Translation Efficiency of Synaptic Proteins and Its Coding Sequence Determinants

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Abstract: The synapse is an organized structure that contains synaptic vesicles, mitochondria, receptors, transporters and stored proteins. About 10% of the mRNAs that are express in mammalian neurons are delivered to synaptic sites, where they are subjected to local translation. While neuronal plasticity, learning and memory occur at the synapse, the mechanisms that regulate post-transcriptional events and local translation are mostly unknown. We hypothesized that evolutional signals that govern translational efficiency are encoded in the mRNA of synaptic proteins. Specifically, we applied a measure of tRNA adaptation index (tAI) as an indirect proxy for translation rate and showed that ionic channels and ligand-binding receptors are specified by a global low tAI values. In contrast, the genuine proteins of the synaptic vesicles exhibit significantly higher tAI values. The expression of many of these proteins actually accompanied synaptic plasticity. Furthermore, in human, the local tAI values for the initial segment of mRNA coding differs for synaptic proteins in view of the rest of the human proteome. We propose that the translation of synaptic proteins is a robust solution for compiling with the high metabolic demands of the synapse.

1 INTRODUCTION

Translation must be tightly controlled for coping with the cell demand and its limited resources. Energetically, translation is the most expensive operation in dividing cells (Arava, *et al.*, 2003; Gingold and Pilpel, 2011; Ingolia, *et al.*, 2009). Thus, an appropriate regulation of the rate of translation reduces the ribosomal drop-off, the translation errors and improves the overall ribosomal allocation (Zhang, *et al.*, 2010).

In unicellular organisms, it has been shown that the genomic tRNA copy number (CN) approximates the levels of intracellular tRNA and thus the codon usage. Moreover, the relative genomic abundance of synonymous codons varies in all organisms from bacteria to mammals (Sharp, *et al.*, 1993), and codon usage among genes tends to be related to their expression levels (dos Reis, *et al.*, 2004; Marais and Duret, 2001; Plotkin and Kudla, 2010; Tuller, *et al.*, 2010). Specifically, highly expressed genes (*e.g,* ribosomal proteins) tend to include codons that are recognized by abundant tRNA molecules, suggesting that the control of the translation process is under a selective pressure.

In all organisms, decoding of mRNA to proteins occurs by tRNAs. The tRNA anticodon recognizes the complementary codon or the wobble-based codon that encodes the same amino acid (Percudani, 2001). In bacteria and fungi, the genomic tRNA CN correlates with the intracellular tRNA levels (Ikemura, 1985; Lucks, *et al.*, 2008). A similar trend is detected in healthy and diseased tissues in human (Mahlab, *et al.*, 2012). Consequently, the tRNA adaptation index (tAI) (dos Reis, *et al.*, 2004) is applied as a measure for ranking the adaptation of a gene in term of translation elongation. The assumption is that the availability of relevant tRNA types has a strong effect on the efficiency and speed of translation (Mahlab, *et al.*, 2012; Tuller, *et al.*, 2010).

Synapses are autonomous structures at nerve terminals that are specified by high metabolic demands, and functional plasticity. Communication across the synapse is mediated by neurotransmitters (NT) and neuropeptides (NP) that are released from synaptic vesicles (SV) as a result of neuronal activity. A success coupling of the action potential to

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exocytosis requires a coordinated action of priming, targeting and docking (Ferro-Novick and Jahn, 1994). Actually, tens of proteins that belong to SVs and secretory granules participate in storing, docking, fusion and recovery in synapses (*e.g.,* worm, fly, human) (Broadie and Richmond, 2002). The intense energetic demand is maximized (Ames, 1992) upon extensive brain activity and experience, which is the basis for synapse plasticity (Nestler, 2001).

Most synaptic proteins belong to the secretory systems. From a cellular perspective, the main sub-compartments include: (i) the trafficking organelles such as the Endoplasmic Reticulum (ER), Golgi, endosomes, lysosomes and secretory granules. (ii) The plasma membrane (PM) with a partition to pre- and post-synaptic sites. (iii) The extracellular space and the synaptic cleft.

Neurons are unique with respect to their ability for local translation (Martin*, et al.*, 2000). A tight regulation of translation is achieved by translation inhibition (Richter and Sonenberg, 2005). Still, 5-10% of the brain transcripts have the potential for a local translation at synaptic sites (Gebauer and Hentze, 2004).

