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Review

Transcription and translation in a package deal: The TISU paradigm

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ABSTRACT

The major strategy for cap dependent translation involves ribosomal scanning. In the scanning mechanism the small ribosomal subunit is recruited to the mRNA through the m7G cap and then scans the 5′ UTR until it reaches an AUG codon. This short review focuses on a recently discovered alternative strategy of cap-dependent translation that operates without scanning, but nonetheless is highly efficient and accurate. This non-scanning translation is directed by the Translation Initiator of Short 5′ UTR (TISU) element. TISU is strictly located close to the 5′ end of the mRNA, resulting in a very short 5′ UTR. It is present in a sizable number of mammalian genes, many of them with fundamental cellular functions. In addition to its unique translational activity, TISU is also a transcription regulatory element that is specifically enriched in TATA-less promoters. Thus TISU represents a prototype regulatory element that links mammalian transcription to a specific mode of translation initiation.

1. Introduction

Expression of protein-encoding genes in eukaryotes is a complicated multi-step process that starts with chromatin remodeling, progresses to transcription and mRNA processing and ends with mRNA translation. Two types of DNA elements, enhancer and core promoter, control transcription of protein-encoding and non-coding genes. Gene specific enhancer elements serve as binding sites for transcription regulatory factors and can be divided into two classes: those that function independently of their position relative to the transcription start site (TSS) and those that can activate transcription only when located proximal to the TSS (Kadonaga, 2004). The core promoter is situated around the TSS and is the site on which RNA polymerase II and general transcription factors (GTFs) assemble into a pre-initiation complex. Some transcription regulatory elements are localized downstream to the TSS and are present in the mRNA as well, often in the 5′ UTR, so they are in a position to influence translation (Dikstein, 2011). In the past few years an increasing number of studies suggests coupling between mRNA and protein synthesis, particularly through factors that accompany the mRNA from the site of synthesis to its cytosolic destination (Bono and Gehring, 2011; Harel-Sharvit et al., 2010; Le Hir and Seraphin, 2008). This short review describes another mechanism to coordinate transcription with translation through regulatory element(s) common to transcription and translation.

As the process of protein synthesis is the final stage of gene expression, it impacts directly on the cell fate and physiology. Therefore mRNA translation, in particular the initiation phase, is tightly controlled, both by distinct translation regulatory factors and by structural elements on the mRNA (for review see Jackson et al., 2010; Kozak, 2005; Marintchev and Wagner, 2004; Sonenberg and Hinnebusch, 2009). The predominant form of eukaryotic translation initiation depends on the m7G cap structure of the mRNA and ribosomal scanning.

2. Mechanism of translation initiation through the m7G cap

Translation initiation begins with the formation of the 43S preinitiation complex composed of the 40S small ribosomal subunit, the ternary complex (eIF2-GTP-Met-tRNAi) and additional translation initiation factors (eIFs) (Jackson et al., 2010; Kozak, 2005; Marintchev and Wagner, 2004; Sonenberg and Hinnebusch, 2009). The preinitiation complex is recruited to the mRNA by eIF4F, a factor that is bound to the m7G cap of the mRNA through its eIF4E subunit. The 43S then scans the mRNA 5′ UTR until it identifies the AUG start codon through base-pairing with the Met-tRNAi. During scanning, eIF2 hydrolyzes its bound GTP with the help of a GTPase activating protein, eIF5. The hydrolyzed phosphate is not released but remains associated with the complex through the action of eIF1, which maintains the scanning complex in an open conformation (Algire et al., 2005; Das and Maitra, 2001; Maag et al., 2005). Recognition of the start codon triggers release of the phosphate and switches the 43S complex to a closed committed conformation (48S) that enables the joining of the 60S large ribosomal subunit to form the 80S initiation complex ready to enter the elongation phase.

