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RESEARCH ARTICLE

Positioning of human chromosomes in murine cell hybrids according to synteny

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Abstract Chromosomes occupy non-random spatial positions in interphase nuclei. It remains unclear what orchestrates this high level of organisation. To determine how the nuclear environment influences the spatial positioning of chromosomes, we utilised a panel of stable mouse hybrid cell lines carrying a single, intact human chromosome. Eleven of 22 human chromosomes revealed an alternative location in hybrid nuclei compared to that of human fibroblasts, with the majority becoming more internally localised. Human chromosomes in mouse nuclei position according to neither their gene density nor size, but rather the position of human chromosomes in hybrid nuclei appears to mimic that of syntenic mouse chromosomes. These results suggest that chromosomes adopt the behaviour of their host species chromosomes and that the nuclear environment is an important determinant of the interphase positioning of chromosomes.

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Introduction

Chromosomes are arranged non-randomly in interphase cell nuclei (Cremer et al. 2006; Foster and Bridger 2005; Meaburn and Misteli 2007). When comparing the position of human chromosomes relative to the nuclear centre-periphery axis, there is a strong correlation with chromosomal gene density (Boyle et al. 2001; Bridger et al. 2000; Cremer et al. 2003; Cremer et al. 2001; Croft et al. 1999). In such correlations, gene dense chromosomes are positioned more internally than gene-poor chromosomes. In certain cell types, such as fibroblasts, correlation to chromosomal size has also been reported, with small chromosomes positioned at the nuclear interior and large chromosomes at the nuclear periphery (Bolzer et al. 2005; Cremer et al. 2001; Sun et al. 2000). Positioning according to size is associated with non-proliferating cells since the radial distribution patterns of human chromosomes differ between proliferating and non-proliferating nuclei in fibroblasts and epithelial cells (Bridger et al. 2000; Meaburn et al. 2007a; Meaburn and Misteli 2008; Mehta et al., 2007; Mehta and Bridger 2008, for submission).

In other animals, radial chromosome positioning correlates with both gene density and chromosome size (Mayer et al. 2005; Mora et al. 2006; Neusser et al. 2007; Tanabe et al. 2002b). Radial distribution patterns of chromosomes are similar, although not identical, between species suggesting evolutionary conservation of positioning. Indeed, homologues of human chromosomes in various primate species and chicken map to similar radial positions to that of their human counterparts (Mora et al. 2006; Neusser et al. 2007; Tanabe et al. 2002a; Tanabe et al. 2005; Tanabe et al. 2002b). Interestingly, the correlation between radial positioning and gene density is considerably weaker in mice compared to humans, possibly a reflection of a far-reduced variation in gene densities in the mouse genome (Mayer et al. 2005).

The precise mechanisms that spatially organise genomes in nuclei are unclear (Foster and Bridger 2005; Meaburn and Misteli 2007; Misteli 2007). Gene density and/or size of a chromosome cannot be single determinants of nuclear position, since chromosomes occupy distinct positions in different cell types (Mayer et al. 2005; Parada et al. 2004), after exit from the cell cycle (Bridger et al. 2000; Meaburn and Misteli 2008; Mehta et al., 2007), during differentiation (Foster et al. 2005; Kim et al. 2004; Kuroda et al. 2004; Stadler et al. 2004) and in disease (Borden and Manuelidis 1988; Meaburn et al. 2007a), where these factors remain unchanged. This suggests that chromosome positioning may be linked to the control of gene expression (Fraser and Bickmore 2007). Indeed, some genes are repositioned before or during activation (Lanctot et al. 2007; Meaburn et al. 2007b; Ragoczy et al. 2003; Szczerbal and Bridger 2008, in preparation). Moreover, the active and inactive alleles of monoallelically expressed glial fibrillary acidic protein exhibit distinct intranuclear positions (Takizawa et al. 2008).

A growing number of studies, however, find no correlation between expression and the spatial positioning of genomic loci (Kim et al. 2004; Kumaran and Spector 2008; Kupper et al. 2007; Meaburn and Misteli 2008; Scheuermann et al. 2004). Consequently, expression alone does not fully account for the radial positioning of the genome and thus other factors in the nuclear or chromosomal environment must influence the spatial organisation of genomes. In support, nucleolar organising region (NOR)-containing chromosomes can associate with nucleoli, irrespective of the activity of ribosomal repeats (Bridger et al. 1998; Sullivan et al. 2001). A role for the nuclear environment is further suggested by the nuclear A-type lamins, which have a key role in the peripheral positioning of specific human and mouse chromosomes (Bridger et al. 2007; Meaburn et al. 2007a; Mehta and Bridger 2008, for submission).

To further explore the mechanisms involved in the intranuclear positioning of human chromosomes, we utilised a panel of stable hybrid cell lines generated by microcell-mediated chromosome transfer (MMCT), which carry a single, intact human chromosome in immortalised mouse fibroblast cells. MMCT enables intact, single chromosomes to be transferred into host cells, to form hybrid cell lines (Fournier and Ruddle 1977; Meaburn et al. 2005b; Schor et al. 1975). Such hybrid cells can stably maintain the additional chromosome, even if this chromosome is from another species (Cuthbert et al. 1995; Tanabe et al. 2000). MMCT has become an important technique in functional gene mapping studies since by the addition of a single chromosome to a mutant cell, complementary genes are present as a single copy and in their native chromatin environment, with their regulatory sequences, allowing assessment of their ability to complement the mutant gene in a physiological setting (Anderson and Stanbridge 1993; Doherty and Fisher 2003; Meaburn et al. 2005b; Tindall et al. 1998).

Interspecies hybrids have the potential to be powerful tools to aid our understanding of the spatial organisation of genomes (Meaburn et al. 2005b). For instance, one of the first demonstrations that chromosomes were discrete entities within interphase nuclei came from human/Chinese hamster hybrids (Schardin et al. 1985). We have here analysed the spatial positioning of single human chromosomes in mouse hybrid cells. We report that many human chromosomes have an altered position in mouse mono-chromosome hybrid nuclei compared to their native position in human nuclei and are not positioned according to their gene density or size. Instead, human chromosome positioning in hybrid nuclei better correlates with synteny to mouse chromosomes. These results suggest that the nuclear environment is an important contributor in determining the spatial positioning of chromosomes.

