CYTOPLASMIC REGULATION OF THE POLY(A) TAIL LENGTH AS A POTENTIAL THERAPEUTIC TARGET

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ABSTRACT

Virtually all mRNAs acquire a poly(A) tail co-transcriptionally, but its length is dynamically regulated in the cytoplasm in a transcript-specific manner. The length of the poly(A) tail plays a crucial role in determining mRNA translation, stability, and localization. This dynamic regulation of poly(A) tail length is widely used to create post-transcriptional gene expression programs, allowing for precise temporal and spatial control. Dysregulation of poly(A) tail length has been linked to various diseases, including cancers, inflammatory and cardiovascular disorders, and neurological syndromes. Cytoplasmic poly(A) tail length is maintained by a dynamic equilibrium between cis-acting elements and cognate factors that promote deadenylation or polyadenylation, enabling rapid gene expression reprogramming in response to internal and external cellular cues. While cytoplasmic deadenylation and its pathophysiological implications have been extensively studied, cytoplasmic polyadenylation and its therapeutic potential remain less explored. This review discusses the distribution, regulation, and mechanisms of Cytoplasmic Polyadenylation Element-Binding Proteins (CPEBs), highlighting their dual roles in either promoting or repressing gene expression depending on cellular context. We also explore their involvement in diseases such as tumor progression and metastasis, along with their potential as targets for novel therapeutic strategies.

Keywords: mRNA stability, mRNA translation, poly(A) tail length, cytoplasmic polyadenylation, RNA-binding proteins, cancer, synaptic plasticity, CPEB, integrated stress response.

INTRODUCTION

Poly(A) tail length plays a crucial role in determining mRNA fate, influencing translation, stability, and localization. Cells modulate the poly(A) tail dynamically to achieve precise temporal and spatial gene expression control, ensuring that protein synthesis occurs at the appropriate time and location to meet cellular and developmental requirements. This dynamic regulation occurs in both the nucleus and cytoplasm through the opposing actions of poly(A) polymerases (PAPs) and deadenylases, whose balance is crucial for maintaining cellular homeostasis. Disruptions in these processes are linked to various diseases, including cancers and neurological disorders (Liu et al. 2023).

Poly(A) tail length is intricately tied to multiple stages of the mRNA life cycle, including transcription termination, subcellular localization, mRNA quality control, translation efficiency, and mRNA decay, thus enabling precise regulation of gene expression. Generally, longer poly(A) tails enhance mRNA stability and increase translation efficiency, leading to upregulated gene expression, whereas shorter tails promote mRNA degradation (Weill et al. 2012; Eckmann et al. 2011; Passmore and Coller 2022; Biziaev et al. 2024).

Polyadenylation, typically occurring co-transcriptionally in the nucleus, involves the addition of non-templated poly(A) tails up to 200–250 nucleotides long to RNA polymerase II (Pol II)-synthesized RNAs, except replication-dependent histone transcripts. This process is mediated by polyadenylation signals (PAS) recognized by the cleavage and polyadenylation machinery, including more than 20 core proteins like the cleavage and polyadenylation specificity factors (CPSFs) and nuclear poly(A) polymerase (PAPα) (Tian and Manley 2017). Many mRNAs contain multiple PAS, leading to alternative cleavage and polyadenylation (APA) events that generate mRNA isoforms with varying 3′ untranslated regions (UTRs). The length of these UTRs influences the inclusion or exclusion of cis-acting elements, such as microRNA (miRNA) binding sites or AU-rich elements (AREs), which recruit deadenylation complexes and regulate mRNA stability and translation (Mitschka and Mayr 2022).

As the poly(A) tail grows, it recruits nuclear poly(A)-binding proteins (PABPN), which enhance PAP's binding and processivity, ultimately mediating the export of mRNA to the cytoplasm (Eliseeva et al. 2013). In the nucleus, the Poly(A)-specific ribonuclease (PARN) functions as a deadenylase involved in quality control, shortening poly(A) tails as needed (Godwin et al. 2013). In the cytoplasm, PABPN1 is replaced by the cytosolic poly(A)-binding protein (PABPC), which protects mRNA from degradation and promotes translation. Cytoplasmic poly(A) tail length continues to be regulated dynamically by cytoplasmic poly(A) polymerases, including atypical enzymes like GLD-2 and PAPD4, and by deadenylases. In mammals, deadenylation proceeds in a biphasic manner, primarily involving two conserved complexes: PAN2-PAN3 and CCR4-NOT (Weill et al 2012; Liu et al. 2023). This cytoplasmic regulation allows for further fine-tuning of mRNA stability and translation efficiency after nuclear export. Enzymes can elongate or shorten the poly(A) tail based on cellular needs, modulating gene expression in response to various signals. This dual-layer regulation ensures that mRNA function is initially established in the nucleus and subsequently refined in the cytoplasm, providing precise control over protein synthesis to adapt to changing cellular conditions.

CYTOPLASMIC REGULATION OF POLY(A) TAIL LENGTH

Cis-acting elements, such as microRNAs (miRNAs) and AU-rich elements (AREs), primarily function to promote mRNA deadenylation and degradation. AREs were initially identified as conserved sequences composed of AUUUA pentamers located within the 3′ UTRs of specific mRNAs, particularly those encoding cytokines. These elements play a significant role in post-transcriptional regulation by influencing mRNA stability. It is estimated that approximately 16% of human protein-coding genes contain AREs, many of which are associated with critical processes like inflammation, immune response, and cancer progression (Gruber et al. 2011; Beisang and Bohjanen 2012; Khabar 2010).

ARE-binding proteins (AUBPs) regulate mRNA stability by either promoting decay or stabilizing transcripts (Otsuka et al. 2019). For example, tristetraprolin (TTP) binds to ARE-containing mRNAs and facilitates degradation by recruiting the CCR4-NOT deadenylase complex, the exosome, and the

Dcp1a/Dcp2 decapping complex, leading to translational repression (Bulbrook et al. 2018; Lykke-Andersen and Wagner 2005). The activity of TTP and similar AUBPs is often modulated by phosphorylation through signaling pathways such as p38/MAPK. Phosphorylation of TTP decreases its affinity for AREs, reducing its ability to target mRNAs for degradation (Tiedje et al. 2012). Conversely, other AUBPs, like HuR, stabilize mRNA transcripts by preventing their degradation (Brennan and Steitz 2001). This regulatory balance is critical for cellular homeostasis and has implications for diseases such as cancer, cardiovascular disorders, and neurodevelopmental diseases (Podszywalow-Bartnicka and Neugebauer 2024; Liu et al. 2023).

CPEB PROTEINS: STRUCTURAL AND FUNCTIONAL DIVERSITY

While numerous RNA-binding proteins (RNABPs) are involved in either promoting mRNA degradation or stabilization, only a select few have the ability to reactivate mRNAs that have been stored in an inactive state with shortened poly(A) tails. Among these, the Cytoplasmic Polyadenylation Element-Binding (CPEB) proteins are the most extensively characterized, and possibly the only RNABPs known to perform this function. CPEBs recognize and bind to cytoplasmic polyadenylation elements (CPEs) located in the 3′ UTRs of approximately 20% of vertebrate genes, modulating mRNA translation through post-transcriptional regulation (Pique et al. 2008; Duran-Arque et al. 2022) **(Figure 1).**

All CPEB proteins possess a conserved C-terminal domain containing two RNA recognition motifs (RRMs), which are crucial for mRNA binding, while their N-terminal domains are variable **(Figure 2)**. This N-terminal region is responsible for their regulatory functions and interactions with other cellular factors, allowing CPEBs to adapt to diverse cellular environments and signaling cues (Fernandez-Miranda and Mendez 2012; Afroz et al. 2014). This structural versatility enables CPEBs to act as key regulators of mRNA stability and translation, thereby influencing numerous cellular processes.

CPEBs are nucleocytoplasmic shuttling proteins that regulate mRNAs at multiple levels **(Figure 3).** In the nucleus, CPEBs participate in the definition of alternative PAS, and therefore alternative 3’ UTRs (Bava et al. 2013). CPEB can also recruit the nuclear deadenylase PARN (Kim and Richter

2006). In the cytoplasm, CPEBs regulate mRNA translation, either by assembling repressor complexes, in condensates with liquid-like properties **(Figure 4)** (LLDs, which include the CCR4-NOT deadenylase that maintains target transcripts translationally silenced or by promoting cytoplasmic polyadenylation and subsequent translation through the recruitment of the atypical poly(A) polymerases GLD-2 or GLD-4 (Igea et al. 2010; Guillen-Boixet et al. 2016; Duran-Arque et al. 2022). Coordinated with the temporal regulation of translation, the CPEB-assembled translational repression complexes can be localized and subsequently locally activated to generate spatial regulation of gene expression (Pascual et al. 2020b; Nagaoka et al. 2012; Huang et al. 2023; Huang and Richter 2004).

The CPEB family consists of four members in vertebrates, classified into two subfamilies: CPEB1, and CPEB2–4. These two groups have differential properties in target/motif recognition, formation and dynamics of large-order complexes and regulation by posttranslational modifications. CPEB1 recognizes canonical CPEs (UUUUA(1-2)U), whereas CPEB2–4 recognize both canonical and G-variant CPEs (UUUUGU). This differential binding implies that, while CPEB1 targets are shared with CPEB2–4, this second CPEB subfamily has also other specific targets. CPEB1 assembles into large ribonucleoprotein (RNP) complexes that exhibit structural stability and associate with the cytoskeleton for mRNA localization, aligning with its interaction with dynein and kinesin (Huang et al. 2003) **(Figure 3)**. On the other hand, condensates assembled by CPEB2–4 showed properties of canonical LLDs in terms of size, morphology and dynamic properties **(Figure 4)**.

Regulation by phosphorylation also defines two subfamilies **(Figure 2)**. Thus, CPEB1 is activated by a single phosphorylation event mediated by Aurore kinase A (AurkA), leading to the remodeling of its mRNP complexes but not their inclusion into LLDs (Duran-Arque et al. 2022). Subsequent multiple proline-directed phosphorylation events promote CPEB1 degradation. On the other hand, CPEB2-4 are activated through multiple proline-directed phosphorylation events (by ERK and Cdk1), which promote the dissolution of LLDs (Duran-Arque et al. 2022). Therefore, the two CPEB subfamilies are regulated through distinct signaling pathways and mechanisms and more importantly, both subfamilies can not be activated simultaneously. Indeed, AurkA is specifically activated at pro-metaphase and upon synaptic stimulation, while

CDK1/ERK are activated starting from anaphase during cell cycle, and ERK as part of the stress responses. This implies that, for cells that co-express the two subfamilies as well as for mRNAs with canonical CPEs, there will be a competitive equilibrium between a CPEB acting as a repressor and other CPEBs acting as activators. mRNAs with non-canonical G/CPEs would not be subjected to these opposing actions. In addition, regulation by multiple phosphorylation sites in CPEB2–4, compared to a single site in CPEB1, enables an ultrasensitive response in the CPEB2–4 subfamily. Multiple cooperative phosphorylation events enhance the sensitivity of successive steps, resulting in a steep, sigmoidal output. Additionally, requiring phosphorylation of only a subset of sites for activation balances low- and high-end thresholds, further improving system responsiveness. (Ferrell and Ha 2014).

Changes in CPEB expression, particularly in epithelial cells, are linked to tumor growth and vascularization, highlighting their involvement in cancer biology (Fernandez-Miranda and Mendez 2012; D'Ambrogio et al. 2013; Ortiz-Zapater et al. 2011; Calderone et al. 2016). The properties of the regulation of poly(A) tail length by CPEBs generate a mechanism to define temporal and spatial windows of gene expression that can be rapidly reprogrammed in response to intracellular and extracellular cues. Accordingly, this dynamic regulation of the poly(A) tail length is extensively utilized to create post-transcriptional gene expression programs where precise temporal or spatial patterns are required.

