ABSTRACT

Despite the capabilities of molecular-biological methods in deciphering the interplay of different biological molecules and molecular complexes, the understanding of respective functions in living cells requires application of *in situ* methods. Obviously, these methods should provide maximal resolution and the best possible preservation of the biological object in a native state, as well as correct statistical evaluation of the spatial characteristics of detected molecular players.

Transmission electron microscopy provides the highest possible resolution for analysis of biological samples. The simultaneous detection of biological molecules by means of indirect immunolabeling provides valuable information about their localization in cellular compartments and their possible interactions in macromolecular complexes. To analyze this, we have developed a complex stereological method for statistical evaluation of immunogold clustering and colocalization patterns of antigens on ultrathin sections, including a user-friendly interface. Functional microarchitecture of DNA replication and transcription sites has been successfully characterized using the developed stereological tools. Our data demonstrate that DNA replication is compartmentalized within cell nuclei at the level of DNA foci and support the view that the synthetic centers are spatially constrained while the chromatin loops are dynamic during DNA synthesis. In HeLa cells, we have ultrastructurally distinguished two morphological types of replication sites - replication bodies and replication foci. Both types contain a set of enzymatic, structural and regulatory proteins, which are known to take part in replication itself or in S-phase regulation. Some regulatory and structural proteins, however, were found only in replication sites of one type. In transcription sites, we have demonstrated on the ultrastructural level the presence of two main cellular molecular motor molecules. Using the model of PHA-stimulated human lymphocytes, the presence of actin was mainly not dependent on the activity status of the cells, while nuclear myosin I shows dynamic behavior upon transcriptional activation.

To extend the technical capabilities for analysis of multiple interactions at the ultrastructural level, we have developed a new system, which allowed us simultaneous immunolabeling of up to five antigens, as compared to only two conventionally available. Gold-silver core-shell nanoparticles, gold nanorods and cubic palladium nanoparticles distinguishable in TEM by their shape were synthesized, and PIP2, B23, actin, Sm protein, and SMC2 were simultaneously detected on ultrathin sections. We have demonstrated that PIP2-positive foci were found in a close contact with Sm–, actin-, and/or SMC2-rich foci, forming complementary 3D domains in the cell nucleus.

Simultaneous ultrastructure and antigen preservation of biological samples is not a trivial task. LR White resin is suitable for sample embedding after both chemical and cryo fixation. However, for some antigens Lowicryl HM 20 could be preferable. Addition of 1.5% water and 0.5% glutaraldehyde into the acetone for freeze substitution improves the antigen preservation in most cases, as shown by immunogold labeling and biochemical quantification of protein amount, while the ultrastructure stays well preserved.