Materials and methods

Cell culture

A human dermal foreskin (HDF) fibroblasts cell line (1HD) (Bridger et al. 1998) and an immortalised mouse fibroblast cell line (A9) were maintained in Dulbecco's modified Eagle's medium (DMEM; Invitrogen) supplemented with 10% (*v*/*v*) foetal bovine serum (FBS; Invitrogen), 20 mM glutamine (Invitrogen) and 2% (*v*/*v*) penicillin/streptomycin (Invitrogen) at 37°C, in a humidified atmosphere containing 5% CO_2. The mono-chromosome hybrid cell lines (A9HyTK) (Cuthbert et al. 1995), A9 cells containing individual human chromosomes, tagged with *HyTK* for selection, were grown in a similar manner to A9 cells, except with the addition of 400 U/ml hygromycin B (CN Biosciences, UK). A9.9delP, an A9 cell line containing human chromosome (HAS) 9 with a deleted p arm (England et al. 1996) and K1H9, a Chinese hamster ovary (CHO) cell line containing whole HSA 9 (Cuthbert et al. 1995) were also utilised. These cells were grown under the same conditions as the other hybrid cell lines. In a specific case, A9.2, A9.3, A9.18 and A9.X cell lines (A9 cells containing human chromosomes 2, 3, 18 or X, respectively) were also routinely maintained for 3 weeks in non-selection media before fixation for 2D interphase fluorescence in situ hybridisation (FISH).

2D interphase fluorescence in situ hybridisation

To delineate human chromosomes, standard 2D FISH was performed as described in detail in (Meaburn et al. 2007a), using directly labelled total human chromosome DNA Probes (Appligene Oncor), or for some preparations, amplified flow-sorted HSA 18 labelled with biotin-16-dUTP (Boehringer Mannheim). To determine if the HDF cells were proliferating or non-proliferating, nuclei were stained for the presence of

pKi-67, as described in Bridger et al. (2000). For visualising mouse chromosomes, directly labelled total mouse chromosome DNA Probes (Appligene Oncor) were used. FISH was performed as for the human probes, with the exception that probes were denatured at 72°C for 5 min and then placed straight on ice until used. After 48 h of hybridisation with the mouse probes, nuclei were washed for 2 min at 72°C, without agitation, in 0.5× SSC, pH 7. Slides were then incubated in 4× SSC at room temperature for 2 min and nuclei were counterstained by mounting in 4′6-diamidino-2-phenylindole (DAPI) containing mounting medium (Vector Laboratories).

Microscopy and analysis

All slides were examined under a ×100 oil immersion objective (Leica), on a Leica fluorescent microscope (Leitz DMRB) and images of random nuclei were collected with a CCD camera (Sensys, Photometrics), using quips pathvysion, SmartCapture VP V1.4 (Digital Scientific, Vysis). Simple erosion analysis was then performed on the captured images as described in (Croft et al. 1999). Briefly, 50–60 2D FISH images were run through a script written by P. Perry (MRC HGU, Edinburgh, a kind gift from Prof. W. Bickmore) in IPlab spectrum software. An exception to this was mouse chromosome X in A9 cells, where 23 nuclei were analysed. The script divides nuclei into five concentric shells of equal area eroded from the periphery (shell 1) to the interior (shell 5), recording 4′,6-diamidino-2-phenylindole (DAPI) and chromosome probe signal in each shell. Background was removed from the FISH signal by subtraction of the mean signal pixel intensity within the nucleus. To normalise the probe signal, the percentage of probe signal in each shell was divided by the percentage of the DAPI signal (total DNA) in that shell. Error bars denote ± standard error of the mean. Statistical analysis was performed using the unpaired, unequal variances, two-tailed Student's *t* test, using Excel software. $P<0.05$ was considered to be significant.

Human chromosome gene densities were calculated by dividing the number of genes by chromosome size (Mbp) using data from http://www.ncbi.nlm.nih.gov/mapview/map_search.cgi?taxid=9606.

Measurement of chromosome territory area

The area of 50 chromosomes delineated by FISH probes and the area of the nuclei they were located in, delineated by DAPI, were measured for HSAs 3, 4, 13, 18 and X, in HDFs and the hybrid cell lines A9.3, A9.4, A9.13, A9.18 and A9.X in Adobe Photoshop 5.0.2. Images were enhanced to ensure the whole nucleus or chromosome territory was being outlined. Since A9 nuclei were generally smaller than HDF nuclei, the area of each chromosome territory was normalised to the nuclear area for that specific nucleus. Average values were calculated from the normalised values. Statistical analysis was performed with the unpaired, unequal variances, two-tailed Student's *t* test, using Excel software.

Indirect immunofluorescence

Indirect immunofluorescence was performed on cells grown to ~70% confluence as previously described in Meaburn et al. (2007a). The antibodies used were: mouse monoclonal anti-lamin A/C (Novocastra) diluted to 1:10; mouse monoclonal lamin A/C (636) sc-7292 (Santa Cruz Biotechnology), diluted to 1:100; mouse monoclonal anti-lamin B2 (Novocastra), diluted to 1:200; mouse monoclonal anti-pRb (Ab-11) (Oncogene) diluted to 1:200 and donkey anti-mouse FITC (Jackson ImmunoResearch) diluted to 1:60. Cells were counterstained by mounting in DAPI-containing mounting medium. Slides were viewed and imaged on the Leica fluorescence microscope. Data were derived from at least three independently treated coverslips per antibody per cell line and a minimum of 200 nuclei were counted per coverslip. Anti-Rb staining was only performed on 4% paraformaldehyde fixed cells and anti lamin B2 was only performed on 1:1 (*v*/*v*) methanol/acetone fixed nuclei, since the other fixation did not work well for these antibodies. For both anti-A-type lamin antibodies, the same distributions and fractions were observed regardless of the fixation or antibody used.

Results

Human chromosomes occupy distinct nuclear locations in hybrid nuclei

We sought to determine the spatial positioning of 22 human chromosomes in a panel of mono-chromosome mouse/human stable A9 mouse fibroblast hybrid cell lines (Figs. 1, 2 and Table 1). Each hybrid cell line contains a single, intact human chromosome, which is tagged with a selectable hygromycin resistance marker (*HyKT*) (Cuthbert et al. 1995). Human chromosomes in A9 cells are functional since several human proteins were detected by immunofluorescence using antibodies that specifically recognised only the human forms of the proteins (Fig. S1, Table S1). Human lamin B2 (HSA19), lamin A/C (HSA1) and retinoblastoma (pRb) (HSA13) proteins were expressed and correctly localised in 8.8±4.0%, 50.6±9.2% and 63.7±12.3% of nuclei in the appropriate hybrid cell line, respectively. The lamin proteins were localised to the murine nuclear envelope and intranuclear foci. pRB is present as discrete foci throughout the nucleoplasm. The localisation of human pRB was found to be similar between HDFs and the hybrid nuclei containing HSA 13, albeit at lower

