CellPress

Trends in Biochemical Sciences

Review

Beyond simple tails: poly(A) tail-mediated RNA epigenetic regulation

Jingwen Liu[1,2] and Falong Lu[1,2,*]

The poly(A) tail is an essential structural component of mRNA required for the latter's stability and translation. Recent technologies have enabled transcriptome-wide profiling of the length and composition of poly(A) tails, shedding light on their overlooked regulatory capacities. Notably, poly(A) tails contain not only adenine but also uracil, cytosine, and guanine residues. These findings strongly suggest that poly(A) tails could encode a wealth of regulatory information, similar to known reversible RNA chemical modifications. This review aims to succinctly summarize our current knowledge on the composition, dynamics, and regulatory functions of RNA poly(A) tails. Given their capacity to carry rich regulatory information beyond the genetic code, we propose the concept of 'poly(A) tail epigenetic information' as a new layer of RNA epigenetic regulation.

Highlights

New methods are enabling comprehensive transcriptome-wide poly(A) tail analysis.

Cytoplasmic dynamics involving the deadenylation and polyadenylation of poly(A) tails regulate gene expression.

Non-A residues are prevalent in the 3′ ends and the internal body of poly (A) tails, where they may play crucial roles in RNA regulation.

Poly(A) tails potentially encode vital epigenetic regulatory information.

Epigenetic regulation of gene expression

In eukaryotic organisms, genetic information is encoded within DNA sequences. However, additional information beyond the DNA sequence controls gene expression, driving diverse biological processes. The term 'epigenetics' historically referred to phenomena not explainable solely by genetic principles [1]. As our understanding of chromatin-mediated gene regulation has advanced, the definition of epigenetics has evolved. It now encompasses the study of molecules and mechanisms that maintain alternative gene activity states in the context of the same DNA sequences. This includes DNA methylation, histone modifications, the incorporation of histone variants, and the structure of chromatin itself [1,2]. In recent advancements, RNA has emerged as a carrier of another layer of epigenetic information, called **RNA epigenetics** (see Glossary), including small RNAs and chromatin-associated non-coding RNAs (ncRNAs) which impact gene expression, as well as RNA editing and RNA chemical modifications (**epitranscriptomics**) which affect the RNA itself [3–6]. The major types of well-accepted RNA epigenetics are summarized in Table 1 [7–12], and they are not discussed further due to space limitations. The essential point is that these epigenetic regulations do not involve changes to the underlying DNA sequence.

Poly(A) tails – long chains of adenine nucleotides attached to the 3′ end of most eukaryotic messenger RNA (mRNA) molecules – represent a fundamental aspect of mRNA architecture. This review aims to concisely summarize our current knowledge on the composition, dynamics, and regulatory roles of RNA poly(A) tails, which underscore the crucial role of poly(A) tails, appended to mRNA without a DNA template, in encoding essential regulatory information; this represents a new layer of RNA-based epigenetic regulation, herein referred to as **poly(A) tail epigenetic information**. Understanding poly(A) tail epigenetic information will uncover key biological mechanisms and advance mRNA biotechnology development.

RNA poly(A) tails

Traditionally, poly(A) tails are considered to be long chains of adenine nucleotides with initial lengths of approximately 70–80 nt in yeast and 200–250 nt in mammalian cells [13,14]. Given

[1]State Key Laboratory of Molecular Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China
[2]University of Chinese Academy of Sciences, Beijing 100049, China

*Correspondence:
fllu@genetics.ac.cn (F. Lu).

their ubiquitous presence in most eukaryotic mRNAs, the poly(A) tail is considered an essential structural component of mRNA required for its life cycle, including stability, translation, and nuclear export [13]. Additionally, poly(A) tails are not exclusive to mRNAs but are also found on many long non-coding RNAs (lncRNAs) [15,16] where they contribute to nuclear export and stability, paralleling their functions in mRNA [17]. Exploring whether poly(A) tails play additional roles in lncRNA metabolism and function represents an interesting future direction.

In mammals, the addition of a poly(A) tail to a pre-mRNA involves two main steps: cleavage by the cleavage and polyadenylation specificity factor (CPSF) complex and polyadenylation by poly (A) polymerase (PAP) (Figure 1). The CPSF complex recognizes and binds to a specific sequence motif called polyadenylation signal (PAS), usually AAUAAA, near the 3′ end of the pre-mRNA. The CPSF complex, together with other protein factors, forms a large cleavage complex that cleaves the pre-mRNA downstream of the PAS [18]. PAP then adds a poly(A) tail, typically 200–250 nt long, without needing a template [19]. The poly(A) tails can then be bound by a group of poly (A) binding proteins (PABPs) [20]. The steady-state length of mRNA poly(A) tails is commonly seen in the range of 20–100 nt, which therefore is unlikely to be determined at the time of synthesis by PAPs, but rather determined by trimming through deadenylation activity after exporting to the cytoplasm; trimming involves PAN2–PAN3 and CCR4–NOT complexes [13,14] (Figure 1). In addition to transcription-coupled polyadenylation in the nucleus, mRNAs can undergo polyadenylation extension or modification through a group of non-canonical PAPs (ncPAPs) called terminal nucleotidyltransferases (TENTs) (Figures 1 and 2). There are 11 TENTs in the human genome. Several of them have been implicated in cytoplasmic poly(A) tail remodeling [21], while the others represent emerging themes to be explored. Due to length constraints, beside brief touches on the TENTs, this review directs readers to more extensive discussions in recent reviews on this topic [21].

Methods of poly(A) tail sequencing and composition of poly(A) tails

Recent technical advancements have enabled the transcriptome-wide investigation of RNA poly (A) tails [22]. Next-generation sequencing (NGS) has made transcriptome sequencing a powerful tool for dissecting gene expression at single-cell resolution. However, sequencing poly(A) tails presents significant challenges due to their homopolymeric nature, which imposes problems for both Sanger and NGS technologies [23]. Therefore, poly(A) tails are normally discarded during the library preparation, sequencing, or data analysis phases of standard RNA sequencing (RNA-seq) methods.

For transcriptome-wide poly(A) tail analysis, several methods have been developed on the Illumina platform using customized sequencing recipes, base-calling algorithms, or data analysis methods, including TAIL-seq [23], mTAIL-seq [24], PAL-seq [25], PAL-seq-v2 [26,27], PAL-seq-v3 [28], PAL-seq-v4 [28], PAT-seq [29], TED-seq [30], and poly(A)-seq [31] (Table 2). Among these methods, TAIL-seq, mTAIL-seq, and various versions of PAL-seq output relatively good measurements of

Glossary

Alternative polyadenylation (APA): a mechanism of gene regulation that allows the production of mRNA isoforms through different polyadenylation sites in the pre-mRNA, resulting in mRNA isoforms with different 3′ untranslated regions from a single gene.
Epitranscriptomics: the study of transcriptome-wide RNA epigenetics. Often it refers to the study of the transcriptome-wide chemical modifications of RNA molecules.
Mixed tailing: in the context of RNA biology, mixed tailing refers to the incorporation of non-adenine nucleotides (such as cytosine, guanine, uracil) into the RNA poly(A) tails in a non-templated manner.
Oocyte-to-embryo transition: the period of development from oocyte maturation and early embryo development to zygotic genome activation. During this period, the developmental control shifts from maternal control to zygotic control.
Poly(A) tail epigenetic information: the regulatory information encoded in the poly (A) tails that is added in a non-templated manner. It includes the length of the poly (A) tail, as well as the type, number, and position of the non-A residues within it. It might also include the chemical modifications within the poly(A) tails.
RNA epigenetics: the study of changes in gene expression that do not alter the DNA sequence but are mediated through RNA molecules. This includes chemical modifications on RNA, editing of RNA, as well as regulatory RNA that influence gene expression, such as small RNAs and chromatin associated non-coding RNAs.
Zygotic genome activation (ZGA): the process in early embryonic development during which the embryonic genome becomes transcriptionally active.

