L1.1.2

Nuclear organization

Note:
orange buttons mean something that you should search for and contribute to in the Methodological Wiki on the Moodle site

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Current Opinion in Cell Biology

Nuclear organization: taking a position on gene expression

Pamela K Geyer, Michael W Vitalini and Lori L Wallrath

Textbook

Eukaryotic genomes are divided into chromosomes that occupy defined regions or territories within the nucleus. These chromosome territories (CTs) are arranged based on the transcriptional activity and chromatin landscape of domains. In general, transcriptionally silent domains reside at the nuclear periphery, whereas active domains locate within the interior. Changes in nuclear position are observed for stress-induced and developmentally regulated tissue-specific genes. Upon activation, these genes move away from a CT to inter-chromosomal space containing nuclear bodies enriched in gene expression machinery. Gene activation is not always accompanied by movement, as positioning is dictated by many determinants, including gene structure and the local genomic environment. Collectively, tissue-specific nuclear organization results from a culmination of inputs that result in proper transcriptional regulation.

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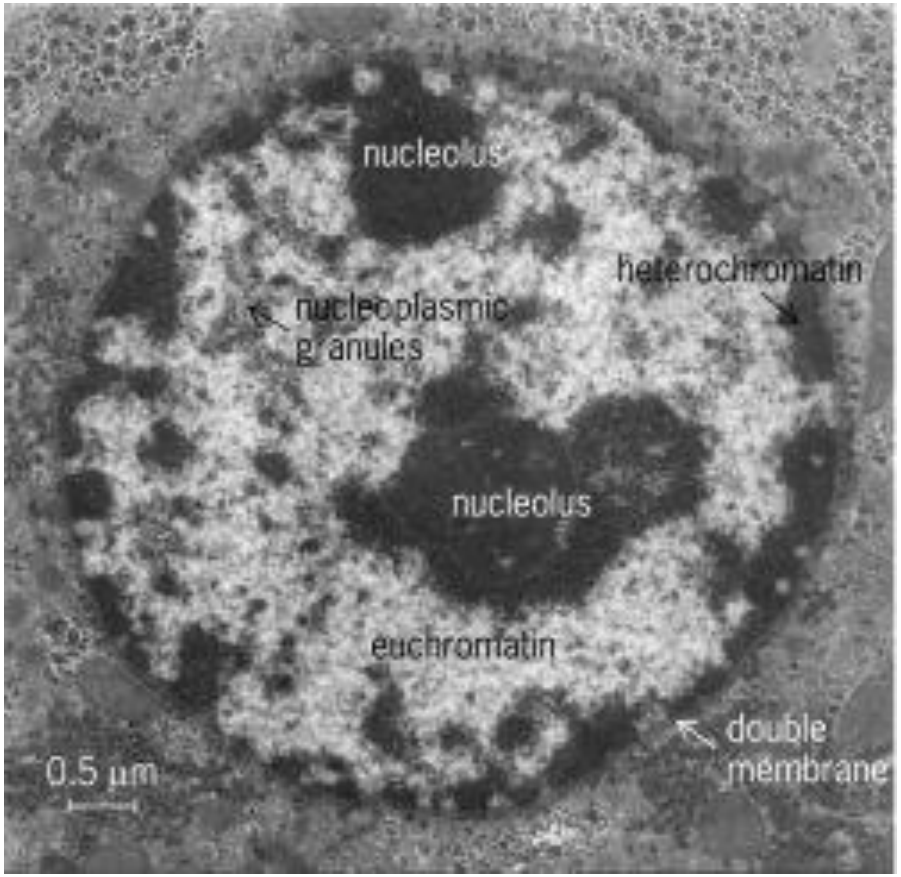
chemical cross-linking and massive parallel sequencing to define genome-wide relationships [3–5]. Results from these studies suggest that the genome is arranged as inter-digitated CTs rather than randomly inter-twined chromosomes [6]. Emerging from these investigations is a picture of the nucleus as an ordered organelle; the consequences of this organization are just being realized.

Nuclear organization during differentiation

Studies have linked nuclear organization to cellular differentiation. Cultured pluripotent mouse embryonic stem (ES) cells possess dispersed chromatin with limited compaction. Upon differentiation, they show changes in chromatin structure that include large-scale compaction of genomic domains [7]. Consistent with these findings, embryonic development proceeds from a single cell embryo possessing a 'featureless' nucleus with dispersed chromatin, to differentiated cells possessing nuclei with peripherally located compact chromatin domains [8]. Interestingly, an extended and dispersed chromatin meshwork was identified in the eight-cell epiblast, reminiscent of nuclear structures defined in cultured ES cells. In contrast to the 'open' chromatin structure in the epiblast nuclei,



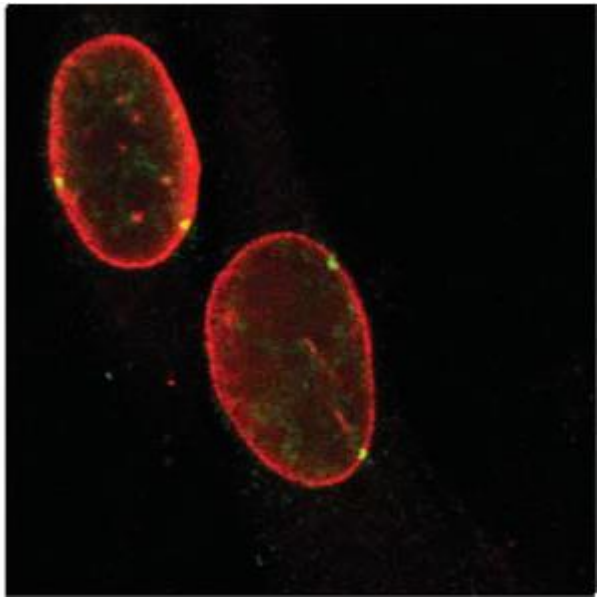
A rat liver cell nucleus at Transmission E.M.

