



ELSEVIER



RNP transport in cell biology: the long and winding road

Carolina Eliscovich^{1,4} and Robert H Singer^{1,2,3}

Regulation of gene expression is key determinant to cell structure and function. RNA localization, where specific mRNAs are transported to subcellular regions and then translated, is highly conserved in eukaryotes ranging from yeast to extremely specialized and polarized cells such as neurons. Messenger RNA and associated proteins (mRNP) move from the site of transcription in the nucleus to their final destination in the cytoplasm both passively through diffusion and actively via directed transport. Dysfunction of RNA localization, transport and translation machinery can lead to pathology. Single-molecule live-cell imaging techniques have revealed unique features of this journey with unprecedented resolution. In this review, we highlight key recent findings that have been made using these approaches and possible implications for spatial control of gene function.

Addresses

¹ Department of Anatomy and Structural Biology, Albert Einstein College of Medicine, Bronx, New York 10461, United States

² Gruss Lipper Biophotonics Center, Albert Einstein College of Medicine, Bronx, New York 10461, United States

³ Janelia Research Campus of the HHMI, Ashburn, VA, 20147, United States

⁴ Current address: Department of Medicine, Albert Einstein College of Medicine, Bronx, New York 10461, United States

Corresponding author: Singer, Robert H (robert.singer@einstein.yu.edu, singerr@janelia.hhmi.org)

Current Opinion in Cell Biology 2017, 45:38–46

This review comes from a themed issue on **Cell regulation**

Edited by **Davide Rugero** and **Reuben Shaw**

<http://dx.doi.org/10.1016/j.ceb.2017.02.008>

0955-0674/© 2017 Elsevier Ltd. All rights reserved.

Introduction

Localization of messenger RNAs (mRNAs) is a conserved mechanism throughout evolution in which gene expression is coupled to its site of function within the cell. This conservation is effected through proteins that have co-evolved to carry these mRNAs to their destinations in a variety of specialized cells and organisms. Although most research has focused on describing mRNA movement, less is known about the unseen actors in the process, the dynamic and changing protein complexes that manage this entire journey from birth to death.

The purpose of this entire process is to regulate the production of proteins in the cell in the place where they function. Many of these proteins are localized post-translationally, asymmetric subcellular distribution of protein is efficiently accomplished by on-site activation of translation after the mRNA is transported and localized in a dormant state. This spatial distribution is a key determinant for many aspects of cell structure and function [1,2]. It has been revealed that a large number of transcripts localize to specific subcellular compartments in polarized cells. High-resolution fluorescence *in situ* hybridization (FISH) during early *Drosophila* embryogenesis showed that over 70% of mRNAs studied (20% of total genes) localize to different subcellular compartments, where they usually colocalize with the proteins they encode [3]. Although this spatial distribution of gene expression was originally thought to be a unique feature of highly specialized and polarized cells such as neurons and germ cells, it has been demonstrated that a subset of mRNAs localize to cell protrusions and focal adhesions in migrating fibroblasts [4–8]. High-resolution microscopy has shown that mRNAs encoding inner-membrane proteins are confined at this membrane in *E. coli* [9,10*]. This indicates that prokaryotes as well as eukaryotes can spatially regulate gene expression modulating the destiny of mRNAs and cell function. Defects in RNA localization machinery have been implicated in disorders ranging from neurodevelopmental and neurodegenerative diseases to cancer [11,12].

In order to understand how RNA moves within the cell, it was imperative to develop high-resolution microscopy technologies directed to follow single molecules of mRNA in living cells from the time they are synthesized in the nucleus until the time they are degraded in the cytoplasm. Intracellular localization of mRNAs was originally observed using *in situ* hybridization in fixed samples [5,13]. It remains the standard tool for examining the distribution of mRNAs in cells, tissues, and even entire organisms at a specific time point. The direct observation of mRNA using the MS2 system in living cells (Box 1) [14–17,18**] provided the dynamics important for ascertaining mechanism. Recent advances in live-cell imaging [19**] have demonstrated that it is possible to follow the mRNA and associated proteins, thereby providing insights into various regulatory steps in the journey.

This Review highlights recent discoveries concerning how mRNPs carry genetic information from the genes to various locations within the cell in order to target protein synthesis to the right place and at right time.

Box 1 The MS2 system.

The MS2 system has been successfully used to visualize mRNAs in living cells and whole organisms due to its simplicity and high sensitivity [14,17,18**,54,89,90]. This binary system relies on tagging RNA with fluorescent proteins (FPs) *in vivo*. It is based on the high-affinity association between a specific and unique RNA stem-loop structure derived from the MS2 bacteriophage genome regulatory element and the bacteriophage MS2 coat protein (MCP) fused to a FP [14,91]. The amplification of the fluorescent signal is achieved by the generation of multimerized MS2 stem-loops or binding sites (MBS) inserted in the gene of interest that can be recognized by an increase number of molecules of MCP-FP, hence, allowing the visualization of single-molecules in living cells due to an increase in the signal-to-noise ratio [92]. Similar to the MS2 system, the interaction between the *Pseudomonas aeruginosa* PP7 bacteriophage coat protein (PCP) and their cognate RNA stem-loop has been characterized [91,93] and has been also used for visualization of single-molecule mRNAs in living cells [73**,76**,77**,94]. MS2- and PP7-engineered transcripts can be used simultaneously for two-color imaging of different mRNA species allowing the study of trafficking dynamics for different mRNA species within the same cell, thus expanding what can be studied in living cells.

Messenger RNPs control the journey from birth to death

After being born in the nucleus, all molecules of mRNAs start a journey to the site(s) of translation and degradation in the cytoplasm (Figure 1). Transcription is the first step of gene expression regulation. Single-molecule live-cell imaging techniques have revealed that gene promoters stochastically transition between active and inactive transcription states generating bursts of nascent transcripts [20–22]. It has also been shown that these promoter sequences can couple transcription with translation and degradation [23–25,26*,27*]. This allows mRNAs to be ‘flagged’ for sorting to specific subcellular locations from the moment of their birth in the nucleus. A large number of proteins associate with pre-mRNAs to promote crucial modifications such as the addition of the cap structure to the 5'-end by cap-binding protein, the addition of poly(A) to the 3'-end by polyadenylation proteins, and the deposition of the exon junction complex (EJC) proteins with the removal of introns during splicing [2,28]. The association of these and other RNA-binding proteins (RBPs) with the transcript during these events, and when the mRNA is exported to the cytoplasm, is essential and directs the mRNA to its next destination [1,29]. For instance, in *Drosophila* oocytes, splicing creates the spliced *oskar* localization element (SOLE) that together with EJC deposition mediate proper mRNA transport to the posterior pole [30–32].

