Re-modelling of nuclear architecture in quiescent and senescent human fibroblasts

Re-modelling of nuclear architecture in quiescent and senescent human fibroblasts

J.M. Bridger*†, S. Boyle*, I.R. Kill‡ and W.A. Bickmore*

Spatial organisation of the genome within the nucleus can play a role in maintaining the expressed or silent state of some genes [1]. There are distinct addresses for specific chromosomes, which have different functional characteristics, within the nuclei of dividing populations of human cells [2]. Here, we demonstrate that this level of nuclear architecture is altered in cells that have become either quiescent or senescent. Upon cell cycle exit, a gene-poor human chromosome moves from a location at the nuclear periphery to a more internal site in the nucleus, and changes its associations with nuclear substructures. The chromosome moves back toward the edge of the nucleus at a distinctive time after re-entry into the cell cycle. There is a 2–4 hour period at the beginning of G1 when the spatial organisation of these human chromosomes is established. Lastly, these experiments provide evidence that temporal control of DNA replication can be independent of spatial chromosome organisation. We conclude that the sub-nuclear organisation of chromosomes in quiescent or senescent mammalian somatic cells is fundamentally different from that in proliferating cells and that the spatial organisation of the genome is plastic.

Addresses: *MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK. ‡Department of Biological Sciences, Brunel University, Uxbridge, Middlesex UB8 3PH, UK.

†Present address: Department of Biological Sciences, Brunel University, Uxbridge, Middlesex UB8 3PH, UK.

Correspondence: W.A. Bickmore
E-mail: Wendy.Bickmore@hgu.mrc.ac.uk

Results and discussion

We have previously shown that the gene-rich human chromosome 19 (HSA19) is located toward the centre of the nucleus. In contrast, the similarly sized, but gene-poor, human chromosome 18 (HSA18) is situated toward the nuclear periphery [2], a site equated with gene silencing in other eukaryotes [1]. This organisation was found in G1, S and G2 phase nuclei from proliferating cells [2].

Here, we investigate whether this level of nuclear compartmentalisation is altered in quiescent or senescent cells.

Human dermal fibroblasts (HDFs; passage 6–11) were made quiescent by serum starvation. Incubation with bromodeoxyuridine (BrdU) for 10 hours was used to detect any cells still able to enter S phase, and proliferating cells were also identified with an antibody recognising the pKi-67 antigen [3]. We first determined the preferred sub-nuclear location of chromosomes 18 and 19 in flattened two-dimensional (2D) preparations of proliferating and quiescent (G0) cells using a simple erosion analysis described previously [2]. As expected, in proliferating cells the distribution of hybridisation signals showed HSA18 and 19 partitioned toward the edge (shell 1) and the centre (shell 5) of the nucleus, respectively ($p < 0.01$; Figure 1a,d,g). In G0 cells (Figure 1b,e,h), however, chromosome 18 had moved away from the nuclear periphery (shell 1; $p < 0.01$), and there was no longer a significant difference between the proportion of HSA18 and 19 signals in the nuclear interior (shell 5; $p < 0.29$; Figure 1b,e,h).

During serial passage, HDFs eventually enter senescence. In passage 8 HDFs, HSA18 and 19 were positioned toward the nuclear periphery and the interior, respectively. After 38 passages, the cessation of further population doublings and the decreased fraction of pKi-67 positive cells (from 67.1% in passage 8 cells to 29.8% by passage 38) indicated that many of the cells had become senescent. Chromosome 18 had moved away from the nuclear periphery (shell 1) in senescent cells ($p < 0.006$), and there was no significant difference in the proportion of HSA18 and 19 signals at the nuclear periphery or in the nuclear interior ($p < 0.62$ for shell 1 and $p < 0.68$ for shell 5; Figure 1c,f,i).

Changes in chromosome position after cell cycle exit were also analysed by confocal laser scanning microscopy (CLSM) in three-dimensionally (3D) preserved nuclei [2]. In proliferating cells, HSA18 territories were significantly closer than HSA19 territories to the lateral and the top or bottom edges of the nucleus (Figure 2a,d). In G0 and senescent cells, HSA18 moved away from these edges of the nucleus (Figure 2b–d). Interestingly, chromosome 18 was not located close to the apical edge of the nucleus, even in proliferating cells. Surprisingly, 96% of signals from HSA19 territories in proliferating cells, and 91% or 88% in G0 or senescent cells, showed some coincidence with nucleolar antigens pKi-67 [4] or fibrillarin [5] (yellow signal in Figure 2e–g), even though this chromosome carries no rRNA genes. In contrast, only 66% of HSA18