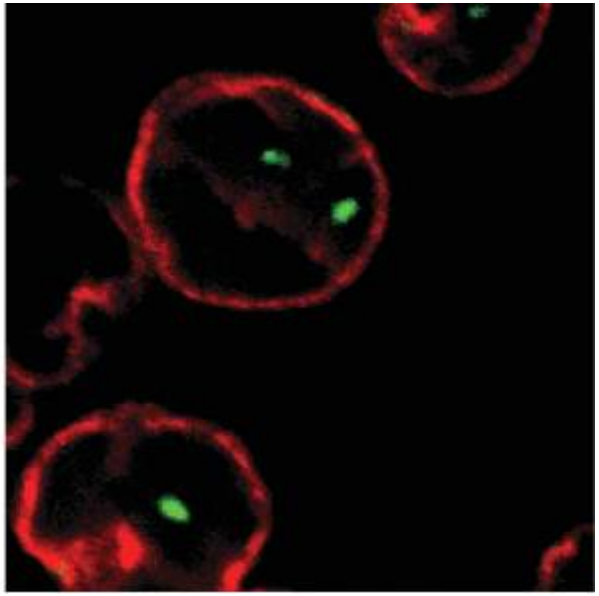
Gene-poor regions and **silenced genes** are frequently found at the **nuclear periphery** (same as heterochromatin)

How do we know this ?

3D DNA immunoFISH of the **Igh** loci

What is this ? Go to NCBI-Gene database





fibroblast

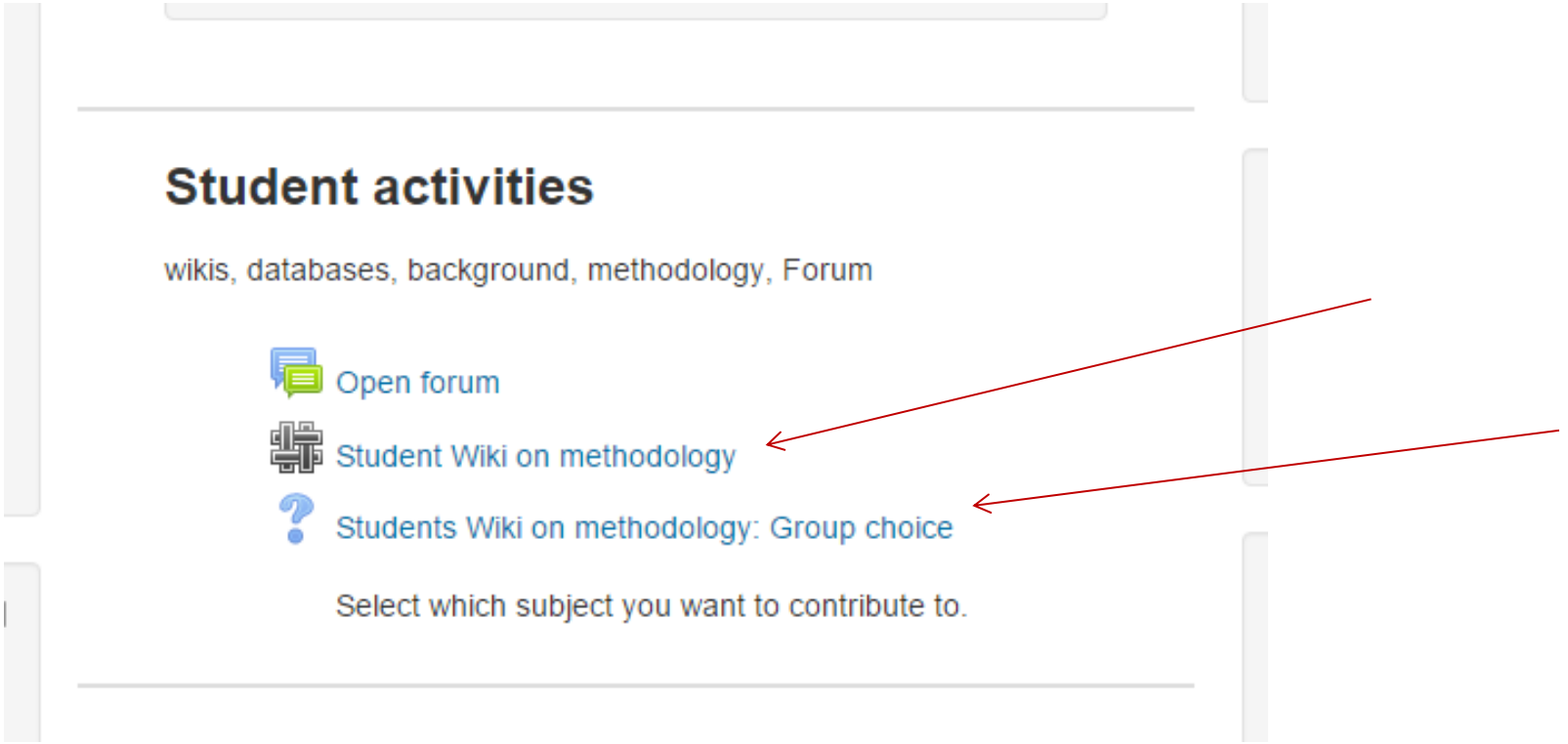
Pro-B cell

Immuno-FISH

From: Reddy et al., 2008 (Research paper 1)

How do researchers make FISH experiments ?

