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Coordinate Regulation of mRNA Decay Networks by GU-rich Elements and CELF1

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Abstract

The GU-rich element (GRE) was identified as a conserved sequence enriched in the 3′ UTR of human transcripts that exhibited rapid mRNA turnover. In mammalian cells, binding to GREs by the protein CELF1 coordinates mRNA decay of networks of transcripts involved in cell growth, migration, and apoptosis. Depending on the context, GREs and CELF1 also regulate pre-mRNA splicing and translation. GREs are highly conserved throughout evolution and play important roles in development of organisms ranging from worms to man. In humans, abnormal GRE-mediated regulation contributes to disease states and cancer. Thus, GREs and CELF proteins serve critical functions in gene expression regulation and define an important evolutionarily conserved posttranscriptional regulatory network.

Keywords

GU-rich element; CELF1; CUG-binding protein 1; RNA-binding proteins; CELF; mRNA degradation; posttranscriptional gene regulation

Introduction

The precise control of gene expression during cellular processes such as activation, proliferation, differentiation, and development requires multiple levels of regulation, including transcriptional and posttranscriptional mechanisms. Steady-state protein levels within a cell correlate poorly with steady-state levels of mRNA, suggesting that large numbers of transcripts undergo post-transcriptional regulation [1]. *Cis*-acting regulatory sequences found in coding regions and in 3′ and 5′ untranslated regions (UTRs) of mRNA allow selective recognition by RNA-binding proteins (RBPs) or microRNAs which direct the fate of the mRNA by controlling posttranscriptional processes such as translation and mRNA degradation (reviewed in references [2]•, [3], [4], [5]). Here, we review the GU-rich element (GRE) as an example of an evolutionarily conserved *cis* element in mRNA that

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controls posttranscriptional gene expression networks through its interaction with the protein CELF1.

Cis Elements in the Coordinate Regulation of mRNA Decay

Cis elements in mRNA function at posttranscriptional levels to coordinately regulate gene expression through their interactions with microRNAs or RBPs. microRNAs are small endogenous RNA molecules that bind to specific sequences in mRNA and regulate translation and/or mRNA degradation during growth and development [6]•, [7]•, [8]. Overexpression of a specific microRNA can lead to the down-regulation of hundreds of mRNAs suggesting that microRNAs play important roles in orchestrating mRNA degradation. In addition to microRNAs, certain RBPs bind to specific mRNA sequences and also coordinately regulate mRNA degradation and/or translation. The combinatorial interplay between various miRNAs and RBPs that bind to a given mRNA transcript control many developmental decisions in a variety of species [9], [10], [11].

Considerable insight into the mechanisms of coordinate mRNA degradation by *cis* elements has come from studies involving the AU-rich element (ARE) and ARE-binding proteins. The ARE is a well characterized *cis*-acting mRNA sequence that regulates mRNA decay by binding to a variety of RBPs depending on the cellular context (for reviews see [12],[13]). Several ARE-binding proteins, including AUF1 [14], BRF1 and TTP [15],[16],[17], and KSRP [18], [19], promote ARE-mediated mRNA decay, whereas other ARE-binding proteins such as HuR [20],[21], HuB, HuC, and HuD stabilize target mRNAs and stimulate their translation [22]. ARE-binding proteins rapidly modulate the stability and/or translation of mRNA during cell proliferation and development [23],[24],[25] •. For example, the destabilizing protein TTP is induced following T cell activation and functions to mediate the degradation of multiple ARE-containing transcripts that encode inflammatory mediators such as interleukin-2 and interferon-gamma [26],[27]. TTP mediates the decay of ARE-containing transcripts by recruiting components of the mRNA decay machinery to the transcript [28],[16],[29]. This mechanism allows for the coordinate down-regulation of multiple genes at the appropriate time following T cell activation. The characterization of the ARE as a cis-acting regulator of mRNA decay led to a more systematic classification of ARE-containing genes, including the construction of ARE databases [30],[31]•,[32] and examination of the mRNA decay rates of ARE-containing transcripts using microarray technology [33]. These methodologies enabled biologists to globally assess the physiological significance of ARE-mediated mRNA decay regulation and to identify coordinate gene expression networks regulated by AREs [34],[35],[13],[36]. This approach can be applied to identify and understand other posttranscriptional regulatory networks.

