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Imaging Real-Time Gene Expression in Living Systems with Single-Transcript Resolution: Construct Design and Imaging System Setup

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Abstract

The most common way for a cell to respond to internal and external signals is to change its gene expression pattern. This requires the synchronization of regulatory steps along the expression pathway. Biological imaging techniques can be used to visualize and measure such processes in individual live cells in real time. This article discusses the use of a fluorescent RNA-binding protein system that allows real-time analysis of gene expression with single-transcript resolution.

OVERVIEW

High-resolution fluorescence in situ hybridization (FISH) provides single-transcript information in fixed cells, but it cannot offer insight into how mRNA traffics through the cell or how the genetic information is then translated into protein. To address these issues, we use a fluorescent RNA-binding protein system to perform real-time gene expression analysis with single-transcript resolution. The system consists of a genetically-encoded tag that allows the direct visualization of mRNAs in vivo using high-resolution fluorescence live-cell imaging. Figure 1 illustrates how this approach has been used in various model systems to visualize the dynamics of mRNA molecules from their birth in the nucleus to their destinations in the cytoplasm. We describe how the MS2 system is designed and how to construct RNA and green fluorescent protein (GFP) reporters for use in various experimental systems. Further, we describe the basic components of a live-cell imaging platform capable of single-transcript detection, provide examples of how to acquire data using it, and explain how to detect and analyze single fluorescent mRNA molecules.

THE MS2 SYSTEM: A GENETIC TAG FOR REAL-TIME mRNA IMAGING

The MS2 system (Bertrand et al. 1998) uses a unique genetically encoded tag that can be inserted into mRNAs of interest. It is recognized by an RNA binding protein fused to GFP, thereby allowing the detection of fluorescently labeled mRNAs (see Fig. 2). The mRNA and GFP reporter are expressed in the cell using transient transfection or stable integration into

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the genome. Genetically expressing the GFP fusion protein with the reporter mRNA allows one to monitor the process from transcription to translation in a single cell in real time. The MS2 system has a signal-to-noise ratio (SNR) far superior to methods that rely on microinjection of labeled mRNA probes, thus allowing greater sensitivity to visualize single molecules and their dynamics.

The RNA tag consists of a 19-nucleotide stem-loop structure that forms the binding site for a single-stranded RNA binding protein required for viral assembly of the RNA bacteriophage MS2 capsid (see Fig. 2A; Peabody and Ely 1992; Valegard et al. 1994). The MS2 capsid protein (termed MCP) binds to this stem-loop with a very high affinity ($K_d = 4 \times 10^{-10}$ M) (Lim and Peabody 1994; Lim et al. 1994), ensuring that the fusion protein does not dissociate from the mRNA (Valegard et al. 1997). The MCP is expressed in the cell as a fusion to a fluorescent protein, which identifies labeled mRNA containing the stem-loop sequence (see Fig. 2B,C). GFP is most often used for the MCP-fusion, but other fluorescent protein derivatives can be used, as long as they provide enough signal for imaging. We therefore refer to these as MCP-*xFP*, where *x* stands for any *F*luorescent *P*rotein fused to the MCP.

The MS2 binding site (MBS) needs to be multimerized (see Fig. 2D) to increase the SNR and allow detection of single mRNAs in the cell. MBSs of different lengths have been optimized for specific applications. Twenty-four MBSs, which have been suggested to bind an average of 33 MCP-GFP molecules (Fusco et al. 2003), are sufficient to detect single mRNAs in vivo (Fusco et al. 2003; Shav-Tal et al. 2004). However, the use of up to 96 MBSs has been reported (Golding et al. 2005). The MS2 system has been used successfully in both prokaryotic (Golding and Cox 2004; Golding et al. 2005) and eukaryotic systems (e.g., Chubb et al. 2006) to follow gene expression in living cells of various types.

Construction of Vectors Expressing MBS-Tagged mRNAs

The first and most important step is the design of the reporter plasmid. Adding 24 MBSs to an mRNA is equivalent to adding 1.4 kilobases (kb), which is often similar to the size of the RNA one wants to study and could potentially influence its functionality. However, insertion of the repeats into the correct position within the mRNA will create a perfectly functional mRNA. Table 1 lists selected MBS-containing reporter constructs that have been used. In general, avoid placing the MBS within the coding region, introns, or known regulatory sequences in the 5′ untranslated region (UTR) or 3′UTR in order to preserve proper pre-mRNA processing. The preferred placement is between the coding sequence and the 3′UTR (Fusco et al. 2003; Dynes and Steward 2007). The MBS can also be inserted near the transcription termination site to avoid interference with regulatory sequences in the 3′UTR.

Sensitivity is achieved through amplification of the binding sites. When single-transcript resolution is required, at least 24 MSBSs should be used. For some applications, such as tracking mRNA “granules” in neurons, situations where multiple mRNAs are present in a large complex, or localizing *ASH1* mRNA particles in yeast, a smaller number of repeats can be used (Bertrand et al. 1998; Beach et al. 1999; Rook et al. 2000; Bannai et al. 2004; Fusco et al. 2004; Dynes and Steward 2007). However, a fraction of the RNA population might be missed due to the lack of sensitivity.

