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Alternative polyadenylation of mRNA precursors

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Abstract

Alternative polyadenylation (APA) is an RNA-processing mechanism that generates distinct 3' termini on mRNAs and other RNA polymerase II transcripts. It is widespread across all eukaryotic species and is recognized as a major mechanism of gene regulation. APA exhibits tissue specificity and is important for cell proliferation and differentiation. In this Review, we discuss the roles of APA in diverse cellular processes, including mRNA metabolism, protein diversification and protein localization, and more generally in gene regulation. We also discuss the molecular mechanisms underlying APA, such as variation in the concentration of core processing factors and RNA-binding proteins, as well as transcription-based regulation.

The transcriptome of eukaryotic cells is produced by three RNA polymerases, each with its own mechanisms for the maturation of the 3' ends of nascent transcripts (reviewed in REF. 1). Protein-coding transcripts, or mRNAs, are transcribed by RNA polymerase II (Pol II). With the exception of the canonical, replication-dependent transcripts encoding histones in metazoans², the maturation of mRNA 3' ends involves endonucleolytic cleavage of the nascent RNA followed by synthesis of a poly(A) tail on the 3' terminus of the cleaved product by a poly(A) polymerase (PAP). These two coupled reactions, collectively referred to as cleavage and polyadenylation or, simply, polyadenylation, are intimately linked to transcription termination¹. Polyadenylation also occurs for some other Pol II products, especially long non-coding RNAs (lncRNAs; non-coding transcripts of ~200 nt or longer).

The sequences in the mRNA precursor and the proteins required for polyadenylation are now well understood. The polyadenylation site, also known as the poly(A) site (PAS), is defined by surrounding RNA sequence elements (BOX 1), which are generally conserved across metazoans with some minor variations (BOX 1 and Supplementary information S1 (box)). However, major distinctions can be found in yeast and plant PASs³ (Supplementary information S1 (box)). Notably, the key protein factors responsible for polyadenylation are conserved throughout eukaryotes, although the machinery in mammals, which comprises more than 20 core proteins (BOX 1), has differences in protein composition and subcomplex organization compared with the machinery in yeast^{4–7}.

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It was first reported more than three decades ago that a gene can give rise to transcripts with multiple PASs and that differential usage of these sites can lead to the formation of distinct mRNA isoforms, a phenomenon termed alternative polyadenylation (APA; early studies were reviewed in REFS 8,9). From early studies using expressed sequence tags^{10,11} and more recent analyses using high-throughput sequencing, we know that APA is very common and occurs most frequently in the 3' untranslated region (3' UTR) of mRNAs, and that it is used frequently in essentially all eukaryotes, from yeast to humans. For example, at least 70% of mammalian mRNA-encoding genes express APA isoforms^{12,13}. Substantial, albeit slightly lower, APA frequencies have been reported in simpler species (Supplementary information S1 (box)). In this Review, we discuss our current understanding of APA from genomic as well as molecular and cellular perspectives, focusing mostly on the mechanisms and consequences of APA in metazoans. Readers are referred to other reviews for discussions of some early studies and of work in other species^{6,14–19}.

APA in 3' UTRs

Most APA sites are located in 3' UTRs. In line with the nomenclature used for alternative splicing, here we refer to the 3' UTR portion upstream of the first, or proximal, PAS as the constitutive UTR (cUTR) and the portion downstream as the alternative UTR (aUTR) (FIG. 1a). APA occurring in the 3' UTR, referred to hereafter as 3' UTR-APA, gives rise to mRNA isoforms with significantly different 3' UTR lengths. For example, for mouse transcripts, the median 3' UTR lengths of shortest and longest APA isoforms differ about sevenfold, at 249 nt and 1,773 nt, respectively¹³. As 3' UTRs contain *cis* elements that are involved in various aspects of mRNA metabolism, 3' UTR-APA can considerably affect post-transcriptional gene regulation in various ways, including through the modulation of mRNA stability, translation, nuclear export and cellular localization, and even through effects on the localization of the encoded protein (FIG. 1b–d). One remarkable feature of 3' UTR-APA is that it can be regulated globally, simultaneously involving numerous transcripts in a cell. This was first shown for different human tissues that display a biased preference for certain APA isoform types (BOX 2) and was later demonstrated in studies of proliferation- and differentiation-based changes in APA profiles (BOX 3).

mRNA stability and translation

Perhaps the best studied consequence of 3' UTR-APA is its effect on microRNA (miRNA) functions. miRNAs are small RNAs (~22 nt) that modulate the stability and/or translation of their target complementary mRNAs²⁰. miRNA target sites are generally located in 3' UTRs. In mammals, more than half of the conserved miRNA target sites are located in aUTRs^{21,22}. Differential targeting of 3' UTR-APA isoforms was first demonstrated in activated T cells and cancer cells, both of which display global 3' UTR shortening compared with non-activated T cells and non-transformed cells, respectively^{21,23}. A recent study showed that APA isoform expression influences about 10% of targeting by miRNAs between any two cell types analysed and, importantly, that the accuracy of target prediction can be improved if the cellular APA profile is considered²⁴. Targeting by miRNAs is often influenced by target site location in the mRNA and by the surrounding sequences²⁰. For example, target sites located near either end of a 3' UTR tend to be more efficient than sites in the middle.

Consistent with this, target sites for certain pro-proliferation miRNAs are enriched in the region immediately upstream of the proximal PASs of pro-differentiation or anti-proliferation mRNAs; the shortening of 3' UTRs during cell proliferation improves the targeting context for these miRNAs and can thus enhance their targeting efficiency and their promotion of cell proliferation²⁵.

3' UTRs are also hotbeds for mRNA destabilization elements, which often function through RNA-binding proteins (RBPs). Well-characterized motifs include AU-rich elements (AREs), GU-rich elements (GREs) and PUF protein-binding elements²⁶. As with miRNA target sites, inclusion or exclusion of these elements by 3' UTR-APA can affect mRNA stability. For example, a genetic polymorphism leading to differential expression of two APA isoforms of human IFN-regulatory factor 5 (IRF5) is linked to the risk of developing systemic lupus erythematosus²⁷ (FIG. 1b). Because of the presence of an ARE in the aUTR, the two isoforms have different decay rates²⁷. In addition, RNA–RNA interactions, such as base pairing between 3' UTR-encoded Alu elements (which are the most abundant transposable elements in the human genome) and lncRNAs can lead to mRNA decay through STAU1-mediated mRNA decay²⁸. Moreover, a long 3' UTR is itself considered to be a feature that causes mRNA degradation through nonsense-mediated mRNA decay²⁹. It is therefore generally believed that, owing to their tendency to harbour destabilizing elements and their sheer size, isoforms with long 3' UTRs are less stable than short isoforms. However, this view has been challenged by a genome-wide study of the role of APA in mRNA decay in mouse cells. Using the transcription inhibitor actinomycin D (ActD) to measure mRNA stability, long isoforms were found to be only slightly less stable than short isoforms³⁰. Possible ActD-related artefacts notwithstanding, this suggests that the fate of 3' UTR-APA isoforms is more complex than was previously thought. For example, additional sequences such as stabilizing elements in aUTRs can also substantially affect mRNA decay^{30–34}. Although our understanding is therefore far from complete, it is nonetheless now clear that many genes produce multiple mRNA isoforms with different decay rates, highlighting the importance of 3' UTR-APA in modulating mRNA stability.