FISH



Another method, non-morphological, to address whether silenced genes are generally at the periphery and active genes in the nucleoplasm ??

Eukaryotic genomes are divided into chromosomes that occupy defined regions or territories within the nucleus. These chromosome territories (CTs) are arranged based on the transcriptional activity and chromatin landscape of domains. In general, transcriptionally silent domains reside at the nuclear periphery, whereas active domains locate within the interior. Changes in nuclear position are observed for stress-induced and developmentally regulated tissue-specific genes. Upon activation, these genes move away from a CT to inter-chromosomal space containing nuclear bodies enriched in gene expression machinery. Gene activation is not always accompanied by movement, as positioning is dictated by many determinants, including gene structure and the local genomic environment. Collectively, tissue-specific nuclear organization results from a culmination of inputs that result in proper transcriptional regulation.

Several new concepts:

Chromosomal territories

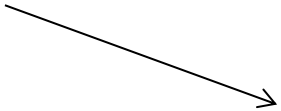
Chromatin landscapes

Transcriptionally silent domains

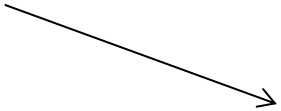
Transcriptionally active domains

Changes in nuclear position (induced genes)

Imput



tissue specific nuclear organization



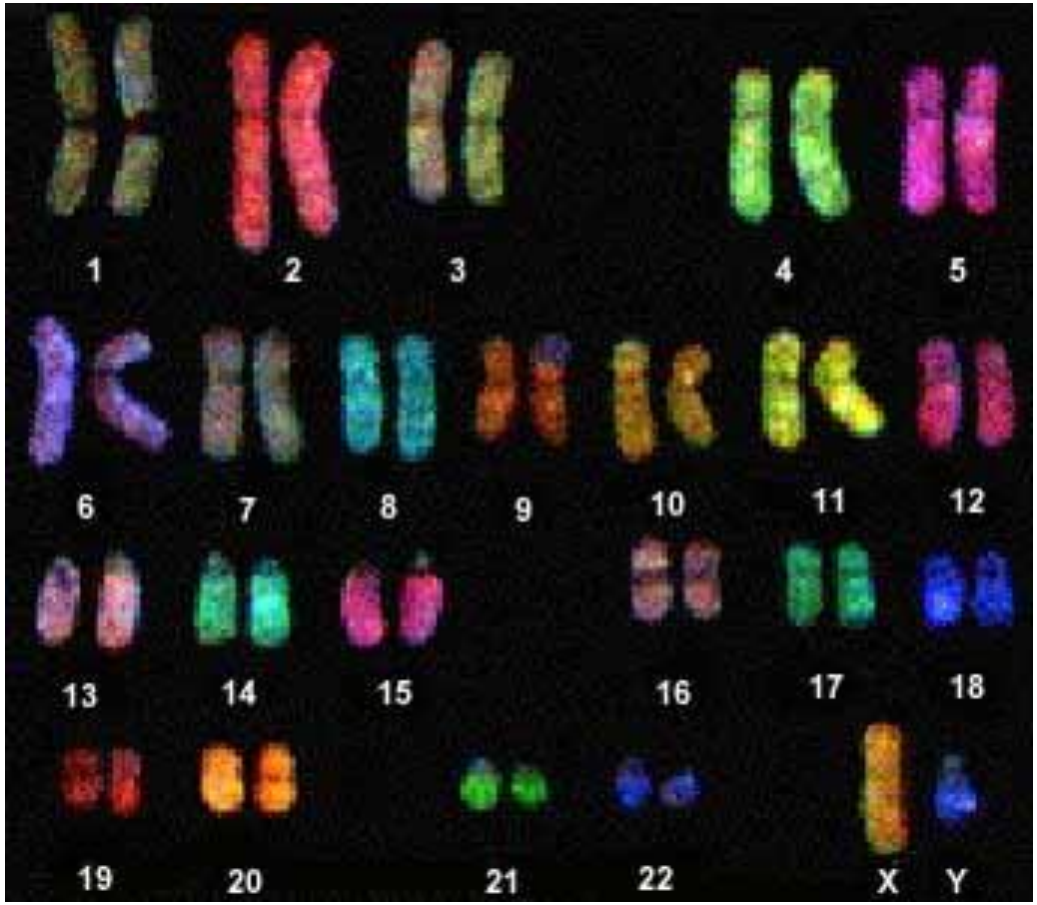
proper transcriptional regulation

Chromosomal territories (CT)

Individual chromosomes can be «painted» and maintain separate territories in interphase nuclei

Chromosome painting techniques can visualize individual chromosomes both at the metaphase and interphase