The cloning of MBS-containing expression vectors requires cloning of repetitive sequences that often recombine in bacteria, leading to the loss of MBSs. To minimize this loss, plasmids must be propagated in *Escherichia coli* strains that have a lower recombination frequency. We suggest using the Stbl2 cell strain (Invitrogen) for plasmid DNA amplification. Each time a plasmid containing MBSs is amplified in bacteria, the integrity of the MBSs should be tested by restriction analysis. If the construct has lost some of its repeats, a sample of the original DNA must be retransformed in bacteria and different colonies screened to find the ones expressing the full number of MBSs.

Plasmids containing different numbers of MBSs are available to begin building your own MBS constructs. The Singer lab has generated three plasmids expressing 6, 12, or 24 MBS stem-loops (pSL-MS2-6X, -12X, -24X) that can be excised with a BamHI/BglII digest (http://www.singerlab.org/requests/). Cloning the repeats as a BamHI/BglII fragment into a BamHI site allows verification of the orientation with a simple restriction digest. Additionally, the MBS cassette inserted in the wrong orientation acts as a negative control. If different restriction sites are required, the repeats can also be amplified by PCR using primers outside the repetitive sequences. Primers within the repeats would anneal all over the 24 MBSs and no full-length product would be obtained. PCR amplification can be problematic, as the repetitive sequences can anneal with each other. PCR amplification of MBSs works well using Platinum *Pfx* DNA polymerase (Invitrogen). High annealing temperatures should be used during PCR cycles to avoid nonspecific primer annealing.

Expression of the correct reporter mRNA in the experimental cells should be tested by Northern blot analysis using probes against the MBSs as well as against the specific mRNA. FISH with probes against the MBSs can also be used. A simple FISH protocol can be found at http://www.singerlab.org/protocols/.

Expressing MCP-xFP

The MCP is encoded on a plasmid and transfected into cells (transiently or stably). Its expression can be controlled by a constitutive promoter (cytomegalovirus [CMV] or mammalian polII). However, one can engineer inducible or tissue-specific promoters for temporal or cell-specific expression. When both the MBS-containing RNA reporter and MCP-xFP plasmids are expressed in the cell, only a small fraction of the MCP-xFP fusion is bound to the MBS-tagged mRNAs at any given time. To ensure that free MCP-xFP fluorescence does not overwhelm the mRNA-MCP-xFP signal, expression levels of the MCP-xFP fusion must be carefully controlled, by altering the level of expression and/or by selectively sequestering the free MCP-xFP to a region of the cell that will not be imaged.

MCP-xFP can be expressed in cells as free cytoplasmic or free nuclear pools, each of which has advantages. Table 2 lists selected MCP-xFP constructs with and without a nuclear localization signal (NLS). For studies of cytoplasmic localization and dynamics of mRNAs, an MCP-xFP fused to an NLS should be used. The MCP-xFP-NLS that is targeted to the nucleus binds to the newly transcribed reporter mRNA and is exported to the cytoplasm. Sequestration of free MCP-xFP in the nucleus results in very low cytoplasmic background fluorescence, thus facilitating single mRNA detection in the cytoplasm. When nuclear and cytoplasmic events are imaged simultaneously, an MCP-xFP without an NLS can be used.

The fusion protein is small enough to enter the nucleus by diffusion, leading to a fairly uniform expression within the nucleus and the cytoplasm. Nuclear events can be monitored with good SNR, but free MCP-xFP levels in the cytoplasm lead to higher background fluorescence. Adjusting the expression level of MCP-xFP can help decrease the background signal. If MCP-xFP expression is too low, one may not saturate all MBSs on the mRNA, which will decrease transcript detection. On the other hand, excess expression leads to a loss of single-transcript resolution, because the background is too strong.

IMAGING SYSTEM CONFIGURATION CAPABLE OF SINGLE-MOLECULE DETECTION IN LIVE CELLS

Ideally, live-cell imaging with single-molecule sensitivity should use a system that acquires high-quality images (high SNR) rapidly enough to record dynamic cellular events with high temporal resolution. In practice, one of these components is usually compromised by the optimization of the other. For example, sufficient SNR often requires longer exposure times at a slower frame rate acquisition, resulting in poor time resolution. Also, phototoxicity can damage the health and viability of the cell. Live-cell imaging requires a fine balance between maintaining the integrity of the cell and capturing the best possible quality images at fast enough speeds to accurately record dynamic events.

Fundamental Components of a Live-Cell Imaging System

The components are shown in Figure 3 and listed in Table 3.

