Turkish Journal of Biology

Volume 40 | Number 3 Article 1

1-1-2016

Mechanisms of mRNA polyadenylation

HIZLAN HINCAL AĞUŞ

AYŞE ELİF ERSON BENSAN

TÜBİTAK

Turkish Journal of Biology

http://journals.tubitak.gov.tr/biology/

Review Article

Turk J Biol
(2016) 40: 529-538
© TÜBİTAK
doi:10.3906/biy-1505-94

Mechanisms of mRNA polyadenylation

Hızlan Hıncal AĞUŞ, Ayşe Elif ERSON BENSAN*
Department of Biology, Arts and Sciences, Middle East Technical University, Ankara, Turkey

Received: 26.05.2015 • **Accepted/Published Online:** 21.08.2015 • **Final Version:** 18.05.2016

Abstract: mRNA 3'-end processing involves the addition of a poly(A) tail based on the recognition of the poly(A) signal and subsequent cleavage of the mRNA at the poly(A) site. Alternative polyadenylation (APA) is emerging as a novel mechanism of gene expression regulation in normal and in disease states. APA results from the recognition of less canonical proximal or distal poly(A) signals leading to changes in the 3' untranslated region (UTR) lengths and even in some cases changes in the coding sequence of the distal part of the transcript. Consequently, RNA-binding proteins and/or microRNAs may differentially bind to shorter or longer isoforms. These changes may eventually alter the stability, localization, and/or translational efficiency of the mRNAs. Overall, the 3' UTRs are gaining more attention as they possess a significant posttranscriptional regulation potential guided by APA, microRNAs, and RNA-binding proteins. Here we provide an overview of the recent developments in the APA field in connection with cancer as a potential oncogene activator and/or tumor suppressor silencing mechanism. A better understanding of the extent and significance of APA deregulation will pave the way to possible new developments to utilize the APA machinery and its downstream effects in cancer cells for diagnostic and therapeutic applications.

Key words: Alternative polyadenylation, mRNA, untranslated region, cancer, cleavage stimulatory factor, cleavage and polyadenylation stimulatory factor

1. Introduction

Polyadenylation, a cotranscriptional process, was first identified in the nuclear extracts of calf thymus as early as the 1960s (Edmonds and Abrams, 1960). All eukaryotic pre-mRNAs undergo polyadenylation, i.e. cleavage and polyadenylation at the 3' untranslated region (UTR), except replication-dependent histone transcripts that are processed by small nuclear ribonucleoproteins (Marzluff et al., 2008).

In essence, a poly(A) tail is added to the 3' UTR of newly synthesized pre-mRNAs by the poly(A) polymerase, followed by the recognition of the poly(A) signal and endonucleolytic cleavage of the pre-mRNA at the poly(A) site. Conserved upstream elements (U-rich elements, UGUA elements) found at ~10–35 nucleotides upstream of the poly(A) site aid the poly(A) signal (AAUAAA, AUUAAA, and other variants) selection. Downstream elements are the U-rich and GU-rich elements (Tian and Graber, 2012). Polyadenylation prolongs mRNA lifespan by protecting the 3' downstream sequences against exosome nucleases and plays roles in export to cytosol, localization, stability, and translation (reviewed by Guhaniyogi and Brewer, 2001; Lutz and Moreira, 2011). Given these functional roles of polyadenylation, variations in 3' UTR processing mechanisms such as alternative polyadenylation (APA) are to be expected to affect the length/reading frame, stability, localization, and translational efficiency (Lutz and Moreira, 2011; Elkon et al., 2013). As polyadenylation is a cotranscriptional process, it is also plausible to consider mRNA processing steps to affect and/or regulate polyadenylation efficiency and specificity (Proudfoot et al., 2002).

While the extent of APA is better understood today with improvements in high-throughput sequencing techniques, isolated cases of APA events were reported much earlier, in the 1980s, for the *IgM* (immunoglobulin M) and *DHFR* (dihydrofolate reductase) genes. Intronic APA-generated isoforms of *IgM* were found to encode two distinct proteins, secreted if the proximal APA site is used or membrane-bound if the distal site is used (Alt et al., 1980; Early et al., 1980; Rogers et al., 1980). *DHFR*, on the other hand, has four isoforms with identical reading frames but different 3' UTR lengths (Setzer et al., 1980). Since then, an improved understanding of the extent of APA events in the genome suggests an impressive 70% of known human genes to harbor multiple poly(A) sites in their 3' UTRs and high conservation of APA sites among different species (Derti et al., 2012).

* Correspondence: erson@metu.edu.tr

While the role of APA-based isoform generation has been linked to normal processes, findings suggest APA to be a novel and important deregulated mechanism in diseases, especially cancer. In this review, we aim to provide an overview of current data on APA and known consequences of altered 3' UTR lengths during the complex events of tumorigenesis.

2. Polyadenylation mechanisms

To better understand the complexity of polyadenylation and APA, proteins known to be involved in these processes will be described with potential roles in cancer development when their expressions are altered. Pre-mRNA 3' UTRs have long been known to be important for the nuclear export, stability, and translational efficiency of mRNAs. With recent developments, 3' UTR-based posttranscriptional processing mechanisms are gaining attention as important modulators of gene expression. APA is an example of how much complexity exists for posttranscriptional regulation, specifically for 3'-end processing. So far, we know that the 3'-end processing machinery is quite complex, comprising approximately 85 proteins, some of which are known to be core elements whereas others may mediate crosstalk with other processes including splicing (Shi et al., 2009). Not surprisingly, the polyadenylation complex is quite large and has a comparable size similar to that of bacterial large ribosomal subunits (1.5 MDa) (Radermacher et al., 1987).

The polyadenylation complex consists of cleavage and polyadenylation stimulatory factor (CPSF), cleavage stimulatory factor (CSTF), cleavage factor Im (CFIm), and cleavage factor IIm (CFIIm), along with the scaffold protein symplekin and poly(A) polymerase (PAP). Known key APA machinery proteins are summarized in Figure. 1.