Therefore, both ARE- and CPE-dependent mechanisms generate a dual regulatory equilibrium in which the poly(A) tail length is dynamically modulated by opposing cis-acting elements, CPEs and AREs, which in turn recruit trans-acting factors competing for opposite outcomes (CPEB1/CPEB2-4 or TTP/HuR) **(Figure 5)**. CPEB proteins and tristetraprolin (TTP) are both RNA-binding proteins that regulate mRNA stability and translation, but they generally exert opposing effects. CPEB and TTP have opposing functions in various cellular contexts. CPEB generally acts to stabilize mRNAs and promote their translation, enhancing protein expression, whereas TTP promotes mRNA degradation and decreases protein expression. Their interplay is critical for fine-tuning gene expression in response to cellular and environmental cues, balancing processes such as inflammation, cell cycle progression, synaptic plasticity, and tumor growth.

These trans-acting factors are themselves regulated, in a coordinated manner, by signal transduction pathways that are, in turn, translationally controlled by CPEs and AREs, establishing self-sustained, through interlinked positive and negative feedback loops, gene expression programs (Belloc and Mendez 2008; Suñer et al. 2022). This coordination ensures that cellular responses are tightly regulated and adaptive, allowing for precise modulation of gene expression in response to external stimuli and maintaining cellular homeostasis.

CPEB PROTEINS IN DISEASE CONTEXTS

Neurological Disorders:

Local protein synthesis at synapses is critical for synaptic plasticity, the cellular basis of learning and memory. Newly synthesized proteins are believed to "tag" activated synapses, distinguishing them from unstimulated ones and thereby establishing a form of cellular memory. This process requires the transport of mRNA from the soma to dendrites, coupled with translational activation at synapses in response to neuronal stimulation. CPEBs are essential for synaptic plasticity and memory, playing key roles in regulating mRNA localization, translation, and stability, which are vital for neuronal function. Dysregulation of CPEB expression or activity has been implicated in cognitive impairments, neurodevelopmental and neurodegenerative diseases, including autism spectrum disorder (ASD), epilepsy, Huntington's disease, Alzheimer's disease, and Fragile X syndrome (FXS) (Huang et al., 2023).

Notably, different CPEB family members can contribute to the same phenotype by participating in distinct sequential steps. For instance, CPEB1 interacts with fragile X mental retardation protein (FMRP), the product of the *FMR1* gene associated with FXS, a neurodevelopmental disorder on the autism spectrum (ASD) and the most common inherited cause of intellectual disability. Interestingly, depletion of CPEB1 has been shown to compensate for mutant FMRP, presumably due to its role in mRNA translational repression and localization (Udagawa et al 2013). CPEB4 also plays a significant role in ASD. Increased skipping of the neuron-specific

exon 4 in CPEB4 (*nCPEB4Δ4*) has been observed in idiopathic ASD. This splicing alteration correlates with reduced poly(A) tail length and diminished expression of several ASD-risk genes. Transgenic expression of *nCPEB4Δ4* in mouse brains induced autistic-like behaviors, including changes in anxiety, vocalization, and social interaction. However, CPEB4 haploinsufficiency or loss does not phenocopy *nCPEB4Δ4*, suggesting that the mutant variant acts as a dominant negative, disrupting the function of all CPEB proteins (Parras et al 2018). Our findings also reveal that maintaining a precise ratio of microexon 4 inclusion is essential for preserving the dynamic state of neuronal CPEB4 (Garcia-Cabau et al. 2024). Neuronal CPEB4 forms condensates that dissolve upon depolarization, transitioning from translational repression to activation. This phase transition is mediated by heterotypic interactions between the microexon and a cluster of histidine residues. These interactions kinetically stabilize the condensates by competing with homotypic interactions that otherwise promote irreversible aggregation. The phase equilibrium of neuronal CPEB4 is regulated by changes in the protonation state of histidine residues in the intrinsically disordered N-terminal domain (NTD) in response to local pH fluctuations during depolarization. These changes modulate intermolecular interactions, promoting reversible condensation. Thus, Microexon 4 plays a crucial role in enhancing both the thermodynamic and kinetic stability of repressive CPEB4 condensates, effectively preventing aggregation. Importantly, even slight variations in microexon 4 inclusion significantly alter aggregation tendencies. In ASD, the observed reduction in microexon 4 inclusion levels leads neuronal CPEB4 to adopt a more solid-like state sequestering CPE-containing mRNAs and other CPEBs present in the same agregates. Intriguingly, exogenous addition of a peptide containing the microexon can restore the dynamic properties of condensates formed by *nCPEB4Δ4* in a dose-dependent manner. This finding suggests potential therapeutic strategies for targeting the pathological effects of microexon 4 skipping in ASD.

Cancer: CPEBs are integral to cell cycle regulation, influencing tumor development and progression by modulating mRNA translation through poly(A) tail length dynamics. During meiotic and mitotic cell cycle transitions, changes in poly(A) tail length facilitate the translation or degradation of

mRNAs encoding critical proteins like cyclins and cyclin-dependent kinases (CDKs). Generally, phosphorylated CPEBs, in conjunction with HuR, promote cell cycle progression, whereas unphosphorylated CPEB1 and TTP act as inhibitors, repressing or destabilizing their target mRNAs to halt cell cycle progression (Villalba et al. 2011; Ivshina et al. 2014; Galloway et al. 2016; Haga et al. 2024; Pascual et al. 2020b).

Both CPEBs and ARE-binding proteins (AREBPs) are implicated in tumor suppression and cancer progression. For example, TTP and HuR, which either destabilize or stabilize prosurvival mRNAs, have been widely studied in cancer biology (Ross et al. 2012; Finan et al. 2023). Cytoplasmic HuR plays a key role in stabilizing and enhancing the translation of prosurvival mRNAs involved in cellular stress responses, including those triggered by hypoxia, radiotherapy, and chemotherapy. Its ability to bind and stabilize these mRNAs enhances the cell's capacity to survive and adapt under stress conditions, which is particularly advantageous for cancer cells. HuR's overexpression and elevated activity in cancer cells, compared to its relatively lower levels in normal cells, make it a promising target for cancer therapy. Targeting HuR could disrupt the survival mechanisms that cancer cells rely on, potentially sensitizing them to treatments and reducing tumor progression (see below).

CPEBs proteins play diverse and context-dependent roles in cancer progression by regulating mRNAs that control cell growth, survival, and tumor suppression (Fernandez-Miranda and Mendez 2012; D'Ambrogio et al. 2013). Depending on the cellular context and specific CPEB isoform involved, they can either promote oncogenesis or act as tumor suppressors. Although their functions in cancer have not yet been systematically analyzed, and there are relatively few cases with established causality, CPEBs have been shown to influence key processes such as cell cycle regulation, cell death, and angiogenesis. This variability highlights the complexity of their roles and suggests that CPEBs could be both potential biomarkers and therapeutic targets, depending on the type and stage of cancer. CPEB1, in particular, is generally associated with tumor suppression, while CPEB4 and CPEB2 are more often linked to malignancy. Studies show that CPEB1, CPEB2, and CPEB4 are implicated in breast cancer, where they either enhance tumor growth or facilitate metastasis (Pascual et al. 2020a; Lu et al. 2017; Nagaoka et al. 2016). Additionally, CPEB1 and

CPEB3 are frequently downregulated in ovarian cancer (Hansen et al. 2009), correlating with the aggressive status of the disease. Both CPEB1 and CPEB4 are involved in glioblastoma development (Boustani et al. 2016; Galardi et al. 2016; Hui et al. 2018; Ortiz-Zapater et al. 2011). In hepatocellular carcinoma, CPEB4 plays a pivotal role in regulating ferroptosis and iron homeostasis, key processes that influence tumor progression (Delgado et al. 2024). Low CPEB4 expression correlates with poor prognosis, reduced ferroptotic sensitivity, and increased tumor aggressiveness in both human and mouse models. Mechanistically, CPEB4 enhances the translation of hepcidin, a key regulator of intracellular iron levels under stress conditions. Its depletion results in reduced hepcidin, elevated ferroportin, lower intracellular iron, and impaired lipid peroxidation, thereby diminishing ferroptotic cell death and providing liver cancer cells a survival advantage (Delgado et al. 2024). CPEB4 also exhibits a biphasic expression pattern, with high levels during early hepatocellular carcinoma transitioning to lower levels in advanced stages (Tsai et al. 2016), highlighting its stage-specific tumor-suppressive functions. These findings suggest that therapeutic strategies aimed at activating CPEB4 or inhibiting its antagonist, CPEB1, hold promise for mitigating liver cancer progression. Furthermore, CPEB4 contributes to gastric cancer progression by promoting growth and metastasis via ZEB1-mediated epithelial-mesenchymal transition (EMT), illustrating its critical role in malignancy enhancement (Cao et al. 2018). CPEB4 also indirectly influences colorectal cancer development through inflammation resolution (Sibilio et al. 2022). In contrast, CPEB3 acts as a tumor suppressor in gastric cancer (Chen et al. 2022), emphasizing the diverse roles of CPEB proteins across different cancers. In pancreatic ductal adenocarcinoma and glioblastoma, aberrant CPEB4 expression promotes metastasis by activating tissue plasminogen activator (tPA) mRNA, which is usually silenced in homeostatic conditions (Ortiz-Zapater et al. 2011). CPEB4 also contributes to melanoma progression by regulating the translation of mRNAs linked to cell migration and invasion (Perez-Guijarro et al. 2016). In contrast, CPEB1 generally acts as an antitumoral agent in various cancers. For example, it has been shown to suppress tumor growth in pancreatic cancer, gastric cancer, and melanoma (Zhang et al. 2024; Shoshan et al. 2015). This complex interplay between CPEB family members underscores their context-specific functions in cancer biology. While CPEB1

often functions as a tumor suppressor, CPEB4 frequently supports tumorigenic processes, highlighting the need for precise therapeutic targeting based on the specific cancer type and CPEB protein involved.

Immune regulation and inflammation: CPEBs are emerging as important regulators in immune responses and inflammation. Poly(A) tail dynamics are key in creating temporal windows for gene expression, which are essential for controlling the tumor inflammatory immune environment. RBPs, such as CPEBs, interact with cis-RNA elements to regulate mRNA stability and translation, thus influencing immune cell function and metabolism (Turner and Diaz-Muñoz 2018).

A well-studied RBP-mediated mechanism related to inflammation involves the p38 MAPK-regulated balance between TTP (which destabilizes mRNAs) and HuR (which stabilizes them), controlling the timing and levels of pro-inflammatory cytokines (Suñer et al. 2022). Disruption in this balance can result in either reduced cytokine production or chronic inflammation, leading to autoimmune diseases or impaired anti-tumor immune responses (Salerno et al. 2018a, 2018b).

