Nuclear organisation in totipotent human nuclei and its relationship to chromosomal abnormality

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Summary

Studies of nuclear organisation, most commonly determining the nuclear location of chromosome territories and individual loci, have furthered our understanding of nuclear function, differentiation and disease. In this study, by examining eight loci on different chromosomes, we tested hypotheses that: (1) totipotent human blastomeres adopt a nuclear organisation akin to that of committed cells; (2) nuclear organisation is different in chromosomally abnormal blastomeres; and (3) human blastomeres adopt a 'chromocentre' pattern. Analysis of in vitro fertilisation (IVF) conceptuses permits valuable insight into the cell biology of totipotent human nuclei. Here, extrapolations from images of preimplantation genetic screening (PGS) cases were used to make comparisons between totipotent blastomeres and several committed cells, showing some differences and similarities. Comparisons between chromosomally abnormal nuclei and those with no detected abnormality (NDA) suggest that the former display a significant non-random pattern for all autosomal loci, but there is a less distinct, possibly random, pattern in 'NDA' nuclei. No evidence was found that the presence of an extra chromosome is accompanied by an altered nuclear location for that chromosome. Centromeric loci on chromosomes 15 and 16 normally seen at the nuclear periphery were mostly centrally located in aneuploid cells, providing some evidence of a 'chromocentre'; however, the chromosome-18 centromere was more peripheral, similar to committed cells. Our results provide clues to the nature of totipotency in human cells and might have future applications for preimplantation diagnosis and nuclear transfer.

Key words: Nuclear organisation, Chromosome, Preimplantation human embryos

Introduction

The spatial and temporal location of chromatin in the interphase nucleus has been implicated in the regulation of genome function and human disease (Bickmore and Bridger, 1999; Cremer and Cremer, 2001). Whole chromosomes are organised non-randomly in 'chromosome territories' (Cremer and Cremer, 2006) and both 'gene-density-related' and 'size-related' organisation models have been proposed (Foster and Bridger, 2005; Cremer et al., 2001; Sun et al., 2000; Bolzer et al., 2005; Croft et al., 1999; Boyle et al., 2001; Cremer et al., 2003; Meaburn et al., 2005a; Mayer et al., 2005). The gene-density model supports the rationale that there is spatial control over the regulation of gene expression, but, although this has been demonstrated for a few individual loci (Lukasova et al., 2002; Scheuermann et al., 2004; Zink et al., 2004; Bartova et al., 2005; Chuang and Belmont, 2005), it has not yet been demonstrated as a genome-wide phenomenon. More recently, another level of nuclear organisation has been reported; namely, that of a 'chromocentre', with the centromeres located towards the nuclear interior and the telomeres towards the periphery. Human sperm heads display this pattern (Zalenskaya and Zalensky, 2004), as do blastomeres from preimplantation mouse embryos (Martin et al., 2006a).

Developmental processes are controlled by changes in gene expression, chromatin re-modelling and other epigenetic alterations. Thus, considerable research effort has gone into determining the nuclear position of chromosome territories and sub-regions in different cell types, cell cycle stages and cell phases. Significant changes in nuclear organisation associated with embryogenesis and organogenesis have been reported: porcine sex-chromosome territories migrate from a predominantly peripheral nuclear location to a more interior one, whereas chromosome 13 does the reverse during spermatogenesis (Foster et al., 2005). Several papers have also reported alterations to chromosome or sub-chromosomal position during differentiation (Brown et al., 1999; Stadler et al., 2004; Chamberyon and Bickmore, 2004; Kuroda et al., 2004; Galiova et al., 2004; Kim et al., 2004; Parada et al., 2004; Bartova et al., 2005; Foster et al., 2005). Kosak et al. identified compartmentalization of immunoglobulin genes during the development of white blood cells (Kosak et al., 2002), and Alcobia et al. provided evidence that centromere positions in haematopoietic cell nuclei were different depending on the cell type and stage of differentiation (Alcobia et al., 2000). Mayer et al. identified that the distribution of chromosome territories (including centromeric loci) changed during differentiation (Mayer et al., 2005) and Hewitt et al. determined that nuclear repositioning of loci is a feature of T-helper-cell differentiation (Hewitt et al., 2004). However, we are aware of no reported evidence of significantly altered chromosome territory position associated with aneuploidy, despite the alteration in gene expression that most ensue in an aneuploid cell (Croft et al., 1999). This is also true for artificially created trisomies (Upender et al., 2004; Sengupta et al., 2006) and polyploidies (Kost-Alimova et al., 2004) generated by micro-cell-mediated chromosome transfer, in which altered gene expression (trisomies) and induced chromosome instability (polyploidies) are seen (Meaburn et al., 2005b).

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To date, little is known about the nuclear organisation in totipotent human cleavage-stage embryos, despite (a) them being key to our understanding of early human development, and (b) stem cells that are derived from them being likely to produce effective culture systems for use in drug discovery and transplantation. However, embryos generated via in vitro fertilisation (IVF; principally for preimplantation genetic diagnosis and screening) allow a valuable insight into nuclear organisation at this stage and perhaps the most significant finding is the high level of chromosome abnormalities that they possess. Between 50 and 70% of human cleavage-stage embryos are thought to contain chromosomally abnormal cells (Delhanty et al., 1997; Munné and Cohen, 1998; Voullaire et al., 2000; Wells and Delhanty, 2000). Chromosomal abnormalities are the most common cause of mental retardation and first-trimester pregnancy loss in humans (reviewed in Griffin, 1996), and the majority of conceptions are lost in humans at the preimplantation stage because of them. Moreover, 'chaos' (multiple and variable chromosome abnormalities with little or no discernable cytogenetic mechanism to explain them) is commonplace in human IVF embryos (Delhanty et al., 1997; Chatzimeletiou et al., 2005b).

It remains unclear whether nuclear organisation is different in totipotent human embryos as compared to more-differentiated cells derived from ex vivo tissues. Diblik et al. observed peripheral locations of chromosomes 18 and X (Diblik et al., 2005). McKenzie et al. (McKenzie et al., 2004) suggest that chromosomes normally found at the nuclear periphery (chromosomes 13, 18, X and Y) in lymphoblasts and primary fibroblasts (Boyle et al., 2001) are found more towards the nuclear interior in human blastomeres (3- to 4-days post-fertilization) and in the same nuclear location as chromosomes 16, 21 and 22. This, the authors purport, would indicate that there might be differences in the organisation of the genome in nuclei prior to differentiation; however, they also suggest that both gene-rich and gene-poor chromosomes would be found in the same nuclear compartment. We have concerns about the design of this study, however, and these are expanded upon in the discussion.

If chromosome-territory position is a reliable marker of 'nuclear health' in preimplantation human embryos, then it follows that significant alterations from 'normal' levels could be indicative of aberrant or arrested development and related to the mechanisms that lead to numerical chromosome abnormalities. In the present study, therefore, we tested the hypotheses that: (a) human totipotent blastomeres have a similar nuclear organisation compared to committed proliferating cells; (b) chromosomally abnormal blastomeres demonstrate a change in nuclear organisation compared with their normal counterparts; and (c) nuclei in human day-3 to day-4 blastomeres adopt a 'chromocentre' pattern, as seen in day-1 to day-2 mouse embryos (Martin et al., 2006a) and human sperm (Zalenskaya and Zalensky, 2004).

Results

Analysis categories

In order to address the hypotheses set out in the Introduction, a total of 626 blastomeres were assayed for the nuclear position of sub-chromosomal regions from day-3 to day-4 human embryos after 68 IVF cycles from 48 couples. Numbers of nuclei per slide varied from 1 to 12, with an average of 3.4. A total of 218 nuclei were observed with no chromosomal aneuploidy and these were thus classified as 'NDA' (no detectable abnormality, see category 5 in the Materials and Methods). There were 408 chromosomally abnormal nuclei, including 333 nuclei that had simple aneuploidy (one, two or three copy-number changes, see category 6 in the Materials and Methods) and 75 grossly aneuploid (chaotic) nuclei in which four or more abnormalities were detected (category 7, see Materials and Methods). A total of 300 nuclei were analysed for loci on the particular chromosome that was involved in the abnormality (category 8, see Materials and Methods).

