Research

Tissue-specific spatial organization of genomes

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Abstract

Background: Genomes are organized *in vivo* in the form of chromosomes. Each chromosome occupies a distinct nuclear subvolume in the form of a chromosome territory. The spatial positioning of chromosomes within the interphase nucleus is often nonrandom. It is unclear whether the nonrandom spatial arrangement of chromosomes is conserved among tissues or whether spatial genome organization is tissue-specific.

Results: Using two-dimensional and three-dimensional fluorescence *in situ* hybridization we have carried out a systematic analysis of the spatial positioning of a subset of mouse chromosomes in several tissues. We show that chromosomes exhibit tissue-specific organization. Chromosomes are distributed tissue-specifically with respect to their position relative to the center of the nucleus and also relative to each other. Subsets of chromosomes form distinct types of spatial clusters in different tissues and the relative distance between chromosome pairs varies among tissues. Consistent with the notion that nonrandom spatial proximity is functionally relevant in determining the outcome of chromosome translocation events, we find a correlation between tissue-specific spatial proximity and tissue-specific translocation prevalence.

Conclusions: Our results demonstrate that the spatial organization of genomes is tissue-specific and point to a role for tissue-specific spatial genome organization in the formation of recurrent chromosome arrangements among tissues.

Background

Chromosomes represent the largest structural units of eukaryotic genomes. The physically distinct nature of each chromosome is clearly visible during mitosis, when chromosomes condense and appear as separate entities. Chromosome-painting techniques have demonstrated that chromosomes are also physically separated during interphase, when each chromosome occupies a well defined nuclear subvolume, referred to as a chromosome territory [1,2]. The positioning of chromosomes during interphase is generally nonrandom [1,3,4]. In cells of plants with large genomes and of *Drosophila melanogaster*, centromeres and telomeres are positioned at opposite sides of the nucleus, giving rise to a chromosome arrangement known as the Rabl configuration [1,3,5]. In mammalian cells this pattern of genome organization is rare; instead, the spatial organization of chromosomes can be described by their radial positioning relative to the center of the nucleus [1,3,6]. In human lymphocytes, the radial positioning of chromosomes correlates with their gene density, with gene-dense chromosomes located towards the center of the nucleus and gene-poor chromosomes preferentially located towards the periphery [7,8].

Remarkably, the preferential radial positioning of at least two chromosomes, 18 and 19, has been evolutionarily conserved over 30 million years [9]. In addition to radial positioning, the nonrandom nature of genome organization is also reflected in the positioning of chromosomes relative to each other [10]. For example, in a lymphoma cell line derived from an ATM$^{-/-}$ mouse, two translocated chromosomes are preferentially positioned in close proximity to each other and the three chromosomes from which the translocations originated from a close-packed cluster in normal lymphocytes [10]. This type of nonrandom relative positioning has been proposed to facilitate formation of translocations by increasing the probability of illegitimate joining of broken chromosome ends of proximally positioned chromosomes [3,11,12].

While it is now well established that chromosomes are nonrandomly positioned [3,13,14], it is unclear how similar the spatial organization of the genome is in different tissues. Analysis of the radial positions of chromosomes 18 and 19 in different cell types failed to find significant differences [15]. Furthermore, a comparison of the distribution of several chromosomes in tissue-cultured fibroblasts and lymphoblasts gave mixed results: the position of several chromosomes appeared to be largely conserved between the two cell types, but on the other hand, chromosomes 6, 8, and 21 were positioned differently [7]. In both studies only radial positioning was used as a single indicator and distributions were not directly compared to each other by statistical means [7,15]. In an attempt to probe the spatial arrangement of chromosomes among tissues more systematically, we report here the comparative mapping of a subset of chromosomes in the cell nucleus of several cell types. From statistical analysis of several positioning criteria, including radial positioning, relative positioning, distance measurements and chromosome cluster analysis, we report evidence for tissue-specificity in the spatial organization of genomes.

Results and discussion

We sought to investigate the nuclear position of chromosomes 1, 5, 6, 12, 14 and 15 in a range of primary cells freshly isolated from mouse tissues. We visualized single chromosomes by fluorescence *in situ* hybridization (FISH) using chromosome-specific probes and analyzed their position in normal interphase cells containing a diploid complement of fluorescent signals (Figure 1). Freshly isolated and minimally cultured primary cell populations were used to prevent potential reorganization of chromosomes during prolonged *in vitro* culture. Qualitative inspection of the distribution of painted chromosomes indicated tissue specificity in chromosome positioning (Figure 1a). For example, chromosome 5 was preferentially found towards the center of the nucleus in liver cells, was predominantly peripheral in small and large lung cells, but was located in an intermediate position in lymphocytes (Figure 1a).