A related question is whether 3' UTR-APA affects mRNA translation. Indeed, the above-mentioned study analysing the effects of APA in mouse cells reported that long isoforms were associated with slightly more ribosomes than were short isoforms³⁰. As with the destabilization effects of longer 3' UTRs, this may be attributable to both translation-enhancing and translation-suppressing elements in aUTRs. However, another study using human cells reported a role for 3' UTR length in suppressing translation and also detailed variable effects of different 3' UTR sequences on translation³⁵. Hence, further work is required to delineate how various *cis* elements and 3' UTR size per se affect the stability and translation of APA isoforms in different cell types and under different conditions, such as cell stress and differentiation.

mRNA nuclear export and localization

Isoforms with a long 3' UTR tend to be more abundant in the nucleus than in the cytoplasm^{36,37}. This was observed initially in a global analysis of all transcribed sequences in human cells³⁷, and a more recent study found that ~10% of all detected 3' UTR-APA

isoforms differed significantly in abundance between nuclear and cytoplasmic fractions³⁶. Although nuclear retention was reported for long isoforms containing certain *cis* elements in the aUTR, such as inverted Alu repeats³⁸, it is still uncertain how much of the differential localization of the long isoforms is due to differences in mRNA stability rather than differences in nuclear export. In addition, if regulation of nuclear export is involved, exactly how *cis* elements in aUTRs and 3' UTR size per se have an impact on export, and what the functional significance of APA might be, remains unclear.

A better understood role of aUTRs in mRNA localization is the control of subcellular localization in the cytoplasm. Such regulated mRNA localization can in turn facilitate localized translation, which is an efficient way to enrich proteins at a specific cellular location³⁹. The relevance of APA for mRNA localization has been demonstrated for several transcripts in neuronal cells, in which localized translation in dendrites and axons is common. For example, a short isoform of the mRNA encoding brain-derived neurotrophic factor (BDNF) is restricted to the cell body, whereas the long isoform localizes to the dendrites, where it is translated⁴⁰ (FIG. 1c). Similarly, long and short isoforms of mRNAs encoding inositol monophosphatase 1 (REF. 41) and RAN⁴² are localized to the axon and cell body, respectively. These reports suggest that long isoforms are more likely to be located in dendrites or axons than are short isoforms. Conversely, a recent study compared mRNA localization in neurites (dendrites and axons) versus the cell body for neuronal cell lines and for primary cortical neurons, and this study found that short and long isoforms are similarly enriched in neurites and in the cell body⁴³. Future investigations are required to delineate the underlying mechanisms involved and to address whether, as in mRNA stability, *cis* elements can function in both enhancing and suppressing subcellular localization of mRNAs.

Protein localization

Sequences in 3' UTRs have been implicated in mRNA localization to the ER to facilitate the expression of membrane proteins^{44,45}. A surprising recent study showed that the 3' UTR can also regulate protein localization independently of mRNA localization⁴⁶ (FIG. 1d). Specifically, the aUTR of the mRNA encoding the transmembrane protein CD47 was found to act as a scaffold for a protein complex containing the RBP Hu antigen R (HUR; also known as ELAVL1) and the phosphatase 2A inhibitor SET; this complex is therefore recruited to the site of translation, resulting in the interaction of SET with the newly translated cytoplasmic domains of CD47 and the subsequent translocation of CD47 to the plasma membrane. The short mRNA isoform, which lacks the sequences necessary for assembly of the HUR–SET complex, gives rise to CD47 that is primarily localized at the ER. Thus, CD47 has a different localization, and hence a function, depending on whether it is translated from the short or long mRNA isoform. This mechanism has also been observed for transcripts encoding several other proteins, including CD44, $\alpha 1$ integrin (ITGA1) and TNF receptor superfamily member 13C (TNFRSF13C)⁴⁶.

APA upstream of the last exon

A sizable fraction of APA sites are located upstream of the last exon, mostly in introns. For simplicity, we refer to this as upstream regions APA (UR-APA). In the mouse genome, for example, more than 40% of genes have PASs of this type¹³. UR-APA leads to the expression of alternative terminal exons and can result in changes to both the coding sequence and 3' UTR of an mRNA. Depending on the configuration of splicing relative to the PAS, the resulting alternative terminal exons can be divided into two subtypes (FIG. 2a): skipped terminal exons, which are alternative upstream exons selected through splicing to be the terminal exons, and composite terminal exons, which are formed by the extension of an internal exon into the adjacent intron through inhibition of the 5' splice site. In addition, a small fraction of PASs can be identified in internal exons, leading to transcripts without an in-frame stop codon, which are likely to be degraded rapidly through the non-stop decay pathway⁴⁷. However, in some rare cases, truncated proteins can be produced when adenosine residues from the poly(A) tail are used to form a stop codon⁴⁸. UR-APA is generally upregulated in proliferating cells and suppressed during cell differentiation^{13,43,49}, mirroring the use of proximal PASs in 3' UTRs, suggesting that UR-APA and 3' UTR-APA are mechanistically related in these conditions. Similar to 3' UTR-APA, UR-APA can also affect gene expression in various ways, and this is addressed below.

Protein diversification

Two classic APA events reported in the early 1980s, involving transcripts from the calcitonin-related polypeptide- α gene (*CALCA*) and the gene encoding the immunoglobulin M (IgM) heavy chain, are well-known examples of UR-APA. In the case of *CALCA*, alternative splicing and the use of a proximal PAS generates a transcript containing a skipped terminal exon, and this mRNA isoform encodes the protein calcitonin, whereas the use of a distal PAS in the 3'-most exon generates an mRNA encoding calcitonin gene-related peptide 1 (CGRP)⁵⁰. The regulation of APA is tissue specific in this case: when comparing expression levels of the two isoforms, the calcitonin-encoding isoform is more highly expressed in the thyroid, whereas the CGRP-encoding isoform predominates in the hypothalamus. In the case of IgM heavy chain mRNA, during B cell activation there is a switch from using a distal PAS in the 3'-most exon to using a proximal PAS in a composite terminal exon, which results in a shift in protein production from a membrane-bound form of the antibody to a secreted form⁵¹. Notably, bioinformatic analysis has identified at least 376 mouse genes that potentially use such a mechanism for regulating membrane anchoring⁵². Manipulation of UR-APA-based protein isoform switching has also been shown to be a promising therapeutic approach. For example, the addition of an antisense RNA that attenuates splicing triggers the activation of an intronic PAS in the mRNA encoding vascular endothelial growth factor receptor 2 (VEGFR2) and thus enforces the expression of a soluble version of VEGFR2, which functions antagonistically to the membrane-bound form and inhibits angiogenesis⁵³.

