

# SINGLE MOLECULE MICROSCOPY OF INTRANUCLEAR mRNP DYNAMICS IN LIVING *C. TENTANS* SALIVARY GLAND CELL NUCLEI

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**KEYWORDS:** single molecule microscopy, fluorescence correlations spectroscopy (FCS), selective plane illumination microscopy (SPIM), mRNA trafficking, intranuclear dynamics, in vivo labeling

Before messenger ribonucleoprotein particles (mRNPs) exit from the nucleus they move randomly within the nucleoplasm [1]. However, until now single particle tracking with artificial mRNPs revealed diffusion coefficients well below the expected values as deduced from their size. At least the unfolded interphase chromatin should be responsible for these deviations from theory. The polytene nuclei in the salivary gland cells of *Chironomus tentans*, however, harbor giant chromosomes. They are separated by vast regions of nucleoplasm, which allows us to study mRNP mobility without interference of interphase chromatin. We have studied the intranuclear movement of a specific endogenous mRNP, the BR2 mRNP, in salivary gland cells in *Chironomus tentans* with high speed single molecule fluorescence microscopy [2]. The particles were fluorescently labeled with microinjected oligonucleotides (DNA or RNA) complementary to BR2 mRNA or with the RNA-binding protein hrp36.

The epifluorescence data were now complemented by fluorescence correlation spectroscopy measurements. Additionally highly sensitive selective plane illumination microscopy (SPIM) [3] was used for the first time to study the mobility of mRNP particles in vivo.

The Balbiani ring (BR) mRNPs moved randomly in the nucleoplasm but, unexpectedly, in a discontinuous manner. Nonattached mobile mRNPs diffused with a diffusion coefficient corresponding to their size. Most of the time however mRNPs were slowed down 10- to 250-fold, but were never completely immobile. We propose that the observed discontinuous movement reflects transient interactions between freely diffusing BR particles and submicroscopic intranuclear structures not containing chromatin.

[1] Gorski SA, Dundr M, Misteli T, „The road much traveled: trafficking in the cell nucleus.”, *Curr Opin Cell Biol.* 2006 Jun;18(3):284-90. Epub 2006 Apr 18.

[2] Siebrasse JP, Veith R, Dobay A, Leonhardt H, Daneholt B, Kubitscheck U., “Discontinuous movement of mRNP particles in nucleoplasmic regions devoid of chromatin.”, *Proc Natl Acad Sci U S A.* 2008 Dec 23;105(51):20291-6. Epub 2008 Dec 12.

[3] Ritter JG, Veith R, Siebrasse JP, Kubitscheck U., “High-contrast single-particle tracking by selective focal plane illumination microscopy.”, *Opt Express.* 2008 May 12;16(10):7142-52.