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Review

Poly(A) polymerase (PAP) diversity in gene expression – Star-PAP vs canonical PAP

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ABSTRACT

Almost all eukaryotic mRNAs acquire a poly(A) tail at the 3′-end by a concerted RNA processing event: cleavage and polyadenylation. The canonical PAP, PAPα, was considered the only nuclear PAP involved in general polyadenylation of mRNAs. A phosphoinositide-modulated nuclear PAP, Star-PAP, was then reported to regulate a select set of mRNAs in the cell. In addition, several non-canonical PAPs have been identified with diverse cellular functions. Further, canonical PAP itself exists in multiple isoforms thus illustrating the diversity of PAPs. In this review, we compare two nuclear PAPs, Star-PAP and PAPα with a general overview of PAP diversity in the cell. Emerging evidence suggests distinct niches of target pre-mRNAs for the two PAPs and that modulation of these PAPs regulates distinct cellular functions.

1. Introduction

In eukaryotes, nuclear mRNA synthesis is a multistep process that begins with transcription and ends with processing at the 3′-UTR [1–5]. The various steps of mRNA synthesis – transcription, splicing, and 3′-end formation are functionally interconnected through a network of synergistic interactions [3,6]. The 3′-end processing of a precursor mRNA (pre-mRNA) is an essential step in eukaryotic gene expression, which is comprised of two steps – cleavage and addition of poly(A) tail [1,4,5,7,8]. Almost all eukaryotic mRNAs are polyadenylated, a step critical for stability, export and translation efficiency of mRNAs [1,4,5,7,9,10]. Pre-mRNAs are polyadenylated by enzymes called poly(A) polymerases (PAPs) which function in a 3′-end processing complex comprised of a large number of protein constituents [11].

The canonical PAP exists in multiple isoforms and at least three forms of canonical PAP – PAPα, PAPβ (PAPT), and PAPγ (neoPAP) have been reported [12–18]. Canonical PAPs were considered the only PAPs that controlled all co-transcriptional polyadenylation in the nucleus. Apart from PAPα, PAPγ also functions in the similar CPSF and AAUAAA signal dependent polyadenylation of pre-mRNAs in the nucleus [13]. Another nuclear non-canonical PAP, Star-PAP [*S*peckle *T*argeted PIPKIα *R*egulated *P*oly(*A*) *P*olymerase] (RBM21, TUT1), was then reported to polyadenylate certain mRNAs involved in various cellular processes such as oxidative stress response and apoptosis [19,20]. Based on similarities in domain architecture, Star-PAP belongs to a subfamily of non-canonical PAPs (ncPAPs). So far, seven known ncPAPs have been reported in humans with diverse cellular functions [21,22].

PAPα and Star-PAP participate in both cleavage and polyadenylation reactions. In addition, Star-PAP exhibits terminal uridylyl transferase activity toward U6 snRNA [23]. Do the two PAPs compete for target mRNAs? Emerging evidence suggests distinct niches of target mRNAs for each PAP and there appears to be no cross regulation of their targets [19,20,24]. Such specificities in target poly(A) site recognition could potentially modulate alternative polyadenylation (APA). This review presents an overview of diverse PAPs in the cell and compares two functionally similar but distinct

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nuclear PAPs, Star-PAP and PAPα. Here, we explore the differences in properties, mechanism, target mRNA selection, and regulation of the two PAPs in 3′-end pre-mRNA processing.

2. 3′-end pre-mRNA processing in gene expression

The 3′-UTR of an mRNA is critical for the regulation of gene expression. During eukaryotic mRNA maturation, the nascent pre-mRNA undergoes processing at the 3′-UTR. This processing at the 3′-end is a two-step event: first, the pre-mRNA is endonucleolytically cleaved at the cleavage site, followed by the addition of poly(A) tail to the upstream fragment of the cleaved RNA, while the downstream fragment is rapidly degraded [1,4,5,7,8,10]. 3′-end processing is intricately coupled to transcription and splicing, and also regulates the type, and the amount of mRNA and protein levels of a particular gene. Thus, mRNA 3′-end formation links transcription of a gene with the translation of its mRNA [2,6].

Mass spectrometry analysis identified ~85 protein factors associated in the 3′-end processing complex [11]. Some of the critical proteins required for the cleavage and polyadenylation reactions include subunits of Cleavage and Polyadenylation Stimulatory Factor (CPSF), Cleavage stimulatory Factor (CstF), Cleavage Factor I_m (CF I_m), and Cleavage Factor II_m; Symplekin, PAP, and the nuclear Poly(A) Binding Protein (PABPN1) (for review [5,25]). Mammalian CPSF consists of six polypeptides – CPSF 160 (CPSF1), CPSF 100 (CPSF2), CPSF 73 (CPSF3), CPSF 30 (CPSF4), hFip1 and WDR33. CPSF 160 recognises the poly(A) signal, PAS (AAUAAA), a sequence located approximately 15–30 nucleotide upstream of cleavage site and interacts with PAP and CstF [26]. Although CPSF 160 binds to the AAUAAA signal and cooperates with other factors, the assembly of the stable cleavage complex requires an intact CPSF complex [26–28]. The CPSF interaction also requires cooperation with CstF and CF I_m for stable association with pre-mRNA [29–32]. Studies suggest that other trans-acting factors such as splicing factor U1 snRNP interacts with CPSF 160 and promotes its binding to PAS on the pre-mRNA [33]. In HIV, CPSF 160 can also interact with sequence element upstream of the poly(A) site other than the classical AAUAAA signal at the 3′-UTR [34,35]. Another subunit of CPSF, CPSF 73, acts as endonuclease, binds directly to the cleavage site in a AAUAAA dependent manner and then cleaves the pre-mRNA at the cleavage site [36,37]. CPSF 30 may cooperate with CPSF 160 in RNA binding [38]. hFip1, one additional CPSF subunit, also binds PAP and directs PAP to the cleavage site [39]. The exact functions of CPSF 100 and WDR33 subunits are yet undefined [11,40].