In addition to the generation of proteins with distinct functions, UR-APA can lead to the expression of truncated proteins with dominant negative functions. For example, retinoblastoma-binding protein 6 (RBBP6) is a recently characterized polyadenylation factor

(BOX 1 and discussed below) that produces several isoforms through differential RNA processing. One of these isoforms arises from the use of an intronic PAS, which generates a severely truncated protein called Iso3 (REF. 54) (FIG. 2b). Iso3, which is downregulated in several human cancers⁵⁵, is able to compete with full-length RBBP6 for association with the remainder of the polyadenylation machinery, thereby inhibiting polyadenylation and regulating APA.

Repression of gene expression

UR-APA can also generate transcripts without apparent functions by utilizing PASs in promoter-proximal introns. For example, the gene encoding the mammalian polyadenylation factor cleavage stimulation factor 77 kDa subunit (CSTF77; also known as CSTF3) (BOX 1) has a highly conserved intronic PAS, the use of which results in a transcript that would produce a severely truncated, probably non-functional, protein⁵⁶. Production of this UR-APA transcript is induced by high cellular levels of full-length CSTF77 protein, thereby forming a negative feedback loop to control the activity of CSTF77, which is important for cell cycle control⁵⁷ (FIG. 2c). This mechanism was originally proposed for the *Drosophila melanogaster* homologue of CSTF77, Suppressor of Forked⁵⁸, and may exist in transcripts encoding other polyadenylation factors, such as PAP⁵⁹. Notably, UR-APA of CSTF77, as well as that of a large fraction of genes containing promoter-proximal intronic PASs, is also regulated by U1 small nuclear ribonucleoprotein (U1 snRNP) (see below), suggesting that the generation of truncated transcripts by UR-APA is a widespread mechanism for the inhibition of gene expression.

Regulation of APA

As the consequences of APA for gene expression and cell function are becoming increasingly clear, it is important to understand the mechanisms that regulate APA. A growing number of APA-regulatory factors have been identified and characterized in the past few years (FIG. 3a); some have global effects on APA, whereas others have an impact on APA of specific genes, as described below.

Polyadenylation factors that promote proximal PAS usage

One important mechanism of APA regulation involves modulation of the expression levels of core polyadenylation machinery components. This was first demonstrated for CSTF64 (also known as CSTF2), the RNA-binding subunit of the trimeric CSTF complex (BOX 1); strong upregulation of CSTF64 during B cell maturation results in higher levels of the complete CSTF complex and increased usage of the weaker upstream intronic PAS in the IgM heavy chain transcript⁶⁰. Consistent with this, siRNA-mediated knockdown of CSTF64 and its paralogue, CSTF64 τ -variant (τ CSTF64; also known as CSTF2T), was found to cause global 3' UTR lengthening in HeLa cells⁶¹. However, it is notable that knockdown of each factor alone was not sufficient to elicit such an effect^{61,62}, indicating that there is at least partial redundancy between the two highly similar proteins. The role of CSTF64 in APA was recently highlighted by a bioinformatics study which revealed that general 3' UTR shortening occurs in seven tested cancer types, five of which also exhibited upregulation of *CSTF64* mRNA expression⁶³. Furthermore, a significant overlap between

the APA events in this study and those of the knockdown experiment in HeLa cells was observed⁶¹, suggesting that CSTF64 has a role in 3' UTR shortening in cancer⁶³.

Changes in the expression of other core polyadenylation factors can also lead to global 3' UTR-APA. Indeed, the expression level of polyadenylation factors as a whole has been shown to correlate inversely with the global relative expression levels of isoforms with long 3' UTRs during cell differentiation and de-differentiation⁶⁴. Consistent with this, genes encoding polyadenylation factors tend to have proliferation factor-binding elements in their promoters, such as binding sites for the E2F transcription factors^{49,64}, which promote cell cycle progression from G1 to S phase. In addition to these global effects, certain transcripts contain APA sites that are sensitive to the levels of specific polyadenylation factors, and such transcripts often display distinct features. For example, PASs regulated by CSTF64 or τ CSTF64 show strong enrichment for U- and GU-rich elements, whereas those regulated by factor interacting with PAP (FIP1) — a cleavage and polyadenylation specificity factor (CPSF) complex subunit, knockdown of which leads to 3' UTR lengthening^{62,65} — are more likely to possess an upstream U-rich sequence^{61,62,65}, consistent in both cases with the respective RNA-binding activities of the proteins (BOX 1). Notably, FIP1 is particularly important for embryonic stem cell (ESC) self-renewal and somatic cell reprogramming, and a FIP1-dependent APA programme correlates with a change in FIP1 expression during ESC differentiation and somatic cell reprogramming⁶⁵. By contrast, APA regulation by PCF11, a cleavage factor II complex subunit that lacks RNA-binding activity, does not appear to involve any specific sequences around the PAS⁶². Interestingly, although knockdown of RBBP6 leads to global 3' UTR lengthening, RBBP6 also appears to be important specifically for the accumulation of its target mRNAs, including those with AU-rich elements in their 3' UTRs, suggesting that there is a connection between mRNA stability and 3' end processing⁵⁴.

Polyadenylation factors that promote distal PAS usage

A model to explain how elevated levels of core polyadenylation factors can enhance the use of proximal PASs was initially proposed as a result of the early IgM studies⁶⁰ and posits that these sites, which are typically weaker than downstream sites¹¹, are used on a 'first come, first served' basis when the core polyadenylation machinery is not limiting. It was therefore unexpected that downregulation of subunits of the core polyadenylation complex cleavage factor I (CFI) would result in increased use of upstream PASs. CFI is a heterodimer consisting of CFI25 (also known as CPSF5) and either of two closely related subunits, CFI68 (also known as CPSF6) or CFI59 (also known as CPSF7). Specifically, knockdown of CFI25 or CFI68, but puzzlingly not CFI59, leads to significant shortening of 3' UTRs^{62,66,67}. PASs in the last intron were also found to be activated⁶², suggesting a role for the CFI complex in defining the terminal exon. An analysis of *cis* elements indicated that UGUA elements, to which CFI25 binds⁶⁸, are highly enriched in the upstream parts of distal PASs affected by a CFI25 or CFI68 deficiency^{62,66,67}. Because the CFI complex exists as a dimer, one possible mechanism for CFI-based APA is that CFI complexes bind two UGUA elements, one upstream and one downstream of a proximal PAS, thereby leading to skipping of this PAS⁶⁹ (FIG. 3b). Whether this in fact occurs and why CFI59 behaves differently require further study. Importantly, CFI25 expression is downregulated in glioblastoma cells,

leading to the usage of upstream PASs and to enhanced tumorigenicity and increased tumour size; conversely, CFI25 overexpression inhibits tumour growth⁷⁰. In addition, copy number variations of *NUDT21* (the gene encoding CFI25) were found in individuals with certain neuropsychiatric syndromes⁷¹. In lymphoblastoid cells of these individuals, increased CFI25 levels led to higher expression of a long isoform of the mRNA encoding methyl CpG-binding protein 2 (MECP2), resulting in reduced production of MECP2, probably owing to the presence of numerous miRNA target sites in its aUTR. Because MECP2 levels need to be tightly regulated in the brain and small fluctuations in abundance can lead to neurological malfunctions, *NUDT21* was suggested to be a candidate gene for causing intellectual disability and neuropsychiatric diseases⁷¹.

