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REVIEWS

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# Canonical and Noncanonical RNA Polyadenylation

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**Abstract**—Polyadenylation is the non-template addition of adenosine nucleotides at the 3'-end of RNA, which occurs after transcription and generates a poly(A) tail up to 250–300 nucleotides long. In the first section of our review, we consider the classical process of mRNA 3'-terminus formation, which involves the cleavage of the transcript synthesized by RNA polymerase II and the associated poly(A) tail synthesis by canonical polyadenylate polymerase. Nucleotide sequences needed for mRNA cleavage and poly(A) tail synthesis, in particular the AAUAAA polyadenylation signal, as well as numerous proteins and their complexes involved in mRNA cleavage and polyadenylation, is described in detail. The significance of the poly(A) tail for prolonging mRNA lifetime and stimulating their translation is discussed. Data presented in the second section demonstrate that RNA transcribed by RNA polymerase III from certain SINEs (Short Interspersed Elements) can undergo AAUAAA-dependent polyadenylation. The structural and functional features of RNA polymerase III determine the unusual character of polyadenylation of RNAs synthesized by this enzyme. The history of recent developments in this area of study have been described in greater detail, in particular the discovery of AAUAAA-dependent polyadenylation of RNA synthesized by RNA polymerase III, which has not been discussed previously. Data on AAUAAA-independent polyadenylation catalyzed by noncanonical TRAMP poly(A)-polymerases (Trf4 and Trf5) have been presented in the third section. These enzymes promote rapid degradation of RNAs by adding a short poly(A) tail to them. This mechanism enables the recognition, poly(A)-marking, and elimination of incorrectly folded noncoding transcripts (e.g. ribosomal and transfer RNAs).

*Keywords*: canonical and noncanonical poly(A) polymerases, signals and elements of polyadenylation, protein factors of polyadenylation, RNA polymerases II and III, SINE

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## CANONICAL POLYADENYLATION

Polyadenylation is the non-template addition of adenosine nucleotides at the 3'-end of RNA, which most commonly occurs as posttranscriptional modification. Pre-mRNA synthesized by eukaryotic RNA polymerase II (RNAP II) still cannot serve as an adequate template for protein synthesis. First of all, it should undergo several modifications, the most important of them being capping, splicing, and polyadenylation. Capping is the addition of 7-methylguanosine at the 5'-end of a pre-mRNA molecule, usually accompanied by the methylation of ribose moieties of the first two nucleotides. Splicing involves the excision of noncoding (intron) sequences from the pre-mRNA molecule and the subsequent joining of the remaining RNA fragments (exons). Polyadenylation affects the 3'-end of pre-mRNA, and the resulting structure is termed poly(A) tail. Usually poly(A) tails are 250–300 nt long. Polyadenylation is preceded by cleavage of the pre-mRNA chain, which in fact generates its 3'-end. RNA cleavage and polyadenylation are coupled events; together they are known as 3'-processing [1, 2]. The poly(A) tail protects mRNA from cell nucleases and thus prolongs its lifetime. In addition, polyadenylation increases the efficiency of mRNA transportation from the nucleus into the cytoplasm. Finally, the poly(A) tail plays an important role in mRNA loop formation, which facilitates the repeated translation of mRNA molecules [3].

The poly(A)-containing RNA fraction was first isolated in the early 1970s by treating RNA with nucleases that hydrolyze phosphodiester bonds immediately after guanine (RNase T1) and pyrimidine (RNase A) nucleotides; after this treatment, poly(A) segments remain intact. In the same years, it was shown that these segments are not encoded by the DNA, and the responsible enzyme, poly(A) polymerase (PAP) was discovered [2]. However, it turned

*Abbreviations*: RNAP II and RNAP III, RNA polymerase II and III, respectively; CTD, C-terminal domain; USE, upstream sequence element; DSE, downstream sequence element; PAS, polyadenylation signal; RRM, RNA recognition motif; CPSF, cleavage and polyadenylation specificity factor; CstF, cleavage stimulation factor; CFIm and CFIIm, mammalian cleavage factors Im and IIm; PAP, poly(A) polymerase; SINEs, short interspersed elements; TRAMP, Trf4/Air2/Mtr4p polyadenylation complex.