Although much less studied, recent data also suggest a role for CPEBs in tumor-associated immune responses. For instance, CPEB4-transduced dendritic cells have been shown to enhance the number of IFN-γ secreting cytotoxic T-cells in gliomas, reducing tumor growth and increasing lifespan in mouse models (Peng et al. 2014). Lipopolysaccharide (LPS)-injected CPEB1 knockout mice produce significantly elevated levels of IL-6 and other pro-inflammatory cytokines, exhibiting enhanced sensitivity to endotoxic shock. These findings indicate that CPEB1 plays a protective role in regulating the innate immune response (Ivshina et al. 2015). Additionally, CPEB4 mediates a novel adaptive mechanism of the unfolded protein response (UPR), aimed to resolve the ER-stress caused by the accumulation of unprocessed proteins in the ER lumen, essential for the function of CD8+ T-cells. Depletion of CPEB4 impaired T-cell-mediated antitumor responses, underscoring its role in sustaining T-cell effector functions by mitigating chronic ER stress generated by the secretory phenotype of effector T cells (Fernandez-Alfara et al. 2023). In macrophages, CPEB4 stabilizes anti-inflammatory mRNAs during inflammatory responses, promoting the resolution of inflammation. Mice lacking CPEB4 show increased inflammatory responses and impaired survival, indicating that

CPEB4 is essential for maintaining macrophage function and preventing excessive inflammation (Suñer et al. 2022). CPEB4's role extends to responses to LPS stimulation, where its absence led to elevated inflammatory signaling and reduced anti-inflammatory cytokines (Suñer et al. 2022). Furthermore, CPEB4's involvement in obesity-related inflammation has also been documented. CPEB4 drives post-transcriptional reprogramming in adipocytes under obesity conditions, affecting macrophage polarization and migration. CPEB4 regulates pro-inflammatory factors such as CCL2 and TLR4, linking its activity to inflammation modulation in adipose tissue and broader immune system dynamics (Pell et al. 2021). CPEB4-deficient mice showed exacerbated inflammation and altered immune responses, including decreased IL-22 expression, which influenced both acute inflammation resolution and tumor progression (Sibilio et al. 2022).

Overall, these findings emphasize the critical role of the CPEB1/CPEB4 balance in immune regulation, inflammation resolution, and tumor dynamics. Its involvement in stress adaptation, macrophage function, and tumor immunity highlights its potential as a therapeutic target for modulating immune responses and addressing inflammatory and cancerous conditions.

Chronic stress responses: Metabolic stress, or a highly secretory phenotype, often triggers endoplasmic reticulum (ER) stress due to the accumulation of unfolded or misfolded proteins, leading to activation of the unfolded protein response (UPR). The UPR consists of three signaling pathways mediated by activating transcription factor-6 (ATF6), inositol-requiring enzyme-1α (IRE1α), and the eIF2α kinase PERK. Phosphorylation of eIF2α reduces overall protein synthesis, while simultaneously allowing the selective translation of specific mRNAs containing short upstream open reading frames (uORFs). These initial stress responses either facilitate the resolution of stress or, if the stress cannot be resolved, promote apoptosis by inducing the transcription factor CHOP (Wang and Kaufman 2014; Wek et al. 2006; Hetz et al. 2020; Harding et al. 2000). The primary goal of these early responses is to manage acute, temporary stress or trigger programmed cell death if necessary.

However, in cases of chronic stress, such as prolonged exposure of hepatocytes to a high-fat diet (HFD), the ER stress is persistent but less severe, and it does not lead to apoptosis. This chronic ER

stress response is partially regulated by CPEB4, which activates the translation of mRNAs containing cytoplasmic polyadenylation elements (CPEs) **(Figure 6).** The proteins produced by these mRNAs help alleviate metabolic ER stress without causing a general shutdown of protein synthesis. Unlike the response triggered by eIF2α phosphorylation, the CPEB4-mediated response can be sustained over long periods without harmful effects on the cell (Maillo et al. 2017). The translation of Cpeb4 mRNA itself is initially activated by upstream open reading frames (uORFs) in its 5′ untranslated region (UTR) (Maillo et al. 2017) and is later maintained by CPE elements in its 3′ UTR through a positive feedback loop (Igea et al. 2010).

CPEB4, unlike other members of the CPEB family, is encoded by a circadian-regulated and uORF-containing mRNA that responds to acute stress and eIF2α phosphorylation. The levels of CPEB4 mRNA fluctuate in a circadian rhythm, allowing the protein to prepare cells for periods of increased ER stress (Balvey and Fernandez 2021). This function of CPEB4 plays a critical role in the adaptation to chronic ER stress, particularly in highly secretory immune cells, where it regulates cytokine production. CPEB4 allows effector CD8+ T cells to maintain their high anabolic and secretory capacity, and thereby supports their antitumor activity (Fernandez-Alfara et al. 2023). Similarly, CPEB4 is essential for other cellular adaptations associated with chronic activation of the integrated stress response, such as in cardiac hypertrophy and heart failure, where it regulates genes involved in cardiac function and stress response (Riechert et al. 2021). Additionally, CPEB1 and CPEB4 work together to regulate the translation of VEGF mRNA, promoting angiogenesis in chronic liver disease (Calderone et al. 2016).

Liver and metabolic disorders: Although the involvement of multiple CPEB proteins in metabolic disorders is not extensively documented, CPEB1 and CPEB4 have been linked to critical metabolic processes. For example, CPEB1 plays a role in regulating insulin signaling pathways (Alexandrov et al. 2012), while CPEB4 influences lipid metabolism and glucose homeostasis (Maillo et al. 2017; Burns and Richter 2008). Notably, CPEB4 is required for adipose tissue expansion and inflammation under obesity conditions. It also modulates gut microbiota, shifting it toward a more pathogenic profile. Studies have shown that CPEB4 depletion in high-fat diet (HFD) models reduces

adipogenesis and attenuates the pro-inflammatory characteristics of both visceral fat depots and the microbiome (Pell et al. 2021).

CPEB4's role in liver disease is complex and stage-dependent, acting as both a protector and a promoter of pathology. In the early stages of liver steatosis, particularly under HFD and obesity conditions, CPEB4 has a protective function. It supports mitochondrial fatty acid oxidation and regulates endoplasmic reticulum (ER) stress, helping to maintain metabolic balance (Maillo et al. 2017). Mice deficient in CPEB4 demonstrate lipid accumulation, hyperglycemia, and impaired stress responses, highlighting CPEB4's critical role in lipid and glucose homeostasis. The impairment of lipid metabolism due to CPEB4 deficiency indirectly disrupts glucose metabolism by inhibiting hepatic insulin signaling, which leads to hepatosteatosis and the accumulation of unfolded proteins (Maillo et al. 2017).

As liver disease progresses to advanced stages, CPEB4's role shifts from protective to detrimental. It becomes a contributor to liver fibrosis by activating hepatic stellate cells (HSCs) through the upregulation of PFKFB3, a key enzyme in glycolysis (Mejias et al. 2020). This shift towards glycolytic reprogramming fuels HSC proliferation and fibrogenic activity, resulting in excessive collagen deposition and compromised liver structure. Molecularly, CPEB4 drives fibrogenesis by binding to cytoplasmic polyadenylation element (CPE) sequences in PFKFB3 mRNA, thereby increasing its translation and promoting HSC activation. This process is a key factor in the excessive extracellular matrix (ECM) production characteristic of liver fibrosis. Furthermore, CPEB4 facilitates pathological angiogenesis in liver cirrhosis by enhancing the translation of vascular endothelial growth factor (VEGF) mRNA, leading to increased endothelial cell proliferation and new blood vessel formation (Calderone et al. 2016). Additionally, CPEB4 protects against liver cancer progression by regulating iron-dependent ferroptotic cell death in liver tumor cells (Delgado et al. 2024). CPEB4 deficiency reduces ferroptosis sensitivity, thereby promoting tumor progression.

The dual role of CPEB4 in liver disease highlights the need for a nuanced therapeutic approach. Targeted modulation of CPEB4 activity could potentially mitigate its harmful effects in fibrosis and pathological angiogenesis, while preserving its beneficial functions in managing ER stress and

maintaining metabolic homeostasis. Such precision therapies could provide a promising strategy to address the multifaceted challenges associated with chronic liver disease.

POLYADENYLATION AND CPEBs AS THERAPEUTIC TARGETS

CPEBs, like many RNA-binding proteins (RBPs), have traditionally been considered challenging drug targets due to their structural properties and the lack of well-defined small molecule binding sites. The conservation of canonical RNA-binding domains (RBDs) and the intrinsically disordered regions (IDRs) present in many RBPs add further complexity to developing small molecule inhibitors. Despite these challenges, recent advancements in high-throughput screening technologies for RBP-small molecule interactions are opening new avenues for therapeutically targeting these regulators of poly(A) tail length.

CPEBs are divided into two subfamilies, each exhibiting distinct, often opposing functions, integrating various cellular signals to modulate gene expression (Duran-Arque et al. 2022). The N-terminal domain of CPEBs is highly variable across the four isoforms, featuring intrinsically disordered regions that likely serve as docking sites for protein-protein interactions. For instance, CPEB1's N-terminal domain contains phosphorylation sites for Aurora A kinase, ERK/Cdk1, and Plk1, which regulate its activity through ubiquitination and subsequent degradation via the PEST box. In contrast, CPEB2-4 harbor similar regulatory phosphorylation sites controlled by ERK/p38 kinases and uniquely regulate the formation of liquid-liquid phase-separated assemblies that sequester inactive mRNAs (Duran-Arque et al. 2022; Guillen-Boixet et al. 2016).

All CPEB proteins can interact with the CCR4-NOT complex to repress translation and, upon phosphorylation, recruit the GLD2 complex to activate translation. Notably, higher-order complexes formed by CPEB1 resemble canonical ribonucleoprotein (RNP) assemblies, requiring mRNA binding for their formation. In contrast, CPEB2-4 are capable of forming liquid-liquid phase-separated (LLD) droplets, which are characterized by their unique size, morphology, and dynamics, independent of RNA binding. This structural and functional divergence highlights the unique distribution of disordered regions within the N-terminal domains of the two subfamilies. Upon co-expression, different CPEBs co-localize into larger aggregates while preserving their individual

characteristics, indicating spatial heterogeneity within the LLDs. This probably reflects fusion events of smaller, and CPEB-specific, aggregates into larger ones. Thus, large aggregates contain sub-domains for each of the CPEBs subjected to local regulation by their specific Co-factors. At the same time, the phase transition dynamic properties of the large aggregates become unified, explaining the dominant negative effect of the CPEB4D4 splice variant explained above. Their proximomes (proteins into close spatial proximity identified by BioID, where a fusion between CPEBs and a mutant biotin ligase catalyzes the covalent attachment of biotin to nearby proteins within a range of 10 nanometers, labeling them for subsequent identification via mass spectrometry), comprise both common and CPEB-specific cofactors, with CPEB1 undergoing remodeling after a single phosphorylation event, while CPEB2-4 are modulated by multiple proline-directed phosphorylation events that govern their phase separation.

The RNA recognition motifs (RRMs) in CPEB2-4 are highly conserved, showing 96% sequence identity, while CPEB1 shares only about 46% identity with the other isoforms. The tandem arrangement of RRMs in CPEB1 and CPEB4 is unique, enabling both domains to bind a continuous RNA oligonucleotide (Afroz et al. 2014). Additionally, CPEB RRMs contain extensions to the canonical RRM fold; for example, CPEB1 has an extension that interacts between the region upstream of RRM1 and the helical face of RRM2, enhancing its specificity for canonical cytoplasmic polyadenylation elements (CPEs; $UUUUA_{(1-2)}U$). In contrast, CPEB2-4 can also bind "G-variants" (UUUUGU), expanding their RNA target specificity.

Integrating mechanistic insights with innovative screening methodologies holds significant potential for discovering novel compounds that target CPEBs. Recent assays targeting RBPs have emerged, such as AlphaScreen® technology, which identifies small molecules that modulate cytoplasmic poly(A) tail length regulation (Julio and Backus 2021; Bertoldo et al. 2023). Understanding the dynamic equilibrium governing polyadenylation and deadenylation opens new therapeutic strategies to selectively enhance or inhibit these processes. In particular, the mechanistic understanding of CPEBs functions, structures and regulation is being exploited in multiple directions:

Proximity-based biotinylation strategies, like Bio-ID labeling, are expanding the range of potential therapeutic targets within polyadenylation and deadenylation complexes. These methods

are beginning to shed light on the complex compositions and dynamics associated with CPEB assemblies, enabling the identification of new molecular players and potential drug targets (Duran-Arque et al. 2022).