The most conserved motif among eukaryotic mRNA polyadenylation signals is AAUAAA, specifically recognized by the multisubunit CPSF complex and therefore providing sequence specificity in the pre-mRNA cleavage site and polyadenylation. CPSF consists of hFip1, WDR33 (WD repeat domain 33), CPSF30, CPSF73, CPSF100, and CPSF160 subunits (Millevoi and Wagner, 2010). CPSF subunits CPSF30 and WDR33 directly contact the AAUAAA region (Chan et al., 2014; Schönemann et al., 2014) and the CPSF73 subunit is the endonuclease positioned on the cleavage site (Mandel et al., 2006). PAP associates with cleavage and polyadenylation specificity factors CPSF160 and CPSF73 subunits and also with the targeted pre-mRNA (Laishram and Anderson, 2010). Evidence suggests the CPSF complex to also crosstalk with transcriptional initiation and elongation steps through interactions with transcription factor IID (TFIID), RNA polymerase II (Dantonel et al., 1997; Nag et al., 2007; Glover-Cutter et al., 2008), and splicing machinery through spliceosomal factor TRAP150 (Kwon et al., 2014).

Another complex, CSTF, recognizes U- and GU-rich sequences and is composed of 3 subunits: CSTF1 (50 kDa), CSTF2 (64 kDa), and CSTF3 (77 kDa) (Shi et al., 2009; Millevoi and Wagner, 2010). CSTF2 (also known as CSTF64) has 557 amino acid residues, including an RNA-binding domain and a pentapeptide repeat region that appears to be necessary for cleavage and polyadenylation (Takagaki et al., 1992; Takagaki and Manley, 1997). While CSTF2 interacts directly with the GU-rich region downstream of the cleavage site (Takagaki and Manley, 2000; Yao et al., 2012), CSTF1 and CSTF3 subunits interact with the C-terminal domain of RNA polymerase II, possibly to facilitate activation of 3' UTR processing proteins (Cevher et al., 2010). Among CSTF subunits, CSTF64 has gained special attention due to its potential role in the selection of alternate (proximal or distal) poly(A) signals. A transcriptome study reported the depletion of CSTF64 (and its paralogue, CSTF64τ) to result in increased usage of distal poly(A) sites in HeLa cells, indicating that, when present, these factors increase the selection of proximal sites, leading to shorter isoform generation (Yao et al., 2012). Interestingly, CSTF64 was also proposed to play a key role in modulating the cell cycle in normal and in embryonic stem cells while also simultaneously controlling histone mRNA 3'-end processing (Romeo et al., 2014).

The UGUA upstream element provides binding sites for the CFI complex (CFIm25, CFIm59, and CFIm68), which facilitates the binding of CPSF (Fip1 subunit) to the UTR (Coseno et al., 2008; Yang et al., 2010). The tetrameric complex consists of the two 25-kDa subunits partnering with either the 59- or 68-kDa subunits (Martin et al., 2010). Structural studies revealed that the two CFIm complexes bind to two UGUA elements in an antiparallel manner and provide the looping of RNA (Li et al., 2011; Yang et al., 2011). It appears that the CFI complex is important for poly(A) site selection as, for several genes, the sites shifted to distal ones when CFIm25 (also known as *NUDT21*) levels were reduced by siRNA in HeLa cells (Kubo et al., 2006; Fukumitsu et al., 2012; Gruber et al., 2012; Martin et al., 2012). In addition, loss of function of CFIm68 and CFIm25 but not of CFIm59 leads to a transcriptome-wide increase in the use of proximal polyadenylation sites in HEK293 cells (Gruber et al., 2012; Martin et al., 2012). CFI proteins are also likely to function in splicing but have different roles as CFIm59 but not CFIm68 was found to interact with splicing factor U2AF65 (Millevoi et al., 2006), whereas CFIm68 interacts with SR proteins (Dettwiler et al., 2004). CFIm68 can also shuttle between the nucleus and cytoplasm during the cell cycle (Cardinale et al., 2007) and possibly participates in mRNA export (Ruepp et al., 2009).

While the detailed mechanisms needs to be further studied, the CFIIm complex seems to be important for