Individual «chromosome painting» is a **FISH technique**

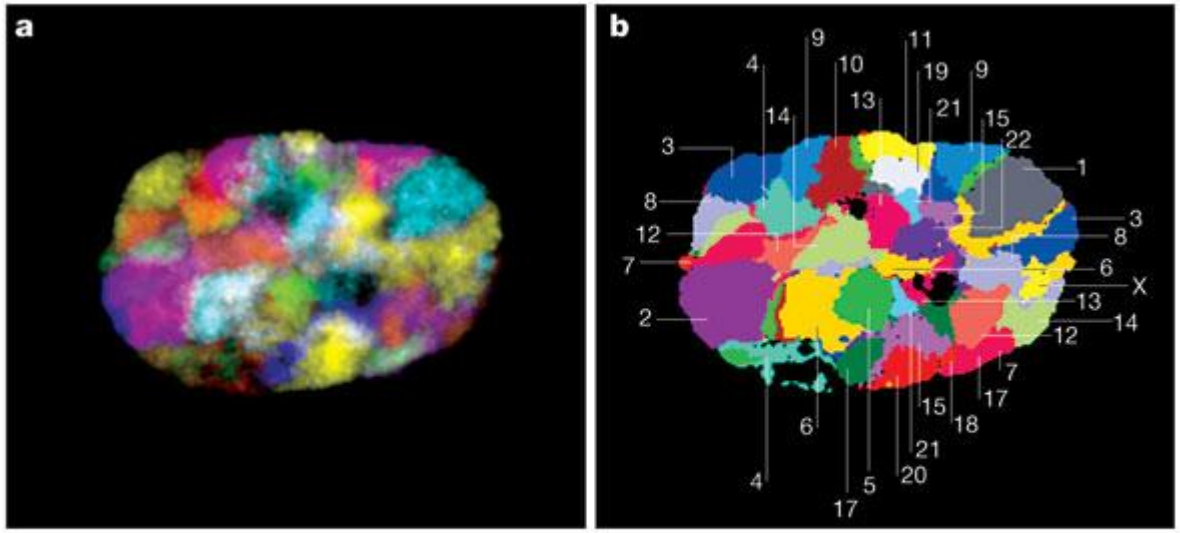


Metaphase human chromosomes

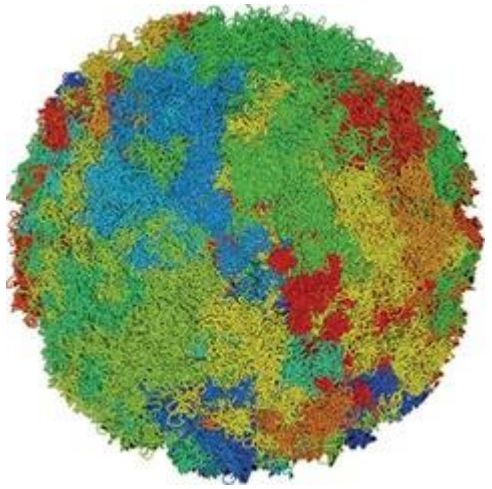
Results from these studies suggest that the genome is arranged as interdigitated CTs rather than randomly inter-twined chromosomes [6]. Emerging from these investigations is a picture of the nucleus as an ordered organelle; the consequences of this organization are just being realized.

Interdigitated, inter-twined or inter-twingled CTs ?

CT = chromosomal territory



Mapping CTs in cell nucleus



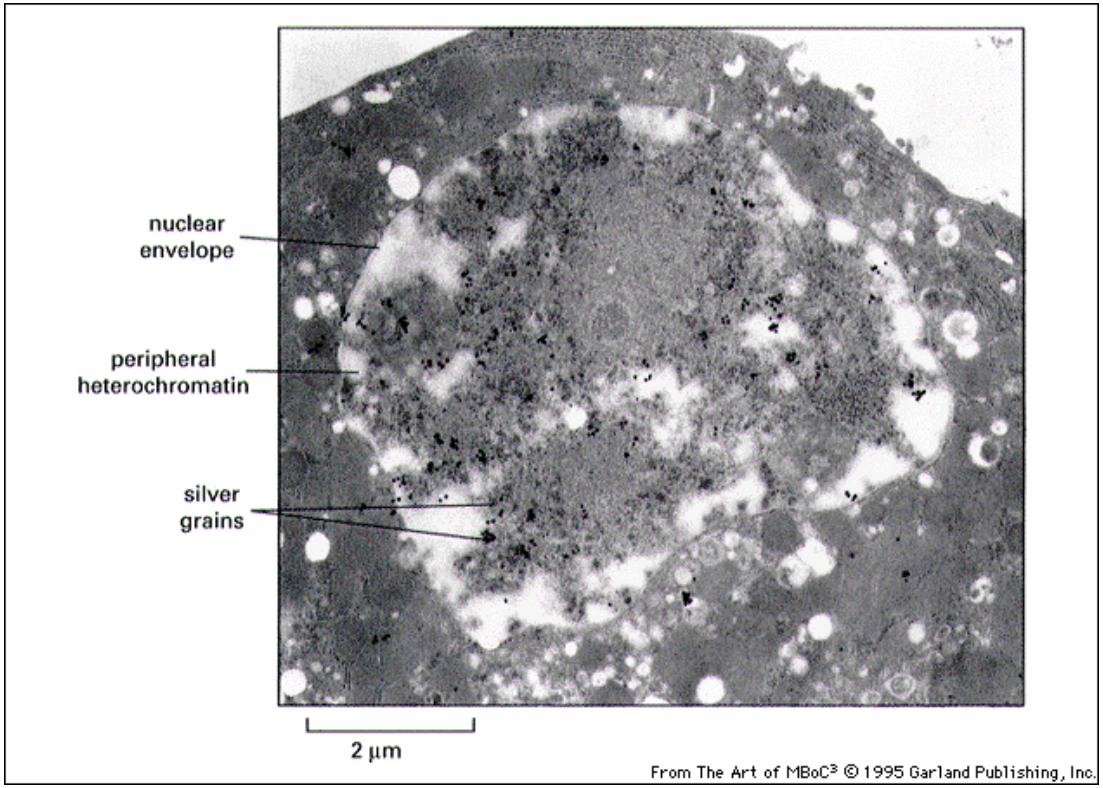
One interesting question: if heterochromatin and CT are established during differentiation...