Abbreviations: 5′ UTR, 5′ untranslated region; TISU, translation initiator of short 5′ UTR; TSS, transcription start site; GTF, general transcription factor; eIF, eukaryotic translation initiation factor; ORF, open reading frame; uAUG, upstream AUG; IRES, internal ribosomal entry site; DBTSS, data base of transcription start site.

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3. The context of the AUG start codon and leaky scanning

In general the 43S scanning ribosomal subunit arrests and initiates at the most 5′-proximal AUG codon. However sometimes it bypasses the first AUG and continues scanning the mRNA, initiating at a more downstream AUG, a phenomenon called leaky scanning (Kozak, 2002). An important determinant that influences the fidelity and efficiency of the translation initiation is the AUG context, a term referring to the sequence that flanks the AUG start codon. In vertebrate mRNAs, the best-characterized translation initiation context is RCCAUGG also known as the Kozak element. The most important nucleotides for the efficiency of translation initiation are the purine (R) in position −3 and G in position +4 relative to the A of the AUG codon which is designated +1 (Kozak, 1994, 1997). When both of these positions match the Kozak element, the AUG context is regarded as strong. When the sequence contains only one of them, the strength is somewhat reduced and when neither one is present the context is considered weak. A weak AUG context allows leaky scanning, whereas a strong one prevents it. The role of the −3 and the +4 positions is to stabilize the 48S following recognition of the start codon (Pisarev et al., 2006).

4. The influence of the 5′ UTR length and structure on cap-dependent translation

Translation initiation is highly sensitive to features found in the 5′ UTR, in particular the length of the 5′ UTR, the presence of stem–loop structures and the presence of AUG(s) upstream of the main ORF (uAUGs). Analysis of the 5′ UTR in various eukaryotic organisms, from yeast to man, shows that the 5′ UTR length is fairly constant, ranging between 100 and 200 nt (Moshonov et al., 2008; Pesole et al., 2001). A 5′ UTR length of at least 20 nucleotides is required for efficient recognition of an AUG with a strong context (Kozak, 1991a) which significantly increases with lengthening of the unstructured 5′ UTR, most likely because the longer 5′ UTR can associate with more 43S ribosomal subunits (Kozak, 1991a). Conversely, a 5′ UTR shorter than 20 nucleotides that precedes a strong AUG context, gives rise to a high proportion of leaky translation initiation (Kozak, 1991a, 1991b; Sedman et al., 1990). The most likely explanation for the 20 nucleotides minimal length is that when the P site of the 40S ribosomal subunit is situated on the AUG codon, the initiation complex forms contacts with 17 nucleotides upstream and 11 nucleotides downstream to the AUG (Pisarev et al., 2008).

The inhibitory effect of a stem–loop on initiation is dependent on its location within the 5′ UTR, its stability and its ability to bind specific regulatory proteins (Kozak, 2002). When a stable stem–loop structure is located near the 5′ cap, translation efficiency is severely reduced due to steric interference with assembly of the preinitiation complex. Located downstream in the 5′ UTR, a stem–loop can present a hurdle for the scanning 43S ribosome which may require unwinding by a ribosome-associated helicase. A weak stem–loop(s) downstream of the AUG may enhance translation fidelity (Elfakess et al., 2011; Kozak, 1990) as it causes 40S subunit pausing which provides sufficient time for the P site to be properly arranged over the AUG codon.

The prevalence of uAUGs in mRNAs is higher than one would expect, as it has been estimated that nearly 50% of all human and Drosophila mRNAs contains uAUG(s) in their 5′ UTR (Davuluri et al., 2000; Medenbach et al., 2011; Pesole et al., 2000; Rogozin et al., 2001; Suzuki et al., 2000). Considering the cap-dependent ribosome scanning mechanism, at least some of these uAUGs would be expected to inhibit translation from the major ORF, and indeed this has been demonstrated in several specific cases (see for example Hinnebusch, 1997; Medenbach et al., 2011; Meijer and Thomas, 2002; Morris and Geballe, 2000). With uAUG(s) in the 5′ UTR there are several ways by which the ribosome can reach the main AUG and these include leaky scanning, reinitiation, and ribosomal shunting (Kozak, 2002; Meijer and Thomas, 2002; Ryabova et al., 2002).

