Alternative polyadenylation: less than meets the eye?

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Abstract

With the advances in deep-sequencing techniques over the last decade, the study of alternative cleavage and polyadenylation (APA) has shifted from individual gene to whole transcriptome analysis. Findings from such global studies have elevated APA to its currently accepted status as a major player in the regulation of eukaryotic gene expression. Although ∼70 % of human genes have been shown to contain multiple cleavage and polyadenylation sites, the extent of the consequences of APA and its role in regulating physiological processes are still largely unknown. The present review aims to summarize the experimental evidence that supports a physiological role of APA and highlights some of the shortcomings that need addressing to substantiate the widely proposed claim that APA is a key player in global gene regulation.

Alternative cleavage and polyadenylation

Cleavage and polyadenylation is a pre-mRNA processing reaction that matures the 3′-end of all pre-mRNAs with the exception of replication-dependent histone genes. The reaction is executed by a multiprotein complex that recognizes the bipartite PAS [poly(A) signal] present on pre-mRNA. The actual processing reaction can be divided into two steps, the cleavage of the pre-mRNA at the CPA (cleavage and polyadenylation) site and the subsequent polyadenylation of the newly created 3′-end of the transcript.

A large proportion of eukaryotic, and in particular mammalian, genes contain multiple CPA sites. The alternative usage of these CPA sites, a process referred to as alternative cleavage and polyadenylation (APA), creates distinct mRNA isoforms that can differ in 3′-UTR length, and potentially their coding capacity. Thus, in analogy to alternative splicing, APA can result in multiple distinct transcript isoforms being produced from a single gene, increasing the diversity of the transcriptome, and in some cases the proteome.

Depending on the location of the different CPA sites within the gene, APA can be classified as either untranslated-region APA (UTR-APA) or coding region APA (CR-APA) as summarized in Figure 1. UTR-APA occurs when multiple CPA sites are present within the same 3′-UTR of a gene. Thus, by default, UTR-APA does not affect the coding capacity of the APA mRNA isoforms, but results in mRNA isoforms that differ solely in 3′-UTR length, with the longer isoform containing an additional aUTR (alternative UTR). The regulatory potential of UTR-APA lies in the presentation of different regulatory modules in this aUTR, including miRNA target sites and AU-rich elements, that can potentially affect the stability, translation efficiency and subcellular localization of that isoform.

CR-APA, on the other hand, describes APA events that involve the usage of CPA sites that are located upstream of the 3′-UTR, therefore altering the coding capacity of the transcript and potentially leading to the production of multiple protein isoforms with distinct C-termini.

CR-APA can be categorized further into three separate classes: exonic CR-APA, whereby a CPA site is located within the coding region itself, composite intronic CR-APA, where the CPA site is located within an intron and can arise when a 5′-splice site becomes silent, and skipped intronic CR-APA, where alternative splicing results in the inclusion of a separate terminal exon containing a CPA site [1] (Figure 1).

Current opinions on the importance of APA

APA was described as early as 1980, with initial studies identifying CR-APA in the immunoglobulin heavy chain *IgM* gene, which leads to the production of both membrane-bound and secreted IgM [2]. Individual gene studies have continued from the 1980s to date and describe a large number of APA events which result in the production of APA isoforms with either differential mRNA stability [3,4] or encoding different proteins [5,6]. Recent advances in deep sequencing technologies have allowed this analysis to move to a global scale, with a plethora of techniques capable of quantitatively sequencing solely the terminal 3′-ends of polyadenylated transcripts [7–11].

These techniques have been used for both the mapping of CPA sites within an organism and quantitatively measuring the relative usage of CPA sites between different cell states or cell types. Mapping studies have found that ∼70 % of all human genes contain multiple CPA sites in their 3′-UTRs [12], and large-scale shifts in these observed APA

Key words: alternative cleavage and polyadenylation, cleavage, 3′-end processing, gene expression, post-transcriptional regulation, RNA metabolism.

Abbreviations: APA, alternative cleavage and polyadenylation; aUTR, alternative UTR; CFI_m, mammalian cleavage factor 1; CPA, cleavage and polyadenylation; CPEB1, cytoplasmic polyadenylation-binding protein 1; CR-APA, coding-region APA; MGMT, *O*-6-methylguanine-DNA methyltransferase; SERT, serotonin transporter; UTR-APA, untranslated region APA.