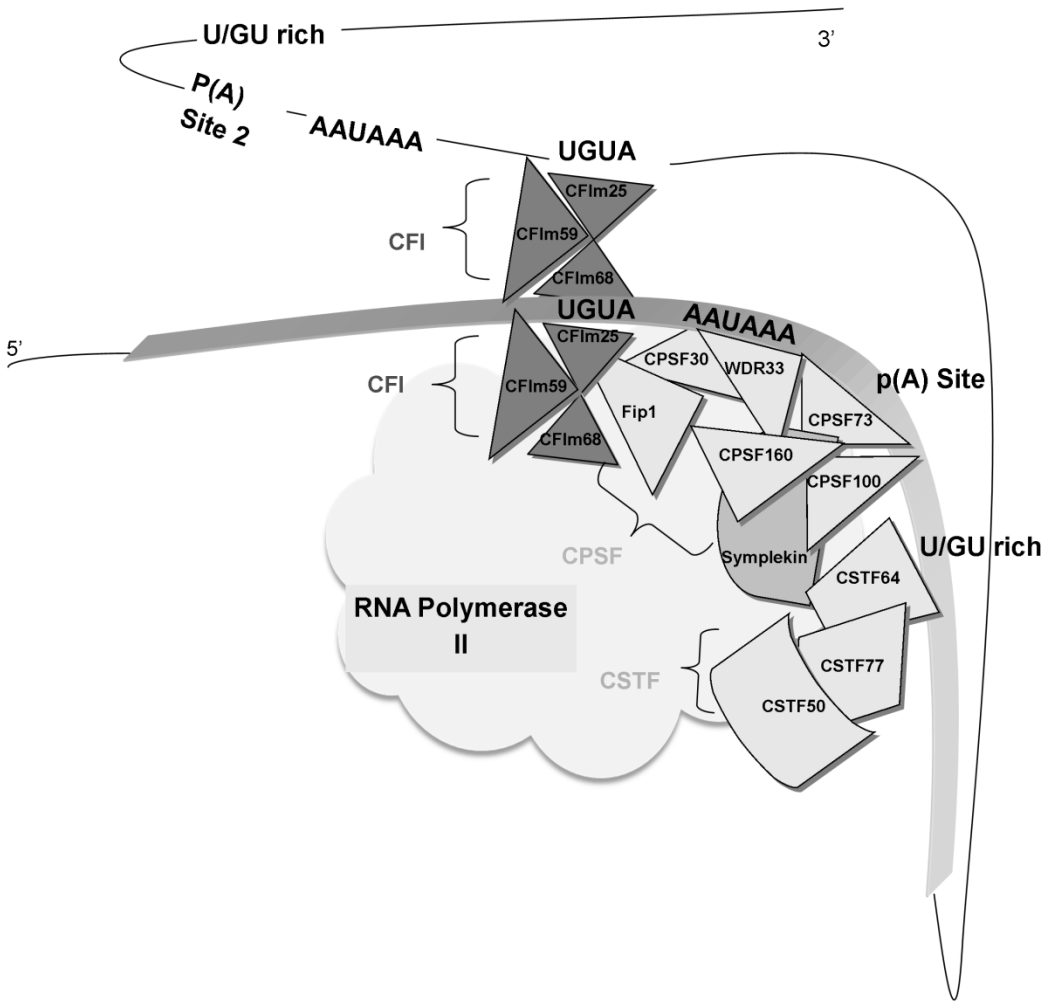

Figure 1. 3' UTR cleavage and polyadenylation machinery. CPSF complex consists of Fip1, WDR33, CPSF30, CPSF73, CPSF100, and CPSF160. Binding of CFI on UGUA upstream sequences facilitates assembly of CPSF complex on the cleavage site. Two CFIm complexes bind to two UGUA elements in an antiparallel manner and provide the looping of RNA. CPSF subunits CPSF30 and WDR33 directly contact the AAUAAA region and CPSF160 and CPSF73 interact with poly(A) polymerase (PAP) (not shown). Symplekin interacts with both CPSF73 and CSTF64 and provides a connection between polyadenylation factors. When recognized, the polyadenylation site (p(A) site) is cleaved by the endonucleolytic activity of CPSF73. CSTF77 acts as a bridge between CSTF64 and CSTF50. The interaction between CSTF50-CSTF77 and the C-terminal domain of RNA polymerase II probably facilitates activation of RNA polymerase II mediated 3'-end processing and assembly of other complexes. After cleavage, PAP starts to polymerize the adenine tail.

the efficient degradation of the 3' product of poly(A) site cleavage and transcriptional termination (West and Proudfoot, 2008). Symplekin, as an essential polyadenylation factor, was shown to bind to CPSF73 and CSTF64 at the early steps of the polyadenylation process, possibly to function as a scaffold protein (Barnard et al., 2004; Ruepp et al., 2010).

3. APA and cancer

Following the pivotal work of Sandberg et al. (2008), describing the increase in expression of shortened mRNA 3' UTR isoforms after activation of primary murine $CD4^+$ T lymphocytes, various other studies demonstrated APA changes in normal and/or cancer cells by delineating functional APA sites using EST databases, microarray data, and modified RNA-seq techniques. These studies provided a link between proliferation/APA and cancer, as well as between development and tissue-specific polyadenylation patterns. For example, transcripts in the nervous system and brain and during mouse embryonic development are very specifically characterized by distal poly(A) site usage, whereas induced pluripotent stem cells (iPSCs) from differentiated cells are accompanied by global 3' UTR shortening (Ji et al., 2009).

Given the specific APA patterns in normal cells, defective global alternative polyadenylation was reported for numerous cancers including colorectal carcinomas, esophageal cancers, breast cancers, and small intestinal neuroendocrine tumors, suggesting APA as a new target for both diagnosis and treatment options (Mayr and Bartel, 2009; Fu et al., 2011; Morris et al., 2012; Rehfeld et al., 2014; Sun et al., 2014). One of the recent findings on APA and tumorigenesis comes from glioblastomas. Approximately 1500 genes with shortened 3' UTRs were discovered after CFIm25 knockdown followed by a marked increase in the expression of several known oncogenes, including *CYCLIN D1*. Furthermore, a subset of APA-deregulated genes was discovered in the low-CFIm25-expressing glioblastomas (Masamha et al., 2014). A functional link between APA and glioblastoma was also reported based on downregulation of CFIm25 expression in glioblastoma cells and enhanced tumorigenic properties.

In addition to the high-throughput approaches demonstrating a global bias towards generation of short or long isoforms, individual mRNA-APA studies have also been imperative to improve the understanding of the significance of APA in cancers. For example, *ECE-1* (endothelin-converting enzyme-1) is responsible for producing active endothelin-1, an overexpressed mitogenic peptide in cancers especially associated with poor prognosis and aiding the acquisition of androgen independence in prostate cancers. APA results in the production of *ECE-1* transcripts with shorter 3' UTRs, which promote elevated protein expression as a means to activate oncogenes (Whyteside et al., 2014). In line with earlier findings, our group reported the role of hormones in APA, and specifically estradiol, in breast cancers to cause 3' UTR shortening of *CDC6*, possibly through E2Fs, transcription factors involved in cell cycle regulation. CDC6 itself is also a major regulator of the S phase and 3' UTR shortening of *CDC6* contributes to the consequent proliferation of ER(+) breast cancer cells in response to estradiol (Akman et al., 2012). In connection, earlier, a role for E2F was proposed for the transcriptional regulation of some APA proteins, providing a mechanistic link between APA and proliferation (Elkon et al., 2012). Another example important for cellular transformation processes is VEGFR2 (vascular endothelial growth factor receptor 2), controlled by intronic APA. The *VEGFR2* gene can transcribe a functional receptor using the distal poly(A) signal or a soluble decoy protein, if the proximal intronic poly(A) signal is preferred in connection with the splicing machinery (Vorlová et al., 2011). Furthermore, as VEGFR2 mediates important angiogenic signaling, inhibitory antisense oligonucleotides to the nearest upstream splicing site of the intronic poly(A) signal results in increased expression of the soluble form of VEGFR2 protein and blockade of angiogenic signals, which may indicate the potential therapeutic implication of APA (Vorlová et al., 2011).

Taken together, it is interesting to observe how APA may provide an additional level of gene expression control. However, our understanding of how and why a specific polyadenylation site is selected over another is far from complete. Transcriptional rate, levels of key poly(A) proteins, and/or alternate targeting of isoforms by either RNA-binding proteins (RBPs) and/or microRNAs are found to be contributing factors to APA. In essence, APA (and its regulation) can be affected by many internal and external factors such as proliferation signals, hormones, developmental stages, epigenetic modifications, and even UV-induced DNA damage (Kleiman and Manley, 2001). Potential internal and external regulatory signals for APA are shown in Figure. 2. Hence, we would like to a give an overview of reported APA events that are linked to the above cellular events given their significance in tumorigenesis.