In this review, we describe how knowledge about coordinate gene regulation by conserved *cis* sequences in mRNA led to the identification of the GRE, which defines a posttranscriptional regulatory network that has been conserved through evolution. By using bioinformatic sequence motif discovery methods, in conjunction with gene expression clustering, the GRE was identified as a highly conserved sequence that was enriched in the 3′ UTR of mRNA transcripts with short half lives and was shown to function in human cells as a regulator of mRNA decay [37] ••. In human cells, the GRE is a target of CELF1, also known as CUG-binding protein 1 (CUGBP1), a member of the CELF (CUGBP and embryonically lethal abnormal vision-type RNA binding protein 3-like factors) family of RNA-binding proteins. CELF1 has been implicated as a regulator of alternative splicing [38],[39],[40], translation [41], deadenylation [42], and mRNA degradation [37]••,[43] ••. Together, the GRE and CELF1 define an evolutionarily conserved posttranscriptional regulatory network.

GREs as Regulators of mRNA Decay

The GRE consensus sequence, UGUUUGUUUGU, was identified as a sequence that was highly enriched in the 3′ UTR of short-lived transcripts expressed in primary human T cells [37] ••. The GRE is a bona fide mRNA decay element because it conferred instability upon reporter transcripts when it was inserted into their 3′ UTR. The CELF1 protein binds to GREs, and knockdown of CELF1 leads to stabilization of GRE-containing transcripts, indicating that CELF1 is essential for GRE-mediated mRNA decay. More recently, RNA-immunoprecipitation (RNA-IP) was performed in cytoplasmic extracts from HeLa cells using an anti-CELF1 antibody, and CELF1-associated transcripts were identified using oligonucleotide microarrays. A bioinformatics search for conserved sequences in immunoprecipitated transcripts, using the program BioProspector (and overrepresentation algorithm), found the previously described UGUUUGUUUGU sequences as well as the GU-repeat sequence UGUGUGUGUGU sequences to be overrepresented [43] ••. Interestingly, the GU-repeat sequence was previously identified through systemic evolution of ligands exponential enrichment (SELEX) as a CELF1-binding sequence [44], and CELF1 binds with high affinity to GU-repeat sequences [45],[46]. Insertion of a GU-repeat sequence into the 3′ UTR of a reporter transcript conferred instability to the reporter construct, demonstrating that this GU-repeat sequence functioned as a decay element [43]. Because the UGUUUGUUUGU sequence and the GU-repeat sequence both bound to CELF1 and functioned as decay elements, the GRE was redefined to contain both of these sequences ([43] ••, see Table 1). In myoblasts, a similar RNA-IP approach using an anti-CELF1 antibody identified GRE hexamers to be significantly overrepresented in short-lived transcripts that co-immunoprecipitated with CELF1 [47] ••. In this system, knockdown of CELF1 led to the stabilization of certain GRE-containing targets, confirming that CELF1 regulated the stability of those transcripts. In *Xenopus*, target transcripts identified by RNA-IP using an antibody against embryo deadenylation element binding protein (EDEN-BP), the CELF1 orthologue, were enriched in GU-rich sequences, very similar to GREs and the 15 nucleotide consensus motif (UGU/UG)n was predicted to be a target of CELF1 [48]••,[49]. Overall, these studies revealed that GU-rich sequences function as mRNA decay elements and serve as binding sites for CELF1 in a manner that has been conserved through evolution.

Evolutionary Conservation of GREs and CELF Proteins

Translation and mRNA decay are often coupled with one another to control of gene expression in response to environmental and developmental changes. In several organisms, translation is regulated by deadenylation, which is also an early step in the mRNA decay pathway. The deadenylation and translation of genes important in development are regulated by GU-rich sequences and CELF proteins across diverse species [50],[51]. In *Xenopus*, the CELF1 orthologue, EDEN-BP, binds to the GU-rich EDEN element, which functions as a deadenylation signal in *Xenopus* embryos after fertilization and regulates translational activation [52],[53]. In Drosophila, the CELF1 orthologue, Bru-3 (Bruno-3), binds specifically to $(UG)_{15}$ repeats to regulate translation of proteins involved in embryogenesis and organogenesis [54], [55], [56]. The *Zebrafish* orthologue, Bru-l, also binds preferentially to GU-rich RNAs and regulates development [57]. NMR-based solution studies demonstrated that human CELF1 RNA recognition motifs bound specifically to RNA UGUU or UGUG sequences [58]•,[59] •.

CELF proteins are essential post-transcriptional regulators of development in lower organisms such as *Xenopus* where they regulate deadenylation and translation [60]. Whereas GRE-mediated deadenylation often regulates translation in lower organisms, the deadenylation is usually the first step leading to mRNA degradation in mammalian cells.

