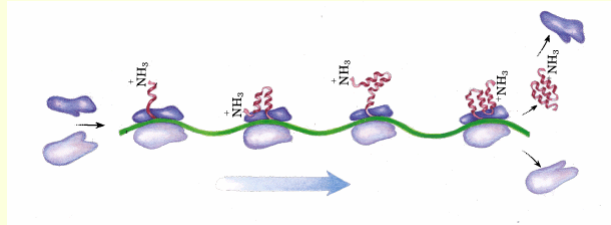


Determinants of “polysome size”

- *Size of the ORF*
- *Rate of ribosome loading*



**Conclusion of transcriptome-wide analysis:
Every transcript is unique**

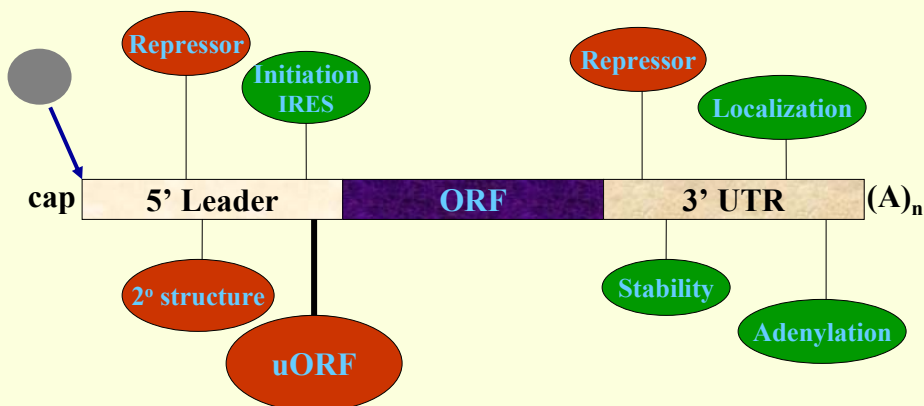
Lecture 2

Cis-acting Elements and Transcript-Specific Translational Control

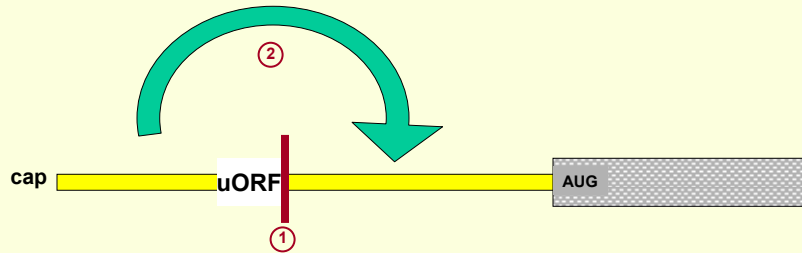
Lecture 2

- Regulation through uORFs
Role of eIF2 α kinases
- Regulation through 2 $^{\circ}$ 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