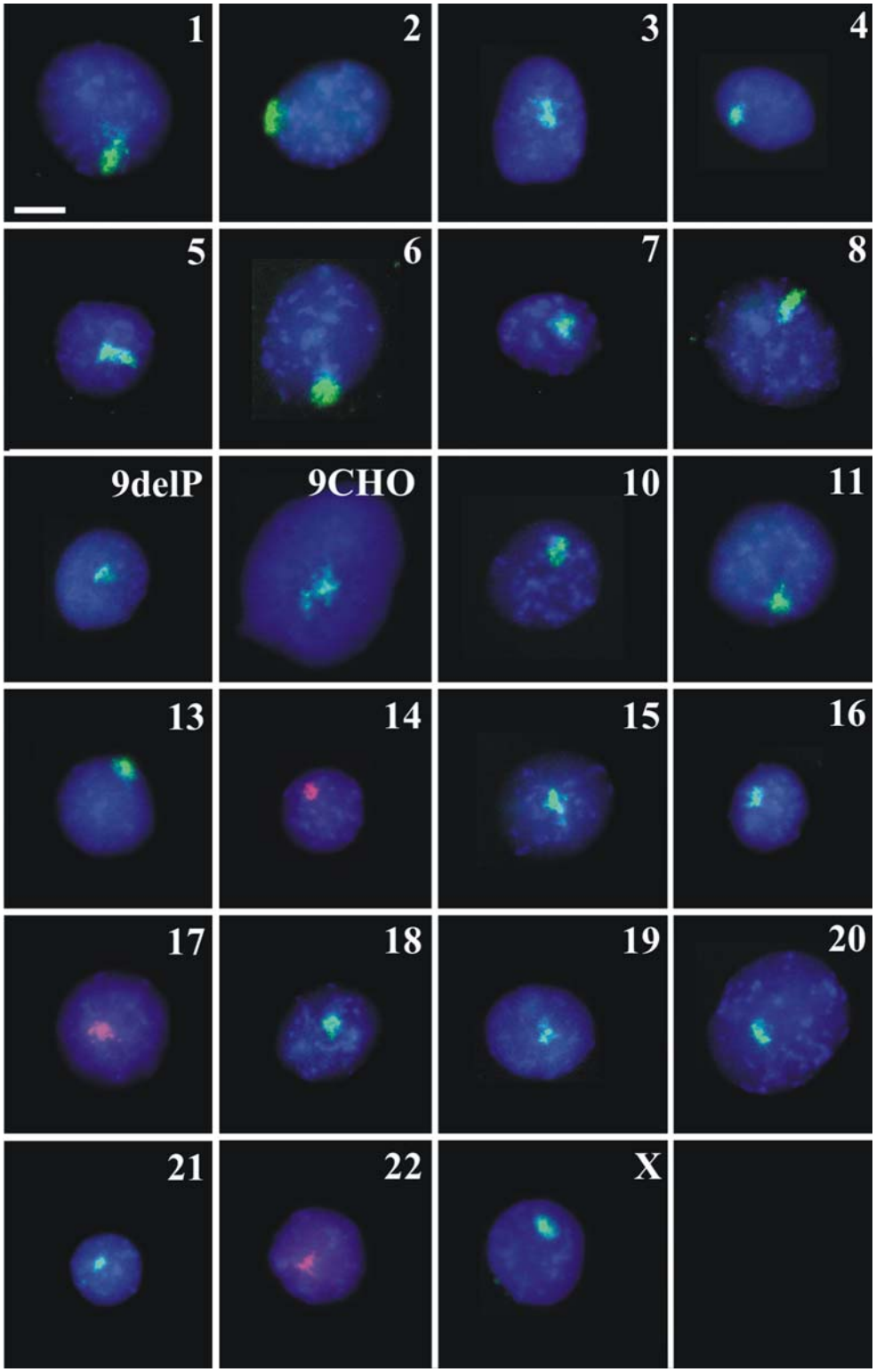

Fig. 1 The nuclear location of human chromosomes in monochromosomal human/mouse hybrid nuclei. Human chromosomes (*green* or *red*) were delineated with whole chromosome probes by standard 2D FISH and nuclei were counter-stained with DAPI (*blue*). The *number* or *letter* above the representative nucleus denotes the human chromosome (HSA). Whole HSA 9 has not successfully been incorporated into A9 nuclei. Instead, HSA 9 with a deleted p arm in A9 nuclei (*9delP*) and whole HSA 9 in CHO cells (*9CHO*) were positioned. *Scale bar* = 10 μm

staining intensities in the hybrid nuclei (Fig. S1, Meaburn et al. 2007a).

Analysis of chromosome positioning was performed by dividing nuclei into five concentric shells of equal area and assessing the normalised proportion of chromosome probe distributed in each shell for 50–60 nuclei for each chromosome as previously described ("Materials and methods"; Bridger et al. 2000; Croft et al. 1999; Meaburn et al. 2007a; Meaburn et al. 2005a). We find that human chromosomes had preferential radial positions in the hybrid nuclei (Figs. 1, 2 and Table 1). HSAs 2, 6 and 13 were positioned peripherally in the A9 nuclei, with enrichment of the chro-

