Determinants of “polysome size”

- Size of the ORF
- Rate of ribosome loading

Conclusion of transcriptome-wide analysis:
Every transcript is unique

Lecture 2

Cis-acting Elements and Transcript-Specific Translational Control
Lecture 2

- Regulation through uORFs
  Role of eIF2α kinases

- Regulation through 2° structure in 5’UTR
  Role of eIF4E
  4E-BPs; signal transduction pathways

- Translational repressors
  Ferritin regulation – IRP
  miRNAs – lin4 and let7
  Tethered cap-binding protein – bicoid

mRNA cis Elements Located in Non-Protein-Coding Regions
Characteristics of Translational Control by uORFs

1. Block to downstream translation
2. Regulated release from blockade
   - Regulated ribosome stalling
   - Interaction between uORFs

Interaction between uORFs is mediated by the level of active eIF2

Rate of recharging with tRNA\text{met} is slowed
Multiple eIF2α Kinases in Eukaryotes

- **GCN2** (nutrient starvation, UV irradiation)
- **PKR** (double-stranded RNA)
- **PERK** (ER stress, unfolded protein response)
- **HRI** (heme, reticulocytes)

Regulation of transcription factor ATF4 by eIF2α kinases in mammalian cells

[Diagram showing the regulation of ATF4 by eIF2α kinases under conditions of ER stress and nutrient deprivation.]

Harding and Ron
CONCLUSION

Regulation of protein synthesis by eIF2α kinases is global, but can be made gene-specific through the appropriate arrangements of uORFs

GENERALITY
Interplay between structural characteristics of an mRNA (e.g. uORFs) and the activity of a general initiation factor (e.g. eIF2α kinases) can convert global to gene-specific regulation

ANOTHER EXAMPLE:
Secondary structure in the 5’ leader inhibits scanning of the preinitiation complex to the initiator AUG
Role of eIF4E in Translation Initiation

- Cap-binding
- Recruitment of eIF4G
- Limiting component
- Overexpression is oncogenic
- Stimulates structured mRNAs
- Phosphorylated
- Regulatory binding proteins

eIF4E has inhibitory binding proteins that are phosphorylated through growth signaling pathways.

growth factors, hormones, mitogens, cytokines, GPCR agonists

PI3-K

PDK1/2

Akt

rapamycin→FRAP/mTOR

4E-BP1

ATP → ADP

4E-BP2

ATP → ADP

4E-BP3

ATP → ADP
MODEL: Level of active eIF4E regulates translation specifically of those transcripts with highly structured 5’ leaders.

PROBLEM: Where are the regulated genes? Only been shown with artificial transcripts.

Translational Repressor Molecules Bind to mRNAs at Either the 5’ or 3’ End

Depending on the mRNA, the repressor could be:
- A transcript-specific binding protein
- A complementary miRNA
A TRANSLATION REPRESSOR PROTEIN

Regulation of Ferritin Expression by IRP

Ferritin translation is turned on when cells are exposed to excess iron.

Mechanism: Blockade of cap

miRNAs Act Through Complementarity to Sequences in 3’UTRs

miRNA

cap  5’ Leader  ORF  3’ UTR  (A)_n
Regulation of Heterochronic Genes by miRNAs in *C. elegans*

Targets of *lin-4* and *let-7* are in the 3’ UTRs

Interaction of *lin-4* and *let-7* miRNAs with Their Target Sequences

- Ubiquity of miRNAs
- Protein complex involved (Argonaute family)
- How do they control translation?
How do Repressors that Bind to the 3’ UTR Inhibit Translation?

- Trigger assembly of mRNP particles
- Interfere with interactions at pA
- Tether inhibitory proteins

The *bicoid* gene is a key determinant in anterior patterning in *Drosophila* development

*Ephrussi & St. Johnston (2004)*
Bicoid has two mechanisms of action

The Caudal 3' UTR Contains a Bicoid Response Element
Bicoid inhibits caudal translation through interaction with an eIF4E homolog, d4EHP

**d4EHP IPs with Bcd**

**Mutating the 4EHP-Bcd interface**

Caudal protein

WT

Mutant

Cho et al. (2005)

Transcript-independent versus specific competition at the 5’ cap

**Transcript-independent inhibition by 4E-BPs**

**Transcript-specific inhibition by mRNA-bound bicoid**
Other 3’-tethered repressors are 4E-BPs that complete with eIF-4G

<table>
<thead>
<tr>
<th>Target mRNA</th>
<th>4E-BP</th>
<th>RNA-BP</th>
</tr>
</thead>
<tbody>
<tr>
<td>oskar</td>
<td>Cup</td>
<td>Bruno</td>
</tr>
<tr>
<td>nanos</td>
<td>Cup</td>
<td>Smaug</td>
</tr>
<tr>
<td>cyclin B1*</td>
<td>Maskin</td>
<td>CPEB</td>
</tr>
</tbody>
</table>

*Xenopus

Mechanisms of transcript-specific translational control

• Interplay between structural characteristics of an mRNA (uORFs, secondary structure) and the activity of a general initiation factor (e.g. eIF2, eIF4E) can convert global to gene-specific regulation

• Translational repressors (proteins, microRNAs) that bind to specific cis elements
mRNA cis Elements Located in Non-Protein-Coding Regions

- Repressor
- 5' Leader
- ORF
- 3' UTR
- (A)$_n$
- 2° structure
- uORF
- Localization