Methods 48 (2009) 294–300

Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

Transcriptome-wide measurement of mRNA polyadenylation state

Traude H. Beilharz [a,b,1], Thomas Preiss [a,b,c,*]

[a] Molecular Genetics Division, Victor Chang Cardiac Research Institute (VCCRI), Lowy Packer Building, 405 Liverpool Street, Darlinghurst (Sydney), NSW 2010, Australia
[b] St Vincent's Clinical School, University of New South Wales, Sydney, NSW 2052, Australia
[c] School of Biotechnology & Biomolecular Sciences, University of New South Wales, Sydney, NSW 2052, Australia

ARTICLE INFO

Article history:
Accepted 9 February 2009
Available online 20 February 2009

Keywords:
Poly(A) tail
Polyadenylation
Deadenylation
Translational control
mRNA decay
Post-transcriptional regulation
Polysome
Microarray
Transcriptome
Proteome

ABSTRACT

The 3′ poly(A) tail has important roles throughout the eukaryotic mRNA life cycle. A characteristic aspect of poly(A) tail function is furthermore that it can be modulated by changes in its length. This is in turn a well-recognised cellular means to regulate both, mRNA translation and stability, and a positive correlation has often been found between the efficiency of mRNA translation and the length of its poly(A) tail. Here we describe methodology to measure mRNA polyadenylation state in a transcriptome-wide manner, using separation of cellular mRNA populations on poly(U) sepharose in combination with microarray analysis of the resulting fractions. We further detail methods for bulk and mRNA-specific poly(A) tail length measurements to monitor the efficiency of initial mRNA separation and to verify candidates selected from the microarray data. Although detailed here for the study of yeast mRNAs, these methods are adaptable to the investigation of any cellular context in which poly(A) tail length control is known or suspected to operate.

1. Introduction

All nuclear-encoded eukaryotic mRNAs exhibit a 5′ $m^7G(5')ppp(5')N$ cap structure and with few exceptions, also a 3′ poly(A) tail. Both, cap and tail are known to influence mRNA splicing, transport, translation, and stability. In particular, the processes of mRNA translation and canonical decay both require access to these two mRNA end modifications [1]. Canonical mRNA decay starts by poly(A) tail shortening, followed by decapping and exonucleolytic degradation [2]. Conversely, efficient initiation of translation depends on the formation of a closed-loop conformation of the mRNA involving the cap and tail as well as their cognate binding proteins [3–5]. Control of translation is frequently achieved through an interference with the formation of this closed-loop [6]. The poly(A) tail is of particular interest, as dynamic variations in its length afford a tuneable mode of mRNA-specific control. microRNAs employ this type of regulation [7–11] and it has long been studied using the case of 'stock-piled' maternal mRNAs in Xenopus oocytes. These mRNAs are stored in an under-adenylated form in arrested oocytes, but they become re-adenylated and translationally active during oocyte maturation and early embryonic divisions [12–14]. Numerous similar examples have further documented a positive correlation between the efficiency of mRNA poly(A) tail length and translation efficiency.

The poly(A) tail length is thus a surrogate parameter through which to assess the functional state of an mRNA, rather like the measurement of its polysome association by density gradient ultracentrifugation. A combination of this classic method of translational control research with microarray analysis, referred to as translation state array analysis (TSAA) has been developed and successfully applied to many cellular conditions [15,16]. We have employed the TSAA approach in previous work [17] and as an extension of this work we devised a method, termed polyadenylation state array (PASTA) analysis, which combines separation of cellular mRNA on poly(U) sepharose with subsequent microarray analysis. We probed the transcriptomes of *S. cerevisiae* and *S. pombe* with the PASTA methodology and found a widespread correlation between poly(A) tail length and translation in both cases, as well as several other global co-regulatory tendencies [18,19].

Here we detail our method for the fractionation of cellular mRNA based on poly(A) tail length using step-wise thermal elution from a $poly(U)_{100}$ sepharose matrix and describe some approaches to the microarray analysis of the resulting fractions. We further provide protocols for bulk and mRNA-specific poly(A) tail length measurements, as they are required for control and verification purposes.

* Corresponding author. Address: Molecular Genetics Division, Victor Chang Cardiac Research Institute (VCCRI), Lowy Packer Building, 405 Liverpool Street, Darlinghurst (Sydney), NSW 2010, Australia. Fax: +61 2 9295 8601.
E-mail addresses: t.beilharz@victorchang.edu.au (T.H. Beilharz), t.preiss@victorchang.edu.au (T. Preiss).
[1] Fax: +61 2 9295 8601.

1046-2023/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.ymeth.2009.02.003

2. Description of method

2.1. Overview

We isolate total yeast RNA by the hot acid phenol method and bind it to poly(U)$_{100}$ sepharose beads in suspension. This is done by heat-denaturing an aliquot of RNA in the presence of poly(U)$_{100}$ sepharose, followed by cooling to anneal poly(A) tails to poly(U) tracts on the beads. Following washes at low temperature, RNA is batch-eluted from the beads by step-wise increases in temperature (Fig. 1). To test fractions for the degree of tail length separation, aliquots of the eluted RNA are labelled at the 3′ end with α-[^{32}P] pCp followed by digestion of the mRNA body with RNases T1 and A. This leaves only poly(A) tracts intact, which can be resolved by denaturing PAGE (Fig. 2A). The RT-PCR-based Ligation-mediated Poly(A)-Test (LM-PAT) is a sensitive assay yielding product sizes that reflect the poly(A) tail lengths present on a specific mRNA (see schematic in Fig. 2B). It can be used to monitor the efficacy of the poly(U) chromatography step (Fig. 2C) as well as to verify tail lengths on candidate mRNAs. These candidates are selected from dual-colour microarray data that are generated by either comparing each poly(U) sepharose eluate separately against a common reference (in a high resolution approach depicted in Fig. 1B), or by combining fractions into a low temperature and a high temperature eluate pool, which are directly compared with each other (in a pool comparison approach depicted in Fig. 1C). Several strategies may be used to rank or otherwise select mRNA candidates based on the microarray-generated measure of their polyadenylation state (Fig. 3).