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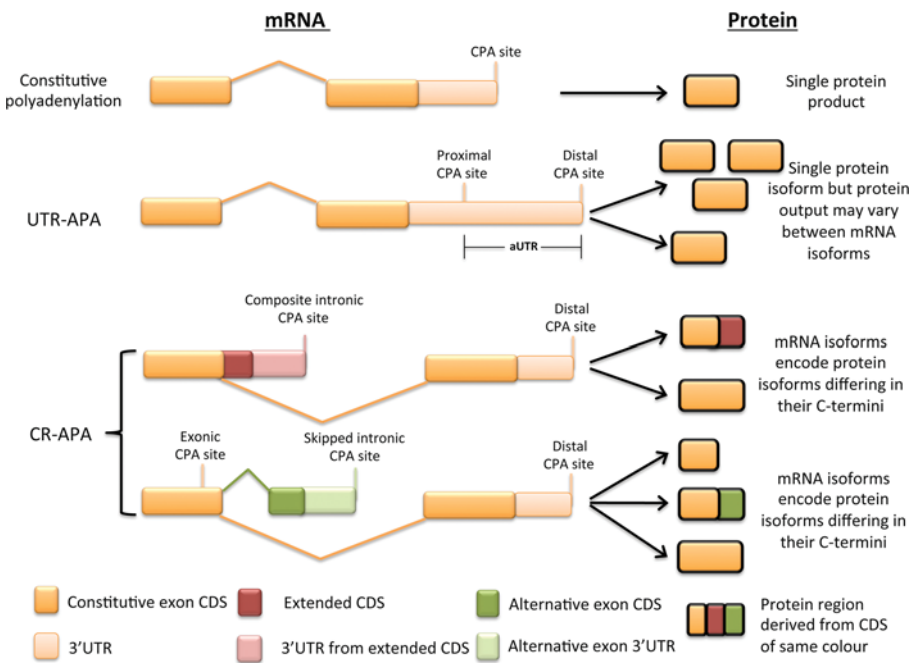
Figure 1 | Schematic representation of types of polyadenylation

Top: constitutive polyadenylation, which occurs when a gene contains a single CPA site, resulting in one mRNA isoform and one protein isoform. Middle: UTR-APA, which occurs when several CPA sites occur within the same 3′-UTR, resulting in several APA mRNA isoforms. Since these mRNA isoforms only differ in their 3′-UTR length, they encode the same protein, but may have altered mRNA stability or translational efficiency, potentially resulting in differential protein output. The region located between the proximal and distal CPA sites is known as the aUTR. Bottom: CR-APA, which occurs when a CPA site is located upstream of the terminal 3′-UTR. This can occur upon intron retention, in the case of composite-intronic CR-APA, or within an alternatively spliced exon in the case of skipped-intronic CR-APA. Exonic CR-APA occurs when a CPA site is located within the coding sequence (CDS) of a gene. CR-APA results in either C-terminus truncation of the resulting protein, in the case of exonic APA, or a different C-terminus, in both composite and skipped intronic CR-APA. Based on data from [52].



profiles have been seen between tissues [12–14], in cancer [15–18], cellular proliferation [19] and differentiation [20,21]. From these observations, it was extrapolated that global APA may contribute significantly to the establishment of the appropriate gene expression profiles associated with different cell states and disease phenotypes.

These bioinformatic studies provide invaluable data to help us to understand the key concepts behind APA. However, one crucial caveat of these studies is that it is difficult to distinguish cause from consequence. The fact that these shifts occur does not necessarily qualify them as key driver events. Furthermore, concerns have recently been raised that global studies may focus too much on the bioinformatic correlations and give too little consideration to their biological significance [22].

Evidence for effects of UTR-APA on transcript stability

In UTR-APA, the lengthening or shortening of the 3′-UTR through APA can potentially affect the post-transcriptional regulation of the APA mRNA isoforms through altering their relative stability, localization and translational efficiency. As the 3′-UTR is generally considered to have an overall repressive nature [16], use of the proximal CPA site was originally thought to result in increased mRNA stability.