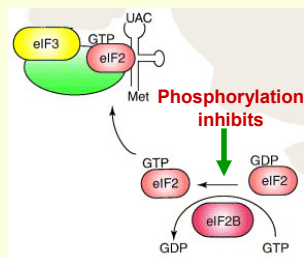


Characteristics of Translational Control by uORFs



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***

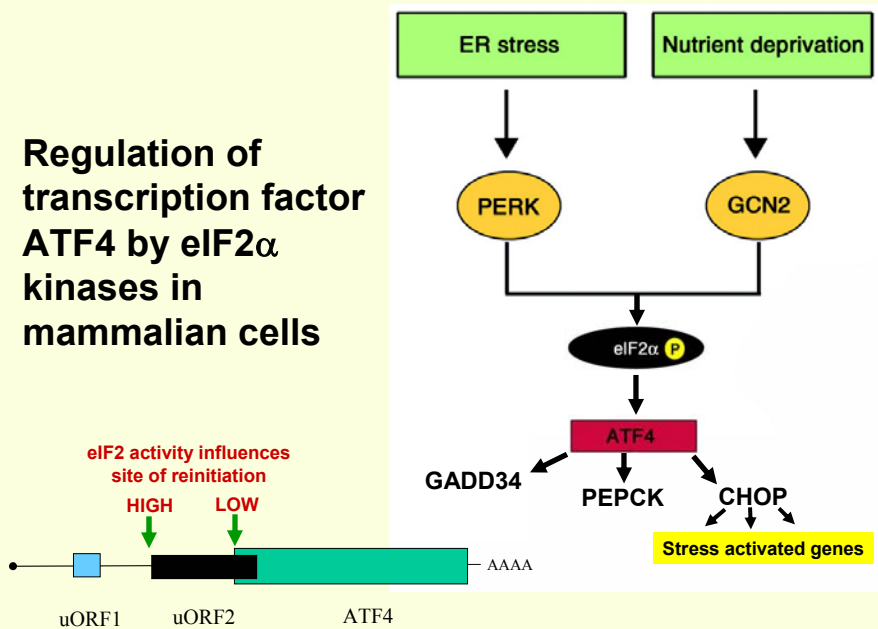


**Rate of recharging with
tRNA^{Met} is slowed**

Multiple eIF2 α 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 eIF2 α kinases in mammalian cells



Harding and Ron

CONCLUSION

Regulation of protein synthesis by eIF2 α 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. eIF2 α kinases) can convert global to gene-specific regulation

ANOTHER EXAMPLE:

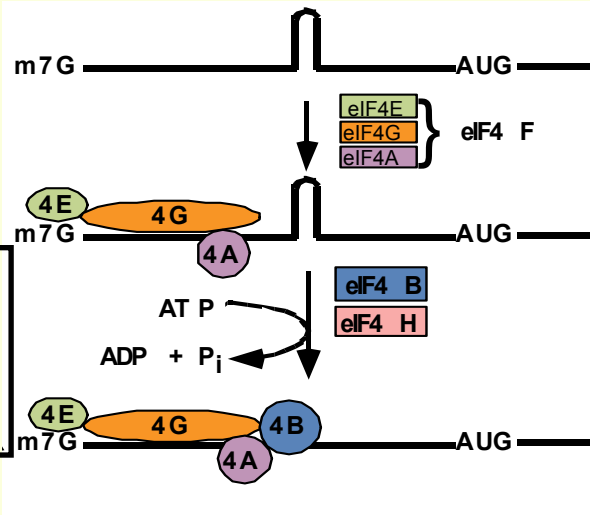
Secondary structure in the 5' leader inhibits scanning of the preinitiation complex to the initiator AUG



