

## PROTEIN DISTRIBUTION AND DYNAMICS IN NUCLEAR COMPARTMENTS

Jasmin Speil & Ulrich Kubitscheck  
Institute of Physical and Theoretical Chemistry  
Rheinische Friedrich-Wilhelms University Bonn  
Wegelerstraße 12, 53115 Bonn, Germany  
E-mail: speil@pc.uni-bonn.de

**KEY WORDS:** Fluorescence microscopy, single molecule microscopy, nucleus, nucleoli, dynamics, diffusion coefficient.

The nucleus is the center of direction and coordination of the cell's metabolic and reproductive activities. In recent years it was found that it is formed by many functionally specialized domains. These subnuclear structures are not surrounded by membranes as the cytoplasmic organelles and their function is mostly not well understood. To obtain insights into the structural organisation of the nuclear interior we focus on the most prominent structure, the nucleoli and the remaining nucleoplasm. We used fluorescently labeled Ovalbumin-Atto647N, an inert protein, to examine the physical properties of nucleoli in comparison to the nucleoplasm. Ovalbumin-Atto647N was microinjected into the cytoplasm of HeLa cells, and after diffusion into the nucleus the protein distribution in the two nuclear compartments was measured. The location of the nucleoli was determined by differential interference contrast. As was detected before for dextran molecules of varying size, a clearly reduced concentration of Ovalbumin in the nucleoli compared to the nucleoplasm was determined as an intensity ratio of nucleolar (No) and nuclear (Nu) regions No/Nu ratio [1, 2]. The ratio approached 0.6 about 30 to 60 min after microinjection. Measurements of Ovalbumin mobility were used to characterize the physical properties of nucleoli and nucleoplasm in terms of possible binding or trapping of the probe molecule [2, 3]. Single molecule tracking of Ovalbumin-Atto647N was performed at almost 200 Hz using an electron-multiplying CCD. In this manner it was possible to observe and analyze single molecule trajectories within nucleoli and nucleoplasm by jump distance analysis [4]. With this method we identified four different fractions with diffusion coefficients ranging from  $D = 13 \mu\text{m}^2/\text{s}$ ,  $D = 2 \mu\text{m}^2/\text{s}$ ,  $D = 0,5 \mu\text{m}^2/\text{s}$  and an immobile fraction. Surprisingly, the mobility of Ovalbumin in the two different compartments did not differ significantly. A slightly larger probability of binding or trapping was found in the nucleoplasm in comparison to the nucleoli.

[1] Sabine M. Görisch, Karsten Richter, Markus O. Scheuermann, Harald Herrmann, and Peter Lichter. "Diffusion-limited compartmentalization of mammalian cell nuclei assessed by microinjected macromolecules," *Exp. Cell Res.* **289**, 282-204 (2003).

[2] Korie E. Handwerker and Joseph G. Gall. "Subnuclear organelles: new insights into form and function," *Trends Cell Biol.* **16** (2005).

[3] Grünwald, D, R.M. Martin, V. Buschmann, D.P. Bazett-Jones, H. Leonhardt, U. Kubitscheck, and M.C. Cardoso. "Probing Intranuclear Environments at the Single Molecule Level," *Biophys. J.* **94**, 2847-58 (2008).

[4] Jan Peter Siebrasse, Roman Veith, Akos Dobay, Heinrich Leonhardt, Bertil Daneholt and Ulrich Kubitscheck. "Discontinuous movement of mRNP particles in nucleoplasmic regions devoid of chromatin," *Proc. Natl. Acad. Sci. USA* **105**, 20291-20296 (2008).