Control nuclei

Control nuclei were chosen to represent a range of committed cell types for comparison with blastomeres. Lymphocytes were chosen as a typical committed diploid cell type and, in order to address the question of whether there was an effect of an extra chromosome in the cell, a trisomy-13 lymphoblastoid cell line was assessed. Amniocytes were chosen as representatives of an embryonic cell type that had undergone a degree of differentiation (i.e. they are, in embryological terms, somewhere between blastomeres and lymphocytes) and sperm nuclei were chosen as cells in which we expected to see a chromocentric pattern.

Our results of shell analysis depicted in Fig. 1 demonstrate that all loci studied in human lymphocyte, trisomy-13 lymphoblast and amniocyte nuclei show a significant non-random radial distribution (Fig. 2A-C, Table 1), with two exceptions. That is, excluding the exceptions, in each case, chi-squared tests revealed a significant ($P<0.05$) or highly significant ($P<0.01$) non-random distribution (Fig. 2A-C, Table 1); the two exceptions were in trisomy-13 lymphoblasts, in which a non-random distribution was not found for 13q14 and 21q22 (Fig. 2B, Table 1). In lymphocytes, after normalisation for the nuclear counterstain, Xc, Yc, 13q14, 16q12, 18c and 15c were found most often at the nuclear periphery (shell 1, see Fig. 1). Locus 22q11.2 was found most often in shell 5 (centre of the nucleus) and locus 21q22 was found most often in an intermediate-interior nuclear position – i.e. shells 3-5 (Fig. 2A, Table 1). Trisomy-13 lymphoblasts showed a pattern similar to lymphocytes, except for the loci on chromosomes 13 and 21 (as explained above – Fig. 2A-D, Table 1). Amniocyte nuclei were similar to lymphocytes for 15c, 21q11.2, Xc and Yc but different for the other loci. That is, 13q14, 16q12 and 21q11 were more centrally located (all found most often in shell 5), whereas 18c and Yc showed 'bimodal' distributions, with signals found most often at the opposite extremes – in shells 1 and 5 (Fig. 2C, Table 1). Sperm nuclei displayed a 'chromocentre' pattern, with all centromeric loci (including 16q12) occupying a central position. A significant non-random pattern for the other loci (13q14, 21q22 and 22q11.2) was not observed (Fig. 2D, Table 1).

Blastomere nuclei

With regard to our first hypothesis (see Introduction), in NDA nuclei, unlike the control groups, no loci showed a significant distribution different to that predicted by the DAPI density measurements (at $P<0.05$) (Fig. 2E, Table 1).

With regard to our second hypothesis (see Introduction), among aneuploid nuclei, a non-random distribution of loci (compared with that predicted by the nuclear counterstain) was apparent for all six autosomal loci, which displayed significant ($P<0.05$) or highly significant ($P<0.01$) non-random patterns. The highest peak(s) in the histogram was the same or similar to that seen in lymphocytes for four loci (i.e. peripheral for 13q14 and 18c, and central or central/medial for 21q22 and 22q11.2). Differences from the pattern seen in control cells include 15c and 16q12, which were more peripheral in lymphocytes and

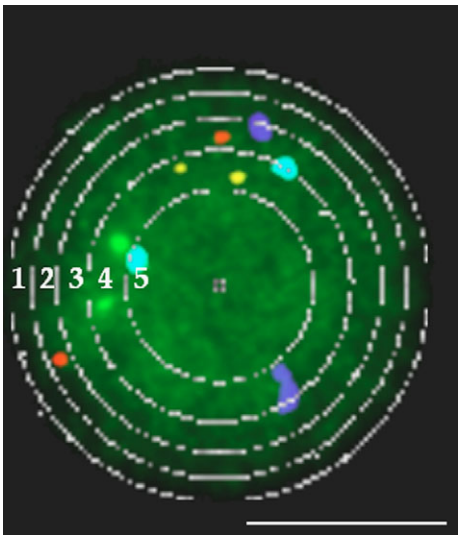

Fig. 1. Representative image of a human blastomere nucleus probed for loci 13q14 (red), 16q12 (light blue), 18c (dark blue), 21q22 (red) and 22q22.1 (yellow). A transparent five-ringed template (with each shell 1-5 of equal area) is overlaid to determine the relative position of the loci. Scale bar: 5 μm.

lymphoblasts (and amniocytes for 15c); however, signals were found most often in a central position in aneuploid blastomeres (Fig. 2F, Table 1). It is also noteworthy that locus 18c, normally seen most often in shell 1 (peripheral) in lymphocytes and lymphoblasts, was found most often in shell 2 (peripheral/medial) in aneuploid blastomeres (Fig. 2F, Table 1). For the sex-chromosome centromeres, a significant distribution different to that predicted by the DAPI counterstain measurements was not seen (Fig. 2F, Table 1).

In the 'chaotic' group, no loci showed a significant distribution different to that predicted by the DAPI density measurements, possibly suggesting a random distribution; however, again, numbers were small for some loci (Fig. 2G, Table 1).

Nuclear position of loci and its relationship to trisomy of the chromosome on which it resides

In order to address the issue of whether trisomy for an individual chromosome led to a different nuclear position in relation to a disomic equivalent, the location of 13q14 was compared in trisomy-13 and disomy-13 nuclei; this exercise was repeated for the seven other regions of interest. Only loci on chromosomes 13 and 21 showed a distribution significantly different to that predicted by the DAPI density measurements (at $P<0.05$) (Fig. 2H, Table 1). Locus 13q14 was observed most often in shells 1-2 (peripheral). For 21q22, the highest bar on the histogram was in shell 5 (central location), but this was of similar height to shell 2, suggesting a bimodal distribution (Fig. 2H, Table 1). All other loci did not display a non-random pattern.

Nuclear positions of centromeric sequences and presence of a chromocentre

With regard to our final hypothesis (see Introduction), for the NDA and chaotic groups the positions of the centromeric loci provide no evidence of the presence of a chromocentre in human day-3 to day-4 blastomeres (Fig. 2E,G). The peripheral position of the centromere of chromosome 18 as well as the distributions seen for the centromeres of the sex chromosomes also argue against the presence, such as is seen in day-1 to day-2 mouse embryos (Martin et al., 2006a), of a chromocentre in humans. Despite this, 15c and 16q12 were centrally located in aneuploid blastomeres but peripheral in lymphocytes and lymphoblasts (Fig. 2A,B,F, Table 1), suggesting that some centromeres are more prone to be centrally located in certain human blastomeres.

