Translational Initiation

Lecture Outline

1. Process of Initiation
2. Alternative mechanisms of Initiation
3. Key Experiments on Initiation
4. Regulation of Initiation

Translation is a process with three phases

1. Translational Initiation – delivery of ribosome and first tRNA to the start codon of the mRNA.

2. Translation Elongation – formation of peptide bonds to generate a growing polypeptide as the ribosome moves along the mRNA using tRNA's to decode the open reading frame.

3. Translation Termination – release of the newly synthesized polypeptide and disassociation of the ribosome mRNA complex.

Cellular Components required for Translation Initiation

- mRNA
- Ribosome
  - 40S subunit
  - 60S subunit
- $tRNA_i$
- 11 eIF's (eukaryotic initiation factors)
  - Transient protein factors that are required for initiation but are not part of the translation machinery during elongation or termination

Cheat Sheet for Initiation Factors

eIF2 – binds $tRNA_i$

eIF2b – activates eIF2

eIF1 and eIF1a – promote formation of stable 48S complex

eIF3 – stabilizes 40S subunit and interacts with eIF2 and eIF4

eIF4e – binds mRNA CAP

eIF4g – adaptor molecule that associates the 43S complex with proteins bound to mRNA (eIF4e, eIF4a, eIF3 and pab1p)

eIF4a – RNA helicase which unwinds secondary structure in mRNA

eIF4b – aides eIF4b

eIF5 – promotes hydrolysis of GTP bound to eIF2, necessary for eIF2 release from 48S complex

eIF5b – promotes recruitment of 60S subunit to 48S complex

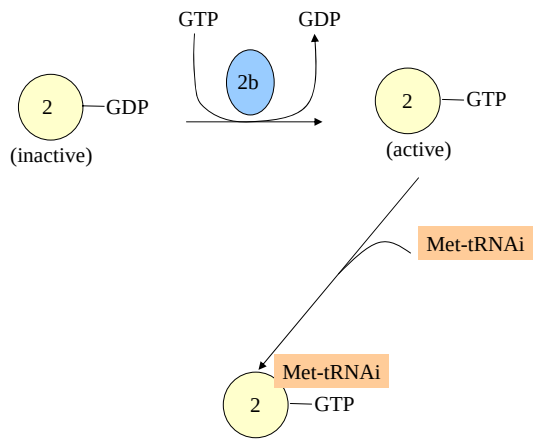
eIF6 – Stabilizes 60S complex

[Pab1p – polyA binding protein – interacts with eIF4g]

Translational Initiation – delivery of ribosome and first tRNA to the start codon of the mRNA.

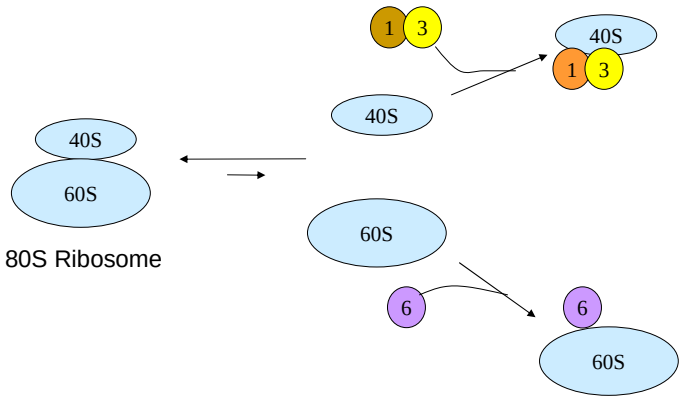
1. Assembly of ternary complex with initiator tRNA ($tRNA_i$)
2. Disassociation of 80S Ribosome
3. Assembly of 43S complex – association of ternary complex with small subunit of ribosome (40S)
4. Assembly of eIF4 CAP complex
5. Assembly of 48S complex by binding of 43S complex to CAP structure of mRNA
6. Scanning to Start Codon
7. Association of the large ribosomal subunit (60S)