4. Transcriptional kinetics

Several lines of evidence have linked APA to transcriptional activity (initiation or elongation). In mice and humans, short 3' UTR isoforms are more abundant for highly expressed genes, whereas long 3' UTR isoforms are more abundant for lower expression genes, possibly due to RNA polymerase II activity and/or chromatin structure (Ji et al., 2011). Since short 3' UTRs are generally more stable due to avoidance of negative regulatory elements in the 3' UTRs (Mayr and Bartel, 2009), more production of short 3' UTR isoforms would make the overall expression level of proteins higher, consistent with oncogene expression cases in cancer cells. Given that highly proliferating cancer cells require increased protein synthesis, transcriptional kinetics and APA connections become interesting for further examination.

In addition to transcription initiation, transcriptional elongation may also be another factor to determine poly(A) signal selection. Supporting evidence comes from *Drosophila* mutants with a 50% decrease in RNA polymerase II elongation rates. In mutants, proximal poly(A) site selection was higher compared to wild-type animals (Pinto et al., 2011). Another interesting example is from yeast. In normally growing yeast cells, the proximal poly(A) site is preferred for the *RPB2* (RNA polymerase II subunit B2) gene. However, after UV damage, transcription of *RPB2* is initially inhibited. As transcription recovers, the distal poly(A) site is preferentially used instead, producing more of a longer form of *RPB2* mRNA (Yu and Volkert, 2013).

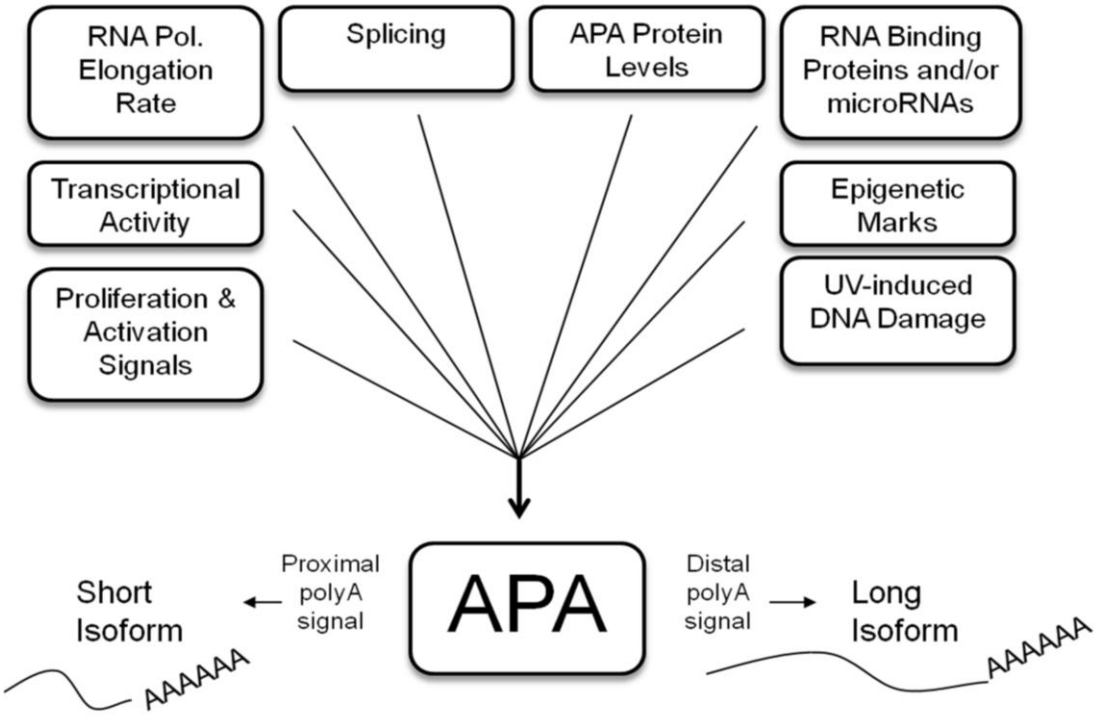

Figure 2. Potential regulatory signals for APA. APA is potentially affected by many factors including proliferation and activation signals, transcriptional activity, RNA polymerase elongation rate, splicing, epigenetic marks, APA protein levels, RNA-binding proteins, microRNAs, and DNA damage. These APA-inducing mechanisms are likely to crosstalk with each other. Following the APA decision, the short or long isoform of a transcript is generated. These isoforms may have the same coding sequence as different 3' UTRs or different reading frames based on the position of the poly(A) site.

5. Splicing

U1 is a ubiquitously expressed small nuclear ribonucleoprotein and is a major part of the spliceosome complex. Interestingly, morpholino oligonucleotide-induced knockdown of U1 in HeLa cells results in generation of premature cleavage and polyadenylation in numerous pre-mRNAs at cryptic polyadenylation signals in introns (Kaida et al., 2010). In connection to its role in polyadenylation, U1 was later proposed as a determinant of mRNA length and a regulator of different isoform expression patterns (Berg et al., 2012). Given the polyadenylation process being highly dependent on transcriptional activity, it is not surprising to observe potential interactions between the splicing machinery and APA. It is clear that we will see more examples of how APA and splicing may coregulate generation of specific UTR isoforms.

6. Epigenetic marks

Transcriptional activity is also known to affect epigenetic features; therefore, regulation of APA by transcriptional activity also correlates with differences in nucleosome positioning and histone methylation patterns around alternative poly(A) sites. Indeed, preferred poly(A) sites are strongly depleted of nucleosomes, whereas neighboring regions can be enriched for nucleosomes (Spies et al., 2009). Given the high conservation of APA signals among species and epigenetic alterations in cancer cells, further studies on mammalian systems will be of interest to investigate the depth of the connections among transcriptional kinetics, associated epigenetic marks, and APA.

