

# IMAGING OF ENDOGENOUS $\beta$ -ACTIN mRNA DURING NUCLEOCYTOPLASMIC TRANSPORT.

**D. Grünwald, Robert H. Singer**  
**Anatomy & Structural Biology (ASB)**  
**Albert Einstein College of Medicine (AECOM)**  
**Yeshiva University**  
**Golding 601, 1300 Morris Park Ave, Bronx NY, 10461 – USA**  
**E-mail: dgruenwa@aecom.yu.edu**

Transport through the Nuclear Pore Complex (NPC) is the only link between nucleus and cytoplasm of a cell. Proteins, RNAs, signaling cascades and ions travel along this largest known multi-protein machine. Knowledge of physical properties and dynamics of the transport process are crucial for our understanding of cellular function and may provide insights into regulatory processes unknown today.[1] The life cycle of mRNA includes a number of posttranscriptional modification steps and export from the nucleus before it can be transcribed by the ribosome machinery. Mobility of mRNAs in the nucleoplasm have been measured by different means revealing probabilistic movement with diffusion coefficients from  $0.03 \mu\text{m}^2/\text{s}$  to  $4 \mu\text{m}^2/\text{s}$  [2-5]. mRNA is found in the cytoplasm within ~10 minutes after being polyadenylated and ~20 minutes after transcription in pulse-chase experiments [6], but the dynamics of mRNA export are largely unknown, as well as its interaction with the NPC. Interactions of transport factors and the NPC are weak, making biochemical analysis difficult [7] and limits access by EM studies [8]. Live cell microscopy provides access to the undisturbed system at physiological concentration providing kinetic and spatial information. While conventionally fluorescent imaging of molecular process is limited by optical resolution, imaging of single molecules in living cells with sufficient time resolution and in multiple channels allowed us to follow mRNA export interactions with the NPC with nm precision in real time, circumventing the problem of synchronizing the export process artificially. In this work we were able to employ a marker for an endogenous mRNA overcoming the limitations of probe delivery for export studies in living cells and to combine this with a stable NPC marker allowing nm precise registration between mRNA and NPC signals. While the physical structure and dynamic compositions of FG-Nups within the central channel of the NPC have been a major subject of discussion [7-11], mRNA during export only spends little time within the central channel. Our data reveal the export dynamics and interactions sides of mRNA during export from the nucleus to the cytoplasm, to our knowledge for the first time.

- [1.] J. A. Hurt, P. A. Silver, *Dis Model Mech* **1**, 103 (Sep, 2008).
- [2.] J. P. Siebrasse *et al.*, *Proceedings of the National Academy of Sciences* **105**, 20291 (December 23, 2008, 2008).
- [3.] J. Braga, J. G. McNally, M. Carmo-Fonseca, *Biophys J* **92**, 2694 (Apr 15, 2007).
- [4.] Y. Shav-Tal *et al.*, *Science* **304**, 1797 (Jun 18, 2004).
- [5.] J. C. Politz, E. S. Browne, D. E. Wolf, T. Pederson, *Proc Natl Acad Sci U S A* **95**, 6043 (May 26, 1998).
- [6.] B. Lewin, in *Gene Expression*. (John Wiley & Sons, New York, 1980), vol. 2, pp. 730-761.
- [7.] S. S. Patel, B. J. Belmont, J. M. Sante, M. F. Rexach, *Cell* **129**, 83 (Apr 6, 2007).
- [8.] N. Pante, U. Aebi, *Science* **273**, 1729 (Sep 20, 1996).
- [9.] S. Frey, D. Gorlich, *Cell* **130**, 512 (Aug 10, 2007).
- [10.] S. Frey, R. P. Richter, D. Gorlich, *Science* **314**, 815 (Nov 3, 2006).
- [11.] R. Y. Lim *et al.*, *Science* **318**, 640 (Oct 26, 2007).