Targeting kinase pathways that regulate CPEBs also represents a promising approach. Both ARE and CPE elements are co-regulated by proline-directed kinases like p38 and ERK, which activate CPEB4 and HuR, while inhibiting TTP and CPEB1 function (Suñer et al. 2022; Duran-Arque et al. 2022). Inhibitors targeting these kinases could therefore shift the balance towards deadenylation and inactivation of mRNAs associated with these elements, including those encoding pro-inflammatory cytokines and other critical proteins.

High-throughput screening technologies, such as proximity-based fluorescent assays (e.g., AlphaScreen®), are proving effective in identifying small compounds that can disrupt RBP-RNA interactions. The distinctive RNA recognition mechanisms employed by CPEB RRMs may enhance selectivity for drug candidates. The structural differences among CPEB RRMs, alongside the specific binding patterns of ARE-binding proteins (ARE-BPs) and CPE-binding proteins (CPE-BPs), suggest the feasibility of identifying compounds that can selectively tilt the balance towards polyadenylation or deadenylation. For instance, HuR, a prominent ARE-binding protein known for stabilizing mRNAs involved in cancer, inflammation, and stress responses, has several small-molecule inhibitors in development that reduce levels of HuR-regulated mRNAs in an ARE-dependent manner. These include MS-444 (blocks HuR dimerization), KH-3 (prevents HuR from binding to AREs), deltonin (downregulates HuR expression), dehydromutactin (disrupts HuR-mRNA interactions) and azaphilone derivatives (inhibit HuR's function) (Bertoldo et al. 2023; Li and Kang 2023).

Beyond small molecules, *RNA-PROTACs* offer a novel approach. These compounds utilize small RNA mimetics to target the RNA-binding site of RBPs, facilitating their proteasomal degradation via a conjugated E3-recruiting peptide (Ghidini et al. 2021). The specificity of cis-acting element recognition for RBPs controlling cytoplasmic poly(A) tail length allows for targeted interventions against the ARE-BPs TTP and HuR (AUUUA), CPEB1-4 (UUUUAU) or CPEB2-4 (UUUUGU), potentially correcting dysregulated gene expression associated with diseases.

Exploiting the gene expression reprogramming linked to imbalanced CPEB expression in various pathological conditions offers promising avenues for therapeutic intervention. Targeting CPEBs may allow for the ectopic expression or suppression of factors that influence cellular function, survival, or apoptosis, depending on the disease context. For instance, pancreatic ductal adenocarcinoma (PDA) cells exhibit a dependency on elevated levels of CPEB4 compared to normal tissue (Ortiz-Zapater et al. 2011). This dependency can be leveraged therapeutically by engineering oncolytic adenoviruses with CPE regulatory sequences in the 3′ untranslated region of the E1A gene. Such viruses achieve selective efficacy in targeting cancer cells (oncoselectivity) while minimizing off-target effects on normal cells (Villanueva et al. 2017). This approach underscores the potential of CPEBs to direct tissue-specific therapies with reduced systemic toxicity.

The exploration of CPEBs and other RNA-binding proteins (RBPs) as therapeutic targets represents a complex but promising opportunity for drug development. Advancing our understanding of their mechanistic roles—especially how they regulate mRNA stability, translation, and poly(A) tail dynamics—can facilitate the identification of new intervention points. Innovative screening technologies, including high-throughput assays and proximity-based biotinylation strategies, are critical tools that can uncover selective compounds or RNA-based therapies targeting these RBPs. By combining mechanistic insights with these state-of-the-art screening methodologies, the therapeutic potential of CPEBs can be harnessed to address a variety of diseases, including cancers, inflammatory disorders, and neurodegenerative conditions.

CONCLUSION

CPEBs appear to have evolved exclusively in multicellular organisms, likely associated with meiotic cell divisions (Fernandez-Miranda, 2012). The initial CPEB was likely CPEB1 (Orb in *Drosophila*), with the other subfamily members arising through gene duplication (CPEB2-4 in vertebrates, Orb2 in *Drosophila*). The first duplication event into two subfamilies allowed for an expansion of mRNA targets and the diversification of regulation through different kinases and phase transitions (Duran Arque et al., 2022). The subsequent duplication, from Orb2 to CPEB2-4, had a more subtle impact. Although CPEB2-4 proteins appear to have distinct functions and tissue-specific

expression patterns, they are largely functionally interchangeable, suggesting that their specialization is more related to their tissue-specific expression than to their individual molecular functions. Throughout evolution, CPEBs seem to have developed roles that help overcome the physiological constraints of transcriptional regulation. The original function of CPEBs likely involved the regulation of maternal mRNAs during meiosis, occurring when chromatin is fully condensed and transcription is inhibited. A similar scenario arises during the mitotic M-phase (Pascual et al., 2020). CPEB4, in particular, mediates the chronic stress response by preventing the transcription of new mRNAs under stress conditions, where transcription errors are more likely. This allows for the reactivation of pre-existing, stored mRNAs. A prominent example of this is seen in synaptic translation in neurons, where CPEBs regulate the repression, localization, and local activation of mRNAs. This regulation holds significant potential for determining cellular polarity and functional compartmentalization, processes that cannot be achieved through transcriptional mechanisms alone. This specialization in particular physiological processes, in turn, determines the pathological scenarios where CPEBs have been implicated.

CPEB proteins play diverse and complex roles across various disease contexts, often exhibiting opposing functions that depend on the cellular environment and specific isoform involved. This functional dichotomy presents both challenges and opportunities for therapeutic intervention. For instance, while CPEB1 generally acts as a tumor suppressor by promoting apoptosis and inhibiting cell proliferation, CPEB4 often has the opposite effect, enhancing cell proliferation and inhibiting apoptosis, thereby supporting tumor growth and survival. These contrasting functions highlight the difficulty in developing targeted therapies, as the roles of CPEBs can vary dramatically depending on the context.

Despite these challenges, the intricate regulatory mechanisms of CPEBs present unique opportunities for therapeutic innovation. By precisely targeting the functional antagonisms within CPEB isoforms, it may be possible to develop effective treatments that exploit these dual roles to address specific pathological conditions. Understanding the balance between CPEB isoforms in different diseases could lead to the development of tailored therapeutic strategies, offering a pathway toward precision medicine.

Integrating transcriptomic and proteomic approaches is critical to identifying CPEB target genes and proteins and to unraveling their involvement in processes such as cell proliferation, stress response, and immune regulation. This comprehensive analysis will be instrumental in discovering novel biomarkers and therapeutic targets, paving the way for more precise and effective interventions.

Looking ahead, several key research directions need to be pursued to fully unlock the therapeutic potential of CPEB proteins. A deeper understanding of the context-dependent roles of CPEBs, such as the pro-oncogenic activity of CPEB4 in cancers and its involvement in liver diseases, is essential. Future studies should focus on elucidating the specific molecular mechanisms through which CPEBs influence disease progression and how they interact with other signaling pathways. Advanced in vivo models and emerging technologies will also be crucial for translating these insights into clinical applications.

Overall, a more refined understanding of CPEBs will be instrumental in developing targeted therapies for a range of conditions. By addressing these complexities, future research has the potential to harness the full therapeutic potential of CPEBs, contributing to the advancement of precision medicine.

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REFERENCES

Afroz T, Skrisovska L, Belloc E, Guillen-Boixet J, Mendez R, Allain FH. 2014. A fly trap mechanism provides sequence-specific RNA recognition by CPEB proteins. *Genes Dev* **28**: 1498–1514. doi:10.1101/gad.241133.114

Alexandrov IM, Ivshina M, Jung DY, Friedline R, Ko HJ, Xu M, O'Sullivan-Murphy B, Bortell R, Huang YT, Urano F, et al. 2012. Cytoplasmic polyadenylation element binding protein deficiency stimulates PTEN and Stat3 mRNA translation and induces hepatic insulin resistance. *PLoS Genetics* **8**: e1002457. doi:10.1371/journal.pgen.1002457

Balvey A, Fernandez M. 2021. Translational Control in Liver Disease. *Front Physiol* **12**: 795298. doi:10.3389/fphys.2021.795298

Bava FA, Eliscovich C, Ferreira PG, Minana B, Ben-Dov C, Guigo R, Valcarcel J, Mendez R. 2013. CPEB1 coordinates alternative 3'-UTR formation with translational regulation. *Nature* **495**: 121–125. doi:10.1038/nature11901

Beisang D, Bohjanen PR. 2012. Perspectives on the ARE as it turns 25 years old. *Wiley Interdiscip Rev RNA* **3**: 719–731. doi:10.1002/wrna.1125

Belloc E, Mendez R. 2008. A deadenylation negative feedback mechanism governs meiotic metaphase arrest. *Nature* **452**: 1017–1021. doi:10.1038/nature06809

Bertoldo JB, Müller S, Hüttelmaier S. 2023. RNA-binding proteins in cancer drug discovery. *Drug Discov Today* **28**: 103580. doi:10.1016/j.drudis.2023.103580

Biziaev N, Shuvalov A, Salman A, Egorova T, Shuvalova E, Alkalaeva E. 2024. The impact of mRNA poly(A) tail length on eukaryotic translation stages. *Nucleic Acids Res* **52**: 7792–7808. doi:10.1093/nar/gkae510

Boustani MR, Mehrabi F, Yahaghi E, Khoshnood RJ, Shahmohammadi M, Darian EK, Goudarzi PK. 2016. Somatic CPEB4 and CPEB1 genes mutations spectrum on the prognostic predictive accuracy in patients with high-grade glioma and their clinical significance. *J Neurol Sci* **363**: 80–83. doi:10.1016/j.jns.2016.02.032

Brennan CM, Steitz JA. 2001. HuR and mRNA stability. *Cell Mol Life Sci* **58**: 266–277. doi:10.1007/PL00000854

Bulbrook D, Brazier H, Mahajan P, Kliszczak M, Fedorov O, Marchese FP, Aubareda A, Chalk R, Picaud S, Strain-Damerell C, et al. 2018. Tryptophan-mediated interactions between tristetraprolin and the CNOT9 subunit are required for CCR4-NOT deadenylase complex recruitment. *J Mol Biol* **430**: 722–736. doi:10.1016/j.jmb.2017.12.018

Burns DM, Richter JD. 2008. CPEB regulation of human cellular senescence, energy metabolism, and p53 mRNA translation. *Genes Dev* **22**: 3449–3460. doi:10.1101/gad.1697808

Calderone V, Gallego J, Fernandez-Miranda G, Garcia-Pras E, Maillo C, Berzigotti A, Mejias M, Bava FA, Angulo-Urarte A, Graupera M, et al. 2016. Sequential functions of CPEB1 and CPEB4 regulate pathologic expression of vascular endothelial growth factor and angiogenesis in chronic liver disease. *Gastroenterology* **150**: 982–997. doi:10.1053/j.gastro.2015.11.038

Cao G, Chen D, Liu G, Pan Y, Liu Q. 2018. CPEB4 promotes growth and metastasis of gastric cancer cells via ZEB1-mediated epithelial- mesenchymal transition. *Onco Targets Ther* **11**: 6153-6165. doi:10.2147/OTT.S175428

Chen J, Li L, Liu TY, Fu HF, Lai YH, Lei X, Xu JF, Yu JS, Xia YJ, Zhang TH, et al. 2022. CPEB3 suppresses gastric cancer progression by inhibiting ADAR1-mediated RNA editing via localizing ADAR1 mRNA to P bodies. *Oncogene* **41**: 4591–4605. doi:10.1038/s41388-022-02454-z