Figure 1
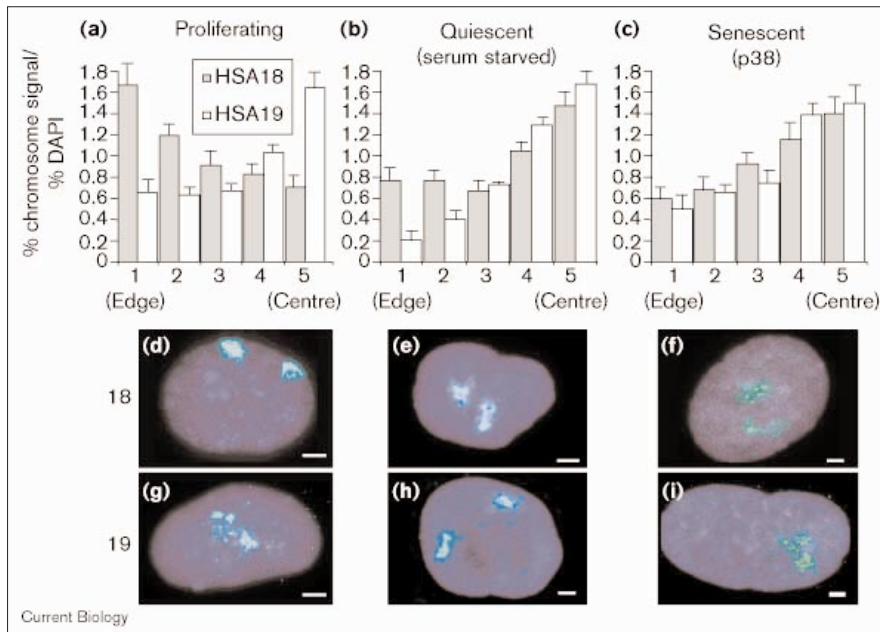
Chromosome position in proliferating, quiescent and senescent fibroblasts. **(a–c)** Mean percentage hybridisation signal, normalised to the percentage of the DAPI signal, of HSA18 (grey) and HSA19 (white) in each shell of nuclei from (a) proliferating 1HD fibroblasts, (b) G0 (quiescent or serum starved) 1HD fibroblasts, and (c) senescent (passage 38) 2DD fibroblasts. The data were analysed by erosion [2] of five concentric shells of 50 nuclei; shells were numbered 1–5 from the edge to the centre. Error bars show the SEM. **(d–i)** Representative images showing the position of HSA18 and 19 territories (green) in DAPI-stained (blue) nuclei. The scale bar represents 2 μm.

territories had any signal coincident with nucleolar antigens in proliferating cells, rising to 90% in G0, and 83% in senescent cells (Figure 2). Although blocks of heterochromatin on non-rDNA-carrying chromosomes have been shown to locate at the nucleolus [6,7], this is the first report of the arms of a non-rDNA-containing chromosome being consistently located at the nucleolus.

In proliferating cells, the territories of HSA18 and 19 differ in their compaction. Despite their similar size (in bp), chromosome 19 occupies a larger proportion of

nuclear area than does HSA18 [2]. This is also the case in quiescent and senescent cells. The more compact structure of HSA18 was seen in all cell states, even when it was no longer located at the nuclear periphery (Figure 2).

It is not clear whether other studies that report differences in nuclear positioning of loci between quiescent and proliferating mammalian cells represent movement of just individual loci or of entire chromosomes [8,9]. Here, we have shown that extensive re-organisation of the nucleus, at the level of whole chromosomes, accompanies the transition