- 1. *A motorized inverted microscope.* There should be a stable automated inverted microscope stand that houses multiple ports for external device coupling. The microscope should be capable of automated focus, stage positioning, shuttering, and fluorescent filter cube positioning. A motorized microscope stand, along with automated auxiliary devices, is critical for multiwavelength time-lapse imaging of cell dynamics.
- 2. *Vibration isolation table.* It should be equipped with damped pneumatic isolators. Vibration isolation results in better spatial resolution when detecting and tracking small particles. The larger and thicker the table is, the better.
- 3. *An ultrasensitive camera.* This is the single most important component. Electron-multiplying charge-coupled device (EMCCD) cameras display less readout noise, higher sensitivity, and faster image acquisition rates (some up to 500 frames per second) compared to CCD cameras that are commonly used for standard fluorescence microscopy.
- 4. *A high precision, fast axial (z-axis) focus controller.* Fine axial positioning is achieved by using a high-speed servo or piezoelectric-driven objective or stage insert, or with a built-in motorized *z*-axis controller housed within the microscope stand. This is important for correcting focus drift (see additional auxiliary devices), measuring axial displacements, and

optically stepping through a sample to obtain image stacks (or *z*-stacks) that can later be deconvolved and reconstructed in 3D.

- 5. *A tunable excitation light source.* Living cells are sensitive to the wavelength and intensity of the excitation light. Neutral density (ND) filters can be placed between the illumination source and the sample to attenuate the light intensity, and highly destructive UV irradiation should be minimized either with glass filters or by using a lamp with low UV emission, such as a xenon arc lamp. For wide-field imaging and subsequent quantitative fluorescence analysis, uniform illumination of the imaging field and output power stability are important, especially when ultrasensitive cameras are used for detection. Thermal fluctuation noise in the excitation light can be attenuated by placing the light source away from the microscope stand. A liquid or crystal light guide can be used to couple the light to the microscope and homogenize the light to create even illumination of the field, thus eliminating “hot spots” generated by mercury arc lamps. Other alternative light sources are lasers, monochromators and galvanometer-controlled devices. These can deliver specific wavelengths of light, provide rapid shuttering between wavelengths and quickly filter or attenuate the light.
- 6. *Temperature regulation.* Many live-cell experiments need constant temperature control above room temperature. Temperature-control devices placed on the stage and objective are necessary for the most efficient heat distribution and to help maintain focus. A Plexiglas environmental chamber that surrounds the microscope stage and objective may help focus drift by thermally stabilizing the axial position of the objective and protecting the sample from air currents and sound vibrations. It can also be used to maintain appropriate levels of CO_2 and humidity.
- 7. *Sample chambers or imaging dishes.* Live cells can be imaged in open or closed dish systems. Open systems are the most convenient, because the cells can be grown in the same chamber or dish that is used for imaging. However, the medium is open to the environment where changes in pH and osmolality can occur. A closed system has a better chance of preventing environmentally induced changes. When it is coupled to a perfusion chamber, buffers can be readily exchanged during imaging without losing *x*, *y*, or *z*positioning.
- 8. *A high numerical aperture (NA) objective (1.4 or greater).* This will improve the quality and resolution of the image and will collect more light from the sample. Some high-NA objectives support advanced imaging applications, such as photoactivation, uncaging, total internal reflection fluorescence (TIRF), UV illumination, and infrared (IR) imaging.
- 9. *Image acquisition and analysis software.* It should be compatible with all microscope, camera, and peripheral devices that require automation. The computer should have a fast microprocessor, large random access

memory, and large hard drive storage capabilities. IPLab (Scanalytics, Inc.) and SlideBook (Intelligent Imaging Innovations) are good software packages for image acquisition and analysis. For more advanced off-line image processing, Imaris (Bitplane, Inc.), Huygens Professional (Scientific Volume Imaging), and MetaMorph (Molecular Devices) offer excellent software capabilities depending on the type of analysis desired, such as 3D volume rendering, deconvolution, or object tracking. In addition, the National Institutes of Health (NIH) offers a free Java-based package, ImageJ (previously called NIH Image), which is also an excellent data analysis tool (http://rsb.info.nih.gov/ij/).

Auxiliary Imaging Devices Useful for Live-Cell Imaging

Automation and digital synchronization of multiple imaging devices greatly decreases the time required for image capture and increases the likelihood of obtaining high quality data. Due to the complexity of coordinating all of the components, the lab or facility should have adequate expertise to engineer such a system from scratch. If so, one can add some of the following devices to the microscope. For examples of devices that can be used, see Table 3.

- 1. *An autofocus mechanism*. Such a mechanism (e.g. Olympus IX81-ZDC equipped with the Zero Drift Compensator [ZDC] or the Nikon TE2000-PFS) and CRIFF-2000 Continuous Reflective-Interface Feedback Focus System (Applied Scientific Instruments, Inc.) can greatly improve the quality of images taken in long time-lapse movies.
- 2. *High speed electromechanical shutters.* These block excitation light and can control the duration of light exposure to cells, which helps to decrease photobleaching and to attenuate phototoxicity. Multiple wavelength sources, such as laser lines and transmitted light, can be rapidly switched during time-lapse imaging.
- 3. *Automatic stage.* Precise *x*, *y*, and *z* positioning is useful for returning to positions while imaging multiple fields in a sample or for visiting multiple fields during time-lapse imaging. With the *z*-axis servomotor engaged, *z*-series stacks can be taken of multiple cells in one sample dish or slide, allowing for 3D and 4D (3D with time) imaging.
- 4. *Multichannel imaging device.* A Dual-View or Dual-Cam (Photometrics) can record multiple wavelengths simultaneously without changing filters. This provides excellent time resolution during time-lapse experiments, but the imaging field is restricted. An automated external filter wheel is an alternative if the full field of view is necessary; however, temporal resolution is decreased. Dual and triple filter cubes can be placed in the microscope turret to define excitation wavelengths and the external filter wheel can rapidly switch between multiple emission filters. If this is added to an existing system, ensure that the focal plane is maintained so that the exiting light is properly focused on the camera. The multichannel

imaging device can also induce optical aberrations and wavelength disturbance.

