

Interphase Chromosome Conformation and Chromatin-Chromatin Interactions in Human Epithelial Cells Cultured under Different Gravity Conditions.

Ye Zhang¹, Michael Wong^{2,3}, Megumi Hada^{2,4}, and Honglu Wu².

¹NASA Kennedy Space Center, Houston, TX, USA, ²NASA Johnson Space Center, Houston, TX, USA, ³Texas Southern University, Houston, TX, USA, ⁴Wyle Laboratories, Houston, TX, USA,

Microgravity has been shown to alter global gene expression patterns and protein levels both in cultured cells and animal models. It has been suggested that the packaging of chromatin fibers in the interphase nucleus is closely related to genome function, and the changes in transcriptional activity are tightly correlated with changes in chromatin folding. This study explores the changes of chromatin conformation and chromatin-chromatin interactions in the simulated microgravity environment, and investigates their correlation to the expression of genes located at different regions of the chromosome. To investigate the folding of chromatin in interphase under various culture conditions, human epithelial cells, fibroblasts, and lymphocytes were fixed in the G1 phase. Interphase chromosomes were hybridized with a multicolor banding in situ hybridization (mBAND) probe for chromosome 3 which distinguishes six regions of the chromosome as separate colors. After images were captured with a laser scanning confocal microscope, the 3-dimensional structure of interphase chromosome 3 was reconstructed at multi-mega base pair scale. In order to determine the effects of microgravity on chromosome conformation and orientation, measures such as distance between homologous pairs, relative orientation of chromosome arms about a shared midpoint, and orientation of arms within individual chromosomes were all considered as potentially impacted by simulated microgravity conditions. The studies revealed non-random folding of chromatin in interphase, and suggested an association of interphase chromatin folding with radiation-induced chromosome aberration hotspots. Interestingly, the distributions of genes with expression changes over chromosome 3 in cells cultured under microgravity environment are apparently clustered on specific loci and chromosomes. This data provides important insights into how mammalian cells respond to microgravity at molecular level.

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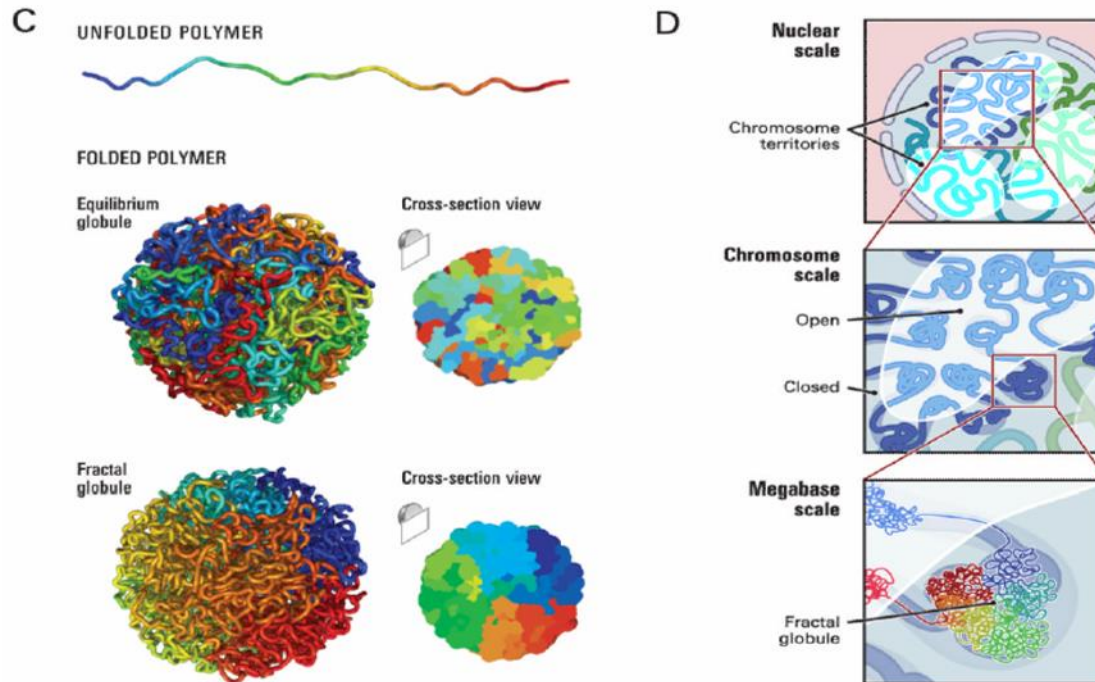
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Chromatin Folding Models

Random walk/polymer model of chromatin folding on 0.5-5 mbp scales



Lieberman-Aiden et al., Science, 2010

Non-randomness of chromatin folding on multi-megabase scales is contributed by:

- Confinement of chromatin fibers within chromosome territory
- Regulation of genes and gene densities

Chromosome 3

- Chromosome 3 spans about 200 million base pairs, representing about 6.5 percent of the total DNA in human cells, and containing 1,980 genes
- Aberrations involving chromosome 3 are associated with a number of known cancer types



Topography	Case	Case with Ch 3 Aberration	%	Case Analyzed
Bone and Soft Tissues	3406	576	16.9	500
Breast	1128	293	25.9	245
Cadiovascular	48	2	4.2	1
CNS	2511	208	8.3	175
Digestive System	2285	543	23.8	492
Endocrine System	605	115	24.8	107
Female Genital Organs	1552	266	17.1	225
Male Genital Organs	609	94	15.4	63
Respiratory System	1128	365	32.4	319
Skin	331	76	22.9	71
Urinary Tract	2166	640	29.5	569
Overall	15769	3178	20.2	2767