The consequences of deadenylation differ in different organisms, although the mechanism of deadenylation appears to be evolutionarily conserved. For example, a GU-rich sequence from human c-jun mRNA substituted for the EDEN element as a deadenylation signal in *Xenopus* extracts [61]. Furthermore, human CELF1, which has 88% identity with EDEN-BP, was able to functionally substitute for EDEN-BP to mediate transcript deadenylation in *Xenopus* extracts [53], suggesting that the deadenylation function of GU-rich sequences and CELF proteins were conserved in diverse species. Human CELF1 was shown to associate with poly A ribonuclease (PARN) and to stimulate poly A tail shortening in a cell-free assay using S100 extracts from human cells, suggesting that CELF1 mediates mRNA decay through deadenylation [42]. Thus, the deadenylation function of GREs and CELF1 is conserved through evolution and may be responsible for coordinated mRNA decay in mammalian cells.

In addition to regulating deadenylation and translation, CELF proteins regulate alternative splicing in diverse species by binding to GU-rich or U-rich sequences (reviewed in [62] •, [4]). CELF-mediated regulation of alternative splicing is necessary for maintenance of normal muscle structure and function [63],[64],[65]. Recently, a RNA cross-linking immunoprecipitation (RNA-CLIP) approach was used to identify 315 CELF1 RNA targets in whole cell extracts from mouse hindbrain [66] ••. RNA binding targets for CELF1 were enriched in UG repeat sequences with 64% of target sequences found in introns and 25% found in 3′ UTR sequences. Thus, by binding to GU-rich sequences, CELF1 may function to regulate pre-mRNA splicing, translation, and/or deadenylation/decay, depending on the context.

Coordinate Regulation of the GRE/CELF1 Network in Cellular Activation and Differentiation

In primary human T cells, GREs and CELF1 appear to regulate rapid changes in gene expression following T cell receptor-mediated activation. Figure 1 shows a network of short-lived GRE-containing transcripts that are involved in T cell signaling. Many of these GRE-containing transcripts were expressed transiently following T cell activation and then rapidly disappeared [67], suggesting that GRE-mediated mRNA decay plays a central role in the coordinate down-regulation of these genes following T cell activation [37]••,[68] ••. Thus, GRE-mediated mRNA decay appears to be an important regulatory step in the early stages of T cell activation.

In mouse myoblasts, RNA-IP followed by microarray analysis identified a variety of CELF1 target transcripts that contained GU-rich sequences, including networks of transcripts that regulate cell cycle and intracellular signaling cascades involved in intracellular transport and cell survival (Figure 2) [47] ••. Many of these CELF1 target transcripts were found to be significantly stabilized in CELF1 knockout myoblasts [47] ••, suggesting CELF1 mediates the decay of a network of transcripts that may be involved in myoblast growth and differentiation. Interestingly, many of the CELF1 target transcripts in mouse myobasts were also found to be target transcripts of EDEN-BP in *Xenopus tropicalis* extracts (Figure 2), providing further evidence that GRE/CELF1 posttranscriptional networks were conserved through evolution.

The GRE/CELF1 Posttranscriptional Network in Human Diseases

CELF1 and its GRE-containing target transcripts define posttranscriptional regulatory networks that functions to control cellular growth, activation, and differentiation. Disruptions in GRE-mediated mRNA regulation may play a role in developmental pathology [69],[62] or cancer. CELF1 was found in a transposon-based genetic screen in

mice to be one of the top ten genes to drive tumorigenesis if mutated and/or dysregulated [70] •, suggesting that CELF1/GRE networks may be regulated abnormally in cancer. RNA-IP followed by microarray analysis to identify CELF 1 target transcripts in human Hela cells (carcinoma cell line), revealed that numerous target transcripts play roles in processes important for cancer development including cell growth, apoptosis, and cell migration [43] ••. For example, CELF1 targets in HeLa cells included numerous transcripts encoding regulators of G-protein signaling pathway and G-protein coupled receptor ligands (Figure 3). These pathway activate/or repress cell-cell interaction, cell migration and invasion, and thereby play important roles in cancer development and metastasis. Thus, CELF1/GRE networks may be aberrantly regulated in malignant cells.