D'Ambrogio A, Nagaoka K, Richter JD. 2013. Translational control of cell growth and malignancy by the CPEBs. *Nature Reviews Cancer* **13**: 283–290. doi:10.1038/nrc3485

Delgado ME, Naranjo-Suarez S, Ramirez-Pedraza M, Cardenas BI, Gallardo-Martinez C, Balvey A, Belloc E, Martin J, Boyle M, Mendez R, Fernandez M. 2024. CPEB4 modulates liver cancer progression by translationally regulating hepcidin expression and sensitivity to ferroptosis. *JHEP Reports* (in press). doi:10.1016/j.jhepr.2024.101296

Duran-Arque B, Cañete M, Castellazzi CL, Bartomeu A, Ferrer-Caelles A, Reina O, Caballe A, Gay M, Arauz-Garofalo G, Belloc E, et al. 2022. Comparative analyses of vertebrate CPEB proteins

define two subfamilies with coordinated yet distinct functions in post-transcriptional gene regulation. *Genome Biol* **23**: 192. doi:10.1186/s13059-022-02759-y

Eckmann CR, Rammelt C, Wahle E. 2011. Control of poly(A) tail length. *Wiley Interdiscip Rev RNA* **2**: 348–361. doi:10.1002/wrna.56

Eliseeva IA, Lyabin DN, Ovchinnikov LP. 2013. Poly(A)-binding proteins: structure, domain organization, and activity regulation. *Biochemistry (Mosc)* **78**: 1377–1391. doi:10.1134/S0006297913130014

Fernandez-Alfara M, Sibilio A, Martin J, Tusquets Uxo E, Malumbres M, Alcalde V, Chanes V, Cañellas-Socias A, Palomo-Ponce S, Batlle E, et al. 2023. Antitumor T-cell function requires CPEB4-mediated adaptation to chronic endoplasmic reticulum stress. *EMBO J* **42**: e111494. doi:10.15252/embj.2022111494

Fernandez-Miranda G, Mendez R. 2012. The CPEB-family of proteins, translational control in senescence and cancer. *Ageing Research Reviews* **11**: 460–472. doi:10.1016/j.arr.2012.03.004

Ferrell JE Jr, Ha SH. 2014. Ultrasensitivity part II: multisite phosphorylation, stoichiometric inhibitors, and positive feedback. *Trends Biochem Sci* **39**: 556–569. doi: 10.1016/j.tibs.2014.09.003

Finan JM, Sutton TL, Dixon DA, Brody JR. 2023. Targeting the RNA-binding protein HuR in cancer. *Cancer Res* **83**: 3507–3516. doi:10.1158/0008-5472.CAN-23-0972.

Galardi S, Petretich M, Pinna G, D'Amico S, Loreni F, Michienzi A, Groisman I, Ciafre SA. 2016. CPEB1 restrains proliferation of Glioblastoma cells through the regulation of p27(Kip1) mRNA translation. *Sci Rep* **6**: 25219. doi:10.1038/srep25219

Galloway A, Saveliev A, Łukasiak S, Hodson DJ, Bolland D, Balmanno K, Ahlfors H, Monzon-Casanova E, Mannurita SC, Bell LS, et al. 2016. RNA-binding proteins ZFP36L1 and ZFP36L2 promote cell quiescence. *Science* **352**: 453-459. doi:10.1126/science.aad5978.

Garcia-Cabau C, Bartomeu A, Tesei G, Cheung KC, Pose-Utrilla J, Picó S, Balaceanu A, Duran-Arqué B, Fernández-Alfara M, Martín J, *et al.* 2024 Mis-splicing of a neuronal microexon promotes CPEB4 aggregation in ASD. Nature. doi: 10.1038/s41586-024-08289-w.

Ghidini A, Clery A, Halloy F, Allain FHT, Hall J. 2021. RNA-PROTACs: Degraders of RNA-binding proteins. *Angew Chem Int Ed Engl* **60**: 3163–3169. doi:10.1002/anie.202012330

Godwin AR, Kojima S, Green CB, Wilusz J. 2013. Kiss your tail goodbye: the role of PARN, Nocturnin, and Angel deadenylases in mRNA biology. *Biochim Biophys Acta* **1829**: 571–579. doi:10.1016/j.bbagrm.2012.12.004

Gruber AR, Fallmann J, Kratochvill F, Kovarik P, Hofacker IL. 2011. AREsite: a database for the comprehensive investigation of AU-rich elements. *Nucleic Acids Res* **39**: D66–D69. doi:10.1093/nar/gkq990

Guillen-Boixet J, Buzon V, Salvatella X, Mendez R. 2016. CPEB4 is regulated during cell cycle by ERK2/Cdk1-mediated phosphorylation and its assembly into liquid-like droplets. *eLife* **5**: e19298. doi:10.7554/eLife.19298

Haga Y, Bandyopadhyay D, Khatun M, Tran E, Steele R, Banerjee S, Ray R, Nazzal M, Ray RB. 2024. Increased expression of long non-coding RNA FIRRE promotes hepatocellular carcinoma by HuR-CyclinD1 axis signaling. *J Biol Chem* **300**: 107247. doi:10.1016/j.jbc.2024.107247

Hansen CN, Ketabi Z, Rosenstierne MW, Palle C, Boesen HC, Norrild B. 2009. Expression of CPEB, GAPDH and U6snRNA in cervical and ovarian tissue during cancer development. *APMIS* **117**: 53–59. doi:10.1111/j.1600-0463.2008.00015.x

Harding HP, Zhang Y, Bertolotti A, Zeng H, Ron D. 2000. Perk is essential for translational regulation and cell survival during the unfolded protein response. *Mol Cell* **5**: 897–904. doi:10.1016/s1097-2765(00)80330-5

Hetz C, Zhang K, Kaufman RJ. 2020. Mechanisms, regulation and functions of the unfolded protein response. Nat Rev Mol Cell Biol 21: 421–438. doi:10.1038/s41580-020-0250-z

Huang YS, Carson JH, Barbarese E, Richter JD. 2003. Facilitation of dendritic mRNA transport by CPEB. *Genes Dev* **17**: 638–653. doi:10.1101/gad.1053003

Huang YS, Richter JD. 2004. Regulation of local mRNA translation. *Curr Opin Cell Biol* **16**: 308–313. doi:10.1016/j.ceb.2004.03.002

Huang YS, Mendez R, Fernandez M, Richter JD. 2023. CPEB and translational control by cytoplasmic polyadenylation: impact on synaptic plasticity, learning, and memory. *Mol Psychiatry* **28**: 2728–2736. doi:10.1038/s41380-023-02088-x

Hui X, Zhang S, Wang Y. 2018. miR-454-3p suppresses cell migration and invasion by targeting CPEB1 in human glioblastoma. *Mol Med Rep* **18**: 3965–3972. doi:10.3892/mmr.2018.9386

Igea A, Mendez R. 2010. Meiosis requires a translational positive loop where CPEB1 ensues its replacement by CPEB4. *EMBO J* **29**: 2182–2193. doi:10.1038/emboj.2010.111

Ivshina M, Alexandrov IM, Vertii A, Doxsey S, Richter JD. 2015. CPEB regulation of TAK1 synthesis mediates cytokine production and the inflammatory immune response. *Mol Cell Biol* **35**: 610–618. doi:10.1128/MCB.00800-14

Ivshina M, Lasko P, Richter JD. 2014. Cytoplasmic polyadenylation element binding proteins in development, health, and disease. *Annu Rev Cell Dev Biol* **30**: 393–415. doi:10.1146/annurev-cellbio-101011-155831

Julio AR, Backus KM. 2021. New approaches to target RNA binding proteins. *Curr Opin Chem Biol* **62**: 13–23. doi:10.1016/j.cbpa.2020.12.006

Khabar KS. 2010. Post-transcriptional control during chronic inflammation and cancer: a focus on AU-rich elements. *Cell Mol Life Sci* **67**: 2937–2955. doi:10.1007/s00018-010-0383-x

Kim JH, Richter JD. 2006. Opposing polymerase-deadenylase activities regulate cytoplasmic polyadenylation. *Mol Cell* **24**: 173–183. doi:10.1016/j.molcel.2006.08.016

Li Q, Kang C. 2023. Targeting RNA-binding proteins with small molecules: Perspectives, pitfalls and bifunctional molecules. *FEBS Lett* **597**: 2031–2047. doi:10.1002/1873-3468.14710

Liu Y, Ramkumar N, Vu LP. 2023. RNA deadenylation complexes in development and diseases. *Biochem Cell Biol* **101**: 131–147. doi:10.1139/bcb-2022-0325

Lu R, Zhou Z, Yu W, Xia Y, Zhi X. 2017. CPEB4 promotes cell migration and invasion via upregulating Vimentin expression in breast cancer. *Biochem Biophys Res Commun* **489**: 135–141. doi:10.1016/j.bbrc.2017.05.112

Lykke-Andersen J, Wagner E. 2005. Recruitment and activation of mRNA decay enzymes by two ARE-mediated decay activation domains in the proteins TTP and BRF-1. *Genes Dev* **19**: 351–61. doi:10.1101/gad.1282305

Maillo C, Martin J, Sebastian D, Hernandez-Alvarez M, Garcia-Rocha M, Reina O, Zorzano A, Fernandez M, Mendez R. 2017. Circadian- and UPR-dependent control of CPEB4 mediates a translational response to counteract hepatic steatosis under ER stress. *Nat Cell Biol* **19**: 94–105. https://doi.org/10.1038/ncb3461

Mejias M, Gallego J, Naranjo-Suarez S, Ramirez M, Pell N, Manzano A, Suñer C, Bartrons R, Mendez R, Fernandez M. 2020. CPEB4 increases expression of PFKFB3 to induce glycolysis and activate mouse and human hepatic stellate cells, promoting liver fibrosis. *Gastroenterology* **159**: 273–288. doi: 10.1053/j.gastro.2020.03.008

Mitschka S, Mayr C. 2022. Context-specific regulation and function of mRNA alternative polyadenylation. *Nat Rev Mol Cell Biol* **23**: 779–796. doi:10.1038/s41580-022-00507-5

Nagaoka K, Udagawa T, Richter JD. 2012. CPEB-mediated ZO-1 mRNA localization is required for epithelial tight-junction assembly and cell polarity. *Nat Commun* **3**: 675. doi:10.1038/ncomms1678

Nagaoka K, Fujii K, Zhang H, Usuda K, Watanabe G, Ivshina M, Richter JD. 2016. CPEB1 mediates epithelial-to-mesenchyme transition and breast cancer metastasis. *Oncogene* **35**: 2893–2901. doi:10.1038/onc.2015.350

Ortiz-Zapater E, Pineda D, Martinez-Bosch N, Fernandez-Miranda G, Iglesias M, Alameda F, Moreno M, Eliscovich C, Eyras E, Real FX, et al. 2011. Key contribution of CPEB4-mediated translational control to cancer progression. *Nat Med* **18**: 83–90. doi:10.1038/nm.2540

Otsuka H, Fukao A, Funakami Y, Duncan KE, Fujiwara T. 2019. Emerging evidence of translational control by AU-rich element-binding proteins. *Front Genet* **10**: 332. doi:10.3389/fgene.2019.00332

Parras A, Anta H, Santos-Galindo M, Swarup V, Elorza A, Nieto-Gonzalez JL, Pico S, Hernandez IH, Diaz-Hernandez JI, Belloc E, et al. 2018. Autism-like phenotype and risk gene mRNA deadenylation by CPEB4 mis-splicing. *Nature* **560**: 441–446. doi:10.1038/s41586-018-0423-5