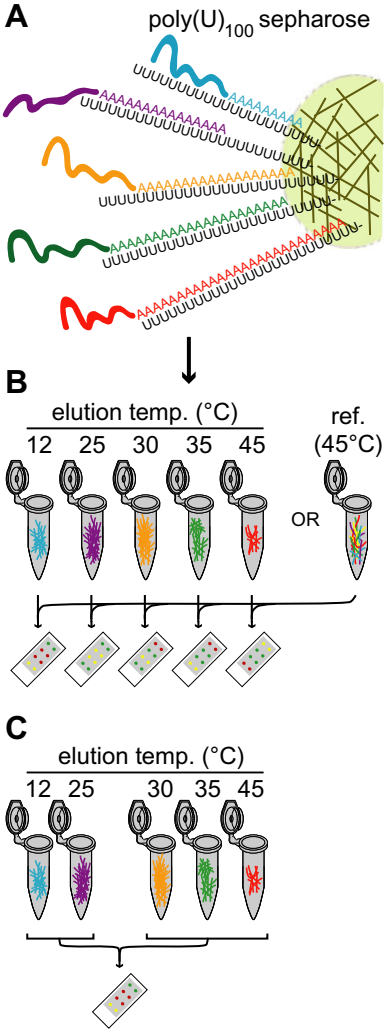

Fig. 1. PolyAdenylation STate Array (PASTA) analysis. (A) mRNA is bound to poly(U)$_{100}$ sepharose beads at 12 °C and then eluted by step-wise increases in temperature as detailed in the main text. (B) For high-resolution PASTA analyses, each temperature eluate (e.g., reverse-transcribed into Cy5-labeled cDNA) is compared against reference mRNA eluted in a single step at 45 °C (e.g., as Cy3-labeled cDNA) on separate dual-colour microarrays. (C) For low-resolution PASTA analyses, pools of elution fractions were compared to each other by microarray (30 °C, 35 °C, and 45 °C [e.g., Cy5-labeled cDNA] versus 12 °C and 25 °C [e.g., Cy3-labeled cDNA]). (A) Reproduced with permission, RNA 13: 982–997 © 2007 by the RNA society. (B) & (C) Reproduced with permission, Microbiology Australia 28: 85–86 © The Australian Society for Microbiology Inc.

2.2. Step-by-step protocols

2.2.1. Preparation of total RNA from yeast

Total RNA of high quality and purity is required so that it remains intact throughout the lengthy manipulations at elevated temperature. It can be rapidly prepared from yeast cells according to the hot acid phenol method. There are several versions of this method in use that differ in subtle ways from the original [20]. The protocol we use is based on that of the Derisi laboratory (http://cat.ucsf.edu/pdfs/TotalRNAIsolation.pdf), with modifications as described below. Total RNA prepared by other methods or from other sources should also be suitable, as long as it is high quality. All solutions are prepared from RNase-free stocks in new or RNase-free plastic or glassware.

1. A yeast overnight culture is diluted 1/50 into 50 ml of fresh media and re-grown for at least 3 generation times to an $OD_{600\ nm}$ between 0.5 and 0.8.
2. Transfer cells to a 50 ml Falcon tube, and collect cells by centrifugation (cooled bench-top clinical centrifuge at 3000*g* for 3 min), then resuspend once in 50 ml ice-cold dH_20 and spin cell down again.
3. Remove supernatant, resuspend pellet in 1 ml ice-cold dH_20 and transfer to a 2 ml screw cap microfuge tube (which helps to prevents phenol leaks, see below). Spin again for 30 s at 10,000*g* in a cooled microcentrifuge and discard supernatant. At this point the cell pellet can be frozen in liquid nitrogen and stored at −80 °C.
4. Resuspend the cell pellet on ice in 400 µl AE Buffer (50 mM sodium acetate pH 5.2, 10 mM EDTA). Add 33 µl of 25% (w/v) SDS and 400 µl acid phenol (Invitrogen Cat # 15594-047), vortex and transfer to a water-bath at 65 °C. Incubate at 65 °C for 20 min with intermittent vortexing, making sure that the phases remain thoroughly mixed. At this temperature there should be no distinction between phases.
5. Transfer microfuge tube to ice and incubate for 5 min to precipitate SDS. Spin at 16,000*g* in a cooled bench-top microcentrifuge.
6. Transfer the supernatant (both phases) into a fresh 2 ml microfuge tube and add 400 µl chloroform. Vortex thoroughly and spin at 16,000*g* in a microcentrifuge for 5 min at room temperature.
7. Remove the aqueous phase to a fresh 1.5 ml microfuge tube, avoiding the inter-phase and re-extract with an equal volume of chloroform:isoamylacohol (24:1, v/v; Fluka Cat # 2566).
8. Transfer aqueous phase to a new microfuge tube containing 1/10th vol of 5 M NaCl and add an equal volume of isopropanol. Mix by inversion, then spin for 10 min at 16,000*g* in a microcentifuge at room temperature. A white pellet should be clearly visible at this stage. Wash the pellet with 80% ethanol, remove all traces of solvent and dry pellet at room temperature.