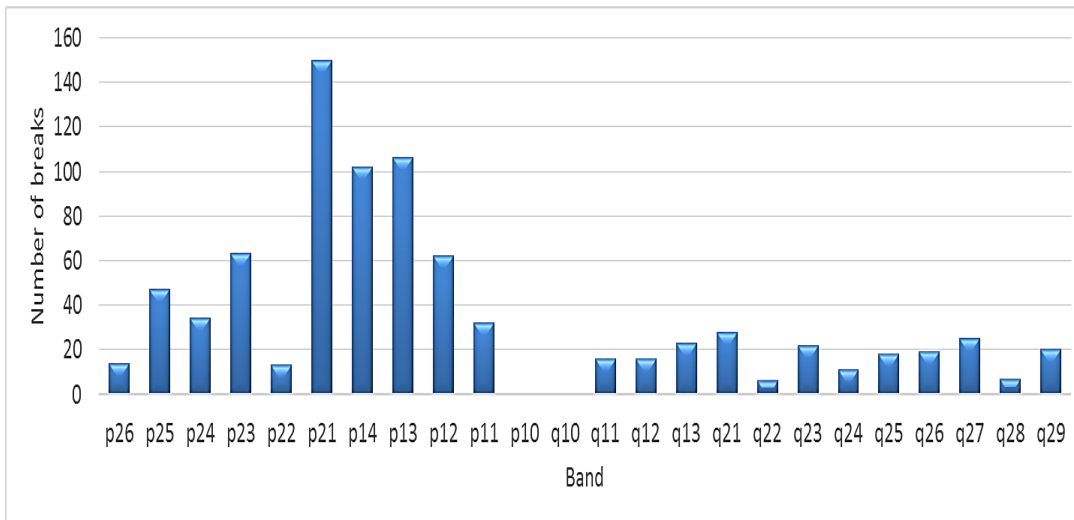
Yunis and Hoffman
1989

Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer (2013). Mitelman F, Johansson B and Mertens F (Ed.), <http://cgap.nci.nih.gov/Chromosomes/Mitelman>

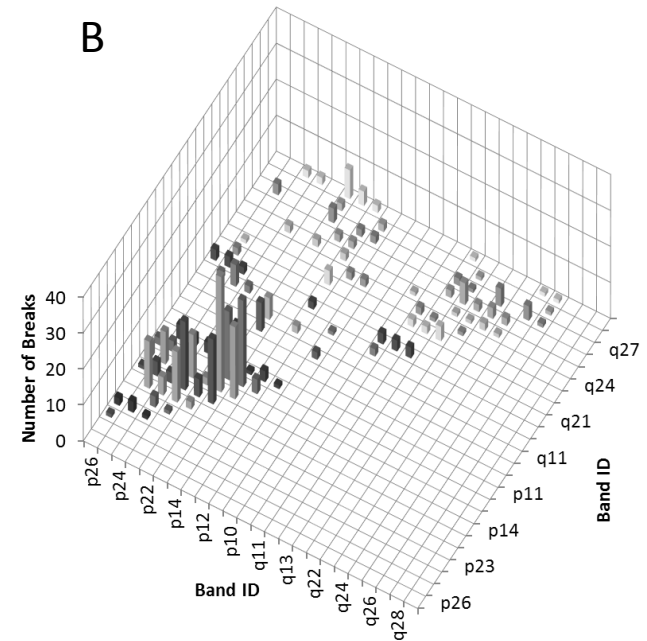
Distributions of intra-chromosome 3 exchanges in cancer patients

Most of the breaks involved in intra-chromosome 3 exchanges are found in the middle of the p-arm. The 3p21.3 region is a known fragile site and contains several tumor suppression genes.

A

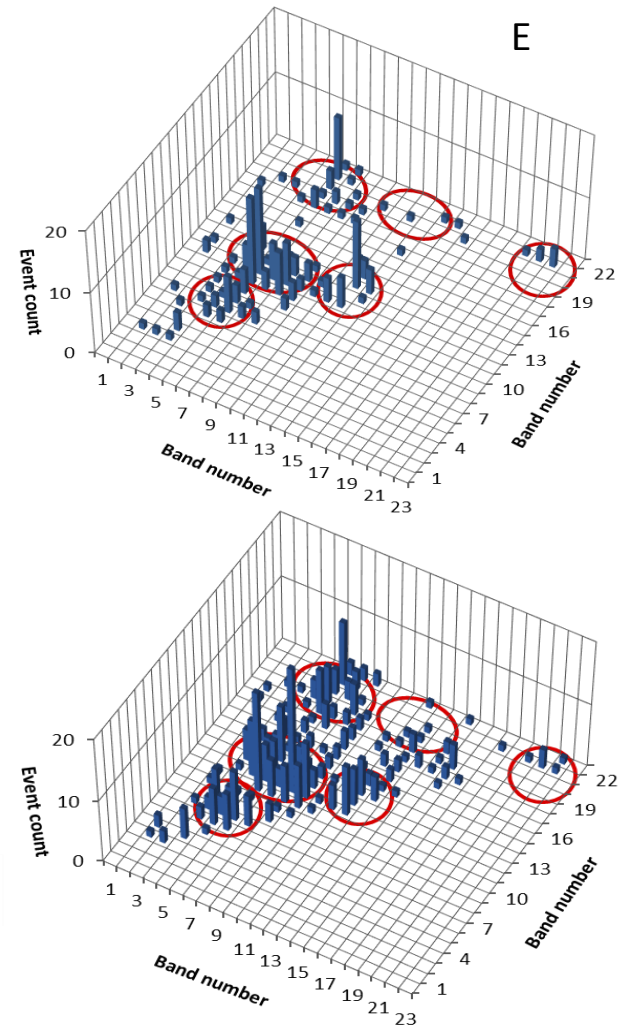
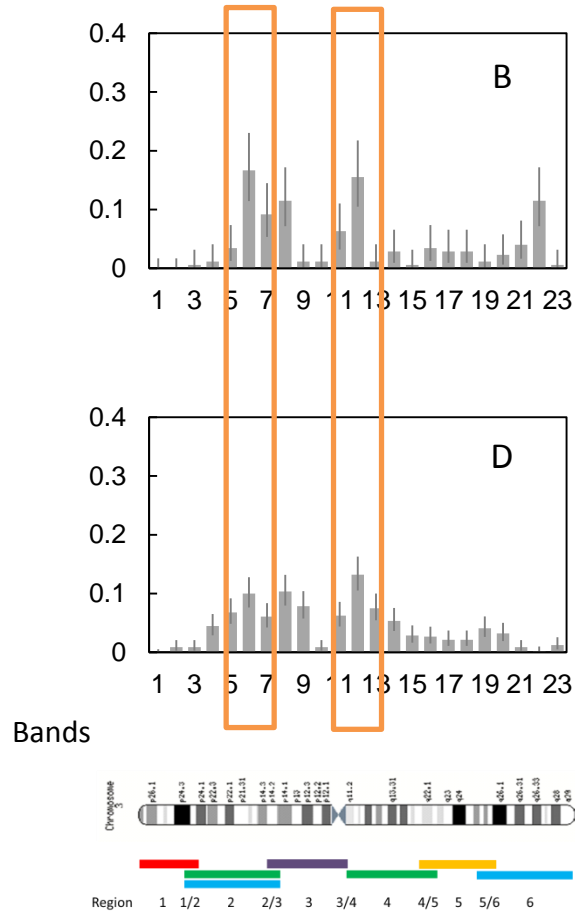
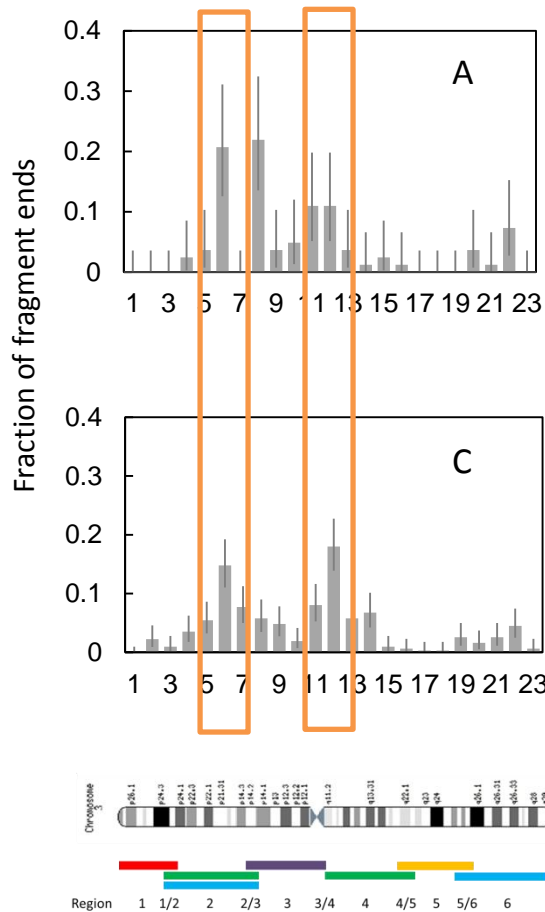