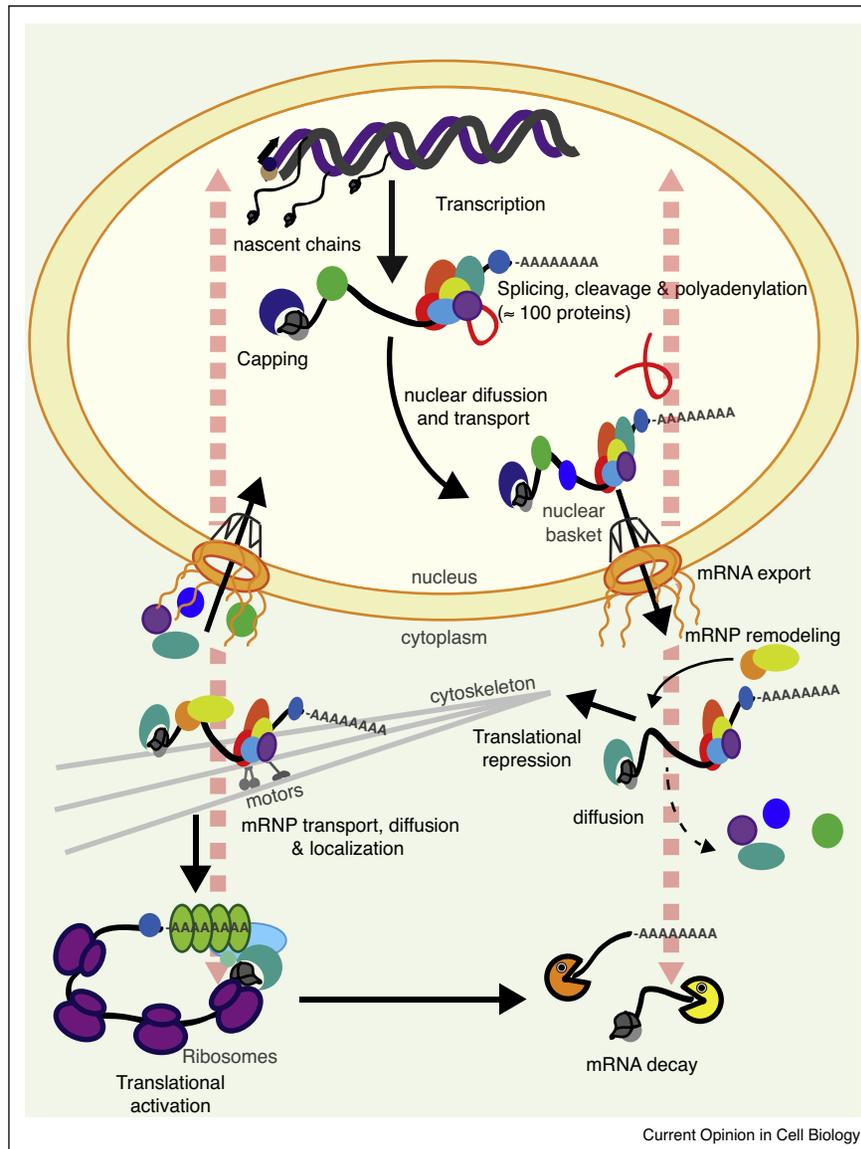
By observing the mRNA tagged with MS2, we can divine how the movement occurs. Surprisingly, most of the movement is by diffusion. Messenger RNPs exhibit Brownian movement and disperse throughout the entire nucleus before they exit, even when the transcription site (TS) is located near the nuclear periphery [33,34]. Once

the mature transcript has been released from the TS it moves randomly until it finds nuclear pores in a time frame of a few minutes [35–37]. The nuclear pore complex (NPC) mediates nucleo-cytoplasmic transport through the nuclear envelope. A superregistration approach using fluorescence microscopy revealed that the kinetics of mRNP export in mammalian cells includes three-steps: docking (80 ms), translocation (5–20 ms), and release (80 ms) [37]. Remarkably, mRNPs remain docked at the nuclear basket for a period of time indicating a rate-limiting step possibly related to quality control [37–39]. This would allow crucial protein rearrangements before export. In budding yeast, mRNAs scan the nuclear periphery before being exported, presumably by a receptive pore complex [40*]. This scanning behavior is defective in nuclear basket protein MLP1/2 mutants that shortens the docking time and releases the mRNAs before they can export. It has been shown that not all pores are equally active [37,38], however, pores transporting β -actin mRNA are repeatedly active over time [41*]. Export rate is influenced by mRNPs involved in splicing, alternative polyadenylation and nuclear surveillance as well as the length of the transcript (reviewed in Refs. [29,42,43]). For instance it has been shown that unspliced mRNAs are exported very inefficiently as the recruitment of export factors may be faulty. Export directionality is achieved by mRNP remodeling as the mRNA moves through the NPC [40*,44*]. The nuclear export factor 1 (NXF1) and NTF2-related protein 1 (NXT1) interact with different adaptor proteins mediating sequential mRNA maturation and export [45]. It has been suggested that in neurodegenerative disorders, where deposition of aggregates of misfolded proteins accumulates in the cytoplasm, nucleo-cytoplasmic RNA export could be affected due to a sequestration and mislocalization of export/import factors [46].

The NPC may not be the only nucleo-cytoplasmic exit pathway. In *Drosophila* neurons, large mRNP complexes appear to leave the nucleus via budding [47,48], a similar mechanism previously shown for nuclear exit of herpes-type virus [49,50].

