Imaging the life and death of RNAs in single cells

Prof. Jeffrey A. Chao
FMI, Friedrich Miescher Institute for Biomedical Research
Basel, Switzerland

After transcription, an mRNA's fate is determined by an orchestrated series of events (processing, export, localization, translation and degradation) that is regulated both temporally and spatially within the cell. In order to more completely understand these processes and how they are coupled, it is necessary to be able to observe these events as they occur on single molecules of mRNA in real-time in living cells. To expand the scope of questions that can be addressed by RNA imaging, we are developing multi-color RNA biosensors that allow that status of a single mRNA molecules (e.g. translation or degradation) to be directly visualized and quantified.

In order to image the first round of translation, we have developed TRICK (translating *R*NA imaging by coat protein knock-off) which relies on the detection of two fluorescent signals that are placed within the coding sequence and the 3′UTR. In this approach, an untranslated mRNA is dual labeled and the fluorescent label in the coding sequence is displaced by the ribosome during the first round of translation resulting in translated mRNAs being singly labeled. A conceptually similar approach was used for single-molecule imaging of mRNA decay, where dual-colored mRNAs identify intact transcripts, while a single-colored stabilized decay intermediate marked degraded transcripts (TREAT, 3′ RNA end accumulation during turnover). We are using these tools to characterize localized translation and degradation during normal cell growth and stress.