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The emerging theme of 3'UTR mRNA isoform regulation in reprogramming of cell metabolism

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Abstract

The 3' untranslated region (3'UTR) of mRNA plays a key role in post-transcriptional regulation of gene expression. Most eukaryotic protein-coding genes express 3'UTR isoforms owing to alternative cleavage and polyadenylation (APA). The 3'UTR isoform expression profile of a cell changes in cell proliferation, differentiation, and stress conditions. Here we review the emerging theme of regulation of 3'UTR isoforms in cell metabolic reprogramming, focusing on cell growth and autophagy responses through the mTOR pathway. We discuss regulatory events that converge on the Cleavage Factor I complex, a master regulator of APA in 3'UTRs, and recent understandings of isoform-specific m^6A modification and endomembrane association in determining differential metabolic fates of 3'UTR isoforms.

Introduction

Cleavage and polyadenylation (CPA) of precursor RNA is responsible for 3' end maturation of almost all eukaryotic mRNAs [1,2]. About 70-80% of protein-coding genes in the human genome harbor multiple CPA sites (also known as polyA sites or PASs), leading to alternative polyadenylation (APA) isoforms [3]. Most APA sites are located in the last exon, resulting in expression of mRNA isoforms with different 3'UTR sizes. The 3'UTR isoform expression profile is highly variable across cell or tissue types [4-6]. For example, brain and blood cells tend to express long and short 3'UTR isoforms, respectively [4]. In addition, substantial, global 3'UTR size changes have been found in cell proliferation [7], cancer cell transformation [8], embryonic development [9], cell differentiation [10], and stress conditions [11,12]. About 20% of human genes, typically those with large introns, also display intronic polyadenylation (or IPA) [3]. IPA events are also dynamically controlled and often correlated with 3'UTR APA events [4-6], indicating common underlying regulatory mechanisms.

3'UTR APA isoforms are believed to have distinct mRNA metabolic fates, such as stability, translational efficiency, and subcellular localization, due to variable sequence and structure motifs embedded in different isoforms [13]. For simplicity, the 3'UTR portion subject to alternative polyadenylation (APA) regulation is named alternative 3'UTR, or aUTR (Figure 1). Here we review recent advances in understanding mechanisms and consequences of

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3’UTR isoform regulation in the context of cell metabolic reprogramming, including cell growth and autophagy. Readers are referred to other recent reviews for more comprehensive coverage of APA [14-16].

Core CPA factors mediate APA regulation

The PAS is defined by surrounding motifs [17] (Figure 1). In mammals, upstream PAS motifs include the A[A/U]UAAA hexamer or their close variants, U-rich sequences, and UGUA sequences; downstream PAS motifs include U-rich, GU-rich, and G-rich sequences [17] . The cleavage reaction typically takes place after an adenosine [3,18]. The strength of a PAS is determined by all these motifs in a combinatory fashion [19,20]. In general, due to motif composition differences, distal PASs, especially those at the 3’-most position of a gene, are stronger than proximal PASs [19]. For example, the canonical motifs AAUAAA and UGUA in particular are conspicuously enriched at the 3’-most PASs [3].

The mammalian CPA machinery is composed of over 20 core proteins [1,21,22](Figure 1), most of which are engaged in forming four sub-complexes, including the Cleavage and Polyadenylation Specificity Factor (CPSF), the Cleavage stimulation Factor CstF, the Cleavage Factor I (CFI, also known as CFIm) and the Cleavage Factor II (CFII, also known as CFIIm). CPSF comprises two functional modules [21], namely, the mammalian polyadenylation specificity factor (mPSF), composed of CPSF-160, CPSF-30, WDR33, and FIP1, and the mammalian cleavage factor (mCF), composed of CPSF-100, CPSF-73, and Symplekin. CPSF proteins have binding affinities to the core PAS motifs, while CstF, CFI and CFII factors have affinities to auxiliary motifs for CPA (illustrated in the CPA machinery inset of Figure 1). In addition, RBBP6, Poly(A) polymerase (PAP), Poly(A) binding protein PAPN1, and RNA polymerase II (Pol II) are also key components of the CPA machinery.

