Biochimica et Biophysica Acta 1829 (2013) 654–665

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

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

Review

RNA decay *via* 3′ uridylation ☆

Daniel D. Scott, Chris J. Norbury *

University of Oxford, Sir William Dunn School of Pathology, South Parks Road, Oxford, OX1 3RE, UK

ARTICLE INFO

Article history:
Received 29 September 2012
Received in revised form 22 January 2013
Accepted 24 January 2013
Available online 4 February 2013

Keywords:
microRNA
Piwi-interacting RNA
Lin28
Decapping
mRNA degradation
TUTase

ABSTRACT

The post-transcriptional addition of non-templated nucleotides to the 3′ ends of RNA molecules can have a profound impact on their stability and biological function. Evidence accumulated over the past few decades has identified roles for polyadenylation in RNA stabilisation, degradation and, in the case of eukaryotic mRNAs, translational competence. By contrast, the biological significance of RNA 3′ modification by uridylation has only recently started to become apparent. The evolutionary origin of eukaryotic RNA terminal uridyltransferases can be traced to an ancestral poly(A) polymerase. Here we review what is currently known about the biological roles of these enzymes, the ways in which their activity is regulated and the consequences of this covalent modification for the target RNA molecule, with a focus on those instances where uridylation has been found to contribute to RNA degradation. Roles for uridylation have been identified in the turnover of mRNAs, pre-microRNAs, piwi-interacting RNAs and the products of microRNA-directed mRNA cleavage; many mature microRNAs are also modified by uridylation, though the consequences in this case are currently less well understood. In the case of piwi-interacting RNAs, modification of the 3′-terminal nucleotide by the HEN1 methyltransferase blocks uridylation and so stabilises the small RNA. The extent to which other uridylation-dependent mechanisms of RNA decay are similarly regulated awaits further investigation. This article is part of a Special Issue entitled: RNA Decay mechanisms.

1. Introduction

Template-independent addition of nucleotides to RNA 3′ ends was first described in the early years of modern molecular biology, through the biochemical purification of nucleotidyl transferase activities and, following the advent of the necessary methodologies, comparison of cDNA and genomic sequences. The literature in this area soon came to be dominated by studies of polyadenylation, once the pivotal role for this modification in eukaryotic mRNA function became apparent, even though clear evidence for the modification of cytoplasmic RNA in other ways – and specifically by 3′ uridylation – had been described as early as the 1960s [1]. A resurgence of interest in RNA uridylation, and the recognition that this modification is widespread among eukaryotes, came some forty years later, as a consequence of studies into small RNA (sRNA) biology, along with the development of mRNA 3′ end sequencing methods that are not reliant on oligo(dT)-primed reverse transcription. It is now clear that uridylation may contribute to RNA function through conferring stability, as in the case of the small nuclear RNA U6, or through RNA editing, as observed in the mitochondria of trypanosomes. These roles have been reviewed elsewhere [1]; here we instead focus on the evidence that implicates 3′ uridylation in RNA decay, and explore other instances where the role of this modification is currently less clear.

2. Uridylation associated with RNA decay

2.1. Uridylation of microRNA-directed 5′ cleavage products

In *Arabidopsis*, microRNAs (miRNAs) or small interfering RNAs (siRNAs) that bind with perfect complementarity to their mRNA template elicit endonucleolytic cleavage by the Argonaute (AGO1) component of the RNA-induced silencing complex (RISC), forming 5′ and 3′ cleavage products that must be degraded. While the 3′ fragment is degraded by the Xrn1-like 5′ to 3′ exonuclease AtXRN4 [2], circularised rapid amplification of cDNA ends (cRACE) sequencing analysis suggested that, rather than being exonucleolytically degraded 3′ to 5′ by the exosome, the 5′ cleavage product can be oligouridylated and consequently may be degraded 5′ to 3′ by decapping and exonucleolysis [3]. Similar uridylation was observed on the 5′ moiety of murine *Hoxb8* mRNA cleaved by *miR-196* [3] and, potentially, on a truncated Epstein-Barr virus (EBV) *Pol* mRNA cleaved by a virally-encoded miRNA prior to polyadenylation [3–5]. The *Chlamydomonas* small sRNA uridyltransferase Mut68 has also been implicated in the oligoadenylation of miRNA 5′ cleavage products [6,7]. Uridylation and/or adenylation of such cleavage products may be a conserved mechanism directing rapid decapping and 5′ to 3′ degradation in order to prevent translation of cleaved mRNAs that could otherwise encode aberrant, C-terminally truncated proteins [3].