B



A. Total fragment ends participating in intra-chromosomal exchanges; B. Number of intra-chromosomal exchange events between two fragment ends identified by the band numbers.

Distributions of radiation-induced intra-chromosomal exchanges in human epithelial cells

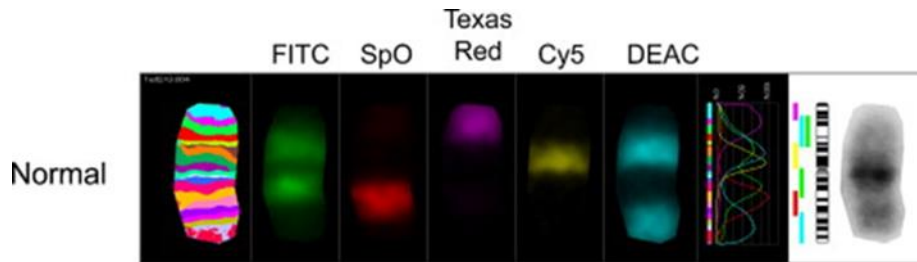
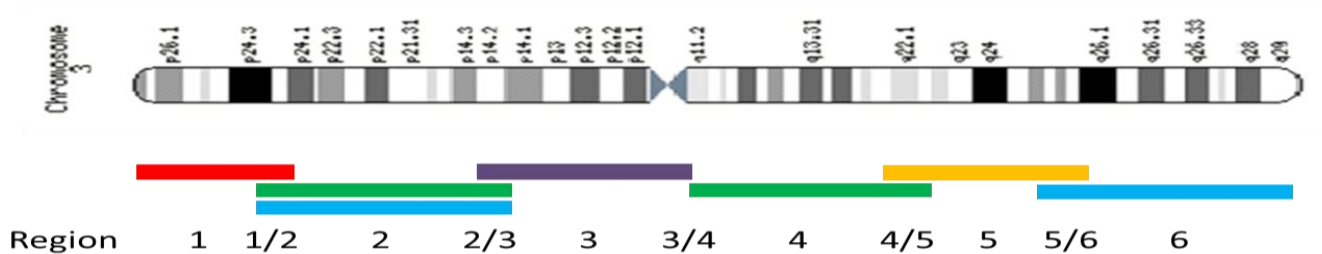


Intra-chromosomal exchanges occur frequently by rejoining one break in mband 6-8 (3p21) and one break in mband 11-12 (3q12)

Hada *et al.*, *Rad Res.* 176:25-37, 2011

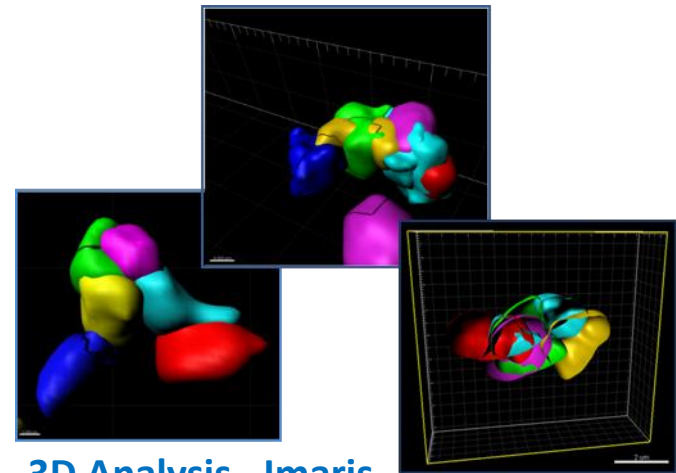
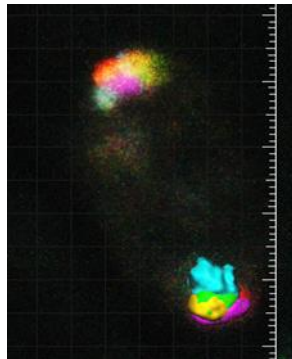
Proximity within an interphase chromosome contributes to the breakpoint distribution in radiation-induced intra-chromosomal exchanges.