Dysregulation of several CPA factors have been implicated in human developmental diseases and cancers [23-26]. One contributing reason to disease etiology is that the expression level of several core CPA factors has a substantially impact on APA site selection [27-31]. For example, decreased expression levels of FIP1, CstF-64/CstF-64τ or PCF11 all lead to a global shift to distal APA site usage, resulting in 3’UTR lengthening for most genes [27,28]. Conversely, overexpression (OE) of PCF11 results in an opposite trend [32,33]. The underlying reason for these global APA site shifts is the fact that proximal APA sites are typically weak, making their usage highly responsive to the CPA activity. On the other hand, these findings highlight the importance of individual CPA factors in determining the overall CPA activity.

By contrast, KD of CFI-25 or CFI-68 leads to drastic 3’UTR shortening for nearly every gene that has APA sites in the 3’UTR [27,29,34,35]. Since the UGUA motif, which CFI binds, is enriched at distal PASs, CFI is believed to have an enhancing role biased to distal PAS usage. Interestingly, OE of CFI-25 or CFI-68 has only a limited effect on 3’UTR size globally [34], indicating that these proteins are likely to be in sufficient amounts under normal conditions. The mechanism by which CFI enhances CPA was revealed by Zhu et al., who showed that the arginine-serine repeat (RS)-like domain of CFI-59/68 directly interacts with the arginine-aspartate/glutamate (RE/D) domain of FIP1 [36]. Therefore, CFI could help

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recruit FIP1-contianing CPSF for CPA [35]. Notably, despite protein sequence similarities between CFI-59 and CFI-68, the former has a much weaker function in enhancing CPA than the latter [37], which explains why CFI-59 KD does not lead to widespread 3'UTR shortening as does KD of CFI-25 or CFI-68 [27,34,35].

In addition to its direct function in CPA, CFI-68 has been implicated in mRNA nuclear export. The protein has the property of shuttling between nucleus and cytoplasm [38] and physically interacts with the nuclear export factor NXF1 [38,39]. Notably, NXF1 KD also leads to global 3'UTR shortening as well as cumulation of Pol II around the 3' end of genes, indicating that nuclear export is tightly connected with CPA, plausibly through CFI-68 [39]. Related to this, a recent study by Tang et al. showed that long 3'UTR isoforms can be further processed into short 3'UTR isoforms in the nuclear matrix [40], opening the possibility that some of the 3'UTR shortening effect of CFI-25 or -68 KD might be due to decreased nuclear export of long 3'UTR isoforms, which allows more time for CPA at proximal PASs. All in all, it is now well established that CFI-25/68 are master regulators of the 3'UTR size of mRNAs.

3'UTR isoform regulation by the mTOR pathway

The mammalian target of rapamycin (mTOR) pathway is a key player in cell proliferation and growth [41]. In keeping with the notion that 3'UTR isoform expression is coupled with cell proliferation and differentiation [7,42], activation of mTOR was found to cause 3'UTR shortening in multiple human and mouse cells [43]. Based on selective KD of Raptor or Rictor, components of the two distinct mTOR complexes in the cell, the same study found that mTORC1, not mTORC2, is the one responsible for 3'UTR isoform regulation. Interestingly, genes with certain functions, such as protein processing in endoplasmic reticulum and ubiquitin-mediated proteolysis, appeared to be particularly affected by mTORC1-mediated 3'UTR shortening [43,44]. Notably, similar Gene Ontology terms, such as protein transport and cellular response to stress, were among the top biological processes associated with CFI-25/68-regulated APA events in HEK293 cells [34], indicating a potential role of CFI in mTORC1-mediated APA. In the same vein, a recent phosphoproteomics study using HEK 293 cells with OE or KD of CFI-25 or CFI-68 highlighted the potential roles of CFI-25/68 in regulation of several kinases involved in cell metabolism [34], such as ULK1 and ERK1/2 [45].