Assembly of ternary complex with initiator tRNA ($tRNA_i$)

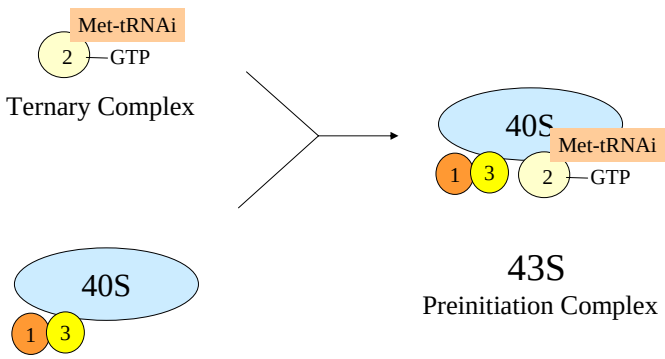


Ternary Complex

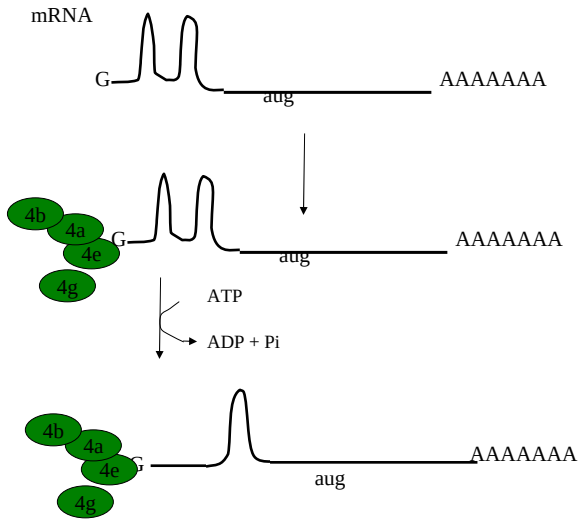
Disassociation of 80S Ribosome



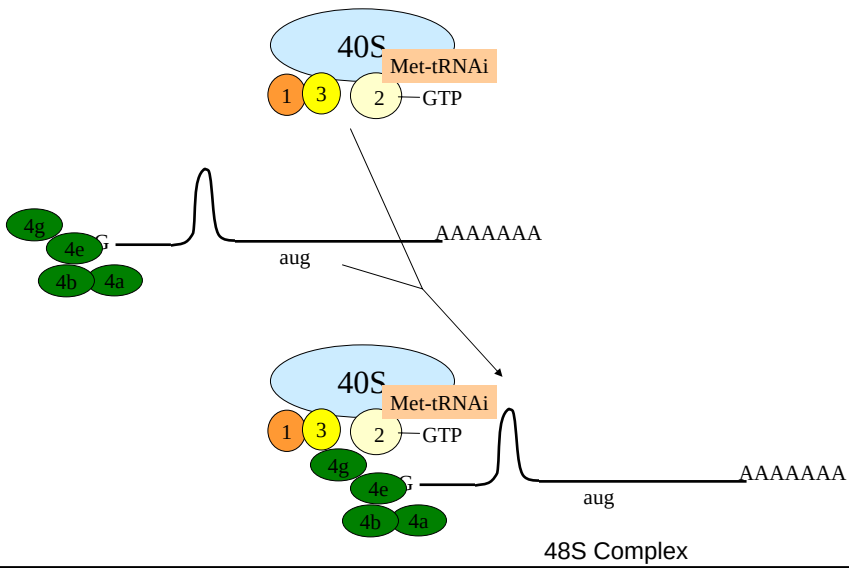
Assembly of 43S complex – association of ternary complex with small subunit of ribosome (40S)