Abbreviations: Ago, Argonaute; ncPAP, non-canonical poly(A) polymerase; RISC, RNA-induced silencing complex; RITS, RNA-induced transcriptional silencing; TRAMP, Trf4/Air1/2/Mtr4 polyadenylation

☆ This article is part of a Special Issue entitled: RNA Decay mechanisms.

* Corresponding author. Tel.: +44 1865 275540; fax: +44 1865 275501.
E-mail addresses: daniel.scott@path.ox.ac.uk (D.D. Scott), chris.norbury@path.ox.ac.uk (C.J. Norbury).

1874-9399/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbagrm.2013.01.009

2.2. Uridylation and degradation of mRNAs

For most eukaryotic organisms studied, the rate-limiting step of mRNA degradation is removal of the poly(A) tail by one or more deadenylases [8,9], which in turn promotes degradation of the mRNA by stimulating decapping and 5′ to 3′ exonucleolysis or by recruiting the exosome for 3′ to 5′ exonucleolytic decay [8]. However, the recent identification of deadenylation-independent degradation pathways involving cytoplasmic uridyltransferases suggests that regulation of mRNA degradation by 3′ modification may be more complicated than previously suspected.

The *cid1* gene of the fission yeast *Schizosaccharomyces pombe* was initially identified in an overexpression screen through its ability to suppress DNA replication checkpoint defects, and was subsequently found to play subtle roles in the maintenance of this checkpoint in response to genetic or pharmacological inhibition of DNA replication [10,11]. Initial bioinformatic and biochemical analyses suggested that Cid1 was a cytoplasmic non-canonical poly(A) polymerase (ncPAP) [11,12]; however, subsequent work demonstrated that Cid1 possesses robust terminal uridyltransferase activity, which outcompetes its poly(A) polymerase activity *in vitro* and *in vivo* [13]. Surprisingly, Cid1 is able to add mono- or di-uridyl tails to mature polyadenylated mRNA transcripts *in vivo* in a manner independent of poly(A) tail length [13]. These tails are found mainly on decapped mRNAs in unperturbed cells but, upon inactivation of decapping factors Lsm1 and Dcp1, accumulate on capped transcripts, demonstrating that Cid1-mediated uridylation participates in a novel, deadenylation-independent pathway of mRNA decay [13,14]. Importantly, Cid1-dependent uridylation also accumulated upon inactivation of the major deadenylase Ccr4, while poly(A) tails on decapped transcripts were significantly shorter in *cid1* deletion strains, suggesting that uridylation and deadenylation operate redundantly to promote degradation of mRNAs. In support of this interpretation, deletion of either *cid1* or *ccr4* partially stabilised the uracil-responsive *urg1* transcript but neither did so to the same extent as deletion of the gene encoding the common decapping activator Lsm1 [14]. The uridylation-dependent pathway may be particularly important in *S. pombe*, in which mean poly(A) tail lengths are characteristically short by comparison with other eukaryotes, and in which decapping frequently occurs without further erosion of the poly(A) tract [14]. One surprising observation was the extremely short nature of the uridyl tails added to mRNA by Cid1 (almost exclusively U_{1-2}), in contrast to the long poly(U) tails observed *in vitro* [13]. However, short oligo(U) tails are selectively bound by the Lsm1-7 decapping complex, and even a U_1 tail is sufficient for some enhancement of decapping *in vitro* [15]; such a tail on the end of a poly(A) sequence may be sufficient to override the preference of Lsm1-7 for oligo(A) sequences over poly(A) sequences [16] and facilitate efficient decapping of mRNAs with mature poly(A) tails. Alternatively, cell cycle- or transcript-specific potentiation of Cid1 activity could facilitate the addition of longer oligo(U) tails, though such regulation has not so far been observed.

A recent study in *Aspergillus nidulans* illuminated a similar pathway, wherein the previously uncharacterised ncPAP CutA (CU nucleotidyltransferase) was able to add heteropolymeric oligo (C/U) tails with a consensus CUCU sequence specifically to deadenylated mRNAs [17]. Deletion of the gene encoding this enzyme impaired bulk mRNA decay [17] and resulted in the disassembly of P-bodies [18], suggesting that CUCU addition is an important step in decapping-mediated degradation of mRNAs subsequent to deadenylation. Similar to observations in *S. pombe* [14], in cells lacking deadenylase enzymes this modification was enriched and could be found on mRNAs possessing mature poly(A) tails [17].

