Single-molecule Imaging and Quantification of mRNAs in a Living Cell

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In eukaryotic cells, pre-mRNA molecules contain multiple intron sequences that are removed by splicing reactions. Truncated *ftz* pre-mRNA containing one intron and two exons, which mimics RNA under the post-transcriptional splicing, was synthesized and labeled with a fluorescent dye *in vitro* and then injected to the nucleus of cos7 cell. The injected pre-mRNAs accumulated in 'speckles' in an intron-dependent manner and were spliced and exported to the cytoplasm with a half-time of about 10 min. Dissociation of the accumulated pre-mRNAs in speckles exhibited rapid diffusion and slow dissociation of about 100 s. The slow dissociation required metabolic energy of ATP. Some pre-mRNAs shuttled between speckles and nucleoplasm, suggesting that pre-mRNAs repeatedly associated with and dissociated from speckles until introns were removed. Next, endogenous poly(A)$^+$ RNA was visualized by injecting Cy3-labeled 2'*O*-methyl oligo(U)$_{22}$ probes. Some poly(A)$^+$ RNA also shuttled between speckles and nucleoplasm. These results suggest that speckles function as a checkpoint for whether or not mRNAs are appropriately processed. Next, mature mRNAs of truncated β-globin were synthesized, fluorescently labeled *in vitro*, and injected to the nucleus. The trajectories of single mRNA molecules in the nucleus were visualized using video-rate confocal microscopy. Approximately half the mRNAs moved by Brownian motion in the nucleoplasm, except the nucleoli, with an apparent diffusion coefficient of 0.2 μm^2/s, about 1/150 of that in water. The remaining mRNAs were stationary with an average residence time of about 30 s. These results indicate that mRNAs are transported to nuclear pores by Brownian motion.