This principle has been demonstrated directly in quiescent muscle stem cells, where 3′-UTR shortening by APA in the myogenic regulator *PAX3* mRNA results in the removal of an *miR-206* target site and consequently enables *PAX3* to escape *miR-206*-mediated repression [23]. Conversely, 3′-UTR lengthening in the DNA repair enzyme *MGMT* (*O*-6-methylguanine-DNA methyltransferase) in glioblastomas serves as an example of where 3′-UTR lengthening results in the inclusion of several miRNA-target sites and the subsequent repression of *MGMT* expression [4].

Although many of these individual events have been shown to be physiologically significant, the pervasiveness of UTR-APA effecting stabilization on a global scale is much less evident. If the large-scale shifts in UTR-APA described in the numerous global studies performed were to have a significant impact on the RNA stability, it would be expected that the overall mRNA abundances of these genes would either increase in the case of 3′-UTR shortening or decrease on

3′-UTR lengthening. However, this expected pattern is not consistently seen. De Klerk and Venema [24] found that ∼45 % of genes that showed general 3′-UTR shortening were accompanied by changes in transcript levels, but only a slight bias towards overall up-regulation is observed. Similarly, in several other studies only modest, if any, changes in transcript levels were observed in the genes that show a shift in CPA site choice [16,25,26].

Finally, a recent analysis that directly assesses the relative mRNA stabilities of APA isoforms globally draws similar conclusions. In NIH 3T3 mouse fibroblast cells, of 3463 detected genes that produce several APA isoforms, only approximately 500 differ in relative mRNA stability [27]. Interestingly, in ∼40 % of these APA events where significant changes in stability are seen, the longer isoform is actually more stable than the shorter transcript, suggesting the presence of stabilizing *cis*-elements in the aUTR. A similar association between longer UTRs and increased stability has also been described in human adipocyte stem cells [28]. Global studies in yeast, however, suggest that here APA can have a more profound impact on the stability of transcript isoforms, even in isoforms differing by a single nucleotide [29,30].

Nevertheless, in mammalian cells and tissues, even though APA is widespread, relatively few events appear to have a significant impact on overall mRNA stability. Furthermore, there is little evidence that these changes in mRNA isoform stability affect the final protein output to a point that could have a physiological impact.

Differential translational efficiencies of APA mRNA isoforms

As with stability, a number of studies have identified examples where APA events lead to alteration in translational efficiency [15,31–34]. With the help of reporter assays, protein output of short and long UTRs of *CCND2* (cyclin D2), *IMP-1*(insulin-like growth factor 2 mRNA-binding protein 1) and *DICER1* were compared and 3′-UTR shortening was found to be generally associated with increased translational efficiency, and thus increased expression [15]. However, this view has become more complex in recent years as opposite effects have also been seen where the distally polyadenylated transcript is actually translated with a higher efficiency [27,31,33].

One of the most well characterized examples of the latter is the anxiety-related gene *SERT* (serotonin transporter). Here, hnRNPK (heterogeneous nuclear ribonucleoprotein K) binding in the aUTR of the distally polyadenylated *SERT* transcript prevents *miR-16* binding and promotes the translation of the distal isoform relative to the proximal isoform [31]. A similar situation is also seen in the *Drosophila* cell cycle gene *polo*, where its longer APA isoform is translated at three times the rate of the shorter isoform [33].

More global studies have found similar conflicting trends regarding correlations between APA mRNA isoforms and translational efficiency. Studies in HEK (human embryonic kidney)-293T cells show a bias towards a greater polyribosomal association of the shorter UTR-APA mRNA isoforms [35]. This is somewhat in contrast with a recent study using ribosomal profiling assays performed in mouse NIH 3T3 cells, where increases in ribosomal association of the distal APA isoform relative to their proximal counterpart were seen [27]. However, these effects were minimal, with a median <3 % increase in ribosomal association of the distally polyadenylated transcript. This latter study concludes that the large majority of the APA events are unlikely to have a physiologically significant effect on either transcript stability or translational efficiency, at least in NIH 3T3 cells.