The observation of residual uridylation of mRNAs in *S. pombe cid1* deletion strains suggests that another of the six Cid1 family members in *S. pombe* [11,19] may also possess mRNA uridyltransferase activity [13]. Low-level homopolymeric oligo(U) tails were also observed in *cutA* deletion strains of *A. nidulans* [17]. This is surprising given that the *A. nidulans* genome encodes only one other ncPAP family member, AN5694.2 [17], which exhibits extensive similarity to Trf4/5-like nuclear poly(A) polymerases (Table 1). Though the source of the residual uridyltransferase activity in these species is currently unknown, the existence of multiple mRNA uridyltransferase enzymes could allow different regulatory pathways of mRNA uridylation and/or redundant activity on mRNAs, potentially masking more severe phenotypes that might result from complete loss of mRNA uridylation.

Though deadenylation-independent pathways of decapping have been reported in *S. cerevisiae*, which lacks ncPAP family members with uridyltransferase activity, these are either gene-specific pathways regulated by *cis*-acting elements [20,21] or occur in mutants compromised for stable mRNP formation and, potentially, for efficient protection of the 5′ cap [22]. However, most other eukaryotes with fully sequenced genomes possess a large complement of ncPAPs, many of which have been demonstrated to possess uridyltransferase activity ([19]; see Fig. 1, Table 1), suggesting that the mRNA uridylation observed in fungi may be more widely conserved than previously suspected. Importantly, the use of oligo(dT) primers in microarray and deep sequencing studies necessarily prevents the detection of uridylation distal to the poly(A) tail, suggesting that this mRNA modification may have gone unobserved in many previous studies.

In mammals, the cell cycle-regulated ('replication-dependent') histone mRNAs are unique in that they are not polyadenylated, terminating instead with a conserved stem-loop structure which mediates pre-mRNA processing, export and translation [23–25]. The stem-loop is also a *cis*-regulatory element necessary and sufficient for the rapid degradation of histone mRNAs upon the conclusion of S-phase or inhibition of DNA replication [26]; failure of this degradation disturbs normal DNA:histone stoichiometry and results in genome instability and chromosome loss [27]. The stem-loop forms a ternary complex with stem-loop binding protein (SLBP, also known as hairpin-binding protein, HBP) [23–25] and 3′hExo/ERI1 [28], a conserved ssRNA exoribonuclease implicated in sRNA degradation [29] and biogenesis [30,31] as well as in the maturation of the 5.8S rRNA [32,33]. Both members of this ternary complex, the structure of which has recently been solved [34], are required for efficient degradation of histone mRNAs [24,28,35], as are an interaction with SLBP of the nonsense-mediated decay (NMD)-related helicase Upf1 [36,37] and proximity of the stem-loop/SLBP/3′hExo complex to a translating ribosome [38].

While early analyses of histone mRNA degradation suggested that degradation was achieved solely in the 3′➔5′ direction [39], more recent sequencing [40] and kinetic [41] analyses have shown that mRNAs can be degraded both 3′➔5′ and 5′➔3′, and that both activities may act on any given mRNA. Strikingly, sequencing of mRNAs following DNA replication inhibition with hydroxyurea revealed post-transcriptional oligouridylation both of mature and of 3′-truncated mRNAs [40]; inhibition of this oligouridylation through 3′ terminal addition of 3′-deoxyadenosine (cordycepin) [41] or by depletion of uridyltransferase(s) [40–42] slowed degradation in both directions, demonstrating a key role for oligouridylation in both the decapping and the 3′➔5′ exonucleolysis of histone mRNAs. While the stabilisation of histone mRNAs upon depletion of decapping factors Lsm1 and hDcp2 [40–42] or addition of a non-hydrolysable mRNA cap [41] suggests that uridylation facilitates 5′➔3′ degradation *via* a decapping-dependent mechanism similar to that reported for fungal mRNAs, Hoefig et al. [35] report an entirely novel mechanism by which Lsm1-7, recruited by the oligouridyl tail, is able to interact directly with 3′hExo and enhance its catalytic activity, facilitating exonucleolysis of the stem-loop structure and exposing the single-stranded mRNA body to subsequent exosomal degradation. The addition of oligouridyl tails to mRNAs exhibiting 3′ truncation which removes part, but not all, of the stem-loop suggests that mRNAs may undergo multiple rounds of oligouridylation and 3′hExo-mediated exonucleolysis before the stabilising stem-loop structure is fully degraded [35,40].