Conclusions

Posttranscriptional regulation of gene expression is controlled through a highly dynamic and combinatorial interaction of RBPs, microRNAs, and mRNA that forms complex ribonucleoprotein particles. Sequences and structures within a given mRNA species may interact with numerous regulatory proteins and microRNAs that function together to determine the fate of the transcript. Networks of transcripts may share regulatory sequences, such as the GRE, that allow for coordinated expression during cellular activation or development. Coordination of mRNA degradation by the GRE in mammalian cells depends on the CELF1 protein, but further work is needed to understand the mechanisms by which CELF1 mediates mRNA decay and how this process responds to environmental signals during cellular activation and differentiation. A better understanding of the molecular mechanisms through which GREs and CELF1 regulate mRNA decay and how this process is disrupted in disease states such as malignancy may provide new avenues for therapeutic modalities.

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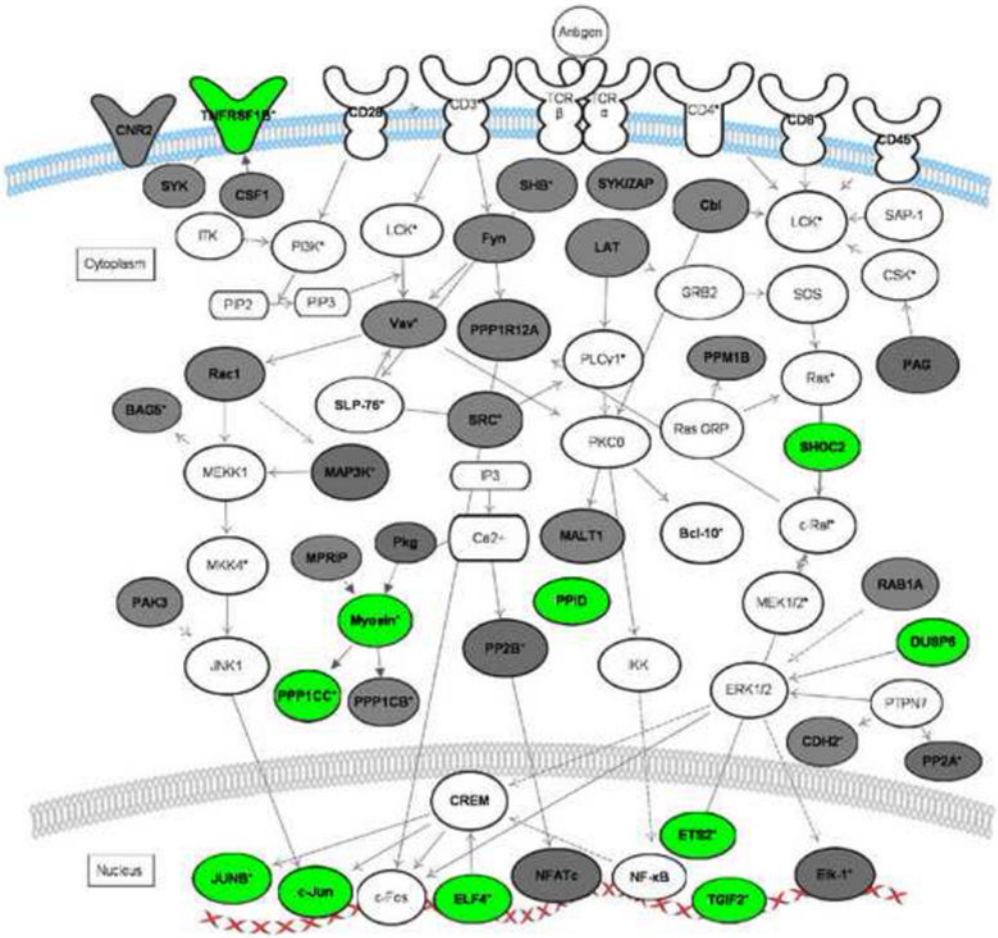

Figure 1. T-cell receptor signaling pathways regulated by GREs

The network diagram depicts the coordinate regulation of GRE-containing transcripts involved in T cell receptor signaling. Transcripts in bold are GRE-containing. Transcripts in grey were identified as CELF1 targets in HeLa cells by RNA-IP [43].

Transcripts in green represent short-lived GRE-containing transcripts expressed in primary human T cells [33], [37]. Transcripts labeled with an asterisk exhibited changes in steady state levels following T-cell receptor stimulation [33]. This network diagram was built using Ingenuity Pathway Assistant Software.