Misfolding of proteins in neurons is the basis for diseases such as Prion, Alzheimer's (AD) and Parkinson's diseases (PD) (Chiti and Dobson, 2006). Other conditions with memory loss are associated with a failure in the balance of synaptic proteins and their proper folding (Ross and Poirier, 2004).

Unlike vesicle trafficking, the SV fusion in synaptic structures is tightly regulated in time and space (Brachya*, et al.*, 2006; Trimble*, et al.*, 1991). The synaptic protein catalogue (Pielot*, et al.*, 2012; Yanay*, et al.*, 2008) allows testing the evolutionary refinement on the translational capacity. In this study, we examine whether the synapse homeostasis is governed by managing a stable production of proteins at the right quantities. We propose a translational dependent strategy to handle the extreme metabolic and proteomic demands of synaptic proteins across model organisms.

In this study, we address the notion of sequence-encoded component of 'speed controls' as shaped by evolution. We hypothesized that the sequences of synaptic proteins, especially those needed at high amounts or under restricted conditions are prone to production failure. We will not elaborate on additional critical factors that directly alter translation elongation such as mRNA folding or translational initiation (Holcik*, et al.*, 2000).

2 METHODS

2.1 Databases

We retrieved sequences from UniProtKB according to the selected organisms and their subcellular localization annotations (Barrell*, et al.*, 2009). We applied the terms "synapse", "pre-synaptic" and "post-synaptic" and "complete proteome". We extracted proteins that are marked as 'fragment'. A partition of proteins to non-disjoint groups was performed using the UniProtKB Sequence Features (FT). We tested features such as 'Signal peptide', 'Transmembrane' (TMD), 'Disulfide-bond' and 'Coiled coil'.

2.2 Computing tAI

tRNA adaptation index (tAI) was computed according to (dos Reis*, et al.*, 2004). The adaptation of tRNAs (tAI) is calculated from the genomic tRNA CN, combined with thermodynamic considerations of the codon-anticodon interaction. While the tAI is associated with each codon, the tAI of a gene is the average of its codons' tAI. This measure gauges the availability of tRNAs for each codon along an mRNA. As codon-anticodon coupling is not unique due to wobble interactions, practically, several anticodons can recognize the same codon, with somewhat different efficiency.

Formally, Let ni be the number of tRNA isoacceptors recognizing codon i. Let tCGNij be the copy number of the jth tRNA that recognizes the ith codon, and let Sij be the selective constraint on the efficiency of the codon-anticodon coupling. We define the absolute adaptiveness, Wi, for each codon i as:

$$Wi = \sum_{j=1}^{ni} (1 - Sij)tCGNij \qquad (1)$$

From Wi we obtain wi, which is the relative adaptiveness value of codon i, by normalizing the Wi values (dividing them by the maximal of all 61 Wi).

2.3 Computing Segmental tAI

Local tAI is calculated by dividing each coding sequence into several overlapping windows (window of 30, overlapping by 15 codons). For sequences that are shorter than 180 amino acids, only local segmental tAI were calculated. This was applied to

avoid overlap between N' and C' terminal windows. The successive windows are marked N1, N2 and a similar notation for C'-terminal. In this study we only focus on the N'-terminal region.

2.4 Statistical Analysis

Statistical significance, correlations, variance and p-values were according to standard MatLab suite. We used the t-test and Kolmogorov–Smirnov (KS)-statistics.

3 RESULTS

3.1 tAI Values in Worm, Fly and Human

The major model organisms for studying neuronal functions include human, worm and fly. These organisms were used to identify the common molecular apparatus for fast and slow transmission in the CNS. We took advantage of the manually compiled SVs and the synaptic proteins catalogue from human, fly and worm in order to find the evolutionary signals that impact their translation efficiency.

Table 1: Correlations of tAI codons in model organisms.

	Hs	Dm	Ce
H. sapiens	1	0.57	0.46
D. melanogaster		1	0.6
C. elegans			1

The tRNAs CN is subjected to evolutionary forces. Thus, it is significantly different along the evolutionary tree. For example, there are 87 tRNAs in *E. coli K12*, 287 in *S. cerevisiae* (budding yeast) and over 3600 in *Bos taurus* (cow). The CN for tRNAs in *D. melanogaster* (Dm, fruitfly) and *C. elegans* (Ce, worm) is 299 and 606, respectively. The variation in tRNA composition for tRNA isoacceptors and the fraction for each isoacceptors from the number of tRNA active genes is converted to tAI values (dos Reis, et al., 2004) (see Methods).
Table 1 displays the correlation between the tAI values of 61 codons (excluding stop codons) of the selected model organisms. For example, for human and fruit fly it is only 0.57 (p-value=6.7e-7) and the correlation between *C. elegans* and *D. melanogaster* is 0.6 (p-value=1.9e-7). Thus, the tAI values *per se* cannot explain any apparent similarity in translation signals across these species.