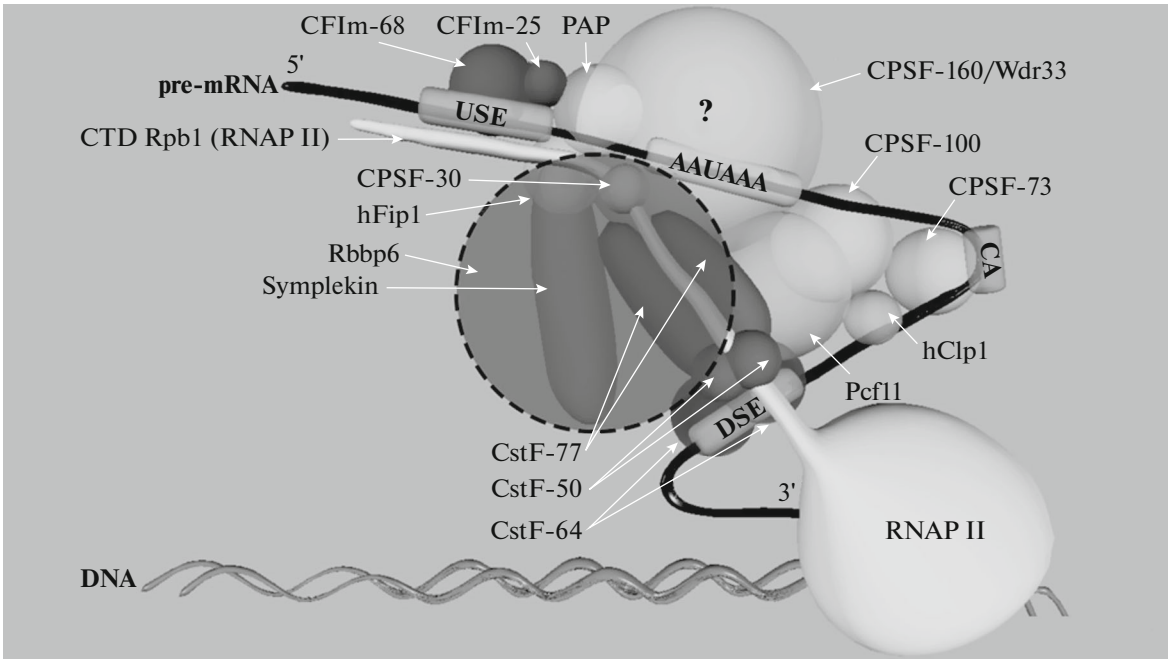


**Fig. 1.** Scheme of the protein complex performing 3'-terminal processing of pre-mRNA. RNAP II synthesizes a transcript, which is cleaved by CPSF-73 at the CA site. Next, PAP performs non-template poly(A) synthesis at the resulting 3'-end of the pre-mRNA. 3'-terminal processing depends on the polyadenylation signal (AAUAAA), as well as on DSE and/or USE. Proteins participating in 3'-terminal processing are shown as spherical or elongated structures and indicated with arrows. Further explanations are provided in the text. Scheme is based on data from [1, 8, 9, 13].

out that pre-mRNA polyadenylation depends not only on PAP itself, but also requires a number of auxiliary protein factors, as well as certain nucleotide sequences in pre-mRNA that should be recognized by these factors.

The most important of these sequences is the polyadenylation signal (PAS), which is a hexamer, most commonly AAUAAA, located 20–30 nt upstream from the 3'-end of mRNA [4]. The hexamer consensus sequence is NNUANA; its variants AAUAAA, A(U/G)UAAA, or UAUAAA are found in 79% of mRNAs with functional hexamers. Not infrequently, a single pre-mRNA contains several rare PAS variants [5]; this was observed in approximately 54% of human genes and 32% of mouse genes. The presence of several, not necessarily canonical, PASs within a single transcript provides a possibility to regulate the length of the resulting mRNA; the choice of the proximal PAS results in a shorter 3'-untranslated region (3'-UTR). These mRNA isoforms can differ in stability, translation rates, and intracellular localization because their 3'-UTRs can contain different regulatory sequences, such as binding sites for microRNAs and regulatory proteins. Thus, an mRNA with a shorter 3'-UTR can sustain a higher level of protein synthesis. These mRNA forms are typical for many actively proliferating cells, including tumor cells [6–9]. It should be mentioned that 12% of polyadenylated RNAs do not seem to contain any known PAS [5].

Apart from the hexamer, the 3'-end can contain two further regions responsible for 3'-processing. The one lying downstream from the polyadenylation site is called a *downstream sequence element* (DSE); it is either a GU-rich sequence (YGUGUUYY) or an oligo-U stretch (UUUUU) [1, 10]. Upstream from PAS, there might be an upstream sequence element (USE); in mammals, a USE is often represented by two (or more) UGUAN pentamers (N = A > U ≥ C/G). In the absence of a canonical PAS, the key role in the assembly of the 3'-processing complex can belong to a USE [2, 11]. A cleavage site, commonly a CA dinucleotide, lies between a DSE and a PAS (within 11–23 nt from PAS and 10–30 nt upstream of DSE) and can affect the cleavage efficacy [12]. The position of the cleavage site is often loosely defined, which can help regulate the transcript length [6]. All of these sequences serve as binding sites for various proteins that act as 3'-processing factors (Fig. 1). In mammals, at least 14 of these proteins have been identified, although 3'-processing complexes can include much more proteins, i.e., up to 85 of them. Importantly, the protein complex that performs the 3' processing of pre-mRNA is assembled in the course of transcription at the C-terminal domain (CTD) of the largest RNAP II subunit [1].

