mRNA stability in soma and neurites of cultured neuronal cells

by

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Abstract

Neurons rely on mRNA localization and local protein synthesis in neurites to regulate synaptic plasticity, axonogenesis and neural signaling. The local regulation of mRNA stability in neurites is poorly understood. To determine mRNA decay rates, we analyzed the subcellular transcriptomes of neural projections and soma of mouse neuronal cells following inhibition of transcription with actinomycin-D. Less stable transcripts were enriched for GU-rich elements in their 3' UTRs. Around 12% of alternative splicing isoform pairs differed in stability, and cassette alternative ("skipped") exons that negatively impact stability are enriched for A-rich sequences. Overall, decay rates were similar across soma and neurites. However, differences in stability between soma and neurites were observed for GC-rich alternative first exon isoforms, which were preferentially stabilized in neurites. Our results suggest that 5' UTRs may play a key role in regulating local mRNA stability in neurites.

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Chapter 1

Introduction

Neurons are highly specialized cells that express complex and dynamic transcriptomes [1, 2, 3, 4, 5]. They are polarized, with projections (neurites) extending far from the cell body (soma), and therefore rely on mRNA localization and local mRNA translation to regulate synaptic plasticity in dendritic spines, to mediate responses of axons to growth cues, and to regenerate axons [6, 7, 8, 9]. Maintaining these complex repertoires of mRNAs requires mechanisms in place that not only regulate mRNA localization and local translation but also local degradation of mRNAs. As such, regulation of mRNA stability plays a critical role in neural development, axonogenesis and other cell-fate decisions [10].

Subcellular mRNA localization has previously been considered a mechanism used by a small subset of transcripts to spatially restrict protein expression within polarized cells. However, new research has shown that thousands of mRNAs are distributed in spatially distinct patterns [5, 11, 12]. A recent study showed that mRNA isoforms that contain gene-distal alternative last exons or gene-proximal alternative first exons are preferentially localized to neurites, and localization of many of these neuronal mRNAs depends on muscleblind proteins [5]. Three common mechanisms are known to be used by cells to localize mRNA [12] - active transport of transcripts to a subcellular site; local entrapment of transcripts that are diffusing throughout the cytoplasm; and local stabilization and regulated degradation of mRNAs.

There are many advantages to localizing mRNA. The production of protein can
be spatially restricted and temporally controlled with expression regulated by local signals, and translation of localized messages provides a rapid and robust response to stimuli as many molecules of protein can be made from a single localized mRNA [13]. Localized translation can also promote assembly of spatially distinct protein complexes and allow for different cell fates of dividing cells [14, 15]. Given the importance of mRNA localization, one would expect cells to have spatially distinct rates of mRNA degradation influenced by local requirements, and have mechanisms in place to degrade aberrantly localized mRNAs.

Studies of individual genes and proteins in different cell types have revealed a number of cases in which mRNA stability is controlled in a spatially restricted manner [16, 17]. Many RNA binding proteins have been shown to regulate both mRNA localization and decay [18, 19, 20], and transcripts being localized are often kept in a translationally repressed state, potentially altering their susceptibility to degradation.

To date, studies on mRNA localization and degradation in neurons have mostly focused on specific transcripts. The spatial heterogeneity of mRNA stability has yet to be studied on a transcriptome-wide scale. Here, we assessed the determinants of mRNA stability in neurons and the prevalence of localized mRNA decay by monitoring the degradation of transcripts in neurites and soma of mouse neuronal cells, showing that mRNA stability is similar across the two fractions and GU-rich and GC-rich sequences play important roles in controlling stability.
Chapter 2

Methods

2.1 Cell culture and fractionation

CAD cells were grown in DMEM/F12 (Gibco) supplemented with 10% FBS. To fractionate, polyethylene terephthalate membranes with 1-mm pores (Millipore PIRP-30R48) were treated on their underside with 0.2% matrigel in DMEM for 30 min at 37°C. Then, 4 mL media were placed in each well of a six-well plate and the membranes were placed in the plate. Next, 2 mL confluent cells (around $10^6$ cells) were plated on the top of the membrane and allowed to adhere for 1 h. The media below the membrane and on top of the cells were then replaced with media lacking serum. The cells were incubated at 37°C for a total of 32 h before fractionation. The cells were treated with 10 μg/mL actinomycin D (Sigma A1410) for the last 0, 1, 2, 4 or 8 hours and then fractionated as described below.