7. APA protein levels

Another potential contributor to APA usage is the changes in APA protein levels in cells. Initial evidence supporting the role of key proteins in APA comes from B-cell differentiation where upregulation of CSTF64 leads to a switch from distal to proximal poly(A) site selection for the IgM heavy chain, leading to the conversion of IgM heavy chain from membrane-bound to secreted form, which is required for B-cell activation (Takagaki and Manley, 1998). CSTF64 was also shown to be upregulated upon T-cell stimulation, leading to shorter isoform generation of the transcription factor, *NFATC*, in effector cells, whereas both short and long isoforms are synthesized in unstimulated cells (Chuvpilo et al., 1999). Lipopolysaccharide stimulation has also been reported to increase CSTF64 expression in macrophages to promote

alternative polyadenylation of several mRNAs (Shell et al., 2005). In addition to proliferative/activatory signals, cell death decisions may also have effects on APA. For example, CPSF73 is translocated from the nucleus to cytosol by CSR1 (cell stress response 1). CSR1 is overexpressed under oxidative stress conditions and induces apoptosis. Consequently, CPSF73 translocation to the cytosol results in a significant inhibition of overall polyadenylation activity in prostate cancers (Zhu et al., 2008).

8. RNA-binding proteins and microRNAs

Perhaps also in connection with some of the above mechanisms, growing lines of evidence have shown that RBPs play roles in the regulation of APA by either directing poly(A) site selection or being part of the APA machinery. RBPs may also regulate translation by interacting exclusively with specific *cis* elements in the 3' UTRs. Some of these *cis* regulatory elements are A+U-rich elements, CU-rich elements, stem-loop destabilizing elements (reviewed by Knapinska et al., 2005), polyadenylation inhibition elements (Clerte and Hall, 2004), and GU-rich elements (Vlasova et al., 2008). While the roles of RBPs binding to these *cis* elements are just beginning to be understood in cancer cells, few of these proteins have been implicated in APA as well. For example, HuR is a member of the human embryonic lethal abnormal vision (ELAV) family of proteins, originally identified in *Drosophila*, and is ubiquitously expressed. HuR has been shown to regulate many cancer-related mRNAs such as tumor suppressors (TP53, p21, and p27), oncogenes (c-fos, c-Myc), cyclins (A, B1, D1), various growth factors (VEGF, TGFβ, and TNFα), and apoptosis-related factors (BCL-2, MCL-1). HuR has also been implicated in cancer-related phenomena such as hypoxia or inflammatory signals (reviewed by Wurth and Gebauer, 2015). Interestingly, HuR overexpression in HeLa cells blocks polyadenylation at poly(A) signals only if they contain U-rich sequences, possibly by blocking the physical interaction between CSTF64 and the mRNA (Zhu et al., 2007). Therefore, alterations in HuR levels may have profound secondary effects on multiple target mRNAs both in terms of its role in stabilizing mRNAs and binding to potential poly(A) sites in cancer cells. Indeed, cytoplasmic localization of HuR has shown to be a poor prognostic factor in several cancer types including breast, lung, colon, esophageal squamous cell carcinoma, and ovarian carcinomas (Wurth and Gebauer, 2015).

PABPN1 (nuclear poly(A) binding protein) appears to be contributing to the regulation of APA by inhibiting proximal polyadenylation. PABPN1 directly interacts with noncanonical and weak proximal poly(A) signals and possibly competes with the APA machinery as loss of PABPN1 leads to a genome-wide 3' UTR shortening pattern (Elkon et al., 2012). Lower expression of PABPN1 is also linked to poor prognosis in nonsmall-cell lung cancers (Ichinose et al., 2014). Another RBP, which regulates mRNA translation, is CPEB1 (cytoplasmic polyadenylation binding protein 1), which interacts with CPSF and splicing factors in the nucleus. CPEB1 mediates shortening of hundreds of mRNA 3' UTRs. In addition to 3' UTR shortening due to alternative polyadenylation, CPEB1 mRNA interaction has also been linked to alternative splicing (Bava et al., 2013).

Interestingly, two cold-induced RBPs, CIRBP and RBM3, have been found to repress proximal poly(A) site usage under cold shock conditions, leading to longer 3' UTR isoforms (Liu et al., 2013). Further investigations revealed that many of these APA events had circadian oscillations depending on the ambient temperature. It will be of interest to investigate the mechanism of this connection.

In addition to RBPs, microRNAs are small, endogenous, noncoding RNAs that also target 3' UTRs. By binding to target mRNAs, microRNAs can degrade, destabilize, delay, or suppress translation of mRNAs (Akman and Erson-Bensan, 2014). While the role of deregulated microRNAs has been well established in cancers, an interesting metaanalysis of 3' UTR lengths and microRNA expression profiles suggested upregulated microRNA expression profiles to be highly correlated with a shift towards shorter isoforms in breast cancer cells, possibly due to increased degradation of the longer isoforms (Liaw et al., 2013). Indeed, when microRNA prediction algorithms are used, there is generally a clear preference for microRNA binding sites for the longer isoforms simply due to the availability of more binding sites. To support this observation, earlier we used several microRNA binding predictions for the short and long *CDC6* isoforms and detected a common microRNA binding profile for the longer isoform. Indeed, western blot analysis and luciferase reporter assays confirmed increased CDC6 levels upon 3' UTR shortening and higher translation of the shorter isoform (Akman and Erson-Bensan, 2014). Cyclooxygenase 2 (*COX-2*), overexpressed in some cancers, has also been shown to be regulated by microRNAs (miR-146) in connection with APA (Cornett and Lutz, 2014). Cellular inflammatory responses, APA, and microRNA connection may be functionally interesting to examine in cancer cells as a potential way to activate oncogenes.

From the perspective of normal cellular events and cancer, it appears that APA is not regulated by a single mechanism and that different physiological states and/or signals can potentially be functionally important for APA-based UTR isoform generation. Considering the hallmarks of cancer, increased proliferation rates and activated signaling cascades, APA may emerge as an additional mechanism to explain certain oncogene activation and/

or tumor suppressor inactivation cases where no other explanation exists. Exciting developments clearly show the significance of UTR shortening and lengthening events in cancers and it is interesting to further evaluate microRNA deregulation in connection with APA. It should also be emphasized that one needs to be careful and take into consideration how RBPs may also bind to short or long isoforms in addition to microRNAs as RBPs may have stabilizing roles. Moreover, 3' UTR-isoform choice and influence on the stability and translational efficiency may not always correlate, as was shown in mouse fibroblasts (Spies et al., 2013).