A key factor of 3'-processing is the cleavage and polyadenylation specificity factor (CPSF), which recognizes PAS, while the second most important protein

of the complex, cleavage stimulation factor (CstF), enhances their interaction (Fig. 1). Along with PAS, these proteins recruit all other 3'-processing factors [1]. CPSF comprises seven components, i.e., CPSF100, CPSF73, and symplekin form its core, while Fip1, CPSF160, Wdr33, and CPSF30 lie on the periphery [13]. It was long believed that the largest subunit, CPSF160, is responsible for PAS recognition, even though it does not contain a canonical RNA recognition motif (RRM), similar to the other components [14]. However, it was recently shown that UV crosslinks cannot be generated between CPSF160 and AAUAAA. In contrast, CPSF160, as well as CPSF73 and CPSF30, can be crosslinked to upstream 3'-UTR regions [8]. Experimental evidence suggests that Wdr-33 and CPSF30 can interact with PAS, while complementing each other [9]. Wdr33 is a fairly large subunit comparable in size to CPSF160. It still cannot be ruled out that CPSF160 is responsible for the hexamer binding in a subset of PAS variants, while Wdr33 replaces it in other variants [9]. CPSF73 contains a β-CASP domain typical for endonucleases. CPSF73 most likely cleaves pre-mRNA, although its activity is low in the absence of other components of the complex [15]. CStF is composed on three subunits: CstF77, CstF50, and CstF64, and most likely functions as a dimer. CstF77 is responsible for dimerization and interaction with CPSF160 and also serves as a platform for two other subunits [16]. CstF64 possesses an RRM and is required to bind to DSE, but it is only in the presence of CPSF, which exhibits the necessary affinity level. Apparently, this is how the presence of PAS and DSE at the 3'-end, as well as the adequate distance between them, is assured. CstF50 is responsible for interaction with the CTD of RNAP II and can also bind symplekin and Fip1 [14, 17].

Two further important agents of 3' processing are cleavage factors Im and IIm (CFIm and CFIIm; Fig. 1). CFIm is composed of two subunits, i.e., a large one that exists in three variants, a 68-kDa form that is the most common, and a small one of 25 kDa; the protein functions as a heterotetramer composed of two large and two small subunits [18, 19]. Although the large subunit contains an RRM, the small subunit is responsible for RNA binding, as it contains a damaged Nudix hydrolase domain that has acquired the ability to bind RNA. CFIm targets are UGUAN-containing USEs. If two such elements are present at a certain distance from each other, CFIm can loop out the interspacing RNA fragment, which probably represents the basic mechanism of selection among alternative polyadenylation sites [20]. CFIm exhibits a higher affinity to distal PASs, as shown in CFIm-68 knockdown experiments. Its expression is commonly downregulated in tumor cells; this phenomenon is associated with enhanced tumor invasiveness [8]. CFIm also plays an important role in the formation of the 3' processing complex and can independently recruit PAP. A further important property of CFIm is its involvement in exporting mRNA from the nucleus (demonstrated for CFIm-68) [8]. CFIIm is composed of two subunits, Pcf11 and hClp1 [14]. Pcf11 can bind to CTD of RNAP II, which forms a bridge between CTD and the transcript and induces the disassembly of the elongation complex; i.e., Pcf11 is responsible for the termination of transcription, which was confirmed by mutation analysis [21]. Another recently identified protein of the 3' processing complex is Rbbp6. It acts in association with CstF and is also involved in the polyadenylation site selection, especially in pre-mRNAs with AU-rich 3'-UTRs. Rbbp6 knockdown downregulates the production of proteins encoded by mRNAs with such 3'-UTRs, as well as promotes the selection of more distal PASs in pre-mRNAs [9].

PAP is composed of only one subunit; its catalytic domain is located in the N-terminal part, while the C-terminal part contains regulatory elements. For example, hyperphosphorylation of the C-terminal PAP domain represents one of the principal ways of arresting protein synthesis in mitosis. In the middle part of the molecule, there is an RNA-binding domain, which, however, interacts with RNA nonspecifically. The correct functioning of the enzyme within the 3'-processing complex crucially depends on CPSF and CFIm. When the poly(A) tail synthesized by PAP reaches a certain length, it binds to the poly(A)-binding protein, PABPN1, which results in a considerable increase in PAP activity. PABPN1 is also responsible for the termination of poly(A) tail synthesis and participates in the mRNA export from the nucleus [14]. In the cytoplasm, the poly(A) tail binds PABPC1, which interacts with eIF4G elongation factor and some other proteins in the course of translation and serves for mRNA loop formation [22].