- 5. *Digital Micromirror Device (DMD).* A DMD is an optical semiconductor device that can modulate excitation light by electronically controlling the tilt of mirrors in the light path. This allows more precise control over the amount (intensity), spatial location, and angle of incident light. This device is useful when uncaging and bleaching small areas of a cell and for light intensity modulation when using wide-field illumination.

CONCLUSIONS

The MS2-GFP tagging system is a powerful tool that allows us to follow the fate of one mRNA molecule as it travels throughout the entire cell. By using the MS2 system in combination with other novel imaging techniques, like the FlAsH/ReAsH system (Gaietta et al. 2002; Rodriguez et al. 2006), which labels newly synthesized proteins, we will soon be able to follow real time gene expression pathways in their entirety.

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References

Bannai H, Fukatsu K, Mizutani A, Natsume T, Iemura S, Ikegami T, Inouie T, Mikoshiba K. An RNA-interacting protein, SYNCRIP (heterogeneous nuclear ribonuclear protein Q1/NSAP1) is a component of mRNA granule transported with inositol 1,4,5-trisphosphate receptor type 1 mRNA in neuronal dendrites. J. Biol. Chem. 2004; 279:53427–53434. [PubMed: 15475564]

Beach DL, Salmon ED, Bloom K. Localization and anchoring of mRNA in budding yeast. Curr. Biol. 1999; 9:569–578. [PubMed: 10359695]

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. [PubMed: 9809065]

Bi J, Hu XL, Loh HH, Wei LN. Mouse κ-opioid receptor mRNA differential transport in neurons. Mol. Pharmacol. 2003; 64:594–599. [PubMed: 12920195]

Bi J, Tsai N-P, Lin Y-P, Loh HH, Wei L-N. Axonal mRNA transport and localized translational regulation of κ-opioid receptor in primary neurons of dorsal root ganglia. Proc. Natl. Acad. Sci. 2006; 103:19919–19924. [PubMed: 17167054]

Chubb JR, Trcek T, Shenoy SM, Singer RH. Transcriptional pulsing of a developmental gene. Curr. Biol. 2006; 16:1018–1025. [PubMed: 16713960]

Dynes JL, Steward O. Dynamics of bidirectional transport of Arc mRNA in neuronal dendrites. J. Comp. Neurol. 2007; 500:433–447. [PubMed: 17120280]

Forrest KM, Gavis ER. Live imaging of endogenous RNA reveals a diffusion and entrapment mechanism for nanos mRNA localization in Drosophila. Curr. Biol. 2003; 13:1159. [PubMed: 12867026]

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. [PubMed: 12546792]

Fusco D, Bertrand E, Singer RH. Imaging of single mRNAs in the cytoplasm of living cells. Prog. Mol. Subcell. Biol. 2004; 35:135–150. EINSTEIN LIBRARYMedlineGoogle Scholar. [PubMed: 15113083]

Gaietta G, Deerinck TJ, Adams SR, Bouwer J, Tour O, Laird DW, Sosinsky GE, Tsien RY, Ellisman MH. Multicolor and electron microscopic imaging of connexin trafficking. Science. 2002; 296:503–507. [PubMed: 11964472]

Golding I, Cox EC. RNA dynamics in live Escherichia coli cells. Proc. Natl. Acad. Sci. 2004; 101:11310–11315. [PubMed: 15277674]

Golding I, Paulsson J, Zawilski SM, Cox EC. Real-time kinetics of gene activity in individual bacteria. Cell. 2005; 123:1025–1036. [PubMed: 16360033]

Lim F, Peabody DS. Mutations that increase the affinity of a translational repressor for RNA. Nucleic Acids Res. 1994; 22:3748–3752. 10.1093/nar/22.18.3748. [PubMed: 7937087]

Lim F, Spingola M, Peabody DS. Altering the RNA binding specificity of a translational repressor. J. Biol. Chem. 1994; 269:9006–9010. [PubMed: 8132638]

Peabody DS, Ely KR. Control of translational repression by protein-protein interactions. Nucleic Acids Res. 1992; 20:1649–1655. 10.1093/nar/22.7.1649. [PubMed: 1579455]

Rodriguez AJ, Shenoy SM, Singer RH, Condeelis J. Visualization of mRNA translation in living cells. J. Cell Biol. 2006; 175:67–76. [PubMed: 17030983]

Rook MS, Lu M, Kosik KS. CaMKIIalpha 3' untranslated region-directed mRNA translocation in living neurons: Visualization by GFP linkage. J. Neurosci. 2000; 20:6385–6393. [PubMed: 10964944]

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. [PubMed: 15205532]

