Neuroscience Letters 635 (2016) 71–76

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

Rat *NEURL1* 3′UTR is alternatively spliced and targets mRNA to dendrites

Madis Jaagura[1], Kati Taal, Indrek Koppel[2], Jürgen Tuvikene, Tõnis Timmusk*, Richard Tamme*

Department of Gene Technology, Tallinn University of Technology, Akadeemia tee 15, 12618, Tallinn, Estonia

HIGHLIGHTS

- The 3′UTR of *Neuralized-like1* (*NEURL1*) mRNA exists in two splice variants (1743 and 1477 nt in length).
- The *NEURL1* mRNA with spliced 3′UTR is expressed at low levels, reaching up to 1.65% of the levels of total *NEURL1* transcripts.
- The spliced *NEURL1* 3′UTR is a target of nonsense-mediated decay.
- *NEURL1* transcripts with spliced and full-length 3′UTRs are enriched in the neurites of primary hippocampal neurons.
- Both spliced and full-length *NEURL1* 3′UTRs can direct reporter mRNAs to the dendrites in primary hippocampal neurons.

ARTICLE INFO

Article history:
Received 6 May 2016
Received in revised form 15 October 2016
Accepted 21 October 2016
Available online 22 October 2016

Keywords:
Neuralized-like 1
Dendritic targeting
Alternative splicing in 3′UTR
Nonsense-mediated decay

ABSTRACT

Neuralized, an E3 ubiquitin ligase, interacts with and positively modulates the Notch pathway by promoting ubiquitination and subsequent endocytosis of its ligands. *NEURL1* mRNA is dendritically localised in the dentate gyrus of an adult rat brain, implying that it may be locally translated, but its transport mechanisms remain unstudied.

Here, we report the presence of a previously unknown, shorter splice-variant of rat *NEURL1* 3′UTR (1477 bp in length), and identify it as a potential target of nonsense-mediated decay. We show that endogenous *NEURL1* mRNAs with both longer and shorter 3′UTRs are enriched in the neurites of cultured rat primary hippocampal neurons. Both *NEURL1* 3′UTRs can mediate transport of reporter mRNAs into dendrites in primary hippocampal neurons. By analysing the dendritic trafficking capacity of reporter mRNAs linked to various regions of longer or shorter *NEURL1* 3′UTR, we localise the dendritic targeting element (DTE) of spliced version of *NEURL1* 3′UTR to its first half, corresponding to the nucleotides 1-148 and 416-914 of the full-length 3′UTR. In contrast, the dendritic targeting capacity of the full-length *NEURL1* 3′UTR is abolished by splitting its 3′UTR in two halves (nt 1-914 and nt 915-1744), suggesting that slightly different DTE might mediate dendritic transport of the two transcripts.

1. Introduction

Neuralized (Neur) acts as an E3 ubiquitin ligase for Delta and other Notch ligands [1] and CPEB3, a cytoplasmic adenylation element binding protein [2]. Only ubiquitinated Notch ligands can be efficiently endocytosed, allowing the Notch receptor to be sequentially proteolytically processed, resulting in nuclear translocation of its intracellular domain and activation of target genes in conjunction with transcriptional coactivators, e.g. Su(H) and Mam [1]. Evolutionarily conserved Notch signalling is crucial both during development and adulthood and its dysregulation has been linked to numerous pathologies [3,4].

Mammalian Neuralized E3 ubiquitin protein ligase 1 (*NEURL1*) transcripts, but not the transcripts of another mammalian *Neuralized* family member, *NEURL1B* [5] are transported to the dendrites of the dentate gyrus of the rat hippocampal formation, implying

* Corresponding authors.
E-mail addresses: tonis.timmusk@ttu.ee (T. Timmusk), richard.tamme@ttu.ee, tamme.richard@gmail.com (R. Tamme).
[1] Current address: Competence Centre for Food Technologies, Tallinn, 12618, Estonia.
[2] Current address: Department of Biological Chemistry, Weizmann Institute of Science, Rehovot, 76100, Israel.

http://dx.doi.org/10.1016/j.neulet.2016.10.041
0304-3940/

that they could be locally translated [6]. Memory formation as well as late phases of long-term potentiation and long-term depression, the electrophysiological manifestations of synaptic plasticity, require new protein synthesis [7]. Such protein synthesis can occur in the neuronal cell bodies (somas) and in the dendrites near synapses, allowing the proteins mediating synaptic plasticity modification to be synthesised synapse-specifically. Localised translation can confer partial independence to synapses since the proteins required for synaptic plasticity such as components of signalling pathways, neurotransmitter receptors and scaffolding proteins can be translated rapidly without relying on protein transport from the soma. Many transcripts encoding proteins important for the protein-synthesis dependent stages of synaptic plasticity and memory formation, such as *αCAMKII*, *Arc* (or *Arg3.1*), *dendrin*, are trafficked from the somas into the dendrites [8]. Dendritic transport of mRNAs relies on dendritic targeting elements (DTEs) – *cis*-acting sequence elements or secondary structures formed by them that mostly reside in the 3′ untranslated regions (3′UTRs) of the respective mRNAs. RNA-binding proteins (e.g. hnRNPA2) that directly associate with the DTEs and various adaptor and motor proteins mediate the dendritic transport of mRNAs along the cytoskeleton to the vicinity of appropriate synapses [8]. While the DTEs of different dendritic mRNAs are usually not conserved on a sequence level, certain conserved structures have been identified. For instance, G-quadruplex motif ($(G_3N_{1-7})_4$) has been bioinformatically identified in approximately 30% of dendritically localised mRNAs, including human *NEURL1* [9]. A G-quadruplex from *αCaMKII* and *PSD-95* mRNAs' 3′UTR can target a reporter transcript to the dendrites [9]. However, the DTE(s) of *NEURL1* have not been previously characterised.