Figure 2
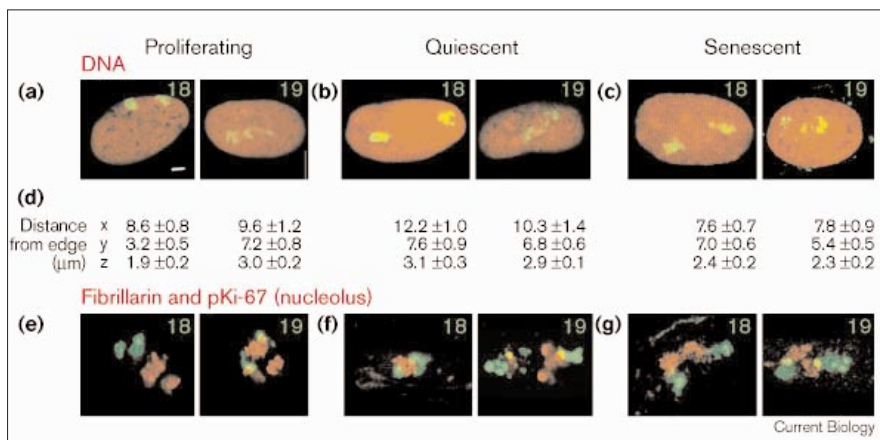
Three-dimensional analysis of nuclear positioning in proliferating, quiescent and senescent cells. Spatial positioning of HSA18 and 19 (marked with a chromosome paint detected with fluorescein-isothiocyanate, green) relative to **(a–c)** the nuclear periphery (as defined by propidium iodide staining, red), or **(e–g)** the nucleolus, delineated by either anti-pKi-67 or anti-fibrillarin staining (red), in 3D preparations of (a,e) proliferating, (b,f) quiescent and (c,g) senescent fibroblasts. Slides were sectioned at 1 μm intervals by CLSM, but only confocal midsections are shown here. **(d)** Measurements were made of the mean distance (in μm ± SEM) from the centre of chromosome territories to the closest edges of the nucleus in the x (apical), y (lateral) and z (top–bottom) planes ($n = 20$). The scale bar represents 2 μm.

from proliferative to G0 or senescent states. To investigate whether these changes in nuclear architecture are reversible, G0 fibroblasts were re-stimulated with serum. BrdU was added coincident with serum to follow subsequent cell cycle events. The sub-nuclear localisation of HSA18 and 19 was analysed in the original proliferating culture, the G0 cells (0 hours), and at 8, 24, 32 and 36 hour after re-stimulation. Erosion analysis of 2D preparations showed that HSA18 moved into the nuclear interior (shell 5) upon quiescence, and remained there at 8 hours ($p < 0.001$; Figure 3a). Only 1% of cells incorporated BrdU at this time point, indicating that the cells had not yet entered S phase. At 24 hours, 30% of cells were BrdU-positive and, after 32 hours the first mitoses appeared, but HSA18 was still located in an internal nuclear position relative to that in proliferating cells (Figure 3a). Similarly, in 3D analyses by CLSM, the extensive coincidence of hybridisation signals from HSA18 at the nuclear periphery, seen in proliferating cells, was absent from the G0 cells and from re-stimulated cells at the 8 and 24 hour time points (data not shown). The percentage of chromosome 18 territories that had any signal coincident with nucleolar antigens rose from 69% in proliferating cultures to 89–91% in G0 and 8 and 24 hour samples. At 32 hours, this figure dropped back down to 67%.

By 36 hours, a large fraction of the cells had exited mitosis and entered the next G1 phase. Erosion analysis now revealed movement of HSA18 away from the nuclear interior and back toward the nuclear periphery, to a location not significantly different from that seen in proliferating cells ($p < 0.936$ for shell 1 and $p < 0.626$ for shell 5).

Also, in 3D analyses, cells with chromosome 18 abutting the nuclear periphery began to reappear in the 32 and 36 hour samples. Re-positioning of HSA18 back toward nuclear periphery was not complete in all G1 cells at 36 hours. At this time point, cells that had only just exited mitosis (many small speckles of pKi-67 — type Ia staining) [6], still had similar proportions of HSA18 and 19 signals in the nuclear interior ($p < 0.193$). When more pKi-67 had reached the nucleolus (type Ib staining; early to mid G1 phase), HSA18 signals became depleted from the nuclear interior ($p < 0.02$) and enriched at the nuclear periphery ($p < 0.016$; Figure 3b,c). A delay in chromosome positioning immediately after mitosis was also seen in early (type Ia) G1 cells of a proliferating culture (data not shown). Re-positioning of labelled chromosome domains has also been reported in nuclei of early G1 CHOC 400 cells [10]. Hence, there is a time