Nuclear poly(A)-binding protein 1 (PABPN1; also known as PABP2) controls poly(A) tail length⁷². Surprisingly, given that PABN1 was not thought to participate in PAS choice, knockdown of PABPN1 induced global 3' UTR shortening^{73,74}. Ectopic expression of trePABPN1(A17), which is a short trinucleotide-repeat expansion mutant found in patients with autosomal-dominant oculopharyngeal muscular dystrophy (OPMD), led to a similar trend of 3' UTR shortening in cultured cells and mouse tissues, suggesting a connection between 3' UTR regulation and the aetiology of OPMD⁷³. Indeed, PABPN1 can inhibit 3' cleavage of target transcripts *in vitro*⁷³, but whether this occurs *in vivo* is unknown. It is worth noting that, although a PABPN1 deficiency elicits 3' UTR shortening, there is no apparent correlation, at least in mouse myoblasts, between the aUTR size and the degree of APA regulation, whereas such a correlation is a common feature for APA regulation by other polyadenylation factors⁶². Thus, how PABPN1 affects APA remains to be determined. Knockdown of PABPN1 also led to increased expression of RNA species using PASs near gene promoters, in both sense and antisense directions, with the latter being more prominent⁶². This function is likely to be related to the role of PABPN1 in hyperpolyadenylation, which leads to RNA degradation by the nuclear exosome^{75,76}. Indeed, a similar phenotype was observed following knockdown of the exosome factors RRP44 and RRP6 (also known as EXOSC10)⁶². Interestingly, hyperpolyadenylation was found to involve the canonical enzymes PAP α and PAP γ , rather than the non-canonical PAPs that are usually associated with exosome activity⁷⁷. It was suggested that hyperpolyadenylation is an important nuclear RNA decay pathway that is responsible for the removal of transcripts that have been poorly spliced or retained in the nucleus. An outstanding question is how PABPN1 has different roles at the 5' and 3' ends of genes, and whether the apparent effects on APA may instead reflect differential nuclear RNA decay.

Another poly(A)-binding protein that functions in APA is polyadenylate-binding protein 1 (PABP1), which shuttles between the nucleus and cytoplasm and is possibly the major poly(A) tail-binding protein in the cytoplasm. Knockdown of PABP1 was also found to modulate 3' UTR length⁶². Exactly how poly(A) tail-binding proteins function in APA, and whether their involvement is direct or indirect, needs to be further studied.

It is clear that core polyadenylation factors have substantial roles in APA regulation. Notably, although some polyadenylation factors seem to fall into either proximal-PAS-promoting or distal-PAS-promoting activity groups, this bifurcation may be oversimplifying the situation for other polyadenylation factors. For example, similar numbers of proximal

and distal PASs were found to be regulated by CSTF77 knockdown⁵⁷, and CPSF30 of *Arabidopsis thaliana* regulates a large number of APA events without a clear preference for proximal or distal PAS usage during the response to oxidative stress⁷⁸.

The splicing connection and the role of U1 snRNP in APA

Splicing and polyadenylation are frequently interconnected, and this connection was initially suggested as a mechanism to facilitate the definition of 3'-terminal exons⁷⁹. In the case of intronic PASs, splicing and polyadenylation are likely to be in competition with each other, as large introns with weak 5' splice sites undergo polyadenylation at their internal PASs to a greater extent than other introns⁸⁰, and inhibition of splicing — for example, by ablation of the U2 snRNP component splicing factor 3B subunit 1 (SF3B1) — generally activates intronic PASs⁶². Multiple protein–protein interactions exist between core splicing factors and core polyadenylation factors, such as between U1 snRNP and CPSF 160 kDa subunit (CPSF160; also known as CPSF1)⁸¹, U2 snRNP and the CPSF complex⁸², and U2 auxiliary factor 65 kDa subunit (U2AF65; also known as U2AF2) and the CFI complex⁸³. However, U1 snRNP, which recognizes 5' splice sites, seems to have an active and, in many cases, probably distinct role in APA regulation. In early studies, U1 snRNP was shown to suppress PAS usage through inhibition of PAPα⁸⁴. More recently, inhibition of U1 snRNP was found to result in the activation of cryptic PASs near transcription start sites⁸⁵, implying that U1 snRNP normally represses the use of such PASs, and mild attenuation of this inhibitory function caused increased usage of proximal PASs in 3' UTRs⁸⁶. This process, dubbed telescripting, involves the inhibition of polyadenylation by U1 snRNP binding to canonical 5' splice sites, or similar sites, throughout the nascent RNA⁸⁷. The existence of telescripting provides an answer to the long-standing question of why U1 snRNP is present at a much higher abundance than other snRNPs⁸⁸. Telescripting has also been implicated in the global transcript shortening that occurs during transient transcription upregulation upon activation of neurons⁸⁶. A similar phenomenon was recently observed in the human colon carcinoma cell line RKO after exposure to UV damage⁸⁹, such that 5' intronic PASs were substantially activated, probably owing to reduced levels of U1 snRNA. Notably, 3' UTR shortening was not obvious in these RKO cells. Whether the difference is due to cell specificity or some other factors remains to be seen.

Regulation of APA by other RBPs

A growing number of RBPs have been found to interact with regions near PASs and to regulate PAS usage. RBPs already known to regulate splicing are very often also found to be regulators of APA, and they typically regulate PAS usage in a context-dependent manner, as was first shown for the neuronal RBP NOVA2 (REF. 90): binding of NOVA near the PAS is inhibitory, whereas binding distantly from the PAS enhances PAS usage. Below, we highlight some recent findings that exemplify both general rules and novel mechanisms. Readers are referred to other reviews⁹¹ for more exhaustive information.

The ELAV (embryonic-lethal abnormal visual) proteins constitute an extensively studied family of RBPs that function in several aspects of mRNA metabolism, including APA. For example, *D. melanogaster* Elav was shown to mediate neuron-specific 3' UTR lengthening by suppressing the use of proximal PASs⁹² (FIG. 3c). Interestingly, this involves the

recruitment of Elav to paused Pol II near the promoter of the Elav-responsive gene, indicative of a link between APA and transcription (see below)⁹³. In mammals, the Hu proteins, which are Elav homologues, inhibit the use of PASs with U-rich elements⁹⁴. The mRNA encoding HUR, a ubiquitously expressed Hu protein, is also subjected to APA, either by HUR itself⁹⁵ or by neuron-specific Hu proteins, HUB (also known as ELAVL2), HUC (also known as ELAVL3) and HUD (also known as ELAVL4)⁹⁶. This process balances the pro-differentiation activity of the neuron-specific Hu proteins with the pro-proliferation activity of HUR.