Discussion

Results in comparison to proposed hypotheses

Our first and second hypotheses were that totipotent human blastomeres adopt a nuclear organisation akin to that of committed cells and that that organisation is different in chromosomally abnormal nuclei. The evidence suggests that blastomeres with aneuploidy do display a pattern of nuclear organisation similar to that of some control cells but no such pattern was seen in blastomeres that were apparently chromosomally normal. One possible explanation for a failure to identify non-random patterns for certain loci is that the locus is randomly distributed throughout the nucleus; however, other possibilities exist, such as the presence of a mixed population of cells, each with different nuclear locations or the possibility that numbers were not sufficiently large to reach statistical significance. In the latter case it should be noted that some of the 'not significant' graphs were of similar shapes to their 'significant' counterparts and perhaps, therefore, with more

Table 1. Summary of results indicating the nuclear position of each locus in each cell type

	Nuclear position in which each locus was found more commonly (after adjustment for nuclear counterstain)							
Cell type	13q14	15c	16q12	18c	21q22	22q11.2	Xc	Yc
Chromosomally normal lymphocytes	Peripheral	Peripheral*	Peripheral	Peripheral*	Central/medial	Central*	Peripheral*	Peripheral
Trisomy-13 lymphoblasts	NS	Peripheral*	Peripheral	Peripheral*	NS	Central*	Peripheral*	Peripheral*
Amniocytes	Central*	Peripheral*	Central*	Bimodal	Central*	Central*	Peripheral*	Biomodal*
Spermatozoa	NS	Central*	Central*	Central*	NS	NS	Central*	Central*
NDA blastomeres	NS	NS	NS	NS	NS	NS	NS	NS
Aneuploid blastomeres	Peripheral*	Central	Central	Peripheral/medial*	Central*	Central*	NS	NS
Chaotic blastomeres	NS	NS	NS	NS	NS	NS	NS	NS
Blastomeres tisomic for chromosome measured	Peripheral*	NS	NS	NS	Bimodal*	NS	NS	NS

Peripheral, shell 1 or 1/2; Medial, shell 3, 2/3 or 3/4; Central, shell 5 or 4/5; Peripheral/medial, shell 2 or 1-3; Central/medial, shell 4 or 3-5; Bimodal, roughly equally high peaks in two non-adjacent shells; NS, not significant. *Highly significant, i.e. $P<0.01$; significant, $P>0.05$. All significance values at $P<0.05$.

numbers, might become significant in time. Indeed, some graphs, e.g. for 13q14 and 22q11.2 in the NDA group, were significantly different from each other although not individually from the pattern predicted by a random distribution. The presence of a chromocentre for some loci but not others in aneuploid human blastomeres (re. our third hypothesis) suggests some fundamental differences in the nuclear organisation of humans and mice at this stage of development.

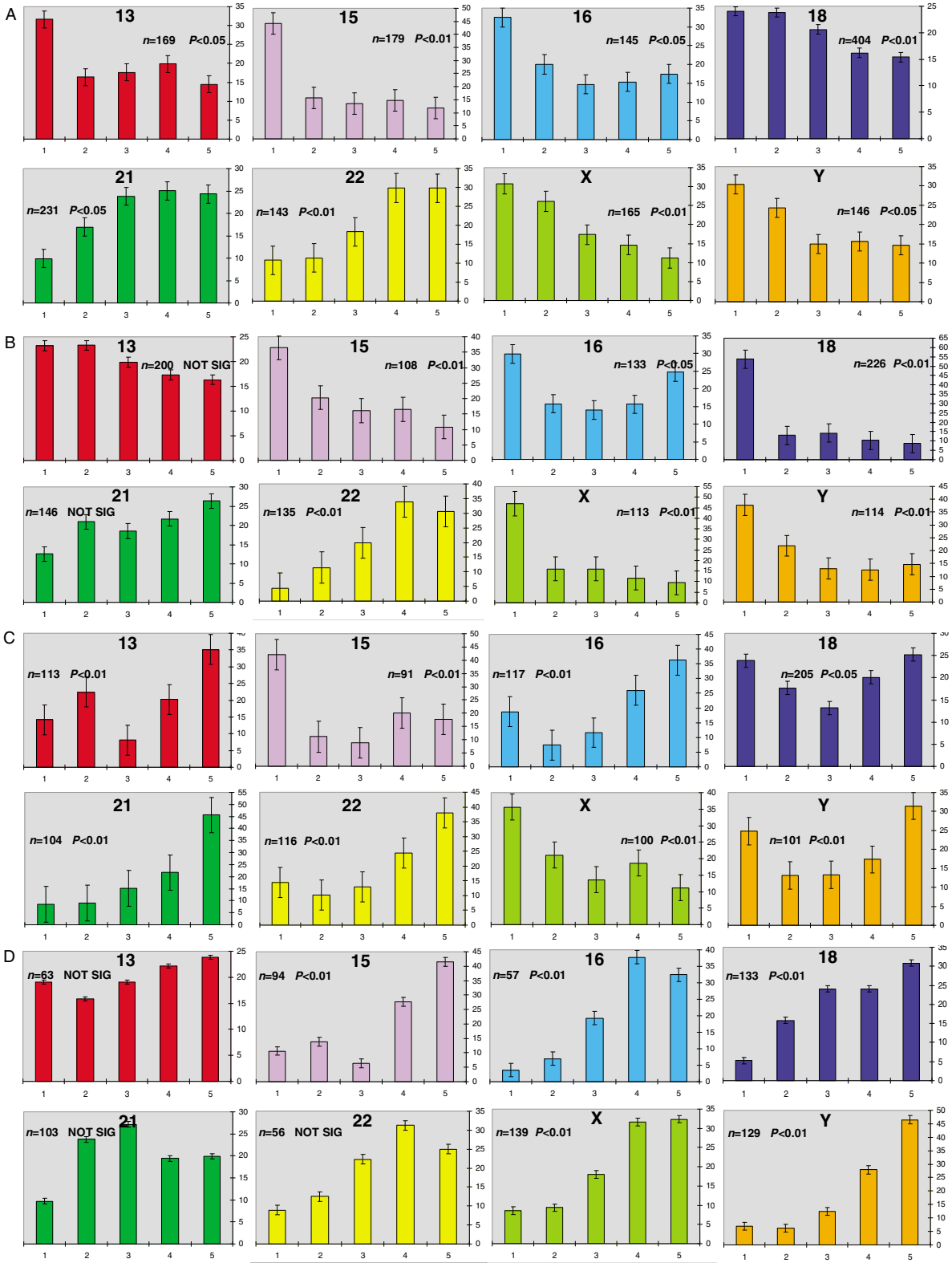

Fig. 2. See next page for legend.

