Letter to the Editor

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Universal scanning-free initiation of eukaryote protein translation– a new normal

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Abstract: A unique feature of eukaryote initiation of protein translation is a so-called scanning of 5'-untranslated region (5'-UTR) by a ribosome initiation complex to enable bound Met-tRNA_i access to the initiation codon located further downstream. Here, we propose a universal scanning-free translation initiation model that is independent of 5'-UTR length and applicable to both 5'-m⁷G (capped) and uncapped mRNAs.

Keywords: 5'-untranslated region; eukaryote; ribosome translation initiation complex; ribosome scanning; translation initiation site.

A unique feature of eukaryote protein translation initiation process is the notion of “scanning” by an initiation complex (IC) comprising 40S ribosome (40S) containing Met-tRNA_i and initiation factors (eIF1-5), which “moves along” or “brings in” downstream 5'-untranslated region (5'-UTR) to allow interaction of Met-tRNA_i anticodon with the initiation codon (AUG) located beyond ribosome (R) IC initial location at the 5'-m⁷G (capped) mRNA terminus [1]. This process arises owing to most eukaryote mRNAs having 5'-UTR lengths (~150 nt) that are longer than the width (50-70 nt) of the 40S. This canonical scanning property was first suggested in 1978 by Kozak and Shatkin [2] to explain their earlier observation of the protection from T₁ RNase digestion of reovirus mRNA regions downstream to 5'-cap that are contained within wheat germ 40S initiation complexes [3]. The key experiment was the presence in a sucrose density gradient of unusual polysome-like complexes composed of 40S when a wheat

germ cell-free translation system was treated with edeine, an antibiotic that blocks recognition of the downstream initiation AUG codon by 40S-associated Met-tRNA_i, allowing the 40S to move beyond this normal *stop* site [2]. However, in the absence of the antibiotic, poly-40S present on the 5'-UTR would not be discernible in the sucrose gradient.

Subsequent studies have employed electron microscopy to visualize 40S strung along the putative 5'-UTR (hardly visible in the micrographs) [4] and “ribosome foot printing”. The latter process involves treatment with formaldehyde (RNA-protein cross-linker) of polysomes isolated from human, yeast or zebra fish cells, and then followed by digestion with RNase, separation of individual ribosomes in a sucrose density gradient and final sequencing of 40S-associated mRNA to observe a preferred binding of 40S across the whole 5'-UTR [5-7]. However, owing to the few amounts of free 40S during active translation, it is questionable whether the data actually reflect a scanning process or artifacts generated during the preparation by random binding of free 40S to unoccupied 5'-UTR regions. In addition, this canonical scanning property is not always compulsory, e.g. for capped mRNAs containing short 5'-UTRs or uncapped viral mRNAs where 40S may slot into an internal ribosome entry site (IRES) [8]. Despite acceptance for decades of 40S scanning in eukaryote translation initiation, its actual mechanism remains unclear, as opposed to the universal necessity of a two-subunit intact ribosome for translocating mRNA [9].

We applied Occam's razor for interpreting these findings, and resulted with a universal model of eukaryote translation initiation that dispenses with the 40S scanning function altogether. In our proposed universal “new normal” model, RIC binds straight onto a region where the translation initiation site (TIS) is located, singled out by a flanking Kozak motif and other contiguous sequences [10]. The intervening 5'-UTR sequence (int5'-UTR) between 5'-m⁷G terminus and RIC proximal boundary comprises a secondary structure, which requires this region to remain outside RIC, but not interfering with mRNA-RIC association. This stage is pivotal in locating the 40S at its

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Figure 1: Diagram of (A) proposed new normal eukaryote scanning-less and (B) canonical scanning translation initiation model. A. 48S, human 48S translational initiation ribosome [9], is positioned at the cognate translation initiation site flanked by a Kozak (KOZAK) motif with Met-tRNA_i at the P site and the int-5'-UTR intervening between 5'-m⁷G (●) (bound to eukaryote initiation factor eIF4F complex) and proximal 48S boundary is located in the aqueous environment. B. 48S is tethered to 5'-m⁷G (●) and stochastic movement brings the cognate translation initiation site into 48S. The diagram is based on that depicted in [11]. Although the mRNA is depicted as a linear molecule, *in vivo* the 3' polyA terminus is attached to 5'-m⁷G terminus via interaction of a polyA binding protein with eIF4F [14].

cognate TIS (Figure 1A). This also recapitulates the role of upstream mRNA secondary structure in defining the location of IRES in uncapped viral mRNAs [8].

The proposed model is compatible with a recent 3 Å three-dimensional structure obtained by cryo-EM of human 48S translational initiation ribosome, which reveals a 5'-m⁷G mRNA-tethered eIF4F complex bound (via eIF3) to the 43S ribosome preinitiation complex [11]. Although the structure provides no clue how 40S scans 5'-UTR, the authors suggest stochastic shift of 48S assisted by ATPase activity of eIF4A located at the mRNA exit channel that provides a backstop enabling a 5' to 3' unidirectional movement to draw downstream TIS into the 40S interior. Although the three-dimensional 48S structure cannot reveal the presence of RNA, it is reasonable to surmise that if 48S remains tethered to 5'-m⁷G terminal (through binding with eIF4E) 5'-UTR emerging from the channel exit would be deposited in the external aqueous environment without perturbing the existing tethered

organization (Figure 1B): The new normal model simply leapfrogs this process. In addition, cryo-EM reveals an “open” 48S structure that does not involve initiation codon-anticodon base pairing, interpreted likely as that of a scanning 48S and a “closed” structure where Met-tRNA_i is correctly positioned in the P site as has been already described [1, 8]: Our near normal model interprets the “open” structure as that of 48S with mRNA first located in 40S channel but not correctly positioned to allow correct codon-anticodon interaction at the P site, but next stochastic movement of 48S (of short distances along the inserted mRNA) eventually results in the “closed” state. Binding of 60S completes the translation initiation step and protein translation then proceeds.

For re-initiation, RIC just slots into the vacant TIS region, a minimalist mechanism obviating an alternative mechanism proposed before, which requires re-threading of mRNA through a 40S entry channel at every round of re-initiation [12]. Stability of int5'-UTR secondary

structure plays a crucial role as this will affect alacrity of translation initiation and the extent of “leaky” translation at improper TISs. Factors with helicase activity, such as yeast Ded1 (see references in [8]), formerly assigned to facilitate scanning, could instead be involved in removing mRNA secondary structures impeding slotting of RIC into TIS region.

Hayek H, et al. [13] supported our notion by using a combination of cross-linking and ribonucleoprotein immunoprecipitation in their *in vivo* and *in vitro* experiments. The authors demonstrated that eIF3 binds to a stem-loop structure located within the coding region of H4 histone mRNA, which is a direct tethering of RIC to a specific H4 mRNA structure, facilitating re-initiation of H4 mRNA translation during the short period of the cell cycle S phase. Moreover, the authors observed binding of eIF3 to H1, H2A, H2B and H3 histone mRNAs but at distinct mRNA sites depending on histone type (not unexpected as a different stem-loop structure would be expected for each unique mRNA). Although this type of interaction has been so far shown for histone mRNAs (which contain short 5'-UTRs), similar experiments should be conducted using other mRNA species (with longer 5'-UTRs where scanning is expected to occur) to demonstrate an “exception that proves the rule”.

In conclusion, the universal new normal model of scanning-free translation initiation proposes positioning of RIC directly at mRNA TIS, followed by attachment of 60S and subsequent protein biosynthesis, thus relegating translocation along an mRNA only to the universal intact two-subunit ribosome.

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