Differential localization of APA mRNA isoforms

Only a small number of examples that demonstrate a role of UTR-APA in regulating the subcellular localization of mRNA isoforms have been presented to date. Inclusion of inverted *Alu* repeats in the distal APA isoform of *NICN1* (nicolin 1) has been shown to lead to its nuclear retention, whereas the proximal APA isoform, which lacks these sequences, is freely exported to the cytoplasm [36]. The general role of 3′-UTRs in the localization of mRNAs in highly polarized cells is well known [37], and differential localization of APA mRNA isoforms in neurons has been reported. aUTRs play a critical role in regulating the localized translation of *BDNF* (brain-derived neurotrophic factor) APA mRNA isoforms in neurons where the distal APA isoform localizes to the dendrites and the proximal APA isoform is restricted to the somata [38]. 3′-UTR lengthening in the brain is one of the most striking examples of tissue-specific APA [13], which may serve as a platform to control the localization of these transcripts. However, to date, there have been no global studies that look at the effect of APA on subcellular localization so, although it appears that APA may control differential APA isoform localization in the brain, its prevalence is currently unknown.

Conservation of APA

Comparative genomics has been used in a number of studies to assess the conservation of APA between species. Global features, such as the general sequence composition surrounding the cleavage site and the presence of multiple CPA sites at a significant portion of genes, are conserved between humans and mice [39]. However, when the conservation of individual CPA sites between mice and humans are considered, only ∼500 genes contain conserved tandem CPA sites, and consequently it was suggested that "in general, alternative poly(A) sites are species-specific and involve minor, non-conserved sites that are unlikely to play essential roles" [40].

Derti et al. [12] found a similar lack of conservation of CPA sites. Of the 439390 CPA sites that they identified in humans, only 2590 (0.6 %) were found to have CPA sites within the equivalent 30 base region in all four other mammals considered (rhesus monkey, dog, mouse and rat)

[12]. Interestingly, it was found that, of the conserved CPA sites, the read numbers were more similar in equivalent tissues across species, rather than between tissues in the same species [12]. However, this approach is looking at absolute read numbers over individual CPA sites and not looking at the relative usage of tandem CPA sites, so this may simply reflect a conservation of tissue-specific gene expression rather than the conservation of APA patterns itself.

Of the tandem CPA sites that are conserved, it is the distal CPA sites that are conserved at a higher level than the proximal CPA sites [41], and, in general, it is these sites that have the highest processing efficiency [42]. This may therefore suggest a mechanism by which the distal CPA site serves as the default CPA site and functions to ensure efficient 3′-end processing and transcription termination, whereas the proximal CPA site functions as a regulatory site that can potentially be activated to remove any downstream regulatory *cis*-elements.

Conclusions and perspectives

With the recent wave of global studies, APA is now considered to be a key regulator of gene expression, but there are several issues that need to be addressed in future studies. Most importantly, more direct physiological evidence needs to be presented that supports the proposed global role of APA in establishing tissue- and proliferation-specific gene expression signatures.

A clear distinction needs to be drawn between cause and effect of APA. Since APA mRNA isoforms potentially have various levels of stability, observed shortening of 3′-UTRs could be the result of two separate mechanisms: (i) a change in CPA site choice, resulting in an increased production of the shorter transcript, or (ii) an increase in post-transcriptional regulation targeted to the aUTR, which would result in the selected degradation of solely the longer transcript [43]. This introduces potential complications in studies that aim to address the mechanism of APA regulation at the point of CPA site choice.

It is still largely unclear how APA is regulated and how CPA site choice is determined. This is highly critical in order to distinguish between stochastic events that may result from fluctuations in the levels of key CPA factors and APA events that are indeed the result of targeted regulation. A number of knockdown studies have identified several key polyadenylation and splicing factors whereby APA profiles are altered by their depletion, notably CFI_m68 (mammalian cleavage factor 1 of 68 kDa) [41], CFI_m25 (mammalian cleavage factor 1 of 25 kDa) [44,45], PABPN1 [poly(A)-binding protein nuclear 1] [46] and U1 snRNP (small nuclear ribonucleoprotein) [47]. However, linking these potential regulators to physiological processes that are driven by changes in CPA site choice is far from complete. It is not clear how regulation of APA through such global factors could achieve specificity.