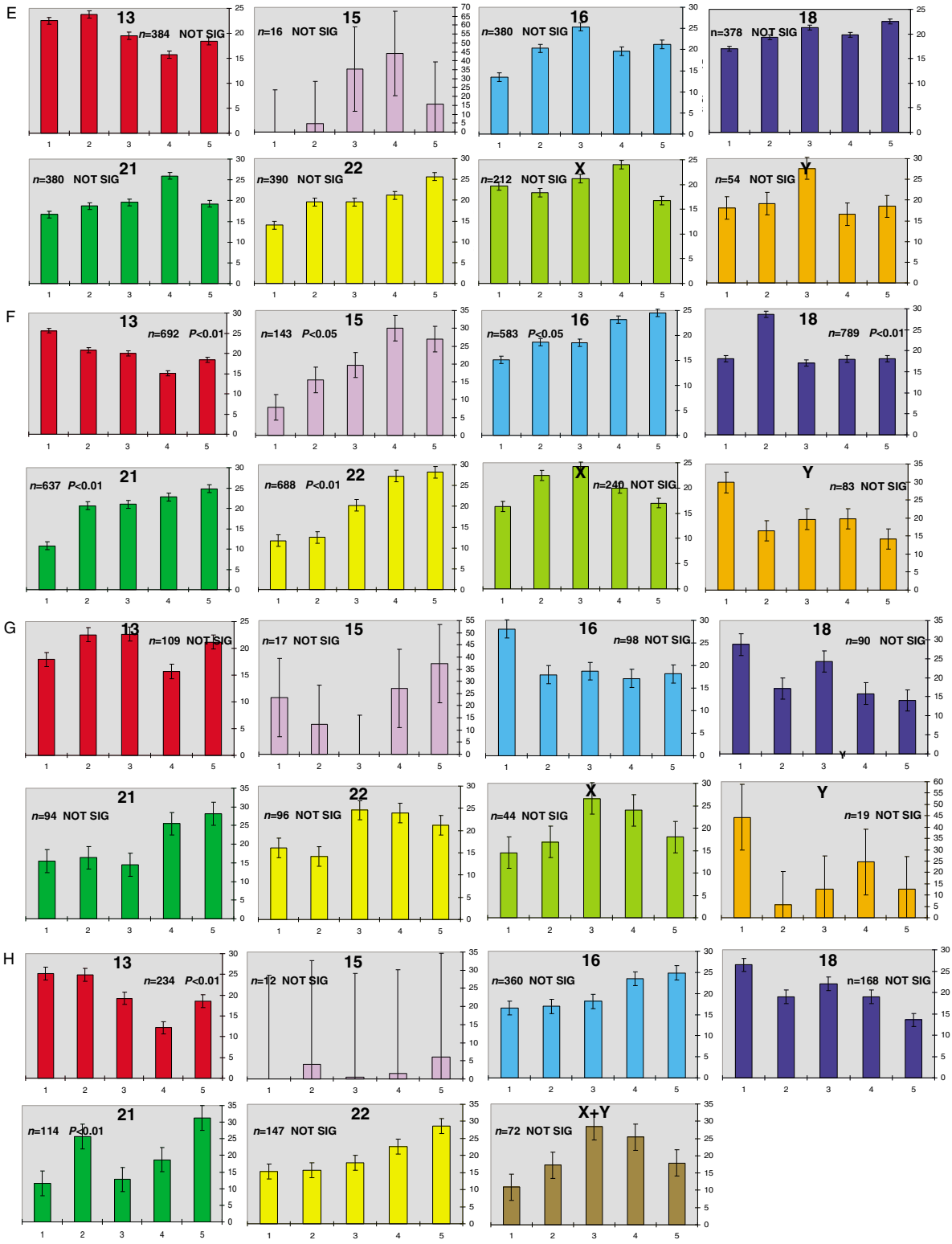

Fig. 2. The distribution of the eight loci (13q14, 15c, 16q12, 18c, 21q22, 22q11.2, Xc and Yc) in the nuclei of various cell types. The *y*-axis represents the percentage of signals represented in each shell (1-5) after normalisation to take into account the nuclear counterstain (excluding sperm). Shell 1 (*x*-axis) represents the outermost shell and shell 5 the innermost (see Fig. 1). Thus, a graph with the highest peak in shell 1 compared with the other shells represents a signal found most often at the nuclear periphery; a graph with the highest peak in shell 5 represents a signal found most often at the nuclear centre. *n*, the number of signals scored. *P* values represent the results of chi-squared tests to establish whether a significant non-random pattern could be identified. $P<0.05$, significantly non-random ($P<0.01$, highly significant); NOT SIG, not significant (i.e. $P>0.05$). (A) Normal lymphocyte nuclei. (B) Nuclei of trisomy 13 lymphoblastoid cells. (C) Nuclei of cultured cells from amniotic fluid (chromosomally normal). (D) Sperm-head nuclei (chromosomally normal, fertile male). (E) Blastomere nuclei with no detectable abnormality (NDA group). (F) Aneuploid blastomere nuclei. (G) Chaotic blastomere nuclei. (H) Blastomeres trisomic for the chromosome measured (e.g. position of chromosome-13 locus in trisomy-13 cells).

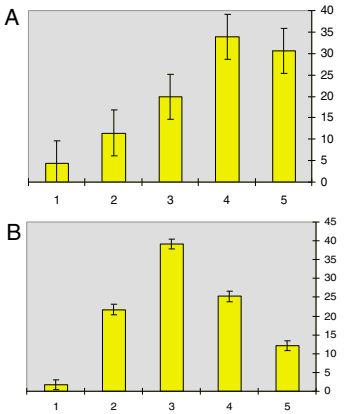

Fig. 3. (A) The distribution of locus 22q11.2 in our data set (see also Fig. 2b). A central location is apparent. (B) The same data set analysed according to McKenzie et al. (McKenzie et al., 2004) – the graph suggests a more medial location. *x*-axis depicts the shell number.

Nuclear addresses in control cells compared to previous studies

Results of previous studies of nuclear organisation that involved the detection of whole-chromosome territories of proliferating lymphoblast and fibroblast nuclei (e.g. Croft et al., 1999; Boyle et al., 2001; Cremer et al., 2003; Meaburn et al., 2005a; Mayer et al., 2005) and our current data set on control nuclei (which involve the detection of specific loci, e.g. centromeres) are not necessarily comparable given the differences in cell types and probes used. Nevertheless, there are interesting similarities – this should not be entirely unexpected, because the loci of interest in this study should, by definition, lie within or near the chromosome territory from which they derive. Previous papers report whole-chromosome territories X, Y, 13 and 18 as peripherally located, chromosome territories 16 and 21 as in an intermediate location, and 15 and 22 territories as more interior (Croft et al., 1999; Boyle et al., 2001; Cremer et al., 2003; Meaburn et al., 2005a; Mayer et al., 2005). Our data for individual loci are largely consistent with this pattern, with the exception of chromosome 16, in which we identified a more peripheral location. This discrepancy might be explained by the fact that the probe itself hybridises to a large heterochromatic block below the centromere that might be expected to occupy a more peripheral position relative to the rest of the territory. For the other loci, however, the pattern of locus position and the fact that they were mostly comparable with previous studies gave us sufficient confidence that our approach was a robust one to apply to blastomeres. In trisomy-13 lymphoblasts it was noteworthy that one of the chromosomes on which we did not identify a non-random distribution was the chromosome (13) that was involved in the trisomy. To the best of our knowledge, this is the first time that such an association has been seen and warrants further investigation using multiple loci and greater numbers of cells. The results also provide evidence for interesting differences in nuclear organisation in amniocytes, in which 13q14 is more centrally located than in lymphocytes, and centromeric loci on chromosomes 18 and Y are unusually bimodal, with the highest peaks in both shells 1 and 5. One possible explanation for this distribution is that amniocytes are a mixed population of cells with some nuclei more similar to lymphocytes in terms of their nuclear organisation but others more chromocentric, at least for chromosomes 18 and Y. For the sperm cells, our results are consistent with those of Zalenskaya and Zalensky (Zalenskaya and Zalensky, 2004), who reported a chromocentre pattern of nuclear organisation in human sperm.

Nuclear organisation of totipotent blastomeres compared to committed cells

The major finding of this work is the suggestion that nuclei that are known to be aneuploid show a pattern of nuclear organisation that is similar to that seen in committed cells; i.e. clear nuclear addresses can be determined. Such an arrangement, however, was not observed for nuclei in which no chromosome abnormality could be found (NDA group), suggesting a unique pattern of nuclear organisation. One possible interpretation of these findings, therefore, is that totipotent, chromosomally normal nuclei are characterised by a more 'relaxed' state of nuclear organisation and that copy-number change is also accompanied by an early entry into a state more closely associated with committed cells. If this is the case, then future nuclear transfer and preimplantation protocols might need to take into account issues of 'nuclear health', such as nuclear organisation, in addition to simple copy-number differences.

Our findings for chaotic nuclei are indicative of a random pattern of nuclear organisation. It is possible that there are too few nuclei on which to generate statistically significant results (e.g. for centromeres of chromosomes 15 and Y); however, it seems more likely that a chaotic cell would not have a discernable pattern of nuclear organisation because of the fact that (as revealed by G-banding studies) chromosomes are not only grossly aneuploid but severely structurally rearranged and different in karyotype from cell to cell (Clouston et al., 2002).