For quantitative analysis of positioning, we first measured the distance between the nuclear center and the center of mass of each chromosome signal as an indicator of its radial position in two-dimensional (2D) projections of three-dimensional (3D) image stacks as previously described (Figure 1b; see also Materials and methods) [8,11]. The distribution profiles of chromosomes showed considerable differences among tissues (Figure 1b). Statistical analysis of pairwise comparisons of the distribution of single chromosomes using contingency table analysis among all tissues revealed highly significant differential radial positioning (Figure 1c). Differential positioning in at least three cell types was found for all chromosomes analyzed (Figure 1b,c). Out of 71 pairwise comparisons, 34 were statistically significant at the $p < 0.05$ level (Figure 1c). Most cell types shared positioning of some, but not other chromosomes. For example, small lung cells and liver cells shared the position of chromosomes 12 and 14 (all p-values > 0.5), but not of chromosomes 5, 6 and 15 (Figure 1b,c; all p-values $< 3.1 \times 10^{-4}$). The most similar distribution of chromosomes was found in cell types sharing common differentiation pathways. Large and small lung cells shared the distribution of all chromosomes, and lymphoblasts and myeloblasts only differed in the positioning of chromosome 5 ($p = 0.02$) (Figure 1b,1c). As previously reported, the radial distribution within a cell type differed significantly among all chromosomes and most radial distributions were distinct from a uniform random distribution [7,8] (Figure 1b). Similar results were obtained in cells fixed with either paraformaldehyde or methanol. We conclude from these comparisons that the radial positioning of chromosomes within the interphase nucleus is tissue-specific.

To investigate the relative spatial relationship between chromosomes in different cell types, we first measured distances between the closest pair of nonhomologous chromosomes in each cell nucleus (Table 1, Figure 2). Absolute distances between chromosomes were measured in 2D projections of 3D image stacks and expressed as relative distances normalized to the nuclear diameter to take into account the variations in nuclear size among tissues. We find significant differences in the physical separation of six out of seven chromosome pairs among cell types (Table 1, Figure 2). For example, the closest homologs of chromosomes 12 and 14 were separated by 24.5% of the nuclear diameter in lymphocytes, whereas they were only separated by 19.4% in liver cells. This difference is statistically highly significant in a Kolmogorov-Smirnov test ($p = 1.1 \times 10^{-6}$). Similarly, chromosomes 5 and 6 were separated by 25.0% of nuclear diameter in small lung cells, but by only 17.7% in liver cells ($p = 1.3 \times 10^{-3}$) (Table 1, Figure 2). While most chromosome pairs had statistically distinct separation distances in several tissues, chromosomes 1 and 12 appeared to be more conserved in their relative positions (Figure 2). The differences in chromosome distances were not due to differences in the size or shape of the cell nucleus, as indicated by the shorter interchromosomal distances in kidney or large lung cells compared to lymphocytes,

whose nuclei are significantly smaller (Table 1) [15]. Furthermore, no correlation between distances between chromosomes and nucleus size was observed between large and small lung cells (Table 1).

A second, more direct criterion to test the relative positioning of chromosomes relative to each other was used. Chromosomes 12, 14 and 15 have previously been reported to have nonrandom relative positioning in lymphocytes where they form a close-packed triplet cluster [10]. We used the spatial clustering of these chromosomes to further probe the positioning of chromosomes relative to each other among tissues. For each tissue, we determined the percentages of cells containing: no 12-14-15 triplet clusters (Figure 3a); a single triplet cluster containing exactly one chromosome 12, 14 and 15 (Figure 3b); a single cluster made up of a pair of homologs and one additional chromosome (Figure 3c); or a cluster containing a pair of homologs and more than one additional chromosome (Figure 3d). As previously described, a cluster was defined as a triplet where all chromosomes are separated by less than 30% of the nuclear diameter [10]. Statistically significant quantitative differences in the occurrence of the four types of chromosome clusters were detected among tissues by contingency table analysis (Figure 3a-d, and see Additional data file 1).

Formation of distinct types of clusters was also evident by qualitative inspection (Figure 3e). Almost 50% of large and small lung cells contained no obvious clusters (Figure 3a). In contrast only around 20% of kidney and liver cells did not contain clusters (Figure 3a). About 30% of lymphocytes and myeloblasts did not contain clusters (Figure 3a). As previously reported, clusters containing exactly one copy of chromosome 12, 14 and 15 were prevalent in lymphocytes with 35% of cells containing such a cluster, but only 13% of liver cells, 17% of small lung cells and 19% of kidney cells contained such a cluster (Figure 3b; $0.01<p<0.05$). Clusters containing one homolog pair were more evenly distributed among tissues, but significant differences were still found. Almost 30% of liver cells, but only 12-15% of small and large lung cells and lymphocytes contained this type of arrangement (Figure 3c;