On the other hand, several examples of mutated poly(A) sites were found to be associated with other diseases such as neonatal diabetes, type I and II diabetes, preeclampsia, fragile X-associated premature ovarian insufficiency, ectopic Cushing syndrome, and myotonic dystrophy type I (Rehfeld et al., 2013; Batra et al. 2014). For example, an A-to-G conversion was shown to drive the polyadenylation complex to a distal poly(A) site, which resulted in destabilization of *FOX3P* mRNA and decreased protein levels in IPEX (immune dysfunction, polyendocrinopathy, enteropathy, X-linked) patients (Di Giammartino et al., 2011). Aberrant spermatogenesis was also reported when testis-specific CSTF64 was disrupted (Dass et al., 2007).

In summary, the extent and functionality of APA deregulation in disease states including cancer must be established so as to pave the way to new developments to utilize APA machinery and its downstream effects for diagnostic and therapeutic applications.

Acknowledgment
Hızlan Hıncal Ağuş was funded by TÜBİTAK grants 112S478 and 114Z884.

References

Akman BH, Can T, Erson-Bensan AE (2012). Estrogen-induced upregulation and 3'-UTR shortening of CDC6. Nucleic Acids Res 40: 10679–10688.

Akman HB, Erson-Bensan AE (2014). Alternative polyadenylation and its impact on cellular processes. MicroRNA 3: 2–9.

Alt FW, Bothwell AL, Knapp M, Siden E, Mather E, Koshland M, 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.

Barnard DC, Ryan K, Manley JL, Richter JD (2004). Symplekin and xGLD-2 are required for CPEB-mediated cytoplasmic polyadenylation. Cell 119: 641–651.

Batra R, Manchanda M, Swanson MS (2015). Global insights into alternative polyadenylation regulation. RNA Biol 12: 597–602.

Bava FA, Eliscovich C, Ferreira PG, Miñana B, Ben-Dov C, Guigó R, Valcárcel J, Méndez R (2013). CPEB1 coordinates alternative 3'-UTR formation with translational regulation. Nature 495: 121–125.

Berg MG, Singh LN, Younis I, Liu Q, Pinto AM, Kaida D, Zhang Z, Cho S, Sherrill-Mix S, Wan L et al. (2012). U1 snRNP determines mRNA length and regulates isoform expression. Cell 150: 53–64.

Cardinale S, Cisterna B, Bonetti P, Aringhieri C, Biggiogera M, Barabino SM (2007). Subnuclear localization and dynamics of the pre-mRNA 3' end processing factor mammalian cleavage factor I 68-kDa subunit. Mol Biol Cell 18: 1282–1292.

Cevher MA, Zhang X, Fernandez S, Kim S, Baquero J, Nilsson P, Lee S, Virtanen A, Kleiman FE (2010). Nuclear deadenylation/polyadenylation factors regulate 3' processing in response to DNA damage. EMBO J 29: 1674–1687.

Chan SL, Huppertz I, Yao C, Weng L, Moresco JJ, Yates JR, Ule J, Manley JL, Shi Y (2014). CPSF30 and Wdr33 directly bind to AAUAAA in mammalian mRNA 3' processing. Genes Dev 28: 2370–2380.

Chuvpilo S, Zimmer M, Kerstan A, Glöckner J, Avots A, Escher C, Fischer C, Inashkina I, Jankevics E, Berberich-Siebelt F et al. (1999). Alternative polyadenylation events contribute to the induction of NF-ATc in effector T cells. Immunity 10: 261–269.

Clerte C, Hall KB (2004). Global and local dynamics of the U1A polyadenylation inhibition element (PIE) RNA and PIE RNA–U1A complexes. Biochemistry 43: 13404–13415.

Cornett AL, Lutz CS (2014). Regulation of COX-2 expression by miR-146a in lung cancer cells. RNA 20: 1419–1430.

Coseno M, Martin G, Berger C, Gilmartin G, Keller W, Doublie S (2008). Crystal structure of the 25 kDa subunit of human cleavage factor I-m. Nucleic Acids Res 36: 3474–3483.

Dantonel JC, Murthy KG, Manley JL, Tora L (1997). Transcription factor TFIID recruits factor CPSF for formation of 3' end of mRNA. Nature 389: 399–402.

Dass B, Tardif S, Park JY, Tian B, Weitlauf HM, Hess RA, Carnes K, Griswold MD, Small CL, MacDonald CC (2007). Loss of polyadenylation protein ┬CSTF-64 causes spermatogenic defects and male infertility. P Nat Acad Sci USA 104: 20374–20379.

Derti A, Garrett-Engele P, Macisaac KD, Stevens RC, Sriram S, Chen R, Rohl CA, Johnson JM, Babak T (2012). A quantitative atlas of polyadenylation in five mammals. Genome Res 22: 1173–1183.

Dettwiler S, Aringhieri C, Cardinale S, Keller W, Barabino SM (2004). Distinct sequence motifs within the 68-kDa subunit of cleavage factor Im mediate RNA binding, protein-protein interactions, and subcellular localization. J Biol Chem 279: 35788–35797.

Di Giammartino DC, Nishida K, Manley JL (2011). Mechanisms and consequences of alternative polyadenylation. Mol Cell 43: 853–866.

Early P, Rogers J, Davis M, Calame K, Bond M, Wall R, Hood L (1980). Two mRNAs can be produced from a single immunoglobulin mu gene by alternative RNA. Cell 20: 313–319.

Edmonds M, Abrams RJ (1960). Polynucleotide biosynthesis-formation of a sequence of adenylate units from adenosine triphosphate by an enzyme from thymus nuclei. J Biol Chem 235: 1142–1149.

Elkon R, Drost J, van Haaften G, Jenal M, Schrier M, Vrielink JA, Agami R (2012). E2F mediates enhanced alternative polyadenylation in proliferation. Genome Biol 13: R59.

Elkon R, Ugalde AP, Agami R (2013). Alternative cleavage and polyadenylation: extent, regulation and function. Nat Rev Genet 14: 496–506.

Fu Y, Sun Y, Li Y, Li J, Rao X, Chen C, 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.

Fukumitsu H, Soumiya H, Furukawa S (2012). Knockdown of pre-mRNA cleavage factor Im 25 kDa promotes neurite outgrowth. Biochem Biophys Res Commun 425: 848–853.

Glover-Cutter K, Kim S, Espinosa J, Bentley DL (2008). RNA polymerase II pauses and associates with pre-mRNA processing factors at both ends of genes. Nat Struct Mol Biol 15: 71–78.