Upon arrival into the cytoplasm, the mRNP undergoes rearrangements that promote its journey toward its biological destiny. The mRNP composition is remodeled near the nuclear envelope before cytoplasmic release. For instance, Mex67p is removed from the mRNP complex presumably preventing further interaction of the now cytoplasmic mRNA with the pore complex in budding yeast [44*]. The now cytoplasmic mRNP may gain additional factors that control its mobility and further localization (reviewed in Refs. [29,43]). In addition to RBPs, noncoding RNAs and microRNAs might be components of these mRNPs [51]. In the cytoplasm, the mRNA movements have a directional component. The mRBPs may directly or indirectly bind to motor

Figure 1



Messenger RNPs control the journey from birth to death. Dynamic association with RNA-binding proteins regulates the journey of an mRNA from transcription in the nucleus to translation and degradation in the cytoplasm. In the nucleus, pre-mRNAs are capped, spliced, cleaved and polyadenylated co-transcriptionally but then diffuse to nuclear pore complexes. Quality control mechanisms ensure that only properly processed mRNPs are remodeled and exported to the cytoplasm, where they undergo a series of diffusional and transported steps. Continuous mRNA remodeling allows proper RNA transport and localization to the subcellular sites of translation and degradation. Dotted orange arrows represent

Super registration imaging can reveal these mRNA-protein interactions [95].

proteins (*i.e.*, kinesins, dyneins and myosins) to form high molecular weight mRNP motor complex(es) [29]. In *S. cerevisiae*, *ASH1* mRNA is bound to She2p, which in turn allows the binding of She3p and Myo4p (myosin) to the complex. Once bound, Myo4p transports *ASH1* mRNA to the daughter cell along actin filaments [52,53]. Single-molecule live-cell imaging and tracking analysis have revealed dynamics of mRNAs as they move through the cytoplasm. The movement of mRNAs

can be segmented into stationary, corralled, diffusive, and directed transport [54]. Similar to the nucleus, mRNAs are predominately governed by diffusion movement in the cytoplasm [55,18**]. However, they are continually subjected to cycles of diffusion, direct transport and anchoring as shown in neurons by Hidden Markov Modeling (HMM) [56]. The diffusion coefficient in cytoplasm is faster than in the nucleus because the environment is less restrictive. RNA transport is

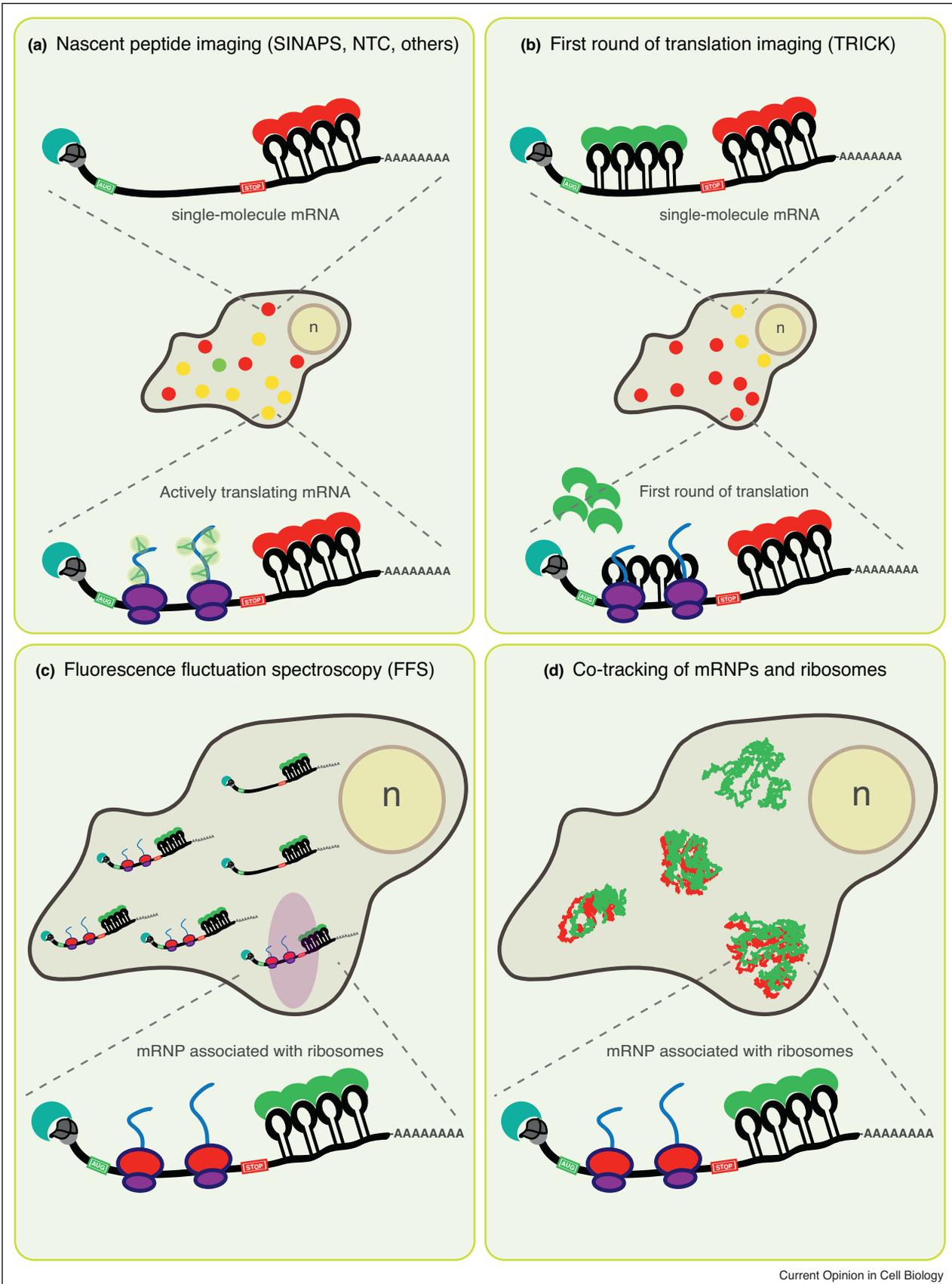
biased by molecular motors that mediate longer trajectories through direct association with the cytoskeleton [57] and hence bring mRNAs into subcellular regions where they are more likely to anchor awaiting the right time to be translated. The ability of some mRNAs to become localized and translated at specific regions has profound implications for cell structure and function. The best example of this is the neuron. Messenger RNPs have to travel from the cell body to synapses that are far away, sometimes meters, to be locally activated to synthesize proteins at a precise moment. This mechanism is the basis of learning and memory [58]. For instance, β -actin mRNA faces this logistic problem in hippocampal neurons by continuously assembling and disassembling large mRNA–protein complexes while traveling toward the base of activated dendritic spines [18^{**},59^{**},60^{**}]. It has been shown that diffusion of β -actin mRNA in neurons is slower than in fibroblasts and 10% of mRNAs are actively transported with a mean speed of 1.3 $\mu\text{m/s}$, equivalent to the assembly with a microtubule motor complex [18^{**}]. Newly synthesized β -actin protein occurs in activated spines [60^{**}]. This local production of actin protein allows enlargement of the cell structures involved in synaptic growth and arborization in neurons [61–64,60^{**}]. It has been shown that this local translation regulation of β -actin mRNA involves translational de-repression by Src kinase-mediated phosphorylation of the zipcode binding protein 1 (ZBP1) [65]. Arc mRNA (which encodes activity-regulated cytoskeleton-associated protein) is also targeted to the base of individual dendritic spines where synapse-specific translation may occur [66]. Similarly, localization of *oskar* mRNA to the posterior pole is essential for germ cell formation during *Drosophila* oogenesis [67]. *oskar* mRNA diffuses randomly, with only 13% being actively transported [68]. The RNA transport toward the posterior pole may be favored by a subtle bias in microtubule orientation. Translation of *oskar* mRNA is repressed by Bruno during transport [69,70] and it is only active when it reaches the posterior pole [71,72,73^{**}].