Valegard K, Murray JB, Stockley PG, Stonehouse NJ, Liljas L. Crystal structure of an RNA bacteriophage coat protein-operator complex. Nature. 1994; 371:623–626. [PubMed: 7523953]

Valegard K, Murray JB, Stonehouse NJ, van den Worm S, Stockley PG, Liljas L. The three-dimensional structures of two complexes between recombinant MS2 capsids and RNA operator fragments reveal sequence-specific protein-RNA interactions. J. Mol. Biol. 1997; 270:724–738. [PubMed: 9245600]

Weil TT, Forrest KM, Gavis ER. Localization of bicoid mRNA in late oocytes is maintained by continual active transport. Dev. Cell. 2006; 11:251–262. [PubMed: 16890164]

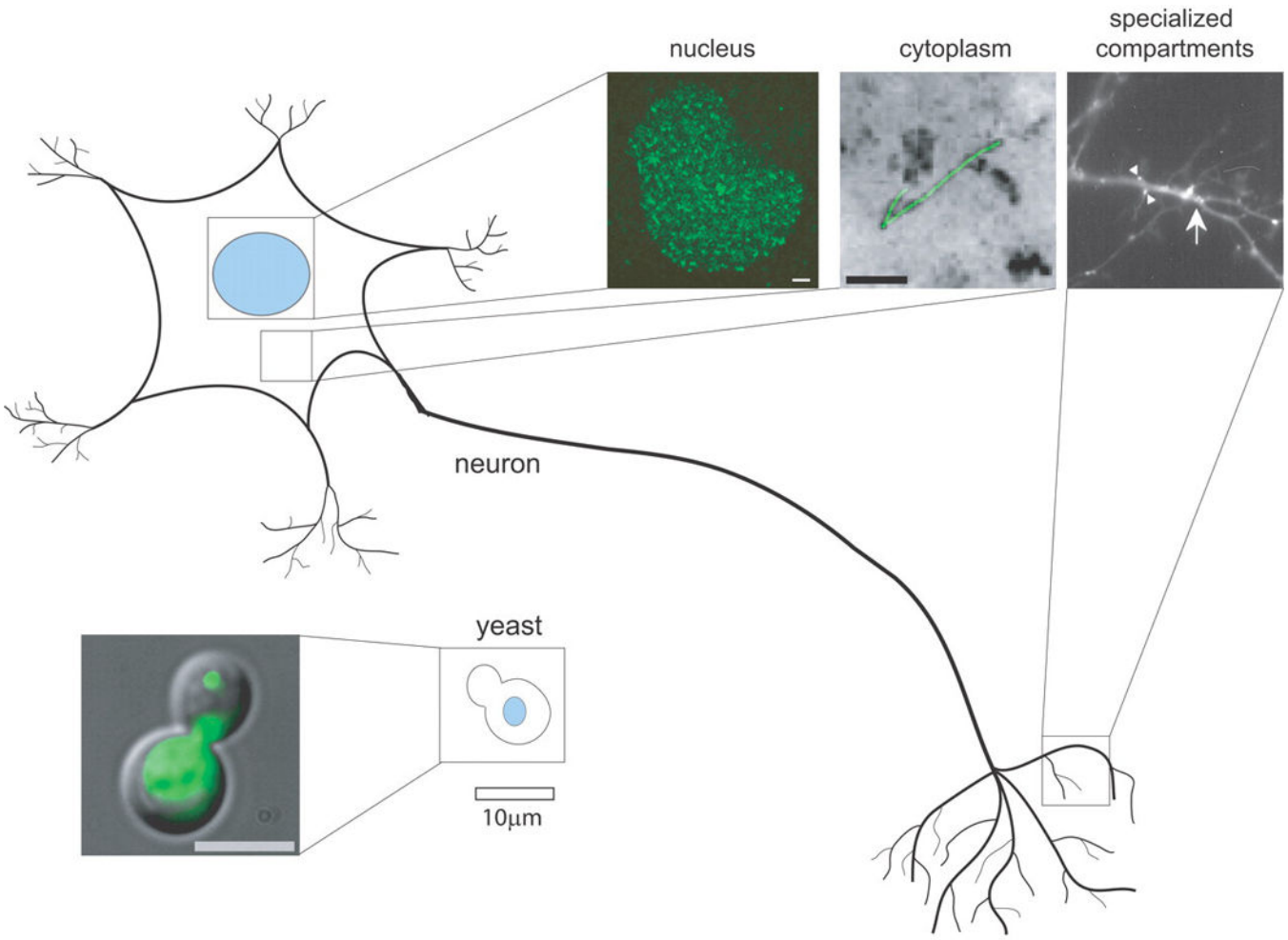

Figure 1.