SR proteins are a family of conserved RBPs that contain RNA-binding domains and sequences rich in Ser-Arg dipeptide repeats (RS domains). First discovered as splicing factors⁹⁷, these proteins are now known to have various roles in mRNA biogenesis and metabolism⁹⁸. Two of the twelve SR proteins, SRSF3 and SRSF7, were also found to regulate 3' UTR length in mouse P19 cells, in which SRSF3 lengthens 3' UTRs and SRSF7 has the opposite effect⁹⁹. SRSF3 binding to the last exon also promotes mRNA nuclear export through an interaction with nuclear RNA export factor 1 (NXF1). Although it is unclear how SRSF3 and SRSF7 alter PAS choice, the observation that SR proteins can regulate APA and mRNA export suggests that there is a connection between these two processes. Indeed, multiple interactions have been reported between nuclear export factors and polyadenylation factors^{100,101}, and all of these interactions have been shown to affect PAS choice.

The usually fatal neurodegenerative disease amyotrophic lateral sclerosis (ALS) has long been linked to defects in RNA processing, in part because it can be caused by mutations in genes encoding RBPs, including TAR DNA-binding protein 43 (TDP43) and FUS¹⁰². Although these proteins have documented roles in splicing, recent studies have pointed to functions in APA as well. FUS binds nascent RNAs and interacts with the CPSF and CSTF complexes, and FUS knockdown leads to changes in APA¹⁰³. FUS also binds the carboxy-terminal domain of the largest subunit of Pol II and prevents inappropriate hyperphosphorylation of this domain¹⁰⁴. Loss of FUS leads to Pol II accumulation at transcription start sites and activation of promoter-proximal PASs (see below). Future studies are needed to elucidate how the function of FUS in Pol II phosphorylation is related to its RNA binding and its interaction with polyadenylation factors. Interestingly, whereas wild-type FUS binds mostly intronic sequences, an ALS-causing FUS mutant binds predominantly to sites in 3' UTRs¹⁰⁵. However, how this is related to FUS-mediated pathology in ALS remains to be determined.

The most frequent known cause of ALS (as well as of a dementia called frontotemporal dementia (FTD)) is an expansion of the hexanucleotide GGGGCC in the gene *C9ORF72*. It is notable that a global shortening of 3' UTRs was observed in the cerebellum of patients with ALS who also had the gene expansion¹⁰⁶. A number of mechanisms have been suggested for how the expansion can lead to ALS and FTD, including the sequestering of multiple RBPs, such as heterogeneous nuclear RNP H (HNRNPH), into nuclear foci¹⁰⁷, but additional studies are needed to investigate how APA is dysregulated in ALS and to discern the possible significance of 3' UTR shortening to the aetiology of the disease. On a related note, sequestering of muscleblind-like RBPs by expanded CUG repeats is known to lead to

the disease myotonic dystrophy and was found to change the adult APA profile to match that seen in neonates in the skeletal muscles of human patients and mouse models¹⁰⁸, highlighting the importance of RBP-mediated APA regulation in development and disease.

Regulation of APA by transcription

Polyadenylation is frequently, if not always, co-transcriptional. It is not surprising, therefore, that various aspects of the transcription process influence PAS choice. Similar to the effects of transcription on alternative splicing (reviewed in REF. 109), transcriptional influences on PAS usage can fall into two categories. First, alterations in the rate of Pol II elongation at the PAS region can control APA. Consistent with this idea, *cis* elements that cause pausing of Pol II, such as G-rich sequences, facilitate PAS usage¹¹⁰. Mutations in the genes encoding the transcription elongation factors TFIIS (also known as Dst1) and Spt5, as well as in the gene encoding Pol II subunit Rpb2, can enhance the usage of upstream PASs in yeast, probably by increasing pausing¹¹¹; furthermore, in mouse plasma cells, the elongation factor ELL2 can modulate usage of the proximal PAS of immunoglobulin heavy chain pre-mRNA¹¹². More direct evidence comes from the study of a mutant *D. melanogaster* strain expressing Pol II with a slower elongation rate¹¹³, in which expression of the short isoform of the *polo* gene was increased. This is physiologically relevant because transgenic flies lacking the long *polo* isoform die at the pupa stage owing to perturbed proliferation of abdominal precursor cells.

A second link between transcription and polyadenylation involves transcription machinery-mediated recruitment of specific factors that can influence PAS choice. Certain transcription activators can promote efficient 3' end processing both *in vivo*¹¹⁴ and in a transcription-coupled 3'-end-processing assay *in vitro*¹¹⁵, and this leads to more frequent use of proximal PASs. Indeed, global analyses indicate that when genes are expressed at high levels, mRNA isoforms with shorter 3' UTRs tend to be more abundant^{116,117} and that Pol II tends to pause at proximal PASs to a greater extent¹¹⁶. The recruitment of polyadenylation factors by transcription-activating factors may be responsible for the presence of several polyadenylation proteins at promoter regions^{118–121}, and can be carried out directly¹²² or through the Pol II-associated factor (PAF) elongation complex¹¹⁵. The CDC73 (also known as parafibromin) subunit of PAF, encoded by a tumour suppressor gene that is mutated in hereditary and sporadic parathyroid tumours, directly interacts with the CPSF and CSTF complexes¹²⁰, and knockdown of CDC73 or another PAF subunit, PAF1, in mouse myoblasts leads to significant shortening of transcripts through activation of intronic PASs and proximal PASs in 3' UTRs¹²³. These findings, as well as the observation that upstream antisense transcripts which use PASs near promoters were also upregulated following PAF knockdown, are consistent with there being a role for PAF in releasing paused Pol II¹²⁴. Also notable is the finding that knockdown of the PAF subunit SKI8, which associates with the exosome, leads only to 3' UTR shortening, with little effect on PASs in upstream regions or around the promoter. How different components of PAF exert different effects at different regions of transcripts is an interesting question that remains to be answered.

Chromatin structure is intimately connected with transcription. Studies from yeast to humans have shown that the region around the PAS is generally depleted of nucleosomes¹²⁵,

presumably at least partially owing to the presence of AT-rich sequences, which are less favourable for nucleosome association, around the PAS¹²⁶. However, the higher affinity of nucleosomes for sequences downstream of highly used alternative PASs¹²⁷ and the correlation between nucleosome levels and the accumulation of Pol II downstream of the PAS¹²⁸ suggest a possible active role of chromatin organization in APA. Consistent with this, variations in nucleosome density and levels of histone H3 Lys36 trimethylation — a histone modification that is enriched at gene 3' ends — between genes expressed at different levels were found to be much greater at proximal PASs than at distal PASs¹¹⁶. Furthermore, a recent study found that a heterochromatin formation that causes Pol II pausing also promotes the usage of proximal PASs³⁶. Conversely, the more open chromatin conformation during spermatogenesis, as indicated by the histone H3 Lys4 trimethylation, was found to correlate with an increased usage of proximal PASs in 3' UTRs¹²⁹, suggesting that open chromatin allows more efficient recruitment of the polyadenylation complex and facilitates PAS usage. Future studies will be needed to delineate and reconcile the data about how chromatin organization affects APA.

Concluding remarks

Numerous advances in the past few years have substantially enriched our knowledge of APA, which is now acknowledged as an important and widespread mechanism for modulating gene expression. We expect that our understanding of APA will continue to grow rapidly in the next few years, as some of the outstanding questions outlined below are answered.