Innovative improvements in reagent design using rapid live fluorescence microscopy made it possible to determine when and where single molecules of mRNAs translate [73^{**},74^{**},75^{**},76^{**},77^{**},78^{**}] (Figure 2a,b). It is now possible, using fluorescence fluctuation spectroscopy (FFS, Figure 2c), to ascertain that the association of a specific RBP (ZBP1) with a specific mRNA (β -actin) in living cells is anti-correlated with the assembly of ribosomes, hence validating its role in translational repression and its dissociation at the periphery where actin protein translation then occurs [19^{**}]. It has been thought that mRNA translation was silenced during transport in neurons but recent tracking indicated that the mRNA can be in the act of translating while moving: 20% of mRNAs are both actively translated and transported along dendrites at 2 $\mu\text{m/s}$ [74^{**}].

Most mRNA–polyribosome complexes (polysomes) are diffusive in the cytoplasm [19^{**},74^{**},77^{**}]. It has also been shown that the association of the transcript with the translation machinery slightly affects their mobility [74^{**}]. This provides another approach to determine when and where mRNPs are translated. Tracking of thousands of mRNA–ribosome trajectories per cell showed that ribosome load slightly slowed down highly translating mRNAs in fibroblasts [55^{*}] (Figure 2d). For endoplasmic reticulum (ER)-associated polysomes the mobility is slower than for free cytoplasmic counterparts as the nascent peptide restricts the movement to membranes [74^{**},77^{**}].

A combination of cell biology, biochemistry and genome-wide approaches have showed that each mRNA is bound by multiple RBPs and that individual RBPs can be associated with hundreds (and possibly thousands) of target mRNAs. Elucidation of the molecular mechanism of RNA transport requires the identification and characterization of these RBPs (and motors) as well as the *cis*-acting regulatory elements present in the mRNA. Many RBPs have been identified and deduced from sequence homology; however, only a handful of them had been validated *in vivo*. A step toward the understanding of protein binding to mRNA is the recent global initiative to validate RBPs (<https://www.encodeproject.org/> [79^{*}]). The RBPs bind to localization elements (LE) located mainly in the 3'-UTR. They can be either primary nucleotide sequences or secondary structures. Less frequently these regulatory elements can be also found in the 5'-UTR, coding sequence or introns (reviewed in Refs. [2,80]). For mRNAs coding for membrane associated proteins, the nascent peptide can target the entire translating mRNP to its final destination: mitochondria or the ER [81,82,74^{**},77^{**}]. LEs show modularity, redundancy and diversity. The combinatorial code of LEs and their binding factors specifies the destiny of the mRNP and its effect on cell physiology. Messenger RNAs that code for functionally related proteins might be transported, localized and translated together. For instance, proteins required for spindle formation and hence for meiosis progression in *Xenopus* oocytes are synthesized synchronously by mRNAs localized on spindle microtubules [83]. Similarly, localization of mRNAs encoding for proteins involved in focal adhesions controls cell adherence and motility of fibroblasts [4,84,55^{*}]. Local translation of β 2B-tubulin mRNA near to microtubule tips in axons and growth cones has been proposed to promote neuron migration [85^{*}]. These events described above all have their respective RBPs controlling their localization and translation. Some of these proteins have been identified. The cytoplasmic polyadenylation binding element protein (CPEB) is involved in the microtubule-localized translation activation of mRNAs encoding for spindle proteins. The ZBP1 has been shown to be essential for motility related mRNAs in fibroblasts and the localization

Figure 2



of mRNAs within dendritic compartments of neurons. The Adenomatous polyposis coli (APC) mediates translation at microtubule ends but is an unconventional RBP in that it does not contain known RNA binding motifs.

Regulation of mRNAs in time and space also requires degradation. mRNA decay includes mechanisms of quality control that eliminate the synthesis of possibly toxic peptides and that shorten mRNA half-life, changing the abundance of a functional protein [86]. For instance, single-molecule imaging revealed that non-sense mediated decay (NMD) takes place immediately after nuclear export [87,88]. Similar to translation, mRNA decay occurs in the cytoplasm. It has been shown that degradation as well as translation can be determined in the nucleus for stress-response and cell cycle-regulated genes in mammalian cells and yeast [23–25,26*,27*]. This suggests that specific mRNPs integrate and regulate events between the cytoplasm and the nucleus. However, the mechanism of this communication remains to be elucidated.

Conclusions

The movements of RNPs from one compartment of the cell progressively to the next and the regulatory events surrounding each step illustrate an elegant integration of spatiotemporal events within a single cell. Over the last few years, it has become clear that this mechanism defies thermodynamics by orchestrating a highly complex pattern of subcellular protein distribution, effected by specific proteins that bind to and regulate the mRNAs that code for these cellular distributions. Single-molecule single-cell imaging has revealed mechanistic features of this RNA localization, transport and translation with unprecedented spatial and time resolution. Despite recent findings, it is still not possible to follow an individual mRNA and the proteins with which it associates in a living cell *all the way* from its site of transcription in the nucleus to the site of translation with precise kinetics for all the steps. Hence, although mRNA behavior is well described in living cells much of the mechanism is still unknown and it includes coordinated regulation by RBPs in time and space, including non-coding RNAs. Future efforts on the characterization and visualization of individual mRNPs for these molecules during their journey

will eventually explain how this is related with cellular function and disease.

Funding

This work was supported by NIH grant NS083085 to R.H.S.

