

**FRAP CONDITIONS INFLUENCE MEASUREMENTS OF D_{eff} AND
MOBILE FRACTION OF FIBRILLARIN**

Viktoria V. Barygina and Olga V. Zatsepina

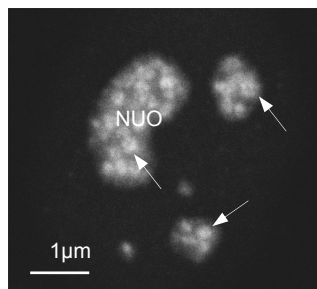
**Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of
Sciences, Moscow, Russia**

E-mail : zatsepina@ibch.ru

KEY WORDS: HeLa cells, nucleolus, fibrillarlin, EGFP, FRAP, mobile fraction, effective diffusion coefficient (D_{eff}), pH of culture medium

Fibrillarlin is a representative protein of early rRNA processing events which take place within the nucleolus and are necessary for ribosome synthesis. Human fibrillarlin (321 aa, pI 8.5) belongs to highly mobile nucleolar proteins, but data on its mobility in cells (i.e. the volume of mobile/immobile fractions and the effective diffusion coefficients, D_{eff}) vary within a wide range [1-3]. The reasons of these discrepancies remain unclear. In the current work, we used HeLa cells stably transfected with a plasmid encoding human full-length fibrillarlin fused to EGFP (Enhanced Green Fluorescence Protein) and photobleaching techniques to study the protein mobility depending on pH of extracellular media and on squares of bleached areas. FRAP (Fluorescence Recovery After Photobleaching) experiments were performed using a confocal microscope LSM510 DuoScanMETA equipped with an Ar and He-Ne lasers and a Plan-Apochromat 63x/1.40 oil objective (Carl Zeiss, Germany). Parts of the nucleolus of $\sim 0.9 \mu\text{m}^2$ or $\sim 3 \mu\text{m}^2$ in square were bleached by 488 nm laser beam parking for up to 250 ms, and single section images were collected every 15 s. Mobile fractions and D_{eff} s were assessed as described in [4]. At least ten independent FRAP measurements were performed for each experimental point.

Typical pattern of fibrillarlin-EGFP location within nucleoli (NUO) of a proliferating HeLa cell nucleus at pH 7.2-7.4 is shown in Figure. The protein fusion is seen in numerous discrete foci (*arrows*) which overlay with rRNA synthesis foci. The localization was essentially the same at pH 7.8-8.0. Singular foci were casually selected for bleaching of the relatively small areas. A part of or an entire nucleolus were selected for bleaching of the larger areas. All experiments were conducted at 37° C. The results are summarized in Table. They show that the D_{eff} and fibrillarlin mobile fraction remain rather stable at various pH. However, both parameters become significantly changed when bleached areas differ in size. Similar results were also obtained when HeLa cells were transiently transfected with a plasmid coding *Nop56-DsRed* – a chimeric protein that is known to co-localize with fibrillarlin-EGFP [5]. We concluded that the size of areas subjected for FRAP experiments should be considered, if protein mobility is analyzed.



Square of bleached areas ($S; \mu\text{m}^2$)	0.85-0.90		2.1-3.4	
	7.2-7.4	7.8-8.0	7.2-7.4	7.8-8.0
$D_{\text{eff}} (\mu\text{m}^2 \cdot \text{s}^{-1})$	0.012±0.003	0.011±0.002	0.030±0.003	0.027±0.002
Mobile fractions (%)	62±5	60±7	28±5	30±5

- [1] R.D. Phair, and T. Misteli. *Nature*. **404**, 604-609 (2000).
 [2] S. Snaar, K. Wiesmeijer, A. Jochemsen, et al. *J Cell Biol*, **151**, 653-662 (2000).
 [3] N. Gurskaya, V. Verkhusha, A. Shcheglov, et al. *Nature Biotech*, **24**, 461-465 (2006).
 [4] D. Axelrod, D. Koppel, J. Schlessinger, et al. *Biophys J*, **16**, 1055-1069 (1976).
 [5] T. Lechertier, V. Sirri, D. Hernandez-Verdun, P. Roussel. *J Cell Sci*. **120**, 265-275 (2006).