Regulation of APA

Novel findings about the regulation of APA continue to emerge. For example, N⁶-methyladenosine (m⁶A) was found to be highly enriched in 3'-most exons, and a reduction of m⁶A levels has been shown to affect APA, mostly causing 3' UTR shortening¹³⁰. But what is the mechanistic basis for this form of APA regulation, and what is its physiological role? More generally, we now know that variations in the expression levels of different polyadenylation factors can alter PAS choice in different ways, but what is the mechanism underlying this? For example, do changes in levels of a complex subunit affect the levels of the intact functional complex, as shown in the initial studies of CSTF64-mediated APA regulation in B cell differentiation⁶⁰? If so, do different cell types or conditions have different rate-limiting polyadenylation factors? Or might these variable effects reflect the existence of heterogeneity in the make-up of the polyadenylation machinery? It is also important to understand the interplay between the polyadenylation complex and the cellular RBPs in control of APA, and the contributions of promoter sequences and U1 snRNP are to APA regulation in different cells. Finally, we still know very little about how the expression of polyadenylation factors is itself regulated through transcriptional and post-transcriptional mechanisms. When all these layers of regulation are better understood, we will be in a position to 'crack the APA code'.

Consequences of APA

The recent findings that 3' UTR-APA can regulate protein localization independently of mRNA localization⁴⁶ and that UR-APA can change mRNA localization through the inclusion of different terminal exons⁴³ are particularly intriguing. How widespread are the roles of 3' UTR-APA and UR-APA in protein localization and mRNA localization, respectively? In conditions under which 3' UTR length is globally regulated, such as in cancer cells, is protein localization also globally remodelled as a consequence? In addition, the impact of APA on mRNA decay and translation needs to be further analysed in more cellular conditions, such as during cell stress and differentiation. How different cell types that have different average 3' UTR lengths, such as neuronal cells, regulate mRNA decay and translation remains to be addressed.

The connection between poly(A) tail length — an important feature for mRNA stability and translation¹³¹ — and PAS choice is still poorly understood. Poly(A) tail synthesis is intimately related to the nuclear cleavage reaction but can be dynamically remodelled in the cytoplasm. For example, cytoplasmic polyadenylation elements (CPEs) near the PAS can modulate poly(A) tail length through CPE-binding proteins and the activity of non-canonical PAPs, as demonstrated in neurons and during early development^{132,133}. Although several methods for measuring poly(A) tail length have been established^{134,135}, they have yet to be exploited to investigate the long-standing issue of how or whether poly(A) tail length is regulated by changing PAS choice. With the recent rapid advances in sequencing technologies, we expect this issue will be elucidated in a genome-wide manner.

Another outstanding question is how APA affects the functions of lncRNAs, which constitute a still-expanding class of transcripts with many structural and regulatory functions (reviewed in REF. 136). These lncRNAs could be extensively regulated by APA¹³. For example, APA isoforms of the lncRNA nuclear-enriched abundant transcript 1 were shown to have different functions in paraspeckle formation¹³⁷.

Clinical implications

The first example showing that human disease can be caused by a malfunction of 3' end processing comes from the identification of a mutation in the AAUAAA sequence of the PAS of the gene *HBA2*, which encodes haemoglobin subunit $\alpha 2$, in patients with α -thalassaemia¹³⁸. The relevance of APA, as opposed to 3' end processing per se, to human health was first demonstrated by the causative correlation between systemic lupus erythematosus and a single nucleotide polymorphism in the *IRF5* gene that affected APA in the transcript²⁷. Similarly, a polymorphic PAS downstream element in the *ATP1B1* gene, encoding the (Na⁺+K⁺)ATPase $\beta 1$ subunit, was found to be associated with high blood pressure¹³⁹. As the feasibility of performing population-wide, whole-genome sequencing improves, we will gain a systemic view of how APA is affected by disease-causing mutations and genetic variations, and more importantly, what the role of APA is in disease aetiology and in shaping human traits. Given the cell type specificity of APA profiles, it is conceivable that information about APA could assist in disease diagnosis, as has been demonstrated in certain cancers^{63,140} and cardiac diseases^{141–143}. Whether and in what way

such approaches could be used in clinical settings and whether APA, or 3' end processing in general, could serve as a therapeutic target remain to be explored.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

PUF protein

(Pumilio and FBF homology family protein). A member of a family of RNA-binding proteins that regulate aspects of mRNA metabolism by binding to specific sequences in 3' untranslated regions

STAU1-mediated mRNA decay

An mRNA decay mechanism in which RNA structures in the 3' untranslated region interact with double-stranded RNA-binding protein Staufen homologue 1 (STAU1) to mediate mRNA decay

AU-rich element-mediated decay

mRNA decay elicited by the presence of AU-rich elements (AREs) in the 3' untranslated region

PIWI-interacting RNAs

Small non-coding RNAs that form RNA–protein complexes with PIWI proteins to silence transposable elements in germline cells of metazoans

Non-stop decay

An mRNA decay mechanism that specifically degrades mRNAs without a stop codon

Exosome

A nuclear or cytoplasmic multiprotein complex that degrades mRNAs through the activity of 3'-to-5' exoribonucleases

Non-canonical PAPs

(Non-canonical poly(A) polymerases). Enzymes that have distinct structural features and are capable of synthesizing poly(A) tails but are not typically associated with the polyadenylation machinery

Paused Pol II

(Paused RNA polymerase II). Pol II that has paused in the promoter-proximal region of the mRNA and is poised for productive elongation

Paraspeckle

A dynamic nuclear compartment composed of RNA-binding proteins and RNAs. The functions of paraspeckles are not entirely clear

Box 1**Core sequence elements and factors involved in cleavage and polyadenylation**

Cleavage and polyadenylation (hereafter referred to as polyadenylation) is controlled by cis elements located upstream and downstream of the polyadenylation site (PAS) (see the figure). In vertebrates, upstream elements include the hexamers A[A/U]UAAA or other close variants, U-rich elements and UGUA elements. Downstream elements include U-rich and GU-rich (typically in the form of GUGU) elements. A CA sequence is often found immediately 5' to the cleavage site (see the figure; indicated by a lightning bolt). In addition, upstream UAUA elements and downstream G-rich sequences can frequently be found near PASs, typically more than 40 nt away¹⁴⁴. The 'strength' of a PAS seems to be defined by these sequence elements in a combinatorial manner¹⁴⁵. A functional PAS in human cells can consist of only an A-rich upstream sequence and strong U-rich downstream elements¹⁴⁶. It is notable that this make-up of the core PAS is analogous to the organization of core RNA polymerase II (Pol II) promoters (discussed in REF. 6).

Not surprisingly, variants of the A[A/U]UAAA sequence are weaker at directing polyadenylation in vitro than the consensus hexamer¹⁴⁷. However, variants are fairly common, especially for upstream APA sites¹¹. For example, AAGAAA is commonly found in upstream (not 3'-most) APA sites¹¹, even though this sequence is essentially inactive in vitro. Other elements, such as upstream UGUA and downstream GU-rich sequences, also are less frequent in upstream APA sites, supporting the idea that such sites have suboptimal strength. This is probably important for the regulation of polyadenylation and could explain global APA regulation during cell proliferation and differentiation, when the concentration of polyadenylation factors changes²². By contrast, 3'-most PASs are typically strong, presumably to ensure proper transcription termination.