Although 3'-processing factors are important for maintaining the complex structure, the central role belongs to CTD of the largest RNAP II subunit (Rpb1). CTD contains 52 seven-residue repeats with a YSPTSPS consensus sequence; its amino acid phosphorylation profile enables it to interact with different proteins involved in transcription [23]. CPSF (CPSF160) and CstF (CstF50 and 64) bind to CTD in the beginning of transcription (at the capping stage) [24, 25]; some data suggest that CPSF is recruited by TFIID. CFIm also binds to CTD before the end of elongation [24–26]. The role of CFIIm in CTD binding was discussed above; this interaction is mediated by the first serine residue of YSPTSPS [21]. Thus, immediately before 3'-processing, CTD of the larger RNAP II subunit is already associated with CPSF, CstF, and CFIm. In the course of transcript synthesis, these three factors detect their target sequences in the RNA molecule and bind to them. Next, they recruit CFIIm and PAP, and then CPSF73 cleaves the transcript [14, 25]. Apparently, by the moment when polyadenylation begins, all 3' processing factors, except for CPSF and PAP have already dissociated from the complex. PAP slowly synthesizes a poly(A)

fragment of 11–15 b, which can then bind PABP. While the poly(A) tail grows to 200–300 b, it forms a loop structure at the end, along with the associated PAP and PABP molecules. PABP and PAP interact with CPSF, arresting the synthesis of the poly (A) tail. At the same time, the 3'-fragment detached from the former pre-mRNA is degraded by Xrn2 exonuclease recruited by PSF, which is most likely also associated with CTD of RNAP II [3, 27]. The 3'-processing complex is ultimately disassembled by the factors responsible for mRNA export from the nucleus; some data suggest that CFIm68 may also be involved in the export [28].

Another process coupled with 3'-processing is splicing. In mammalian genes, the last exon is much longer than the others and undergoes splicing in a considerably different manner. One of the splicing factors, U2AF65, can recruit CFIm59/25. It is known that U2AF65 can stimulate transcript polyadenylation in a CPSF-independent way, and CFIm59/25 most likely mediates this process. This interaction can be especially important in the case of weak polyadenylation sites. The above mechanism is much less efficient when it involves the most common CFIm68/25 form [29]. It is known that 3'-processing is stimulated by CPSF interaction with U2 small nuclear RNA, one of the spliceosome components [14].

The ubiquitous pre-mRNA polyadenylation in the nucleus is not the only example of RNAP II transcript polyadenylation. In some cases, an essentially different polyadenylation apparatus acts in the cytoplasm. An mRNA molecule that has been subjected to all standard posttranscriptional modifications in the nucleus can be deadenylated after export. The corresponding enzyme, PARN 3'-exonuclease, leaves but a small part of the poly(A) tail [30]. These mRNA are stored by the cell and remain in the inactive, dormant state, but can be mobilized at a particular moment. This process is limited to certain cell types; it was first described in oocytes of *Xenopus laevis*. It is mainly typical for oocytes and early embryos of different animals, ranging from insects to mammals. For cytoplasmic polyadenylation, mRNA should possess a PAS that binds CPSF, as occurs during nuclear polyadenylation. The principal sequence characteristic of thee mRNAs is the cytoplasmic polyadenylation element (CPE); there is at least one auxiliary sequence that interacts with poly(C)-binding protein PCBP2. CPEs are recognized by CPE-binding proteins (CPEBs), which possess a zinc finger domain and RRM domains. The target sequence of the most important one, CPEB1, is UUUUAU or UUUUAAU. CPEB proteins regulate the whole process of cytoplasmic polyadenylation; in particular, they mediate the function of the specific PAPD4 (GLD2) polymerase. CPEBs can bind target mRNAs already in the nucleus. If the N-terminal domain of CPEB is not phosphorylated, the protein maintains the deadenylated mRNA state; otherwise, it initiates cytoplasmic polyadenylation, which means that this mRNA can be translated. The functioning of phosphorylated CPEB is supported by PUM2 (Pumilio) protein, which interacts with its own binding site in mRNA, UGUANUAU, and is especially important for noncanonical CPEs [22, 31]. After deadenylation, PARN exonuclease remains bound to mRNA, and so are CPEB, CPSF, and the maskin proteins that inhibit translation. Symplekin and CPSF act as scaffolding proteins supporting all these factors. In response to CPEB phosphorylation, PAPD4 is recruited from the cytoplasm, while deadenylase is excluded from the complex, enabling polyadenylation [32].

Some mRNAs that do not possess CPEs can be polyadenylated in the cytoplasm by a less studied system, i.e., MSI-binding elements (MBEs) in mRNA and Musashi proteins (MSI1 and MSI2) that interact with them. This process is characteristic of early embryogenesis [33]. About 10% of somatic cell mRNAs can also undergo cytoplasmic polyadenylation. It was shown that the functioning and even regulation of CPEB1 and PAPD4 in oocytes and dendrites are similar; in dendrites, they are probably involved in synapse remodeling in learning. It is also possible that the central role here belongs to other CPEB proteins; for instance, in mice, this process involves CPEB3. It is also known that CPEB1 can participate in important processes, such as regulation of mitosis, cell aging, and carcinogenesis. For example, it was shown that CPEB1 knockdown arrests fibroblast aging, while CPEB1 itself interacts with p53 [22].