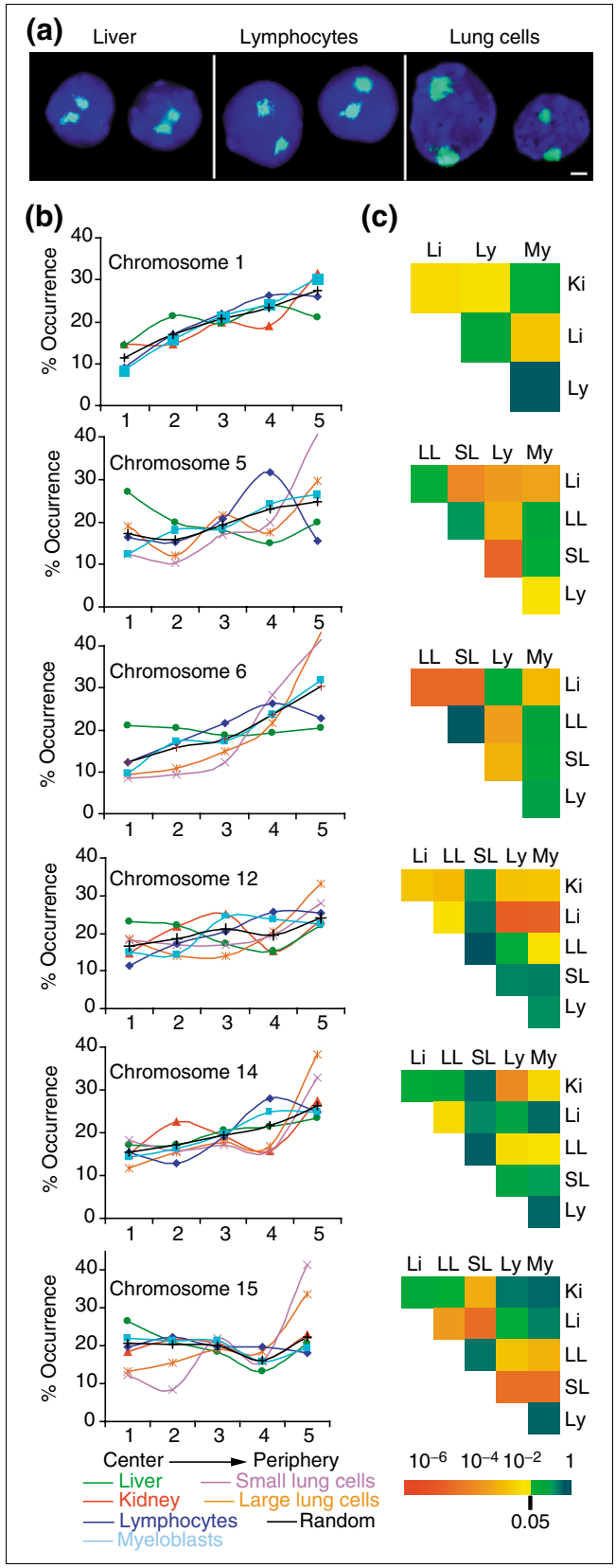

Figure 1

Figure 1
Tissue-specific radial positioning of chromosomes. **(a)** FISH analysis of chromosome 5 (green) in liver, lymphocytes and lung cell nuclei. DNA counterstaining with DAPI is in blue. Chromosome 5 is preferentially enriched in the nuclear interior in liver, within the medial nuclear subvolume in lymphocytes, and at the nuclear periphery in lung cells. Scale bar, 2 μm. **(b)** Quantitation of the radial distribution of chromosomes in different cell types. Data was binned in five concentric shells of equal volume designated 1 to 5 from the center of the nucleus to the periphery. **(c)** Pairwise comparison of chromosome radial distribution using contingency table analysis. *p*-values < 0.05 (yellow/red) were considered significant. Ki, kidney; Li, liver; LL, large lung cells; Ly, lymphoblasts; My, myeloblasts; SL, small lung cells. Between 41 and 180 cells were analyzed per tissue.