In this study we have found that the 3′UTR of rat *NEURL1* mRNA is alternatively spliced and that the shorter, spliced transcript constitutes a substrate for nonsense-mediated decay (NMD). Both endogenous *NEURL1* mRNAs, with spliced and unspliced 3′UTR, are transported into the dendrites of primary hippocampal neurons. To characterise the DTE(s) of *NEURL1* mRNA, we performed deletion analysis of the *NEURL1* mRNA's 3′UTR regions. We found that the DTE of *NEURL1* mRNA resides within the nucleotides 1-148 and 416-914 of its 3′ UTR (numbering corresponds to the longer 3′UTR).

2. Materials and methods

RNA isolation, reverse transcription and qPCR were carried out as described in [10]. The primers used for PCR analysis and cloning of various *NEURL1* 3′UTR expression constructs are listed in Supplementary Table 1. Rat primary neurons were cultured, transfected and treated with wortmannin as described in [11]. Cloning of *NEURL1* 3′UTR and the reporter constructs used are detailed in Supplementary data and Materials. *In situ* hybridisation was carried out essentially as described by [12]. Detailed description of the Materials and Methods is provided in the Supplementary data.

2.1. Quantitative imaging and statistical analysis of dendritic targeting

Images of neurons carrying the ISH signal were acquired with an Olympus BX61 microscope equipped with an Olympus DP70 CCD camera and analysed using Photoshop 7 software (Adobe InDesign). Dendrite ISH signals were traced and measured using the ruler tool up to a point where ISH labelling was still visually distinguishable from the background. To calculate the statistical significance of differences of dendritic labelling between the different reporter mRNAs used, arcsine square-root transformation of data was carried out followed by one-way ANOVA analysis and Tukey's post-hoc multiple comparison test (GraphPad Prism 6). The differences were considered statistically significant if the p-value was equal to or lower than 0.05.

2.2. Bioinformatical analysis

Rat *NEURL1* mRNA's 3′UTR sequence was analysed for the presence of G-quadruplexes using the QGRS Mapper (http://bioinformatics.ramapo.edu/QGRS/index.php) with default settings limiting the length of any putative G-quadruplexes to 30 nucleotides.

3. Results and discussion

3.1. *Rat* NEURL1 *mRNA 3′UTR has two alternatively spliced isoforms*

We focussed on the *NEURL1* 3′UTR region only since in most dendritic transcripts studied the DTEs reside within their 3′UTRs [8]. PCR cloning of *NEURL1* 3′UTR from rat cortical cDNA yielded a product with expected size, 1743 bp according to GenBank and the UCSC Genome Browser as well as a previously unrecognised shorter product of 1477 bp (data not shown). Sequence analysis revealed that the 1743 bp product has an intron-like insertion encompassing 3′UTR positions 149-415 whose 5′ and 3′ ends harbour a putative donor splice site (AAG-GUUUG) and an acceptor splice site (CAG-CGG). Moreover, the putative intron contains sequences similar to a branch point and a polypyrimidine tract (Supplementary Fig. 1). This suggests that *NEURL1* 3′UTR may be alternatively spliced. Semi-quantitative PCR-based analysis of rat cortical cDNA using primers recognising both mRNA species showed that the transcript with the shorter 3′UTR was less abundant than the one with longer 3′UTR (Fig. 1A).

3.2. *Rat* NEURL1 *mRNA with spliced 3′UTR is expressed at low levels in both developing and adult brain*

To measure the relative expression levels of both *NEURL1* transcripts in different developmental stages and adult brain regions, we performed qPCR-based analysis using cDNAs derived from the rat embryonic brain (E13 and E21.5) and different adult brain regions. The levels of spliced *NEURL1* 3′UTR relative to the total *NEURL1* transcripts were highest in adult midbrain (1.65%), cortex (1.6%), medulla (1.48%), thalamus (1.46%), and embryonic (E13) brain (0.98%), whereas in E21.5 brain and other adult brain regions splicing occurs less frequently (Fig. 1B). Statistical analysis revealed that the relative levels of spliced *NEURL1* 3′UTR are significantly higher in adult midbrain and cortex *vs* hippocampus and striatum and in medulla and thalamus *vs* hippocampus (Fig. 1B). However, our analysis of the two *NEURL1* transcripts' levels is based on qPCR that does not reveal the relative differences of their levels in different neuronal compartments, e.g. near synapses. Thus, it cannot be ruled out that the *NEURL1* mRNAs with shorter 3′UTR are present in higher levels in some cellular locations.

3.3. *Spliced* NEURL1 *3′UTR is a target for nonsense mediated decay*

We reasoned that the considerably smaller expression levels of the *NEURL1* mRNA with spliced 3′UTR might be at least partly explained by the fact that splicing in a given mRNA's 3′UTR, i.e. downstream of the stop codon, could lead to such transcript's nonsense-mediated decay. For instance, *Arc*, a dendritic transcript containing two introns within its 3′UTR, constitutes a substrate for NMD [13].

Therefore, we treated primary hippocampal rat neurons with wortmannin, a known inhibitor of NMD [11], and measured