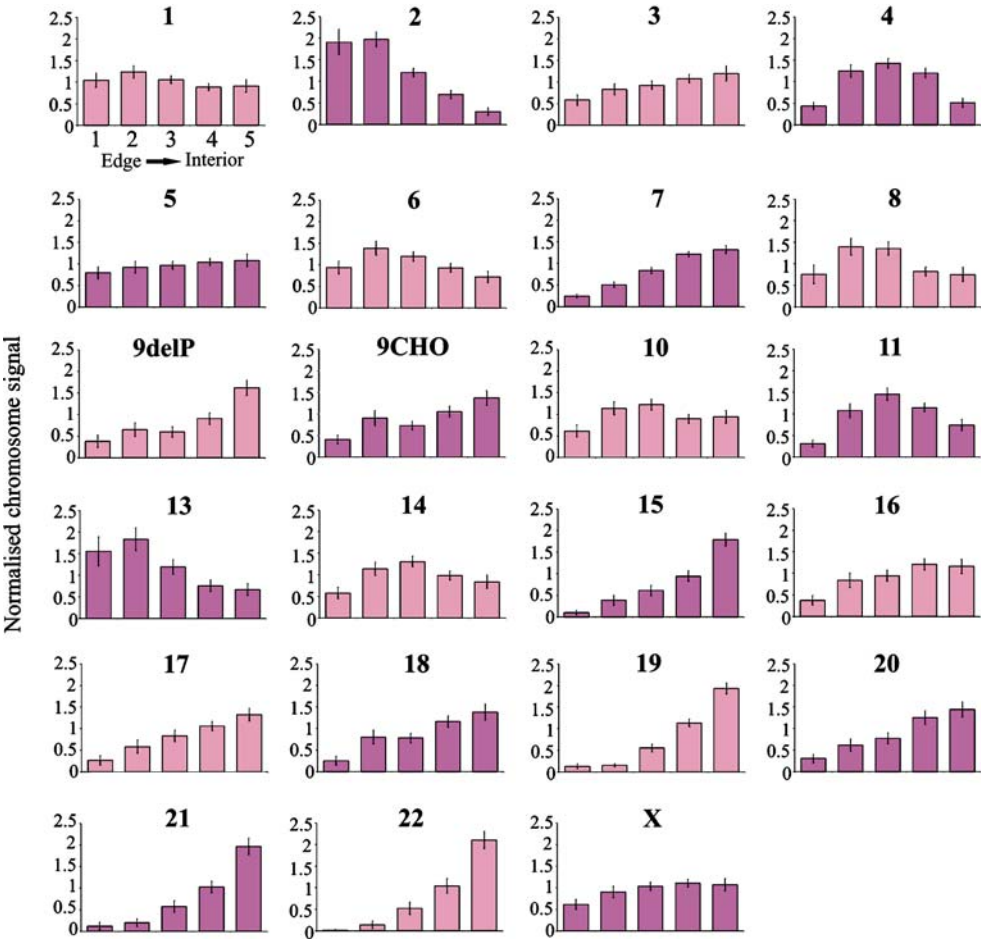

Fig. 2 The radial positions of human chromosomes in monochromosomal human/mouse hybrid nuclei. After standard 2D FISH, 50–60 nuclei per chromosome were subjected to erosion analysis (see "Materials and methods"). The normalised chromosome signal (mean [% of probe signal/% of DAPI signal]) for each of the five shells was plotted as a histogram. *Error bars* show standard error of the mean. The *number* or *letter above a graph* indicates which human chromosome has been analysed. 9delP denotes HSA 9 with deleted p arm in A9 nuclei. 9CHO denotes whole HSA 9 in CHO hybrid nuclei

mosome in shells 1 and 2. Conversely, HSAs 3, 7 and 15–22 located mostly to the nuclear interior, with enrichment in shells 4 and 5. HSAs 4, 8, 10, 11 and 14 were intermediately positioned, as determined by a bell-shaped distribution curve, which peaked in shell 3, whereas HSAs 1, 5 and X had an equal distribution of probe signal, where each shell contained similar amounts of normalised chromosome signal.

Stable A9 hybrid cell lines containing whole HSA 9 have not successfully been generated (Cuthbert et al. 1995; Koi et al. 1989). To position HSA 9 in a foreign nuclear environment, we used an A9 hybrid cell line in which HSA 9 with a deleted p arm was present (A9.9delP) (England et al. 1996) or CHO cells in which whole HSA 9 was present (K1H9) (Cuthbert et al. 1995). In both cases, HSA 9 located to the nuclear interior, with no significant difference in the distribution of normalised HSA 9 signal between the two cell lines ($P>0.05$, t test).

Chromosome positioning in hybrid cells does not correlate with chromosome gene density or size

The radial position of chromosomes in human nuclei has been reported to correlate with either chromosomal gene density or size whereby the gene-rich or the small chromosomes are positioned internally (Foster and Bridger 2005; Meaburn and Misteli 2007). In the mouse nuclear background, however, we found no general correlation between the gene density of the human chromosome and its nuclear position (Table 1). Gene-rich chromosomes (e.g. HSAs 19 and 17; 26.5 and 18.6 genes/Mbp, respectively), gene-poor chromosomes (e.g. HSAs 18 and 3; 5.7 and 7.3 genes/Mbp, respectively) and chromosomes with intermediate gene densities (e.g. HSAs 7 and 15; 9.1 and 9.5 genes/Mbp, respectively) could be found positioned internally. The human chromosomes that located to the periphery of mouse nuclei were either gene-poor (HSA 13; 4.8 genes/Mbp) or had an intermediate gene density (HSAs 2 and 6; 7.8 and 8.8 genes/Mbp, respectively), yet the remaining nine chromosomes with gene densities between that of HSAs 13 and 6 (HSAs 18, 4, 8, 5, 3, 21, 10, 9, X) were positioned away from the nuclear periphery in A9 nuclei, and had variable radial positions relative to each other (Figs. 1, 2 and Table 1).

Furthermore, human chromosomes with similar gene densities could be positioned very differently. For example, HSAs 21 and 2 have similar gene densities (7.5 and 7.8 genes/Mbp, respectively); however, HSA 2 located to the nuclear periphery and HSA 21 located to the nuclear interior. HSAs 13, 18 and 4 were the three most gene-poor chromosomes studied (4.8–6.0 genes/Mbp), yet while HSA 13 was

Table 1 Spatial positioning of human chromosomes in normal proliferating human fibroblasts and in mouse/human mono-chromosome hybrid cell lines

HSA	Normal proliferating HDF	Hybrid	Gene density (genes/Mbp)
1	IM*	E	11.3
2	P*	P	7.8
3	P	I	7.3
4	P#	IM	6.0
5	IM	E	7.0
6	IM*	P	8.8
7	P	I	9.1
X	P*#	EI	8.6
8	IM*	IM	6.7
9	P	I (delP)	–
9	P	I (CHO)	8.2
10	IM	IM	8.2
11	IM	IM	13.8
13	P*#	P	4.8
14	I*	IM	12.0
15	P*	I	9.5
16	I*	I	12.5
17	I*	I	18.6
18	P^*#	I	5.7
19	I^*	I	26.5
20	I	I	11.9
22	I*	I	14.8
21	I*	I	7.5

The position of human chromosome territories in human dermal fibroblasts (HDF) and mouse/human mono-chromosome hybrid fibroblast cell lines (hybrid). Human chromosomes are listed according to size. In some cases, the positions of chromosomes in proliferating HDF were derived from published data: ^ (Bridger et al. 2000); * (Boyle et al. 2001); # (Meaburn et al. 2007a).

Stable A9 hybrid cell lines containing whole HSA 9 have not successfully been generated (Cuthbert et al. 1995; Koi et al. 1989), instead, HSA 9 with a deleted p arm in the mouse nuclei (delP) (England et al. 1996) or Chinese hamster ovary (CHO) cells containing whole HSA 9 (Cuthbert et al. 1995) were utilised. Whole human chromosome gene densities were calculated by dividing the number of genes by chromosome size (Mbp) using values from http://www.ncbi.nlm.nih.gov/mapview/map_search.cgi?taxid=9606.

P peripheral, *I* internal, *IM* intermediate, *E* equal distribution in each shell of probe signal.

peripherally positioned, HSA 4 was intermediately positioned and HSA 18 located to the murine nuclear interior.