Table 1. Well-accepted RNA epigenetics implicated in regulation of gene expression

RNA epigenetics	Major types	Short description of the function	Reviews for in-depth reading
Small RNAs	miRNA, piRNA, siRNA, snRNA	Target degradation, translational repression, transcriptional regulation	Chen and Rechavi [11]
Non-coding RNAs	lncRNAs, eRNAs, circRNAs	Transcriptional and post-transcriptional control of gene expression	Li and Fu [10] Yang, L. *et al.* [12]
RNA editing	A to I, C to U, U to C	Alters the nucleotide sequence of RNA molecules which might lead to protein-coding substitution; involved in innate immunity	Eisenberg and Levanon [9]
RNA chemical modifications	m6A, m1A, m7G, m5C, hm5C, ac4C, Ψ…	Change the structures and biochemical properties of RNA which leads to subsequent impact of mRNA stability and translation	Roundtree *et al.* [8] Delaunay *et al.* [7]

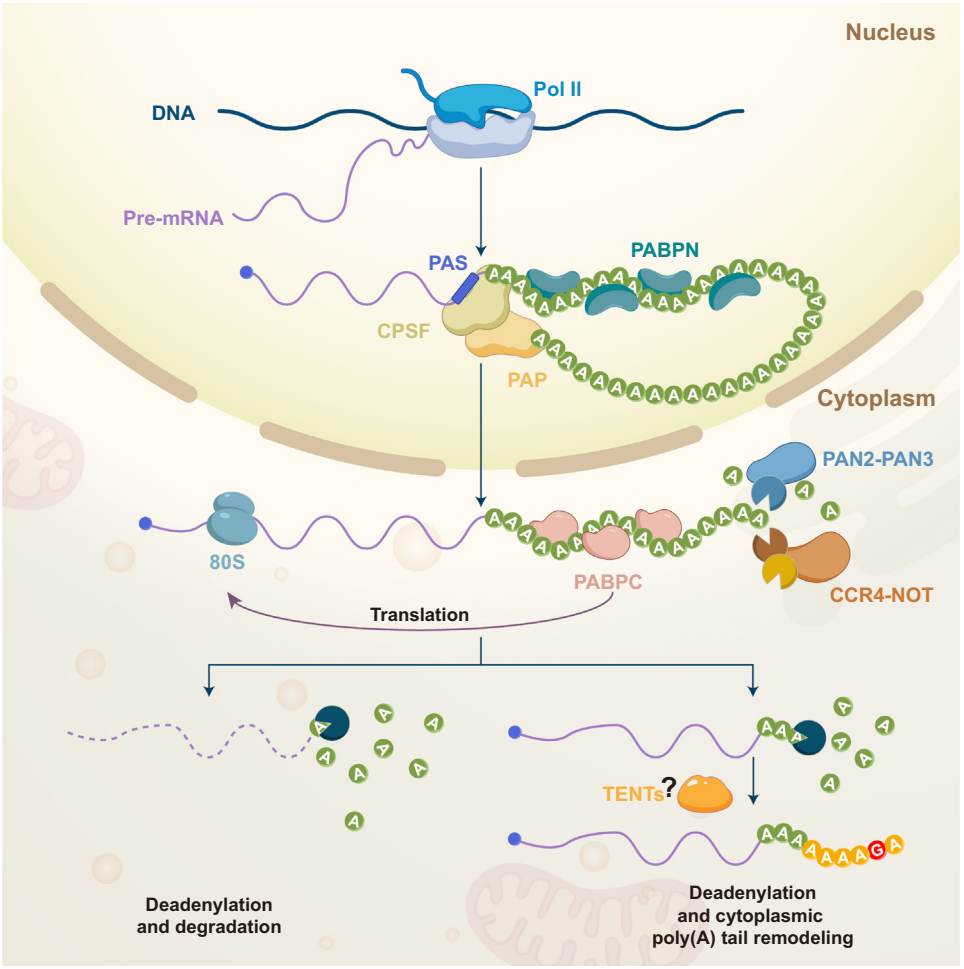

Figure 1. Overview of mRNA poly(A) tail metabolism. Pre-mRNAs are transcribed by RNA polymerase II (Pol II) and undergo co-transcriptional processing where the poly(A) tails are synthesized. This involves recognition of the polyadenylation signal (PAS) by the cleavage and polyadenylation specificity factor (CPSF), followed by cleavage and polyadenylation by canonical poly(A) polymerases (PAPs). In the nucleus, poly(A) tails are bound by nuclear poly(A)-binding protein (PABPN), preparing mRNAs for export to the cytoplasm. Once in the cytoplasm, poly(A) tails undergo trimming by the PAN2–PAN3 and CCR4–NOT complexes. Cytoplasmic poly(A)-binding protein (PABPC) binds the poly(A) tails, enabling mRNA translation by the 80S ribosomes. Further metabolism of mRNA poly(A) tails in the cytoplasm includes both degradation and remodeling. Abbreviation: TENTs, terminal nucleotidyltransferases.

poly(A) tails. PAL-seq utilizes biotin-conjugated dUTP to fluorescently quantify the relative amount of U incorporated at poly(A) tail sites, providing an estimate of poly(A) tail length [25]. By contrast, TAIL-seq, mTAIL-seq, PAL-seq-v2, PAL-seq-v3, and PAL-seq-v4 leverage customized base-calling algorithms to parse raw images from Illumina sequencers, enabling quantification of the number of A nucleotides [23,24,26–28]. Interestingly, TAIL-seq identifies non-adenine residues (U, C, G) at or near the 3′ ends of poly(A) tails [23], while mTAIL-seq, PAL-seq-v2, PAL-seq-v3, and PAL-seq-v4 allow for the identification of 3′ end U residues [24,26–28].

The third-generation sequencing technologies, PacBio and Nanopore platforms, exhibit reduced sensitivity to long homopolymeric sequences, making them exceptionally suitable for accurate poly(A) tail sequencing. For example, PacBio sequencing in HiFi mode excels in accurately