Does heterochromatin i.e. nuclear organization,

differ

from one cell type to another?

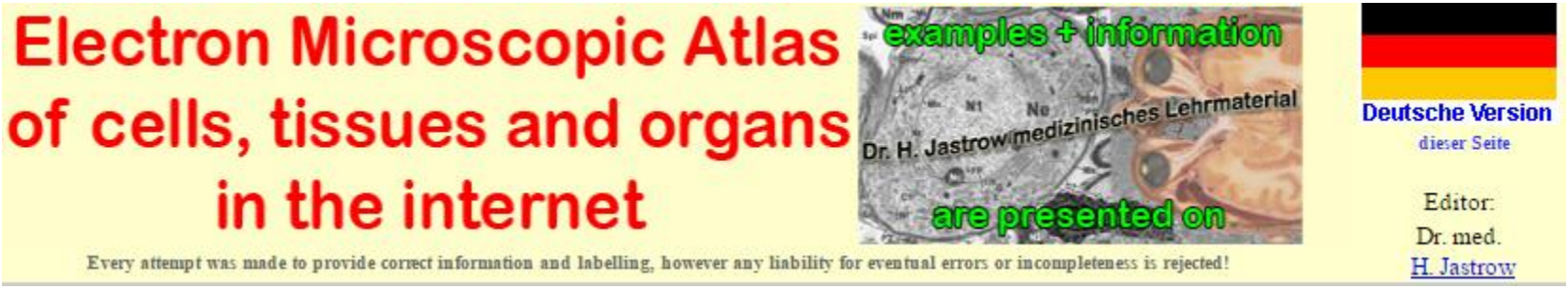
This is quite old concept, indeed….



Macroscopic

http://www.google.it/search?q=nucleus+electron+microscopy&hl=it&client=firefox-a&hs=rE0&rls=org.mozilla:it:official&prmd=imvns&source=lnms&tbm=isch&ei=IJ1dT-SSF9HoOYWWlfYM&sa=X&oi=mode_link&ct=mode&cd=2&ved=0CBQQ_AUoAQ&biw=1330&bih=647

Another interesting collection of E.M. images of different nuclei is found **here**



Do macroscopic differences reflect different (macro)-molecular organization ?

Open Access

Research

Tissue-specific spatial organization of genomes

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Abstract

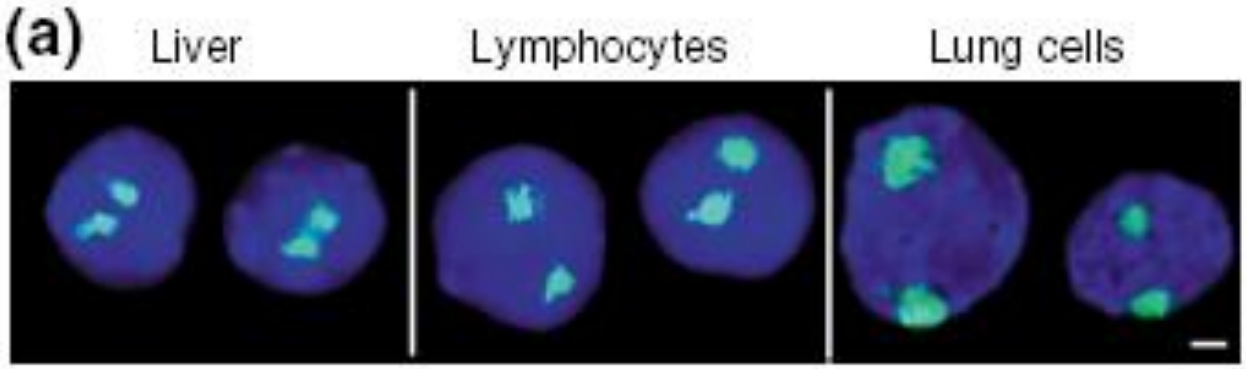
Background: Genomes are organized *in vivo* in the form of chromosomes. Each chromosome occupies a distinct nuclear subvolume in the form of a chromosome territory. The spatial positioning of chromosomes within the interphase nucleus is often nonrandom. It is unclear whether the nonrandom spatial arrangement 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.

