mRNAs scan themselves out

Two single-molecule imaging studies reveal new details of how mRNAs are exported from the nucleus.

In eukaryotic cells, mRNAs are transcribed in the nucleus and must then be exported across the nuclear envelope before they can be translated in the cytoplasm. Multiple factors bind to nuclear mRNAs and mediate their translocation through the nuclear pores, but it is difficult to observe the rapid export of individual RNA molecules in real time. Two papers now track the movements of single mRNAs in budding yeast, yielding important new insights into the export process (1, 2).

Daniel Zenklusen, Mark-Albert Saroufim, and colleagues at the University of Montreal visualized individual mRNAs by adding multiple PP7-binding sites to different transcripts and expressing them together with GFP-tagged PP7 and an mCherry-tagged nuclear pore protein (1). The mRNAs seemed to “scan” the inner surface of the nuclear envelope before they were translocated to the cytoplasm or released back into the nucleoplasm. RNAs remained at the nuclear periphery for up to a second, apparently moving between neighboring nuclear pore complexes (NPCs). Similar mRNA scanning behaviors have previously been observed in mammalian cells (3).

Once they have diffused to the nuclear periphery, RNAs and their associated proteins encounter the nuclear basket, a structure formed by eight protein filaments that protrude from the nuclear side of NPCs. The filaments are formed by two myosin-like proteins, Mlp1 and Mlp2, and, although the basket isn’t required for mRNA export, it appears to have an important quality control function, preventing ribonucleoprotein (RNP) complexes from accessing the pore until they have been remodeled into their mature, export-ready form (4). Saroufim et al. found that, when they completely removed the baskets by deleting MLP1 and MLP2, mRNAs were quickly released into the nucleoplasm, instead of being retained at the nuclear periphery. The researchers saw a similar effect when they specifically abrogated the interaction between Mlp1’s C-terminal domain and the mRNA-binding protein Nab2. “So the nuclear basket is required for this scanning behavior, probably by serving as an interaction platform for RNPs at the nuclear periphery,” Zenklusen explains. “This could allow quality control steps to happen, and help RNAs stay at the periphery until pores become available to translocate them.”

Meanwhile, researchers from the laboratories of Karsten Weis (ETH Zürich), David Grunwald (University of Massachusetts, Worcester), and Ben Montpetit (University of Alberta, Edmonton) discovered that, by removing the yeast cell wall to reduce background fluorescence levels, they could image single, PP7-labeled mRNAs at frame rates quick enough to follow the export event itself (2). Led by Carlas Smith, Azra Lari, and Carina Derrer, the team found that GFA1 transcripts took ~200 ms to cross the nuclear envelope, similar to the export times measured in mammalian cells (3). In contrast to the mRNAs analyzed by Saroufim et al., GFA1 usually didn’t scan the inner nuclear membrane before it was translocated. This could be an inherent property of GFA1, or might reflect the fact that Gfa1, a chitin synthase, is urgently needed to replace the yeast’s missing cell wall.

“GFA1 mRNPs did, however, scan the cytoplasmic surface of the nuclear envelope after their transit through NPCs, perhaps as they are remodeled in preparation for translation. One factor removed from RNPs in the cytoplasm is the export receptor Mex67, which binds to nuclear mRNAs and mediates their association with NPCs. Previous studies have suggested that RNAs are retained in the nucleus of MEX67 mutants but, when Smith et al. tracked mRNA export in yeast with a partial loss of Mex67 function, they saw that some mRNPs would translocate through NPCs but remain associated with the cytoplasmic surface of the nuclear envelope. These mRNPs then moved back through the pores into the nucleus, indicating that Mex67 maintains the directionality of mRNA export by promoting cytoplasmic mRNA release. “No technique other than single-molecule imaging would have been able to predict that,” Grunwald explains. Montpetit agrees, and says that marrying live imaging with “the awesome power of yeast genetics” will provide a wealth of new information. “We can start to put together a picture of mRNA export dynamics,” he says.