Sci. Bull. (2016) 61(14):1071–1073
DOI 10.1007/s11434-016-1116-9

www.scibull.com
www.springer.com/scp

News & Views

Tracking translation of single mRNA molecule in live cells

Wenjuan Wang · Chunlai Chen

Published online: 17 June 2016

Proteins are the major players in cellular functions. To produce proteins, genetic information stored in DNA needs to be firstly transcribed into mRNA, which is then translated by the ribosome. No doubt, protein expression can be regulated at both transcriptional and translational levels. Previous study has suggested that protein abundance is predominantly controlled at translational level [1]. Transcription and movements of individual mRNAs can be visualized and tracked in real time in vivo using fluorescent protein fused with mRNA stem-loop-binding proteins [2]. However, methods to track translation at single molecule level in vivo have been lacking. On May 5th 2016, four independent groups [3–6] reported a set of novel tools to capture translational dynamics of individual mRNAs in live cells.

All four independent groups employ very similar fluorescence based concepts to visualize translation of mRNA at single molecule level. In brief, the mRNA of interest is modified so that both the mRNA and nascent peptide can be tagged by different fluorescent proteins or organic fluorophores in vivo (Fig. 1). Short hairpins are introduced into 3′ untranslated region (UTR, Fig. 1a), which can be specifically recognized by fluorescence tagged MS2 coated protein (MCP) or PP7 bacteriophage coat protein (PCP) with high affinity (Fig. 1b). In addition, an array of HA, FLAG, or SunTag epitopes are introduced before the gene of interest. mRNA–ribosome complexes, which undergo active translation, produce these epitopes to recruit antibody tagged either by another type of fluorescence protein or organic fluorophore. In addition, multiple copies (10–24 repeats) of hairpins and epitopes are introduced to amplify fluorescence signals and to enable single molecule imaging with high signal-to-noise ratio in live cells.

Fast freely-diffusing antibody cannot be visualized individually, whereas antibody recognized mRNA–ribosome complexes produce bright slow diffusing fluorescent spots which represent individual active translating mRNA–ribosome complexes. By analyzing location, intensity, and colocalization of the fluorescence spots representing individual mRNAs and active translation complexes, the following information can be extracted, including: (1) initiation and elongation rates, (2) distribution of the number of ribosomes bound to single mRNAs, (3) percentage of active translating mRNAs, (4) transition between translating and non-translating states for each individual mRNA, (5) diffusion models of translation complexes in cells, and (6) ribosome stalling.

What are the advantages of single molecule imaging in live cells? Behaviors of individual molecule are strongly influenced by their intrinsic stochastic nature, which means identical molecules may stay different amounts of time on the same state or go through different states. Although the smallest live cell is formed by billions of molecules,

W. Wang (✉) · C. Chen (✉)
School of Life Sciences, Tsinghua University, Beijing 100084, China
e-mail: wangwenjuan@biomed.tsinghua.edu.cn