However, it is important to note that mechanisms of APA regulation by several more specific RNA-binding factors are emerging, such as the case for CPEB1 (cytoplasmic polyadenylation-binding protein 1) [48]. This RNA-binding protein has been shown to shuttle to the nucleus, where, through its co-localization with splicing factors, it regulates the 3′-UTR shortening of, specifically, mRNAs which contain CPEs (cytoplasmic polyadenylation elements), the CPEB1-binding sites [48]. Similarly, the cold-induced RNA-binding proteins Cirbp and Rbm3 have also been linked to the control of circadian gene expression through the modulation of APA events [49]. Furthermore, the RNA-binding proteins FPA and FCA have been shown to play an essential role in the control of seasonal flowering in *Arabidopsis* through the modulation of 3′-end formation [50].

There is no doubt that APA can play a key regulatory role in contributing to the control of gene expression. The most compelling evidence that modulation of APA can indeed affect gene expression in a physiological context is from a recent study where CFI_m25 levels in glioblastomas were found to influence the 3′-UTR length at a subset of genes and ultimately glioblastoma tumorigenicity [45].

However, the current view that APA plays a global role in regulating gene expression in association with tissue specificity and proliferation has been seriously challenged [27]. Furthermore, considering that, on average, >90 % of overall reads come from the dominant (most utilized) CPA site of a gene [12], it is challenging to explain how minor changes in stability and translational efficiency of mRNA isoforms may be of biological significance.

Perhaps APA will follow a similar path to alternative splicing, whereby even though >75 % of genes are capable of alternative splicing, most of these events may be a result of stochastic noise and potentially of no functional significance [51]. Overall, future studies on global APA analysis should concentrate more on the biological significance of observed APA events, rather than statistical significance. Only then will we uncover the true physiological consequences of APA.

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References

1 Edwalds-Gilbert, G., Veraldi, K.L. and Milcarek, C. (1997) Alternative poly(A) site selection in complex transcription units: means to an end? Nucleic Acids Res. **25**, 2547–2561 CrossRef PubMed

2 Alt, F.W., Bothwell, A.L., Knapp, M., Siden, E., Mather, E., Koshland, M. and Baltimore, D. (1980) Synthesis of secreted and membrane-bound immunoglobulin mu heavy chains is directed by mRNAs that differ at their 3′ ends. Cell **20**, 293–301 CrossRef PubMed

3 Lazarov, M.E., Martin, M.M., Willardson, B.M. and Elton, T.S. (1999) Human phosducin-like protein (hPhLP) messenger RNA stability is regulated by *cis*-acting instability elements present in the 3′-untranslated region. Biochim. Biophys. Acta **1446**, 253–264 CrossRef PubMed

4 Kreth, S., Limbeck, E., Hinske, L.C., Schütz, S.V., Thon, N., Hoefig, K., Egensperger, R. and Kreth, F.W. (2013) In human glioblastomas transcript elongation by alternative polyadenylation and miRNA targeting is a potent mechanism of *MGMT* silencing. Acta Neuropathol. **125**, 671–681 CrossRef PubMed

5 Helfman, D. and Cheley, S. (1986) Nonmuscle and muscle tropomyosin isoforms are expressed from a single gene by alternative RNA splicing and polyadenylation. Mol. Cell. Biol. **6**, 3582–3595 PubMed

6 Flavell, S.W., Kim, T.-K., Gray, J.M., Harmin, D.A., Hemberg, M., Hong, E.J., Markenscoff-Papadimitriou, E., Bear, D.M. and Greenberg, M.E. (2008) Genome-wide analysis of MEF2 transcriptional program reveals synaptic target genes and neuronal activity-dependent polyadenylation site selection. Neuron **60**, 1022–1038 CrossRef PubMed

7 Haenni, S., Ji, Z., Hoque, M., Rust, N., Sharpe, H., Eberhard, R., Browne, C., Hengartner, M.O., Mellor, J., Tian, B. and Furger, A. (2012) Analysis of *C. elegans* intestinal gene expression and polyadenylation by fluorescence-activated nuclei sorting and 3′-end-seq. Nucleic Acids Res. **40**, 6304–6318 CrossRef PubMed