Imaging gene expression. mRNA transcripts undergo many regulatory events from synthesis to degradation. Live-cell imaging has been used to describe the dynamics of many of those steps. (*Top left*) Visualization of single mRNA particle movement in the nucleus of U2OS cells. Bar, 2 μm. (Reprinted from Shav-Tal et al. 2004, © 2004 American Association for the Advancement of Science.) (*Top middle*) Movement of β-actin mRNAs in the cytoplasm of COS-7 cells. Green line shows the track of an mRNA particle. Bar, 2 μm. (Reprinted from Fusco et al. 2003, with permission from Elsevier © 2003.) (*Top right*) Transport of mRNA particles in dendrites of neuronal cells. (Reprinted from Rook et al. 2000, with permission from the Society for Neuroscience © 2000.) (*Bottom*) Localization of the daughter-cell-specific *ASH1* mRNA to the bud tip of a daughter cell in *S. cerevisiae*. Bar, 5 μm. (Reprinted from Bertrand et al. 1998, with permission from Elsevier © 1998.)

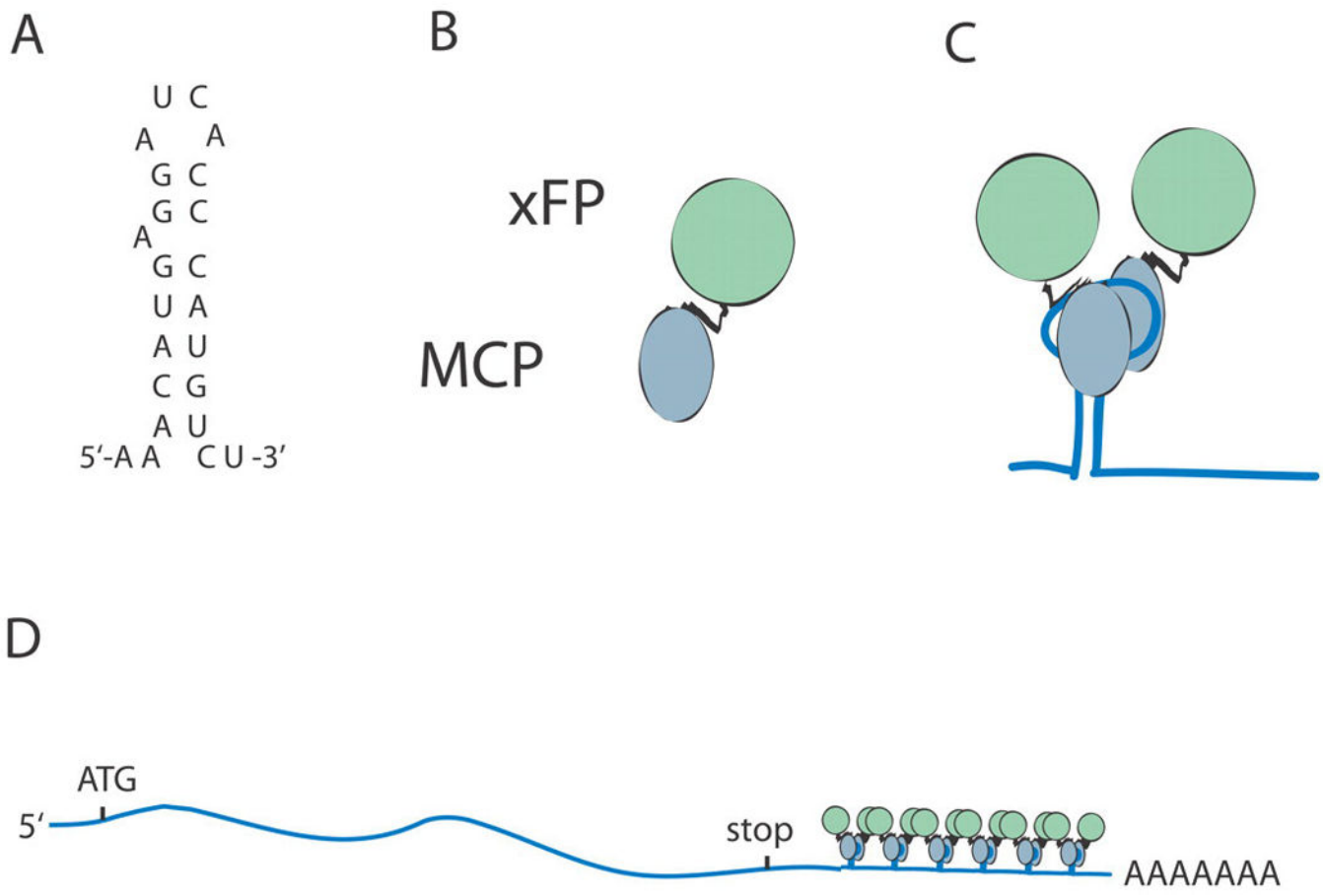

Figure 2.

The MS2 system, a genetic tag to detect RNA in living cells. (*A*) A 19-nucleotide RNA stem-loop is used as a genetic tag to label RNAs (MBS). (*B,C*) The stem-loop structure is bound by the MS2 bacteriophage coat protein. The coat protein (termed MCP) is fused to fluorescent proteins (xFPs). Binding of the MCP-xFP fusion to the MBS leads to a fluorescently labeled RNA. (*D*) To detect MS2-tagged mRNAs, the MBS is multimerized and inserted into an RNA of interest.