The PI3K/AKT signaling pathway plays a major role in mTORC1 regulation [46]. PTEN, a tumor suppressor that is frequently mutated in cancers [46,47], is a key regulator of PI3K/AKT signaling. The *PTEN* gene produces multiple 3'UTR isoforms with variable contributions to the overall protein expression [48]. CFI-68 and CFI-59 were recently found to have opposing roles in PTEN regulation [47], with the former promoting the expression of longer 3'UTR isoforms and the latter functioning in the opposite direction. The authors further indicated that many factors in the PI3K/AKT signaling pathway are subject to similar 3'UTR isoform regulations by CFI-68 and CFI-59, raising the possibility of a concerted regulatory scheme. One of the reasons that 3'UTR isoform regulation is critical for *PTEN* expression is because of the multiple miRNA target sites embedded in its 3'UTRs [49]. Interestingly, the general 3'UTR shortening in cancer cells, which could remove miRNA target sites from 3'UTRs of mRNAs in a global fashion, was found to enhance the functions of the miRNAs that target

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PTEN mRNAs, a phenomenon also known as competing endogenous RNA or ceRNA [50]. Another facet of 3'UTR APA regulation of *PTEN* comes from the Mayr group, who found that deletion of an enhancer region at the *PTEN* promoter alters the relative expression levels of 3'UTR isoforms in breast cancer cell line MCF7 [51]. Whether CFI factors are involved in this enhancer-mediated 3'UTR APA scheme of PTEN would be interesting to explore in the future.

mTORC1 is inhibited in cells under nutrient starvation, eliciting catabolic processes such as autophagy [52]. Using an autophagy-induced *Drosophila* model, involving overexpression of the key autophagy gene *Atg1* (homolog of *ULK1/2* in mammals) in the eye, Tang et al. identified multiple CPA factors as autophagy regulators, including CFI-68, CstF-64, WDR33, CPSF-160 and PCF11 [53]. The authors delineated a mTORC1-CDK8/CLK2 (DOA in *Drosophila*)-CFI-68 axis in autophagy regulation, in which inhibition of mTORC1 leads to phosphorylation of the RS domain CFI-68 by CDK8/CLK2, resulting in nuclear localization of CFI-68 and enhanced distal APA site selection. Importantly, this mechanism regulates both *Atg1* and another autophagy gene *Atg8a* (homolog of *LC3* genes in mammals) by switching short 3'UTR isoform expression to long 3'UTR isoform expression in starvation. Because mRNA stability of these long 3'UTR isoforms is greater than that of short 3'UTR isoforms, CFI-68-mediated lengthening of 3'UTR in starvation increases their protein expression and thus enhances their functions in autophagy response [53]. Interestingly, in the Tang et al. study, CPSF and CstF KDs showed similar phenotypes to CFI-68 KD, while PCF11 KD showed an opposite trend. It remains to be addressed whether the distinct function of PCF11 in the autophagy context is due to different directions of APA events for the same genes [27] or different sets of genes are regulated by PCF11 [32,33]. It is also worth noting that hyperphosphorylation of the RS domain of CFI-68 was found to inhibit its interaction with FIP1 in human cells [37]. Therefore, further studies are needed to reconcile two seemingly opposing consequences of RS domain phosphorylation of CFI-68.

3'UTR regulation has also been studied for other genes involved in autophagy. Using a murine pro-B cell line Ba/F3 that encodes U2AF35^{S34F}, a mutant, oncogenic form of the core splicing factor U2AF35, Park et al. showed that CFI-59 and CFI-68 interact differently with U2AF35^{S34F}, leading to a switch to distal 3'UTR PAS usage in the autophagy gene *ATG7* [54]. Because its long 3'UTR isoform is less efficiently translated than the short 3'UTR isoform, the APA regulation results in downregulation of ATG7 protein and hence autophagy defects, which predispose cells to additional mutations that cause cell transformation.