Zhang *et al.*, *LSSR* 2:23-28, 2014



mBAND - Metaphase

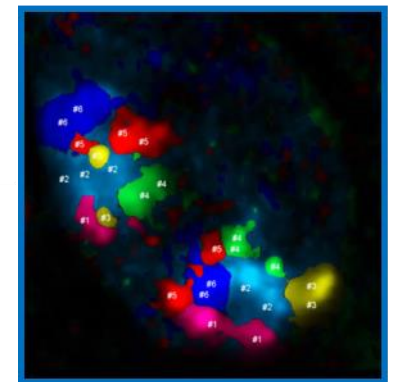
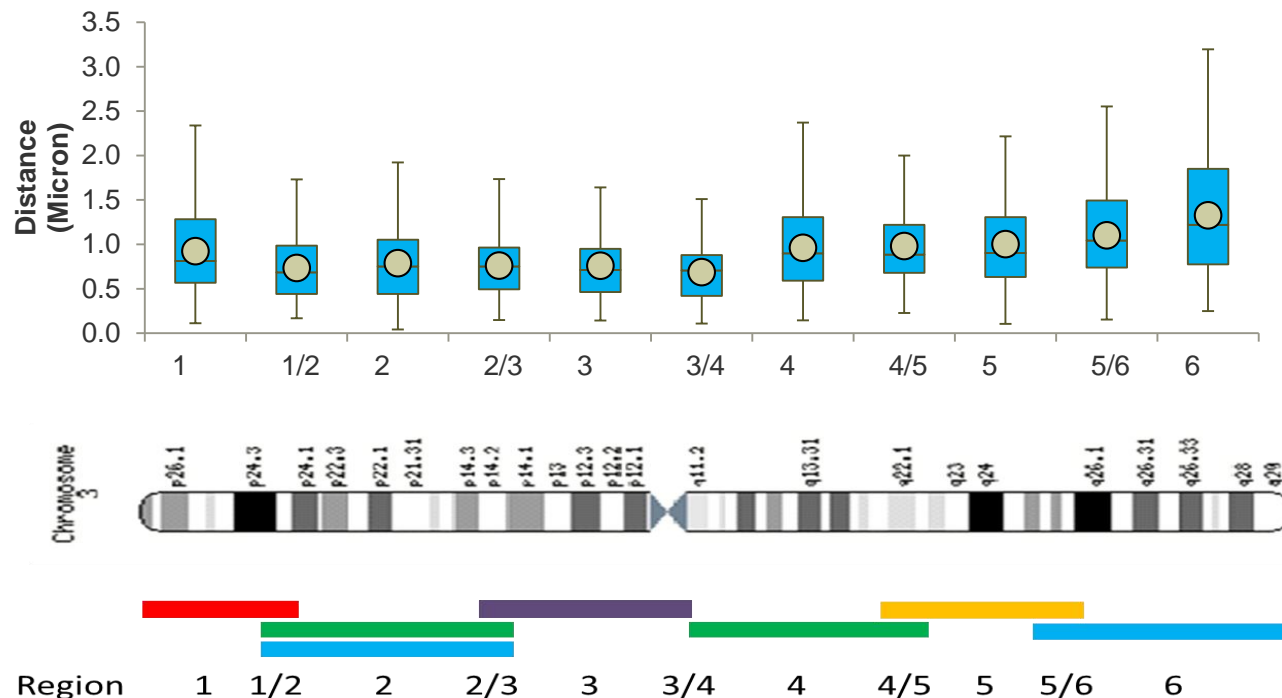
Confocal Image



3D Analysis - Imaris

The three dimensional structure of chromosome 3 in each individual cell was examined using the multicolor chromosome banding (mBAND) techniques and confocal microscopy.

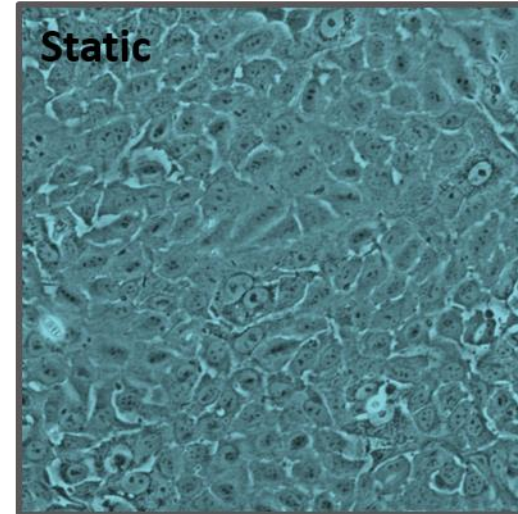
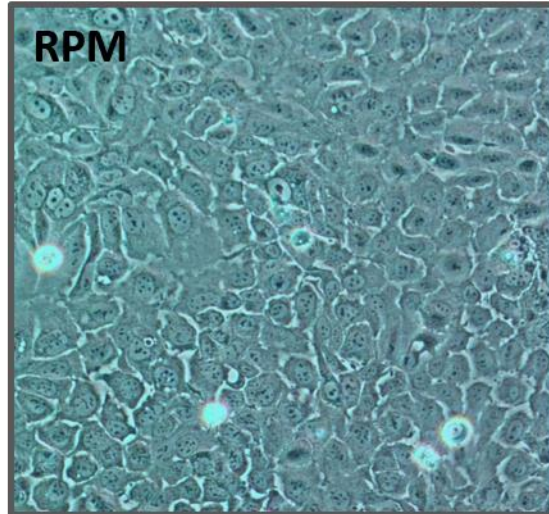
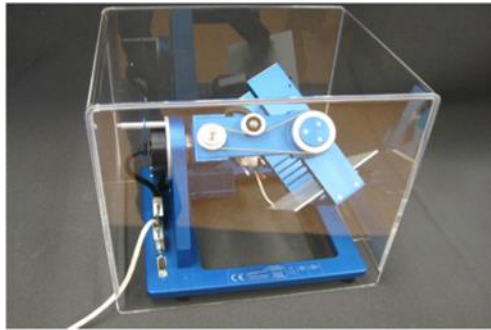
Distances between the center of each region and the center of the chromosome domain



The telomere regions (Regions 1 and 6), as well as the q-arm of the chromosome (Regions 4-6) appeared to be located towards the exterior of the chromosome domain. Most of the regions in the p-arm of the chromosome (Regions 1/2 -3), as well as the centromere region (Region 3/4), however, were closer to the center of the chromosome domain.