Figure 3
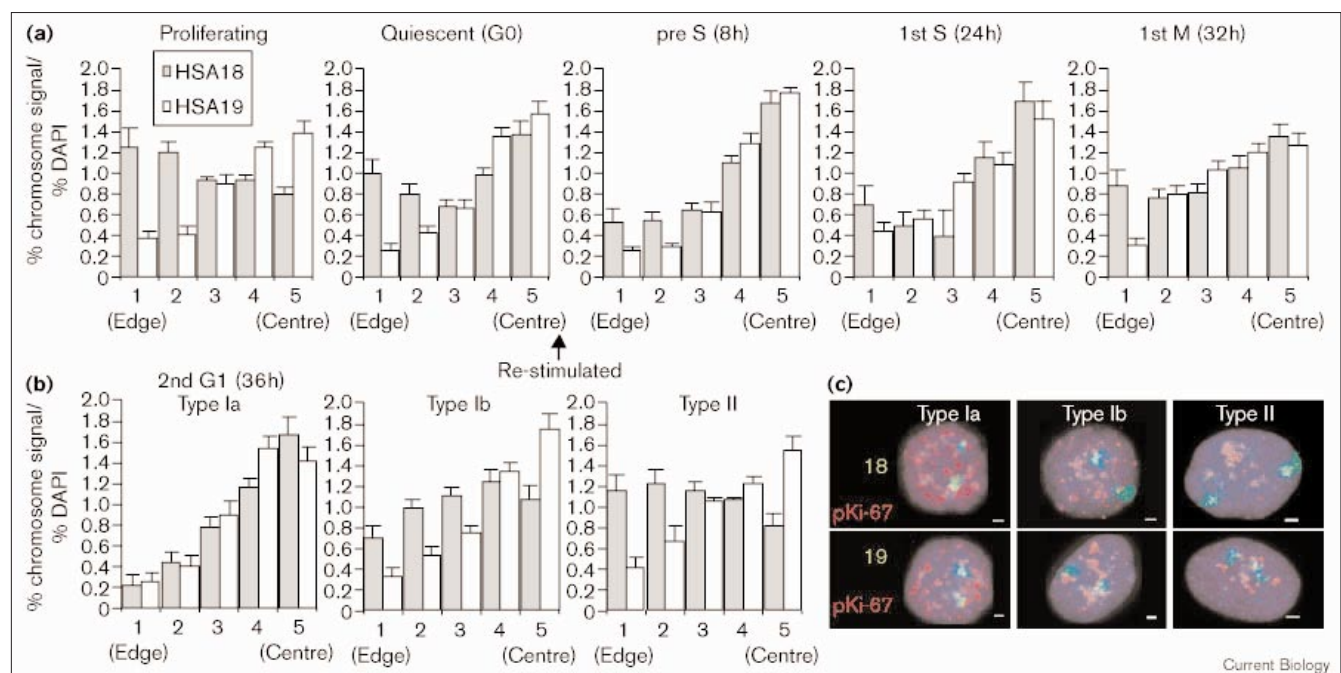
Dynamics of chromosome re-positioning after serum stimulation of G0 cells. Mean percentage hybridisation signals, normalised to DAPI staining, of HSA18 (grey) and 19 (white) in nuclei of **(a)** proliferating 1HD fibroblasts, 1HD fibroblasts in G0, and 1HD cells at 8, 24 and 32 h after serum stimulation and **(b)** 1HD fibroblasts in G1 36 h after re-stimulation. Cell cycle stage was judged by BrdU incorporation and the mitotic index, and only BrdU-positive cells were analysed at 24 and 32 h. The data were analysed by erosion [2] of five concentric shells of 50 nuclei; shells were numbered 1–5 from the edge to the centre. Error bars show the SEM. In (b) the stage of G1 (type Ia, early; type Ib, early/mid; or type II, mid/late) was assessed from the pKi-67 staining pattern [6]. **(c)** Representative images of HSA18 or 19 territories (green) in DAPI-stained (blue) nuclei of cells with a type Ia, Ib, or II distribution of pKi-67 (red). The scale bar represents 2 μm.

window of *2–4* hours at the beginning of G1 when this nuclear architecture is elaborated.

Re-positioning of HSA18 to the nuclear periphery after re-entry into the cell cycle requires passage through both an S phase and an M phase (Figure 3). Passage through an S phase might allow a replication-coupled chromatin assembly process to package chromosomes 18 and 19 into different chromatin states, leading to their differential localisation in the next cell cycle [11]. Nuclear envelope breakdown and re-assembly during mitosis, and the changes in A-type lamins that occur after exit of fibroblasts from quiescence (but that do not take place until after the first mitosis), might then provide the opportunity for the re-modelling of nuclear organisation [12].

Replication foci at mid to late S phase concentrate toward the nuclear periphery of HDFs [13]. If replication time is linked to spatial organisation of the nucleus, the relative periods during S phase when sequences from HSA18 and 19 are replicated might be altered in the first S phase after re-stimulation of G0 cells because the chromosomes are not in their usual locations at this stage (24 hours; Figure 3a) [10]. Using BrdU pulses at different times before harvesting of mitotic chromosomes, we assayed for the presence of early- and late-replicating DNA in HSA18 and 19 from proliferating fibroblasts and from fibroblasts at the first mitosis after exit from quiescence (data not shown). In both cases, BrdU that was incorporated during earlier stages of S phase labelled almost the entirety of HSA19 and lower levels of BrdU incorporation were seen into HSA18 (data not shown). Conversely, when BrdU was present during later stages of S phase, there was intense and extensive labelling of HSA18 and sparse labelling of HSA19 (data not shown). Replication times of other human chromosomes in the first S phase after re-stimulation from quiescence also seemed to be similar to that reported in proliferating lymphocytes (data not shown) [14]. We conclude that sequences from HSA18 are generally replicated later than sequences on HSA19, in the first S phase after serum re-stimulation. This suggests that temporal control of DNA replication can be independent of spatial positioning of chromosomes in the nucleus [10].