5. TISU — a translation initiator directing cap-dependent translation initiation without scanning

Using a bioinformatics approach to search for regulatory elements in the proximal promoter region, an over-represented element with the sequence SAASATGGCGGC (where S is C or G), called Translation Initiation of Short 5′ UTR (TISU) was identified (Elfakess and Dikstein, 2008). TISU is present in ~4.5% of mammalian protein encoding genes and is specifically enriched in TATA-less promoters. It has a strict location downstream of the transcription initiation site (TSS) from +5 up to +30 relative to the TSS (Fig. 1). Characterization of TISU as a transcriptional element revealed that it is essential for transcription and that its activity in transcription is mediated, at least in part, by the YY1 transcription factor (Elfakess and Dikstein, 2008).

The TSS downstream location of TISU along with the fact that its core sequence consists of an invariable ATG sequence, led us to examine whether it also has a role in translation. Indeed it was found that the ATG of TISU functions as the AUG start codon in 64% of the genes bearing it. In these genes the initiating AUG is preceded by an extremely short 5′ UTR with a median length of only 12 nucleotides. Detailed comparison of TISU to other strong AUG contexts, including the Kozak element (Table 1), established it as an element optimized to direct efficient translation initiation from mRNAs with an extremely short 5′ UTR (Elfakess and Dikstein, 2008; Elfakess et al., 2011). Besides the −3 A and the +4 G, the additional ATG flanking sequences, including positions −2 and −1 and the nucleotide sequence in positions +5 to +8 are unique to TISU and cooperate to direct accurate and efficient translation initiation from short 5′ UTR mRNAs. When the 5′ UTR length was reduced to the minimal functional size of 5 nucleotides translation initiation and ribosomal binding directed by TISU was unaffected suggesting a non-scanning mechanism of initiation. In contrast, shortening the 5′ UTR of strong initiators, such as the Kozak element, resulted in predominant leaky scanning, i.e. reduced translation initiation efficiency.

These characteristics of TISU raised the question of whether the translation mediated by TISU is cap-dependent. Using several assays, based on RNA transfection, we established that TISU-mediated translation is cap-dependent: analysis of translation efficiency of mRNA containing the unmethylated cap analog (ApppG) resulted in 90% reduction in translation efficiency; placing a secondary structure between the m7G cap and TISU diminished translation; analyzing the site of translation initiation when two TISU elements are placed in tandem showed that translation initiated exclusively from the TISU element adjacent to the m7G cap; finally TISU was found to be highly sensitive to amino acid starvation and rapamycin, conditions in which

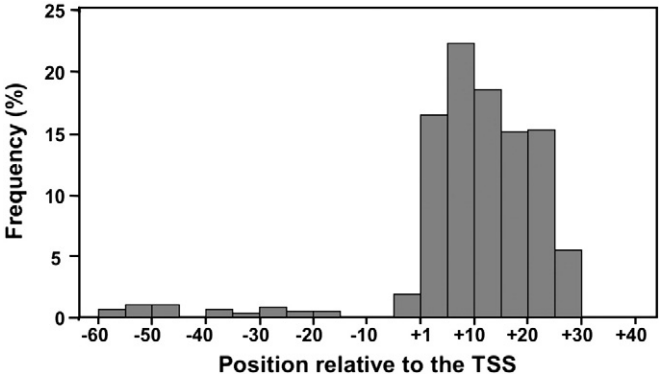

Fig. 1. The distribution of TISU at 5 nt intervals throughout the proximal promoter region (−60 to +40 relative to the TSS) as determined by the database of transcription start site (DBTSS).