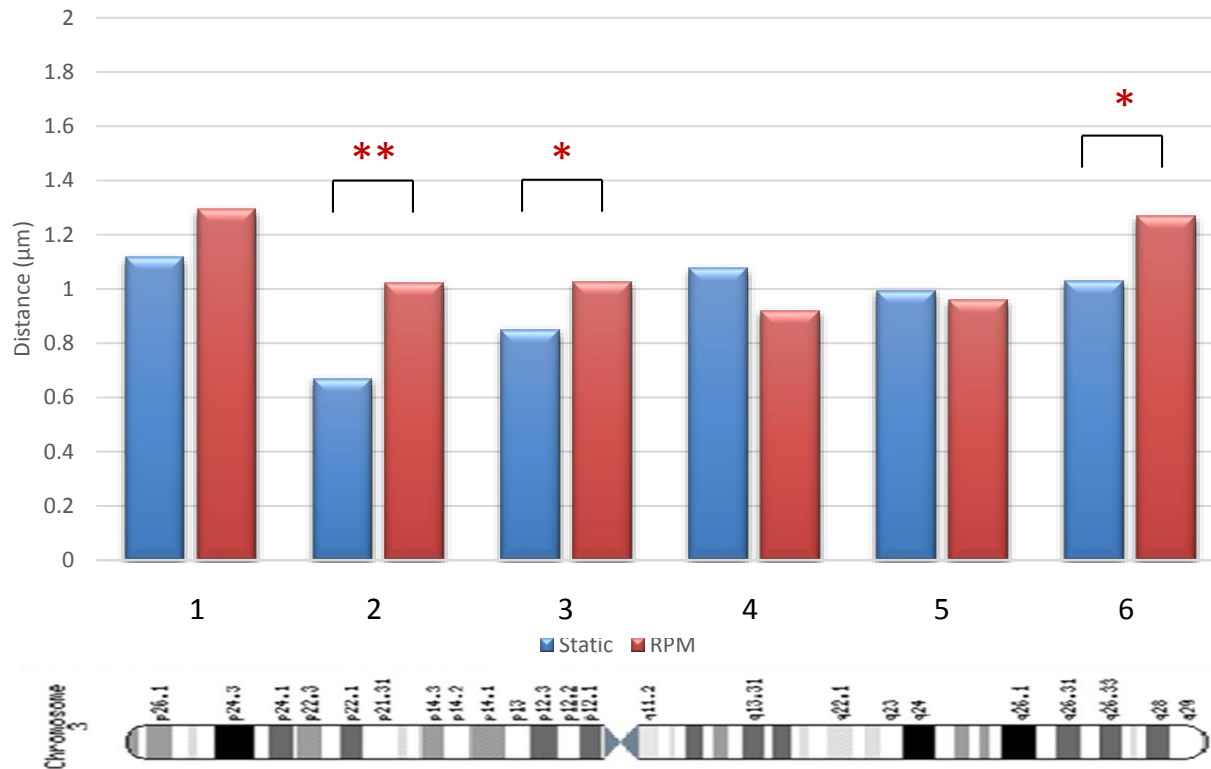
Q: Does microgravity alter the chromosome conformation?

Microgravity simulator: Random Positioning Machine (RPM)



Epithelial cells were cultured on the RPM or statically for 5 days to reach contact-inhibition. No morphological changes were detectable in the cells cultured under both conditions.

Distances between the center of each region and the center of the whole chromosome 3 domain

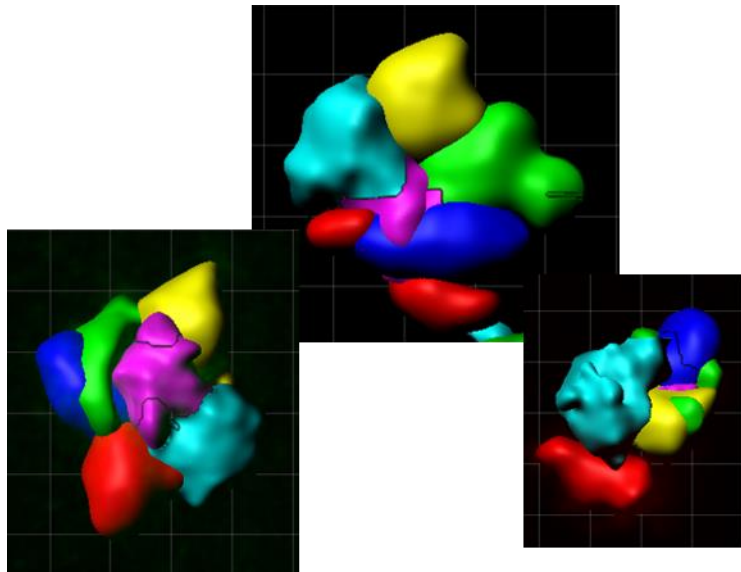


In the cells cultured under static conditions, the mid-p-arm and the centromere regions of the chromosome (Regions 2 and 3) were closer to the center of the chromosome domain in the static cell. After exposure to simulated microgravity, these regions moved away from the center of the chromosome domain.