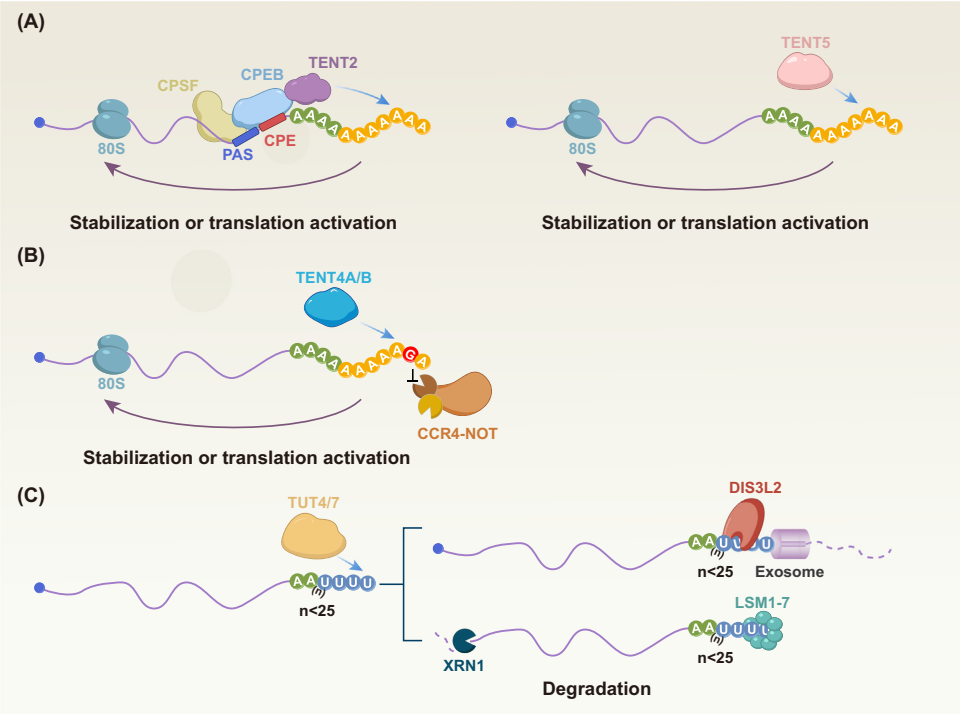

Figure 2. Cytoplasmic poly(A) tail remodeling and poly(A) tail epigenetic regulation. (A) Cytoplasmic polyadenylation by terminal nucleotidyltransferase 2 (TENT2) and TENT5. mRNAs that contain both a cytoplasmic polyadenylation element (CPE) and a polyadenylation signal (PAS) are recognized by the cleavage and polyadenylation specificity factor (CPSF) and the CPE-binding protein (CPEB), which recruits TENT2 to catalyze the extension of poly(A) tails. This process enhances mRNA stability or facilitates translational activation. Similarly, TENT5 can also target mRNAs for cytoplasmic polyadenylation, contributing to the stabilization or activation of these transcripts. (B) TENT4A/B catalyzes mixed tailing, incorporating non-A residues, particularly G residues, into poly(A) tails. These non-A residues, such as G, inhibit the deadenylation activity of the CCR4–NOT complex, thereby contributing to the stabilization and activation of the transcripts. (C) Terminal uridylyltransferase 4 (TUT4) and TUT7 catalyze the addition of U residues at the 3′ ends of poly(A) tails that are shorter than 25 nucleotides or fully deadenylated. The notation 'n' represents the number of adenine (A) residues. These 3′ uridylated transcripts are subject to either 3′-to-5′ degradation by DIS3L2 and the exosome or 5′-to-3′ degradation by the LSM1-7 complex and exonuclease 1 (XRN1).

measuring homopolymeric poly(A) tail sequences by repeatedly sequencing a single molecule to derive a consensus sequence [32–34]. Methods such as PAIso-seq [35–37], PAIso-seq2 [38], FLAM-seq [39], FLEP-seq [40], and FLEP-seq2 [41] on the PacBio HiFi platform have proved effective in sequencing poly(A) tails (Table 2). Notably, their accuracy facilitates the detection of non-A residues within the body of poly(A) tails [37–39], highlighting a complexity beyond mere stretches of A nucleotides. The Nanopore platform has the advantage of directly sequencing mRNA molecules (direct RNA sequencing, DRS) [42–46], as well as sequencing complementary DNA (cDNA) libraries that include the entire poly(A) sequence as evidenced by FLEP-seq, FLEP-seq2, and Nano3P-seq applications [40,41,47] (Table 2). Interestingly, Nanopore sequencing also uncovers non-A residues within the poly(A) tails through both cDNA sequencing [47] and DRS utilizing a neural network-based poly(A) tail non-A base-calling tool, Ninetails (https://github.com/LRB-IIMCB/ninetails). Unlike reverse transcription-based methods, DRS analyzes native RNA molecules and has the potential to detect chemical modifications in them. It is noteworthy that while PAIso-seq, PAIso-seq2, and FLAM-seq libraries may also be sequenced on Nanopore platforms at a lower sequencing cost, the accuracy in measuring internal non-A residues might not match that of the PacBio HiFi platform.

Table 2. Methods for transcriptome-wide poly(A) tail sequencing

Methods	Platform	Minimal input	rRNA removal	Tail preserving principle	Full-length isoform	Length	3′-End non-A residues	Internal non-A residues	Refs
TAIL-seq	Illumina	50 μg total RNA	rRNA depletion by Ribo-Zero	3′ Adaptor ligation	No	231 nt	Yes	Very close to 3′ end	Chang *et al.* [23]
mTAIL-seq	Illumina	1 μg total RNA	–	3′ Splinted ligation	No	231 nt	3′-end U	No	Lim *et al.* [24]
PAL-seq	Illumina GA II	1 μg total RNA	–	3′ Splinted ligation	No	300 nt	No	No	Subtelny *et al.* [25]
PAL-seq V2	Illumina	25 μg total RNA	–	3′ Splinted ligation	No	250 nt	3′ end U	No	Eisen *et al.* [26]
PAL-seq V3	Illumina	500 ng total RNA	–	3′ Splinted ligation	No	250 nt	3′ end U	No	Xiang and Bartel [28]
PAL-seq V4	Illumina	500 ng total RNA	–	3′ Splinted ligation	No	250 nt	3′ end U	No	Xiang and Bartel [28]
PAT-seq	Illumina	1 μg total RNA	–	Templated end extension	No	80 nt	Yes	Very close to 3′ end	Harrison *et al.* [29]
TED-seq	Illumina	N.A.	Poly(A)+ selection by oligo(dT) Dynabead	3′ Adaptor ligation	No	300 nt	No	No	Woo *et al.* [30]
Poly(A)-seq	Illumina	5.1 μg total RNA	Poly(A)+ selection by oligo(dT)	3′ Adaptor ligation	No	300 nt	Yes	Short tails	Yu, F. *et al.* [31]
FLAM-seq	PacBio HiFi	500 ng total RNA	Poly(A)+ selection by oligo(dT)	GI tailing	Yes	No limitation	No	Yes	Legnini *et al.* [39]
FLEP-seq	Oxford Nanopore or PacBio HiFi	3 μg total RNA	rRNA depletion by RiboMinus Plant or riboPOOLs	3′ Adapter ligation	Yes	No limitation	Yes	Yes	Long *et al.* [40]
FLEP-seq2	Oxford Nanopore or PacBio HiFi	500 ng total RNA	rRNA depletion by riboPOOLs	3′ Adapter ligation	Yes	No limitation	Yes	Yes	Jia *et al.* [41]
PAIso-seq	PacBio HiFi	0.5 ng total RNA	–	Templated end extension	Yes	No limitation	No	Yes	Liu *et al.* [37]
PAIso-seq2	PacBio HiFi	100 ng total RNA	rRNA depletion or Cas9-guided digestion of rDNA	3′ Adaptor ligation	Yes	No limitation	Yes	Yes	Liu *et al.* [38]
Nano3P-seq	Oxford Nanopore	50 ng total RNA	rRNA depletion by riboPOOLs	3′ Template switch	Yes	No limitation	Yes	Yes	Begik *et al.* [47]
Direct RNA sequencing	Oxford Nanopore	100 ng poly(A)+ RNA	Poly(A)+ selection by oligo(dT)	3′ splinted ligation	Yes	No limitation	No for standard DRS	No for standard DRS	Krawczyk *et al.* [46] Garalde *et al.* [42] Parker *et al.* [43] Workman *et al.* [44] Roach *et al.* [45]

As methods on third-generation platforms can sequence full-length cDNA isoforms together with the poly(A) tails, these methods provide opportunities to investigate the interplay between poly (A) tail length, non-A residues within poly(A) tails, **alternative polyadenylation (APA)**, alternative splicing, and possibly RNA chemical modifications. These technologies offer powerful tools for delving into the dynamic regulation and functionality of RNA poly(A) tails across a variety of biological processes.

Poly(A) tail length-mediated gene expression regulation

The regulatory function of poly(A) tails in gene expression has been characterized from the studies of dynamic poly(A) tail length, which influences both the stability and the translation of mRNA molecules. This regulation occurs through mechanisms such as deadenylation and cytoplasmic polyadenylation.

Deadenylation precedes decapping in 5′-to-3′ RNA decay, and it also takes place prior to exosome in the 3′-to-5′ RNA decay [13,26,48,49]. Therefore, deadenylation effectively signals that an mRNA is destined for degradation, positioning deadenylation as a crucial mechanism for downregulating gene expression [13,50]. For example, active deadenylation mediated by the CCR4–NOT complex is crucial for clearing mRNAs associated with the naïve pluripotent regulatory network during the exit of naïve pluripotency [51]. The PAN2–PAN3 and CCR4–NOT complexes are primarily responsible for deadenylation [50]. It is generally understood that PAN2–PAN3 targets long poly(A) tails, with minimal effect on the transcriptome, whereas the CCR4–NOT complex is responsible for the degradation of a majority of the poly(A) tails [50,52,53]. Interestingly, not all mRNAs subjected to deadenylation are destined for immediate degradation; some are stably preserved within the cytoplasm. These transcripts can later be reactivated for translation through cytoplasmic polyadenylation or other types of poly(A) tail remodeling mediated mainly by TENTs [21,54,55] (Figure 2A,B). This dual role of deadenylation underscores its significance in both mRNA turnover and the dynamic regulation of gene expression.

