**SJ2-2**

**Imaging of Single mRNA Molecules Moving within a Nucleus of Living Cell**

Takashi Funatsu  
(Department of Physics, School of Science and Engineering, Waseda University)

Messenger RNA is transcribed and processed in the nucleus and transported to the cytoplasm. In order to clarify the molecular mechanism of these processes, we have developed video-rate confocal microscopy that enabled us to visualize individual single fluorescence molecules. As an application of this technique, movement of individual mRNA molecules within living cell was visualized to distinguish whether mRNA is directed by active transport or moving around by diffusion. For these purpose, Cy3-labeled truncated human b-globin gene mRNA molecules were prepared in vitro. When Cy3-labeled mRNA was micro-injected into the nucleus of Xenopus A6 cell, mRNA could be observed except nucleoli, indicating that mRNA could access most of the space, excluding nucleoli, in the nucleus. Some population of mRNA moved around in diffusion, and some remained stationary, and others moved and stopped. Statistical analysis of individual mRNA trajectories revealed that about 50% of mRNA is moving at the diffusion constant of 0.4 [μm²/s]. This value is about 1/100 of that in solution. Our results support the mechanism that mRNA reach nuclear pores by diffusion process.

---

**SJ2-3**

**TGN-endosome-plasma membrane trafficking visualized in living cell**

Satoshi Waguri  
(Department of Cell Biology and Neuroscience (A), Osaka University Graduate School of Medicine, Suita, Japan)

The mannose 6-phosphate receptors (MPRs) play an important role in the intracellular transport of lysosomal enzymes by cycling between the trans-Golgi network (TGN), endosomes and the plasma membrane. To visualize the MPR transport in living cells, we constructed a chimeric protein made of green fluorescent protein (GFP) fused to the transmembrane and cytoplasmic domain of the cation-independent MPR (GFP-MPR), which was transfected into HeLa cells.

At steady state, the bulk of this chimeric protein was localized in the TGN but small amounts were also detected in peripheral, tubulo-vesicular structures which occasionally contained endocytosed transferrin. The GFP-labeled chimeric protein was ubiquitously detected in long membrane tubules emanating from the TGN. These tubular elements also contained the endogenous MPR and were coated with AP-1 and clathrin patches. Time-lapse videomicroscopy revealed that tubular elements detached from the TGN and moved toward the cell periphery, where they were occasionally broken into smaller tubular elements. These structures showed highly dynamic movement, fusing with each other as well as with endosomes containing internalized transferrin. The data support the notion that tubular elements mediate MPR transport from the TGN to a peripheral tubulo-vesicular network, which is dynamically connected with the endocytic pathway.

To define the transport intermediate (TI) more clearly, the cells expressing GFP-MPR were recorded with a confocal microscope after their peripheral regions were photobleached. TIs reaching a membrane domain stayed the place for a while, and transiently increased the fluorescence intensity, which was rapidly spread out on the plasma membrane. To detect TIs that recycle back to the TGN, the TGN region was then photobleached. Vesicular structures moved into the TGN, remained stationary, and dispersed into the TGN. Moreover, by conducting a FRAP (fluorescence recovery after photobleaching) experiment, in which the TGN-pool of GFP-MPR was selectively photobleached, the TGN-fluorescence began to recover with time and reached a plateau within approximately 15 min. From the recovery curve, we could predict that GFP-MPR cycles the TGN and other compartments every 21.5 ± 5.1 min. These observations directly confirm that TGN-derived TIs containing MPR are transported to the plasma membrane as well as early endosomes, and that MPR cycles constitutively between the TGN and the peripheral compartments.