Pascual R, Martin J, Salvador F, Reina O, Chanes V, Millanes-Romero A, Suñer C, Fernandez-Miranda G, Bartomeu A, Huang YS, et al. 2020a. The RNA binding protein CPEB2 regulates hormone sensing in mammary gland development and luminal breast cancer. *Sci Adv* **6**: eaax3868. doi:10.1126/sciadv.aax3868

Pascual R, Segura-Morales C, Omerzu M, Bellora N, Belloc E, Castellazzi CL, Reina O, Eyras E, Maurice MM, Millanes-Romero A, et al. 2020b. mRNA spindle localization and mitotic translational regulation by CPEB1 and CPEB4. *RNA* **27**: 291–302. doi:10.1261/rna.077552.120

Passmore LA, Coller J. 2022. Roles of mRNA poly(A) tails in regulation of eukaryotic gene expression. *Nat Rev Mol Cell Biol* **23**: 93–106. doi:10.1038/s41580-021-00417-y

Pell N, Garcia-Pras E, Gallego J, Naranjo-Suarez S, Balvey A, Suñer C, Fernandez-Alfara M, Chanes V, Carbo J, Ramirez-Pedraza M, et al. 2021. Targeting the cytoplasmic polyadenylation element-binding protein CPEB4 protects against diet-induced obesity and microbiome dysbiosis. *Mol Metab* **54**: 101388. doi:10.1016/j.molmet.2021.101388

Peng W, Nan Z, Liu Y, Shen H, Lin C, Lin L, Yuan B. 2014. Dendritic cells transduced with CPEB4 induced antitumor immune response. *Exp Mol Pathol* **97**: 273–278. doi:10.1016/j.yexmp.2014.06.001

Perez-Guijarro E, Karras P, Cifdaloz M, Martinez-Herranz R, Cañon E, Graña O, Horcajada-Reales C, Alonso-Curbelo D, Calvo TG, Gomez-Lopez G, et al. 2016. Lineage-specific roles of the cytoplasmic polyadenylation factor CPEB4 in the regulation of melanoma drivers. *Nat Commun* **7**: 13418. doi:10.1038/ncomms13418

Pico S, Parras A, Santos-Galindo M, Pose-Utrilla J, Castro M, Fraga E, Hernandez IH, Elorza A, Anta H, Wang N, et al. 2021. CPEB alteration and aberrant transcriptome-polyadenylation lead to a treatable SLC19A3 deficiency in Huntington's disease. *Sci Transl Med* **13**: eabe7104.

Pique M, Lopez JM, Foissac S, Guigo R, Mendez R. 2008. A combinatorial code for CPE-mediated translational control. *Cell* **132**: 434–448. doi:10.1016/j.cell.2007.12.038

Podszywalow-Bartnicka P, Neugebauer KM. 2024. Multiple roles for AU-rich RNA binding proteins in the development of haematologic malignancies and their resistance to chemotherapy. *RNA Biol* **21**: 1–17. doi:10.1080/15476286.2024.2346688

Riechert E, Kmietczyk V, Stein F, Schwarzl T, Sekaran T, Jürgensen L, Kamuf-Schenk V, Varma E, Hofmann C, Rettel M, et al. 2021. Identification of dynamic RNA-binding proteins uncovers a Cpeb4-controlled regulatory cascade during pathological cell growth of cardiomyocytes. *Cell Rep* **35**: 109100. doi:10.1016/j.celrep.2021.109100

Ross CR, Brennan-Laun SE, Wilson GM. 2012. Tristetraprolin: roles in cancer and senescence. *Ageing Res Rev* **11**: 473–484. doi:10.1016/j.arr.2012.02.005

Salerno F, Engels S, van den Biggelaar M, van Alphen FPJ, Guislain A, Zhao W, Hodge DL, Bell SE, Medema JP, von Lindern M, et al. 2018a. Translational repression of pre-formed cytokine-encoding mRNA prevents chronic activation of memory T cells. *Nat Immunol* **19**: 828–837. doi:10.1038/s41590-018-0155-6

Salerno F, Guislain A, Freen-Van Heeren JJ, Nicolet BP, Young HA, Wolkers MC. 2018b. Critical role of post-transcriptional regulation for IFN-γ in tumor-infiltrating T cells. *Oncoimmunology* **8**: e1532762. doi:10.1080/2162402X.2018.1532762

Shoshan E, Mobley AK, Braeuer RR, Kamiya T, Huang L, Vasquez ME, Salameh A, Lee HJ, Kim SJ, Ivan C, et al. 2015. Reduced adenosine-to-inosine miR-455-5p editing promotes melanoma growth and metastasis. *Nat Cell Biol* **17**: 311–321. doi:10.1038/ncb3110

Sibilio A, Suñer C, Fernandez-Alfara M, Martin J, Berenguer A, Calon A, Chanes V, Millanes-Romero A, Fernandez-Miranda G, Batlle E, et al. 2022. Immune translational control by CPEB4 regulates intestinal inflammation resolution and colorectal cancer development. *iScience* **25**: 103790. doi:10.1016/j.isci.2022.103790

Suñer C, Sibilio A, Martin J, Castellazzi CL, Reina O, Dotu I, Caballe A, Rivas E, Calderone V, Diez J, et al. 2022. Macrophage inflammation resolution requires CPEB4-directed offsetting of mRNA degradation. *eLife* **11**: e75873. doi:10.7554/eLife.75873

Tian B, Manley JL. 2017. Alternative polyadenylation of mRNA precursors. Nat Rev Mol Cell Biol **18**: 18–30. doi:10.1038/nrm.2016.116

Tiedje C, Ronkina N, Tehrani M, Dhamija S, Laass K, Holtmann H, Kotlyarov A, Gaestel M. 2012. The p38/MK2-driven exchange between tristetraprolin and HuR regulates AU-rich element-dependent translation. *PLoS Genet* **8**: e1002977. doi:10.1371/journal.pgen.1002977

Tsai LY, Chang YW, Lee MC, Chang YC, Hwang PI, Huang YS, Cheng CF. 2016. Biphasic and stage-associated expression of CPEB4 in hepatocellular carcinoma. *PloS One* **11**: e0155025. doi:10.1371/journal.pone.0155025

Turner M, Diaz-Muñoz MD. 2018. RNA-binding proteins control gene expression and cell fate in the immune system. *Nat Immunol* **19**: 120–129. doi:10.1038/s41590-017-0028-4

Udagawa T, Farny NG, Jakovcevski M, Kaphzan H, Alarcon JM, Anilkumar S *et al.* Genetic and acute CPEB1 depletion ameliorate fragile X pathophysiology. *Nat Med* 2013; **19**(11)**:** 1473-1477

Villalba A, Coll O, Gebauer F. 2011. Cytoplasmic polyadenylation and translational control. *Curr Opin Genet Dev* **21**: 4527. doi:10.1016/j.gde.2011.04.006

Villanueva E, Navarro P, Rovira-Rigau M, Sibilio A, Mendez R, Fillat C. 2017. Translational reprogramming in tumour cells can generate oncoselectivity in viral therapies. *Nat Commun* **8**: 14833. doi:10.1038/ncomms14833.

Wang M, Kaufman RJ. 2014. The impact of the endoplasmic reticulum protein-folding environment on cancer development. *Nat Rev Cancer* **14**: 581–597. doi:10.1038/nrc3800

Weill L, Belloc E, Bava FA, Mendez R. 2012. Translational control by changes in poly(A) tail length: recycling mRNAs. *Nat Struct Mol Biol* **19**: 577–585. doi:10.1038/nsmb.2311

Wek RC, Jiang HY, Anthony TG. 2006. Coping with stress: eIF2 kinases and translational control. Biochem Soc Trans **34**: 7–11. doi:10.1042/BST20060007

Zhang S, Huang J, Lan Z, Xiao Y, Liao Y, Basnet S, Huang P, Li Y, Yan J, Sheng Y, et al. 2024. CPEB1 controls NRF2 proteostasis and ferroptosis susceptibility in pancreatic cancer. *Int J Biol Sci* **20**: 3156–3172. doi:10.7150/ijbs.95962

FIGURE LEGENDS

Figure 1: Schematic representation of a CPE-binding protein (CPEB) bound to the cytoplasmic polyadenylation element (CPE) in the 3' UTR of the target mRNA. The structure of the two RNA recognition motifs (RRMs) and the ZZ-box is adapted from Afroz et al. (2014). The intrinsically disordered N-terminal half of CPEBs is indicated, along with the 5' and 3' untranslated regions (UTRs) of the mRNA, the open reading frame (ORF), and the poly(A) tail.

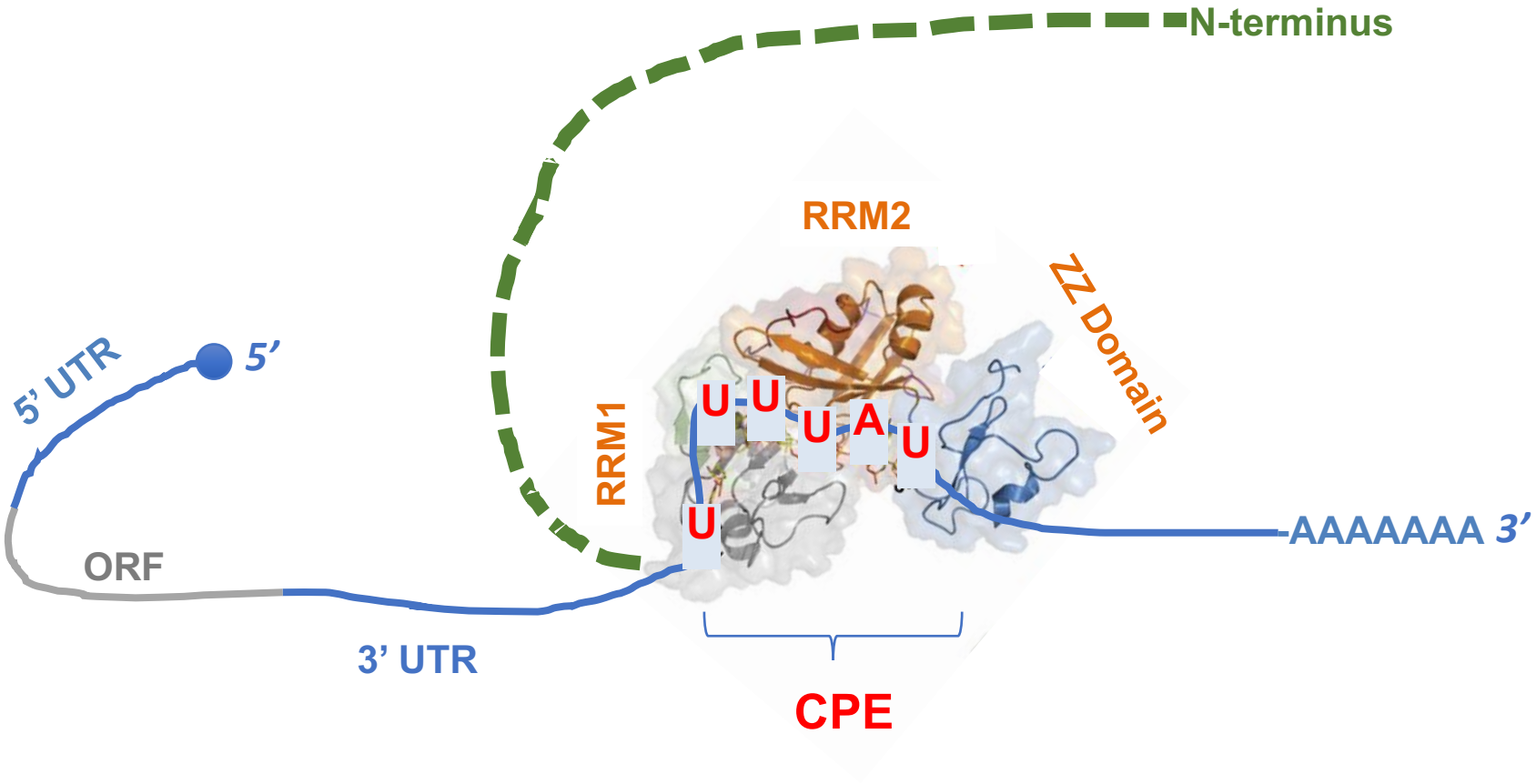
Figure 2: CPEB domains and regulatory phosphorylations. **Left**: All four CPEBs share a similar structure, with a highly ordered C-terminal domain comprising two RNA recognition motifs (RRMs) in tandem and a ZZ-box responsible for binding to their targets, and a highly variable N-terminal domain, which contains regulatory phosphorylation sites (activating sites in green, repressing sites in red; kinases indicated). CPEBs can also undergo alternative splicing, generating isoforms with potentially different behaviors and functions. **Right**: CPEBs 2–4 are predicted to be intrinsically disordered and contain low-complexity regions (LCRs) within their variable N-terminal domains. POND-score profiles of CPEBs 1–4 show stretches of predicted disordered regions.