Cytoplasmic polyadenylation has been extensively explored in various biological contexts such as **oocyte-to-embryo transition**, inflammation, synaptic activity, and processes underlying learning and memory [13,56–59]. This process facilitates the rapid synthesis of proteins in specific spatial or temporal patterns in response to external or internal cues. For example, during mammalian oocyte-to-embryo transition, global mRNA poly(A) tail deadenylation takes place during oocyte maturation [60–65], while select mRNAs undergo polyadenylation to increase the lengths of their poly(A) tails [58,65–69]. The cytoplasmic polyadenylation is crucial for the production of factors important for reproduction, such as CCNB1, a cyclin critical for meiosis [70,71], and BTG4, CNOT6L, and CNOT7, components of the CCR4–NOT deadenylase complex [61,62,64]. The translational activation during oocyte maturation is driven primarily by cytoplasmic polyadenylation element (CPE, mostly the UUUUA element)-dependent cytoplasmic polyadenylation [57–59,66,68,69,72].

In the global view, the changes in poly(A) tail lengths during oocyte maturation are highly associated with mRNA translational efficiency, with longer poly(A) tails generally promoting more effective translation [58,65,66,73]. This phenomenon is a conserved mechanism across various species, including mammals, fish, frogs, and flies [24,25,28,73]. However, this positive association does not extend to somatic cell lines, indicating a cell type specificity for such a link [28,74]. Dysfunctions in poly(A) tail-mediated regulation during the oocyte-to-embryo transition leads to compromised or unsuccessful reproduction [58,60–62,64,75]. For comprehensive knowledge about cytoplasmic polyadenylation and deadenylation-mediated gene expression regulation, including detailed discussions on biochemical mechanisms and biological functions, readers are encouraged to consult focused reviews [13,56].

The regulation of gene expression through poly(A) tail length involves not only factors that enhance deadenylation or polyadenylation activity, but also regulators that inhibit the CCR4–NOT complex [76], or possibly TENTs, thereby fine-tuning poly(A) tail length. Such precise control of gene expression allows for timely protein synthesis, which is essential for development and the cellular response to environmental changes. Future research, empowered by advanced sequencing technologies, will advance our insights into the complex dynamics of poly(A) tail lengths in cellular physiology and disease mechanisms.

Non-A residues within poly(A) tails encode potential epigenetic information

Recent advancements in sequencing technologies – including TAIL-seq, PAIso-seq, PAIso-seq2, and FLAM-seq – have revealed the presence of non-adenine residues both at the 3′ ends and within the bodies of poly(A) tails [23,37–39,47]. These findings challenge the long-standing knowledge of the poly(A) tail as merely a sequence of adenine nucleotides at the mRNA's 3′ end. The discovery of non-adenine residues, combined with the variability in poly(A) tail length, introduces a potential complex layer of post-transcriptional regulation. This complexity, reflected in the diversity, abundance, and distribution of the non-A residues, represents a significant encoding capacity with the potential to code for significant RNA regulatory information beyond the genetic information inscribed within genomic DNA. We suggest conceptualizing this capacity as 'poly(A) tail epigenetic information', representing a new layer in our understanding of gene expression regulation. We hope that the evidence presented in the following sections will help substantiate the concept's validity for our readers.

Non-A residues at the 3′ ends of poly(A) tails

In somatic cells, U residues frequently appear at the 3′ ends of poly(A) tails. Around half of all mRNA species have more than 5% of their poly(A) tails with U residues at 3′ ends [23]. The 3′ end uridylation predominantly occurs in mRNA transcripts with very short poly(A) tails (typically less than approximately 25 nt) [23,77,78], suggesting a link with the process of deadenylation. Indeed, the rate of 3′ end uridylation negatively associates with mRNA half-lives [23]. Mechanistically, mRNA targeted for degradation is first deadenylated to fewer than 25 nt, followed by loss of PABP binding and subsequent 3′ end uridylation, catalyzed by terminal uridylyl transferases 4/7 (TUT4/7), two non-canonical poly(A) polymerases (ncPAPs) [79] (Figure 2C). mRNAs with uridylated poly(A) tails can then be degraded by the exonucleases in both the 5′-to-3′ and 3′-to-5′ directions [79]. This mechanism has proved crucial for degrading unnecessary transcripts during mammalian oocyte growth [80,81]. Importantly, impaired uridylation-mediated mRNA degradation results in defective germinal vesicle (GV) oocytes, which are subsequently unable to complete the reproductive process [80,81]. In addition, this process is also observed in plants, where it facilitates degradation of mRNAs or cleaved mRNA fragments [82,83].

Therefore, 3′ end U residues are added by TUT4/7 to serve as mRNA regulatory information, marking the mRNA for subsequent degradation. DIS3L2 and LSM1-7 are potential readers of these marks, recognizing and acting upon the uridylated tail [79] (Figure 2C). Furthermore, this regulatory mechanism might be conserved beyond mammals, with 3′ end U residues also found in *Arabidopsis thaliana* and other eukaryotes, suggesting a broad biological relevance [23,77,78,84]. These findings illustrate how 3′ end U residues of mRNA poly(A) tails encode epigenetic information, which can primarily be read as a mark for subsequent mRNA degradation.

By contrast with 3′ end U residues, 3′ end G residues are associated with longer poly(A) tails (>40 nt) [23]. In line with this observation, 3′ end guanylation rate is positively associated with mRNA half-lives [23]. Mechanistically, G residues are incorporated into poly(A) tails in a **mixed tailing** mechanism by TENT4A/B, another two ncPAPs [85] (Figure 2B). TENT4A/B catalyze the

incorporation of mostly A residues followed by G residues, as well as a smaller amount of C and U residues [85]. The presence of a relatively high abundance of G residues at the 3′ ends of poly (A) tails is likely the result of a deadenylation process that preferentially stops at the non-A residues, especially at G residues, which are poor substrates for the CCR4–NOT complex [85,86] (Figure 2B). Experimental data support this, showing that G, C, and U residues effectively slow down the deadenylation process *in vitro* when the fully reconstituted human CCR4–NOT complex is used [86]. This characteristic leads to deadenylation preferentially halting at non-A residues during CCR4–NOT-mediated deadenylation, explaining the preferential seen of G residues at the 3′ ends of steady-state poly(A) tails.

Therefore, G, C, and U residues introduced by mixed tailing into poly(A) tails serve as mRNA regulatory information, marking the mRNA for enhanced stability against active deadenylation. The ability of the CCR4–NOT complex to differentiate between non-A and A residues highlights its role as a potential reader of these marks within poly(A) tails [85,86]. TENT4A/B have demonstrated a capacity for mixed tailing. Whether there are other ncPAPs capable of mixed tailing into poly(A) tails remains an interesting question to be explored. These findings illustrate how G, C, and U residues within poly(A) tails encodes epigenetic information, which can primarily be read as marks for longer mRNA half-life.

Non-A residues within the body of poly(A) tails

In addition to regulating mRNA half-life, the frequency of G residues within poly(A) tails has been shown to negatively impact PABP binding and translational efficiency in *Arabidopsis thaliana* [87]. However, the impact of these residues on translation in mammals presents a more complex picture. Studies using synthetic mRNAs with poly(A) tails containing non-A residues have shown that C residues within poly(A) tails can increase target protein production, potentially by stabilizing mRNA against CCR4–NOT-mediated deadenylation [88]. However, this effect was not observed with G residues, despite their known role in protecting poly(A) tails from deadenylation. In the reconstituted human CCR4–NOT-mediated deadenylation system, C residues have a more pronounced negative effect than G or U residues [86]. This might partially explain the more obvious effect of C residues over G residues in the poly(A) tails to increase protein production through increased stability. Alternatively, non-A residues might have additional roles in regulating mRNA translational efficiency. An independent study using synthetic poly(A) tails interspersed with non-A residues has revealed that these can increase translational efficiency independently of their effects on mRNA stability [89]. Therefore, the relationship between non-A residues within poly(A) tails and their impact on mRNA translation remains unclear. It would also be intriguing to determine whether this relationship varies across different cells, tissues, or species. This complexity underscores the necessity for further investigation to fully decipher the epigenetic information encoded by non-A residues within poly(A) tails, which may potentially mark the mRNA for translational regulation in diverse biological contexts.