The Localization of Region 2 that Contains 3p21

Static condition

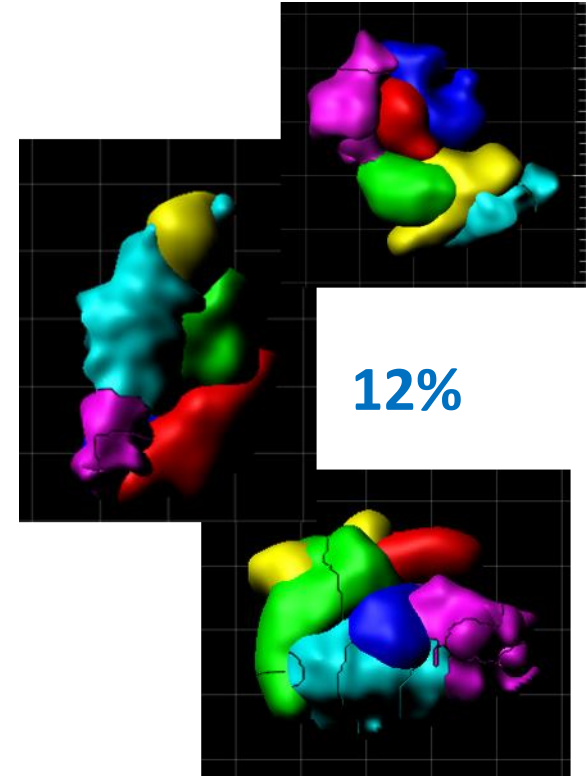
35%



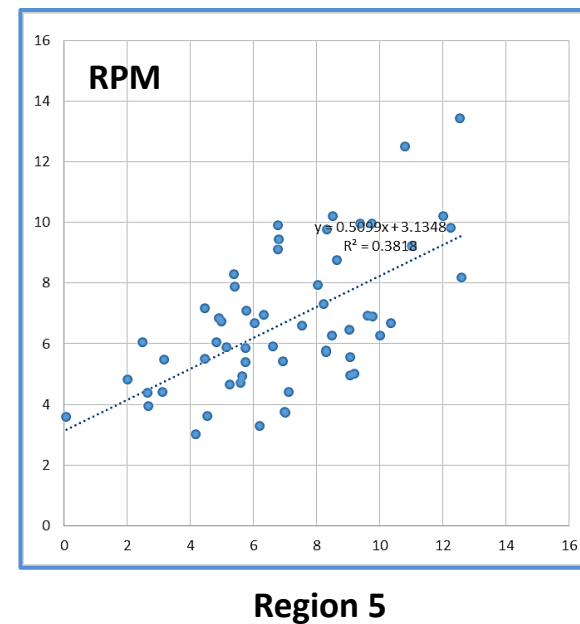
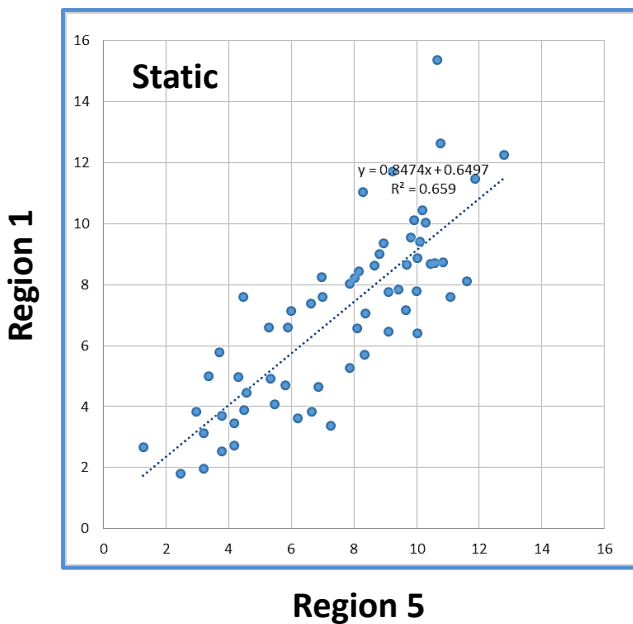
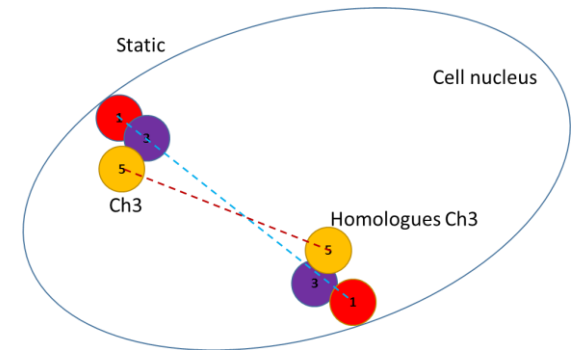
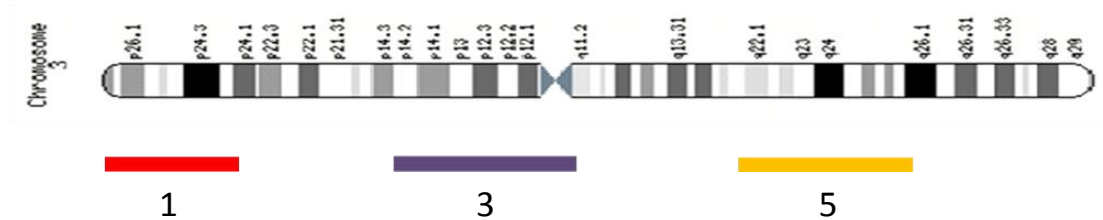
In the static condition, region 2 tended to localize close to the center in a significant number of chromosomes. In the cells exposed to simulated microgravity, region 2 localized at a random position.