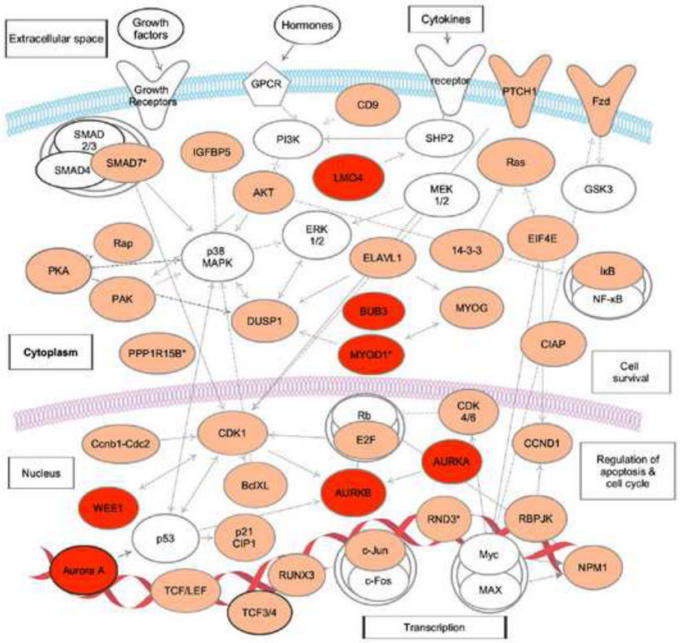

Figure 2. A posttranscriptional network of CELF1 target transcripts in mouse myoblasts

Transcripts shown in orange are CELF1 targets in mouse myoblasts [47]. Transcripts shown in red are CELF1 targets in mouse myoblasts and are also targets of EDEN-BP in *Xenopus tropicalis* extracts [48]. Transcripts, marked with asterisk (*), were stabilized in CELF1 knockout myoblasts. This network diagram was built using Ingenuity Pathway Assistant Software.

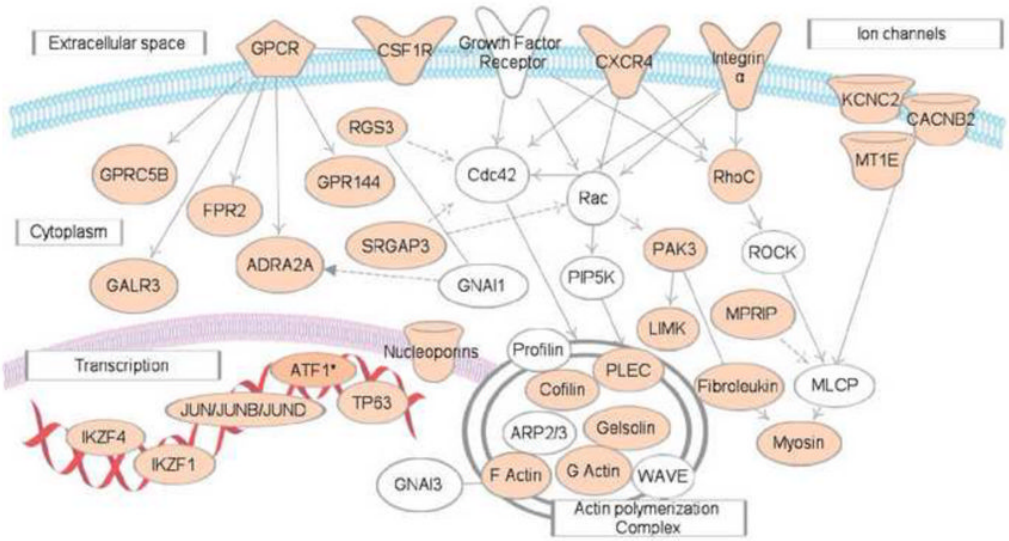

Figure 3. A posttranscriptional network of CELF1 target transcripts in malignant cells

This network represents transcripts that involved in G protein coupled receptor signaling pathways. Transcripts depicted in tan represent GRE-containing CELF1 target transcripts in HeLa cells [43]. This network diagram was built using Ingenuity Pathway Assistant Software.

Table 1

GU-rich motifs known to bind CELF1.

Sequence	Assay	Reference
UGUUUGUUUGU	In vitro binding	[37]
UGUGUGUGUGU	RNA-IP	[43]
(UGU/C)n	SELEX	[44]
(GUU/GUG)n	RNA-IP	[48]
UGUUGU, UUUUUU	RNA-IP	[47]
GU-rich, U-rich	RNA-CLIP	[66]
UGU/(U/G)n	RRM Crystal Structure	[58]
$(UG)_{15}$	Surface Plasmon Resonance	[46]