Similarly, no correlation was detected between the size of a human chromosome and its position in hybrid nuclei (Figs. 1, 2 and Table 1). Although the smaller human chromosomes (HSAs 15–22) did locate to the murine nuclear interior, larger human chromosomes such as HSAs 3 and 7 also located to the nuclear interior. HSAs 2 and 6 were the only large chromosomes to position to the nuclear periphery, along with intermediately sized HSA 13. Moreover, chromosomes of similar size could occupy very distinct positions relative to each other; for example, the four largest chromosomes HSAs 1–4 were found equally distributed, peripherally, internally and intermediately positioned, respectively (Figs. 1, 2 and Table 1). Taken together, these results suggest that chromosome gene density and size do not determine chromosome positioning of heterologous chromosomes in hybrid nuclei.

Human chromosomes occupy distinct positions in hybrid nuclei compared to their native environment

The fact that human chromosomes in hybrid nuclei are positioned according to neither gene density nor size implies that at least some are alternatively positioned in a hybrid nucleus compared to their native environment in human cells. To assess this, we directly compared the position of human chromosomes in hybrid nuclei with their location in human nuclei. Since the spatial organisation of the genome is cell-type and differentiation status dependent (Foster and Bridger 2005), the positions of human chromosomes in hybrid mouse fibroblast nuclei were compared to that of human fibroblast (HDF) nuclei. Immortalised hybrid cell lines are highly proliferative (data not shown) and because the position of chromosomes can be dependent on cell cycle status, only HDF nuclei positive for the proliferation marker pKi-67 were analysed. We determined the radial position of HSAs 3, 5, 7, 9, 10, and 11 in proliferating HDF because they have not previously been positioned in proliferating HDF and HSA 20 because it had only been positioned in proliferating HDF by an alternative analysis method (Bolzer et al. 2005; Boyle et al. 2001). We found that HSAs 3, 7 and 9 were peripherally positioned, HSAs 5, 10 and 11 were intermediately positioned and HSA 20 was internally positioned (Fig. 3, Table 1).

Unpaired *t* tests were performed to assess significant differences between the normalised human chromosome signals in the hybrid mouse cell lines and to the HDF cell line for chromosomes (HSAs) 3, 4, 5, 7, 9, 10, 11, 13, 18, 20 and X (Fig. 3) (Meaburn et al. 2007a). The analysis of HSAs10, 11, 13 and 20 revealed no significant alteration in the radial distribution of chromosome signal, whereas HSAs 3, 4, 5, 7, 9, 18 and X had significantly altered chromosome distributions. Both A9.9delP and K1H9 hybrid cell lines had a similar significantly different location of HSA 9 compared to HDF, implying that the deletion of the P arm of HSA 9 has not led to a change in nuclear location of HSA 9 in the mouse background (Figs. 1, 2, 3 and Table 1). For the remaining 11 chromosomes (HSAs 1, 2, 6, 8, 14–17, 19, 12 and 22), the shape of the normalised probe distribution in the graphs for human chromosomes in A9 nuclei was compared to that of proliferating HDF previously published by Boyle et al. (2001), which used identical analysis methods (Table 1) (Boyle et al. 2001). From these comparisons, HSAs 1, 6, 14, and 15 were found to have an altered distribution of normalised chromosome signal.