## AAUAAA-DEPENDENT POLYADENYLATION OF TRANSCRIPTS SYNTHESIZED BY RNA POLYMERASE III

RNAP III is one of three principal eukaryotic RNA polymerases [34] and serves in the transcription of short RNAs from genes that do not encode proteins. It should be noted that, in addition to mRNAs, RNAP II also synthesizes different short noncoding RNAs. RNAP III synthesizes numerous tRNAs, 5S rRNA, U6, 7SL, 7SK, Y, vt (vault RNA), as well as viral RNAs (VA1, EBER1, and EBER2) [35]. These RNAs play an important role in protein synthesis, as well as in transcription, splicing, and replication. None of them undergo classical polyadenylation, which is not at all surprising. Indeed, the structure and regulation of genes transcribed by RNAP II and RNAP III differ significantly. In particular, in the latter group, the promoter is usually located not before, but within the gene [34, 36]. For example, tRNA gene promoters are composed of two 11-bp-long regions, box A, located 10–12 bp downstream from the transcription start site, and box B, located 33–35 bp downstream from box A [36]. Only two factors, TFIIIB and TFIIIC, are required for RNAP III to associate with a tRNA gene and initiate its transcription [34, 36]. RNAP III-dependent transcription is terminated at a block of five or more T res-

idues, and the enzyme dissociates from the gene after transcribing the first two or three [37]. In other words, producing the 3'-end of a primary RNAP III transcript (in contrast to RNAP II transcripts) does not depend on the presence of the AATAAA hexamer, DSE, or USE, and does not in any way involve RNA cleavage. Finally, RNAP III lacks a domain that is structurally and functionally similar to CTD of the large RNAP II subunit, which recruits CPSF into the polyadenylation complex and thus determines the functioning of the polyadenylation machinery. Therefore, it is unclear how RNAP III could initiate the polyadenylation of its transcripts in the absence of such a structure as CTD.

Many of the above facts were still unknown in mid-1980s. At the time, two research group independently developed similar constructs composed of the promoter of the RNAP III-dependent adenoviral $VA_I$ RNA gene and the herpesvirus thymidine kinase gene, which lacks its own RNAP II promoter [38, 39]. It was expected that this chimeric construct, which includes the thymidine kinase gene, would be transcribed by RNAP III. Lewis and Manley [38] asserted that many of the resulting transcripts were cleaved at the expected mRNA cleavage site and correctly polyadenylated. However, Sisodia et al. [39] reached the opposite conclusion; the mRNA-like RNA transcribed by RNAP III was not polyadenylated, even though it carried all the signal elements necessary and sufficient for its 3′-cleavage and polyadenylation in the case of RNAP II transcription. Apparently, this conclusion was correct; indirect evidence suggests that the polyadenylation reported in [38] was observed for RNA transcribed from random (hidden) RNAP II promoters [39]. Another similar study was performed in 1995; the gene that encodes HIV-1 protein Tat, which lacks its own RNAP II promoter, was inserted into *VAI* downstream from its RNAP III promoter [40]. It was clearly shown that RNA transcribed by RNAP III from this chimeric gene was neither capped nor polyadenylated. Thus, most data indicate that RNAs normally synthesized by RNAP II cannot be polyadenylated when they are transcribed by RNAP III.

In the early 1980s, one of the authors (D.K.) participated in the discovery of a special class of DNA repeat sequences [41–44], called *short interspersed elements* (SINEs) [45]. It was found that the mouse genome contains a great number ($(1–5) \times 10^5$) of SINEs that represent two families, B1 and B2 [41, 46]. The human genome contains up to $1.1 \times 10^6$ of Alu SINEs [47]. Later it became clear that the overwhelming majority of multicellular organisms possess SINEs [48]. Depending on the moment of their evolutionary origin, different SINE families are characteristic of different groups of related species, e.g., B2 elements are only found in four rodent families and Alu repeats are present in all primates. SINE copies of the same family are not identical; usually, their sequences exhibit 75–95% homology. SINEs of most families are 150–300 bp long [48]. SINEs are nonautonomous mobile genetic elements that proliferate in the genome due to activity of the reverse transcriptase encoded by another type of mobile elements, long interspersed elements (LINEs) [49, 50]. Most SINE families originate from different tRNAs [48, 51], but there are also those that evolved from 7SL RNAs, e.g., B1 and Alu [48, 52]. The third small class of SINEs is related to 5S rRNA [48, 53]. In all cases, sequences derived from small RNAs are located in the head part of SINEs, which also contains the RNAP III promoter [48, 54]. The promoter of SINEs related to tRNAs and 7SL RNAs is composed of boxes A and B described in the beginning of this section. The head is followed by the SINE body and, finally, by the tail part. In most SINE families, body sequences show no similarity and their origin is unknown. In great many SINEs, the tail is an A-rich sequence, i.e., it is strongly predominated by adenosine bases. This is especially typical for placental mammals, where most SINEs proliferate using reverse transcriptase encoded by LINE-1) [48, 50, 55]; in other SINE families, tails are composed of 3–5-bp-log tandem repeats [48, 49].