Acknowledgement

We apologize to any authors whose work may be relevant but omitted.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
 - of outstanding interest
1. Buxbaum AR, Haimovich G, Singer RH: **In the right place at the right time: visualizing and understanding mRNA localization.** *Nat Rev Mol Cell Biol* 2015, **16**:95-109.
 2. Eliscovich C, Buxbaum AR, Katz ZB, Singer RH: **mRNA on the move: the road to its biological destiny.** *J Biol Chem* 2013, **288**:20361-20368.
 3. Lecuyer E, Yoshida H, Parthasarathy N, Alm C, Babak T, Cerovina T, Hughes TR, Tomancak P, Krause HM: **Global analysis of mRNA localization reveals a prominent role in organizing cellular architecture and function.** *Cell* 2007, **131**:174-187.
 4. Katz ZB, Wells AL, Park HY, Wu B, Shenoy SM, Singer RH: **beta-Actin mRNA compartmentalization enhances focal adhesion stability and directs cell migration.** *Genes Dev* 2012, **26**:1885-1890.
 5. Lawrence JB, Singer RH: **Intracellular localization of messenger RNAs for cytoskeletal proteins.** *Cell* 1986, **45**:407-415.
 6. Mardakheh FK, Paul A, Kumper S, Sadok A, Paterson H, McCarthy A, Yuan Y, Marshall CJ: **Global analysis of mRNA, translation, and protein localization: local translation is a key regulator of cell protrusions.** *Dev Cell* 2015, **35**:344-357.
 7. Mili S, Moissoglu K, Macara IG: **Genome-wide screen reveals APC-associated RNAs enriched in cell protrusions.** *Nature* 2008, **453**:115-119.
 8. Mingle LA, Okuhama NN, Shi J, Singer RH, Condeelis J, Liu G: **Localization of all seven messenger RNAs for the actin-polymerization nucleator Arp2/3 complex in the protrusions of fibroblasts.** *J Cell Sci* 2005, **118**:2425-2433.
 9. Nevo-Dinur K, Nussbaum-Shochat A, Ben-Yehuda S, Amster-Choder O: **Translation-independent localization of mRNA in *E. coli*.** *Science* 2011, **331**:1081-1084.
 10. Moffitt JR, Pandey S, Boettiger AN, Wang S, Zhuang X: **Spatial organization shapes the turnover of a bacterial transcriptome.** *Elife* 2016, **5**.

(Figure 2 Legend) Single-molecule live-cell imaging techniques that monitor mRNP dynamic translation. **(a)** Nascent peptide imaging techniques (SINAPS, NTC and others) [74**,75**,76**,77**,78**] visualize translation in real time. RNA reporter construct includes a labeling tag for the nascent peptide (SunTag or FLAG) in the coding sequence and MS2 or PP7 stem-loops in the 3'-UTR for labeling the mRNA. Co-expression of fluorescent antibodies that interact with SunTag and FLAG (green) allows the visualization of nascent peptide chains (blue). Fluorescent proteins (FPs) fused with the MCP or PCP (red) bind to the 3'-UTR and enables mRNA tracking. Actively translating mRNAs are visualized as yellow spots. **(b)** Translating RNA imaging by the coat protein knock-off (TRICK) method [73**] discriminates between mRNAs that have undergone the first round of translation (red spots) and mRNAs that have never been translated (yellow spots) by tagging both the coding sequence and 3'-UTR with RNA stem-loops that bind distinct FPs (green and red). The FP bound to the tags in the coding region is knocked-off by the translating ribosome. **(c)** Fluorescence fluctuation spectroscopy (FFS) [19**] allows spatiotemporal quantification of the association between fluorescent labeled ribosomes (red) and MS2-tagged mRNAs (green) as they pass through a femtoliter volume illuminated by a two-photon spot. **(d)** Co-tracking of fluorescent labeled ribosomes (red) and mRNAs using the MS2 system (green) shows translation 'hot-spots' and dynamics of polysomes in living cells [55*].

This paper shows that, like in eukaryotes, different mRNAs in *E. coli* are organized at different locations by combining a super-resolution microscopy technique (STORM) with FISH.

11. Brinegar AE, Cooper TA: **Roles for RNA-binding proteins in development and disease.** *Brain Res* 2016, **1647**:1-8.
12. Wurth L, Gebauer F: **RNA-binding proteins, multifaceted translational regulators in cancer.** *Biochim Biophys Acta* 2015, **1849**:881-886.
13. Singer RH, Ward DC: **Actin gene expression visualized in chicken muscle tissue culture by using in situ hybridization with a biotinylated nucleotide analog.** *Proc Natl Acad Sci U S A* 1982, **79**:7331-7335.
14. Bertrand E, Chartrand P, Schaefer M, Shenoy SM, Singer RH, Long RM: **Localization of ASH1 mRNA particles in living yeast.** *Mol Cell* 1998, **2**:437-445.
15. Beach DL, Salmon ED, Bloom K: **Localization and anchoring of mRNA in budding yeast.** *Curr Biol* 1999, **9**:569-578.
16. Shepard KA, Gerber AP, Jambhekar A, Takizawa PA, Brown PO, Herschlag D, DeRisi JL, Vale RD: **Widespread cytoplasmic mRNA transport in yeast: identification of 22 bud-localized transcripts using DNA microarray analysis.** *Proc Natl Acad Sci U S A* 2003, **100**:11429-11434.
17. Lionnet T, Czaplinski K, Darzacq X, Shav-Tal Y, Wells AL, Chao JA, Park HY, de Turris V, Lopez-Jones M, Singer RH: **A transgenic mouse for in vivo detection of endogenous labeled mRNA.** *Nat Methods* 2011, **8**:165-170.
18. Park HY, Lim H, Yoon YJ, Follenzi A, Nwokafor C, Lopez-Jones M, ●● Meng X, Singer RH: **Visualization of dynamics of single endogenous mRNA labeled in live mouse.** *Science* 2014, **343**:422-424.

This paper describes the development of the first transgenic mouse in which all endogenous β -actin mRNAs are fluorescently labeled with MCP-GFP and MBS.

19. Wu B, Buxbaum AR, Katz ZB, Yoon YJ, Singer RH: **Quantifying protein-mRNA interactions in single live cells.** *Cell* 2015, **162**:211-220.

The authors combine endogenous single-molecule mRNA and protein detection with two-photon fluorescence fluctuation analysis to measure mRNA-protein associations at specific subcellular locations within living cells.