The media was aspirated and both sides of the membrane were rinsed with PBS, and 1 mL PBS was placed on the top of the membrane. Cell bodies were scraped in the PBS using a cell scraper. The membrane, still containing cell projections, was then cut out and incubated with RLT lysis buffer (QIAGEN) at 4°C for 15 min. Twelve membranes from two six-well plates were combined and used as a single neurite preparation, and cell bodies from a single well of a six-well plate were used as a soma preparation. 1 μL of ERCC RNA Spike-In Mix 1 (1:10 dilution) was added to the soma preparation and 1 μL of Spike-In Mix 1 (1:100 dilution) was added to
the neurite preparation [21]. RNA was purified from both fractions using a QIAGEN RNeasy Micro Kit. Approximately 1.5 to 2 μg total RNA was collected from neurite fractions and 8-12 μg from the soma factions in each preparation.

2.2 RNA-seq

Strand-specific, polyA-selected libraries were constructed using the dUTP incorporation method and sequenced on an Illumina NextSeq sequencer with paired-end 75 base reads. We used a total of 5 time points, with 2 replicates per time point, and 2 fractions per replicate. Sequencing yielded approximately 60-80 million read pairs per sample.

2.3 Estimating gene and isoform expression

Transcript expression levels (TPMs) were quantified using kallisto [22] and the transcriptome annotations from mouse GENCODE release M12. The reads were mapped to the mm9 genome using STAR [23]. The replicates were then combined and the relative levels of alternative isoforms (“Percent Spliced In” or PSI values) were determined using MISO [24].

2.4 Gene ontology enrichment

Gene ontology enrichments of genes differing in stability were performed using clusterProfiler in R [25].

2.5 mRNA half-life estimation

The set of TPM values across the time course was used to estimate relative mRNA stability in the soma and neurite fractions. An exponential curve was fit to these TPM values to generate relative decay parameter estimates. A positive exponential
constant indicates that the mRNA is decaying slower than the bulk rate of decay, while
a high negative constant indicates that the mRNA is decaying faster than average.

Using 6 h as an estimate of the median mRNA half-life in soma, mRNA half-lives
were estimated from the relative decay constants.

2.6 Monotonicity Z-score calculation for AS isoforms
and LR values

To identify splicing events that change monotonically over time, a permutation-based
method that could be applied to time course data was used [26]. The samples were
ordered chronologically, and for each isoform pair, all pairs of samples from different
time points were compared, and the number of comparisons representing a signifi-
cant increase or significant decrease in PSI were tallied, at a minimum Bayes factor
(BF) cutoff of 5. A quantity called M, the number of significant positive ΔPSI values
minus the number of significant negative ΔPSI values, was calculated. To assess sta-
tistical significance, M was recalculated after randomly permuting the sample labels.
Repeating this process 1000 times, a null distribution of M-scores was generated,
and the “monotonicity Z-score” (MZ) was defined as MZ = (M − μ)/σ was derived,
where μ and σ are the mean and standard deviation of the distribution of M values
of permuted data. Large positive or negative MZ values indicate consistent increase
or decrease, respectively, in the inclusion of an alternative exon over the time course.

The above method was also adapted to generate LR-MZ-scores using the Local-
ization Ratios computed for each gene. Large positive or negative LR-MZ-scores
indicate consistent increase or decrease, respectively, in the enrichment of an mRNA
in the neurite relative to the soma, indicating greater or lesser stability in the neurite
compared to soma, respectively.
2.7 kmer enrichment

To identify oligonucleotides of length $k$ (kmer) enriched between test and control sequences, the number of occurrences of each kmer in the test and control sequences was counted, and significantly enriched kmers were identified using Fisher’s exact test. The resulting p-values were then corrected using the Benjamini-Hochberg-Yekutieli procedure.

Control sequences for 3’ UTRs from less stable mRNAs were selected from 3’ UTRs of mRNAs matched to have similar expression, length and GC-content. Control sequences for AFEs that differed in stability were selected from AFEs with an MZ-score of 0, matched for PSI, gene expression and transcript GC-content.
Chapter 3

Results

3.1 mRNA stability is similar across soma and neurites of mouse neuronal cells

To estimate mRNA half-lives in soma and neurites of neuronal cells, we treated mouse CAD cells with actinomycin-D to inhibit transcription, and then mechanically fractionated the cells using porous membranes that allow growth of cell projections into pores [27] (Figure 1A, S1B). CAD cells - a mouse brain-derived neuronal cell line - extend neurites in serum-free media, and exhibit many of the molecular and morphological characteristics of primary neurons [28] (Figure S1A). PolyA-selected RNA from both fractions was isolated over 5 time points after transcription inhibition and subjected to strand-specific paired-end RNA sequencing (RNA-seq).