The polyadenylation machinery in metazoans is composed of ~20 core proteins, including four protein complexes and several single proteins¹⁴⁸. The complexes are cleavage and polyadenylation specificity factor (CPSF), which contains CPSF 160 kDa subunit (CPSF160; also known as CPSF1), CPSF100 (also known as CPSF2), CPSF73 (also known as CPSF3), CPSF30 (also known as CPSF4), FIP1 (factor interacting with PAP) and WDR33; cleavage stimulation factor (CSTF), which contains CSTF 77 kDa subunit (CSTF77), CSTF50 (also known as CSTF1) and either CSTF64 or its paralogue, τ CSTF64; cleavage factor I (CFI), which contains CFI 25 kDa subunit (CFI25) and either CFI68 or CFI59; and cleavage factor II (CFII), which contains PCF11 and CLP1. Single proteins include symplekin, poly(A) polymerase (PAP), retinoblastoma-binding protein 6 (RBBP6) and RNA polymerase II (Pol II), specifically the Pol II regulatory carboxy-terminal domain (CTD). Nuclear poly(A)-binding protein 1 (PABPN1) (not shown) is important for synthesis of an appropriately sized poly(A) tail and thus might also be considered to be a core factor.

As expected, many polyadenylation factors are RNA-binding proteins and display sequence-specific RNA binding. CPSF makes multiple RNA contacts, including with the AAUAAA element through CPSF30 and WDR33 (REFS 149,150), and with U-rich

sequences through FIP1 (REF 151). CFI25 binds the UGUA element¹¹⁹, CFI68 and CFI59 also contact RNA, and CSTF64 and τ CSTF64 interact with U- and GU-rich downstream elements^{152,153}. CPSF73 is the endonuclease and has a preference for a CA dinucleotide at the cleavage site¹⁵⁴. Some proteins that do not bind RNA have scaffolding functions, such as symplekin and the Pol II CTD. Both CFI and CSTF function as dimers in the polyadenylation machinery^{155,156}.

Despite considerable divergence between yeast and mammals in the core RNA sequences that constitute the PAS (see Supplementary information S1 (box)), nearly all mammalian polyadenylation factors have homologues in yeast, with the exception of the CFI proteins and CSTF50. However, there seems to be some variation in the make-up of subcomplexes in yeast⁵, and the yeast polyadenylation factor Hrp1p, which interacts with UA-rich elements, is missing from metazoans. The polyadenylation machinery in plants is similar to that in metazoans, but with substantial gene (and thus protein) duplications¹⁵⁷.

Box 2**Tissue-specific APA patterns**

Alternative polyadenylation (APA) patterns are, to a great extent, tissue specific. Corroborating early analyses^{158,159}, deep-sequencing analyses have detailed the existence of tissue-specific APA profiles^{12,160}. Some human tissues have a global tendency to favour certain APA isoform types¹⁵⁸; for example, neuronal tissues favour isoforms that use distal polyadenylation sites (PASs) in 3' untranslated regions (3' UTRs), whereas the use of proximal PASs is favoured in blood cells and testis tissue^{158,161}. The phenomenon in which mRNAs with extremely short or long 3' UTRs are expressed in testis and brain, respectively, also occurs in flies¹⁶². Comparative analysis of the tissues of five different mammals indicated that APA profiles in different tissues are well conserved across species¹². Importantly, ubiquitously expressed genes are more likely to express APA isoforms in different tissues than genes with a restricted tissue expression¹⁶⁰, raising the possibility that APA in the 3' UTR (referred to as 3' UTR-APA) has an important role in tissue-specific regulation of these genes. Consistent with this theory, genes that are evolutionarily old, which tend to be more widely expressed, are more likely to undergo APA than new genes¹⁶³.

Recent studies have shed some light on the mechanisms of expression of long 3' UTR-APA isoforms in the brain. First, in *Drosophila melanogaster*, the RNA-binding protein (RBP) Embryonic-lethal abnormal visual (Elav) inhibits proximal PAS usage^{92,93} (see main text). Second, during neuronal differentiation, when 3' UTRs generally lengthen¹⁶⁴, AU-rich element-mediated decay (which targets isoforms with longer 3' UTRs, as they are more likely to contain AU-rich elements (AREs)) seems to be suppressed¹⁶⁵. Consistent with this, tristetraprolin, an RBP with a role in ARE-mediated decay, is downregulated by the microRNA (miRNA) miR-9 during neurogenesis¹⁶⁵. Thus, the combined activity of RBPs that favour preferential selection of distal PASs and of those that stabilize mRNAs boost the abundance of long isoforms in neuronal cells, and this is important for neuronal cells presumably because of 3' UTR-mediated mRNA localization in dendrites and axons (see main text).

In a recent study of 3' UTR-APA isoform expression during spermatogenesis, 3' UTRs were found to drastically shorten as spermatocytes differentiate into spermatids¹²⁹. This too can be attributed to both regulation of PAS choice and mRNA stability. First, genes producing transcripts with shorter 3' UTRs are more likely to undergo transcriptional upregulation and to reside in open chromatin, suggesting that APA is regulated by these features¹²⁹. This is consistent with previous studies showing that genes transcribed at high rates tend to undergo more efficient polyadenylation and, presumably as a result, use more promoter-proximal PASs (see main text)^{115,116}. This mechanism may function in conjunction with the unique regulation of polyadenylation factor expression during spermatogenesis, such as the regulation suggested for cleavage stimulation factor 64 kDa subunit τ -variant (τ CSTF64 τ)¹⁶⁶ and cleavage factor I¹⁶⁷. Second, mRNA decay mechanisms are highly potent and responsible for the global elimination of RNAs during the transition from spermatocytes to spermatids, which contain ~12 pg and ~2.5 pg total RNA per cell, respectively¹⁶⁸. Thus, long isoforms that contain destabilizing elements are

rapidly degraded, probably by multiple decay mechanisms, including the degradation of mRNAs containing transposable elements, mediated by PIWI-interacting RNAs^{129,169–171}, and the nonsense-mediated decay pathway, which degrades transcripts with abnormally long 3' UTRs (among others)^{172,173}. Importantly, transcripts with short 3' UTRs, which lack these destabilizing elements or features and thus escape mRNA degradation, are thought to be stored for translation at a later developmental stage, when transcription is globally inhibited.

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Box 3**Global regulation of APA**

Alternative polyadenylation (APA) can be globally regulated in response to changes in cell proliferation and differentiation. General shortening of 3' untranslated regions (3' UTRs) during T cell activation (which is accompanied by cell proliferation) was the first example of such regulation in response to changes in cell proliferation status²¹. It was proposed that transcripts with shorter 3' UTRs evade targeting by microRNAs (miRNAs) and thus increase the protein output from a transcript. However, a more recent study using 3' end sequencing and mass spectrometry showed that although 3' UTR shortening during T cell activation is conserved between humans and mice, orthologous genes do not exhibit similar APA profiles¹⁷⁴. In addition, 3' UTR shortening was not accompanied by changes in mRNA and protein levels, suggesting that APA can have limited effects on overall protein output.