20. Chen H, Larson DR: **What have single-molecule studies taught us about gene expression?** *Genes Dev* 2016, **30**:1796-1810.
21. Coleman RA, Liu Z, Darzacq X, Tjian R, Singer RH, Lionnet T: **Imaging transcription: past, present, and future.** *Cold Spring Harb Symp Quant Biol* 2015, **80**:1-8.
22. Lionnet T, Singer RH: **Transcription goes digital.** *EMBO Rep* 2012, **13**:313-321.
23. Haimovich G, Choder M, Singer RH, Trcek T: **The fate of the messenger is pre-determined: a new model for regulation of gene expression.** *Biochim Biophys Acta* 2013, **1829**:643-653.
24. Haimovich G, Medina DA, Causse SZ, Garber M, Millan-Zambrano G, Barkai O, Chavez S, Perez-Ortin JE, Darzacq X, Choder M: **Gene expression is circular: factors for mRNA degradation also foster mRNA synthesis.** *Cell* 2013, **153**:1000-1011.
25. Trcek T, Larson DR, Moldon A, Query CC, Singer RH: **Single-molecule mRNA decay measurements reveal promoter-regulated mRNA stability in yeast.** *Cell* 2011, **147**:1484-1497.
26. Zid BM, O'Shea EK: **Promoter sequences direct cytoplasmic localization and translation of mRNAs during starvation in yeast.** *Nature* 2014, **514**:117-121.

The authors show that subcellular localization and translation of mRNAs can be determined at the time of transcription through the activity of stress-responsive promoter sequences in yeast.

27. Vera M, Pani B, Griffiths LA, Muchardt C, Abbott CM, Singer RH, ● Nudler E: **The translation elongation factor eEF1A1 couples transcription to translation during heat shock response.** *Elife* 2014, **3**:e03164.

This paper shows a coordinated mechanism of gene expression where eEF1A1 regulates not only the transcriptional activation of heat shock genes but also their mRNA stability, transport, and translation in mammalian cells.

28. Hocine S, Singer RH, Grunwald D: **RNA processing and export.** *Cold Spring Harb Perspect Biol* 2010, **2**:a000752.
29. Muller-McNicol M, Neugebauer KM: **How cells get the message: dynamic assembly and function of mRNA-protein complexes.** *Nat Rev Genet* 2013, **14**:275-287.
30. Ghosh S, Marchand V, Gaspar I, Ephrussi A: **Control of RNP motility and localization by a splicing-dependent structure in oskar mRNA.** *Nat Struct Mol Biol* 2012, **19**:441-449.
31. Hachet O, Ephrussi A: **Splicing of oskar RNA in the nucleus is coupled to its cytoplasmic localization.** *Nature* 2004, **428**:959-963.
32. Hachet O, Ephrussi A: **Drosophila Y14 shuttles to the posterior of the oocyte and is required for oskar mRNA transport.** *Curr Biol* 2001, **11**:1666-1674.
33. Shav-Tal Y, Darzacq X, Shenoy SM, Fusco D, Janicki SM, Spector DL, Singer RH: **Dynamics of single mRNPs in nuclei of living cells.** *Science* 2004, **304**:1797-1800.
34. Vargas DY, Raj A, Marras SA, Kramer FR, Tyagi S: **Mechanism of mRNA transport in the nucleus.** *Proc Natl Acad Sci U S A* 2005, **102**:17008-17013.
35. Ben-Ari Y, Brody Y, Kinor N, Mor A, Tsukamoto T, Spector DL, Singer RH, Shav-Tal Y: **The life of an mRNA in space and time.** *J Cell Sci* 2010, **123**:1761-1774.
36. Mor A, Suliman S, Ben-Yishay R, Yunger S, Brody Y, Shav-Tal Y: **Dynamics of single mRNP nucleocytoplasmic transport and export through the nuclear pore in living cells.** *Nat Cell Biol* 2010, **12**:543-552.
37. Grunwald D, Singer RH: **In vivo imaging of labelled endogenous beta-actin mRNA during nucleocytoplasmic transport.** *Nature* 2010, **467**:604-607.
38. Grunwald D, Singer RH, Rout M: **Nuclear export dynamics of RNA-protein complexes.** *Nature* 2011, **475**:333-341.
39. Siebrasse JP, Kaminski T, Kubitschek U: **Nuclear export of single native mRNA molecules observed by light sheet fluorescence microscopy.** *Proc Natl Acad Sci U S A* 2012, **109**:9426-9431.
40. Saroufim MA, Bensidoun P, Raymond P, Rahman S, Krause MR, ● Oeffinger M, Zenklusen D: **The nuclear basket mediates perinuclear mRNA scanning in budding yeast.** *J Cell Biol* 2015, **211**:1131-1140.

The authors show that mRNAs are not immediately exported but scan along the nuclear periphery interacting with nuclear pores in yeast using single-molecule live-cell microscopy and subdiffraction particle tracking.

41. Smith CS, Preibisch S, Joseph A, Abrahamsson S, Rieger B, ● Myers E, Singer RH, Grunwald D: **Nuclear accessibility of beta-actin mRNA is measured by 3D single-molecule real-time tracking.** *J Cell Biol* 2015, **209**:609-619.

By combining multifocus microscopy with registration between labeled mRNA, nuclear pores and chromatin, the authors show that β -actin mRNAs freely access the entire volume of the nucleus.

42. Rodriguez-Navarro S, Hurt E: **Linking gene regulation to mRNA production and export.** *Curr Opin Cell Biol* 2011, **23**:302-309.
43. Oeffinger M, Zenklusen D: **To the pore and through the pore: a story of mRNA export kinetics.** *Biochim Biophys Acta* 2012, **1819**:494-506.
44. Smith C, Lari A, Derrerr CP, Ouwehand A, Rossouw A, Huisman M, ● Dange T, Hopman M, Joseph A, Zenklusen D et al.: **In vivo single-particle imaging of nuclear mRNA export in budding yeast demonstrates an essential role for Mex67p.** *J Cell Biol* 2015, **211**:1121-1130.

By using single-molecule imaging and tracking, this paper shows mRNP transport through the nuclear pore and proves that Mex67p plays a key role in cytoplasmic mRNP release and directional transport in yeast.

45. Walsh MJ, Hautbergue GM, Wilson SA: **Structure and function of mRNA export adaptors.** *Biochem Soc Trans* 2010, **38**:232-236.