For each gene, expression was estimated by TPM (transcripts per million). To assess the relationship between all the samples, the datasets were clustered based on the Jensen-Shannon Divergence of their TPM values. The samples cluster by time rather than fraction (Figure 1B), indicating that gene expression over the transcription inhibition time-course is overall similar between soma and neurites.

Relative mRNA decay parameters in soma and neurites for all genes were calculated by fitting an exponential curve to gene TPM values measured across the time course. Half-lives in CAD cells estimated by assuming a median mRNA half-life of
6 hours are comparable to half-lives measured in mouse embryonic stem cells [29] (Figure 1D, S1C), suggesting that this data captures the general features of mouse mRNA stability. Since all mRNA is being degraded, a positive exponential parameter for an individual gene indicates that the gene has a longer half-life than the bulk of the mRNA, while a high negative value indicates that the gene has a shorter half-life than average. Parameter estimation of relatively stable (Gapdh) and unstable (Brix1) genes are shown (Figure 1E, F). These decay estimates are highly correlated between soma and neurites (Figure 1C), suggesting that mRNA stability is similar across the two fractions.

3.2 Less stable mRNAs are enriched for GU-rich elements in their 3' UTRs

To identify the general determinants of mRNA stability in CAD cells, genes were first classified as less stable or more stable than average based on the distribution of mRNA decay parameters in the soma and neurites (Figure 2A). Out of 8800 genes analyzed, 1239 genes were classified as less stable and 1024 genes as more stable. In agreement with previous studies [29, 30], we found that the top gene ontology categories associated with more stable genes were related to translation and metabolism, including members of the ATP Synthase complex like Atp5b and Atp5d, and several ribosomal protein coding genes like Rps2, Rps3, Rpl3 and Rpl4. The most enriched gene ontology categories associated with less stable genes were related to transcription factors, and regulation of protein degradation (Figure 2B), including Sox4, Smad1 and other members of the SMAD family [31], and transcriptional repressor Ctcf.

mRNA stability has been shown to correlate with exon junction density and 3' UTR length [29, 32], and various cis-acting sequences such as AU-rich elements [33], GU-rich elements [34] and Pumilio (PUM) motifs are known to influence mRNA decay. To help understand the determinants of stability in CAD cells, we examined the properties of genes belonging to less stable and more stable classes. Genes that
are more stable are highly expressed, shorter than average and have higher mRNA GC content, while less stable mRNAs have higher AU-content than average (Figure 2C-E). Several U-rich 5mers matching the motifs of GU-rich elements (GREs) [34] are enriched in the 3' UTRs of less stable mRNAs (Figure 2F), suggesting that GREs and their cognate RNA binding proteins play an important role in regulating neuronal mRNA stability.

3.3 Skipped exons that decrease mRNA stability upon inclusion are AU-rich

Most genes in higher eukaryotes give rise to a several mRNA isoforms that can have distinct functions [35, 36]. Detection of differential stability between mRNA isoforms of a gene has excellent potential to uncover regulatory RNA motifs since differences in stability can be attributed to the often short regions unique to a particular isoform, providing good statistical power to identify these elements. To identify isoforms that differ in stability, we used the MISO software for statistical analysis of RNA-seq data [24] to compute percent spliced in (PSI or Ψ) for alternative exons, representing the fraction of a gene’s mRNAs that include the exon. If a pair of alternative isoforms does not differ in stability, the transcripts containing one of the alternative exons decay at the same rate as the transcripts with the other alternative, yielding values of Ψ that remain constant over time. If one isoform is more or less stable than the other isoform then Ψ is expected to increase or decrease with time.