Global 3' UTR regulation according to cellular proliferation and differentiation status has been observed in a number of biological systems and processes, including during embryonic development, the differentiation of myoblasts and of embryonic stem cells^{22,164}, and the de-differentiation of many cell types into induced pluripotent stem cells⁶⁴. As most factors in the polyadenylation complex are highly expressed in proliferative cells compared to their levels in differentiated cells^{22,64}, it was hypothesized that global regulation of polyadenylation activity might underlie the global APA profile changes that are observed when cells alter their proliferation state. This view is consistent with reporter assays showing decreased proximal PAS usage in differentiated mouse myoblasts compared with proliferating cells²².

The correlation between cellular proliferation status and 3' UTR size extends to cancer cells. A meta-analysis of microarray data indicated that cancer cells have shorter 3' UTRs than non-transformed cell lines²³, and a recent bioinformatics study of RNA-seq data from 358 tumour-versus-normal tissue pairs identified 1,346 genes for which the transcripts underwent significant and recurrent APA in seven tumour types⁶³. The majority of transcripts (61–98%) displayed 3' UTR shortening in the tumours. Another study compared cells (BJ primary fibroblast and the mammary epithelial cell line MCF10A) in the proliferating, growth-arrested and transformed states, and found that proliferation is a more important determinant of 3' UTR length than transformation⁴⁹. Notably, although global shortening of 3' UTRs occurs in cancer cells, a substantial fraction of transcripts appear to undergo 3' UTR lengthening, for example in breast cancer cells¹⁷⁵, colorectal cancer cells¹⁷⁶ and lymphoma cells¹⁴⁰. Interestingly, genes producing transcripts that have lengthened 3' UTRs in cancer cells seemed to be enriched for certain functional groups, such as cell–cell adhesion^{140,175,176}. Overall, the APA profile in cancer cells may be more complicated than was initially suggested, and additional studies are needed to dissect the different groups of genes with respect to their APA regulation.

Finally, APA can be globally regulated by specific extracellular cues. For example, isoforms using the proximal PAS, which encode truncated proteins or have short 3'

UTRs, are generally upregulated when neuronal cells are activated by membrane depolarization agents¹⁷⁷, and activation of the mTOR pathway leads to global 3' UTR shortening¹⁷⁸. Although the mechanism underlying this APA is unknown, isoforms with shortened 3' UTRs have greater translational potential as analysed by polysome profiling. This supports the idea that 3' UTR shortening can increase protein output, a view that has been challenged in other settings, as described above. Interestingly, transcripts encoding proteins related to ubiquitin-mediated proteolysis, which is important for cell cycle progression, were most significantly affected by the 3' UTR shortening elicited by mTOR activation.

Figure 1. 3' UTR-APA

a | Alternative polyadenylation (APA) leading to the production of two mRNA isoforms with different 3' untranslated regions (3' UTRs) — termed 3' UTR-APA here — is shown. The 3' UTR region upstream of the proximal polyadenylation site (PAS) is found in both short (top) and long (bottom) isoforms and is denoted the constitutive UTR (cUTR), whereas the downstream region is present in the long isoform only and is termed the alternative UTR (aUTR). Interactions between the aUTR and RNA-binding proteins (RBPs), microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) can have various functional consequences. The poly(A) tail is represented as AAA. **b** | In the case of the gene encoding human IFN-regulatory factor 5 (IRF5), APA of the transcript produces a long 3' UTR isoform that is more rapidly degraded owing to the presence of a destabilizing AU-rich element (ARE) in the aUTR. The ARE and cytoplasmic exosome mediate mRNA decay. In patients with systemic lupus erythematosus (SLE), a single nucleotide polymorphism reducing the use of the proximal PAS leads to the production of long isoforms at the expense of short isoforms, which results in reduced IRF5 levels. **c** | Differential mRNA localization of brain-derived neurotrophic factor (BDNF) 3' UTR-APA isoforms in neurons. The long isoform localizes to dendrites more than the short isoform, and this supports dendrite-localized protein synthesis. **d** | Differential localization of the transmembrane CD47 proteins encoded by long or short APA isoforms. Both isoforms are translated on the ER membrane. The aUTR of the long isoform is bound by the RBP Hu antigen R (HUR), which leads to the localization of CD47 protein to the cell membrane through a cascade of interactions (dashed arrow) involving the phosphatase 2A inhibitor SET and RAC1. The protein generated from the short isoform remains in the ER. CDS, coding sequence.

Figure 2. UR-APA

a | Alternative polyadenylation (APA) in upstream regions (URs) of mRNAs — termed UR-APA here — can lead to the production of isoforms with different 3'-terminal exons and, hence, different coding sequences and 3' untranslated regions (3' UTRs). Three isoforms are shown, with their respective terminal exon types indicated. Splicing is indicated by a dashed line. The 'canonical' isoform (top) is formed by the use of the polyadenylation site (PAS) in the 3'-most exon. The use of a PAS in an alternative exon that is excluded from the canonical isoform generates a transcript containing a skipped terminal exon (middle). Inhibition of splicing at the indicated 5' splicing site (5'SS) results in the inclusion of part of the downstream intron and use of a PAS within that intron; such a transcript is described as containing a composite terminal exon (bottom). Regions not present in the canonical isoform are shown in red. The functional consequences of UR-APA are indicated. **b** | UR-APA of the transcript encoding polyadenylation factor retinoblastoma-binding protein 6 (RBBP6) produces an isoform encoding a dominant negative protein, Iso3. **c** | UR-APA of the mRNA encoding polyadenylation factor cleavage stimulation factor 77 kDa subunit (CSTF77) produces a short isoform that encodes a truncated protein with no apparent functions (not shown). The full-length protein activates the usage of the upstream PAS, thereby increasing the levels of the short mRNA and forming a negative feedback loop. CDS, coding sequence.

Figure 3. Regulation of APA

a | The choice of polyadenylation site (PAS) during alternative polyadenylation (APA) can be influenced by various factors, including the gene promoter at the transcription start site (TSS); recruitment of polyadenylation factors directly or of proteins that influence PAS choice; nucleosome density in the region around the PAS; RNA polymerase II (Pol II)-mediated transcription elongation by the Pol II-associated factor (PAF) complex; the function of various RNA-binding proteins (RBPs) associated with the nascent transcript; the presence of N⁶-methyladenosine (m⁶A); and inhibition of polyadenylation by the splicing factor U1 small nuclear ribonucleoprotein (U1 snRNP). See the main text for more details. **b** | A proposed model for the regulation of APA by the cleavage factor I (CFI) complex. Two UGUA elements upstream and downstream of a proximal PAS are recognized by the heterodimeric CFI complex, which consists of CFI 68 kDa subunit (CFI68) and CFI25, leading to skipping of the PAS. **c** | Regulation of neuronal APA in *Drosophila melanogaster* by the RBP Embryonic-lethal abnormal visual (Elav). Elav is recruited to Pol II at promoter regions that contain a GAGA sequence, which can cause Pol II pausing. Elav inhibits proximal PAS usage, leading to the expression of long APA isoforms during neurogenesis. PA complex, polyadenylation complex.