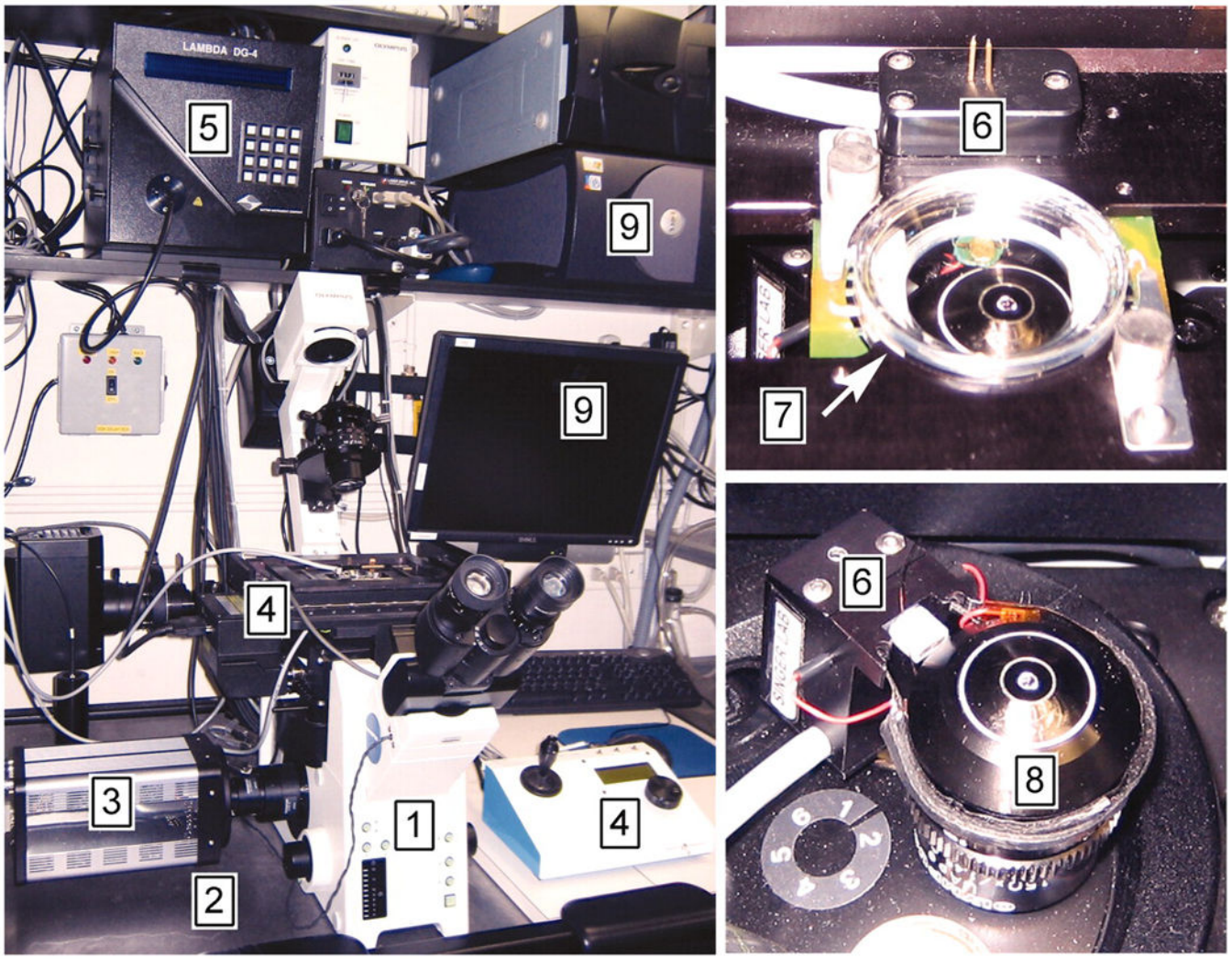

Figure 3.

Illustration of a microscope setup designed for single-molecule fluorescence live-cell imaging. (*Left*) A fully automated microscope system includes a base stand with a (1) built-in autofocus system, (2) vibration isolation table, (3) an ultrasensitive and fast camera, (4) *x*-, *y*-, and *z*-stage positioning, (5) tunable light source, and (9) a multifunctional software package that controls all hardware components and data acquisition. (*Top right*) The Delta T dish system (Bioptechs, Inc.) shows the glass bottom dish (7) securely positioned in its temperature-controlled stage mount (6). (*Bottom right*) A high magnification and NA objective (8) is shown with an objective heater mounted to it (6).