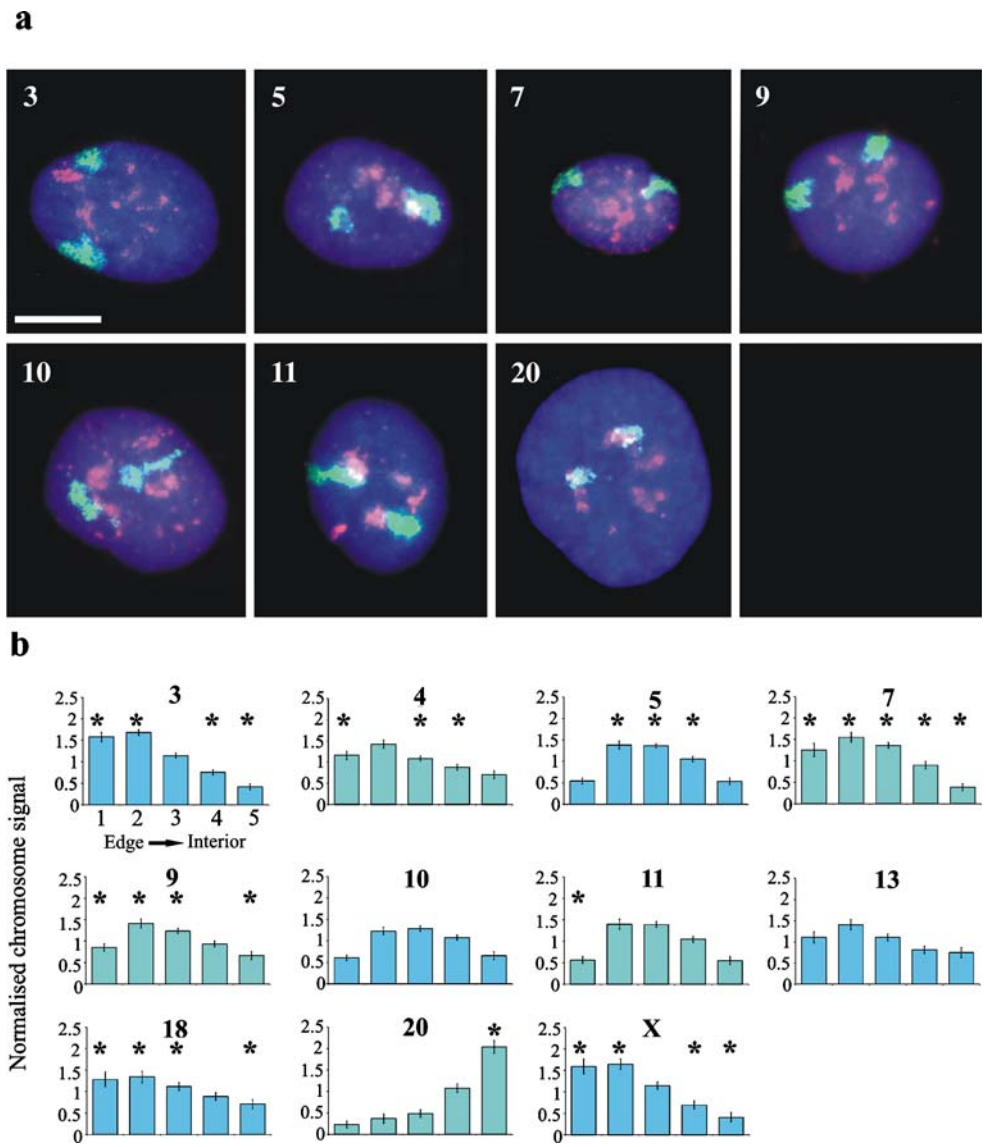

Fig. 3 The radial positions of selected human chromosomes in proliferating human dermal fibroblasts. **a** Human whole chromosome territories (*green*) were delineated in human fibroblasts. Proliferation status was determined by the presence of pKi-67 antigen (*red*) and nuclei were counterstained with DAPI (*blue*). The *number* or *letter* above a nucleus indicates the human chromosome delineated. *Scale bar* = 10 μm. **b** After standard 2D FISH, 50–60 nuclei per chromosome were subjected to erosion analysis. The normalised chromosome signal for each of the five shells was plotted as a histogram. The *number* or *letter above a graph* denotes the human chromosome (HSA). *Error bars* show standard error of the mean. HSAs 4, 13, 18 and X data (Meaburn et al. 2007a). Statistically significant differences, as assessed by Student's *t* test, between the normalised human chromosome signal in each shell of mouse hybrid nuclei as compared to that in HDF are indicated by an *asterisk* ($P<0.05$)

In contrast, HSAs 2, 8, 16, 17, 21 and 22 displayed no shift in chromosome distribution between mouse hybrid and HDF nuclei. To rule out any affect of the presence of selection antibiotic, the nuclear localisation of HSAs 2, 3, 18 and X in hybrid nuclei was also determined in hybrid cells grown in the absence of hygromycin B for 3 weeks. No significant difference in chromosomal distribution between cells grown in the presence or absence of hygromycin B was detected (*t* test, $P>0.05$; Fig. S2). We conclude that 11 out of 22 human chromosomes display an altered positioning in mouse fibroblasts compared to that of HDF. These data suggest that the nuclear environment a chromosome is in is an important determinant of the spatial positioning of whole chromosomes.

Altered compaction of human chromosomes in hybrid nuclei

Since the radial positioning of many human chromosomes was not conserved in the rodent background, we wanted to know whether other features of human chromosome territories were altered in the hybrid cells. To this end, the size of chromosome territories for HSAs 3, 4, 13, 18 and X in both

hybrid and HDF nuclei were measured using their 2D areas in nuclei as a volume indicator as previously described (Croft et al. 1999). These five chromosomes are peripherally positioned in proliferating HDF, but with the exception of HSA 13 are more internally localised in hybrid nuclei (Figs. 1, 2, 3 and Table 1). All five human chromosomes, including HSA 13, which positions similarly in HDF and hybrid nuclei, occupied a significantly smaller area ($P<0.0001$; t test) in mouse fibroblast nuclei compared to HDF (Table 2a). A9 nuclei, however, were generally smaller than the HDF nuclei (18,909 pixels [$n=250$] vs 28938 pixels [$n=162$], respectively ($P<0.0001$)), suggesting that the size of the host nucleus affects the overall condensation state of the introduced chromosome.

To test whether the smaller area of human chromosomes in the hybrid cells was simply a reflection of the overall smaller nucleus, the area of each chromosome territory was normalised to nuclear size (Table 2b). After this normalisation, HSA 3 occupied a larger relative area in the hybrid nuclei compared to HDF (6.2% vs 5.2%, respectively; $P<0.01$); conversely HSA 4 occupied a smaller relative area in the hybrid nuclei to HDF (3.6% vs 6.7%, respectively; $P<0.0001$). The relative nuclear area occupied by HSAs 13, 18 and X, however, was not statistically different in hybrid and HDF nuclei ($P>0.05$). These data suggest chromatin structure is altered when human chromosomes are in murine nuclei, but that nuclear size is not the only factor influencing the relative size of the chromosome territory in foreign nuclei. Moreover, change in the compaction of a specific human chromosome did not influence the likelihood of that chromosome occupying a divergent nuclear position between the human and hybrid nuclei, since although HSAs 3, 4, 18 and X all adopted alternative nuclear positions in the hybrid nuclei, HSAs 3 and 4, but not HSAs 18 or X, occupied an altered relative nuclear area.

Table 2 Area of human chromosomes in human and hybrid nuclei

HSA	HDF (SEM)	Hybrid (SEM)	t test
A: Average area of chromosome territory (in pixels)			
3	1,640 (83.7)	1,235 (80.4)	$P<0.0001$
4	1,429 (61.2)	748 (37.8)	$P<0.0001$
13	988 (54.9)	742 (40.2)	$P<0.0001$
18	765 (44.5)	509 (29.2)	$P<0.0001$
X	1,491 (84.2)	855 (49.1)	$P<0.0001$
B: Percentage of nucleus occupied by individual human chromosomes			
3	5.2 (0.24)	6.2 (0.28)	$P<0.01$
4	6.7 (0.24)	3.6 (0.20)	$P<0.0001$
13	4.3 (0.60)	5.2 (0.99)	NSD
18	3.3 (0.47)	3.2 (0.18)	NSD
X	5.2 (0.20)	5.3 (0.80)	NSD

The area of 50 chromosome territories for HSAs 3, 4, 13, 18 and X in both human fibroblasts (HDF) and hybrid mouse fibroblast nuclei (hybrid) was measured; the averages are displayed in (A), with the standard error of the mean (SEM). (B) To take into account the different sizes of HDF and hybrid nuclei, the percentage of nuclear area occupied by a chromosome territory was also determined. The area of each chromosome territory (pixels) was divided by the area (pixels) of its nucleus. Significance difference between the two cell lines was determined by Student's t test (t test).
NSD no significant difference ($P>0.05$).

Reduced association of human chromosomes to the nuclear periphery in hybrid cells correlates with the positions of syntenic mouse chromosomes

To address if homology to mouse chromosomes could explain the contrasting positions of human chromosomes in the human and mouse background, the nuclear localisation of mouse chromosomes (MMU) 5, 6 and X was analysed in parental A9 nuclei (Fig. 4). The level of synteny varies widely between different human and mouse chromosomes (Murphy et al. 2005; Waterston et al. 2002). MMU X was chosen because chromosome X is highly conserved between human and mouse, and represents the chromosome with the greatest degree of synteny between the two species (Murphy et al. 2005; Waterston et al. 2002). Despite the high level of sequence conservation, the position of chromosome X in fibroblasts derived from these two species was divergent; whereby MMU X was more internal in mouse than HSA X in human (Figs. 3, 4 and Table 1). Our positioning of MMU X is consistent with the positioning of MMU X in mouse fibroblasts previously reported (Mayer et al. 2005). Interestingly, while HSA X was significantly more internally positioned in hybrid nuclei compared to HDF, there was no significant difference between the position of HSA X and MMU X in murine nuclei (Fig. 4 and Table 1, $P>0.05$). This implies that human chromosome X is positioned in the murine nuclei according to synteny with MMU X.