The metabolic fate of 3'UTR isoforms through LC3B and membrane association

Because the 3'UTR is a hotbed for RNA motifs involved in post-transcriptional regulation, 3'UTR size changes could substantially alter mRNA metabolism. While most studies have shown that short 3'UTR isoforms tend to be more stable [3,55] and have higher translational efficiencies [56] than long 3'UTRs, there are many exceptions [57]. This is due likely to the fact that 3'UTRs can harbor both stabilizing and destabilizing motifs [14-16,58]. In addition, while

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long 3'UTR size could have higher affinities to UPF1, a key player of nonsense-mediated decay (NMD) [59,60], a recent study using long-read sequencing of NMD substrates does not support the view that long 3'UTR size *per se* activates NMD [61]. A recent study by Hwang et al., however, indicates that long 3'UTRs may be indeed generally unstable in autophagy conditions [62]. Lipidation of the autophagy regulator LC3B through conjugation with phosphatidylethanolamine is a commitment step in the canonical macroautophagy pathway [63,64]. LC3B-II, the lapidated form of the protein, plays a key role in formation of phagophore, leading to autophagosome maturation [65-67]. Hwang et al. found that LC3B is a *bona fide* RNA-binding protein with specificity to AAUAAA [62], revealing a new mRNA degradation pathway under autophagy conditions. This mechanism, termed LC3B-mediated mRNA decay (LMD), involves interactions of LC3B with mRNAs and subsequent mRNA deadenylation through the CCR4-NOT complex. LMD takes place before lysosome fusion and is thus distinct from lysosome-mediated RNA degradation within the lysosome [68]. Importantly, LC3B lipidation enhances LMD, raising the possibility that membrane-associated mRNAs may be more prone to this degradation pathway. In addition, because the number of AAUAAA generally correlates with 3'UTR size and is more enriched at distal PASs used by long 3'UTR isoforms (our unpublished data), the LMD pathway might differentiate 3'UTR isoforms in mRNA stability control during autophagy (Figure 1).

In addition to stability control, 3'UTRs are critical for mRNA localization, especially in polarized cells, such as neurons [69]. However, it is increasingly clear that 3'UTRs could alter mRNA distribution even in cells not highly polarized. By comparing 3'UTR isoforms in membrane and cytosol fractions of mouse C2C12 myoblast cells and differentiated myotubes, Cheng et al. found that long 3'UTRs promote endoplasmic reticulum (ER) association in a translation-independent manner [70], a mechanism dubbed translation independent ER association (TiERA). 3'UTR length, GC content and structural properties play deterministic roles in TiERA potentials. It is noteworthy that the membrane fraction they isolated contained other organelles as well, leaving open the question as to whether 3'UTRs could also help recruit mRNAs to other organelles. On this note, association of mRNAs with endosomes was found to help localize translating mRNAs in neuronal cells [71]. On the other hand, membrane-associated mRNAs may subject them to a different set of regulatory mechanisms than those in the cytosol. For example, membrane-associated mRNAs may be more prone to LMD or lysosome-mediated mRNA degradation. Related to this, stress granules (SGs), membrane-less protein-RNA aggregates through liquid-liquid phase separation under stress conditions [72], could be formed in both cytosol and ER. Removal of SGs through lysosomes, also known as granulophagy, has implications in many human diseases, including cancer and neuronal degeneration [73]. Importantly, 3'UTR size is an important feature for mRNA recruitment into SGs [74] and long 3'UTRs isoforms were found to be preferentially degraded after SG-inducing arsenic stress in mouse C2C12 myoblast cells and NIH3T3 fibroblast cells [11]. Therefore, it is possible that, due to the differential association with organelles and membrane-less granules, 3'UTR isoforms have distinct metabolic fates during and after stress.

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The connection between m^{6}A and 3’UTR isoform expression

Methylation of the N^6 position of adenosine (m^{6}A) is a widespread modification of mRNA carried out by the m^{6}A methyltransferase complex (MTC) [75]. Recent studies have shown that m^{6}A levels globally increase upon mTORC1 activation, leading to heightened m^{6}A-mediated mRNA metabolism [76 77], promoting cell proliferation and growth as well as suppressing autophagy [78]. m^{6}A levels are typically enriched near the stop codon in the last exon [79,80] and are associated with increased mRNA degradation [81] and higher translational efficiency [82], functions that are mediated by m^{6}A cytoplasmic readers, such as YTHDF proteins [83].