3.2 Global tAI values in synapse

The synapse is an autonomous structure. Schematically, the synaptic proteins can be assigned to the following functionalities: (i) SNAREs, the minimal set of proteins that function in SV docking and fusion. (ii) Direct regulator of SNAREs (iii) Ion channels and transporters. (iv) Enzymes and modifiers (e.g., kinases, phosphatases). (v) Organizers, mainly PDZ and cytoskeleton proteins.

To test whether the above division is recapitulated by the calculated tAI values, we compiled a set of 167 synaptic proteins from *C. elegans* as a test case. This relatively small set is manually curated. For consistency, we maintained identical annotation protocol throughout the study (see Methods). The majority of *C. elegans* synaptic proteins are channels and transporters (class 1.A.9, NT receptor, and Ligand-gated ion channel). The largest group includes >30 LGC (Ligand-gated channel) proteins. These are the homologs of vertebrate Glycine receptor superfamily.

Fig. 1 shows the sorted tAI values of the entire set. About 20% of the genes deviate from the global tAI mean value by >1 s.d. A segregation of functional groups with proteins with relatively high global tAI values was noted (noted as HAT). Specifically, this fraction in enriched with proteins that are associated with SV biogenesis, SNAREs and their direct regulators. The enrichment of low tAI proteins (LATS) includes ligand gated ion channels (13 genes, >1 s.d., Enrichment score p-value=0.0014). None of the ion channel (total 103) was included in this list of 20 proteins with the highest tAI values.
We found that extremely low values of global tAI are associated with ligand gated channels. The possibility that very high sequence similarity explain this observation was discard. Actually, the *C. elegans* ligand gated channels share only 50% similarity at the amino acids and less than 40% at the nucleotide levels. Among the ionotropic NT receptors that are specified by low global tAI values are the Acetylcholine (ACh), Glycine, GABA and Glutamate receptors. Importantly, there is no difference in view of the tAI between cationic channels (e.g., AChR) and the anionic channels (GABA/ Glycine receptors). Note that the overall topology (i.e., N-terminal region facing the extracellular / SV lumen space is common to these channels. A similar distribution in tAI values is noted for all tested organisms (Fig. 1, human).

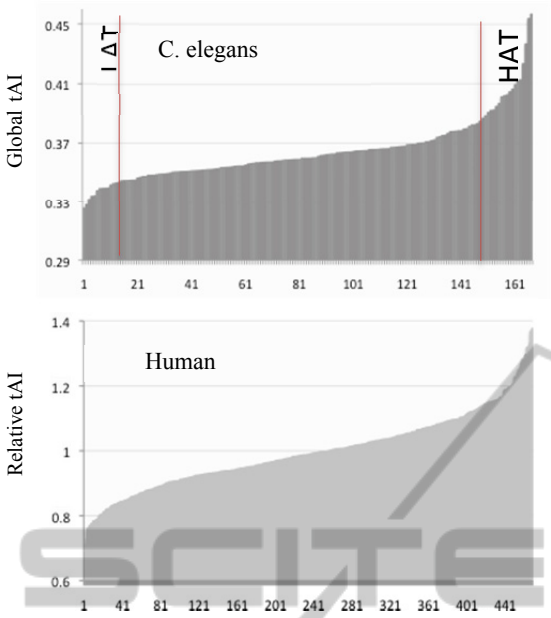

Figura 1: Global tAI of synaptic proteins from *C. elegans* (top) and human (bottom). LAT and HAT are proteins that significantly deviate (>1 s.d.) from the calculated mean tAI value and are associated with low and high values, respectively. Relative tAI measures the deviation relative to the mean which is defined as Relative tAI of 1.0.