Since many human chromosomes became more internally localised in the hybrid cells, we chose to analyse two mouse chromosomes (MMUs 5 and 6) in which the majority of synteny was to human chromosomes that are normally located to the nuclear periphery in proliferating HDF. MMU 5 shares large regions of synteny with HSAs 4 and 7, and much smaller regions with HSAs 2, 1, 22, 12 and 13 (see Fig. 3 in Waterston et al. 2002 and Fig S2 in Murphy et al. 2005). In HDF, all these chromosomes are positioned peripherally, with the exception of HSAs 1 and 22 (Fig. 2 and Table 1). In contrast, MMU 5 was intermediately-to-internally distributed in A9 nuclei (Fig. 4). Only HSAs 2 and 13 locate to the murine nuclear periphery in the hybrid cells; HSA 4 was positioned intermediately, HSAs 7 and 22 were positioned internally and HSA 1 was equally distributed (Fig. 2 and Table 1).

MMU 6 shares large regions of conserved synteny with HSAs 7, 3 and 12 and only small regions with HSA 4, 1, 2 and 10. In HDF, these chromosomes are positioned peripherally, with the exception of HSA 1 and 10 (Fig. 3 and Table 1).

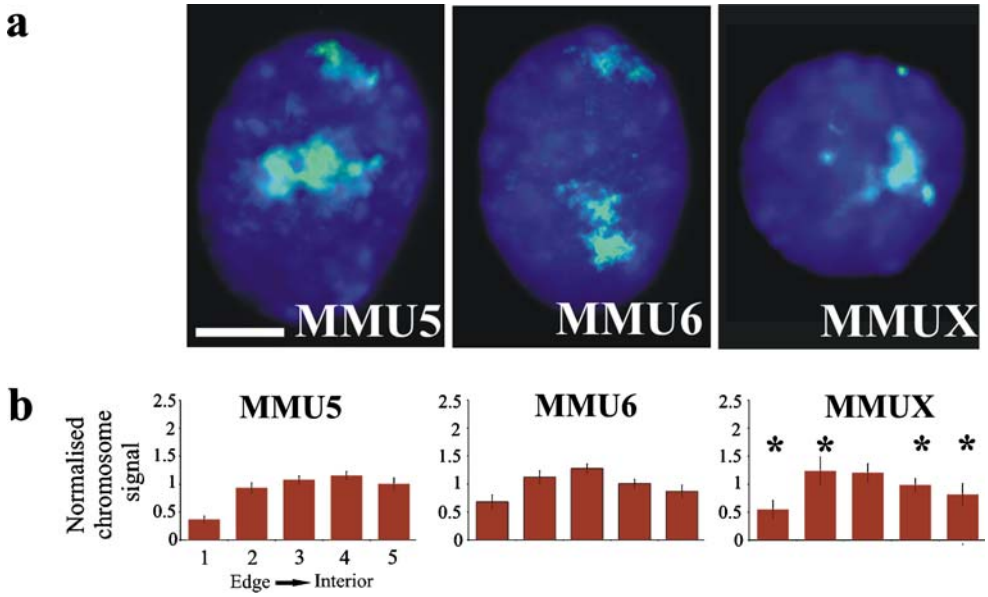

Fig. 4 Radial positioning of mouse chromosomes 5, 6, and X. **a** Parental A9 nuclei were hybridised with the indicated whole mouse chromosome (MMU) paints (*green*) and counterstained with DAPI (*blue*). *Scale bar* = 10 μm. **b** Twenty-three to sixty-one nuclei were subjected to erosion analysis. The normalised chromosome signal for each of the five shells was plotted as a histogram. *Error bars* show standard error of the mean. No significant difference was found between the normalised chromosome distribution of MMU X and human chromosome X in the hybrid cell line, as assessed by Student's *t* test, at the 95% confidence level. The *asterisk* denotes statistical differences between the distribution of MMU X in A9 nuclei and that of HSA X in HDF for each shell

MMU 6 had an intermediate distribution in A9 nuclei (Fig. 4). This is coincidental with a loss of peripheral positioning of many of the human chromosomes with shared synteny in A9 nuclei (intermediate positioning of HSAs 4 and 10 and internal positioning of HSAs 3 and 7; Table 1). Taken together, these data suggest that human chromosomes in hybrid nuclei mimic the position of their syntenic mouse chromatin counterparts.

Discussion

We provide here a systematic characterisation of the position of 22 human chromosomes in a rodent nuclear background. We show that the nuclear environment influences the spatial nuclear position of chromosomes and we find the mechanisms determining the host nuclear spatial organisation to be dominant over the donor chromosome. We provide evidence that human chromosomes position according to synteny with their host.

There are differences in the spatial organisation of the genome between man and mouse. Aside from the alternative positioning of the syntenic X chromosome in fibroblasts (Fig. 4) (Mayer et al. 2005) and a weaker correlation between radial distribution of chromosomes and gene density in mouse (Boyle et al. 2001; Mayer et al. 2005), interphase telomere positioning in lymphocytes is divergent between human and mice (Weierich et al. 2003). The repositioning behaviour of syntenic regions in response to gene activation can also be different between the two species (Brown et al. 2006; Sadoni et al. 2008). Other aspects of the spatial organisation of the genome appear to be more conserved between man and mouse, such as the spatial distribution of late and early replicating DNA (Sadoni et al. 1999) and the relative positions of certain loci to their territories (Mahy et al. 2002a; Mahy et al. 2002b). Regardless, the differences between the species suggest that genomic context or other factors in the nucleus, i.e. the nuclear environment the genome is in, influences the spatial positioning of genomes.

We find half of the human chromosomes studied display an altered position in mouse nuclei as compared to their location within proliferating HDF nuclei. Of these, the chromosomes were predominantly more internally localised in the murine nuclei than expected based on their position in parental human cells. In fact, only three human chromosomes positioned at the nuclear periphery in murine nuclei compared to nine in proliferating HDF. The radial distribution of chromosomes within nuclei is often correlated with their gene density or in some cases size (Cremer et al. 2006; Foster and Bridger 2005; Meaburn and Misteli 2007). Human chromosomes in the hybrid cells followed neither correlation. For instance, human chromosomes mapping to the hybrid nuclear interior were gene-rich, gene-poor and had intermediate gene densities. Although the smaller human chromosomes all positioned towards the interior of mouse nuclei, if chromosome size was a determining factor, an incremental change in location from the largest to smallest chromosomes would be expected. This

was not seen; for example, HSAs 2, 6 and 13 locate to the periphery of the mouse nuclei, whereas HSAs 3 and 7 predominantly locate to the murine nuclear interior.