In the 1980s, the research group of G. P. Georgiev discovered that mouse tumor cells contain large amounts of poly(A)-carrying RNAs 200–500 nt long transcribed from B2 SINE by RNAP III [56–60]. Similar observations were reported by other authors [61]. It was found that the A-rich tail of B2 usually contains one to four AATAAA hexamers and carries the TCTTTT transcription termination signal at the end. It was shown that RNAP III begins the transcription from the first B2 nucleotide and stops at the terminator, which produces RNA of approximately 180 b. The presence of AAUAAA hexamer(s) near the 3'-end of such B2 RNAs suggested that they could be polyadenylated, even though they are synthesized by RNAP III, not by RNAP II. This notion is well supported by the following facts: (1) B2 RNA transcription was stable in cells growing in medium containing 50 μg/mL α-amanitin, an RNAP II inhibitor; (2) B2 RNAs detected in experiments on cDNA cloning and sequencing possessed poly(A) tails; (3) B2 RNAs were heterogeneous in length due to variations in the length of the poly(A) tail, which is presumably synthesized by PAP.

These data were probably not totally convincing because the experiments were performed on specimens of total cell RNA that contained numerous B2 copies. Some of these B2 copies had deficient (truncated or mutated) terminators, and were transcribed far beyond the end of the B2 element. For the same reason, the resulting B2 RNAs could be heterogeneous in length. Furthermore, elongated B2 RNAs usually contain a pure poly (A) stretch 15–20 nt long following the A-rich tail; this stretch is an element of the SINE itself and is produced by transcription. For all these reasons, it was not easy to provide an unambiguous interpretation of the observations. At any rate,

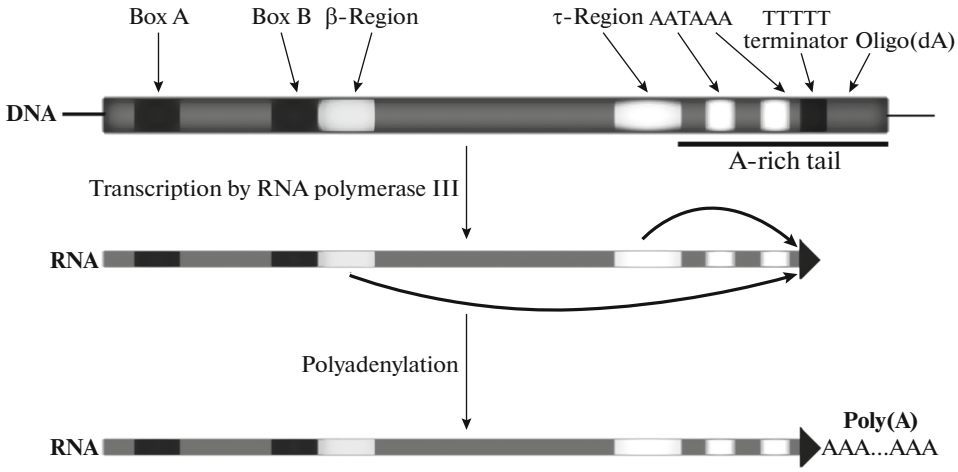


**Fig. 2.** Schematic structure of class T+ SINEs, their transcription by RNAP III, and polyadenylation of the resulting RNA. Curved arrows show stimulating effect of beta and tau regions of transcript polyadenylation. See text for explanations.

the fact that some RNAP III-synthesized transcripts can undergo AAUAAA-dependent polyadenylation was not recognized at that moment.

In the early 2000s, the authors (O.B. and D.K) isolated a series of tRNA-related SINEs from the genomes of different placental mammals [62–65]. By analyzing their nucleotide sequences, together with the sequences of previously described SINEs of the same phylogenetic group, we concluded that all SINEs with A-rich tails can be divided in two groups based on the structural organization of the tail [64]. SINEs of the T+ class possess A-rich tails that feature the following traits:

(1) one or several AATAAA hexamers;

(2) a terminator of RNAP III-dependent transcription (TCTTTT or TTTTT);

(3) a poly $(A)_{10–25}$ stretch at the very end of the SINE (Fig. 2).

The B2 SINE described above represents this class. It should be noted that, in some genomic SINE copies AATAAA hexamers and/or transcription terminators are damaged by mutations; for this reason, the identification of the T+ class was not straightforward. On the other hand, SINEs whose A-rich tails and do not contain AATAAA hexamers and RNAP III terminators at all were grouped into the T– class. RNAs transcribed by RNAP III from such SINEs are not supposed to be polyadenylated because they lack AAUAAA hexamers. Apart from that, the 3'-ends of the produced RNAs might be located rather far downstream from the SINE ends because, in these cases, transcription is terminated at TTTTT blocks that occur in sequences adjacent to SINEs.

At the next stage, polyadenylation of RNAs transcribed by RNAP III was studied using an approach designed for analyzing individual SINE copies [66]. Genomic copies of SINE were cloned in a plasmid vector and introduced into HeLa cells by transitory transfection. In 24 h, total cell RNA was isolated, separated by electrophoresis in a denaturing polyacryamide gel (in early experiments, in an agarose gel), and subjected to Northern hybridization with a $^{32}$P-labeled DNA chain of the target SINE as the probe. SINE RNA was detected by X-ray imaging (qualitatively) and phosphorimaging (quantitatively). Polyadenylated RNAs were recognized as heterogeneous material elongated by 20–300 nt compared to the SINE RNA band. These experiments were first performed with a B2 copy, and it was shown that all RNA detected was transcribed by RNAP III and that elongated B2 RNA possessed poly(A) tails [66]. A C-for-T substitution in both AATAAA hexamers present in this B2 copy resulted in the complete loss of elongated RNA forms, which proved that polyadenylation of B2 RNA was AAUAAA-dependent [66]. Similar experiments were performed with eight different SINEs of the T+ class and showed that all RNAP III-transcribed RNAs could undergo AAUAAA-dependent polyadenylation [67].