The recent cloning of mammals from somatic cells has led to the suggestion that the genome in quiescent nuclei is more amenable to reprogramming than in dividing cells [15]. The change in chromosome positioning in the nuclei of quiescent human somatic cells that we have reported here may be one reflection of this increased plasticity. In future studies of nuclear organisation it will be important to know whether one is analysing proliferating cells, G0/senescent cells, or cells in the early stages of G1.

Supplementary material
Supplementary material including additional methodological details is available at http://current-biology.com/supmat/supmatin.htm.

References
1. Bridger JM, Bickmore WA: **Putting the genome on the map.** *Trends Genet* 1998, **14:**403-409.
2. Croft JA, Bridger JM, Perry P, Boyle S, Teague P, Bickmore WA: **Differences in the localization and morphology of chromosomes in the human nucleus.** *J Cell Biol* 1999, **145:**1119-1131.
3. Gerdes J, Schwab U, Lemke H, Stein H: **Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation**. *Int J Cancer* 1983, **31:**13-20.
4. Verheijen R, Kuijpers HJ, Schlingemann RO, Boehmer AL, van Driel R, Brakenhoff GJ, *et al*.: **Ki-67 detects a nuclear matrix-associated proliferation-related antigen. I. Intracellular localization during interphase.** *J Cell Sci* 1989, **92:**123-130.
5. Aris JP, Blobel G: **Identification and characterization of a yeast nucleolar protein that is similar to a rat liver nucleolar protein**. *J Cell Biol* 1988, **107:**17-31.
6. Bridger JM, Kill IR, Lichter P: **Association of pKi-67 with satellite DNA of the human genome in early G$_1$ cells.** *Chrom Res* 1998, **5:**1-12.
7. Manuelidis L, Borden J: **Reproducible compartmentalization of individual chromosome domains in human CNS cells revealed by in situ hybridization and three-dimensional reconstruction.** *Chromosoma* 1988, **96:**397-410.
8. Brown KE, Baxter J, Graf D, Merkenschlager M, Fisher AG: **Dynamic repositioning of genes in the nucleus of lymphocytes preparing for cell division**. *Mol Cell* 1999, **3:**207-217.
9. Weimer R, Haaf T, Kruger J, Poot M, Schmid M: **Characterization of centromere arrangements and test for random distribution in G$_0$, G$_1$, S, G$_2$, G$_1$ and early S phase in human lymphocytes.** *Hum Genet* 1992, **88:**673-682.
10. Dimitrova DS, Gilbert DM: **The spatial position and replication timing of chromosomal domains are both established in early G1-phase.** *Mol Cell* 1999, **4:**983-993.
11. Shibahara K, Stillman B: **Replication-dependent marking of DNA by PCNA facilitates CAF-1-coupled inheritance of chromatin.** *Cell* 1999, **96:**575-585.
12. Dyer JA, Kill IR, Pugh G, Quinlan RA, Lane EB, Hutchison CJ: **Cell cycle changes in A-type lamin associations detected in human dermal fibroblasts using monoclonal antibodies.** *Chrom Res* 1997, **5:**383-394.
13. Kill IR, Bridger JM, Campbell KHS, Maldonado-Codina G, Hutchison CJ: **The timing of the formation and usage of replicase clusters in S-phase nuclei of human diploid fibroblasts**. *J Cell Sci* 1991, **100:**869-876.
14. Dutrillaux B, Couturier J, Richer CL, Viegas-Pequignot E: **Sequence of DNA replication in 277 R- and Q-bands of human chromosomes using a BrdU treatment**. *Chromosoma* 1976, **58:**51-61.
15. Wilmut I, Campbell KH: **Quiescence in nuclear transfer.** *Science* 1998, **281:**1611.
16. Bridger JM, Kill IR, O'Farrell M, Hutchison CJ: **Internal lamin structures within the G1 nuclei of human dermal fibroblasts**. *J Cell Sci* 1993, **104:**297-306.