RNA export through the nuclear pore complex is directional

Y. Shav-Tal[1]

[1] The Faculty of Life Sciences and the Institute for Nanotechnology and Advanced Materials (BINA), Bar-Ilan University, Ramat Gan, Israel.

Abstract

The organization and compaction of mRNA into an mRNP during nucleo-cytoplasmic transport and export, are key steps in gene expression. Changes in mRNA organization after release from the gene, during nucleoplasmic diffusion and in preparation for export, are unknown. It is assumed that at the nuclear pore complex (NPC), the mRNP unfolds and enters 5'-first into the pore. We revisit this issue in mammalian cells to directly examine if this assumption holds as modus operandi, using single molecule RNA FISH to detect single RNAs (mRNAs and lncRNAs) by advanced microscopy including STED super-resolution microscopy. Thereby, we are able to uniquely and separately detect the fluorescent signatures and the spatial organization of the 5', middle and 3'-ends of the same single long transcripts in human cells. By performing imaging of single molecules in several colors we find that an mRNP is compact during nucleoplasmic travels compared to a more open structure after release from the gene. The mRNP is more open also in the nuclear periphery. Compaction levels of nuclear transcripts could be modulated by varying nuclear levels of RNA-binding proteins and by changing global genome structure. The nuclear mRNPs were mostly rod-shaped with distant 5' and 3'-ends, although for some, the transcript ends were in proximity. The latter was more abundant in the cytoplasm. Modifying the mRNA structure to distance the 5' and 3'-ends was only partly achieved by reducing the energetic status of the cell or inhibiting translation. Exit from the NPC was directly observed by labeling different parts of the same transcript in different colors together with endogenous labeling of single NPCs, exhibiting predominant 5'-first export for both mRNAs and non-translating lncRNAs. This analysis detected 'gene gating' in which several adjacent NPCs were engaged in the export of the same mRNA species. Altogether, using high-resolution microscopy we show that the mRNP is a flexible structure during travels, with 5'-directionality during export.