Since RNAs transcribed by RNAP III from T– SINEs cannot be polyadenylated a priori, seven of them were modified as follows [67, 68]: AATAAA hexamers were inserted into their A-rich tails and a TTTTTT transcription terminator was added at their end. The structure of T– SINEs modified in this way appears similar to the structure of T+ SINEs, and their RNAP III-transcripts could be expected to undergo polyadenylation. However, this idea has in fact been proved wrong. In HeLa cells transfected with modified T– SINEs, their transcripts were not polyadenylated [67]. This result indicated that, in addition to AATAAA, T+ SINEs contain some additional sequences that are required for polyadenylation and absent in T– SINEs. Using different deletion and substitution derivatives of three T+ SINEs (B2 of the mouse, Dip of the jerboa, and Ves of the bat), we established that, along with the A-rich tail,

these SINEs contain two regions important for polyadenylation of their transcripts [67] (Fig. 2). One of them, denoted beta, is located immediately downstream from the promoter box B (and possibly even overlaps with it). Its estimated length is 15–20 bp. The other region, called *tau*, is located upstream from the A-rich tail carrying polyadenylation signals (Fig. 2). In many (7 of 13) T+ SINEs, including Dip and Ves, this region is composed nearly exclusively of C and T residues, i.e., it is a polypyrimidine stretch 30–50 nt long [48, 64, 67]. In B2, the tau region is not enriched in pyrimidines and does not exceed 18 b. Deletion or substitution of beta or tau regions decreased the efficiency of polyadenylation of RNAP III-transcribed SINE RNAs, and the poly(A) tail synthesis was completely arrested if both of these regions were inactivated [67]. Presumably, beta and tau regions of SINE transcripts bind some protein complexes that activate the polyadenylation machinery associated with the AAUAAA hexamer [67]. The beta and tau regions of SINE RNA can probably be compared to USEs, auxiliary elements of pre-mRNA located upstream from polyadenylation signals. Our working hypothesis is that some beta and tau regions bind the CFIm protein complex, which is known to interact with USEs of pre-mRNA and induce its cleavage and polyadenylation. Our preliminary data (Ustyantsev) suggest that there is a specific polypyrimidine tract-binding protein (PTB) that binds to the tau region and seems to be involved in the polyadenylation of SINE transcripts synthesized by RNAP III. Interestingly, PTB has never been identified as a component of the mRNA polyadenylation complex. Most likely, although polyadenylation of RNAP III SINE transcripts is AAUAAA-dependent, its mechanism differs significantly from the mechanism of mRNA polyadenylation.

The fact that T+ SINEs have appeared independently in different branches of the mammalian phylogenetic tree [69] suggests that the ability of RNAP III transcripts of SINEs to undergo polyadenylation developed in the course of parallel evolution. Finally, we should notice that the presence of a poly(A) tail at the end of SINE transcripts prolongs the lifetime of these RNAs [66] and facilitates their reverse transcription, which produces new genomic copies of SINE [55]. It is possible that polyadenylated RNAs transcribed by RNAP III from SINEs will later be found to perform some other cell functions.

## NONCANONICAL POLY(A) POLYMERASES AND POLY(A)-MEDIATED RNA DEGRADATION

Non-template addition of adenosine nucleotides can not only prolong the lifetime of eukaryotic RNAs, but also promote their degradation. This phenomenon was not discovered until in 2000, when van Hoof et al. showed that yeast cells carrying mutations in the nuclear exosome, a complex responsible for degradation of defective or unwanted RNAs, accumulate polyadenylated forms of certain small RNAs, in particular, nuclear U4 RNA and small nucleolar RNAs [70]. Later studies revealed polyadenylation of other RNA substrates and identified a polyadenylating enzyme Trf4p, mutations of which led to an increase in the lifetime of certain tRNAs [71]. Trf4p is a noncanonical PAP; it belongs to the family of beta-type nucleotidyl transferases, which also includes the enzymes attaching CCA at the 3'-end of tRNAs and the cytoplasmic Gld2 PAP mentioned above [72]. It was found that Trf4p is a component of a protein complex that pretreats defective RNAs destined for degradation in the exosome. This complex called TRAMP as an abbreviation of its three components, Trf4p PAP, Air2p RNA-binding protein, and Mtr4p helicase [73]. TRAMP substrates are incorrectly folded tRNAs, small nucleolar RNAs, and small nuclear RNAs, such as U4, U14, and U6, 5S rRNAs, cryptic unstable transcripts (CUTs) [74], as well as large rRNAs and their precursors, i.e., 25S rRNAs and 27S pre-RNAs [75]. Moreover, TRAMP can be recruited to pre-mRNA in the course of transcription where it stimulates splicing by interacting directly with splicing factors, then delivers the excised introns to the exosome, but this pathway does not involve polyadenylating activity [76, 77]. Within the TRAMP complex, Trf4p can be substituted by its Trf5p paralog, and some evidence suggests that this variant of the complex recognizes a somewhat different set of target RNAs, although most of them are treated by Trf4p-containing TRAMP [76]. It has been shown that TRAMP performs the oligo-, rather than polyadenylation of target RNAs and, at least in some cases, adds only three or four adenosine residues [78]. It was also found that Trf4p and the zinc knuckle protein Air2p form the backbone of the complex, while Mtr4p is responsible for the transportation of oligoadenylated RNAs to the exosome. Mtr4p also participates in many other pathways of exosome degradation, not only in the TRAMP-mediated one. According to the recently proposed mechanism of TRAMP functioning (Fig. 3), target RNA is first recognized by Mtr4p and Air2p, then its 3'-end enters the channel within Mtr4p and is simultaneously unwound, thus approaching the active center of Trf4p, where oligoadenylation occurs, and finally the RNA is transported to the exosome [74].