The deviation from the normal distribution of human chromosomes, which was observed in the hybrid nuclei, cannot simply be explained by the presence of additional DNA. Extra copies of whole chromosomes in both human and mouse cells position in accordance with their diploid counterparts (Croft et al. 1999; Parada et al. 2004; Sengupta et al. 2007). Thus, it would seem that the rules governing the nuclear location of the diploid nuclei are maintained with increasing copy number (Meaburn et al. 2005b). Accordingly, human acrocentric chromosomes are able to correctly locate to the murine nucleolus, whereas HSA X, which does not normally co-localise with nucleoli, does not (Sullivan et al. 2001). In this case, it is not clear if the human chromosomes position to mouse nucleoli due to an intrinsic feature of the chromosome or if genome positioning is determined by other factors such as chromatin status.

This latter, intriguing, possibility is suggested by our finding that human chromosomes derived from HDF are organised differently in human and mouse fibroblasts. This interpretation is also in line with our finding that a loss of peripheral positioning of human chromosomes within mouse fibroblasts was coincidental with a more internal positioning of the mouse chromosomes with which they share synteny. This is exemplified by mouse and human chromosome X.

Comparing the positions of human chromosomes in the murine background with the position of mouse chromosomes from the literature also support our finding that the human chromosomes adopt the behaviour of their syntenic mouse counterparts. For example, HSAs 2, 13, 14 and 17 share extensive homology to MMUs 1, 14, 12 and 11, respectively (Murphy et al. 2005; Waterston et al. 2002). The position of these human chromosomes in the mono-chromosome hybrid nuclei is similar to the position of their syntenic mouse counterparts (Mayer et al. 2005; Parada et al. 2004). These correlations do not hold in all cases, however. For example, ~1/3 of HSA 1 is syntenic to MMU 1 (Murphy et al. 2005; Waterston et al. 2002), but the nuclear position of HSA 1 in hybrid nuclei does not match either HDF or MMU 1 (Table 1: Boyle et al. 2001; Mayer et al. 2005). This perhaps simply highlights the complexity rearrangements of the genome between the species makes for these types of comparisons.

The fact that human chromosome have a number of syntenic mouse chromosomes can be utilised in future studies to elucidate the important determining factors when human chromosomes in hybrid nuclei mimic the position of their syntenic mouse chromatin counterparts. HSA 1 also has approximately equal homology with portions of MMUs 3 and 4 to that of MMU 1 (Murphy et al. 2005; Waterston et al. 2002). MMUs 3 and 4 have not been positioned in murine fibroblasts to date. Thus, it is possible that the chromatin of HSA 1 does in fact locate to positions similar to syntenic mouse sequences, or the nearest compromise the confines of a chromosome territory will allow. This could conceivably be at either the level of synteny for the whole HSA or only for the region of the mouse genome it shares the greatest level of synteny with. Local gene density is a good predictor of spatial positioning in humans (Kupper et al. 2007; Murmann et al. 2005), therefore, the position of the human chromosome in hybrid nuclei could also be influenced by the position of the syntenic mouse regions with the most similar gene density or highest/lowest gene density.

It remains to be determined if the gene expression from the human genome in the hybrid nuclei could affect the radial positioning of the HSA. There are a number of studies in which loci move away from the nuclear periphery upon activation (Lanctot et al. 2007; Szczerbal and Bridger 2008, in preparation) and there are a number of studies that reveal silenced genes at the nuclear periphery (Shaklai et al. 2007). However, there are a number of studies that find no correlation between radial positioning of individual genes or larger chromosome regions with gene expression (Kim et al. 2004; Kupper et al. 2007; Meaburn and Misteli 2008). Interestingly, three recent studies tethered loci to the nuclear periphery in an attempt to determine if the radial position of a loci affects gene expression (Finlan et al. 2008; Kumaran and Spector 2008; Reddy et al. 2008). These studies reveal that the expression levels of some, but not all, genes are affected. Thus, it would be interesting in future studies to establish how the different positioning of the human chromosomes in mouse hybrid nuclei may affect the global gene expression pattern of the human chromosomes.

The altered compaction of human chromosomes in the mouse hybrid nuclei suggests it is likely that gene expression is changed significantly. Fittingly, we find a reduced fraction of hybrid cells expressing the human proteins lamin A/C, lamin B2 and pRB compared to control human cells. Most strikingly, human lamin B2 was expressed in only ~10% of hybrid cells that contained HSA 19. The hybrid cells were routinely grown in selection media, under these conditions ~90–95% of the hybrid nuclei contain the human chromosome (data not shown). Consequently, the reduced fraction of hybrid cells with human proteins is not due to an absence of the human chromosome, and reduced expression or increased protein degradation is more likely. It is feasible that the level of human proteins is controlled in conjunction with their syntenic mouse counterparts to ensure that proteins are not overexpressed; such overexpression could be detrimental to the cells function or viability.

It is also possible that the position of a human chromosome in hybrid cells may be influenced by the global epigenetic status of the host genome. It is known that methylation status of a gene can influence its positioning and that of its neighbours within its chromosome territory (Matarazzo et al.

2007) and hypoacetylated chromatin is enriched at the nuclear periphery (Gilchrist et al. 2004; Sadoni et al. 1999). On the contrary, histone hyperacetylation affects the condensation of human chromosome territories, but not the relative spatial locations (Croft et al. 1999), nor does it alter the location of human centromeres (Gilchrist et al. 2004). Similarly, in wheat nuclei, the Rabl configuration of genome organisation was maintained with DNA hypomethylation or histone hyperacetylation, again with decondensation of certain chromatin (Santos et al. 2002). Future studies are required in the mono-chromosome hybrid cells to characterise the epigenetic status differences between the species and in particular to determine if human chromosomes in the hybrid cells have epigenetic markings more similar to those of their native environment or to their syntenic mouse counterparts.

In this study, we focus on the donor chromosome in the foreign host nucleus. It is unclear if the spatial positioning of the mouse genome remains undisturbed by the introduction of a human chromosome. While the addition of an extra chromosome does not affect the position of that chromosome (Croft et al. 1999; Parada et al. 2004; Sengupta et al. 2007), in some cases the position of other chromosomes (Sengupta et al. 2007) or centromeres (Petrova et al. 2007) are affected. When comparing our murine chromosome positioning with findings in the literature, we find that most of our chromosomes fit with data from normal diploid nuclei (Mayer et al., 2005).

In summary, we find that in a murine background human chromosomes are positioned according to their synteny to mouse chromosomes rather than their gene density or chromosome size. These results demonstrate that positioning is not intrinsic to a chromosome, but is strongly influenced by the nuclear environment.

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