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Assessing mRNA nuclear export in mammalian cells by microinjection

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ABSTRACT

The nuclear export of mRNAs is an important yet little understood part of eukaryotic gene expression. One of the easiest methods for monitoring mRNA export in mammalian tissue culture cells is through the microinjection of DNA plasmids into the nucleus and monitoring the distribution of the transcribed product over time. Here we describe how to setup a microscope equipped with a micromanipulator used in cell microinjections, and we explain how to perform a nuclear mRNA export assay and obtain the nuclear export rate for any given mRNA.

1. Introduction

The eukaryotic cell is divided into two compartments: the nucleus where pre-mRNA is synthesized and processed into mature mRNA; and the cytoplasm, where that mRNA is translated into protein.

Although much is known about the regulation of RNA transcription and processing, less is understood about how mRNA nuclear export is regulated and how it contributes to the overall expression profile of the cell. Despite this, it has been observed that the rate of mRNA nuclear export contributes significantly to the overall rate of gene expression [1].

Ultimately, mRNA nuclear export kinetics can be derived using pulse-chase type experiments, which can be performed in several different ways. Many of the initial studies were conducted in *Xenopus* oocytes whose nuclei could be microinjected with radiolabeled mRNA whose distribution could be monitored by autoradiography [2]. In mammalian cells, one of the most effective methods to study mRNA export is by microinjecting plasmid DNA into the nucleus, which permits the expression of a substantial number of mRNAs in as little as 15 min, and then halting further expression by inhibiting transcription with RNA Polymerase II inhibitors such as α-amanitin (see Table 1). Since mammalian cells are a million times smaller than *Xenopus* oocytes, and at most 200 cells can be injected per dish, it is virtually impossible to detect the levels of mRNA by conventional biochemical methods. Instead, the levels of the newly synthesized mRNA in the nucleoplasm and cytoplasm are monitored by fluorescent *in situ* hybridization (FISH). In contrast to microinjection, it takes at the least 6 h to be able to detect mRNA in transiently transfected cells (A. Palazzo, unpublished observations), thus effectively preventing any simple kinetic measurements. Alternatively, it is possible to monitor mRNA produced from an inducible gene, which is either introduced into cells by transfection or by recombination into the genome. These systems allow for a more rapid generation of mRNA than in transfection experiments (e.g., the Tet-responsive element promoter induces the detectable amounts of mRNA after 30–90 min of induction [3,4]); however, as the rate of gene activation and repression is slower than the rate of mRNA nuclear export (5–40 min is the typical half-rate of nuclear mRNA export [5,6]), it is difficult to derive the nuclear export rate in these systems.

Another alternative is to monitor single mRNA particles that are labelled with tethered fluorescent molecules (for example GFP-MS2). This technology has been used to monitor how individual mRNA molecules cross the nuclear pore [6–9]; however, in practice it is hard to track such molecules over long enough timespans to obtain reliable mRNA nuclear export rates. One can dispense with tracking individual molecules and instead derive export rates by observing how the nuclear and cytoplasmic populations of mRNAs change over time [6]. This technique also requires a very sophisticated microscopy setup that is capable of acquiring images at high frame-rates and is also compatible with live cell imaging, which is out of the reach of most labs.

Abbreviations: ELWD, extra-long working distance; FISH, fluorescent *in situ* hybridization; VRC, Vanadyl Ribonucleoside Complex.

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Table 1
Equipment list.