Gruber AR, Martin G, Keller W, Zavolan M (2012). Cleavage factor Im is a key regulator of 3' UTR length. RNA Biol 9: 1405–1412.

Guhaniyogi J, Brewer G (2001). Regulation of mRNA stability in mammalian cells. Gene 265: 11–23.

Ichinose J, Watanabe K, Sano A, Nagase T, Nakajima J, Fukayama M, Yatomi Y, Ohishi N, Takai D (2014). Alternative polyadenylation is associated with lower expression of PABPN1 and poor prognosis in non-small cell lung cancer. Cancer Sci 105: 1135–1141.

Ji Z, Lee JY, Pan Z, Jiang B, Tian B (2009). Progressive lengthening of 3' untranslated regions of mRNAs by alternative polyadenylation during mouse embryonic development. P Natl Acad Sci USA 106: 7028–7033.

Ji Z, Luo W, Li W, Hoque M, Pan Z, Zhao Y, Tian B (2011). Transcriptional activity regulates alternative cleavage and polyadenylation. Mol Syst Biol 7: 534.

Kaida D, Berg MG, Younis I, Kasim M, Singh LN, Wan L, Dreyfuss G (2010). U1 snRNP protects pre-mRNAs from premature cleavage and polyadenylation. Nature 468: 664–668.

Kleiman FE, Manley JL (2001). The BARD1-CstF-50 interaction links mRNA 3' end formation to DNA damage and tumor suppression. Cell 104: 743–753.

Knapinska AM, Irizarry-Barreto P, Adusumalli S, Androulakis L, Brewer G (2005). Molecular mechanisms regulating mRNA stability: physiological and pathological significance. Curr Genomics 6: 471–486.

Kubo T, Wada T, Yamaguchi Y, Shimizu A, Handa H (2006). Knock-down of 25 kDa subunit of cleavage factor Im in Hela cells alters alternative polyadenylation within 3'-UTRs. Nucleic Acids Res 34: 6264–6271.

Kwon C, Tak H, Rho M, Chang HR, Kim YH, Kim KT, Balch C, Lee EK, Nam S (2014). Detection of PIWI and piRNAs in the mitochondria of mammalian cancer cells. Biochem Biophys Res Commun 446: 218–223.

Laishram RS, Anderson RA (2010). The poly A polymerase Star-PAP controls 3'-end cleavage by promoting CPSF interaction and specificity toward the pre-mRNA. EMBO J 29: 4132–4145.

Li H, Tong S, Li X, Shi H, Ying Z, Gao Y, Ge H, Niu L, Teng M (2011). Structural basis of pre-mRNA recognition by the human cleavage factor Im complex. Cell Res 21: 1039–1051.

Liaw HH, Lin CC, Juan HF, Huang HC (2013). Differential microRNA regulation correlates with alternative polyadenylation pattern between breast cancer and normal cells. PLoS One 8: e56958.

Liu Y, Hu W, Murakawa Y, Yin J, Wang G, Landthaler M, Yan J (2013). Cold-induced RNA-binding proteins regulate circadian gene expression by controlling alternative polyadenylation. Sci Rep 3: 2054.

Lutz CS, Moreira A (2011). Alternative mRNA polyadenylation in eukaryotes: an effective regulator of gene expression. Wiley Interdiscip Rev RNA 2: 22–31.

Mandel CR, Kaneko S, Zhang H, Gebauer D, Vethantham V, Manley JL, Tong L (2006). Polyadenylation factor CPSF-73 is the pre-mRNA 3'-end-processing endonuclease. Nature 444: 953–956.

Martin G, Gruber AR, Keller W, 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.

Martin G, Ostareck-Lederer A, Chari A, Neuenkirchen N, Dettwiler S, Blank D, Rüegsegger U, Fischer U, Keller W (2010). Arginine methylation in subunits of mammalian pre-mRNA cleavage factor I. RNA 16: 1646–1659.

Marzluff WF, Wagner EJ, Duronio RJ (2008). Metabolism and regulation of canonical histone mRNAs: life without a poly(A) tail. Nat Rev Genet 9: 843–854.

Masamha CP, Xia Z, Yang J, Albrecht TR, Li M, Shyu AB, Li W, Wagner EJ (2014). CFIm25 links alternative polyadenylation to glioblastoma tumour suppression. Nature 510: 412–416.

Mayr C, Bartel DP (2009). Widespread shortening of 3' UTRs by alternative cleavage and polyadenylation activates oncogenes in cancer cells. Cell 138: 673–684.

Millevoi S, Loulergue C, Dettwiler S, Karaa SZ, Keller W, Antoniou M, Vagner S (2006). An interaction between U2AF 65 and CF I(m) links the splicing and 3' end processing machineries. EMBO J 25: 4854–4864.

Millevoi S, Vagner S (2010). Molecular mechanisms of eukaryotic pre-mRNA 3' end processing regulation. Nucleic Acids Res 38: 2757–2774.

Morris AR, Bos A, Diosdado B, Rooijers K, Elkon R, Bolijn AS, Carvalho B, Meijer GA, Agami R (2012). Alternative cleavage and polyadenylation during colorectal cancer development. Clin Cancer Res 18: 5256–5266.

Nag A, Narsinh K, Martinson HG (2007). The poly(A)-dependent transcriptional pause is mediated by CPSF acting on the body of the polymerase. Nat Struct Mol Biol 14: 662–669.

Pinto PA, Henriques T, Freitas MO, Martins T, Domingues RG, Wyrzykowska PS, Coelho PA, Carmo AM, Sunkel CE, Proudfoot NJ et al. (2011). RNA polymerase II kinetics in polo polyadenylation signal selection. EMBO J 30: 2431–2444.

Proudfoot NJ, Furger A, Dye MJ (2002). Integrating mRNA Processing with Transcription. Cell 108: 501–512.

Radermacher M, Wagenknecht T, Verschoor A, Frank J (1987). Three-dimensional structure of the large ribosomal subunit from Escherichia coli. EMBO J 6: 1107–1114.

Rehfeld A, Plass M, Døssing K, Knigge U, Kjær A, Krogh A, Friis-Hansen L (2014). Alternative polyadenylation of tumor suppressor genes in small intestinal neuroendocrine tumors. Front Endocrinol (Lausanne) 5: 46.

