)-319

SAHF were originally described as a gene-silencing compart-

(Benson et al., 2010

REFERENCES

Lin, A.W., Barradas, M., Stone, J.C., van Aelst, L., Serrano, M., and Lowe, S.W. (1998). Premature senescence involving p53 and p16 is activated in response to constitutive MEK/MAPK mitogenic signaling. Genes Dev. 12, 3008–3019.

López-Otín, C., Blasco, M.A., Partridge, L., Serrano, M., and Kroemer, G. (2013). The hallmarks of aging. Cell 153, 1194–1217.

Macaya, G., Thiery, J.P., and Bernardi, G. (1976). An approach to the organi-zation of eukaryotic genomes at a macromolecular level. J. Mol. Biol. 108, 237–254.

Manukyan, M., and Singh, P.B. (2012). Epigenetic rejuvenation. Genes Cells 17, 337–343.

McCord, R.P., Nazario-Toole, A., Zhang, H., Chines, P.S., Zhan, Y., Erdos, M.R., Collins, F.S., Dekker, J., and Cao, K. (2013). Correlated alterations in genome organization, histone methylation, and DNA-lamin A/C interactions