Our data are not consistent with a previous similar study (McKenzie et al., 2004), in which identical probes to our own were used for chromosomes 13, 18, 21, 22, X and Y, as well as, like us, embryos at 3-days to 4-days post-fertilisation. The authors purported that these chromosomes all occupy the same nuclear compartment, a model that, in itself, we find biologically implausible given the existing literature (e.g. Croft et al., 1999; Boyle et al., 2001; Cremer et al., 2003; Meaburn et al., 2005a; Mayer et al., 2005; Foster and Bridger, 2005; Bridger et al., 2007). Furthermore, it is our belief that this disparity may have arisen because the McKenzie et al. study (McKenzie et al., 2004) did not take into consideration the difference in amount of DNA found in the different regions of the nuclei. That is, they did not normalise their data for chromatin distribution from the edge to the interior: in a flattened nucleus, the nuclear interior contains much more chromatin than the outer regions and thereby increases the probability of a signal being located in the nuclear interior. Rather, they created domains on the basis of circles with regularly increasing diameter sizes (e.g. 1, 2, 3, 4 arbitrary units), leading to shells of differing area; thus, because of its relatively small size, very few signals were seen in the innermost shell. In order to test this hypothesis, we analysed the method described in their paper and applied it as closely as we could to our control human lymphocytes. By way of example, for 22q22.1, using their approach, the locus clearly appears in a more medial position (predominantly in shell 3), which is not consistent with our data, nor that in the pre-existing literature, which overwhelmingly suggests that chromosome 22 is centrally located. McKenzie et al. (McKenzie et al., 2004) also claimed that presence of an extra copy of a chromosome affected a shift towards the nuclear periphery of the corresponding chromosome territory. It the first instance, it is worth noting that, in these assays [both from this study and that of McKenzie et al. (McKenzie et al., 2004)], it is impossible to distinguish pure trisomy (i.e. where only one single chromosome is present in an extra copy) and cells that

have several rearrangements, because only about a third of the chromosome complement is analysed. Results should be viewed with caution, therefore, until a larger number of the chromosomes have been analysed simultaneously. It is perhaps worth mentioning, however, that there was no clear instance in which there was a significant non-random location in Fig. 2H that was different to a corresponding non-random location in Fig. 2E-G. The possible exception to this is 21q22; however, it is debatable whether a bimodal distribution (Fig. 2H) with peaks in shells 2 and 5 is noticeably different from the pattern seen in Fig. 2F for 21q22. In other words, it will be some time before we can make grandiose claims about the presence of and extra or missing chromosome affecting a significant change in the nuclear position of that chromosome compared to a disomic equivalent.

There are interesting parallels to be made from comparison of our work with recent work by Martin et al. (Martin et al., 2006a; Martin et al., 2006b). The first study (Martin et al., 2006a) observed chromocentre formation, describing, at the one-cell stage, a typical pattern seen in mouse cells, with centromeres locating towards the nuclear centre. We found no discernable non-random distribution for any centromere in NDA and chaotic nuclei (as well as for the sex-chromosome centromeres in the aneuploid group). However, in our data set for aneuploid cells, the centromere of chromosome 18 was more peripheral but 15c and 16q12 displayed a central position, suggesting some evidence for a chromocentre in human blastomeres for certain chromosomes. Although the Martin et al. (Martin et al., 2006a) study compared cleavage-stage embryos a few days apart from those used in our own study, our two sets of data might have some parallels because they suggested that, coincident with the activation of the embryonic genome, major rearrangements occurred that were completed by the blastocyst stage (including some previously late replicating pericentric heterochromatin changing its timing and nuclear position). Moreover, the patterns seen in our data set for the centromeres (NDA nuclei) do indeed represent a random distribution. Therefore, our results indicate related findings to those of Martin et al. (Martin et al., 2006a) in that we also demonstrated differences in nuclear distribution between totipotent and committed cells. It remains to be seen whether one- to two-cell human embryos adopt a chromocentre pattern, as do human sperm. Martin et al. (Martin et al., 2006b) also established that embryos derived from transfer of embryonic stem cell metaphasic chromosomes into mouse ooplasm adopt an embryonic-like type of nuclear organisation; therefore, assaying for nuclear organisation might well play a role in nuclear-transfer experimentation in humans.

Possible criticisms

One possible criticism of our work is that embryos generated by IVF might well not reflect the in vivo situation because of artificial in vitro culture conditions and the fact that the embryos were generated from hyper-stimulated ovaries. In the absence of sufficient naturally conceived embryos generated in vivo, however, the results presented here represent a unique and valuable insight into genomic events in the earliest stages of human development. A further criticism is that this is one of the few studies that, rather than analysing whole-chromosome territories, has selected individual loci solely on the basis that images designed to determine copy-number differences were available for analysis. Given that the ethical framework in the UK and most other countries largely precludes the use of fluorescent in situ hybridisation (FISH) on human embryos for reasons other than development or application of diagnostic techniques, then analysis in this way is the only current option. Moreover, as Martin et al. (Martin et al., 2006a; Martin et al., 2006b) demonstrated, examination of the position of specific sequences such as centromeres in this context might well be informative in their own right. Indeed, analysis of individual loci might even be advantageous because, given the complexity of whole-chromosome paints, single loci might make it easier to see changes between cell types. Of course, we also cannot discount the possible effects of the preparation protocols (e.g. hypotonic KCl, HCl, pepsin, paraformaldehyde, formamide, temperature etc.) on nuclear organisation; however, all the cells were treated comparably. In this regard, it could be argued that the majority of microscopy specimens undergo some form of pre-preparation that could influence the interpretation of the data adversely. The fact that, in this study, statistically significant results were produced that compare favourably to pre-existing literature suggests that nuclear organisation was not sufficiently adversely affected to preclude meaningful analysis.

Conclusions and future prospects

The suggestion in this study of an association between normal preimplantation development and nuclear position might indicate sufficient clinical significance that should then, in turn, prompt ethical justification of further investigation of nuclear organisation in human blastomeres in a prospective, rather than a retrospective, way. If/when this is achieved, we will be in a position to study this interesting phenomenon more fully and investigate whether or not it has a role in clinical procedures such as preimplantation diagnosis and nuclear transfer. One obvious initial application of our findings, however, is the realisation that some chromosomes more commonly occupying the nuclear periphery (e.g. chromosome 18) are more likely to be scored as aneuploid than one at the nuclear centre (e.g. chromosome 22) because of the increased likelihood of signal overlap in the latter.

To the best of our knowledge, this is the first study to make comparisons between human blastomeres with other developmental stages using the same probe set. In future experiments we would like (ethical permission accepting) to determine the presence of abnormality for all chromosomes using comparative genomic hybridisation (Wells and Delhanty, 2000) in a proportion of cells from pre-blastocyst embryos while performing FISH on the rest to assay for nuclear organisation. We will also focus on determining whether the origin of the abnormality (i.e. meiotic or post-zygotic) and the presence of chromosomal mosaicism have an impact on nuclear organisation.

Materials and Methods

Control cells: source and preparation

Human lymphocyte and sperm nuclei preparations were made from a chromosomally normal, fertile male with informed consent. Lymphocyte stimulation was achieved through a 72-hour culture of whole blood in KaryoMax Medium (Gibco) (containing phytohaemaglutinin) and preceded colcemid (40 minutes) then hypotonic (75 mM KCl) treatment for 15 minutes at 37°C. Fixation in 3:1 methanol:glacial-acetic-acid (three changes) preceded immobilisation on a glass slide and air-drying. The sperm sample was washed in Tris Buffer (0.1 M, pH 8) three times and fixed in 3:1 methanol:glacial-acetic-acid before immobilisation and air-drying on a glass slide. A rapidly dividing human trisomy-13 lymphoblastoid cell line was colcemid- and hypotonic (75 mM KCl)-treated in a similar way to the lymphocytes and preparations of cultured amniocytes (taken at 15-weeks gestation) fixed in 3:1 methanol:glacial-acetic-acid were a kind gift from Zoë Docherty of Guys and St Thomas's Hospital Trust Cytogenetic Unit (London, UK). These were also air-dried onto glass slides.