C. Chen
e-mail: chunlai@biomed.tsinghua.edu.cn

W. Wang
Technology Center for Protein Sciences, Tsinghua University, Beijing 100084, China

C. Chen
Tsinghua-Peking Joint Center for Life Sciences, Tsinghua University, Beijing 100084, China

C. Chen
Beijing Advanced Innovation Center for Structural Biology, Tsinghua University, Beijing 100084, China

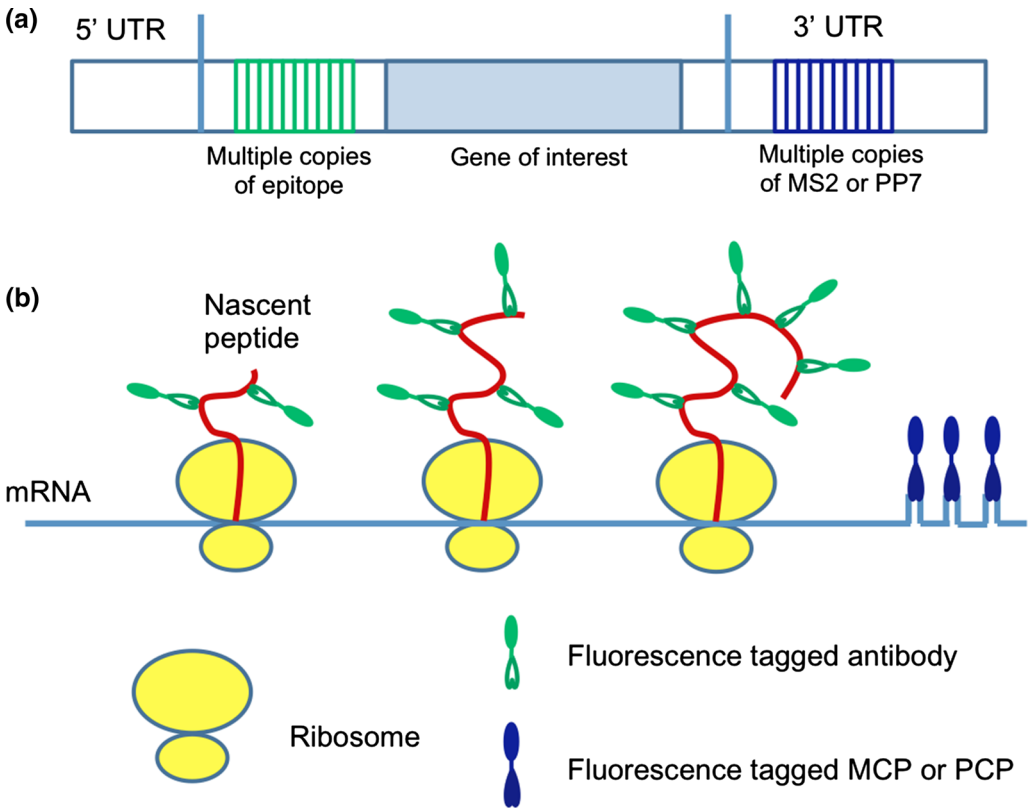

Fig. 1 (Color online) Fluorescence labeling of nascent peptides and mRNAs to visualize translation in live cells. **a** 5' UTR ans 3' UTR of the interested gene; **b** Short hairpins are introduced into 3' UTR and recognized by MCP or PCP, and an array of epitopes are introduced before the gene of interest, mRNA–ribosome complexes then produce these epitopes to recruit fluorescence tagged antibody

cellular phenotypes could be determined by stochastic processes of one or several genes in some occasions [7]. In vitro single molecule tools, such as single molecule tracking and fluorescence resonance energy transfer, optical trap, magnetic trap, and fluorescence correlation spectroscopy, have been rapidly matured and widely used in revealing molecular mechanisms of biological machineries [8]. The ability to capture sub-populations, rare and parallel reaction pathways, and dynamic processes under equilibrium conditions enable single molecule methods to reveal much more comprehensive information than ensemble measurements, which is making greatly influence on how scientists think about biochemical processes. For example, numerous single molecule studies have shown that biomolecules usually transit between two or several conformational states. Binding of ligands or changing of environments are to shift conformational equilibrium rather than to induce an all-or-none conformational change. Now, the newly-developed tools, which enable us to track translation of a single mRNA in live cells, have the potential to reveal unexpected behaviors and to revolutionize our views of translation and its regulation.

Several interesting aspects of translational dynamics of single mRNAs have been discovered. (1) Only a proportion of mRNAs are under active translation, whereas the rest ones are not translated. The percentage of actively translated mRNA varies from 4 % to 86 %, which is positively correlated with the size of transcript [3]. (2) Each individual mRNA can cycle between translating and non-translating states at a time scale of hours, which suggests that mRNAs go through one or several translational shutdown and re-initiation in their lifetime [6]. (3) Even for the transcript, whose overall translational level is highly repressed by its 5′ UTR, has a small fraction (∼2 %) that is highly translated. The amount of ribosomes associated with this subset of the transcript (∼2 %) is higher than the amount of ribosomes associated with the rest of the transcript [6]. (4) Tracking real-time movements of mRNA–ribosome complexes expressing cytosolic proteins reveals three different behaviors: stationary, sub-diffusive, and diffusive. The mRNA–ribosome complexes display a broad distribution of mobility, which is barely correlated with the number of ribosomes on the mRNA. Results suggest that the heterogeneity of mobility is likely caused by interaction between the translating complexes and cellular structures [3–5]. (5) mRNA–ribosome complexes translating secreted and transmembrane proteins have much lower mobility, which is likely caused by anchoring of the complexes onto

the ER [4]. (6) Translation on the mRNA contain a strong ribosome-pausing site also display two different behaviors. Most ribosomes are only transiently paused at the pausing site. However, a small subset of ribosomes are stalled for >10 min before resume translation [6]. In all, translational dynamics of individual mRNAs display heterogeneous behaviors in all different aspects that have been measured in live cells. This is in line with the stochastic nature of single molecules as well as complexity of cellular environments. One can easily image that the uncertainty caused by stochastic behaviors of individual mRNAs would be negligible when overall translational level is high. However, such uncertainty would become significant or even dominant when translational level is very low. How cells cope with such uncertainty under extreme conditions and whether there is a limit beyond which cells cannot tolerate remains unclear. Tracking translational dynamics of single mRNA molecules in live cells is a powerful tool and has a great potential to bridge the gap between cellular behaviors and molecular behaviors.