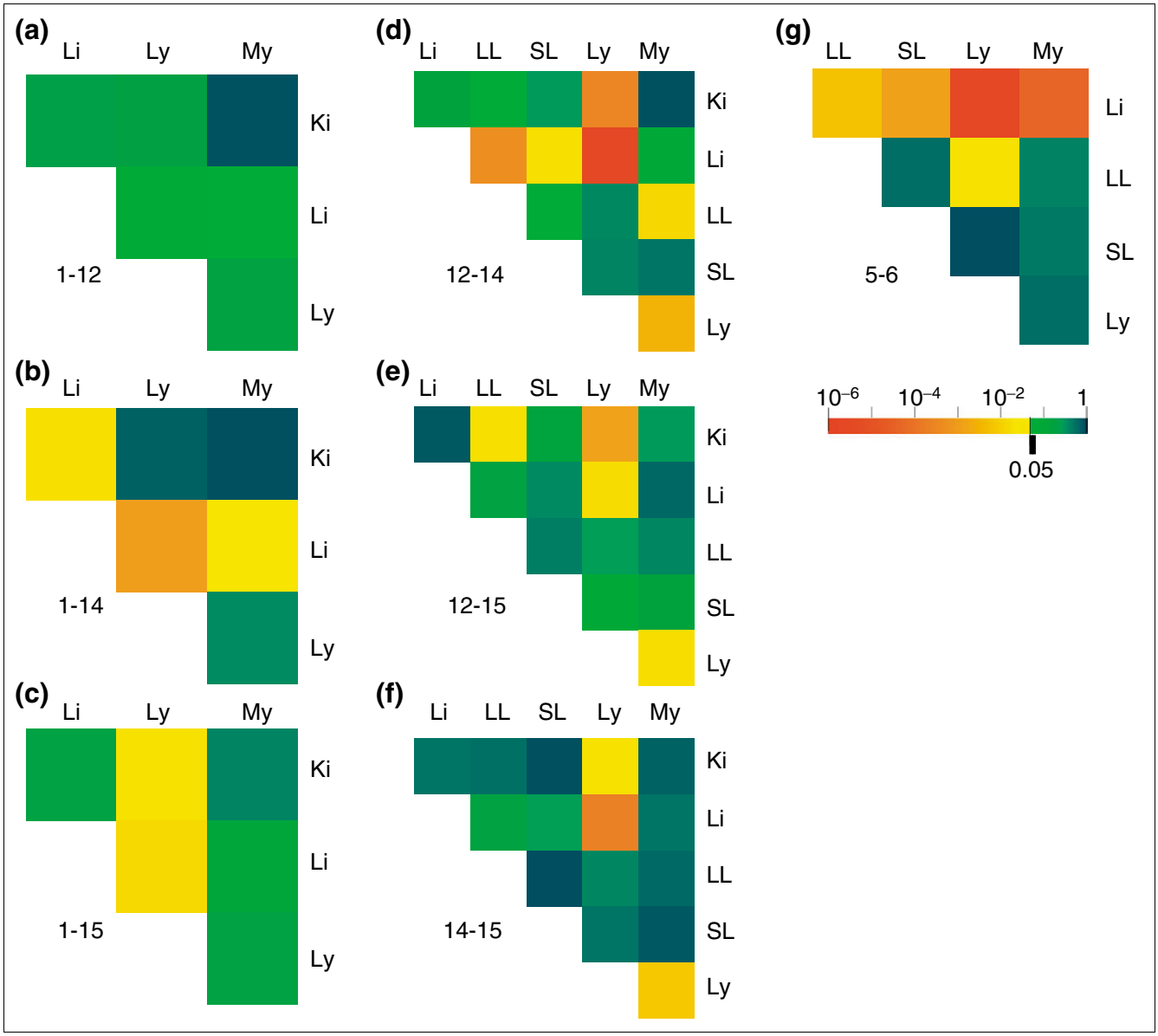

Figure 2
Tissue-specific distances between chromosomes. Average minimum separations between the most proximal pairs of nonhomologous chromosomes were compared pairwise using the Kolmogorov-Smirnov test. **(a)** Chromosome pair 1-12; **(b)** 1-14; **(c)** 1-15; **(d)** 12-14; **(e)** 12-15; **(f)** 14-15; **(g)** 5-6. *p*-values < 0.05 (yellow/red) were considered significant. Abbreviations as in Figure 1. Between 41 and 180 cells were analyzed per tissue.

$p < 0.03$ for all comparisons). Similarly, clusters simultaneously containing a nonhomolog and a homolog pair were present at differentially frequencies among tissues. They were found in around 36% of kidney and liver cells, but only in 11% of large lung cells ($4.2 \times 10^{-4} < p < 5.9 \times 10^{-4}$) and 15% in lymphocytes (Figure 3d; $0.08 < p < 0.09$). Statistical tests for triplet formation by contingency table analysis showed that the majority of triplets were found at frequencies different from those expected on the basis of a random distribution of chromosomes (Figure 3a-e, and see Additional data file 1).

To test whether these differences in chromosome clusters were limited to the 12-14-15 triplet or were a general feature, we analyzed clusters containing chromosomes 1-12-14, 1-14-15 and 1-12-15. Statistically significant differential occurrence of relative positioning of chromosomes in triplets was found for all of these chromosome combinations (see Additional data files 2-4). Similar results were obtained when close chromosome pairs were defined as separated by 20% of the nuclear diameter (data not shown). These observations strongly suggest that the positioning of chromosomes relative to each other differs among tissues.

Table 1

Relative interchromosome distances

		Average minimum separation* (% nuclear diameter)						
Tissue	Area† (μm²)	12-14 (n)	12-15 (n)	14-15 (n)	5-6 (n)	1-12 (n)	1-14 (n)	1-15 (n)
Kidney	252.1	21.7 (144)	20.3 (156)	21.6 (142)	ND	23.9 (134)	23.5 (120)	23.0 (132)
Liver	161.0	19.4 (166)	19.6 (158)	20.3 (167)	17.7 (83)	22.6 (94)	21.0 (103)	20.5 (95)
Lymphocytes	220.0	24.5 (110)	22.5 (88)	22.8 (105)	25.0 (118)	25.2 (94)	24.5 (111)	25.0 (89)
Myeloblasts	164.7	22.2 (157)	20.3 (138)	20.4 (180)	24.0 (124)	24.3 (109)	24.0 (151)	23.0 (132)
Large lung cells	241.9	23.5 (68)	21.1 (68)	21.1 (68)	23.3 (74)	ND	ND	ND
Small lung cells	128.7	20.8 (41)	21.8 (41)	20.8 (41)	25.0 (53)	ND	ND	ND

*Pairwise absolute measurements of distances between the closest pair of non-homologous chromosomes are expressed as relative distances normalized to the nuclear diameter to account for differences in nuclear size. †The nuclear area in an equatorial image plane was used as an indicator of nuclear size. n = number of cells analyzed.