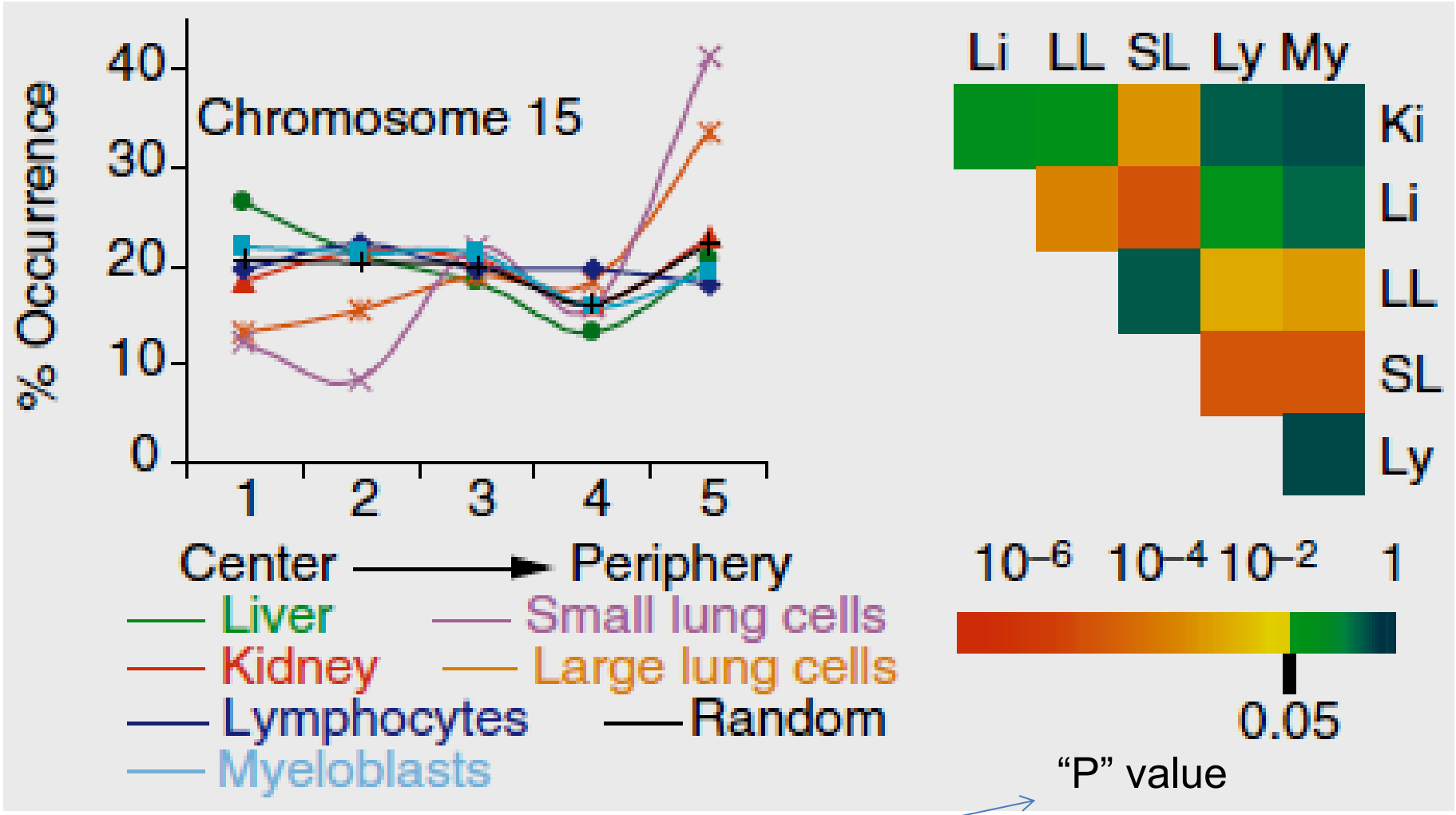
2D and 3D FISH

Introducing nuclear localization of interphase chromosomes



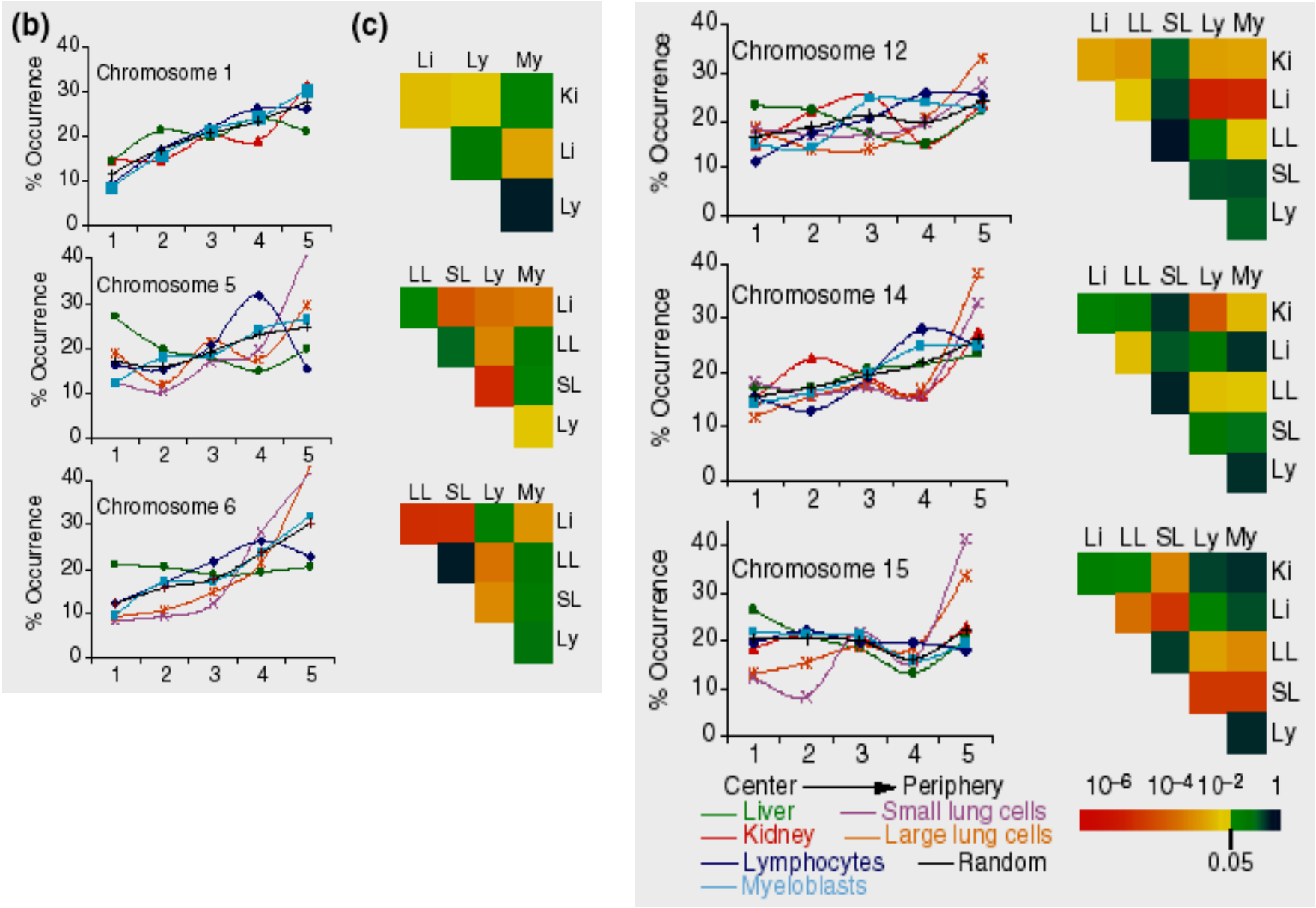
Chromosome 5 painting

From Parada et al., 2004, Genome Biol. 5:R44.



«P-value»