The physiological roles of non-A residues within poly(A) tails are just beginning to be uncovered, thanks to recent advances in poly(A) tail sequencing technologies. Very high abundances of these non-A residues have been observed in mammalian preimplantation embryos prior to **zygotic genome activation (ZGA)** and in matured oocytes [75,84,90,91] (Figure 3). For example, >50% of poly(A) tails in human pre-ZGA embryos contain U residues within their bodies, and around 40% of poly(A) tails in human matured oocytes at the metaphase II (MII) stage feature U residues at the 3′ ends [75]. The poly(A) tail dynamics during human oocyte-to-embryo transition indicate a dynamic regulation of poly(A) tails involving global deadenylation during oocyte maturation, followed by uridylation in matured oocytes, and subsequent re-polyadenylation post-fertilization, that generates poly(A) tails with U residues in the internal parts of poly(A) tails [75] (Figure 3). In addition, a

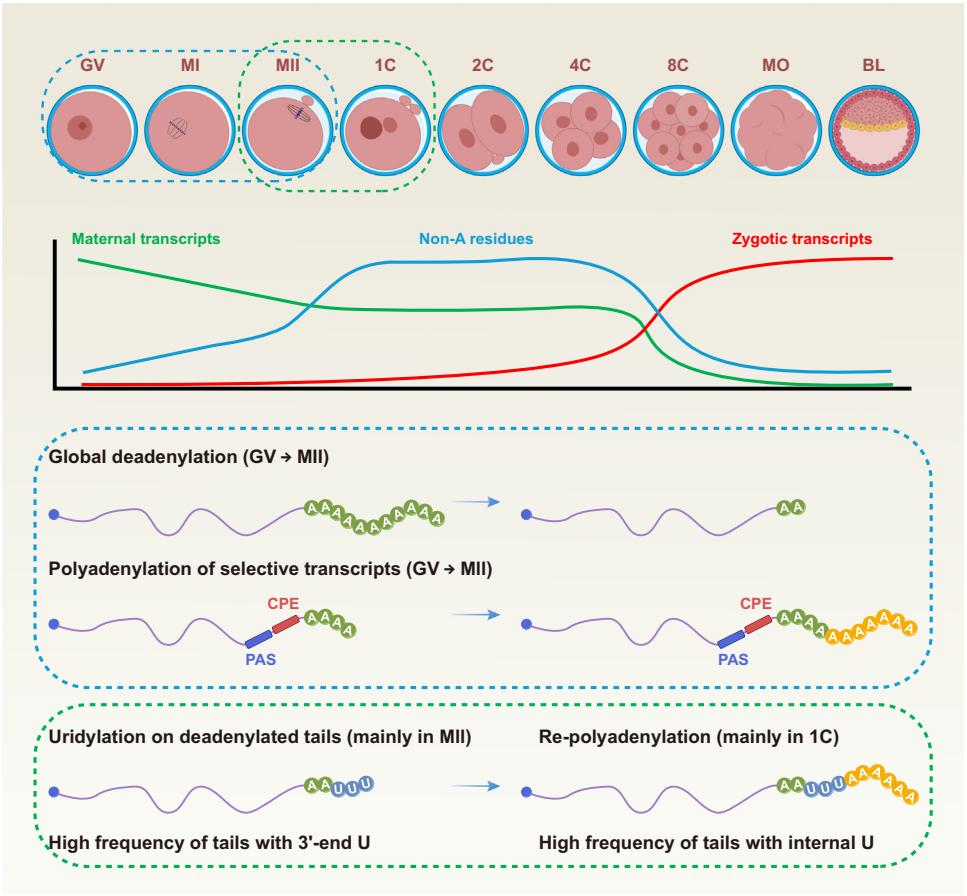

Figure 3. Poly(A) tail remodeling during human oocyte-to-embryo transition (OET). During the human OET, maternal transcripts (green line) are gradually degraded with one wave during oocyte maturation and another wave during zygotic genome activation (ZGA). ZGA (red line) initiates at the late four-cell (4C) stage. Non-A (U, C, and G) residues (blue line) exhibit high dynamics, peaking between the one-cell (1C) and four-cell (4C) stages. During oocyte maturation – from germinal vesicle (GV) to metaphase II (MII) stages – a global deadenylation occurs, while transcripts with proximal cytoplasmic polyadenylation elements (CPEs) and a polyadenylation signal (PAS) undergo selective polyadenylation. The first wave of 3′ uridylation occurs primarily at the MII stage on deadenylated tails, which are then repolyadenylated post-fertilization (at 1C) to generate poly(A) tails with internal U residues.

second wave of terminal incorporation of U residues is seen at the four-cell stage just before ZGA [75]. Although the detailed molecular functions of this global poly(A) tail epigenetic information remodeling remain to be revealed, this remodeling is essential for reproduction, as blocking the re-polyadenylation after fertilization essentially prevents the first cleavage of human zygotes [75]. Similar patterns of poly(A) tail-mediated maternal mRNA remodeling have been observed in mice, rats, and pigs [84], suggesting a conserved mechanism across mammalian species that is crucial for successful reproduction.

Non-A residues have also been found in high abundance at the 3′ ends of poly(A) tails in early embryos of zebrafish and *Xenopus laevis* [47,78], as well as in the body of poly(A) tails in early embryos of zebrafish [47], pointing to a similar poly(A) tail non-A residues dynamics in vertebrates as that seen in mammals. A study focusing on selected genes in starfish oocytes and embryos has revealed a process of deadenylation followed by uridylation and re-polyadenylation [92],

mirroring the patterns observed in mammalian oocyte-to-embryo transition. This suggests that the dynamics of poly(A) tail non-A residues in early embryos may extend beyond vertebrates, raising the possibility that these highly dynamic changes in poly(A) tails around fertilization is a universal feature in metazoans and potentially in other eukaryotes.

Concluding remarks

We believe that this review has demonstrated the potential of poly(A) tails to encode essential epigenetic regulatory information. As a novel aspect of RNA-based epigenetic regulation, this field deserves further exploration of the functions and mechanisms involved in writing, reading, and erasing poly(A) tail epigenetic information (Figure 4). As an area burgeoning with possibilities, poly(A) tail epigenetic information holds significant potential to provide profound insights into the mechanisms of gene expression regulation across diverse biological systems.

The conserved dynamic remodeling of mRNA poly(A) tail epigenetic information during the oocyte-to-embryo transition underscores the critical roles of non-A residues in regulating reproduction. However, the specific functions and mechanisms underlying these poly(A) tail non-A residues in this crucial developmental process await discovery. Given that the final products of this remodeling process, poly(A) tails enriched with non-A residues, can support mRNA translation, it is plausible that this process generates unique poly(A) tails carrying distinct epigenetic information that marks the mRNA to be translated at critical developmental stages. Moreover, these tails might fulfill additional regulatory roles beyond merely influencing spatiotemporal mRNA stability or translational activation. For example, beyond their established role in regulating mRNA stability, 3′ end U residues have been implicated in the repair of deadenylated mRNA ends in *Arabidopsis thaliana* [83], suggesting a complex, multifaceted contribution to mRNA life cycle management.

The oocyte-to-embryo transition represents an excellent model for the study of the mechanism underlying poly(A) tail epigenetic information, particularly since transcription is silent during oocyte-to-embryo transition [93–95], and all changes to poly(A) tails result from remodeling the poly(A) tail epigenetic information rather than poly(A) tails synthesis following new transcription. We envision that this unique developmental context will allow for detailed exploration of the mechanisms involved in writing, reading, and erasing poly(A) tail epigenetic information during early development (Figure 4).