The degree of relative spatial proximity of chromosomes has previously been implicated to be functionally relevant in formation of chromosome translocations in blood cancers [3,10-12,16-18]. As translocations from different tumor tissues, including both blood and epithelial tumors, are frequently characterized by translocations between distinct sets of chromosomes [19], we asked whether the observed differences in spatial chromosome arrangements may explain the formation of tissue-specific translocations. To directly test this hypothesis we took advantage of the differential translocation behavior of chromosome pairs 5, 6 and 12, 15 in mouse lymphocytes and liver. Translocations between chromosomes 5 and 6 frequently occur in mouse hepatomas but are not found in lymphomas; conversely, translocations between 12 and 15 are prevalent in lymphomas but not in hepatomas [20,21]. Qualitative inspection indicated that in hepatocytes, the translocation-prone chromosomes 5 and 6 were more frequently in close physical proximity than non-translocating chromosomes 12 and 15 (Figure 4a). In contrast, chromosomes 12 and 15 were frequently in close physical proximity in lymphocytes but not in hepatocytes (Figure 4a). For quantitative analysis we determined the number of hepatocytes or lymphocytes containing at least one close pair of either 5-6 or 12-15 (Figure 4a). A close pair was defined as two chromosomes separated by less than 20% of nuclear diameter and results were statistically analyzed by contingency table analysis [10]. In hepatocytes, a close pair of the translocation-prone chromosomes 5-6 was found in 69% of cells, whereas only 55% of cells containing a pair of the non-translocating chromosomes 12-15 (Figure 4b). This difference was significant at the $p = 4.0 \times 10^{-2}$ level in a contingency table analysis. Conversely, 50% of lymphocytes contained a pair of translocation-prone chromosomes 12-15 whereas only 33% harbored a close pair of non-translocating 5-6 chromosomes (Figure 4b; $p = 1.4 \times 10^{-2}$). In addition, the frequency of 5-6 pairs in hepatocyte and 12-15 pairs in lymphocytes was above the value expected for uniform randomly distributed chromosomes, but below the expected values in the tissues where these chromosomes do not translocate (Figure 4b). These findings confirm earlier observations of preferential proximal positioning of translocation-prone loci in lymphocytes [11], and extend them by demonstrating a correlation between tissue-specific spatial proximity and tissue-specificity of translocations.

Our observations provide evidence for tissue-specific spatial organization of genomes in the interphase cell nucleus. While we show here that a subset of mouse chromosomes exhibits differential nuclear positioning among tissues, we suspect that differential spatial organization is a general feature of most chromosomes. As radial positioning of some chromosomes is evolutionarily conserved, tissue-specificity is likely to occur in other species as well [9]. Patterns of chromosome arrangements were more similar among tissue types that share differentiation pathways, suggesting that chromosome positioning might be established during differentiation. As previously reported [15], differences in chromosomes positioning were not due to variation in cell size or shape among tissues, as, for example, the morphologically extremely distinct small and large lung cells showed similar distribution patterns. Furthermore, although changes in chromosome positioning have been reported for the G0/G1 transition, our observed differences were unlikely to be due to cell-cycle effects, as chromosome positioning does not significantly change during interphase in cycling cells [22-25]. We suspect that our estimates of the differences in chromosome positions might be an underestimate as it is possible that our isolated cell populations contain several cell types, which might exhibit distinct chromosome positions. A more detailed future analysis of distinct cell types in the context of intact tissues will be insightful to address this issue.

The functional significance of tissue-specific spatial genome organization in gene expression remains unclear. It is possi-