46. Woerner AC, Frottin F, Hornburg D, Feng LR, Meissner F, Patra M, Tatzelt J, Mann M, Winkhofer KF, Hartl FU *et al.*: **Cytoplasmic protein aggregates interfere with nucleocytoplasmic transport of protein and RNA.** *Science* 2016, **351**:173-176.
47. Speese SD, Ashley J, Jokhi V, Nunnari J, Barria R, Li Y, Ataman B, Koon A, Chang YT, Li Q *et al.*: **Nuclear envelope budding enables large ribonucleoprotein particle export during synaptic Wnt signaling.** *Cell* 2012, **149**:832-846.
48. Jokhi V, Ashley J, Nunnari J, Noma A, Ito N, Wakabayashi-Ito N, Moore MJ, Budnik V: **Torsin mediates primary envelopment of large ribonucleoprotein granules at the nuclear envelope.** *Cell Rep* 2013, **3**:988-995.
49. Maric M, Shao J, Ryan RJ, Wong CS, Gonzalez-Alegre P, Roller RJ: **A functional role for TorsinA in herpes simplex virus 1 nuclear egress.** *J Virol* 2011, **85**:9667-9679.
50. Mettenleiter TC, Klupp BG, Granzow H: **Herpesvirus assembly: a tale of two membranes.** *Curr Opin Microbiol* 2006, **9**:423-429.
51. Bramham CR, Wells DG: **Dendritic mRNA: transport, translation and function.** *Nat Rev Neurosci* 2007, **8**:776-789.
52. Long RM, Gu W, Lorimer E, Singer RH, Chartrand P: **She2p is a novel RNA-binding protein that recruits the Myo4p-She3p complex to ASH1 mRNA.** *EMBO J* 2000, **19**:6592-6601.
53. Takizawa PA, Vale RD: **The myosin motor, Myo4p, binds Ash1 mRNA via the adapter protein, She3p.** *Proc Natl Acad Sci U S A* 2000, **97**:5273-5278.
54. Fusco D, Accornero N, Lavoie B, Shenoy SM, Blanchard JM, Singer RH, Bertrand E: **Single mRNA molecules demonstrate probabilistic movement in living mammalian cells.** *Curr Biol* 2003, **13**:161-167.
55. Katz ZB, English BP, Lionnet T, Yoon YJ, Monnier N, Ovrzyn B, Bathe M, Singer RH: **Mapping translation 'hot-spots' in live cells by tracking single molecules of mRNA and ribosomes.** *Elife* 2016, **5**.
- The authors develop a dual-color single-molecule method to track labeled mRNAs and ribosomes using two synchronized EMCCD cameras that results in a high-resolution spatial map of translating mRNAs in fibroblasts.
56. Monnier N, Barry Z, Park HY, Su KC, Katz Z, English BP, Dey A, Pan K, Cheeseman IM, Singer RH *et al.*: **Inferring transient particle transport dynamics in live cells.** *Nat Methods* 2015, **12**:838-840.
57. Gagnon JA, Mowry KL: **Molecular motors: directing traffic during RNA localization.** *Crit Rev Biochem Mol Biol* 2011, **46**:229-239.
58. Holt CE, Schuman EM: **The central dogma decentralized: new perspectives on RNA function and local translation in neurons.** *Neuron* 2013, **80**:648-657.
59. Buxbaum AR, Wu B, Singer RH: **Single beta-actin mRNA detection in neurons reveals a mechanism for regulating its translatability.** *Science* 2014, **343**:419-422.
- The authors show that neurons contain β -actin mRNAs and ribosomes packaged in granules that are impenetrable by RNA FISH probes and how this masks mRNAs until neuronal stimulation.
60. Yoon YJ, Wu B, Buxbaum AR, Das S, Tsai A, English BP, Grimm JB, Lavis LD, Singer RH: **Glutamate-induced RNA localization and translation in neurons.** *Proc Natl Acad Sci U S A* 2016, **113**:E6877-E6886.
- Using single-molecule cell-live imaging and glutamate uncaging techniques, the authors demonstrate that synaptic activity promotes mRNA localization and local translation of β -actin mRNA at the base of spines in hippocampal neurons.
61. Eom T, Antar LN, Singer RH, Bassell GJ: **Localization of a beta-actin messenger ribonucleoprotein complex with zipcode binding protein modulates the density of dendritic filopodia and filopodial synapses.** *J Neurosci* 2003, **23**:10433-10444.
62. Zhang HL, Eom T, Oleynikov Y, Shenoy SM, Liebelt DA, Dichtenberg JB, Singer RH, Bassell GJ: **Neurotrophin-induced transport of a beta-actin mRNP complex increases beta-actin levels and stimulates growth cone motility.** *Neuron* 2001, **31**:261-275.
63. Perycz M, Urbanska AS, Krawczyk PS, Parobczak K, Jaworski J: **Zipcode binding protein 1 regulates the development of dendritic arbors in hippocampal neurons.** *J Neurosci* 2011, **31**:5271-5285.
64. Welshhans K, Bassell GJ: **Netrin-1-induced local beta-actin synthesis and growth cone guidance requires zipcode binding protein 1.** *J Neurosci* 2011, **31**:9800-9813.
65. Huttelmaier S, Zenklusen D, Lederer M, Dichtenberg J, Lorenz M, Meng X, Bassell GJ, Condeelis J, Singer RH: **Spatial regulation of beta-actin translation by Src-dependent phosphorylation of ZBP1.** *Nature* 2005, **438**:512-515.
66. Dynes JL, Steward O: **Arc mRNA docks precisely at the base of individual dendritic spines indicating the existence of a specialized microdomain for synapse-specific mRNA translation.** *J Comp Neurol* 2012, **520**:3105-3119.
67. Ephrussi A, Lehmann R: **Induction of germ cell formation by oskar.** *Nature* 1992, **358**:387-392.
68. Zimyanin VL, Belaya K, Pecreaux J, Gilchrist MJ, Clark A, Davis I, St Johnston D: **In vivo imaging of oskar mRNA transport reveals the mechanism of posterior localization.** *Cell* 2008, **134**:843-853.
69. Kim-Ha J, Kerr K, Macdonald PM: **Translational regulation of oskar mRNA by bruno, an ovarian RNA-binding protein, is essential.** *Cell* 1995, **81**:403-412.
70. Chekulaeva M, Hentze MW, Ephrussi A: **Bruno acts as a dual repressor of oskar translation, promoting mRNA oligomerization and formation of silencing particles.** *Cell* 2006, **124**:521-533.
71. Kim-Ha J, Smith JL, Macdonald PM: **oskar mRNA is localized to the posterior pole of the Drosophila oocyte.** *Cell* 1991, **66**:23-35.
72. Ephrussi A, Dickinson LK, Lehmann R: **Oskar organizes the germ plasm and directs localization of the posterior determinant nanos.** *Cell* 1991, **66**:37-50.
73. Halstead JM, Lionnet T, Wilbertz JH, Wippich F, Ephrussi A, Singer RH, Chao JA: **Translation. An RNA biosensor for imaging the first round of translation from single cells to living animals.** *Science* 2015, **347**:1367-1671.
- The authors develop an RNA biosensor (TRICK) that allows the visualization of the first round of translation during normal growth and stress in mammalian cells and during *Drosophila* oocyte development. The work elegantly shows that mRNAs are translated within minutes after export and that oskar mRNA is only translated when it reaches the posterior pole of the oocyte.
74. Wu B, Eliscovich C, Yoon YJ, Singer RH: **Translation dynamics of single mRNAs in live cells and neurons.** *Science* 2016, **352**:1430-1435.
- The authors develop a novel single-molecule imaging of nascent peptides (SINAPS) that allows quantitative measurement of initiation, elongation and location of translation in mammalian cells and neurons. They also show that mRNAs coding for ER proteins are only translated when they encounter the ER membrane; translation shows a bursting phenomenon. It is the first evidence that mRNAs can be simultaneously transported and actively translated in neurons.
75. Morisaki T, Lyon K, DeLuca KF, DeLuca JG, English BP, Zhang Z, Lavis LD, Grimm JB, Viswanathan S, Looger LL *et al.*: **Real-time quantification of single RNA translation dynamics in living cells.** *Science* 2016, **352**:1425-1429.
- The authors develop a nascent chain tracking (NCT) technique that uses multi-epitope tags and antibody-based fluorescent probes to quantify synthesis of protein dynamics at the single-molecule in living cells. This work shows kinetics of initiation and elongation of translation.
76. Yan X, Hoek TA, Vale RD, Tanenbaum ME: **Dynamics of translation of single mRNA molecules in vivo.** *Cell* 2016, **165**:976-989.
- The authors develop a novel single-molecule imaging method to study synthesis of nascent peptides from individual reporter mRNAs in real time that allows quantitative measurements of ribosome initiation, elongation, and stalling.
77. Wang C, Han B, Zhou R, Zhuang X: **Real-time imaging of translation on single mRNA transcripts in live cells.** *Cell* 2016, **165**:990-1001.