8 Hoque, M., Ji, Z., Zheng, D., Luo, W., Li, W., You, B., Park, J.Y., Yehia, G. and Tian, B. (2013) Analysis of alternative cleavage and polyadenylation by 3′ region extraction and deep sequencing. Nat. Methods **10**, 133–139 CrossRef PubMed

9 Beck, A.H., Weng, Z., Witten, D.M., Zhu, S., Foley, J.W., Lacroute, P., Smith, C.L., Tibshirani, R., van de Rijn, M., Sidow, A. and West, R.B. (2010) 3′-end sequencing for expression quantification (3SEQ) from archival tumor samples. PLoS ONE **5**, e8768 CrossRef PubMed

10 Ozsolak, F., Kapranov, P., Foissac, S., Kim, S.W., Fishilevich, E., Monaghan, A.P., John, B. and Milos, P.M. (2010) Comprehensive polyadenylation site maps in yeast and human reveal pervasive alternative polyadenylation. Cell **143**, 1018–1029 CrossRef PubMed

11 Ma, L., Pati, P.K., Liu, M., Li, Q.Q. and Hunt, A.G. (2014) High throughput characterizations of poly(A) site choice in plants. Methods **67**, 74–83 CrossRef PubMed

12 Derti, A., Garrett-Engele, P., Macisaac, K.D., Stevens, R.C., Sriram, S., Chen, R., Rohl, C.A., Johnson, J.M. and Babak, T. (2012) A quantitative atlas of polyadenylation in five mammals. Genome Res. **22**, 1173–1183 CrossRef PubMed

13 Miura, P., Shenker, S., Andreu-Agullo, C., Westholm, J.O. and Lai, E.C. (2013) Widespread and extensive lengthening of 3′ UTRs in the mammalian brain. Genome Res. **23**, 812–825 CrossRef PubMed

14 Lianoglou, S., Garg, V., Yang, J.L., Leslie, C.S. and Mayr, C. (2013) Ubiquitously transcribed genes use alternative polyadenylation to achieve tissue-specific expression. Genes Dev. **27**, 2380–2396 CrossRef PubMed

15 Mayr, C. and Bartel, D.P. (2009) Widespread shortening of 3′UTRs by alternative cleavage and polyadenylation activates oncogenes in cancer cells. Cell **138**, 673–684 CrossRef PubMed

16 Morris, A.R., Bos, A., Diosdado, B., Rooijers, K., Elkon, R., Bolijn, A.S., Carvalho, B., Meijer, G.A. and Agami, R. (2012) Alternative cleavage and polyadenylation during colorectal cancer development. Clin. Cancer Res. **18**, 5256–5266 CrossRef PubMed

17 Singh, P., Alley, T.L., Wright, S.M., Kamdar, S., Schott, W., Wilpan, R.Y., Mills, K.D. and Graber, J.H. (2009) Global changes in processing of mRNA 3′ untranslated regions characterize clinically distinct cancer subtypes. Cancer Res. **69**, 9422–9430 CrossRef PubMed

18 Lin, Y., Li, Z. and Ozsolak, F. (2012) An in-depth map of polyadenylation sites in cancer. Nucleic Acids Res. **40**, 8460–8471 CrossRef PubMed

19 Sandberg, R., Neilson, J.R., Sarma, A., Sharp, P.A. and Burge, C.B. (2008) Proliferating cells express mRNAs with shortened 3′ untranslated regions and fewer microRNA target sites. Science **320**, 1643–1647 CrossRef PubMed

20 Shepard, P.J., Choi, E.-A., Lu, J., Flanagan, L.A., Hertel, K.J. and Shi, Y. (2011) Complex and dynamic landscape of RNA polyadenylation revealed by PAS-Seq. RNA **17**, 761–772 CrossRef PubMed

21 Wang, L., Dowell, R.D. and Yi, R. (2013) Genome-wide maps of polyadenylation reveal dynamic mRNA 3′-end formation in mammalian cell lineages. RNA **19**, 413–425 CrossRef PubMed

22 Proudfoot, N.J. (2011) Ending the message: poly(A) signals then and now. Genes Dev. **25**, 1770–1782 CrossRef PubMed

23 Boutet, S.C., Cheung, T.H., Quach, N.L., Liu, L., Prescott, S.L., Edalati, A., Iori, K. and Rando, T.A. (2012) Alternative polyadenylation mediates microRNA regulation of muscle stem cell function. Cell Stem Cell **10**, 327–336 CrossRef PubMed