3.2.1 tAI Values across Organisms

We repeated the analysis presented above for synaptic proteins of the fly, worm and human. In human, we collected 469 synaptic proteins according to their 'cellular location' annotation. A striking observation is the abundance of membranous proteins in this set (total of 63%). Some proteins are associated to the membrane through an indirect protein-protein interaction (PPI) network, or via a lipid modification moiety (e.g., GPI anchor).

Functional synapse is strongly dependent on proteins localization, protein state in term of its modification and the abundance of proteins. The membranous proteins in the synapse that contain TMD (single or multi-pass) comprise the majority of the membranous synaptic proteins (71% in human). A large fraction of which includes receptors, ion channels and transporters that are located to PM. An additional set comprises proteins that are secreted. Many of them are short proteins.

We found high correspondence in the list of proteins that have maximal global tAI values across organisms. A good example is the Complexin family (Sudhof and Rothman, 2009). This protein forms a tight PPI for directly regulating the SNARE complex formation. As such, it is a major component in controlling SV exocytosis. Complexins in mammals are composed of 3 related genes (a single gene in fly and worm). Multiple sequence alignment for Complexin from human to chicken and Xenopus showed that the core of the proteins is highly conserved (69.4% amino acid identity). More surprising is the observation that the calculated tAI is extremely high for all tested organisms and the calculated tAI value is within the top 15% of the synaptic proteins (469 in human, 167 in worm and 203 in fly).

The overlap in the list of protein with maximal tAI along the organisms is very significant. These proteins are also among the most abundant proteins of SVs. This set includes the SNAREs (VAMP, Syntaxin, SNAP-25) as well as synaptotagmin, and synaptophysin. In addition we noted that key proteins that participate in endocytosis such as AP-2, Unc-13 and Endophilin share the property of extreme tAI value across organisms. This is a highly significant finding in view of the moderate correlation in tAI codon values for the tested organisms (Table 1).

Enrichment tests with respect to GO-Slim annotations (Barrell*, et al.*, 2009) was performed for the high and low global tAI values quartiles (the complete list of synaptic proteins is used as a background). The enriched terms (p-value <0.01) for the high tAI include SVs, protein transport, membrane docking, membrane fusion, synaptic plasticity. The quartile of the proteins with the lowest tAI values are enriched with regulation of small GTPase, and anion transport.

3.3 Local tAI Values in Synapse

The tAI is an indirect measure that affect the allocation of ribosomes on the transcript. Extreme values of tAI are associated with ion channels (low), SNAREs and their primary regulators (high). The high global tAI of key proteins is in accord of high production. However, it was proposed that the initial segment is a critical feature for ribosomal flow management control.

We evaluate the synaptic proteins in view of the notion of 'speed controls'. Effectively, it is reflected by an unequal distribution of low (low adapted tRNA segments, called LATS) or high tRNA-adapted codons (HATS). The idea tested extensively for yeast and E. coli (Tuller*, et al.*, 2010). In metazoa, the picture is somewhat more complex and the tAI values strongly correlate with codon usage, gene expression, protein expression and GC content (Mahlab*, et al.*, 2012).

We compared the properties of the entire human proteome and those of the synaptic proteome. Table 2 summarizes the global (the entire coding sequence) and local (a segment of the coding sequence) measure of synaptic protein (tAI-local, coined TAIL). The calculated TAIL for the first segment of the coding sequence (N1-TAIL) is reported.

For synaptic proteins we noted that N1-TAIL tAI value is statistically lower than that of the rest of the coding gene, for the tested model organisms. Notably, the average tAI value for all synaptic proteins was used as a reference. Thus, the inherent bias for the synaptic proteins was avoided.

The cytosolic fraction in the human proteome occupies about 70% of all proteins (Fig. 2A, 11,500 proteins). We repeated the test for the local property to the entire human proteome (TAIL, tAI of a selected window).

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Table 2: A global measure of tAI for synaptic protein.

	# Syn[a]	# Proteins	Syn tAI	Syn N1 TAIL	P-value TAIL/tAI
Hs	469	18,433	0.329	0.328	**0.045**
Ce	167	3187	0.364	0.348	**3.42E-8**
Dm	203	3094	0.329	0.312	**1.96E-9**

[a]N1, TAIL for the 30-codon segment of the N-terminal. Syn, synaptic proteins; Hs, Ce and Dm are the proteomes from human, worm and fly, respectively.