The authors develop a novel single-molecule imaging method to monitor transient changes of translation dynamics in response to environmental stresses, characterize polysome mobility in different subcellular compartments, and detect local translation and active transport of polysomes in neurons.

78. Pichon X, Bastide A, Safieddine A, Chouaib R, Samacoits A, Basyuk E, Peter M, Mueller F, Bertrand E: **Visualization of single endogenous polysomes reveals the dynamics of translation in live human cells.** *J Cell Biol* 2016, **214**:769-781.

This work shows a new technique to visualize translation in real time of single endogenous mRNAs in mammalian cells.

79. Sundararaman B, Zhan L, Blue SM, Stanton R, Elkins K, Olson S, Wei X, Van Nostrand EL, Pratt GA, Huelga SC *et al.*: **Resources for the comprehensive discovery of functional RNA elements.** *Mol Cell* 2016, **61**:903-913.

This paper shows the validation of commercially available antibodies that interrogate hundreds unique RBPs. Detailed information about these resources is publicly available at the ENCODE portal (<https://www.encodeproject.org/>).

80. Jung H, Gkogkas CG, Sonenberg N, Holt CE: **Remote control of gene function by local translation.** *Cell* 2014, **157**:26-40.
81. Kilchert C, Spang A: **Cotranslational transport of ABP140 mRNA to the distal pole of *S. cerevisiae*.** *EMBO J* 2011, **30**:3567-3580.
82. Eliyahu E, Pnueli L, Melamed D, Scherrer T, Gerber AP, Pines O, Rapaport D, Arava Y: **Tom20 mediates localization of mRNAs to mitochondria in a translation-dependent manner.** *Mol Cell Biol* 2010, **30**:284-294.
83. Elisovich C, Peset I, Vernos I, Mendez R: **Spindle-localized CPE-mediated translation controls meiotic chromosome segregation.** *Nat Cell Biol* 2008, **10**:858-865.
84. Gu W, Katz Z, Wu B, Park HY, Li D, Lin S, Wells AL, Singer RH: **Regulation of local expression of cell adhesion and motility-related mRNAs in breast cancer cells by IMP1/ZBP1.** *J Cell Sci* 2012, **125**:81-91.
85. Preitner N, Quan J, Nowakowski DW, Hancock ML, Shi J, Tcherkezian J, Young-Pearse TL, Flanagan JG: **APC is an RNA-binding protein, and its interactome provides a link to neural**

development and microtubule assembly. *Cell* 2014, **158**:368-382.

By combining biochemistry, genome-wide and microscopy approaches, the authors show that APC acts as a scaffold for functionally related RNAs and proteins self-organizing the synthesis of its own subunits.

86. Schoenberg DR, Maquat LE: **Regulation of cytoplasmic mRNA decay.** *Nat Rev Genet* 2012, **13**:246-259.
87. Muhlemann O, Lykke-Andersen J: **How and where are nonsense mRNAs degraded in mammalian cells?** *RNA Biol* 2010, **7**:28-32.
88. Trcek T, Sato H, Singer RH, Maquat LE: **Temporal and spatial characterization of nonsense-mediated mRNA decay.** *Genes Dev* 2013, **27**:541-551.
89. JayaNandanan N, Gavis ER, Riechmann V, Leptin M: **A genetic in vivo system to detect asymmetrically distributed RNA.** *EMBO Rep* 2011, **12**:1167-1174.
90. Belaya K, St Johnston D: **Using the mRNA-MS2/MS2CP-FP system to study mRNA transport during *Drosophila* oogenesis.** *Methods Mol Biol* 2011, **714**:265-283.
91. Chao JA, Patskovsky Y, Almo SC, Singer RH: **Structural basis for the coevolution of a viral RNA-protein complex.** *Nat Struct Mol Biol* 2008, **15**:103-105.
92. Wu B, Chao JA, Singer RH: **Fluorescence fluctuation spectroscopy enables quantitative imaging of single mRNAs in living cells.** *Biophys J* 2012, **102**:2936-2944.
93. Lim F, Downey TP, Peabody DS: **Translational repression and specific RNA binding by the coat protein of the *Pseudomonas* phage PP7.** *J Biol Chem* 2001, **276**:22507-22513.
94. Hocine S, Raymond P, Zenklusen D, Chao JA, Singer RH: **Single-molecule analysis of gene expression using two-color RNA labeling in live yeast.** *Nat Methods* 2013, **10**:119-121.
95. Elisovich C, Shenoy SM, Singer RH: **Imaging mRNA and protein interactions within neurons.** *Proc Natl Acad Sci U S A* 2017 <http://dx.doi.org/10.1073/pnas.1621440114>. published ahead of print.