Using the Monotonicity Z-score metric that identifies consistent Ψ changes with time [26], approximately 12% of all detected isoform pairs were found to differ in stability (Figure 3A). A larger fraction of retained introns decrease mRNA stability upon inclusion (>20%), which is expected since retained intron isoforms usually contain premature termination codons and are often targets of nonsense-mediated mRNA decay (NMD) [37]. For instance, Clk4, encoding a tyrosine kinase that phosphorylates splicing factors of the SR protein family, has a large fraction of its transcripts con-
taining retained introns, and these introns are spliced out only under stress [38, 39]. Our data demonstrates that the Clk4 retained intron isoform is less stable than the isoform without the intron, thus ensuring that the mature protein is only translated when the intron is spliced out.

Overall, 819 skipped exon isoform pairs were found to differ in stability using the described MZ-score metric. For example, Mbnl2, a member of the Muscleblind family of proteins known to be involved in pre-mRNA splicing and mRNA localization [5, 40], has two alternatively spliced isoforms that differ by a skipped exon [41]. The longer inclusion isoform appears to be less stable than the shorter isoform in our data.

SEs that decrease mRNA stability have lower GC-content (Figure 3B) and are enriched for A-rich 5mers (Figure 3D). However, neither the position of skipped exons in a gene (UTR vs. coding sequence) or the length of the exon were significantly associated with stability in CAD cells (Figure S2A, B). Genes containing SEs that impact stability have lower average mRNA half-lives than genes with SEs that do not affect stability (Figure 3C). These observations argue that skipped exon inclusion primarily impacts mRNA half-lives by destabilizing the transcript. SEs can decrease stability by altering the codon composition [42] or through the recruitment of RNA-binding proteins. Further experiments need to be carried out to determine the mechanism.

3.4 Alternative first exons that are more stable in neurites are enriched for GC-rich hexanucleotides

mRNA turnover could be spatially controlled by cis-elements that differentially affect stability in different subcellular locations and/or by mRNA decay factors with localization or activity restricted to particular subcellular regions. To assess the prevalence of localized mRNA decay, mRNA stability at the gene level and the isoform level was compared across soma and neurites. At the gene level, a localization ratio (LR) was defined for each gene as the ratio of expression (measured by transcripts per million - TPM) in the neurite fraction divided by expression in the soma fraction. mRNAs
that are more stable in the neurite would show an increase of LR with time, while transcripts destabilized in the neurite would show a decrease of LR with time. To measure the change in LR, a LR-monotonicity Z-score was computed for each gene. This metric identified 254 genes whose transcripts are more stable in the neurite and 291 genes with mRNAs that are less stable in the neurite compared to soma (Figure 4A). Interestingly, genes less stable in neurites are enriched for gene ontology terms related to microtubule motor activity (Figure 4B). These genes include members of the kinesin and dynein families of motor proteins like Kif1a, Kif2a and Dyn1h1, which are known to be involved in transporting various cargoes including mRNAs and synaptic vesicles [43]. Transcripts encoding these proteins could be less stable in neurites to have better temporal control on local synthesis and retrograde transport.

While isoform stability correlated well between the two fractions, a larger percentage of isoform pairs differed in stability in the neurite compared to the soma indicating that mRNAs in the neurite are subject to more regulation (Figure S3A). Alternative first exon (AFE) isoform pairs, which differ mainly in the 5’ UTRs, showed neurite-specific differences in stability. Higher GC-content is associated with stability for both distal and proximal AFEs in the neurite (Figure 4D). AFEs that are more stable are enriched for GC-rich hexanucleotides (Figure 4F, S3C), while less stable AFEs are depleted for similar 6mers (Figure 4E, S3B). Additionally, distal AFEs tend to be more stable than their proximal counterparts in the neurite (Figure 4C). These results demonstrate that GC-rich sequences in 5’ UTRs stabilize transcripts in neurites of CAD cells. Further experiments using a bidirectional Tet-OFF reporter system harboring control and test UTR sequences (Figure S3D) will be done to validate this result and study the effect of 5’ UTR GC-rich sequences on mRNA localization, translation and stability.
Chapter 4

Discussion

Neurons are highly polarized cells with projections extending far from the cell body. These dendritic and axonal projections contain complex and dynamic transcriptomes, and regulation of mRNA stability [10, 44] and local mRNA translation [45] play critical roles in development and maintaining neuronal function. Cis-acting elements can influence mRNA decay by binding to RNA binding proteins which recruit the decay machinery. Here we show that GU-rich elements (GREs), known decay elements that bind to Celf1, are strongly enriched in transcripts that are less stable in mouse CAD cells. GREs, with a consensus sequence of UGUUUGUUUGU, were first identified as conserved sequences enriched in 3'UTRs of unstable transcripts in human T cells [46]. They bind to Celf1, a member of the CELF family of RNA-binding proteins known to be involved in the regulation of alternative splicing, translation, deadenlyation and degradation. Celf1 shares many of its mRNA targets with Muscleblind (Mbnl) proteins in skeletal muscle cells [26, 40]. As Mbnl has been implicated in localizing mRNAs to neurites of CAD cells [5], this suggests that there may be coordinate regulation of mRNA expression and localization by Celf and Mbnl proteins, similar to that previously observed in skeletal muscle.