Table 1

A selective listing of expression vectors for MBS reporter RNAs

Vector	RNA or coding sequence	MBS	Regulatory sequence	Promoter	Species	Reference
pRSV-Z-MS2x24	LacZ	24 MBS	None	RSV	Mammalian	Fusco et al. (2003)
pRSV-Z-MS2x24-β-actin	LacZ	24 MBS	Chicken β-actin 3′UTR	RSV	Mammalian	Fusco et al. (2003)
RSV- β-Gal-MS2bs-CaMKIIα3′UTR	LacZ	8 MBS	CaMKIIα 3′UTR	RSV	Mammalian	Rook et al. (2000)
pCMV-DsRE-6xBS-rArc3′UTR	Rat Arc 3′UTR	6 MBS	Rat Arc 3′UTR	CMV	Mammalian	Dynes and Steward (2007)
pCMV-DsRE-6xBS-no3′UTR	DsRed	6 MBS	No Arc 3′UTR	CMV	Mammalian	Dynes and Steward (2007)
pCMV-bNLS-DsRE-6xBS	DsRed	6 MBS	NLS	CMV	Mammalian	Dynes and Steward (2007)
pCMV- DsRE-6xBS	DsRed	6 MBS	None	CMV	Mammalian	Dynes and Steward (2007)
5′K3′K	Luciferase	3 MBS	κ-opioid receptor (KOR) 3′UTR	Kor	Mammalian	Bi et al. (2003, 2006)
5′K3′SV40	Luciferase	3 MBS	SV40 poly(A)	Kor	Mammalian	Bi et al. (2003)
5′tk3′K	Luciferase	3 MBS	κ-opioid receptor (KOR) 3′UTR	Thymidine kinase (TK)	Mammalian	Bi et al. (2003)
5′tk3′SV40	Luciferase	3 MBS	SV40 polyA	Thymidine kinase (TK)	Mammalian	Bi et al. (2003, 2006)
pCaSbcd-$(ms2)_6$	*Bicoid (bcd)*	6 MBS	*bcd* 3′UTR	Bcd	*Drosophila*	Weil et al. (2006)
pCaS2-nos-$(ms2)_6$	*Nanos (nos)*	6 MBS	*Nos* 3′UTR	*nos*	*Drosophila*	Forrest and Gavis (2003)
dscA-24MS2BS	dscA	24 MBS	dscA	Endogenous promoter	*Dictyostelium*	Chubb et al. (2006)
pGAL-*lacZ*-MS2-ASH1	*lacZ*	6 MBS	*ASH1* 3′UTR	GAL	*S. cerevisiae*	Bertrand et al. (1998)

Table 2

Expression vectors for MCP-xFP fusion proteins

Vector	MCP-fusion	Promoter	Species	Selection	Reference
pMS2-GFP-NLS	MCP-GFP-NLS	pol II	Mammalian	Kan/Neo	Fusco et al. (2003)
GFP-MS2-1xNLS	GFP-MCP-1xNLS	CMV	Mammalian	Kan/Neo	Dynes and Steward (2007)
bNLS-GFP-MS2	bNLS-GFP-MCP[a]	CMV	Mammalian	Kan/Neo	Dynes and Steward (2007)
K89	GFP-NLS-MCP	CMV	Mammalian	Kan/Neo	Bi et al. (2003)
pCaS-P*hsp83*-MCP-GFP/RFP-tubulin 3′UTR	NLS-MCP-GFP/RFP with the α-tubulin 3′UTR	Hsp83	*Drosophila*		Forrest and Gavis (2003); Weil et al. (2006)
pDEXH 82 MS2-GFP	MCP-GFP	*Dictyostelium*Actin 15	*Dictyostelium*	Blasticidin	Chubb et al. (2006)
pG14-MS2-GFP	NLS-MCP-GFP	GDP	*S. cerevisiae*	*ampR,LEU,* 2 μm	Bertrand et al. (1998)
pCP-GFP	GFP-MCP	MET25	*S. cerevisiae*	*ampR,HIS,* CEN	Beach et al. (1999)

[a]Bitartate NLS from *Xenopus* nucleoplasmin protein

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Table 3

Automated microscope imaging system components

	Microscope system feature	Examples of equipment (see Fig. 3)	Manufacturer
1	Inverted microscope stand	IX81	Olympus
2	Vibration isolation table	63–500 series laboratory table	Technical Manufacturing Corporation
3	Ultrasensitive camera	Cascade II 512 EMCCD (16 μm/pixel)	Photometrics
4	Axial position controller	MS-2000-XYLE-PZ	Applied Scientific Instruments Inc.
5	Tunable excitation light source	Lambda DG4/OF30	Sutter Instruments, Inc.
6	Temperature regulation	Sample/stage: Delta T temperature controller Objective: Objective heater controller	Bioptechs, Inc.
7	Sample chambers	Delta T glass bottom dishes	Bioptechs, Inc.
8	High NA objective	U Apochromat 150X TIRF, NA 1.45	Olympus
9	Data acquisition software	IPLab	Scanalytics, Inc.
		SlideBook	Intelligent Imaging Innovations
Optional Devices			
10	Autofocus	Built-in: IX81-ZDC	Olympus
		Retrofit: CRIFF-2000	Applied Scientific Instruments Inc.
11	High-speed shutters	Lambda DG4 and DG5	Sutter Instruments, Inc.
12	Automatic stage (*x, y,* and *z*positioning)	*x, y,* (and optional *z*) automatic servo stage (MS-2000) and controller with an additional linear encoder (800-706-2284) for more accurate reproducibility	Applied Scientific Instruments Inc.
13	Multichannel imaging device	Dual-View	Photometrics

	Microscope system feature	Examples of equipment (see Fig. 3)	Manufacturer
		FW-1000 high-speed filter wheel	Applied Scientific Instruments Inc.
14	Digital micromirror array	Mosaic digital diaphragm system	Photonic Instruments, Inc.