Human embryos: source and consent

Human embryos used in this study were from patients undergoing preimplantation genetic screening for aneuploidy at The London Bridge Fertility Centre (LBFC) and

The University College London (UCL) Centre for Preimplantation Genetic Diagnosis. Levels of blastomere aneuploidy and pregnancy rates are broadly similar between both centres. Pre-existing images from biopsied embryos used for clinical screening purposes and blastomeres from zygotes surplus to embryo-transfer requirements formed the basis of this study. Due to constraints imposed by ethical permission framework, only images of cells that had been previously processed for diagnostic purposes could be used for analysis. All patients gave informed consent for the use of their embryos for research purposes and this work was approved under the auspices of the treatment licence awarded by the HFEA to the London Bridge Fertility Centre and the Research Licence awarded to the UCL Department of Obstetrics and Gynaecology. The Local Research and Ethics committee of the University of Kent have also approved this work.

Human embryos: preparation for cytogenetic analysis

Preimplantation embryos were generated by IVF using standard protocols and embryo biopsy was carried out as previously described, removing one or two cells per embryo that were then processed for FISH analysis (Chatzimeletiou et al., 2005a). Following selection of embryos that appeared chromosomally normal on the basis of the diagnosis from the biopsied cells, embryo transfer proceeded. Embryos not transferred were processed for FISH analysis on day 4. Thus, all images were from cleavage-stage blastomeres at day-3 or -4 post-fertilisation; none of the embryos examined had reached the blastocyst stage.

Embryo nuclei were fixed to poly-L-lysine-coated glass slides through exposure to 0.01 M HCl and 0.1% Tween 20 as described previously (Harper et al., 1994; Chatzimeletiou et al., 2005a). Regular replenishing of this solution until the point that the zona pellucida (if present) and cytoplasm were dissolved preceded air-drying onto the poly-L-lysine slide then PBS washing and dehydration in an ethanol series.

FISH

FISH experiments occurred in two 'layers', i.e. with one experiment using one set of multicolour probes, which (after image capture) were then removed in order to apply a second set. Briefly, all cells were exposed to 1% pepsin/HCl for 20 minutes (39°C), fixed in 4% paraformaldehyde (sperm cells were exposed to 0.1 M DTT for 20 minutes in order to swell the cells), dehydrated and air-dried. The pre-prepared probe was applied to the nuclear DNA under a coverslip, sealed, and both nuclear and probe DNA were denatured simultaneously using a HYBrite (Vysis) hotplate for 5 minutes at 75°C. Hybridisation at 37°C continued for 3-16 hours and post-hybridisation washes consisted of 0.4×SSC/0.3% Tween-20 (Sigma) at 73°C for 3 minutes and 1 minute in 2×SSC/0.1% Tween-20 at room temperature before dehydration in an alcohol series. Slides were then air-dried, mounted in Vectashield anti-fade medium containing DAPI (Vector Laboratories, CA, USA). In order to wash the first layer of probe from the slide, the slides (after examination) were washed in 4×SSC/0.3% Tween-20 for 10 minutes at room temperature, then in PBS for 5 minutes followed by an ethanol series. FISH for the second layer of probes then proceeded as for the first with the addition of Vectashield anti-fade medium with DAPI (Vector Laboratories, CA, USA). The first layer used probes for chromosomes 13, 16, 18, 21 and 22 (Multivysion probe set) (LBFC) or LSI dual probe for 13 and 21 and CEP 18 alpha satellite (D18Z1) (UCL). The second layer used probes for chromosomes X, Y and 18 (part of the Aneuvysion probe set) (LBFC) or 15, 16 and 22 using CEP 16 (D16Z3), CEP 15 (D15Z1) and LSI 22 (UCL). All probes were from Abbott/Vysis UK. The probes for chromosomes 15, 16, 18, X and Y hybridised to peri-centromeric regions (chromosome 16 to 16q12, adjacent to the centromere), whereas the probes for chromosomes 13, 21 and 22 hybridised to regions 13q14, 21q22 and 22q11.2, respectively. It should be noted therefore that the FISH signals might not necessarily be representative of the whole-chromosome territory and thus results pertain to the probed regions alone. FISH images of blastomeres were captured using an epifluorescence microscope and either Cytovysion Software (Applied Imaging) or SmartCapture software (Digital Scientific UK) and exported as JPEG and/or TIFF files to Paint Shop Pro 9.1 for aneuploidy and locus-position analysis.

Scoring and interpretation

Signals were classified as representing separate hybridisation events if two or more similarly sized fluorescent foci could be identified that were farther apart than the diameter of one signal. If less than the diameter of one signal apart, the signal was classed as a 'split signal' and thus scored as a single hybridisation event. Cells in which no chromosomal aneuploidy was observed for the chromosomes assayed were classified 'NDA' (no detectable abnormality). This of course raises the issue of the likelihood of whether these cells are indeed euploid for the rest of the karyotype. On face value, given that only around a quarter of the chromosome complement was examined for copy-number change, then this would imply that the majority of chromosomally abnormal nuclei are not detected by this approach. However, in both this study and in the majority of the literature, approximately 50-70% of human blastomeres are identified as chromosomally abnormal when only around 5-9 chromosome pairs are assayed (e.g. Delhanty et al., 1997; Munné and Cohen, 1998; Daphnis et al., 2005). A simplistic extrapolation would therefore suggest that all human blastomeres are chromosomally abnormal; nevertheless, this cannot be true because the success rate of euploid live births after preimplantation genetic screening (PGS) is 20-25%. This is in good agreement with data from comparative genomic hybridisation (CGH) analysis of every cell from two series of cleavage-stage embryos in which the copy number of all chromosomes was assayed (Voullaire et al., 2000; Wells and Delhanty, 2000). In both series, 25% of embryos were completely euploid. The most likely explanation therefore is that blastomeres aneuploid for one chromosome pair have an increased likelihood of being aneuploid for one or more others. In other words, a more reasonable extrapolation from existing data would suggest that blastomeres with no detected abnormality for 6-7 chromosome pairs (the NDA category in this study) have a 60-80% chance of being chromosomally normal for the whole of the karyotype. Here, we made comparisons with nuclei in which at least one chromosomal abnormality was detected, which, by definition, have a 0% chance of being chromosomally normal. Chromosomally abnormal cells were further sub-divided into those that had simple aneuploidy (one, two or three copy-number changes) and grossly aneuploid nuclei, in which four or more aneuploidies were detected. Analysis of other cells from the same embryo (where present; data not shown) strongly suggests that this latter category were, for the most part, from 'chaotic' embryos by virtue of the fact that the cells were grossly abnormal and came from embryos that had multiple chromosome abnormalities with no discernable cytogenetic mechanism to explain the abnormality (Delhanty et al., 1997). For the purposes of brevity, these are classified as 'chaotic'. Evidence suggests that such nuclei also carry numerous structural as well as numerical abnormalities (Clouston et al., 2002). Finally, analysis was performed on nuclei from which that particular chromosome was involved in the abnormality; for instance, the position of the chromosome-18 centromere was assayed in trisomy-18 cells.

Analysis categories

The analysis categories were therefore as follows:

(1) Karyotypically normal human lymphocytes
(2) Trisomy-13 lymphoblasts
(3) Cultured amniocytes (16-weeks gestation)
(4) Sperm cells
(5) NDA blastomeres (which we estimate have a 60-80% chance of being chromosomally normal – see above)
(6) Aneuploid nuclei (with one, two or three copy-number changes – see above)
(7) Chaotic nuclei (with four or more abnormalities – see above)
(8) Nuclei trisomic for chromosomes 13, 15, 16, 18, 21, 22 and sex chromosomes. In this case, only the chromosome involved in the trisomy was assayed.

Analysis of locus position in interphase nuclei

In order to address the hypotheses set out in the Introduction, we used adaptations of previously published approaches for 3D extrapolations from 2D data, analysing captured images from the above preimplantation screening cases (using our eight locus-specific probes); drawing comparisons between equivalent experiments on nuclei from a range of committed cells.

