A transgenic mouse for *in vivo* detection of endogenous labeled mRNA

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Live-cell single mRNA imaging is a powerful tool but has been restricted in higher eukaryotes to artificial cell lines and reporter genes. We describe an approach that enables live-cell imaging of single endogenous labeled mRNA molecules transcribed in primary mammalian cells and tissue. We generated a knock-in mouse line with an MS2 binding site (MBS) cassette targeted to the 3′ untranslated region of the essential β-actin gene. As β-actin–MBS was ubiquitously expressed, we could uniquely address endogenous mRNA regulation in any tissue or cell type. We simultaneously followed transcription from the β-actin alleles in real time and observed transcriptional bursting in response to serum stimulation with precise temporal resolution. We tracked single endogenous labeled mRNA particles being transported in primary hippocampal neurons. The MBS cassette also enabled high-sensitivity fluorescence *in situ* hybridization (FISH), allowing detection and localization of single β-actin mRNA molecules in various mouse tissues.

The mRNA molecule is the first intermediate of gene expression[1]. At every stage of its lifetime it is regulated both in space and time: transcription at the gene locus[2], export through the nuclear pore[3], diffusion and transport through the cytoplasm that in some cases result in localization of mRNA[4] and eventually decay, perhaps in specialized bodies[5]. These types of regulation are involved in many biological processes and diseases[6]. To completely understand the regulation of gene expression in physiological conditions as well as in the context of disease, ideally one would visualize single mRNA molecules in real time and over their lifetime in a living organism.

Such an experiment has been out of reach because it has not been possible to follow specific mRNAs transcribed from genes in their endogenous genomic context in primary cells or tissues. Therefore, the vast majority of our knowledge about mRNA regulation is derived from cultured cells. In cell culture, imaging of various stages of the mRNA life cycle has been possible using fluorescence microscopy techniques such as fluorescence recovery after photobleaching, fluorescence correlation spectroscopy or widefield microscopy[7]. These are particularly powerful when coupled with the *in vivo* mRNA imaging approach using the MS2 system[8]. In this technique, a sequence derived from the bacteriophage MS2 genome is inserted into a gene of interest. When transcribed, the RNA immediately folds into a hairpin that forms the MS2 binding site (MBS) for the bacteriophage MS2 capsid protein (MCP). When cells express both a gene of interest carrying the MBS and a fusion of MCP with a fluorescent protein (MCP-FP), mRNAs are fluorescently labeled from the moment they are transcribed (**Fig. 1a**). Insertion of multiple MBS copies (24 copies) increases the signal-to-noise ratio so that single mRNAs can be amplified over the background of freely diffusing MCP-FPs[8].

The MS2 system has been used in various contexts, such as bacteria[9], yeast[8], amoeba[10], fruit fly[11] and mammalian cells[12–16]. These studies have yielded a wealth of information about transcription kinetics (such as on-off pulsing of gene expression or the dynamics of elongation, including pausing), the dynamics of mRNA-protein complexes (mRNPs) and their intracellular localization. So far the MS2 system has only been applied to higher eukaryotes in the context of artificial reporter genes, usually inserted in many copies (10–1,000 copies) within a random locus[17]. Imaging artificial reporters in live cultured cells constitutes a useful experimental model but presents some limitations. In those systems consisting of many copies of the same gene, it is not possible to extract events happening at the single-gene level owing to the unsynchronized interactions taking place at the other genes in the locus. A recent improvement of the technique makes it possible to specifically insert single gene copies into the genome of a host cell line[18]. However, reporter genes might be prone to regulation artifacts. Notably, the use of immortalized cell lines introduces an unknown factor in the analysis of gene expression, in which regulatory events such as cell cycle controls are overshadowed by the transformed phenotype[19]. Finally, these systems do not permit the study of mRNA expression in primary cells or in tissue. As improvements in microscopy techniques

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