The ultrastructural localization of various antigens in a cell using antibodies conjugated to gold particles is a powerful instrument in biological research. However, statistical or stereological tools for testing the significance of non-random location of gold particles are missing. We have therefore developed methods which allow one

1, to detect clustering or colocalization of antigens/gold particles using the distribution of distances between them (1) and

2, to delineate the borders of cellular compartments, which are defined by immunogold labelling of specific molecules even if they are morphologically inconspicuous (2).

Furthermore, we have developed plug-ins for the Ellipse program (www.ellipse.sk) that form a set of tools for processing and evaluation of immunogold labeling results. They allow one to detect reliably gold particles in EM images, to evaluate statistically the observed immunogold labelling patterns (clustering, colocalization, compartments) and to produce a convenient output for publications of results. The plug-ins are useful addition for any image analysis software which accompanies most of the modern CCD cameras for electron microscopes. These plug-ins are available at our web-site http://nucleus.biomed.cas.cz/gold. Above-described methods were successfully used in our studies on nuclear actin and myosin. Previously, we produced and affinity purified polyclonal antibodies to adrenal myosin 1. In addition to adrenal myosin 1 (116 kDa), these antibodies recognized by Western blot a 120 kDa protein in extracts prepared from many different cell lines. Based on the properties of the protein, we proposed that the 120 kDa protein is a previously undescribed myosin 1 isoform that is an intranuclear actin based molecular motor. The presence of actin and nuclear myosin I (NMI) in the nucleus suggested their role in nuclear functions. Here we demonstrate that both proteins are associated with rRNA genes and with RNA polymerase I (Pol I) during transcription. Chromatin immunoprecipitation revealed the association of NMI and actin with rDNA and abortive transcription assays showed that actin functions in elongating Pol I complex. Depletion of NMI or actin inhibits Pol I transcription in vivo and in vitro, while over expression of NMI augments pre-rRNA synthesis. NMI associates with Pol I via the transcription initiation factor TIF-IA and this association requires phosphorylation of TIF-IA at serine 649 (3, 4). Those, actin and NMI are apparently required for efficient transcription of rDNA, and possible models will be discussed. Additional improvements in immunogold detection will be discussed using high-pressure freezing, cryo-substitution, and 3D-analysis of gold particles distribution based on tomography data.

(2) Schofer et al., J. Struct. Biol. 147 (2004), 128-135
(3) Philimonenko et al., Nature Cell Biol. 6 (2004), 1165-1172
(4) Hofmann et al., Nature Cell Biol. 6 (2004), 1094-1101
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