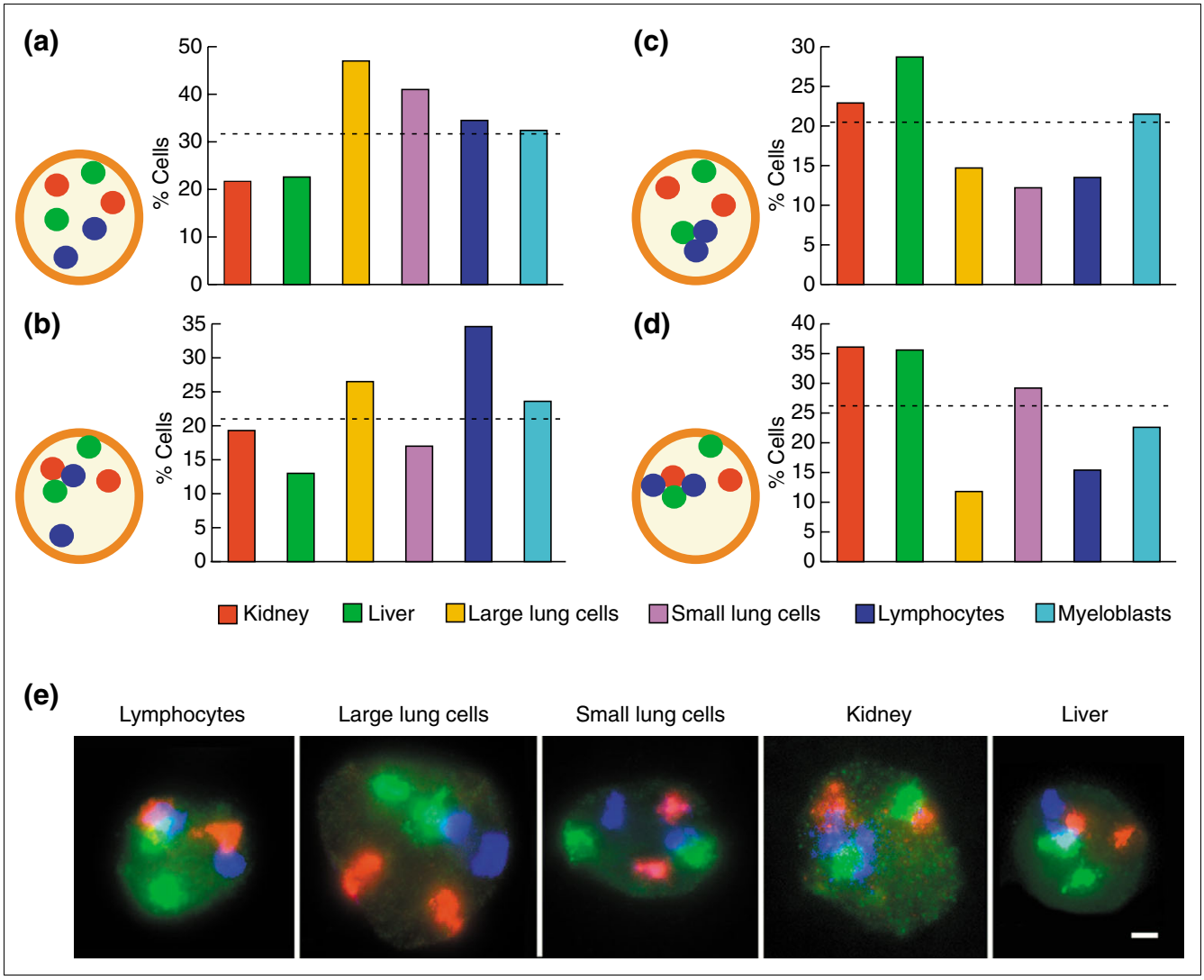

Figure 3
Tissue-specific relative positioning of chromosomes 12, 14 and 15. Quantitation of triplet cluster formation by determining for each tissue type the percentage of cells containing **(a)** no 12-14-15 triplet clusters, **(b)** a single triplet cluster of exactly one chromosome 12, 14 and 15, **(c)** a single cluster of a pair of homologues and one additional chromosome, or **(d)** a cluster of homologues and more than one additional chromosome. Expected values based on random distribution of chromosomes are indicated by a dashed line. Between 41 and 180 cells were analyzed per tissue. **(e)** FISH analysis of different cell types for chromosome 12 (red), 14 (blue), and 15 (green). Distinct preferential cluster types are found in different cell types. Scale bar, 1.8 μm.

ble that the positioning of chromosomes into particular nuclear neighborhoods might expose gene loci to local concentrations of specific regulatory factors or might place loci into a transcriptionally silent heterochromatic environment [3,26,27]. This model is consistent with the observed peripherization of immunoglobulin loci during lymphocyte development and repositioning of several differentiation-stage-specific gene loci away from centromeres during B-cell differentiation [28-30]. Although a previous analysis of radiation-induced random translocations in human lymphocytes yielded no positive evidence for preferential relative positioning of chromosomes [4], several reports support a functional link between nonrandom relative positioning and formation of translocations. A number of translocation-prone chromosomes and gene loci have been shown to be in preferential spatial proximity to their translocation partners in normal cells before translocation events [10-12,14,16,17]. Our observations support this notion and extend it by demonstrating a correlation between tissue-specific spatial organization and tissue-specific translocation frequency. This result suggests that the distinct spatial organization of genomes in normal tissues contributes to the tissue-specificity of prevalent translocations.