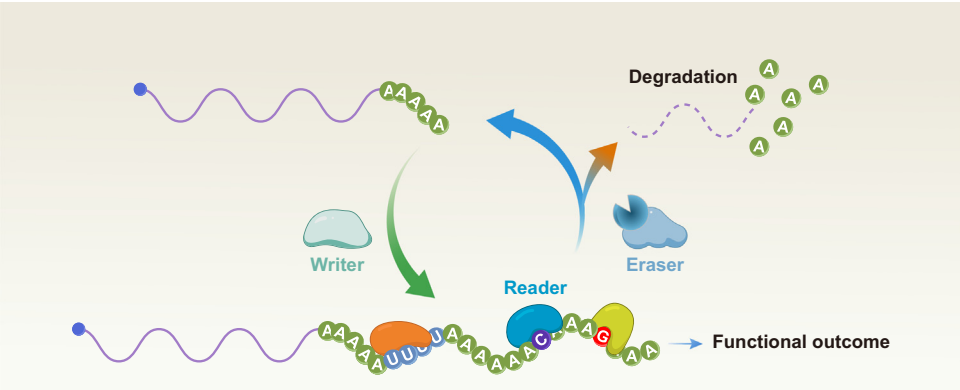

Figure 4. A general model of poly(A) tail epigenetic regulation. Poly(A) tail epigenetic information is written into poly(A) tails by writers. This epigenetic information is subsequently read by readers, which interpret it to execute specific functional outcomes. Eventually, erasers can remove this epigenetic information, resulting in poly(A) tails devoid of such marks or leading to transcript degradation.

Outstanding questions

Many recent advances have been made to enable the transcriptome-wide sequencing of the homopolymeric poly(A) tails. How can analysis of poly(A) tails be performed at the high-throughput single-cell level?

Sequencing of poly(A) tails has revealed that non-A residues are found at the 3′ end of poly(A) tails and within the body of poly(A) tails. While non-A residues at the 3′ ends regulate the stability of RNA transcripts, what functions do non-A residues within the body of poly(A) tails serve?

The mechanisms underlying poly(A) tail regulation involving non-A residues is an emerging theme to be explored. What mechanisms are responsible for the incorporation of non-A residues into poly(A) tails, and what is the specificity of terminal nucleotidyltransferases (TENTs) in this process? Are there enzymes other than TENTs involved? Which factors specifically recognize non-A residues within poly(A) tails to read the encoded epigenetic information, and what mechanisms are involved in the removal of non-A residues from poly(A) tails, effectively erasing the epigenetic information?

The poly(A) tail-mediated post-transcriptional regulation has been recognized to be critical for oocyte maturation and early embryo development. As poly(A) tails are universal to most eukaryotic mRNAs, what are the physiological roles of poly(A) tail epigenetic regulation in other diverse development and diseases processes?

Poly(A) tails are found not only on mRNAs, but also on lncRNAs. What roles do poly(A) tail epigenetic modifications play in the regulation of lncRNAs?

Moreover, since poly(A) tails are ubiquitous across most mRNAs and lncRNAs in eukaryotes, and poly(A) tail non-A residues are also observed in various tissues and cell types, we believe that poly (A) tail epigenetic regulation extends beyond reproduction processes. It likely plays a significant role in a wide range of biological and pathological processes wherever mRNAs and lncRNAs are crucial. However, studying poly(A) tail epigenetic regulation outside of the oocyte-to-embryo transition is challenging, as it is difficult to distinguish between newly synthesized and remodeled poly (A) tails. Developing tools and methods to differentiate these will be essential for advancing our understanding of poly(A) tail epigenetic regulation (see Outstanding questions). Furthermore, the cell type specificity of poly(A) tail-mediated epigenetic regulation calls the development of high-throughput, transcriptome-wide poly(A) tail profiling methods at the single-cell level.

In recent years, mRNA technologies have become a major focus in the pharmaceutical industry [96,97], highlighted by the tremendous success of mRNA vaccines against the global COVID-19 pandemic [98,99]. Extensive efforts have focused on enhancing mRNA stability and translational efficiency, including better 5′ cap modifications, optimization of 5′ and 3′ UTRs, codon optimization, and the use of modified nucleosides [96]. Interestingly, the poly(A) tails are remodeled after mRNA injection into tissues [46]. As discussed earlier, poly(A) tail epigenetic information plays crucial roles in regulating mRNA stability and translational efficiency. The development of technologies that leverage poly(A) tail epigenetic mechanisms or even unnatural branched synthetic poly(A) tails [100] promises to promote mRNA vaccine and drug designs. These new technologies, distinct from current methods, can be seamlessly integrated into existing development pipelines. Therefore, further exploration of poly(A) tail epigenetic information and remodeling mechanisms holds immense potential to advance mRNA-based biotechnologies.

Ultimately, poly(A) tail epigenetic regulation represents a new layer of complexity in gene regulation, highlighting the poly(A) tails beyond their traditional view as mere structural elements of mRNAs and lncRNAs. Better understanding of these principles will not only reveal fundamental biological mechanisms but also promote the development of novel mRNA-based vaccines and therapeutic strategies, leveraging the power of poly(A) tail epigenetic information.

Acknowledgments

This work was supported by the National Key Research and Development Program of China (2020YFA0804000), the Strategic Priority Research Program of the Chinese Academy of Sciences (XDA24020203), the National Natural Science Foundation of China (32170606), and the CAS Project for Young Scientists in Basic Research (YSBR-012).

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT in order to improve the readability of the language. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of interests

F.L. is a named inventor on a patent (number: ZL201910837492.2) covering PAIso-seq method and a patent (number: ZL202210410067.7, PCT/CN2022/093735) covering poly(A) tail non-A residues on mRNA translation filed by the Institute of Genetics and Developmental Biology. J.L. declares no conflicts of interest.

References

1. Goldberg, A.D. *et al.* (2007) Epigenetics: a landscape takes shape. *Cell* 128, 635–638
2. Cavalli, G. and Heard, E. (2019) Advances in epigenetics link genetics to the environment and disease. *Nature* 571, 489–499
3. Roundtree, I.A. and He, C. (2016) RNA epigenetics – chemical messages for posttranscriptional gene regulation. *Curr. Opin. Chem. Biol.* 30, 46–51
4. Holoch, D. and Moazed, D. (2015) RNA-mediated epigenetic regulation of gene expression. *Nat. Rev. Genet.* 16, 71–84
5. Liu, N. and Pan, T. (2015) RNA epigenetics. *Transl. Res.* 165, 28–35
6. He, C. (2010) Grand challenge commentary: RNA epigenetics? *Nat. Chem. Biol.* 6, 863–865
7. Delaunay, S. *et al.* (2024) RNA modifications in physiology and disease: towards clinical applications. *Nat. Rev. Genet.* 25, 104–122
8. Roundtree, I.A. *et al.* (2017) Dynamic RNA modifications in gene expression regulation. *Cell* 169, 1187–1200