Translational regulation allows cells to rapidly response to environmental changes and extracellular stimuli. In addition, proteins need to be expressed at the right place and right time, especially in polarized cells, such as neurons. With the ability to track movements and translation of individual mRNAs, one can elucidate spatiotemporal translation mechanisms in live cells. Observation of translation in neurons made by Singer's group and Zhuang's group reveals interesting unexpected phenomena [4, 5]. The fraction of translating ribosomes in primary neurons is 2–3 folds lower than the ones in U2OS cells, which indicates a more stringent regulation of translational initiation in neurons. In addition, the percentage of translating mRNAs decrease from ~40 % in proximal dendrites (<30 μm from soma) to ~10% in distal dendrites (>100 μm from soma), which clearly demonstrates strong spatial modulation in translational regulation. It has been proposed that mRNAs are packed into granules and transported to distal dendrites. Translation of mRNAs is speculated to be repressed during such active transportation [9]. However, both groups captured that mRNAs undergoing active translation were transported along dendrites and sampled several anchoring sites during movements. Molecular mechanisms and cellular functions of such unexpected phenomenon remain to be determined. "This single molecule methodology provides a powerful tool to determine the mechanism of local translation of mRNA and particularly its relationship to local neuronal activity", stated by Wu et al [5].

Tracking single molecule fluorescence events in live cells is quite challenging. Two major issues are to maintain high signal-to-noise ratio during long observation time (several hours) and to have no more than one event within typical optical diffraction limit (around 200 nm × 200 nm × 1000 nm volume). Several tricks have been used in the proof-of-principle studies to address these issues, including adding extra tag to facilitate fast degradation of the translated free proteins and focusing on mRNAs under high level of translation, whose fluorescence intensities are significantly higher than individual translated proteins. 10–24 copies of epitope sequences and hairpins are introduced into the target mRNA sequence to amplify the fluorescence signal, which could cause perturbation. Further optimizations need to be made to extend this novel techniques to more general conditions. Together, four groups used three different epitopes and two different mRNA hairpins to specifically label nascent peptides and mRNAs, respectively. We expect to have more orthogonal labeling sites and methods been developed in the future. However, it is extremely challenging to perform four-color live imaging due to spectrum overlap, which limits the observation of complex dynamic systems. Engineering a user-friendly high-throughput single molecule spectrally resolved microscope [10] to break this limit will further accelerate the revolution of cell biology research led by single molecule imaging techniques.

Reference

1. Schwanhausser B, Busse D, Li N et al (2011) Global quantification of mammalian gene expression control. Nature 473:337–342
2. Buxbaum AR, Haimovich G, Singer RH (2015) In the right place at the right time: visualizing and understanding mRNA localization. Nat Rev Mol Cell Biol 16:95–109
3. Morisaki T, Lyon K, DeLuca KF et al (2016) Real-time quantification of single RNA translation dynamics in living cells. Science. doi:10.1126/science.aaf0899
4. Wang C, Han B, Zhou R et al (2016) Real-time imaging of translation on single mRNA transcripts in live cells. Cell 165:990–1001
5. Wu B, Eliscovich C, Yoon YJ et al (2016) Translation dynamics of single mRNAs in live cells and neurons. Science. doi:10.1126/science.aaf1084
6. Yan X, Hoek TA, Vale RD et al (2016) Dynamics of translation of single mRNA molecules in vivo. Cell 165:976–989
7. Choi PJ, Cai L, Frieda K et al (2008) A stochastic single-molecule event triggers phenotype switching of a bacterial cell. Science 322:442–446
8. van Oijen AM, Dixon NE (2015) Probing molecular choreography through single-molecule biochemistry. Nat Struct Mol Biol 22:948–952
9. Doyle M, Kiebler MA (2011) Mechanisms of dendritic mRNA transport and its role in synaptic tagging. EMBO J 30:3540–3552
10. Zhang Z, Kenny SJ, Hauser M et al (2015) Ultrahigh-throughput single-molecule spectroscopy and spectrally resolved super-resolution microscopy. Nat Methods 12:935–938