12%

Simulated gravity condition

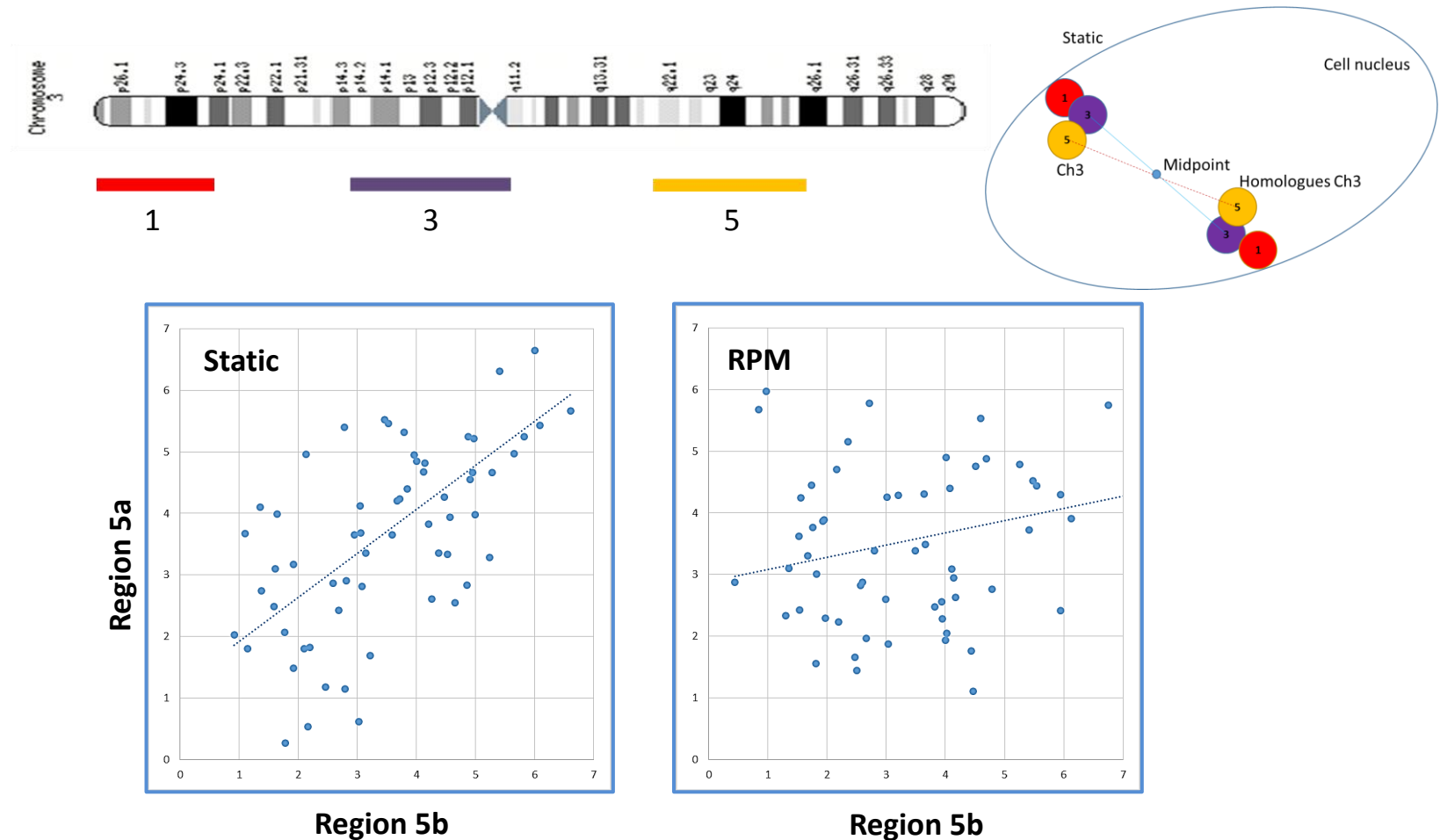


Correlations of the Homologous Pair of Chromosome 3



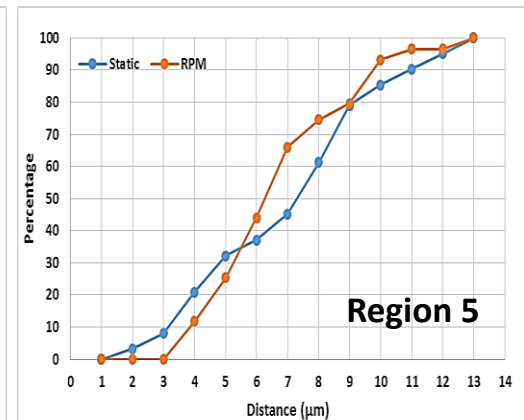
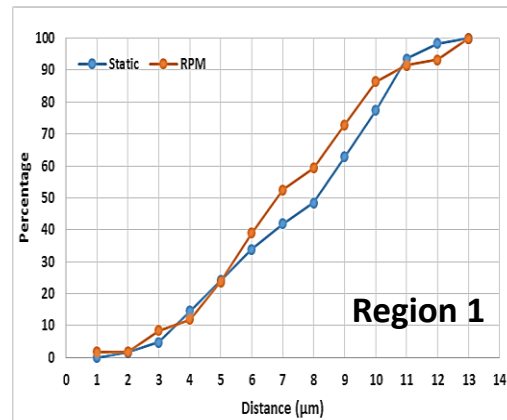
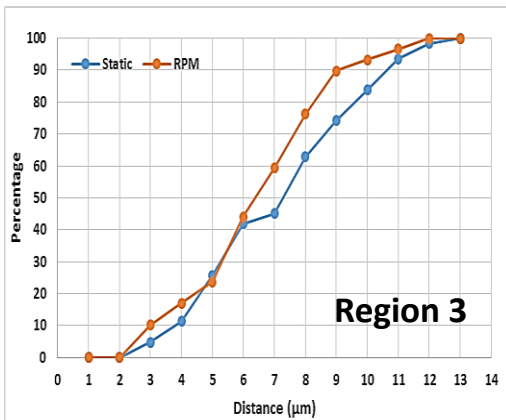
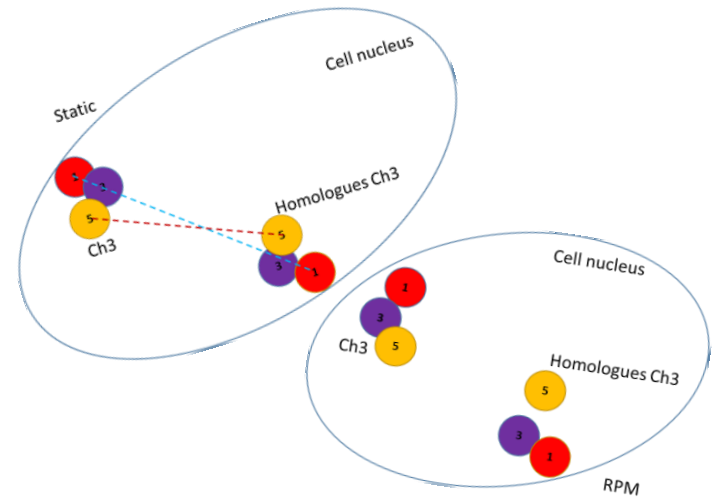
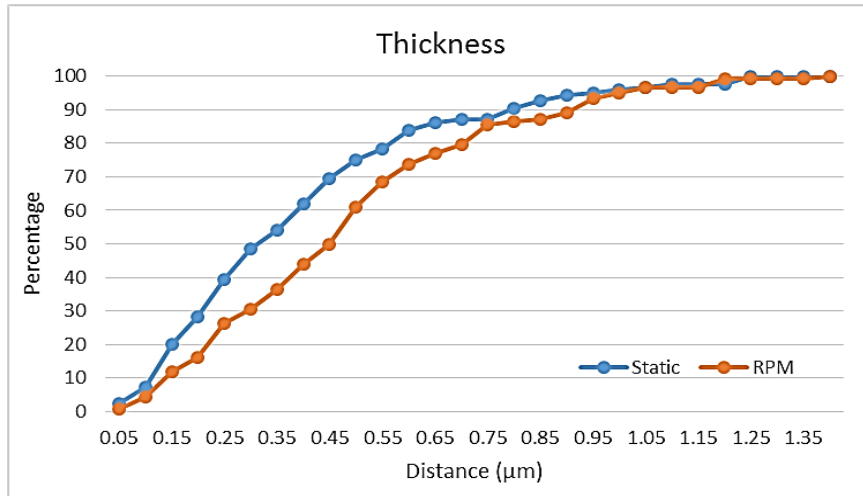
In the cells grown under static conditions, distance between two homologous regions was well correlated with those of other regions. However, in simulated microgravity conditions, these distances were poorly correlated.