9. Eisenberg, E. and Levanon, E.Y. (2018) A-to-I RNA editing – immune protector and transcriptome diversifier. *Nat. Rev. Genet.* 19, 473–490
10. Li, X. and Fu, X.D. (2019) Chromatin-associated RNAs as facilitators of functional genomic interactions. *Nat. Rev. Genet.* 20, 503–519
11. Chen, X. and Rechavi, O. (2022) Plant and animal small RNA communications between cells and organisms. *Nat. Rev. Mol. Cell Biol.* 23, 185–203
12. Yang, L. *et al.* (2022) Biogenesis and regulatory roles of circular RNAs. *Annu. Rev. Cell Dev. Biol.* 38, 263–289
13. Passmore, L.A. and Coller, J. (2022) Roles of mRNA poly (A) tails in regulation of eukaryotic gene expression. *Nat. Rev. Mol. Cell Biol.* 23, 93–106
14. Eckmann, C.R. *et al.* (2011) Control of poly(A) tail length. *Wiley Interdiscip. Rev. RNA* 2, 348–361
15. Cabili, M.N. *et al.* (2011) Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. *Genes Dev.* 25, 1915–1927
16. Dahlgren, A.R. *et al.* (2020) Comparison of poly-A(+) selection and rRNA depletion in detection of lncRNA in two equine tissues using RNA-seq. *Noncoding RNA* 6, 32
17. Chen, L.L. (2016) Linking long noncoding RNA localization and function. *Trends Biochem. Sci.* 41, 761–772
18. Sheets, M.D. *et al.* (1990) Point mutations in AAUAAA and the poly (A) addition site: effects on the accuracy and efficiency of cleavage and polyadenylation *in vitro*. *Nucleic Acids Res.* 18, 5799–5805
19. Bienroth, S. *et al.* (1993) Assembly of a processive messenger RNA polyadenylation complex. *EMBO J.* 12, 585–594
20. Mangus, D.A. *et al.* (2003) Poly(A)-binding proteins: multifunctional scaffolds for the post-transcriptional control of gene expression. *Genome Biol.* 4, 223
21. Yu, S. and Kim, V.N. (2020) A tale of non-canonical tails: gene regulation by post-transcriptional RNA tailing. *Nat. Rev. Mol. Cell Biol.* 21, 542–556
22. Brouze, A. *et al.* (2023) Measuring the tail: methods for poly (A) tail profiling. *Wiley Interdiscip. Rev. RNA* 14, e1737
23. Chang, H. *et al.* (2014) TAIL-seq: genome-wide determination of poly(A) tail length and 3′ end modifications. *Mol. Cell* 53, 1044–1052
24. Lim, J. *et al.* (2016) mTAIL-seq reveals dynamic poly(A) tail regulation in oocyte-to-embryo development. *Genes Dev.* 30, 1671–1682
25. Subtelny, A.O. *et al.* (2014) Poly(A)-tail profiling reveals an embryonic switch in translational control. *Nature* 508, 66–71
26. Eisen, T.J. *et al.* (2020) The dynamics of cytoplasmic mRNA metabolism. *Mol. Cell* 77, 786–799 e710
27. Eisen, T.J. *et al.* (2020) MicroRNAs cause accelerated decay of short-tailed target mRNAs. *Mol. Cell* 77, 775–785 e778
28. Xiang, K. and Bartel, D.P. (2021) The molecular basis of coupling between poly(A)-tail length and translational efficiency. *eLife* 10, e66493
29. Harrison, P.F. *et al.* (2015) PAT-seq: a method to study the integration of 3′-UTR dynamics with gene expression in the eukaryotic transcriptome. *RNA* 21, 1502–1510
30. Woo, Y.M. *et al.* (2018) TED-Seq identifies the dynamics of poly (A) length during ER stress. *Cell Rep.* 24, 3630–3641 e3637
31. Yu, F. *et al.* (2020) Poly(A)-seq: a method for direct sequencing and analysis of the transcriptomic poly(A)-tails. *PLoS One* 15, e0234696
32. Laehnemann, D. *et al.* (2016) Denoising DNA deep sequencing data-high-throughput sequencing errors and their correction. *Brief. Bioinform.* 17, 154–179
33. Ross, M.G. *et al.* (2013) Characterizing and measuring bias in sequence data. *Genome Biol.* 14, R51
34. Hebert, P.D.N. *et al.* (2018) A sequel to Sanger: amplicon sequencing that scales. *BMC Genomics* 19, 219
35. Liu, Y. *et al.* (2024) Sequencing of transcriptome-wide poly(A) tails by PAlso-seq. In *Deadenylation: Methods and Protocols* (Valkov, E. and Goldstrohm, A.C., eds), pp. 215–232, Springer
36. Liu, Y. *et al.* (2022) Transcriptome-wide measurement of poly(A) tail length and composition at subnanogram total RNA sensitivity by PAlso-seq. *Nat. Protoc.* 17, 1980–2007
37. Liu, Y. *et al.* (2019) Poly(A) inclusive RNA isoform sequencing (PAIso-seq) reveals wide-spread non-adenosine residues within RNA poly(A) tails. *Nat. Commun.* 10, 5292
38. Liu, Y. *et al.* (2023) Comprehensive analysis of mRNA poly (A) tails by PAIso-seq2. *Sci. China Life Sci.* 66, 187–190
39. Legnini, I. *et al.* (2019) FLAM-seq: full-length mRNA sequencing reveals principles of poly(A) tail length control. *Nat. Methods* 16, 879–886
40. Long, Y. *et al.* (2021) FLEP-seq: simultaneous detection of RNA polymerase II position, splicing status, polyadenylation site and poly(A) tail length at genome-wide scale by single-molecule nascent RNA sequencing. *Nat. Protoc.* 16, 4355–4381
41. Jia, J. *et al.* (2022) An atlas of plant full-length RNA reveals tissue-specific and monocots-dicots conserved regulation of poly(A) tail length. *Nat. Plants* 8, 1118–1126
42. Garalde, D.R. *et al.* (2018) Highly parallel direct RNA sequencing on an array of nanopores. *Nat. Methods* 15, 201–206
43. Parker, M.T. *et al.* (2020) Nanopore direct RNA sequencing maps the complexity of *Arabidopsis* mRNA processing and m (6)A modification. *eLife* 9, e49658
44. Workman, R.E. *et al.* (2019) Nanopore native RNA sequencing of a human poly(A) transcriptome. *Nat. Methods* 16, 1297–1305
45. Roach, N.P. *et al.* (2020) The full-length transcriptome of *C. elegans* using direct RNA sequencing. *Genome Res.* 30, 299–312
46. Krawczyk, P.S. *et al.* (2022) SARS-CoV-2 mRNA vaccine is re-adenylated *in vivo*, enhancing antigen production and immune response. *bioRxiv*, Published online December 16, 2022. https://doi.org/10.1101/2022.12.01.518149
47. Begik, O. *et al.* (2023) Nano3P-seq: transcriptome-wide analysis of gene expression and tail dynamics using end-capture nanopore cDNA sequencing. *Nat. Methods* 20, 75–85
48. Decker, C.J. and Parker, R. (1993) A turnover pathway for both stable and unstable mRNAs in yeast: evidence for a requirement for deadenylation. *Genes Dev.* 7, 1632–1643
49. Parker, R. and Song, H. (2004) The enzymes and control of eukaryotic mRNA turnover. *Nat. Struct. Mol. Biol.* 11, 121–127
50. Yan, Y.-B. (2014) Deadenylation: enzymes, regulation, and functional implications. *WIREs RNA* 5, 421–443
51. Quan, Y. *et al.* (2022) Cnot8 eliminates naive regulation networks and is essential for naive-to-formative pluripotency transition. *Nucleic Acids Res.* 50, 4414–4435
52. Yi, H. *et al.* (2018) PABP Cooperates with the CCR4-NOT complex to promote mRNA deadenylation and block precocious decay. *Mol. Cell* 70, 1081–1088 e1085
53. Raisch, T. and Valkov, E. (2022) Regulation of the multisubunit CCR4-NOT deadenylase in the initiation of mRNA degradation. *Curr. Opin. Struct. Biol.* 77, 102460
54. Barnard, D.C. *et al.* (2004) Symplekin and xGLD-2 are required for CPEB-mediated cytoplasmic polyadenylation. *Cell* 119, 641–651
55. Liudkovska, V. *et al.* (2022) TENT5 cytoplasmic noncanonical poly(A) polymerases regulate the innate immune response in animals. *Sci. Adv.* 8, eadd9468
56. Weill, L. *et al.* (2012) Translational control by changes in poly (A) tail length: recycling mRNAs. *Nat. Struct. Mol. Biol.* 19, 577–585
57. Reyes, J.M. and Ross, P.J. (2016) Cytoplasmic polyadenylation in mammalian oocyte maturation. *Wiley Interdiscip. Rev. RNA* 7, 71–89
58. Conti, M. and Kunitomi, C. (2024) A genome-wide perspective of the maternal mRNA translation program during oocyte development. *Semin. Cell Dev. Biol.* 154, 88–98
59. Conti, M. and Franciosi, F. (2018) Acquisition of oocyte competence to develop as an embryo: integrated nuclear and cytoplasmic events. *Hum. Reprod. Update* 24, 245–266
60. Zheng, W. *et al.* (2020) Homozygous mutations in BTG4 cause zygotic cleavage failure and female infertility. *Am. J. Hum. Genet.* 107, 24–33
61. Sha, Q.Q. *et al.* (2018) CNOT6L couples the selective degradation of maternal transcripts to meiotic cell cycle progression in mouse oocyte. *EMBO J.* 37, e99333
62. Yu, C. *et al.* (2016) BTG4 is a meiotic cell cycle-coupled maternal–zygotic-transition licensing factor in oocytes. *Nat. Struct. Mol. Biol.* 23, 387–394
63. Wu, D. and Dean, J. (2016) BTG4, a maternal mRNA cleaner. *J. Mol. Cell Biol.* 8, 369–370