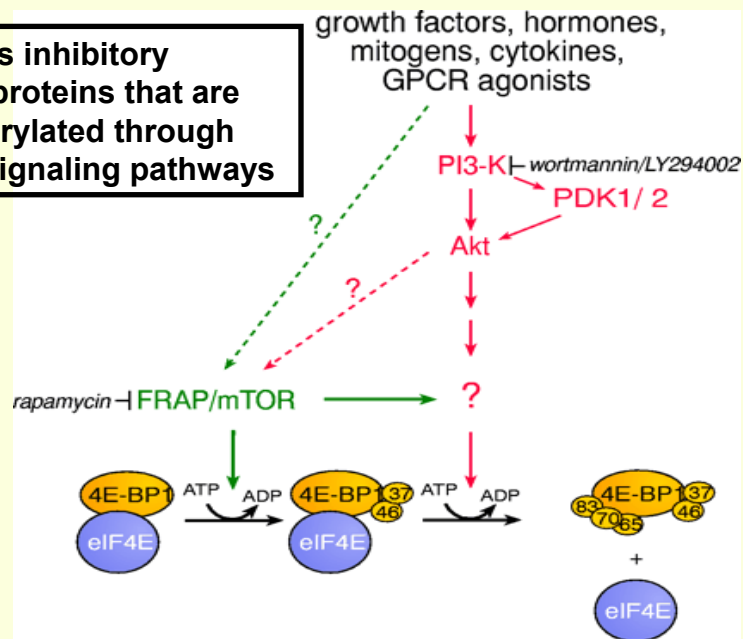
Role of eIF4E in Translation Initiation

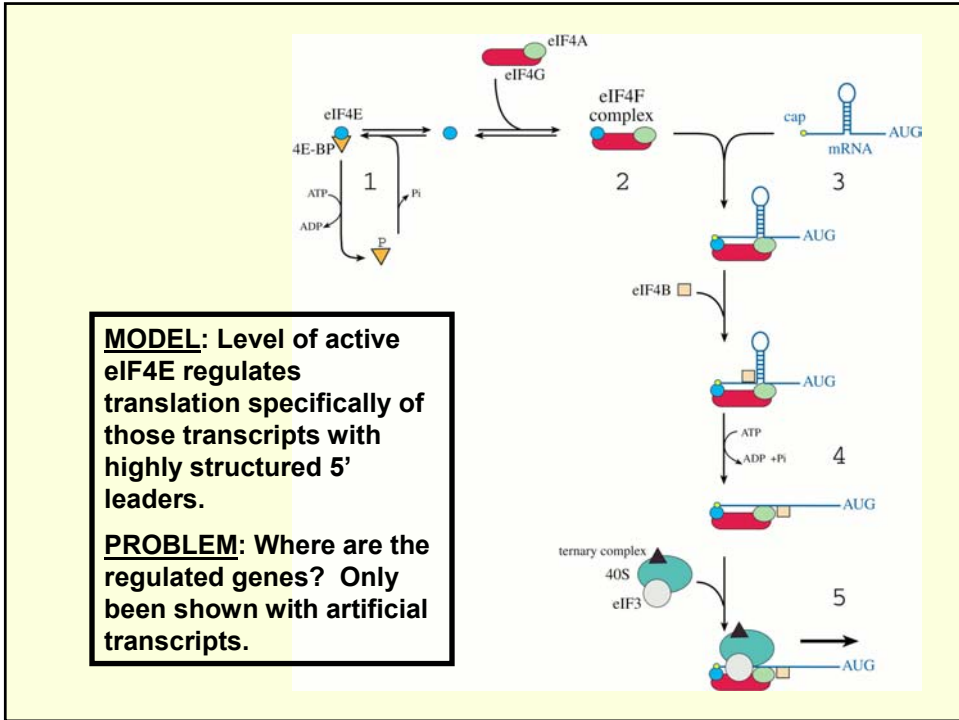
- cap-binding
- recruitment of eIF4G

- Limiting component
- Overexpression is oncogenic
- Stimulates structured mRNAs
- Phosphorylated
- Regulatory binding proteins



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





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



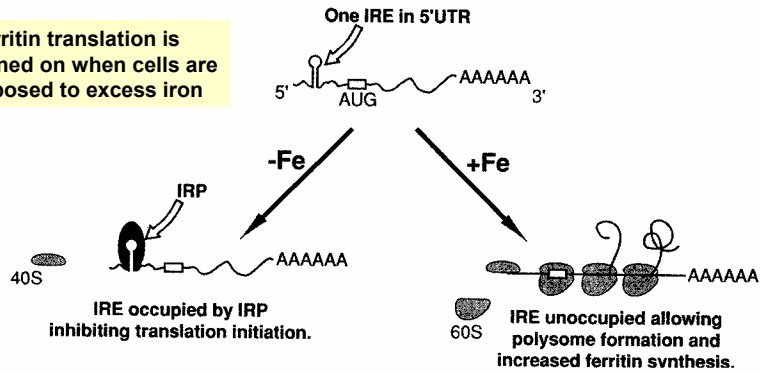
Depending on the mRNA, the repressor could be:

- 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

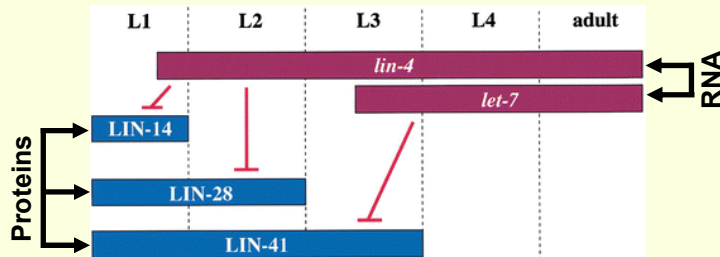


Mechanism: Blockade of cap

miRNAs Act Through Complementarity to Sequences in 3'UTRs

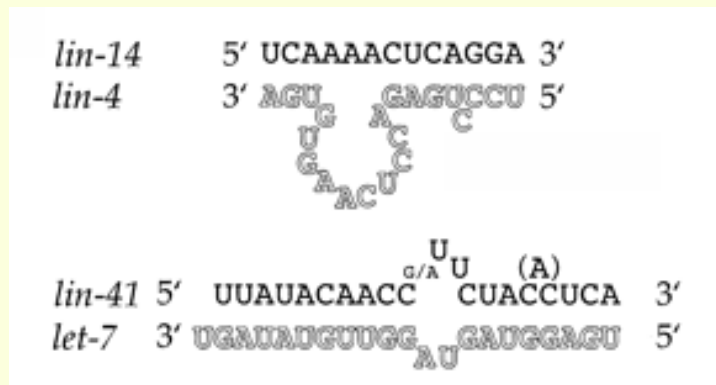


Regulation of Heterochronic Genes by miRNAs in *C. elegans*



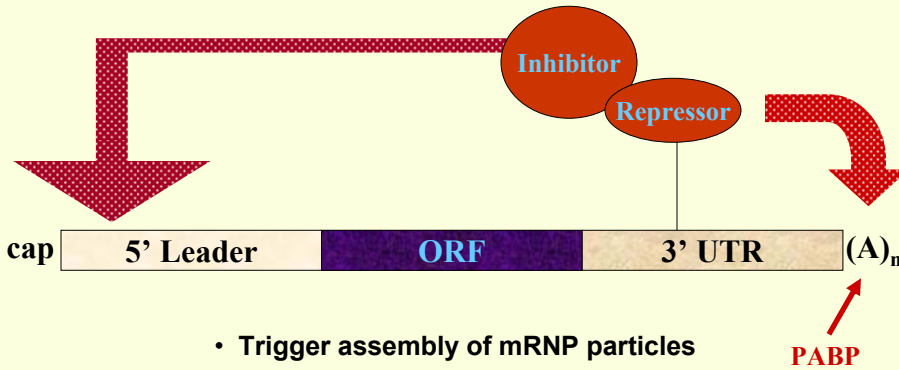
Targets of *lin-4* and *let-7*
are in the 3' UTRs

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



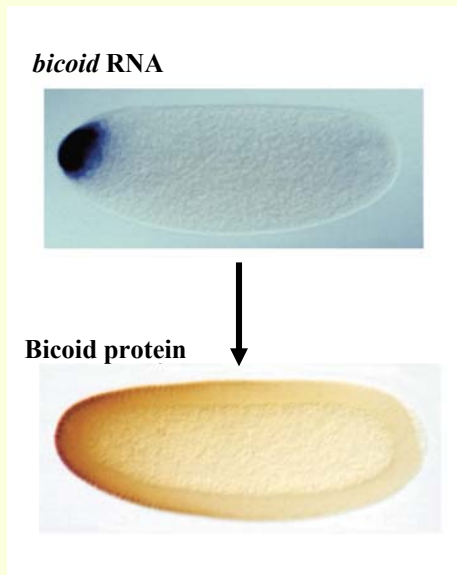
- Ubiquity of miRNAs
- Protein complex involved (Argonaute family)
- How do they control translation?

How do Repressors that Bind to the 3' UTR Inhibit Translation?