Figure 3: Proposed model of nucleocytoplasmic functions of CPEB1. In the nucleus, CPEB1 facilitates the recruitment of CPSF (Cleavage and Polyadenylation Specificity Factor) to suboptimal (upstream) polyadenylation sites (PAS), thereby promoting the generation of shorter 3′ untranslated regions (3′UTRs). In the cytoplasm, non-phosphorylated CPEB1 assembles a repression complex by recruiting deadenylases and associated cofactors, effectively blocking the mRNA cap and rendering the mRNA translationally silent. These mRNPs (messenger ribonucleoprotein particles) can associate with motor proteins, enabling their subcellular localization along microtubules. Upon phosphorylation, CPEB1 undergoes activation, leading to the remodeling of the mRNP complex. This remodeling facilitates the recruitment of CPSF and the cytoplasmic poly(A) polymerases Gld2 or Gld4, which extend the poly(A) tail. Simultaneously, CPEB1 recruits cap-ribose methyltransferases, promoting the formation of CapI and CapII structures, thereby enhancing mRNA stability and translational efficiency.

Figure 4: The activity of CPEBs 2–4 as translation regulators depends on phase separation. Unphosphorylated CPEBs undergo liquid-liquid phase separation, repressing the translation of target mRNAs. Hyperphosphorylation by ERK2 and Cdk1 induces changes in the charge pattern, leading to condensate disassembly and promoting translational activation of CPEB4 targets. Phase separation properties rely on the intrinsically disordered N-terminal domain. Image adapted from Guillen-Boixet et al. (2016).

Figure 5: Cytoplasmic regulation of poly(A) tail length is controlled by a double equilibrium of cis-acting elements (AREs vs. CPEs) and trans-acting factors (TTP vs. HuR and CPEB1 vs. CPEB4). The activity of the trans-acting factors is, in turn, regulated by kinases acting in a coordinated manner on ARE-binding proteins (AREBPs) and CPEBs.

Figure 6: Model of the adaptive response to chronic stress. Sustained unfolded protein response (UPR) occurs in two sequential phases: **Acute phase**: Transient activation via eIF2α phosphorylation, allowing the permissive translation of uORF-containing mRNAs, including CPEB4 mRNA. **Chronic phase**: Mediated by translational activation and increased stability of CPE-containing transcripts, including CPEB4.


N-terminus
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ZZ Domain
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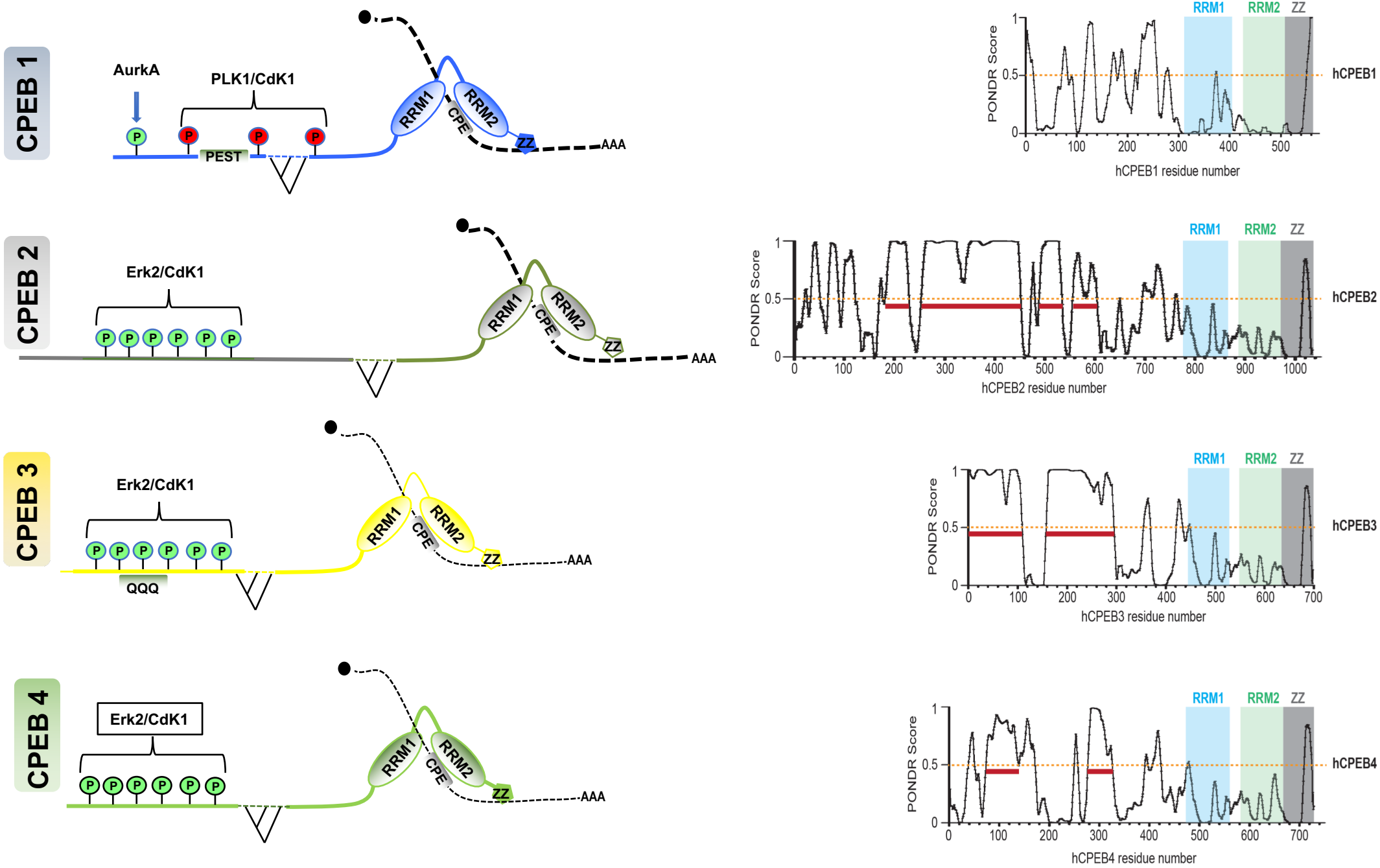
5'
U
U
U
A
U
U
AAAAAAA 3'
ORF
3' UTR
CPE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AurkA
PLK1/CdK1
P
PEST
RRM1
RRM2
CPE
ZZ
AAA
RRM1
RRM2
ZZ
PONDR Score
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0
0
100
200
300
400
500
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CPEB 2
Erk2/CdK1
RRM1
RRM2
CPE
ZZ
AAA
RRM1
RRM2
ZZ
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0
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600
700
800
900
1000
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hCPEB2 residue number
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RRM2
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hCPEB3
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RRM2
CPE
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RRM2
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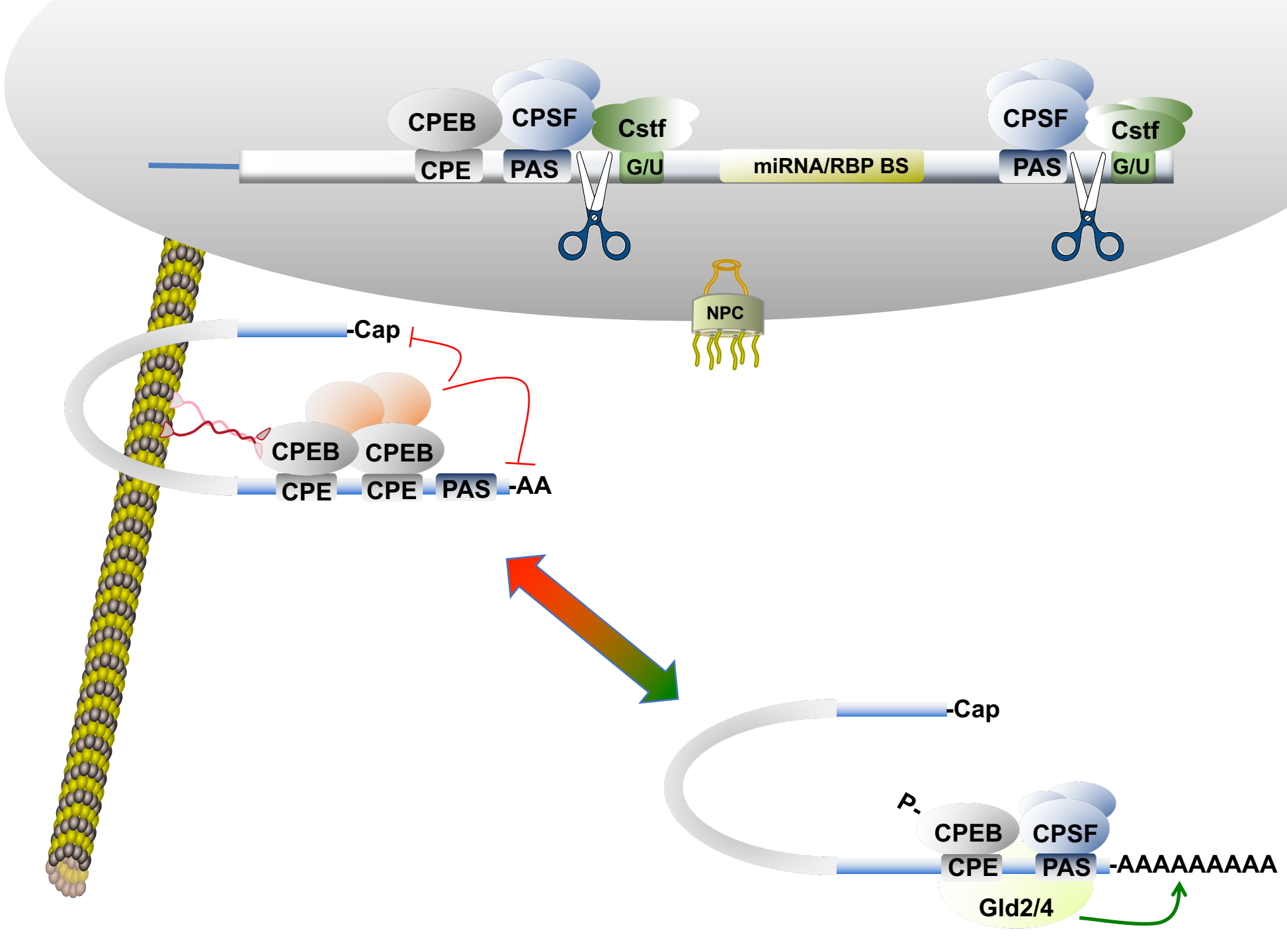
hCPEB4 residue number