It is unclear at present how patterns of chromosome arrangements are established and maintained. One possibility is that the nucleus contains specific structural components that determine genome organization. Tissue-specificity of genome

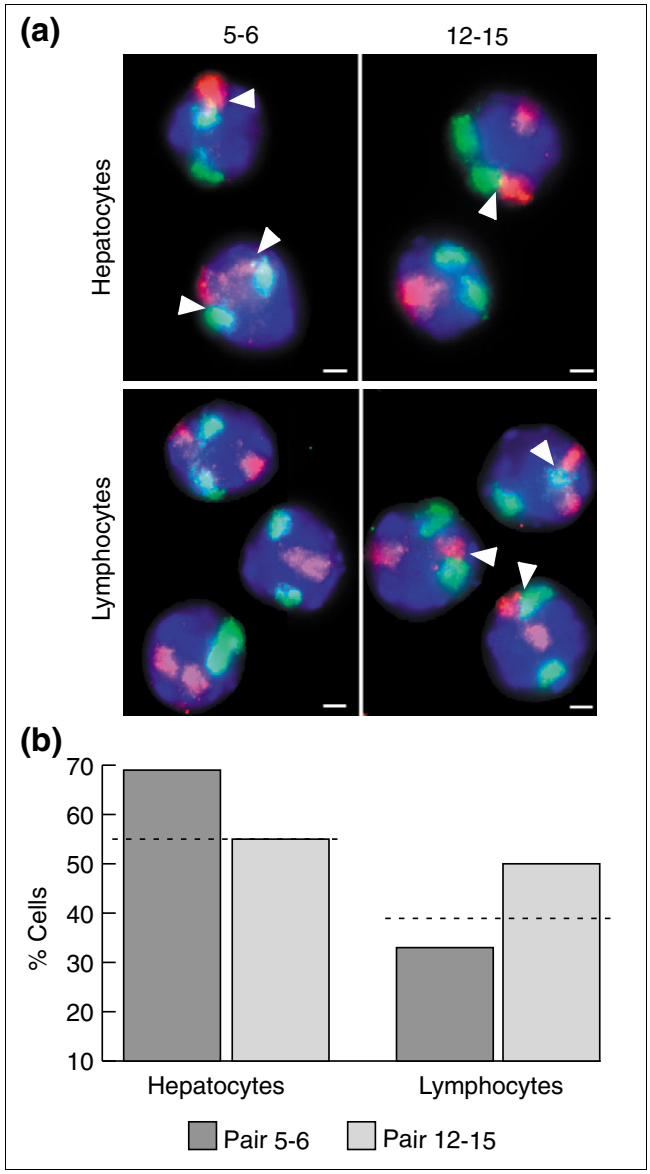

Figure 4
Tissue-specific relative chromosome positioning correlates with tissue specific translocation frequency. **(a)** FISH analysis of chromosome 5 (green), 6 (red), 12 (green) and 15 (red) in hepatocytes or lymphocytes. Arrowheads indicate proximal pairs. Scale bar, 2 μm. Pairs of chromosomes 5-6 are more frequent than pairs of chromosomes 12-15 in hepatocytes, whereas the opposite is true in lymphocytes. **(b)** Determination of the percentage of hepatocytes or lymphocytes containing at least one 5-6 or 12-15 pair. The likelihood of pair formation correlates with the observed tissue-specific translocation frequency among these chromosomes. Dotted lines represent expected values based on a uniform random distribution. Note that the expectation value of contingency tables is dependent on the number of analyzed cells. For analysis of 5-6 pairing, 83 hepatocytes and 118 lymphocytes were analyzed. For analysis of 12-15 pairing, 158 hepatocytes and 88 lymphocytes were analyzed.

organization could be established by regulated expression of structural proteins such SATB1, a thymocyte-specific protein that has been proposed to regulate thymocyte-specific genes by tethering chromatin regions onto a structural nuclear scaffold [31]. An intriguing alternative possibility is that the transcriptional status of chromosome regions affects their structural properties and that the collective transcriptional activity of a genome thus determines its arrangement in a self-organizing manner based on the physical properties of the chromatin and the interacting polymerases [32]. In our case, chromosomes 12 and 15 contain nucleolar-organizing centers which might facilitate the preferential nonrandom association of these chromosomes. Our observation of similarities in genome organization in cell types with shared differentiation pathways is consistent with such a self-organization model. Regardless of how the patterns are established, our description of tissue-specific spatial genome patterns should be of use in further experimental tests of these models as well as in understanding the functions and mechanisms of nonrandom spatial genome organization.