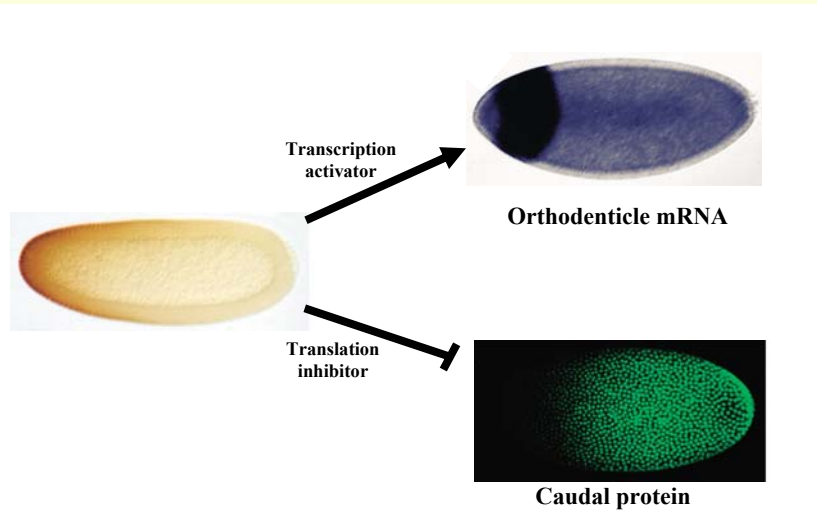


- Trigger assembly of mRNP particles
- Interfere with interactions at pA
- Tether inhibitory proteins

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

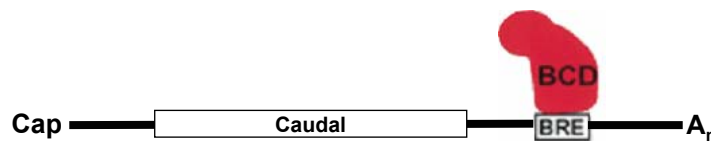


Bicoid has two mechanisms of action



Ephrussi & St. Johnston (2004)

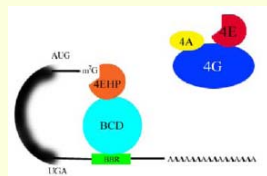
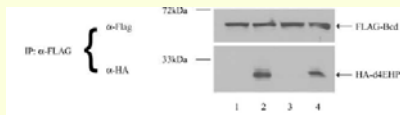
The Caudal 3' UTR Contains a Bicoid Response Element



Bicoid inhibits caudal translation through interaction with an eIF4E homolog, d4EHP

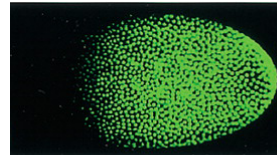
Mutating the 4EHP-Bcd interface

d4EHP IPs with Bcd

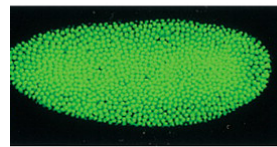


Caudal protein

WT



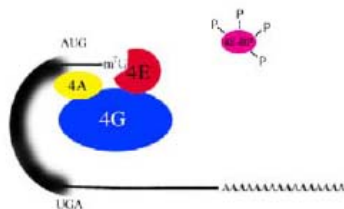
Mutant



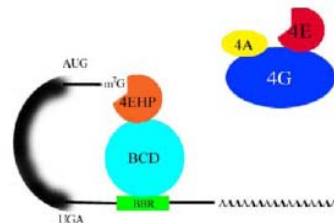
Cho et al. (2005)

Transcript-independent versus specific competition at the 5' cap

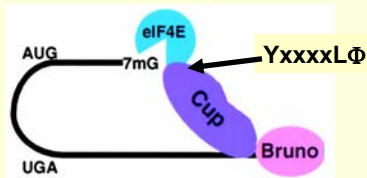
Transcript-independent inhibition by 4E-BPs



Transcript-specific inhibition by mRNA-bound bicoid



Other 3'-tethered repressors are 4E-BPs that compete with eIF-4G



Target mRNA	4E-BP	RNA-BP
<i>oskar</i>	Cup	Bruno
<i>nanos</i>	Cup	Smaug
cyclin B1*	Maskin	CPEB

*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