The CstF complex recognises the GU/U rich downstream sequence element (DSE) and cooperates with CPSF. CstF has three subunits – 50 (CSTF1), 64 (CSTF2) and 77 (CSTF3) KDa of which CstF 64 binds the GU/U rich downstream sequence element (DSE) [30,41,42]. CstF 77 functions as a homodimer and bridges the 64 and 50 KDa subunits and cooperates with CPSF 160; CstF 50 interacts with the RNA Polymerase II (Pol II) C-terminal domain (CTD) [26,43,44]. The interaction of CstF and CPSF complexes and their corresponding associations with DSE and PAS is considered the most significant event in defining the cleavage site. CF I_m is a heterotetramer with two 25 KDa subunits (CPSF5 or NUDT21) that forms the core of the complex along with two larger polypeptides of 68 KDa (CPSF6) and/or 59 KDa (CPSF7) subunits. CPSF5 binds pre-mRNA upstream of PAS to a sequence element that contains the U(G/A)UA motif. In addition, CF I_m cooperates with CPSF for RNA binding and enhances the recognition of the cleavage site [31,45–47] CF I_m can also direct a sequence-specific AAUAAA-independent polyadenylation by recruiting the CPSF subunit hFip1 and PAP in vitro [48]. CF II_m consists of two subunits, hPcfl 1 and hClp1, and possibly links CF I_m and CPSF within the cleavage complex [49]. Symplekin is a scaffolding protein that putatively joins a large number of proteins together in the complex [50–53]. CPSF, CstF, Symplekin and CF I_m interact with each other stabilising the 3′-end processing complex assembled on pre-mRNA and promotes recruitment of PAPα. Mammalian PAPα is also required for the cleavage reaction, however, the mechanism as to how PAPα is involved in cleavage is not precisely defined [5,25]. After cleavage of the transcript, PAPα adds a poly(A) tail to the upstream fragment of cleaved RNA. The nuclear poly(A) binding protein (PABPN1) binds the nascent poly(A) tail, confers processivity to PAP and controls poly(A) tail length. PABPN1 also interacts with PAPα and CF I_m and enhances the efficiency of polyadenylation [54–60]. Thus, a large number of protein factors cooperate with each other and assemble at the 3′-UTR to accomplish cleavage of the transcript followed by polyadenylation.

3. Polyadenylation

Polyadenylation is a process of template-independent addition of a long poly(A) tail to the 3′-end of an mRNA. Polyadenylation activity was first identified some 50 years ago from calf thymus nuclei extracts [61]. However, it was only a decade later that poly(A) tails were recognised as a product of post-transcriptional processing of the mRNA 3′-UTR [62–64]. Almost all mammalian mRNAs have a poly(A) tail at their 3′-end, with the exception of histone mRNA which ends after a highly conserved RNA stem-loop structure, and lacks a poly(A) tail [65]. The length of a nascent polyadenylated tail on an mRNA in mammalian cells varies from 200 to 300 adenosine residues [1,9]. In the nucleus, the poly(A) binding protein, PABPN1 helps to define the length of the newly synthesised poly(A) tail during de novo mRNA synthesis [9,54,56]. PABPN1 interacts with the first 11 polyadenosine residues added, stimulates PAPs affinity for RNA substrate, and in presence of CPSF induces PAP from its distributive mode to a processive polyadenylation [54,60,66]. When polyadenylation reaches ~250 residues, PAP switches back to its distributive mode resulting effectively in termination of polyadenylation [58]. The precise mechanism of this length control is not fully known, it appears to occur through the formation of a ~20 nm spherical structure involving the poly(A) tail and the bound PABPN1 disrupting the tripartite, CPSF-PAP-PABPN1 processive polyadenylating complex [56,67]. Additionally, a role of multi-functional protein nucleophosmin (NPM1) in poly(A) tail length determination has also been proposed [68,69]. However, a recent poly(A) tail profiling indicated much shorter average lengths of poly(A) tails from various eukaryotic species (<100 in mammalian cells) [70] likely due to the shortening in the cytoplasm [71]. Another genome wide measurement of poly(A) tail length also demonstrated a median tail length of 50–100 adenosine nucleotides in HeLa and NIH 3T3 cells [72]. The observed length of mammalian poly(A) tails is at least influenced or maybe determined by the shortening reaction in the cytoplasm. In addition, there was diversity in the tail length not only among the transcripts from different individual genes but also within different mRNA transcripts from the same gene. Intriguingly, shorter tails were observed for mRNAs encoding ribosomal or housekeeping proteins [70]. In general, poly(A) tails play crucial roles in maintaining mRNA stability and turnover, transport of message from nucleus to cytoplasm, and translation efficiency of mRNA [1,5,9,10]. Moreover, defective polyadenylation has been linked to various human diseases [73].

3.1. Alternative polyadenylation (APA)

In humans, pre-mRNAs are polyadenylated in several different ways due to the existence of more than one polyadenylation site, allowing a single gene to encode multiple mRNA transcripts

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