Human cells were found to contain two protein complexes similar to TRAMP. They have a common component, hMtr4p, which is homologous to the yeast protein, but their location and composition are different. The so-called nuclear exosome targeting complex (NEXT) complex is located in the nucleus outside the nucleoli and contains, in addition to hMtr4p, the zinc knuckle protein ZCCHC8 and the RRM-containing protein RBM7. The substrates it delivers to the exosome are mainly promoter upstream transcripts (PROMPT), analogs of yeast CUTs; importantly, NEXT does not adenylate them [79, 80].

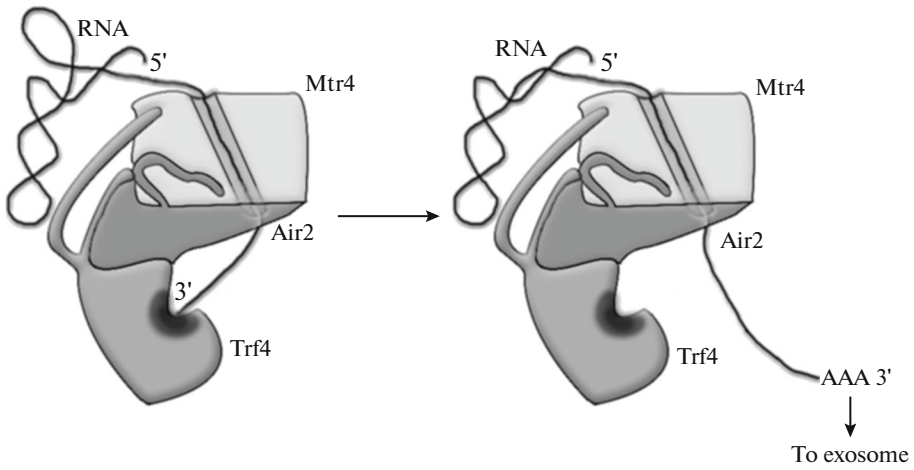


**Fig. 3.** Scheme of yeast TRAMP complex that recognizes and binds RNAs with incorrect structure. This RNA is oligoadenylated by the Trf4 subunit, which stimulates its degradation by the exosome.

The nucleoli contain a different protein complex, hTRAMP, which is more similar to TRAMP; along with hMtr4p, it comprises PAPD5 (hTRF4-2) and ZCCHC7 (hAIR2). This complex exhibits polyadenylating activity and mediates the degradation of incorrectly folded pre-mRNAs in the exosome. It was also shown that PAPD5 knockdown causes an increase in the concentration of carcinogenic miR-21 microRNA [79, 81].

It was revealed relatively recently that polyadenylation was implicated in RNA degradation and not stabilization in eukaryotes; however, it has long been known that in prokaryotes polyadenylation serves to mark degrading RNAs. In particular, *Escherichia coli* cells contain PAPI and polynucleotide phosphorylase (PNPase) adding a random nucleotide sequence only a half of which are adenosines. Poly(A) tails synthesized by PAPI are relatively short, usually 15–30 nt long, and their length may depend on the RNA type. In *E. coli*, both coding RNAs (usually with Rho-independent terminators) and noncoding RNAs (at least 2% of total RNA), especially defective ones, can undergo polyadenylation and, in 90% of cases, this reaction is mediated by PAPI. In contrast to canonical polyadenylation in eukaryotic cells, it leads to a more rapid RNA degradation. A poly(A) tail of prokaryotic RNA can be recognized by the RNase E component of the degradosome, a complex functionally similar to the exosome, and possibly by RNase R. A significant increase in PAPI concentration is toxic for bacterial cells, either because of degradation of vital RNAs, or due to ATP overconsumption. Interestingly, mitochondria possess their own PAPs, and mitochondrial RNAs have poly(A) tails up to 40–60 nt long. The most likely also mark RNA molecules that should be degraded [82].

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