LABORATORY OF CELL BIOLOGY

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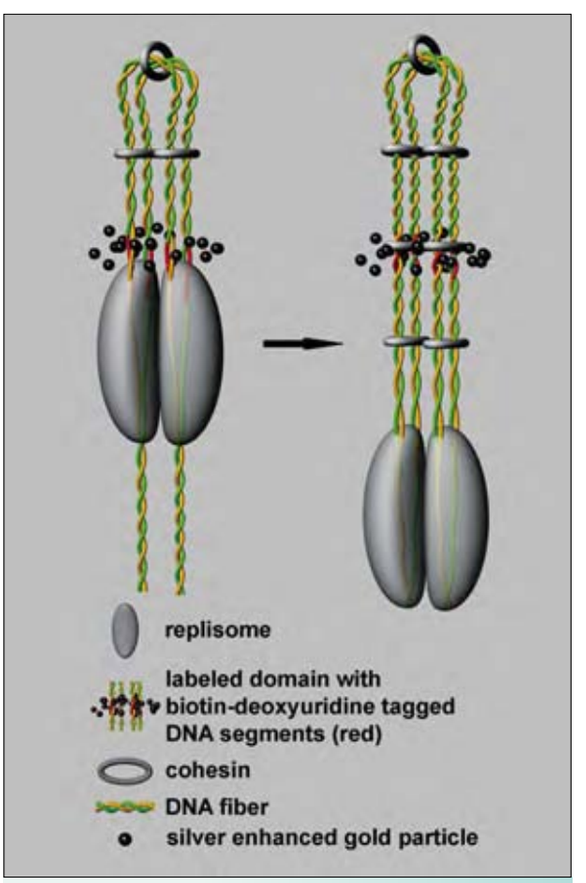
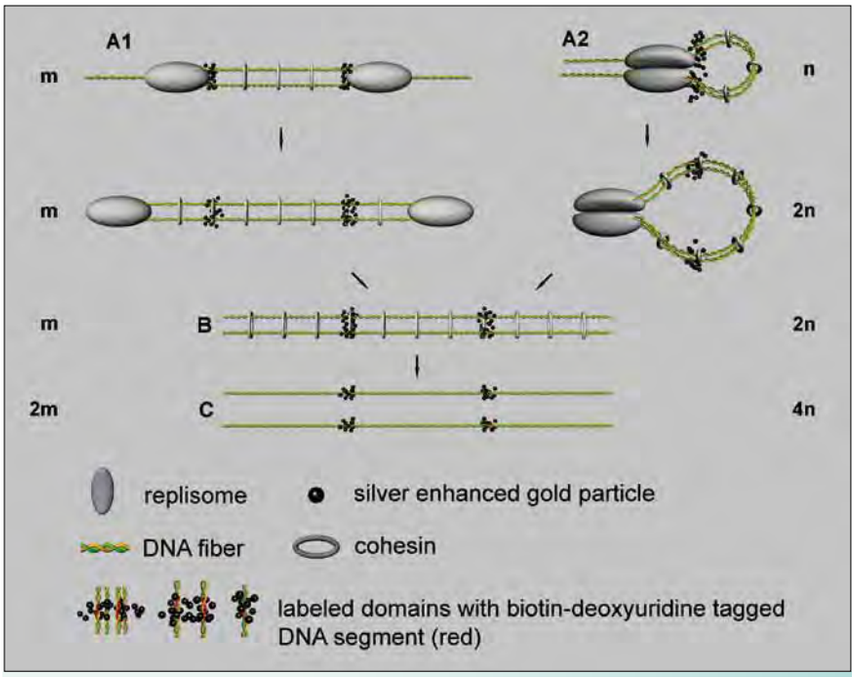
RESEARCH TOPICS

Research in the Laboratory of Cell Biology focuses on DNA replication and the organization of human chromatin. DNA replication of the two template strands at DNA replication forks is a highly coordinated process comprising the functioning of many factors that ensures accurate and efficient duplication of the eukaryotic genome. According to the general paradigm, proper DNA duplication from each replication origin is ensured by two protein complexes termed replisomes. In this respect, our comparative analysis of short segments of replicons labeled in pulse-chase experiments of various lengths showed that replisomes in HeLa cells are organized into couples during DNA replication.



The model of zipping loops.
Zipping of a DNA loop is shown. During replication, replisome couples produce a loop with the associated (zipped) arms probably in the form of four tightly associated 30 nm fibers. According to this model, "sister" pairs of biotin-dU-tagged segments of chromatids do not separate before the termination of the DNA synthesis of the replicon and the relaxation of the zipped arms. Immediately after labeling, the four tagged segments are present in one labeled domain (the left part of the image). Such an organization of the tagged segments persists during the synthesis of the whole replicon (the right part of the image). Although the mutual changes of the replisome position between the left and right parts of the Figure can result in the impression of movement of the replisome along the DNA, this model does not reflect whether the DNA or the replisome complex is moving during replication.