From Parada et al., 2004, Genome Biol. 5:R44.



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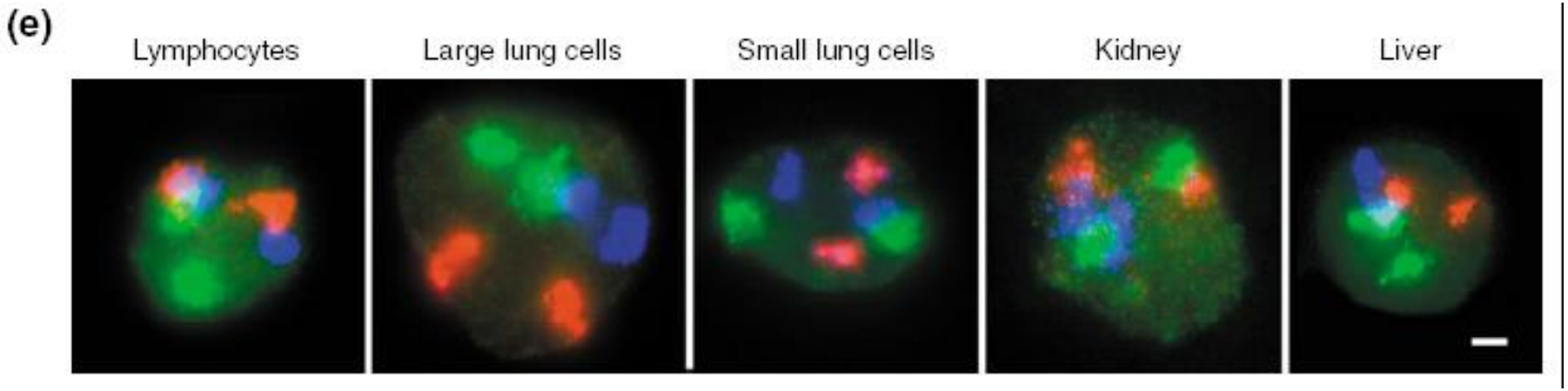

Tissue-specific **relative positioning** of chromosomes 12, 14 and 15

From Parada et al., 2004, Genome Biol. 5:R44.