Materials and methods

Cell preparation

C57BL/6 mice (4-8 weeks old) were sacrificed and the relevant tissues recovered. For lung and kidney samples the tissue was washed in RPMI 1640 medium and the tissue minced with scalpels and enzymatically disaggregated by collagenase type IV. The resulting cell suspension was washed twice by centrifugation in RPMI 1640 medium. Cells were successively plated four times at 60-min intervals in medium consisting of Dulbecco's MEM, supplemented with 10% fetal bovine serum, penicillin (100 IU/ml), streptomycin (0.2 mg/ml), hydrocortisone (0.5 μg/ml), dibutyryl cAMP (10 nM), and 1% insulin/transferrin/sodium selenite. The epithelial-enriched cell suspension was seeded onto glass coverslips. After 24 h the coverslips were processed for FISH analysis. Lymphocytes and granulocytes were isolated from spleen and thymus by differential centrifugation in a gradient of Ficoll/sodium diatrizoate. Hepatocytes were isolated by two-step collagenase perfusion of the liver followed by isodensity centrifugation in Percoll. Cells were washed by centrifugation in isotonic phosphate-buffered solution and cytocentrifuged (10^5 cells/ml) onto glass slides and fixed in 4% paraformaldehyde.

Chromosome painting

Detailed protocols for FISH and probe generation are available online [33]. Briefly, slides were hybridized for 48 h at 37°C in a moist chamber with a combination of three painting probes at a time. Probes were prepared from flow-sorted chromosomes by degenerate oligonucleotide-primed polymerase chain reaction (PCR) using biotin, digoxigenin or Spectrum Red deoxyuridine nucleotides for labeling. Biotin and digoxigenin-labeled probes were detected with streptavidin conjugated to Cy5 and FITC-conjugated sheep anti-digoxigenin antibodies respectively. Specimens were examined with a Nikon Eclipse E800 microscope equipped with epifluorescence optics and a Photometrics MicroMax cooled CCD

camera (1,300 × 1,300 array, 6.7 μm pixel size, 5 MHz, image pixel size 80 nm).

Positioning measurements

Images of triple-labeled cell nuclei were generated and analyzed using MetaMorph Imaging System 4.6 (Universal Imaging). All measurements were performed on maximum projections of 10 focal planes covering the entire nucleus. All measurements were done on unprocessed images without thresholding. For distance analysis the perimeter of each cell as well as each chromosome were manually drawn on the maximum projection and the center of mass determined automatically using MetaMorph software. Chromosomes in the projections were visually compared to the single focal planes to verify that the regions were representative of the entire chromosome. The mean nuclear radii, and the center of mass of each chromosome and nucleus were measured using MetaMorph software. Nuclear radii (R_n) and diameters (D_n) were calculated from $R_n = (A/pi)^{0.5}$, where (A) is the nuclear pixel area. Radial chromosome positions were calculated as $[(Ch:C)/R_n \times 100]$, where (Ch:C) is the distance from the center of an individual chromosome to the nuclear center. Cell nuclei were subdivided in five concentric shells of equal volume designated 1 to 5 from the center of the nucleus to the periphery. To measure relative positioning, the absolute spatial separations between chromosome pair centers of mass (Ch_1:Ch_2) were normalized as a fraction of nuclear diameter $[(Ch_1:Ch_2)/D_n \times 100]$ to account for natural variations in nuclear size. For measurements of radial positioning both homologs were scored separately. For distance measurements the closest non-homolog pair was measured for each possible chromosome combination.

Statistical analysis

Analysis of radial and relative positioning were essentially performed as previously described [10,11]. To characterize specific trends in the radial distribution of chromosomes, we conducted contingency table analysis with the null hypothesis that a given chromosome has the same radial distribution in the two cell types being compared [10,11,34,35]. The bins used to generate contingency tables were defined by the following boundaries: 0.0-33.98%, 33.98-48.79%, 49.79-61.51%, 61.61-73.91% and > 73.91% nuclear radius. Bins defined in this manner represent volumes with equal probability of containing the same number of chromosomes assuming a uniform random distribution of 40 spherical chromosomes of excluding volume with a radius of 10% of the nuclear volume in a spherical nucleus. To test for differences in average minimum separation of chromosome pairs between cell types we applied the Kolmogorov-Smirnov test [36]. To test for differences in proximal triplet formation, we constructed contingency tables for the frequencies of the experimentally observed four categories of chromosome arrangements [34,35]. Triplets were defined as a collection of three chromosome pairs all separated by less than 30% of nuclear diameter [10]. The contingency table analysis tested the null hypothesis that all four categories of chromosome arrangements are equally likely and independent of the cell type. To determine tissue-specific proximity of translocation partners we determined the frequencies of cells containing at least one close pair of either 5-6 or 12-15 in lymphocytes and hepatocytes as previously described [11]. We defined a close pair as two chromosomes located at a distance not larger that 20% of the nuclear diameter. Frequencies of pair formation were analyzed analogously to the triplet analysis using the null hypothesis that the number of close pairs in a given cell type is independent of the identities of the chromosomes. All analyses were done using standard algorithms coded in Java. For all experiments data from at least three independent experiments was pooled.

Additional data files

The following additional files are available with the online version of this paper: contingency tables for chromosomes 12, 14, and 15 triplet formation (Additional data file 1); chromosomes 1, 12 and 14 triplet formation (Additional data file 2); chromosomes 1, 12 and 15 triplet formation (Additional data file 3); and chromosomes 1, 14 and 15 triplet formation (Additional data file 4).

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