TRANSLATION (III): INITIATION AND TRANSLATION CONTROL

MAJOR POINTS

1. In prokaryotes, mRNAs that encode a product whose primary function is to bind RNA are often autogenously regulated. When the primary target is saturated, the protein shuts off translation of its secondary target, its own mRNA.

2. Eukaryotic ribosomes lack the SD sequence and are (generally) constrained to enter the mRNA at the capped 5' end. The 40S ribosome scans in an ATP-dependent search for the initiator codon. In 90% of mRNAs, this is the first (5'-proximal) AUG. Upon termination, ribosomes generally dissociate; in some cases, they can continue scanning and re-initiate at a downstream AUG.

3. Global control of translation is frequently exerted by regulating the phosphorylation or availability of initiation factors. Two of the most well-known examples are the regulation of eukaryotic initiation factor (eIF) 4E availability by 4E-binding proteins (4E-BPs), and the modulation of the levels of active ternary complex by eIF2 phosphorylation. Modulation of translation rates accompanies major biological processes including proliferation, differentiation, and development. A key initiation factor in regulation of translation is eIF4E. Phosphorylation of this factor leads to an increase in translation.

4. Certain viruses inactivate host translation factors (like eIF-4G) and use cap-independent Internal Ribosome Binding Sites (IRES). Several cellular mRNA also use IRESes. Selective activation of IRES-mediated translation can allow protein synthesis during M phase of the cell cycle (where overall translation drops 25%). eIF4G is also cleaved during programmed cell death, few proteins, important for the control of cell death, are translated by IRES-dependent mechanism.

5. The poly (A) binding protein (PABP1) interacts with eIF4G and increases the affinity of the eIF4E for the cap. PABP1 also stimulates 60S joining step and protects the mRNA from degradation. Interaction of PABP1 and the eIF4G effectively circularizes the mRNA. Circularization of the mRNA helps to explain how regulatory factors that bind within the 3'UTR control initiation of
translation at the 5’end. However, in certain cases regulatory factors bind at the 5’UTR (IRE/IRP1).

6. mRNA-specific translational control is driven by RNA sequences and/or structures that are commonly located in the 5’ and 3’ untranslated regions of the transcript. These features are usually recognized by regulatory proteins or micro RNAs (miRNAs).

7. Quasi-circularization of mRNAs can be mediated by the cap structure and the poly (A) tail via the eIF4E–eIF4G–polyA-binding-protein (PABP) interaction. Such interactions between the 5’ and the 3’ ends of mRNAs could provide a spatial framework for the action of regulatory factors that bind to the 3’ untranslated region (UTR). However, other forms of 5’–3’-end interactions are likely to occur as well.

8. Many regulatory proteins target the stable association of the small ribosomal subunit with the mRNA. These factors function by steric hindrance (for example, iron-regulatory protein; IRP), by interfering with the eIF4F complex (for example, Maskin, Bicoid, Cup) or by as-yet-unknown, distinct mechanisms to control translation initiation (sex-lethal; SXL).
The initiator codon AUG is also used to specify methionine internally. The initiator methionyl tRNA is specialized to interact with an Initiation Factor (IF2) instead of EFTu and to bind to the P site instead of the A site. (Graffe et al., 1992; Hui & de Boer, 1987; Simonetti et al., 2008; Varshney, Lee, & RajBhandary, 1993)

The initiator codon is constrained to lie a short distance downstream of a polypurine-rich sequence which base-pairs with the 3' end of 16S rRNA (the Shine-Dalgarno (SD) interaction). Internal initiation sites can be used as independent ribosome entry sites (if they are not occluded).
mRNAs that encode a product whose primary function is to bind RNA are often autogenously regulated. When the primary target is saturated, the protein shuts off translation of its secondary target, its own mRNA.