Fig. 2A shows the distribution of the TAIL values for cytosolic human proteins relative to the same analysis for the synaptic proteome (Fig. 2B). Plotting the results for N1, N2 and N3 (each sequential segment is 30 codons, no overlap) indicates that the N1 TAIL is higher than the following segments (N1>N2≥N3). Thus, the human proteome is signifies by a N1 that has a tendency for tRNA-adapted codons relative to the tAI of these genes. However, for the Synaptic proteins, the opposite trend holds with a calculated TAIL values in which N1<N2<N3. While the synaptic proteins display higher global tAI values (vertical dashed line, Fig. 2), the N1-segment uses codons that are slightly less adapted. The statistical analysis between the two sets (Fig. 2) reveals that the variance in the values of the synaptic proteome is considerably lower than that of the entire human proteome (mean, median and statistical error).

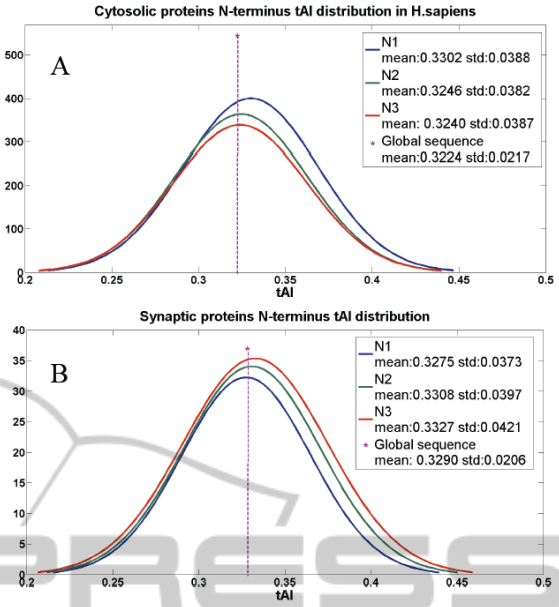

Figura 2: TAIL values of human cytosolic and synaptic proteomes. (A) Distribution of the TAIL for N1, N2 and N3. Each window covers 30 non-overlapping codons. (B) Distribution of the TAIL for N1, N2 and N3 for synaptic proteins. The global tAI is marked as a dashed line. The x-axis range is identical for Fig. 2A and 2B.

4 DISCUSSION

Based on the results from this study, we test whether the synaptic proteome is uniquely marked with translation 'speed controls' signals. Several properties signify synaptic proteins: the high tendency of membranous proteins, diverse cytosleletal proteins, precise subcellular localization and accurate allocation of proteins to organelles (e.g. SVs, endosomes, recycling vesicles). Many of the proteins of the synapse share properties such as the abundance of disulfide bonds, coiled-coil and membrane association.

The SV is an autonomous organelle that accounts for a substantial fraction of the protein mass in the synapse. For example, synaptophysin and VAMP together, account for 5% of all synaptic proteins. The SV anatomy supports such load. A pre-synaptic structure in the human or mouse hippocampus contains about 300 SVs. Each of the SVs is composed of tens of proteins. The quantitative composition of the SVs was revisited using mass spectrometry (Takamori, *et al.*, 2006) and quantitative antibodies imaging tools (Mutch, *et al.*, 2011). Each of the key proteins (VAMP, Rab3, SV2 and Synaptophysin) appears with 5-30 copies per

SV. Consequently, the mass and protein packing in SV is maximal.

5 CONCEPTUAL REMARKS

We postulate that variants in N1-TAIL are attractive to cope with changing metabolic and activity status of the synapse.

We suggest that in addition to the regulation for uORFs (upstream ORFs) and activation of alternative splicing, the translation regulation is an additional mode for fine-tuning the overall protein production. Investigating translational signals along the transcripts of synapse is only in its infancy. We expect methods such as ribosomal profiling (Ingolia*, et al.*, 2009) to provide quantitative data for translation speed and efficiency.

An open question that we began to explore challenges the translational management with respect to ribosomal subunits in dendrites (and other neuronal compartment) (Sutton and Schuman, 2006). We postulate that local translation is critical for fast and efficient translation under conditions of restricted resources. Recently, it was shown that hundreds of mRNAs are localized to neuronal compartments such as synaptic neuropils (Cajigas*, et al.*, 2012). Translation of such mRNA must be highly regulated at the levels of transcript accessibility and the translation efficiency. Here, we provide a glimpse on an overlooked evolutionary encoded signal for managing translation of synaptic proteins.

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