64. Liu, Y. *et al.* (2016) BTG4 is a key regulator for maternal mRNA clearance during mouse early embryogenesis. *J. Mol. Cell Biol.* 8, 366–368
65. Lee, K. *et al.* (2024) An extended wave of global mRNA deadenylation sets up a switch in translation regulation across the mammalian oocyte-to-embryo transition. *Cell Rep.* 43, 113710
66. Xiong, Z. *et al.* (2022) Ultrasensitive Ribo-seq reveals translational landscapes during mammalian oocyte-to-embryo transition and pre-implantation development. *Nat. Cell Biol.* 24, 968–980
67. Sha, Q.Q. *et al.* (2019) A story of birth and death: mRNA translation and clearance at the onset of maternal-to-zygotic transition in mammalsdagger. *Biol. Reprod.* 101, 579–590
68. Dai, X.X. *et al.* (2019) A combinatorial code for mRNA 3′-UTR-mediated translational control in the mouse oocyte. *Nucleic Acids Res.* 47, 328–340
69. Xiang, K. *et al.* (2024) Control of poly(A)-tail length and translation in vertebrate oocytes and early embryos. *Dev. Cell* 59, 1058–1074 e11
70. Yang, Y. *et al.* (2017) Maternal mRNAs with distinct 3′ UTRs define the temporal pattern of Ccnb1 synthesis during mouse oocyte meiotic maturation. *Genes Dev.* 31, 1302–1307
71. Takahashi, N. *et al.* (2023) CPEB1-dependent disruption of the mRNA translation program in oocytes during maternal aging. *Nat. Commun.* 14, 416
72. Luong, X.G. *et al.* (2020) Genome-wide analysis reveals a switch in the translational program upon oocyte meiotic resumption. *Nucleic Acids Res.* 48, 3257–3276
73. Liu, Y. *et al.* (2021) Poly(A) tail length is a major regulator of maternal gene expression during the mammalian oocyte-to-embryo transition. *bioRxiv*, Published online August 31, 2021. https://doi.org/10.1101/2021.08.29.458052
74. Park, J.E. *et al.* (2016) Regulation of poly(A) tail and translation during the somatic cell cycle. *Mol. Cell* 62, 462–471
75. Liu, Y. *et al.* (2023) Remodeling of maternal mRNA through poly (A) tail orchestrates human oocyte-to-embryo transition. *Nat. Struct. Mol. Biol.* 30, 200–215
76. Park, J. *et al.* (2023) Short poly(A) tails are protected from deadenylation by the LARP1-PABP complex. *Nat. Struct. Mol. Biol.* 30, 330–338
77. Sement, F.M. *et al.* (2013) Uridylation prevents 3′ trimming of oligoadenylated mRNAs. *Nucleic Acids Res.* 41, 7115–7127
78. Chang, H. *et al.* (2018) Terminal uridylyltransferases execute programmed clearance of maternal transcriptome in vertebrate embryos. *Mol. Cell* 70, 72–82 e77
79. Lim, J. *et al.* (2014) Uridylation by TUT4 and TUT7 marks mRNA for degradation. *Cell* 159, 1365–1376
80. Wu, D. *et al.* (2023) DIS3L2 ribonuclease degrades terminal-uridylated RNA to ensure oocyte maturation and female fertility. *Nucleic Acids Res.* 51, 3078–3093
81. Morgan, M. *et al.* (2017) mRNA 3′ uridylation and poly(A) tail length sculpt the mammalian maternal transcriptome. *Nature* 548, 347–351
82. de Almeida, C. *et al.* (2018) RNA uridylation and decay in plants. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 373, 20180163
83. Zuber, H. *et al.* (2016) Uridylation and PABP cooperate to repair mRNA deadenylated ends in *Arabidopsis*. *Cell Rep.* 14, 2707–2717
84. Liu, Y. *et al.* (2021) Conservation and divergence of poly(A) tail regulation during the mammalian oocyte-to-embryo transition. *bioRxiv*, Published online August 31, 2021. https://doi.org/10.1101/2021.08.29.458065
85. Lim, J. *et al.* (2018) Mixed tailing by TENT4A and TENT4B shields mRNA from rapid deadenylation. *Science* 361, 701–704
86. Lee, Y.S. *et al.* (2024) Deadenylation kinetics of mixed poly (A) tails at single-nucleotide resolution. *Nat. Struct. Mol. Biol.* 31, 826–834
87. Zhao, T. *et al.* (2019) Impact of poly(A)-tail G-content on *Arabidopsis* PAB binding and their role in enhancing translational efficiency. *Genome Biol.* 20, 189
88. Li, C.Y. *et al.* (2022) Cytidine-containing tails robustly enhance and prolong protein production of synthetic mRNA in cell and *in vivo*. *Mol. Ther. Nucleic Acids* 30, 300–310
89. Liu, Y. *et al.* (2021) Enhancement of synthetic mRNA translation efficiency through engineered poly(A) tails. *bioRxiv*, Published online August 31, 2021. https://doi.org/10.1101/2021.08.30.458298
90. Liu, Y. *et al.* (2021) Abundant non-A residues in the poly(A) tail orchestrate the mouse oocyte-to-embryo transition. *bioRxiv*, Published online August 31, 2021. https://doi.org/10.1101/2021.08.29.458077
91. Zhang, C. and Zhang, Y. (2023) Start of life controlled by poly (A) tail-mediated remodeling. *Trends Genet.* 39, 340–341
92. Yamazaki, H. *et al.* (2022) Recycling of uridylated mRNAs in starfish embryos. *bioRxiv*, Published online April 07, 2022. https://doi.org/10.1101/2022.04.07.487435
93. Clegg, K.B. and Piko, L. (1983) Poly(A) length, cytoplasmic adenylation and synthesis of poly(A)+ RNA in early mouse embryos. *Dev. Biol.* 95, 331–341
94. Piko, L. and Clegg, K.B. (1982) Quantitative changes in total RNA, total poly(A), and ribosomes in early mouse embryos. *Dev. Biol.* 89, 362–378
95. Jukam, D. *et al.* (2017) Zygotic genome activation in vertebrates. *Dev. Cell* 42, 316–332
96. Sahin, U. *et al.* (2014) mRNA-based therapeutics – developing a new class of drugs. *Nat. Rev. Drug Discov.* 13, 759–780
97. Kariko, K. (2019) *In vitro*-transcribed mRNA therapeutics: out of the shadows and into the spotlight. *Mol. Ther.* 27, 691–692
98. Baden, L.R. *et al.* (2021) Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N. Engl. J. Med.* 384, 403–416
99. Polack, F.P. *et al.* (2020) Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *N. Engl. J. Med.* 383, 2603–2615
100. Chen, H. *et al.* (2024) Branched chemically modified poly(A) tails enhance the translation capacity of mRNA. *Nat. Biotechnol.*, Published online March 22, 2024. https://doi.org/10.1038/s41587-024-02174-7