Correlations of the Homologous Pair of Chromosome 3



In addition, the distances between two homologous region 5's to the midpoint of two homologous region 3's (containing the centromeres) were well correlated (0.77) in the static cells, but not in the cells cultured on RPM.

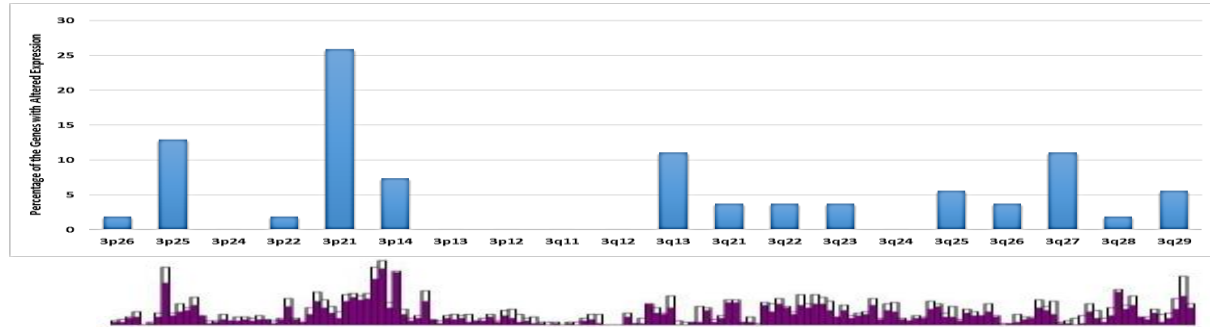
Chromosome Domain Thickness and the Distance Between Two Homologous Regions



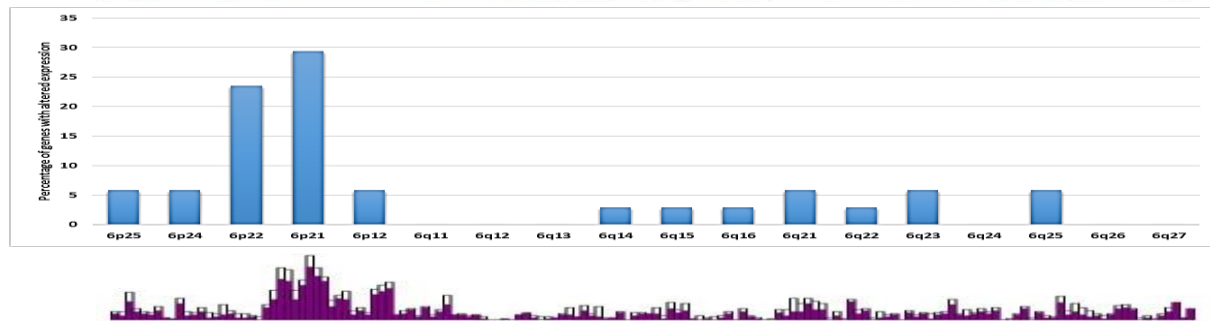
The cells cultured on the RPM had much thicker chromosome domains, with correspondingly shorter distances between two homologous regions, compared to the static cells.

The Gene Expression Profiles of Individual Chromosome of Fibroblasts Cultured on ISS vs on Ground

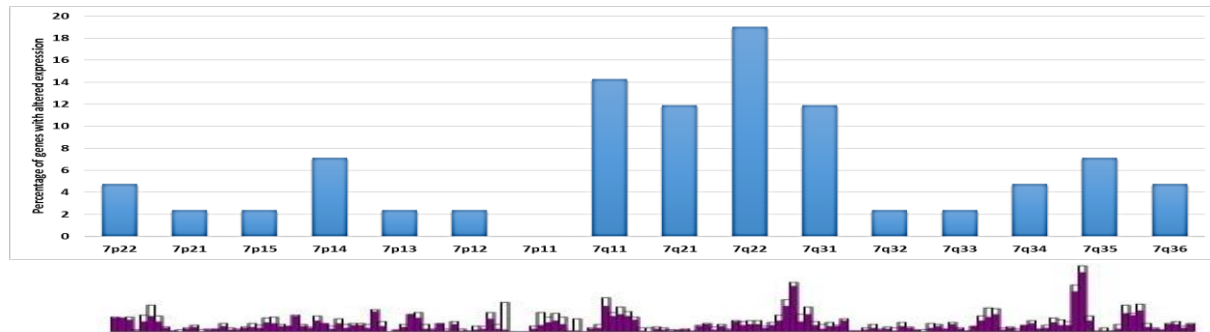
Chromosome 3



Chromosome 6



Chromosome 7



Non-random distribution of genes with altered expression over individual chromosomes were found in the cells flown on the ISS.

Conclusion

- On a multi-megabase pair scale of the DNA, the arrangement of chromatin in M10 epithelial cells was found to be non-random.
- In human epithelial cells, both telomere regions tended to be located towards the exterior of the chromosome domain, whereas the remaining p-arm chromatin region was located towards the interior. In contrast, most of the q-arm of the chromatin was found in the periphery of the domain.
- Chromosome conformation in a significant number of cells was found to be altered under simulated microgravity condition.
- The alterations included rounder shape of the nucleus, poor correlations of two homologous chromosomes and their regions, and changes in the relative location of region 2 that contains the 3p21 hotspot in a significant number of chromosome 3s.
- The gene expression changes in the cells flown on the ISS displayed non-random distribution of genes with altered expression over individual chromosomes, indicating unique chromosome conformation or gene clusters may be involved in spaceflight induced gene expression regulation.

Further Investigations:

- **Whether changes of chromosome conformation in simulated microgravity correlate with gene expression changes under the simulated microgravity culture condition.**
- **Whether the changes of chromosome conformation alter the gene expression profiles in response to radiation induced damage.**
- **Whether the changes of chromosome conformation alter the breakpoint distribution in radiation-induced intrachromosomal exchanges, which may subsequently alter the long-term outcome induced by radiation exposure.**
- **Whether non-random conformation of Chromosome 3 exists at the tissue level, or only within the cultured cells in the static condition.**
- **Whether other chromosomes or chromosomes in other cell types have their own unique conformations.**

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Thank you!