An approach very similar to that of Croft et al. (Croft et al., 1999) and Boyle et al. (Boyle et al., 2001) was used to assess chromosome location using 2D images. Within Paint Shop Pro 9.1, a transparent five-ringed template was created that had five concentric 'shells' of equal area. This was then transposed over the image of the blastomere nucleus and scaled for size and shape (which was roughly circular in both flattened lymphocytes and blastomeres) in order to best fit each individual nucleus (Fig. 1). Signals for each chromosome were assigned according to the shell in which they were seen: shell 5 being innermost and shell 1 being at the periphery. In cases in which a signal was seen to span two or more shells, it was assigned according to where the majority of signal was observed.

Statistical analysis

In order to address the issue of whether the distributions seen were consistent with a non-random pattern, we first determined the relative density of the DNA counterstain in each shell by densitometry measurements of images of DAPI-stained nuclei. On average, in blastomere nuclei for instance, the mean of the relative DAPI densities was 8.2, 15.5, 21.5, 25.6 and 29.2% for shells 1-5, respectively. In other words, we surmised that, if the distribution of 100 signals in a nucleus were random, we would expect to see, on average, 8.2, 15.5, 21.5, 25.6 and 29.2 signals in shells 1-5, respectively. In testing the null hypothesis that distributions were not significantly different from what would be observed from a random pattern, our 'expected' values for each shell were derived from the above numbers adjusted for the number of signals that were actually scored (e.g. if 200 signals were observed, the expected number of signals in a random distribution would be 16.4, 31.0, 43.0, 50.1 and 58.4 for shells 1-5, respectively). For each locus in each cell type, our raw data (i.e. the number of signals that were actually scored) constituted our 'observed' values. From there, a chi-squared test was used to determine the presence of non-random distributions. Given that there were five individual values (one for each shell) that made up the subsequent chi-squared total, P-values were determined at four degrees of freedom. Distributions were considered significantly different when compared to the nuclear counterstain at $P \leq 0.05$ and highly significantly different when $P \leq 0.01$. Distributions significantly different from those predicted by the nuclear counterstain measurements were thus determined statistically by comparing the observed value of the number of signals seen in each shell with the expected value of the signal distribution as determined by the DAPI density measurements. Of course. DAPI densities (and hence

expected values) differed between cells and cell types – e.g. from sperm cells, measurements revealed highly DAPI-dense nuclei with little discrimination between nuclear periphery and centre. This approach is a minor adaptation of one with precedent from several studies in the literature (e.g. Croft et al., 1999; Bridger et al., 2000; Boyle et al., 2001; Meaburn et al., 2005a).

Histogram presentation of results

In order to present histograms that reflected deviations (or otherwise) from the random pattern predicted by the nuclear counterstain (e.g. ones in which a random distribution would appear as five bars of equal height), raw data was normalised according to the relative density of the DAPI counterstain (see Figs 2 and 3). Relative positions were therefore initially calculated as 'standard arbitrary units' (i.e. total number of signals observed divided by the relative density of the DAPI counterstain) then converted to '% relative observed frequency' in order to allow direct comparisons between graphs (Figs 2 and 3). Therefore, in addition to the chi-squared data, the shape of the graphical distributions was taken into account, including analysis of standard error of the mean (s.e.m.) (as depicted by the standard error bars in the histograms – Figs 2 and 3).

References

Alcobia, I., Dilao, R. and Parreira, L. (2000). Spatial associations of centromeres in the nuclei of hematopoietic cells: evidence for cell-type-specific organizational patterns. *Blood* **95**, 1608-1615.

Bartova, E., Harnicarova, A., Pachernik, J. and Kozubek, S. (2005). Nuclear topography and expression of the BCR/ABL fusion gene and its protein level influenced by cell differentiation and RNA interference. *Leuk. Res.* **29**, 901-913.

Bickmore, W. A. and Bridger, J. M. (1999). A sense of time and place-meeting report of HUGO Spatial Organisation of the Genome workshop, Edinburgh, 14-15 May 1999. Human Nuclear organisation. *Chromosome Res.* **7**, 425-429.

Bolzer, A., Kreth, G., Solovei, I., Koehler, D., Saracoglu, K., Fauth, C., Muller, S., Eils, R., Cremer, C., Speicher, M. R. et al. (2005). Three-dimensional maps of all chromosomes in human male fibroblast nuclei and prometaphase rosettes. *PLoS Biol.* **3**, e157.

Boyle, S., Gilchrist, S., Bridger, J. M., Mahy, N. L., Ellis, J. A. and Bickmore, W. A. (2001). The spatial organization of human chromosomes within the nuclei of normal and emerin-mutant cells. *Hum. Mol. Genet.* **10**, 211-219.

Bridger, J. M., Boyle, S., Kill, I. R. and Bickmore, W. A. (2000). Re-modelling of nuclear architecture in quiescent and senescent human fibroblasts. *Curr. Biol.* **10**, 149-152.

Bridger, J. M., Foeger, N., Kill, I. R. and Herrmann, H. (2007). The nuclear lamina – a structural framework involved in nuclear organisation. *FEBS J.* **274**, 1354-1361.

Brown, K. E., Baxter, J., Graf, D., Merkenschlager, M. and Fisher, A. G. (1999). Dynamic repositioning of genes in the nucleus of lymphocytes preparing for cell division. *Mol. Cell* **3**, 207-217.

Chambeyron, S. and Bickmore, W. A. (2004). Chromatin decondensation and nuclear reorganization of the HoxB locus upon induction of transcription. *Genes Dev.* **18**, 1119-1130.

Chatzimeletiou, K., Morrison, E. E., Panagiotidis, Y., Prapas, N., Prapas, Y., Rutherford, A. J., Grudzinskas, G. and Handyside, A. H. (2005a). Comparison of effects of zona drilling by non-contact infrared laser or acid Tyrode's on the development of human biopsied embryos as revealed by blastomere viability, cytoskeletal analysis and molecular cytogenetics. *Reprod. Biomed. Online* **11**, 697-710.

Chatzimeletiou, K., Morrison, E. E., Prapas, N., Prapas, Y. and Handyside, A. H. (2005b). Spindle abnormalities in normally developing and arrested human preimplantation embryos in vitro identified by confocal laser scanning microscopy. *Hum. Reprod.* **20**, 672-682.

Chuang, C. H. and Belmont, A. S. (2005). Close encounters between active genes in the nucleus. *Genome Biol.* **6**, 237.

Clouston, H. J., Herbert, M., Fenwick, J., Murdoch, A. P. and Wolstenholme, J. (2002). Cytogenetic analysis of human blastocysts. *Prenat. Diagn.* **22**, 1143-1152.

Cremer, M., von Hase, J., Volm, T., Brero, A., Kreth, G., Walter, J., Fischer, C., Solovei, I., Cremer, C. and Cremer, T. (2001). Non-random radial higher-order chromatin arrangements in nuclei of diploid human cells. *Chromosome Res.* **9**, 541-567.

Cremer, M., Kupper, K., Wagler, B., Wizelman, L., von Hase, J., Weiland, Y., Kreja, L., Diebold, J., Speicher, M. R. and Cremer, T. (2003). Inheritance of gene density-related higher order chromatin arrangements in normal and tumor cell nuclei. *J. Cell Biol.* **162**, 809-820.

Cremer, T. and Cremer, C. (2001). Chromosome territories, nuclear architecture and gene regulation in mammalian cells. *Nat. Rev. Genet.* **2**, 292-301.

Cremer, T. and Cremer, C. (2006). Rise, fall and resurrection of chromosome territories: a historical perspective. Part I. The rise of chromosome territories. *Eur. J. Histochem.* **50**, 161-176.

Croft, J. A., Bridger, J. M., Boyle, S., Perry, P., Teague, P. and Bickmore, W. A. (1999). Differences in the localization and morphology of chromosomes in the human nucleus. *J. Cell Biol.* **145**, 1119-1131.