Most genes in higher eukaryotes give rise to a large number of mRNA isoforms [35, 36]. Conservative estimates predict 2-12 isoforms for most mammalian genes [24]. Previous work has shown that some mRNA isoforms that differ in their 3' ends are degraded at different rates [32, 47], but these studies did not look at the half-lives
of other types of mRNA isoforms. Here, we identified such mRNA isoforms that exhibit differential stability in neuronal cells, and characterized the RNA elements that bring about this difference. Approximately 800 SE isoform pairs were found to differ in stability, and an enrichment of As is associated with decreased stability. These cassette exons could impact stability by altering the codon optimality of the transcript [42], as some A-rich codons are classified as non-optimal.

Almost half of all mammalian genes use alternative promoters to generate isoforms carrying alternative first exons (AFEs) [48]. Individual alternative promoters tend to be regulated by specific transcription factors, and the isoforms produced can encode distinct 5’ UTRs and even different protein isoforms with distinct functional activities [49]. Here, we show that GC-rich AFEs, particularly distal AFE isoforms, are more stable than their partners in neurites of CAD cells. As distal AFEs tend to have higher GC-content than proximal AFEs overall, this data suggests that the neurite microenvironment promotes the stabilization of transcripts with GC-rich sequences in their 5’ UTRs. These sequence elements could either directly increase the stability of the transcript in the neurite, or could influence translation thereby indirectly stabilizing the transcript.

To evaluate the effects of GC-rich 5’ UTRs on mRNA localization, translation and stability, we designed a bidirectional-promoter reporter harboring control and GC-rich UTRs (Figure S3D). As mRNA encoding EGFP and that encoding EBFP are equally transcribed, any differences in expression of these transcripts observed via qPCR would indicate differences in mRNA stability. The proteins translated from these mRNA differ in size and both contain N-terminal FLAG tags. Anti-FLAG western blotting can be done to observe differences in translation from the two mRNAs. The impact of GC-rich sequences on mRNA localization can also be studied by comparing mRNA expression across soma and neurites. Follow-up experiments with knockdown and overexpression of candidate proteins can then help identify the mechanism involved in stabilizing GC-rich 5’ UTRs. The RNA binding motif 4 (Rbm4) proteins are mammalian orthologs of the Drosophila RNA-binding protein Lark, and are known to be involved in regulating alternative splicing, translation, and miRNA-
mediated regulation of gene expression [50, 51, 52, 53]. Rbm4 is highly expressed in the brain [54] and regulates expression of proteins important for neuronal function [55, 56, 57, 58]. It has also been shown to bind a GC-rich motif [59, 60], making it an ideal candidate for stabilizing transcripts in neurites.

mRNA localization can be achieved through active transport, passive diffusion and local entrapment, or through local stabilization/degradation of transcripts. The overall mRNA stability was found to be similar across the soma and neurite fractions, suggesting that local regulation of mRNA stability is not a primary mechanism to achieve mRNA localization in neurons. However, our data shows that transcripts with GC-rich 5’ UTRs are stabilized in the neurites, and that Celf and Mbnl proteins may coordinate mRNA degradation and localization to neurites. While 3’ UTRs determine the localization of mRNAs, our data suggests that 5’ UTRs may be involved in regulating local translation and stability of mRNA after localization.
Appendix A

Figure legends

A.1 Figure 1. Transcription inhibition and cellular fractionation followed by sequencing reveals mRNA half-lives in CAD cells

A Mouse CAD cells are grown on porous membranes, enabling physical fractionation of cells after growth of neurites through the pores. Cells are treated with actinomycin-D prior to fractionation and RNA isolation.

B Complete-linkage hierarchical clustering of samples based on TPM values of genes.

C Scatterplot of exponential decay constants of gene TPMs in soma and neurite fractions.