Couple of chromosomes

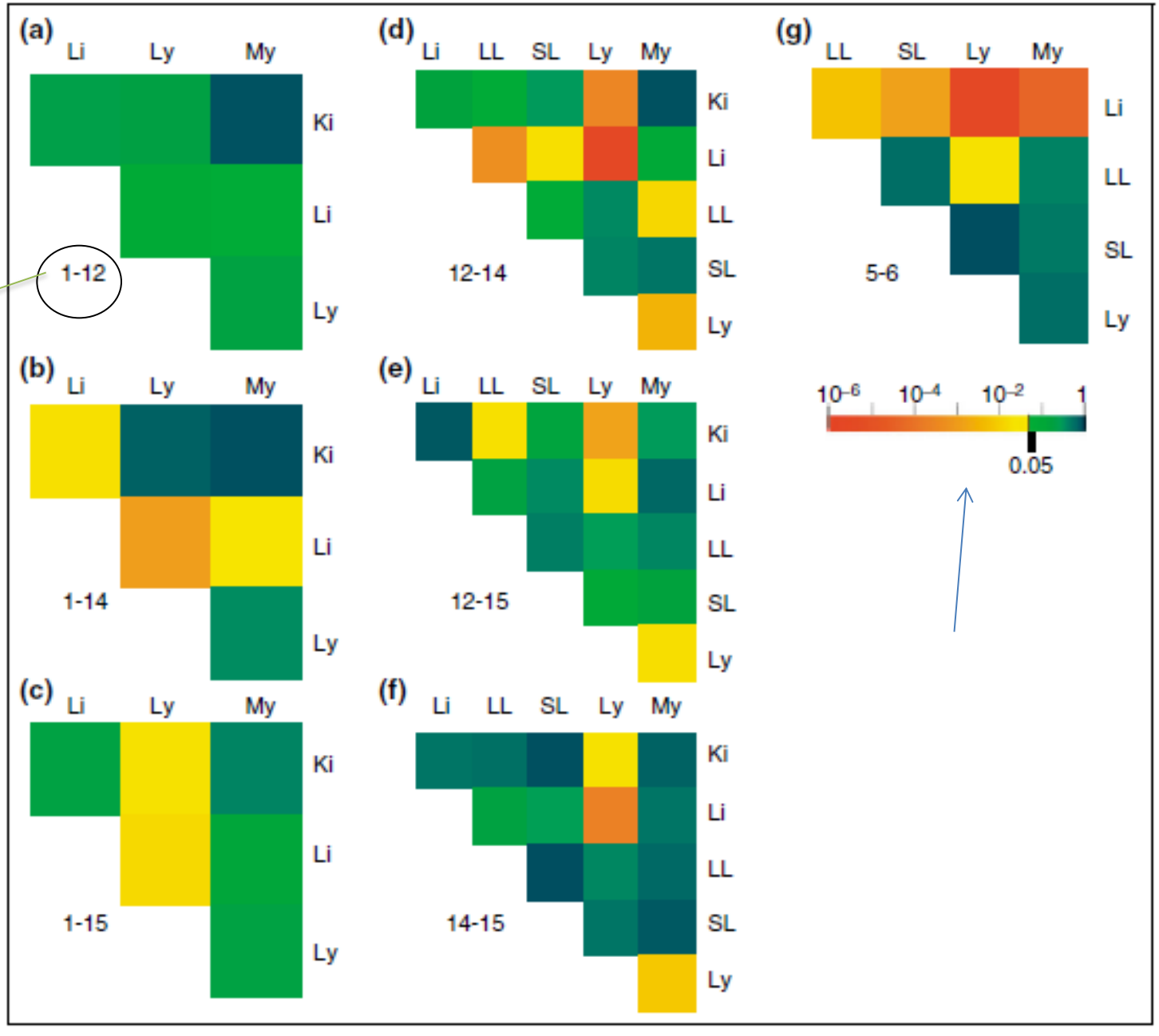

Figure 2

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.

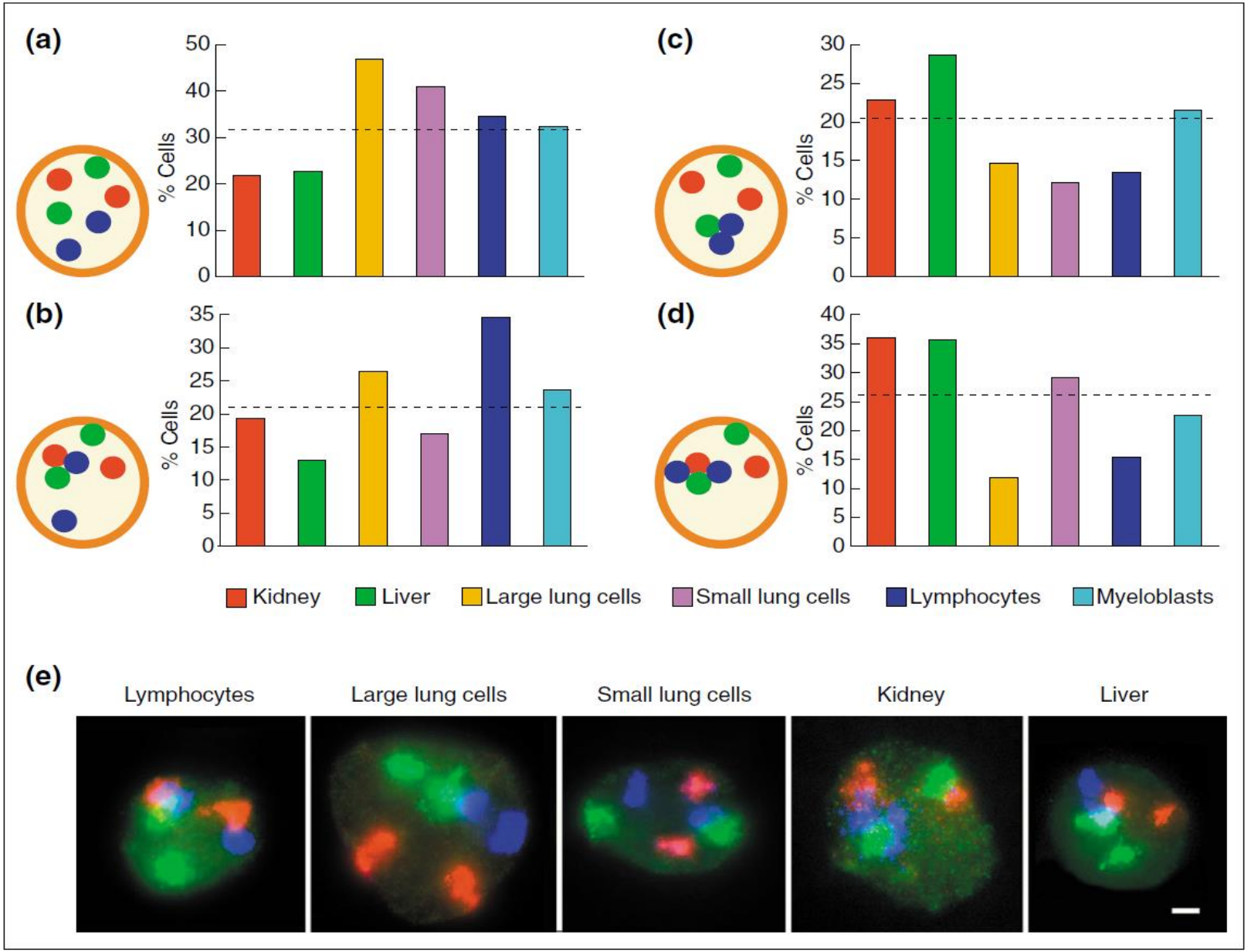

Figure 3

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.

Conclusions:

- Interphase chromosomes occupy discrete “territories” within the nucleus
- Position of interphase chromosomes is cell-specific
- Relative positioning is also cell type-specific