CPEB
CPSF
Cstf
CPE
PAS
G/U
miRNA/RBP BS
CPSF
Cstf
PAS
G/U
NPC
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CPEB
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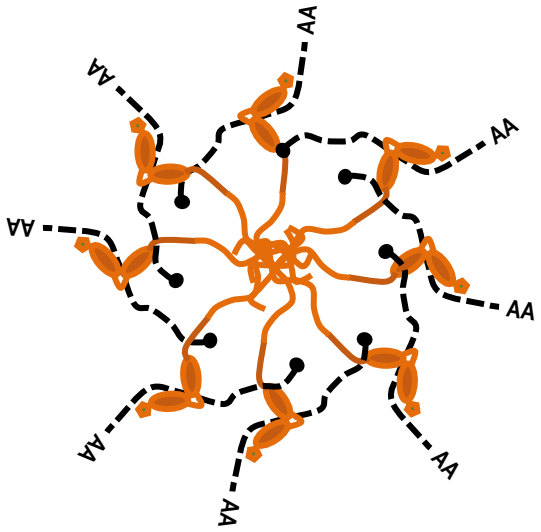
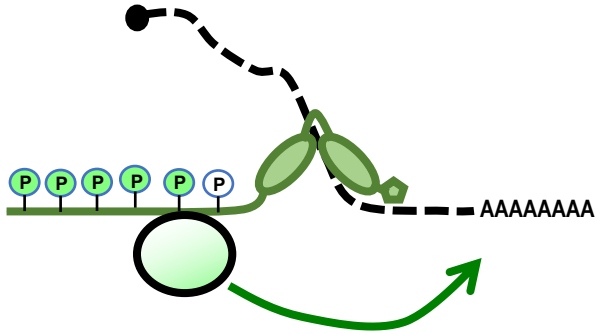
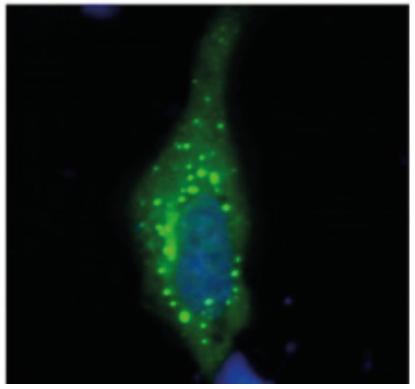
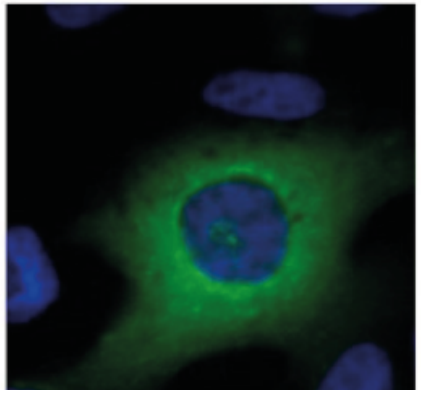
CPE
PAS
-AAAAAAAAA
Gld2/4



Erk2/CdK1

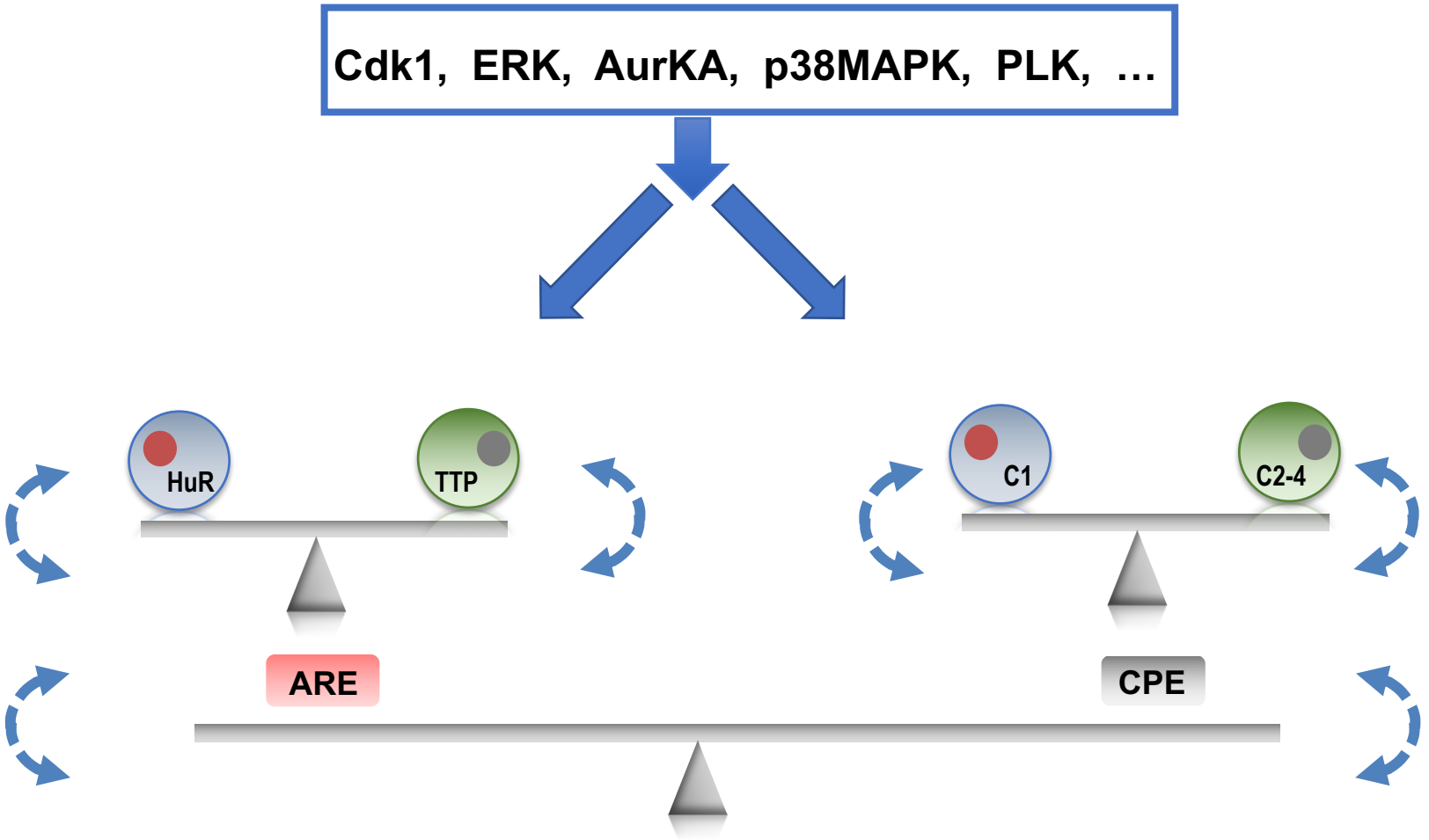




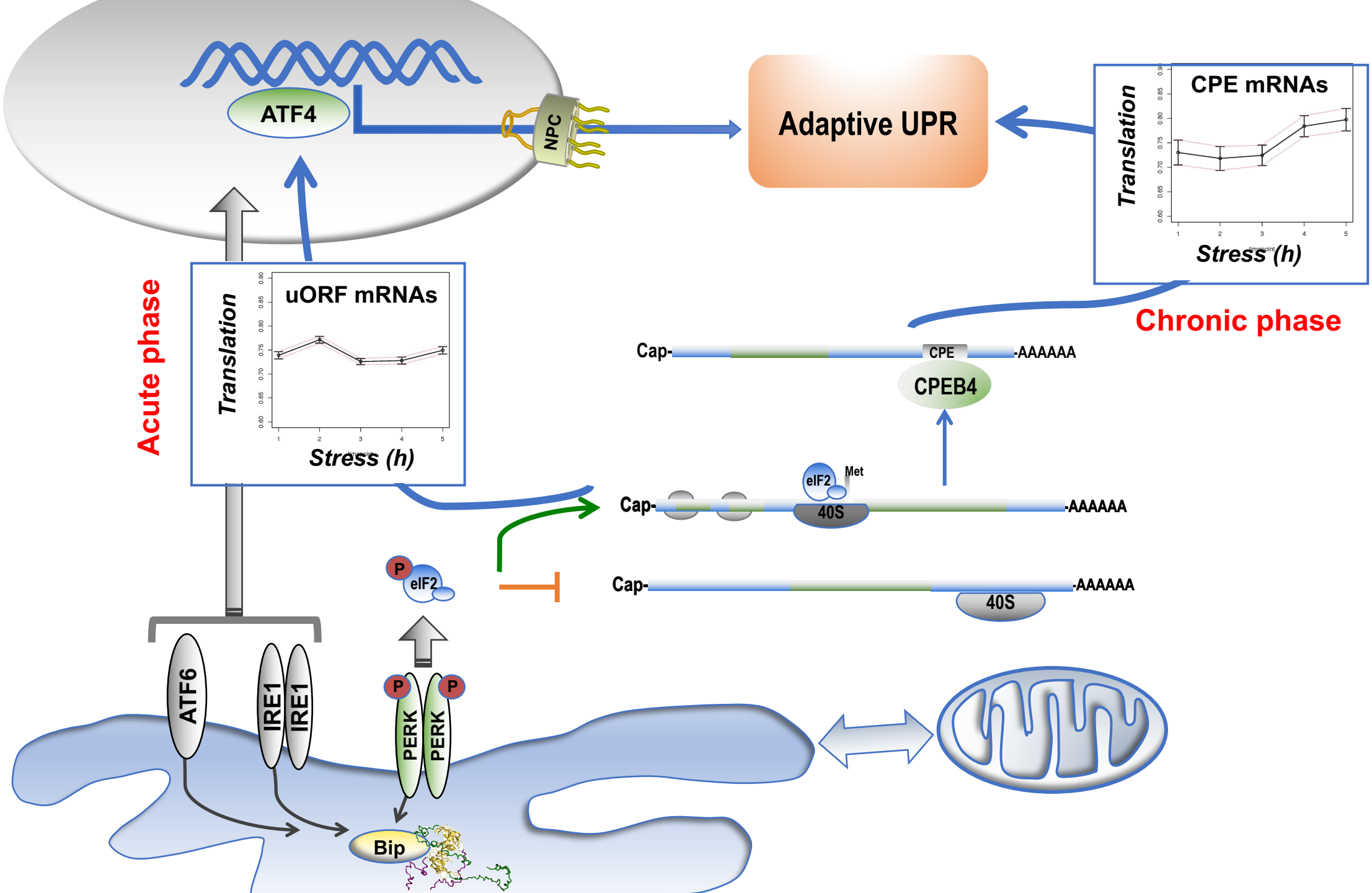


Repression of translation

Translation activation



mRNA Stability and Translation


ATF4
NPC
Adaptive UPR
CPE mRNAs
Translation
Stress (h)
Chronic phase
Acute phase
uORF mRNAs
Translation
Stress (h)
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CPE
-AAAAAA
CPEB4
eIF2
Met
40S
Cap-
-AAAAAA
P
eIF2
Cap-
-AAAAAA
40S
ATF6
IRE1
IRE1
P
P
PERK
PERK
Bip

Cytoplasmic Regulation of the Poly(A) Tail Length as a Potential Therapeutic Target

Mercedes Fernandez and Raul Mendez

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