Using anti-m^{6}A RNA immunoprecipitation (RIP) and human H1 embryonic stem cells, Molinie et al. found that short 3’UTR isoforms are more likely to have higher levels of m^{6}A than long 3’UTR isoforms from the same gene [84]. In addition, Yue et al. found that the m^{6}A motif GGACU was enriched, albeit modestly, around the proximal PAS site in genes with multiple APA sites in the last exon [85]. These findings support the notion that 3’UTR isoforms could have different m^{6}A contents, leading to distinct metabolic fates.

A key question concerning m^{6}A deposition is whether APA site selection process could influence the level of m^{6}A in 3’UTR. The VIRMA component of MTC was found to physically interact with the CFI-25/68 complex [85]. Interestingly, while VIRMA KD globally suppresses m^{6}A enrichment in the 3’UTR and around the stop codon, CFI-25 KD leads to expression of short 3’UTR isoforms with increased m^{6}A in these regions [85]. This finding indicates that CFI controls both the size and m^{6}A content of 3’UTR. The connection between m^{6}A and 3’UTR isoform expression is further supported by the observations that long 3’UTR isoforms are relatively more abundant after ablations of m^{6}A eraser FTO [86] or nuclear m^{6}A reader YTHDC1 [87]. Notably, YTHDC1, which is required for embryo viability and germline development in mice [87], was found to physically interact with CFI-68, SRSF3, and SRSF7 in HEK293T cells [87]. The authors indicated that these interactions support a role of m^{6}A in regulation of APA and splicing. Another study by Chen et al., however, showed that YTHDC1 could also interact with FIP1, thereby hindering its interaction with CPSF-30 [88] and hence causing PAS usage suppression. Therefore, while it is indisputable that m^{6}A and 3’UTR isoform expression are well connected, the detailed mechanism(s) for the interplay between m^{6}A deposition and APA site selection, especially concerning the role of YTHDC1, still require further experimentation.

Conclusions and future perspectives

3’UTR isoform regulation is increasingly appreciated as a widespread mechanism that modulates gene expression. A growing number of physiological and pathological conditions have been found to impact 3’UTR isoform expression in the cell, such as cell growth, differentiation, and stress. The mTORC1-CFI axis is emerging as a key signaling pathway leading to 3’UTR size control. The consequences of 3’UTR size changes could be multifold. Endomembrane association, AAUAAA-mediated LMD, and 3’UTR m^{6}A content are recently uncovered mechanisms that can differentiate the metabolic fates for 3’UTR

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isoforms. These studies raise many interesting questions that require further investigation. Due to space limitation, we list three of them here:

i. The mTORC1-CFI axis needs to be further examined in different metabolic and stress conditions. Because CFI factors have a global effect on almost every gene containing 3'UTR APA sites, how specific genes are targeted for 3'UTR size regulation needs to be addressed. In addition, why CFI-59 and CFI-68, two CFI proteins with similar sequences, appear to have substantial differences APA regulation and how these two proteins have differential impacts on cell signaling require further investigation.

ii. Long 3'UTR isoforms tend to be enriched on ER and perhaps other organelles as well. The underlying mechanism(s) are still elusive. Whether some RBPs can be lipidated, similar to LC3B, and serve as receptors for 3'UTR sequences or structures need to be explored. In addition, the difference in mRNA metabolism for cytosolic vs. membrane-bound mRNAs is an interesting subject to study, especially in cells with markedly different 3'UTR isoform expression patterns, such as blood cells and neurons.

iii. The m^6A content has a clear role in mRNA metabolism. The cause and consequence of m^6A content difference between 3'UTR isoforms need more rigorous examination. The role of YTHDC1 in 3'UTR isoform regulation could be further analyzed by comparing nascent pre-mRNAs and newly made mature RNAs. To what extent YTHDF proteins, which can promote stress granule formation [89], contribute to differential recruitment of 3'UTR isoforms with different m^6A contents to stress granules needs to be firmly established.