24 de Klerk, E. and Venema, A. (2012) Poly(A) binding protein nuclear 1 levels affect alternative polyadenylation. Nucleic Acids Res. **40**, 9089–9101 CrossRef PubMed

25 Elkon, R., Drost, J., van Haaften, G., Jenal, M., Schrier, M., Oude Vrielink, J.A. and Agami, R. (2012) E2F mediates enhanced alternative polyadenylation in proliferation. Genome Biol. **13**, R59 CrossRef PubMed

26 Fu, Y., Sun, Y., Li, Y., Li, J., Rao, X., Chen, C. and Xu, A. (2011) Differential genome-wide profiling of tandem 3′ UTRs among human breast cancer and normal cells by high-throughput sequencing. Genome Res. **21**, 741–747 CrossRef PubMed

27 Spies, N., Burge, C. and Bartel, D. (2013) 3′ UTR-isoform choice has limited influence on the stability and translational efficiency of most mRNAs in mouse fibroblasts. Genome Res. **23**, 2078–2090 CrossRef PubMed

28 Spangenberg, L., Shigunov, P., Abud, A.P.R., Cofré, A.R., Stimamiglio, M.A., Kuligovski, C., Zych, J., Schittini, A. V, Costa, A.D.T., Rebelatto, C.K. et al. (2013) Polysome profiling shows extensive posttranscriptional regulation during human adipocyte stem cell differentiation into adipocytes. Stem Cell Res. **11**, 902–912 CrossRef PubMed

29 Geisberg, J.V., Moqtaderi, Z., Fan, X., Ozsolak, F. and Struhl, K. (2014) Global analysis of mRNA isoform half-lives reveals stabilizing and destabilizing elements in yeast. Cell **156**, 812–824 CrossRef PubMed

30 Gupta, I., Clauder-Münster, S., Klaus, B., Järvelin, A.I., Aiyar, R.S., Benes, V., Wilkening, S., Huber, W., Pelechano, V. and Steinmetz, L.M. (2014) Alternative polyadenylation diversifies post-transcriptional regulation by selective RNA–protein interactions. Mol. Syst. Biol. **10**, 719 CrossRef PubMed

31 Yoon, Y., McKenna, M.C., Rollins, D.A., Song, M., Nuriel, T., Gross, S.S., Xu, G. and Glatt, C.E. (2013) Anxiety-associated alternative polyadenylation of the serotonin transporter mRNA confers translational regulation by hnRNPK. Proc. Natl. Acad. Sci. U.S.A. **110**, 11624–11629 CrossRef PubMed

32 Tranter, M., Helsley, R.N., Paulding, W.R., McGuinness, M., Brokamp, C., Haar, L., Liu, Y., Ren, X. and Jones, W.K. (2011) Coordinated post-transcriptional regulation of *Hsp70.3* gene expression by microRNA and alternative polyadenylation. J. Biol. Chem. **286**, 29828–29837 CrossRef PubMed

33 Pinto, P.A.B., Henriques, T., Freitas, M.O., Martins, T., Domingues, R.G., Wyrzykowska, P.S., Coelho, P.A., Carmo, A.M., Sunkel, C.E., Proudfoot, N.J. and Moreira, A. (2011) RNA polymerase II kinetics in polo polyadenylation signal selection. EMBO J. **30**, 2431–2444 CrossRef PubMed

34 Akman, B.H., Can, T. and Erson-Bensan, A.E. (2012) Estrogen-induced upregulation and 3′-UTR shortening of CDC6. Nucleic Acids Res. **40**, 10679–10688 CrossRef PubMed

35 Sterne-Weiler, T. and Martinez-Nunez, R. (2013) Frac-seq reveals isoform-specific recruitment to polyribosomes. Genome Res. **23**, 1615–1623 CrossRef PubMed

36 Chen, L.-L., DeCerbo, J.N. and Carmichael, G.G. (2008) *Alu* element-mediated gene silencing. EMBO J. **27**, 1694–1705 CrossRef PubMed