Daphnis, D. D., Delhanty, J. D., Jerkovic, S., Geyer, J., Craft, I. and Harper, J. C. (2005). Detailed FISH analysis of day 5 human embryos reveals the mechanisms leading to mosaic aneuploidy. *Hum. Reprod.* **20**, 129-137.

Delhanty, J. D., Harper, J. C., Ao, A., Handyside, A. H. and Winston, R. M. (1997). Multicolour FISH detects frequent chromosomal mosaicism and chaotic division in normal preimplantation embryos from fertile patients. *Hum. Genet.* **99**, 755-760.

Diblik, J., Macek, M., Sr, Magli, M. C., Krejci, R. and Gianaroli, L. (2005). Topology of chromosomes 18 and X in human blastomeres from 3- to 4-day-old embryos. *J. Histochem. Cytochem.* **53**, 273-276.

Foster, H. A. and Bridger, J. M. (2005). The genome and the nucleus: a marriage made by evolution. Nuclear organisation and nuclear architecture. *Chromosoma* **114**, 212-229.

Foster, H. A., Abeydeera, L. R., Griffin, D. K. and Bridger, J. M. (2005). Non-random chromosome positioning in mammalian sperm nuclei, with migration of the sex chromosomes during late spermatogenesis. *J. Cell Sci.* **118**, 1811-1820.

Galiova, G., Bartova, E. and Kozubek, S. (2004). Nuclear topography of beta-like globin gene cluster in IL-3-stimulated human leukemic K-562 cells. *Blood Cells Mol. Dis.* **33**, 4-14.

Griffin, D. K. (1996). The incidence, origin, and etiology of aneuploidy. *Int. Rev. Cytol.* **167**, 263-296.

Harper, J. C., Coonen, E., Ramaekers, F. C., Delhanty, J. D., Handyside, A. H., Winston, R. M. and Hopman, A. H. (1994). Identification of the sex of human preimplantation embryos in two hours using an improved spreading method and fluorescent in-situ hybridization (FISH) using directly labelled probes. *Hum. Reprod.* **9**, 721-724.

Hewitt, S. L., High, F. A., Reiner, S. L., Fisher, A. G. and Merkenschlager, M. (2004). Nuclear repositioning marks the selective exclusion of lineage-inappropriate transcription factor loci during T helper cell differentiation. *Eur. J. Immunol.* **34**, 3604-3613.

Kim, S. H., McQueen, P. G., Lichtman, M. K., Shevach, E. M., Parada, L. A. and Misteli, T. (2004). Spatial genome organization during T-cell differentiation. *Cytogenet. Genome Res.* **105**, 292-301.

Kosak, S. T., Skok, J. A., Medina, K. L., Riblet, R., Le Beau, M. M., Fisher, A. G. and Singh, H. (2002). Subnuclear compartmentalization of immunoglobulin loci during lymphocyte development. *Science* **296**, 158-162.

Kost-Alimova, M., Fedorova, L., Yang, Y., Klein, G. and Imreh, S. (2004). Microcell-mediated chromosome transfer provides evidence that polysomy promotes structural instability in tumor cell chromosomes through asynchronous replication and breakage within late-replicating regions. *Genes Chromosomes Cancer* **40**, 316-324.

Kuroda, M., Tanabe, H., Yoshida, K., Oikawa, K., Saito, A., Kiyuna, T., Mizusawa, H. and Mukai, K. (2004). Alteration of chromosome positioning during adipocyte differentiation. *J. Cell Sci.* **117**, 5897-5903.

Lukasova, E., Kozubek, S., Kozubek, M., Falk, M. and Amrichova, J. (2002). The 3D structure of human chromosomes in cell nuclei. *Chromosome Res.* **10**, 535-548.

Martin, C., Beaujean, N., Brochard, V., Audouard, C., Zink, D. and Debey, P. (2006a). Genome restructuring in mouse embryos during reprogramming and early development. *Dev. Biol.* **292**, 317-332.

Martin, C., Brochard, V., Migne, C., Zink, D., Debey, P. and Beaujean, N. (2006b). Architectural reorganization of the nuclei upon transfer into oocytes accompanies genome reprogramming. *Mol. Reprod. Dev.* **73**, 1102-1111.

Mayer, R., Brero, A., von Hase, J., Schroeder, T., Cremer, T. and Dietzel, S. (2005). Common themes and cell type specific variations of higher order chromatin arrangements in the mouse. *BMC Cell Biol.* **6**, 44.

McKenzie, L. J., Carson, S. A., Marcelli, S., Rooney, E., Cisneros, P., Torskey, S., Buster, J., Simpson, J. L. and Bischoff, F. Z. (2004). Nuclear chromosomal localization in human preimplantation embryos: correlation with aneuploidy and embryo morphology. *Hum. Reprod.* **19**, 2231-2237.

Meaburn, K. J., Levy, N., Toniolo, D. and Bridger, J. M. (2005a). Chromosome positioning is largely unaffected in lymphoblastoid cell lines containing emerin or A-type lamin mutations. *Biochem. Soc. Trans.* **33**, 1438-1440.

Meaburn, K. J., Parris, C. N. and Bridger, J. M. (2005b). The manipulation of chromosomes by mankind: the uses of microcell-mediated chromosome transfer. *Chromosoma* **114**, 263-274.

Munné, S. and Cohen, J. (1998). Chromosome abnormalities in human embryos. *Hum. Reprod. Update* **4**, 842-855.

Parada, L. A., Sotiriou, S. and Misteli, T. (2004). Spatial genome organization. *Exp. Cell Res.* **296**, 64-70.

Scheuermann, M. O., Tajbakhsh, J., Kurz, A., Saracoglu, K., Eils, R. and Lichter, P. (2004). Topology of genes and nontranscribed sequences in human interphase nuclei. *Exp. Cell Res.* **301**, 266-279.

Sengupta, K., Upender, M. B., Barenboim-Stapleton, L., Nguyen, Q. T., Wincovitch, S. M., Sr., Garfield, S. H., Difilippantonio, M. J. and Ried, T. (2007). Artificially introduced aneuploid chromosomes assume a conserved position in colon cancer cells. *PLoS ONE* **2**, e199.

Stadler, S., Schnapp, V., Mayer, R., Stein, S., Cremer, C., Bonifer, C., Cremer, T. and Dietzel, S. (2004). The architecture of chicken chromosome territories changes during differentiation. *BMC Cell Biol.* **5**, 44.

Sun, H. B., Shen, J. and Yokota, H. (2000). Size-dependent positioning of human chromosomes in interphase nuclei. *Biophys. J.* **79**, 184-190.

Upender, M. B., Habermann, J. K., McShane, L. M., Korn, E. L., Barrett, J. C., Difilippantonio, M. J. and Ried, T. (2004). Chromosome transfer induced aneuploidy results in complex dysregulation of the cellular transcriptome in immortalized and cancer cells. *Cancer Res.* **64**, 6941-6949.

Voullaire, L., Slater, H., Williamson, R. and Wilton, L. (2000). Chromosome analysis of blastomeres from human embryos by using comparative genomic hybridization. *Hum. Genet.* **106**, 210-217.

Wells, D. and Delhanty, J. D. (2000). Comprehensive chromosomal analysis of human preimplantation embryos using whole genome amplification and single cell comparative genomic hybridization. *Mol. Hum. Reprod.* **6**, 1055-1062.

Zalenskaya, I. A. and Zalensky, A. O. (2004). Non-random positioning of chromosomes in human sperm nuclei. *Chromosome Res.* **12**, 163-173.

Zink, D., Amaral, M. D., Englmann, A., Lang, S., Clarke, L. A., Rudolph, C., Alt, F., Luther, K., Braz, C., Sadoni, N. et al. (2004). Transcription-dependent spatial arrangements of CFTR and adjacent genes in human cell nuclei. *J. Cell Biol.* **166**, 815-825.