	Company	Catalogue Number/ Model	Comments
Microinjection scope	Nikon	Eclipse Ti-S	
Micromanipulator	Nashigne International	NT-88-V3MSH	
Fluorescence Microscope	Nikon	Eclipse Ti-U	
Imaging Camera	Photometrics	CoolSNAP HQ2	
Air table	Kinetic Systems		
mMESSAGE mMACHINE® T7 Transcription Kit	ThermoFisher	AM1344	
Poly(A)-tailing kit	ThermoFisher	AM1350	
PureLink® RNA Mini Kit	ThermoFisher	12183020	
1× Injection Buffer			100 mM KCl and 10 mM HEPES, pH 7.4 (Make 10× as a stock and dilute accordingly)
Oregon Green 488-70 kD Dextran	ThermoFisher Scientific	D7173	10 mg/ml stock in injection buffer can be stored at – 80 °C for long periods of time
Borosilicate Microcapillary tubes	World Precision Instruments Inc.	1B100F-3	
Needle Puller	Sutter Instruments Co.	Model P-97	
Heating filament (for needle puller)	Sutter Instruments Co.	FB245B	
23 × 23 mm Glass coverslips, 0.15 mm Thickness	VWR International	470019-004	
23 × 23 mm Glass coverslips, with etched grid, 0.15 mm Thickness	VWR International	100489-446	
35 mm Tissue Culture Dish			
Gel Capillary Loading Tips	Eppendorf	022351656	
Glass syringe	Becton Dickinson	512311	
Dow Corning High-vacuum silicone grease	Sigma	Z273554-1EA	
Parafilm	ThermoFisher Scientific	13-374-12	
α-amanitin	Sigma	A2263-1MG	
1× PBS Buffer			137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 2 mM KH2PO4, pH 7.420× stock can be stored in – 20 °C for long periods of time
1× SSC Buffer			150 mM NaCl, 15 mM sodium citrate, pH 7.420× stock can be stored in – 20 °C for long periods of time
37% Microfiltered Paraformaldehyde	Electron Microscopy Sciences	15686	Stock of 37% paraformaldehyde is diluted to 4% in 1× PBS
Triton X-100 (Surfact-Amps)	ThermoFisher Scientific	28314	Stock of 10% Triton X-100 is diluted to 1% in 1× PBS
Formamide	Bioshop	FOR001	
Dextran Sulfate	Bioshop	DEX001	
Yeast tRNA	Life Technologies	15401011	
Ribonucleoside Vanadyl Complex (VRC)	New England BioLabs	Item #S1402S	
Alexa-546 labelled ssDNA probe	Integrated DNA technologies		
Whatman filter paper	VWR International	28298-020	
DAPI Fluoromount G-stain	Southern Biotech	0100-20	

One benefit of microinjection-based experiments is that they allow the researcher to introduce *in vitro* synthesized mRNA directly into cells, which can be used to address particular questions. This has been used to demonstrate that particular mRNA features, such as the 5′cap and the 3′poly(A)-tail, are required for efficient nuclear export [5,10,11]. In addition, microinjection of *in vitro* synthesized mRNA has also been used to demonstrate that mRNAs must acquire nuclear factors for them to be properly translated in the cytoplasm [12].

Microinjection experiments can be quite challenging. First they require specialized equipment, most notably a micromanipulator hooked up to an inverted microscope that contains specialized objectives and condenser lenses. It is also worthwhile noting that although the act of physically injecting fluid into nuclei is quite easy, the success of a microinjection experiment relies quite heavily upon having an optimized setup that is ready to use. Optimizing small procedures, such as how to find the needle while preparing the microinjection setup, and how to locate the injecting cells while imaging, will ultimately save the researcher time and energy.

Here we will guide you through the process of microinjection. Much of this protocol was previously published [13], however in the protocol that follows we provide extra information and point out small tweaks and tricks that will greatly aid any researcher who plans to setup a microinjection station with the goal of measuring mRNA nuclear export kinetics.

2. Materials and methods

2.1. Determining whether to study the export of microinjected mRNA or in vitro synthesized mRNA

First it is important to identify whether one wants to measure the export kinetics of exogenously introduced mRNA or mRNA synthesized *in vivo* from microinjected DNA plasmids. In mammalian cells, the export rate of microinjected mRNA is 2-3× faster than endogenously transcribed mRNA from microinjected plasmids, for reasons that are not clear [5]. Furthermore, it appears that these two forms of mRNA have different requirements for export. For example, microinjected *fushi tarazu* mRNA requires splicing or a specialized *cis*-element to be well exported, whereas the *in vivo* transcribed version of the very same mRNA is exported regardless of splicing or any special *cis*-elements [5,14]. Moreover, the 3′ terminal sequences of the mRNA (just prior to the poly(A)-tail) can also significantly alter the kinetics of export for microinjected, but not *in vivo* transcribed, mRNA (A. Palazzo, unpublished observations). We generally only