The correct messenger RNA start site and the reading frame are selected when, with the help of initiation factors IF1, IF2 and IF3, the fMet-tRNAfMet is deliver into the P-site. IF1 binds to A site. IF3 bindsto the E site and prevent 50s joint. IF2-fMet-tRNAfMet-GTP deliver the tRNA ot the P-site. GTP hydrolysis triggered 50S joining. Importantly, GTP-binding domain of IF2 directly faces the GTPase-activated centre of the 50S subunit. (Simonetti et al., 2008)
Initiation in eukaryotes

<table>
<thead>
<tr>
<th>Eukaryotic Factor</th>
<th>Prokaryotic Factor</th>
<th>Archaeal Factor</th>
<th>Function</th>
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</thead>
<tbody>
<tr>
<td>elf1</td>
<td>IF3*</td>
<td>a-elf1</td>
<td>Fidelity of AUG codon recognition</td>
</tr>
<tr>
<td>elf1A</td>
<td>IF1</td>
<td>a-elf1A</td>
<td>Facilitate Met-tRNA&lt;sup&gt;Met&lt;/sup&gt; binding to small subunit</td>
</tr>
<tr>
<td>elf2</td>
<td>IF1</td>
<td>a-elf2</td>
<td>Bind Met-tRNA&lt;sup&gt;Met&lt;/sup&gt; to 40S subunit; GTPase</td>
</tr>
<tr>
<td>elf2B</td>
<td></td>
<td></td>
<td>Guanine-nucleotide exchange factor for elf2</td>
</tr>
<tr>
<td>elf3</td>
<td></td>
<td>a-elf4A</td>
<td>Promote Met-tRNA&lt;sup&gt;Met&lt;/sup&gt; and mRNA binding to 40S subunit</td>
</tr>
<tr>
<td>elf4A</td>
<td></td>
<td></td>
<td>DEAD-box helicase</td>
</tr>
<tr>
<td>elf4B</td>
<td></td>
<td></td>
<td>Promote elf4A activity</td>
</tr>
<tr>
<td>elf4E</td>
<td></td>
<td></td>
<td>m&lt;sup&gt;7&lt;/sup&gt;GppX cap binding protein</td>
</tr>
<tr>
<td>elf4F</td>
<td></td>
<td></td>
<td>Cap binding complex of elf1 4A, 4E, and 4G</td>
</tr>
<tr>
<td>elf4G</td>
<td></td>
<td></td>
<td>Adaptor protein interacts with many other factors</td>
</tr>
<tr>
<td>elf4H</td>
<td></td>
<td></td>
<td>Similar to elf4B</td>
</tr>
<tr>
<td>elf5</td>
<td>IF2</td>
<td>a-elf5B</td>
<td>AUG recognition and promote elf2 GTPase activity</td>
</tr>
</tbody>
</table>

*The proposed grouping of elf1 and IF3 is based on their common function to insure accurate Met-tRNA<sup>Met</sup> and AUG codon selection, and structural similarity of elf1 (Fletcher et al., 1999) to the C-terminal domain of IF3 (Biou et al., 1995).
Cis-acting elements involved in controlling initiation of translation

(Hinnebusch et al., 2016)

Close loop model and two point of regulation: $m^7$G (cap) recognition and initiator met tRNA delivery by eIF2. 4E-BP is selectively phosphorylated by mTOR (Dever, 2002; Pause et al., 1994)
How does the 40s recognize start codon? Initiator tRNA and the start codon of mRNA should be positioned in the ribosomal P site. In eukaryotes, factors, eIF1 and eIF1A are essential in the process, they facilitate tRNA binding, allows scanning of mRNA, and maintains fidelity of start codon recognition. eIF1 and eIF1A promote an open, scanning-competent preinitiation complex that closes upon start codon recognition and eIF1 release to stabilize ternary complex binding and clamp down on mRNA. (Cigan, Feng, & Donahue, 1988; Jaramillo, Dever, Merrick, & Sonenberg, 1991; Konarska, Filipowicz, Domdey, & Gross, 1981; Kozak, 1986; Passmore et al., 2007; Paterson & Rosenberg, 1979; Pause & Sonenberg, 1992; Pestova, Borukhov, & Hellen, 1998)

**Concerted action of polyA and Cap:** selection of the first AUG

Cap and polyA are signatures of intact mRNA. mRNAs emerging from the nucleus. Monitoring those elements ensures that the translation machinery will engage with the correct messengers.

In addition, the formation of a “closed loop” structure facilitate that, upon scanning, the first AUG is used to initiate translation.

(Preiss & Hentze, 1998)
Cap and polyA binding proteins: affinity for the 5’cap is modulated the the formation of complex involving the mRNA, 4E, 4G and PABP.