37 Andreassi, C. and Riccio, A. (2009) To localize or not to localize: mRNA fate is in 3′UTR ends. Trends Cell Biol. **19**, 465–474 CrossRef PubMed

38 An, J.J., Gharami, K., Liao, G.-Y., Woo, N.H., Lau, A.G., Vanevski, F., Torre, E.R., Jones, K.R., Feng, Y., Lu, B. and Xu, B. (2008) Distinct role of long 3′ UTR *BDNF* mRNA in spine morphology and synaptic plasticity in hippocampal neurons. Cell **134**, 175–187 CrossRef PubMed

39 Tian, B., Hu, J., Zhang, H. and Lutz, C.S. (2005) A large-scale analysis of mRNA polyadenylation of human and mouse genes. Nucleic Acids Res. **33**, 201–212 CrossRef PubMed

40 Ara, T., Lopez, F., Ritchie, W., Benech, P. and Gautheret, D. (2006) Conservation of alternative polyadenylation patterns in mammalian genes. BMC Genomics **7**, 189 CrossRef PubMed

41 Martin, G., Gruber, A.R., Keller, W. and Zavolan, M. (2012) Genome-wide analysis of pre-mRNA 3′ end processing reveals a decisive role of human cleavage factor I in the regulation of 3′ UTR length. Cell Rep. **1**, 753–763 CrossRef PubMed

42 Beaudoing, E. (2000) Patterns of variant polyadenylation signal usage in human genes. Genome Res. **10**, 1001–1010 CrossRef PubMed

43 Liaw, H.-H., Lin, C.-C., Juan, H.-F. and Huang, H.-C. (2013) Differential microRNA regulation correlates with alternative polyadenylation pattern between breast cancer and normal cells. PLoS ONE **8**, e56958 CrossRef PubMed

44 Kubo, T., Wada, T., Yamaguchi, Y., Shimizu, A. and Handa, H. (2006) Knock-down of 25 kDa subunit of cleavage factor I_m in HeLa cells alters alternative polyadenylation within 3′-UTRs. Nucleic Acids Res. **34**, 6264–6271 CrossRef PubMed

45 Masamha, C.P., Xia, Z., Yang, J., Albrecht, T.R., Li, M., Shyu, A.-B., Li, W. and Wagner, E.J. (2014) CFI_m25 links alternative polyadenylation to glioblastoma tumour suppression. Nature **509**, 412–416 PubMed
46 Jenal, M., Elkon, R., Loayza-Puch, F., van Haaften, G., Kühn, U., Menzies, F.M., Oude Vrielink, J.A.F., Bos, A.J., Drost, J., Rooijers, K. et al. (2012) The poly(A)-binding protein nuclear 1 suppresses alternative cleavage and polyadenylation sites. Cell **149**, 538–553 CrossRef PubMed
47 Kaida, D., Berg, M.G., Younis, I., Kasim, M., Singh, L.N., Wan, L. and Dreyfuss, G. (2010) U1 snRNP protects pre-mRNAs from premature cleavage and polyadenylation. Nature **468**, 664–668 CrossRef PubMed
48 Bava, F.-A., Eliscovich, C., Ferreira, P.G., Miñana, B., Ben-Dov, C., Guigó, R., Valcárcel, J. and Méndez, R. (2013) CPEB1 coordinates alternative 3′-UTR formation with translational regulation. Nature **495**, 121–125 CrossRef PubMed
49 Liu, Y., Hu, W., Murakawa, Y., Yin, J., Wang, G., Landthaler, M. and Yan, J. (2013) Cold-induced RNA-binding proteins regulate circadian gene expression by controlling alternative polyadenylation. Sci. Rep. **3**, 1–11
50 Hornyik, C., Terzi, L.C. and Simpson, G.G. (2010) The spen family protein FPA controls alternative cleavage and polyadenylation of RNA. Dev. Cell **18**, 203–213 CrossRef PubMed
51 Melamud, E. and Moult, J. (2009) Stochastic noise in splicing machinery. Nucleic Acids Res. **37**, 4873–4886 CrossRef PubMed
52 Di Giammartino, D.C., Nishida, K. and Manley, J.L. (2011) Mechanisms and consequences of alternative polyadenylation. Mol. Cell **43**, 853–866 CrossRef PubMed

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