Rehfeld A, Plass M, Krogh A, Friis-Hansen L (2013). Alterations in polyadenylation and its implications for endocrine diseases. Front Endocrinol (Lausanne) 4: 53.

Rogers J, Early P, Carter C, Calame K, Bond M, Hood L, Wall R (1980). Two mRNAs with different 3' ends encode membrane-bound and secreted forms of immunoglobulin mu chain. Cell 20: 303–312.

Romeo V, Griesbach E, Schümperli D (2014). CstF64: Cell cycle regulation and functional role in 3' end processing of replication-dependent histone mRNAs. Mol Cell Biol 34: 4272–4284.

Ruepp MD, Aringhieri C, Vivarelli S, Cardinale S, Paro S, Schümperli D, Barabino SM (2009). Mammalian pre-mRNA 3' end processing factor CF I_m68 functions in mRNA export. Mol Biol Cell 20: 5211–5223.

Ruepp MD, Vivarelli S, Pillai RS, Kleinschmidt N, Azzouz TN, Barabino SM, Schümperli D (2010). The 68 kDa subunit of mammalian cleavage factor I interacts with the U7 small nuclear ribonucleoprotein and participates in 3'-end processing of animal histone mRNAs. Nucleic Acids Res 38: 7637–7650.

Sandberg R, Neilson JR, Sarma A, Sharp PA, Burge CB (2008). Proliferating cells express mRNAs with shortened 3' untranslated regions and fewer microRNA target sites. Science 320: 1643–1647.

Schönemann L, Kühn U, Martin G, Schäfer P, Gruber AR, Keller W, Zavolan M, Wahle E (2014). Reconstitution of CPSF active in polyadenylation: recognition of the polyadenylation signal by WDR33. Genes Dev 28: 2381–2393.

Setzer DR, McGrogan M, Nunberg JH, Schimke RT (1980). Size heterogeneity in the 3' end of dihydrofolate reductase messenger RNAs in mouse cells. Cell 22: 361–370.

Shell SA, Hesse C, Morris SM, Milcarek C (2005). Elevated levels of the 64-kDa cleavage stimulatory factor (CstF-64) in lipopolysaccharide-stimulated macrophages influence gene expression and induce alternative poly(A) site selection. J Biol Chem 280: 39950–39961.

Shi YS, Di Giammartino DC, Taylor D, Sarkeshik A, Rice WJ, Yates JR, Frank J, Manley JL (2009). Molecular architecture of the human Pre-mRNA 3' processing complex. Mol Cell 33: 365–376.

Spies N, Burge CB, Bartel DP (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.

Spies N, Nielsen CB, Padgett RA, Burge CB (2009). Biased chromatin signatures around polyadenylation sites and exons. Mol Cell 36: 245–254.

Sun M, Ju H, Zhou Z, Zhu R (2014). Pilot genome-wide study of tandem 3' UTRs in esophageal cancer using high-throughput sequencing. Mol Med Rep 9: 1597–1605.

Takagaki Y, Macdonald CC, Shenk T, Manley JL (1992). The human 64-kDa polyadenylylation factor contains a ribonucleoprotein-type RNA-binding domain and unusual auxiliary motifs. P Natl Acad Sci USA 89: 1403–1407.

Takagaki Y, Manley JL (1997). RNA recognition by the human polyadenylation factor CstF. Mol Cell Biol 17: 3907–3914.

Takagaki Y, Manley JL (1998). Levels of polyadenylation factor CstF-64 control IgM heavy chain mRNA accumulation and other events associated with B cell differentiation. Mol Cell 2: 761–771.

Takagaki Y, Manley JL (2000). Complex protein interactions within the human polyadenylation machinery identify a novel component. Mol Cell Biol 20: 1515–1525.

Tian B, Graber JH (2012). Signals for pre-mRNA cleavage and polyadenylation. Wiley Interdiscip Rev RNA 3: 385–396.

Vlasova IA, Tahoe NM, Fan D, Larsson O, Rattenbacher B, Sternjohn JR, Vasdewani J, Karypis G, Reilly CS, Bitterman PB et al. (2008). Conserved GU-rich elements mediate mRNA decay by binding to CUG-binding protein 1. Mol Cell 29: 263–270.

Vorlová S, Rocco G, Lefave CV, Jodelka FM, Hess K, Hastings ML, Henke E, Cartegni L (2011). Induction of antagonistic soluble decoy receptor tyrosine kinases by intronic polyA activation. Mol Cell 43: 927–939.

West S, Proudfoot NJ (2008). Human Pcf11 enhances degradation of RNA polymerase II-associated nascent RNA and transcriptional termination. Nucleic Acids Res 36: 905–914.

Whyteside AR, Turner AJ, Lambert DW (2014). Endothelin-converting enzyme-1 (ECE-1) is post-transcriptionally regulated by alternative polyadenylation. PLoS One 9: 83260.

Wurth L, Gebauer F (2015). RNA-binding proteins, multifaceted translational regulators in cancer. Biochim Biophys Acta 1849: 881–886.

Yang Q, Gilmartin GM, Doublié S (2010). Structural basis of UGUA recognition by the Nudix protein CFI_m25 and implications for a regulatory role in mRNA 3' processing. P Natl Acad Sci USA 107: 10062–10067.

Yang Q, Gilmartin GM, Doublié S (2011). The structure of human cleavage factor I_m hints at functions beyond UGUA-specific RNA binding: a role in alternative polyadenylation and a potential link to 5' capping and splicing. RNA Biol 8: 748–753.

Yao CG, Biesinger J, Wan J, Weng LJ, Xing Y, Xie XH, Shi YS (2012). Transcriptome-wide analyses of CstF64-RNA interactions in global regulation of mRNA alternative polyadenylation. P Natl Acad Sci USA 109: 18773–18778.

Yu L, Volkert MR (2013). UV damage regulates alternative polyadenylation of the RPB2 gene in yeast. Nucleic Acids Res 41: 3104–3114.

Zhu H, Zhou HL, Hasman RA, Lou H (2007). Hu proteins regulate polyadenylation by blocking sites containing U-rich sequences. J Biol Chem 282: 2203–2210.

Zhu ZH, Yu YP, Shi YK, Nelson JB, Luo JH (2008). CSR1 induces cell death through inactivation of CPSF3. Oncogene 28: 41–51.