D Comparing mRNA half-lives measured in mouse embryonic stem cells [29] to half-lives estimated in CAD cells (n=4747 genes).

E-F Estimation of relative decay parameters for Gapdh and Brix1 mRNAs in soma.
A.2 Figure 2. Unique features of mRNAs that differ in stability

A Classification of mRNAs as less stable or more stable based on their relative decay parameters.

B Gene ontology categories of less stable and more stable genes.

C-E Abundance, length and GC-content of different classes of mRNAs.

F Enrichment of 5mers in 3' UTRs of less stable mRNAs relative to 3' UTRs of control mRNAs matched to have similar GC-content and expression levels.

A.3 Figure 3. mRNA stability differences between alternative splicing isoforms

A Percent of alternative mRNA isoforms that differ in stability. MZ-score is calculated for every detected AS event (AFE = 6286, SE = 15388, RI = 1040, Tandem 3aÅZ UTR = 4117, ALE = 11154). Events with an absolute value of MZ >1.5 are considered to differ in stability.

B GC-content of different classes of skipped exons.

C Cumulative distribution of estimated half-lives of mRNAs containing skipped exons.

D Enrichment of 5mers in SEs that decrease mRNA stability upon inclusion, relative to SEs that do not affect stability.
A.4 Figure 4. Comparing mRNA stability between soma and neurites shows that AFEs display unique properties

A Scatterplot of exponential decay constants of gene TPMs in soma and neurite fractions, with genes colored by their LR-MZ-scores. A high LR-MZ value suggests that the mRNA is more stable in the neurite, while a high negative value suggests that the mRNA is more stable in the soma.

B Gene ontology categories of genes less stable in neurites.

C MZ-score distributions of alternative first exons in soma and neurite fractions.

D GC-content of different classes alternative first exons.

E-F Enrichment of 6mers in distal AFEs that are less (E) or more (F) stable than their proximal counterparts in neurites, relative to control distal AFEs.

A.5 Supplementary Figure 1. Related to Figure 1

A CAD cells before and after serum withdrawal. CAD cells generate neuronal projections upon serum withdrawal.

B Soma and neurite lysates for all samples were immunoblotted for the presence of beta-actin, a marker of both soma and neurite fractions, and histone H3, a marker of soma fractions.

C Pearson correlation of mRNA half-lives estimated in multiple studies. Data obtained from references [32, 61, 62, 63, 29].
A.6 Supplementary Figure 2. Related to Figure 3

A MZ-score distributions of skipped exons (soma), categorized by their position in a gene. No significant differences are observed.

B MZ-score distributions of skipped exons (soma), categorized by the length of the exon. No significant differences are observed.

C Enrichment of 5mers in SEs that increase mRNA stability upon inclusion, relative to SEs that do not affect stability. No significant enrichment is seen.

A.7 Supplementary Figure 3. Related to Figure 4

A MZ-score distributions of alternative splicing isoforms in soma and neurite fractions.

B-C Enrichment of 6mers in proximal AFEs that are less (B) or more (C) stable than their distal counterparts in neurites, relative to control distal AFEs.

D Schematic of AFE reporter construct. The construct consists of a bidirectional tet-OFF promoter that transcribes equally on both sides in the absence of doxycycline. On one side with the test 5' UTR, is a destabilized version of a FLAG-tagged enhanced green fluorescent protein (d2EGFP [64]) followed by 4 HA-tags. The other side of the promoter contains the control UTR, followed by a destabilized version of FLAG-tagged EBFP, with a stop codon before the HA tags. The two transcripts therefore code for proteins of different sizes, while having nearly identical mRNA sequences (the mRNA sequences differ in their 5' UTRs, and at 3 nucleotides in the rest of the sequence). mRNA stability will be measured in the neurites and soma for different control and test UTR sequences.
Appendix B

Figures
Figure 1. Transcription inhibition and cellular fractionation followed by sequencing reveals mRNA half-lives in CAD cells.
Figure 2. Unique features of mRNAs that differ in stability
Figure 3. mRNA stability differences between alternative splicing isoforms
Figure 4. Comparing mRNA stability between soma and neurites shows that AFEs display unique properties.
Supplementary Figure 1. Related to Figure 1
Supplementary Figure 2. Related to Figure 3
Supplementary Figure 3. Related to Figure 4
Bibliography