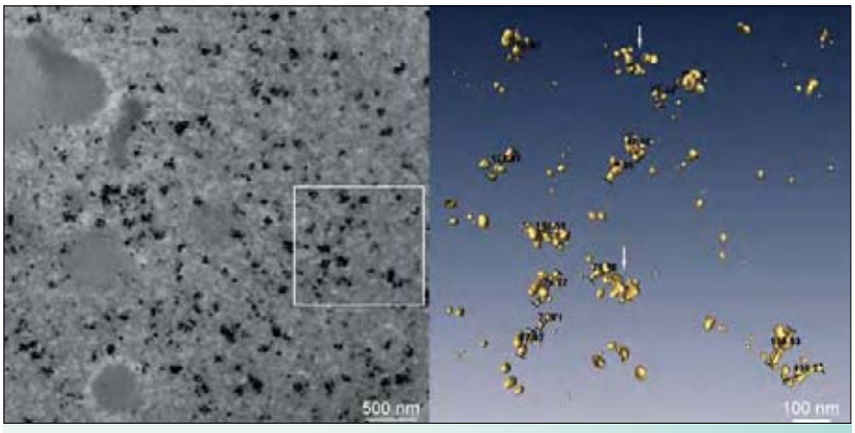


Two models of the arrangement of "sister" replisomes in HeLa cells and the effect of different organizations of biotin-dU-tagged segments on the number of labeled domains during various pulse-chase experiments.
The scheme shows the expected results of the consecutive mapping (indicated by arrows) of segments tagged during a short pulse of biotin-dUTP in the early S phase followed by chase periods of different lengths from the time immediately after the pulse (the upper part of the scheme) to the complete mitotic segregation of the sister chromatids (the lower part of the scheme).

Moreover, our data suggested a new model of the organization of replicated DNA. According to this model, replisome couples produce a loop with the associated arms in the form of four tightly associated 30 nm fibers.

The Laboratory's research is also focused on changes in chromatin arrangement during the cell cycle, the timing of DNA replication, epigenetically inherited chromatin remodeling, and the large scale organization of chromosomal territories.

Concurrently, the Laboratory participates in a project focused on the development of new techniques for the delivery of bioactive molecules into human cells. Two basic research lines are followed in this respect: the development of methods based on the use of viral kinases for the efficient phosphorylation of chosen nucleosides and the development of methods based on the modification of nucleotide precursors.



3D reconstruction of the labeled domains.
The original image of a 200 nm-thick section of a cell nucleus is shown on the left (Scale bar: 500 nm), whereas a 3D reconstruction of the labeled domains reconstructed from the insert is shown on the right (Scale bar: 100 nm). The length measurement is demonstrated. The arrows indicate labeled domains traversing the section faces.

Techniques that are employed include cell and bacterial cultivation, affinity cytochemistry involving immunocytochemical and in situ hybridization techniques, light and confocal microscopy, electron microscopy including electron tomography, stereology approaches, protein, RNA and DNA purification and analysis, immunoprecipitation and GFP technology.

CURRENT GRANTS SUPPORT

GA AS CR, KAN200520801, Targeted expression and transport of bioactive molecules, 2008–2012.

GA CR, 206/07/0233, Biochemical, ultrastructural and partial proteomic analysis of the penetration apparatus of bird Schistosoma cercariae, 2007–2010.

GA CR, 305/08/0535, Organization of human nuclear chromatin, 2009–2011.

GA AS CR, KJB500390701, Replication of the mammalian genome – 4D view, 2007–2009.

SELECTED RECENT PUBLICATIONS

1. Koberna K, Ligasová A, Malínský J, Pliss A, Siegel AJ, Cvačková Z, Fidlerová H, Mašata M, Fialová M, Raška I, Berezney R. (2005). Electron microscopy of DNA replication in 3-D: Evidence for similar-sized replication foci throughout S-phase. J Cell Biochem 94: 126–138.

2. Li J, Santoro R, Koberna K, Grummt I. (2005) the chromatin remodeling complex NoRC controls replication timing of rRNA genes. EMBO J 24: 120–127.

3. Stavreva DA, Kavasaki M, Dundr M, Koberna K, Muller WG, Tsujimura-Takahashi T, Komatsu W, Hayano T, Isobe T, Raška I, Misteli T, Takahashi N, McNally JG. (2006). Potential role for ubiquitin and the proteasome during ribosome biogenesis. Mol Cell Biol 26: 5131–5145.

4. Kalmárová M, Smirnov E, Mašata M, Koberna K, Ligasová A, Popov A, Raška I. (2007). Positioning of NORs and NOR-bearing chromosomes in relation to nucleoli. J Struct Biol 160: 49–56.

5. Ligasová A, Raška I, Koberna K. (2009) Organization of human replicon: singles or zipping couples. J Struct Biol 165: 204–213.