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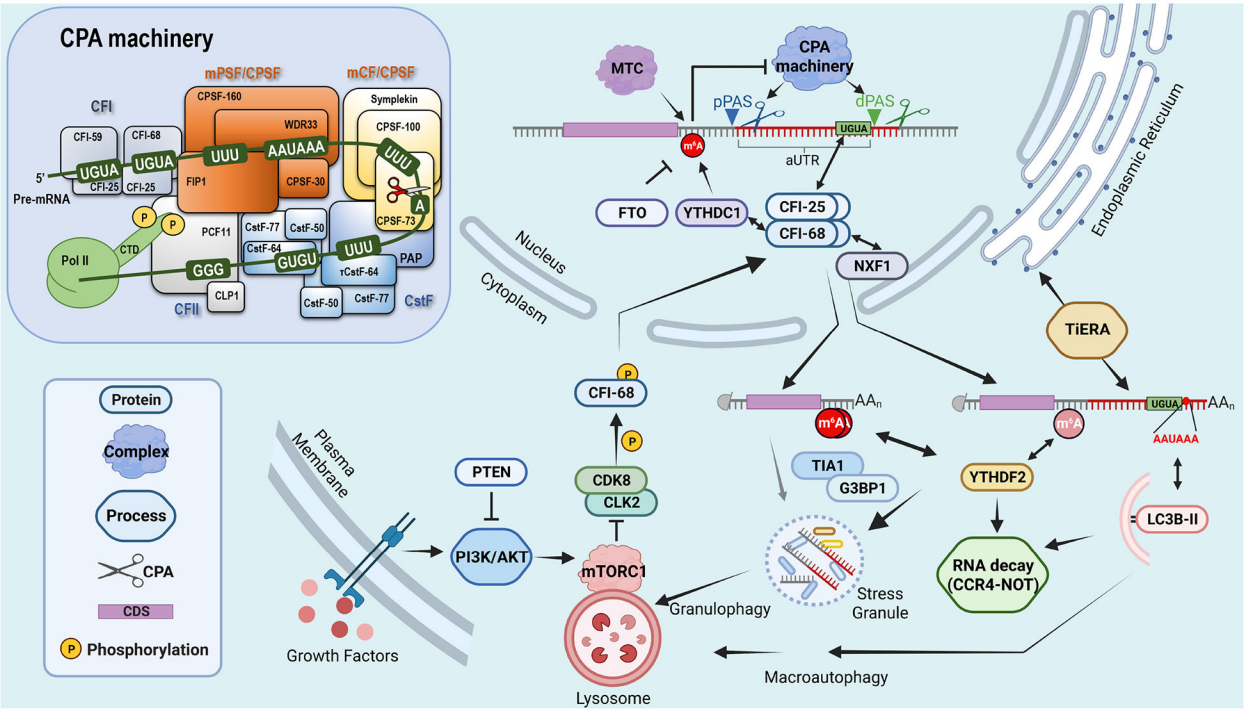

Figure 1.

Regulation of alternative polyadenylation (APA) site choice and differential mRNA metabolism between 3'UTR isoforms. A hypothetical gene with two 3'UTR APA sites are shown.

pPAS, proximal PAS; dPAS, distal PAS. MTC, m^{6}A methyltransferase complex; LC3B-II, lipidated LC3B. CCR4-NOT carries out deadenylation of mRNA, often the first step for mRNA degradation. TiERA is translation-independent ER association. The core factors of CPA machinery and PAS motifs are also shown in the inset. Four subcomplexes in the machinery and two functional modules within CPSF are noted. CPSF-73 is the endonuclease responsible for precursor RNA cleavage, WDR33 and CPSF-30 collectively interact with the A[A/U]UAAA motif, and FIP1 binds to U-rich binding sequences. CstF exists as a dimer, each containing CstF-50, CstF-64/CstF-64τ and CstF-77. CstF-64 and CstF-64τ, two paralogs in the genome, have binding affinities to U-rich and GU-rich (GUGU or UGUG) motifs. CFI exists as a tetramer, comprising two molecules of CFI-25 together with two alternative larger subunits, While both CFI-59 and CFI-68 are RNA-binding proteins, CFI-25 has binding specificity to the UGUA motif. CFII includes CLP1 and PCF11, with the latter having binding affinity to G-rich RNA sequences [90].