Affinity was used to purify mRNA forming closed loop structures. RIP-seq data reveal that mRNAs that associate highly with the closed loop complex have short open reading frames and are highly translated. (Pyronnet et al., 1999; Thompson & Gilbert, 2017)

Translational enhancement and regulation via the closed loop.
Closed loop mRNAs are translated at higher level than linear mRNAs due to higher de novo initiation rates intrapolyosomal ribosome recycling. The mutually reinforcing network of interactions between the cap, eIF4E, eIF4G, and PABP on closed loop mRNAs decreases the dissociation rate of the complex. eIF4E binding to closed loop mRNAs is stabilized by protein–protein interactions. Closed loop mRNAs may benefit from reduced competition for limiting translation factors. c Under repressing conditions, 4E-BPs disrupt translation. Sub-saturating levels of 4E-BPs repress translation of linear mRNAs, but do not affect closed loop mRNAs. (Thompson & Gilbert, 2017)
Global regulation by eIF2a phosphorylation

eIF2 is an essential factor for protein synthesis that forms a ternary complex (TC) with GTP and the initiator Met-tRNA\textsubscript{Met}. eIF2 activity is regulated by a mechanism involving both guanine nucleotide exchange and phosphorylation. Phosphorylation takes place at the α-subunit, which is a target for a number of serine kinases that phosphorylate serine 51. Those kinases act as a result of stress such as amino acid deprivation (GCN2), ER stress (PERK), the presence of dsRNA (PKR) heme deficiency (HRI), or interferon. Once phosphorylated, eIF2 shows increased affinity for its Guanine nucleotide exchange factor eIF2B. However, eIF2B is able to exchange GDP for GTP only if eIF2 is in its unphosphorylated state. Phosphorylated eIF2, however, due to its stronger. (Sonenberg & Hinnebusch, 2009)

(Dever, 2002)
Global translation downregulation and the activation of some mRNAs

High levels of active eIF2 causes initiation at upstream reading frames (uORF) (green, red, purple, bleu boxes), this lead to ribosome encountering stop codons (at the end of those short uORF) and the ribosome never reach the authentic AUG. The AUG context in uORF is different than GCN4 ORF (lower dependency of eIF2).

(Hinnebusch, 2005)

Interferon regulating gene IRF7

4E-BP1 -/- 4E-BP2 -/- double knockout mouse embryonic fibroblasts result in upregulation of interferon regulatory factor 7 (Irf7) messenger RNA translation.

4E-BPs are negative regulators of type-I IFN production, via translational repression of Irf7 mRNA.

A safety measure to prevent too much INF response…which is not good for you…!

(Colina et al., 2008)
Global regulation by eIF2α phosphorylation

Translation regulation by iron: cytoplasmicaconitase

Aconitases are iron-sulfur proteins that require a 4Fe-4S cluster for their enzymatic activity, in which they catalyze conversion of citrate to isocitrate. Iron regulates RNA binding affinity to two distinct elements. This, in turn, regulates translation or stability of specific mRNAs.

(Muckenthaler, Gray, & Hentze, 1998; Paraskeva, Gray, Schläger, Wehr, & Hentze, 1999)

Small RNA 5' UTR modifications controlling translation in specific cell types: m6As

(Meyer et al., 2015)
Large structures at the 5’ end controlling translation during stress: Internal ribosomal entry sites (IRESs)

All initiation factor required, but eIF4F

Only needs eIF3

No scanning (like in bacteria)

Does not uses eIF2

(Mailliot & Martin, 2017)
Regulation by factors interacting with the 3’UTR of the mRNA

(Sonenberg & Hinnebusch, 2009)
Paip 2 is a PABP-interacting protein, which disrupts the PABP-poly(A) interaction and consequently inhibits translation, reducing translation of a reporter mRNA by 80%. Ectopic overexpression of Paip2 affects cell growth. Paip2 activity is regulated by phosphorylation.

Untether factors and factors interacting with the 3′UTR of the mRNA: Competitor inhibitors of eIF4E

eIF3–eIF4G–eIF4E interaction. Initiation is disrupted by 4E-BP, which binds and sequesters eIF4E by interacting with eIF4G; this process occurs on a number of mRNAs because 4E-BP is not tethered to any particular sequence. Two examples of tethered 4E-BPs are represented by Maskin (Xenopus) and Cup (Drosophila). Through its association with CPEB, Maskin interacts with the eIF4E only on RNAs that contain a Bruno response element (BRE).

(Andreev et al., 2017; Gebauer & Hentze, 2004; Preiss, Muckenthaler, & Hentze, 1998)
Nanos mRNA distribution

(Mohr & Richter, 2001; Niessing, 2013; Xing & Bassell, 2012)