Assembly of eIF4 CAP complex

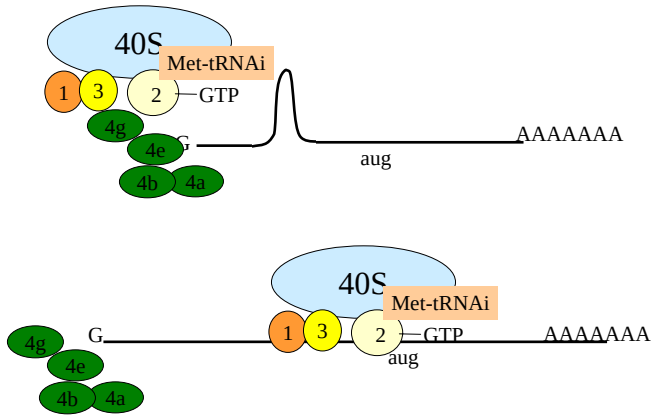


Assembly of 48S complex by binding of 43S complex to CAP structure of mRNA

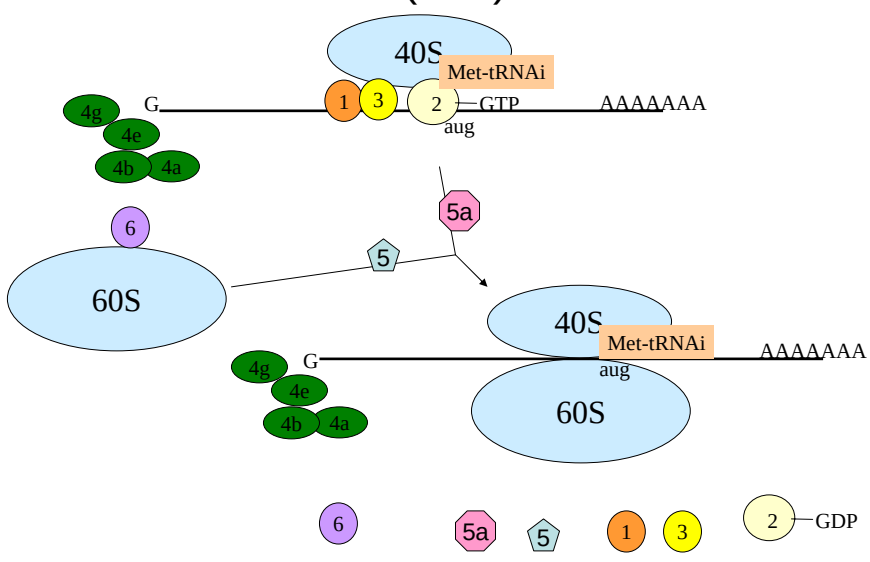


48S Complex

Scanning to Start Codon



Association of the large ribosomal subunit (60S)



Central Role of eIF4g

Hentz Science 1997

Key Experiments

- Kozak – Kozak's Rules
- Kozak – Testing rules
- Kozak - ribosome scanning
- Kozak – Early AUG's
- Kozak – effect of secondary structure
- Donahue – the role of $tRNA_i$ start codon identification
- Gallie – CAP PolyA tail Interaction

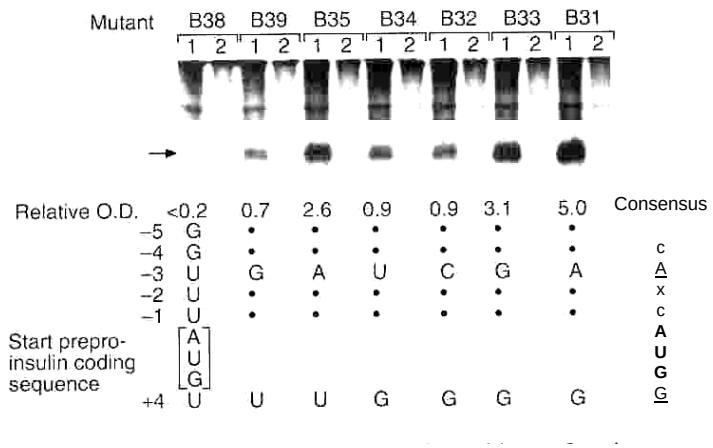
Kozak Rule

Observed that typically that first codon was used in most mRNA. However about 5% of the time the second codon was used. Question what determined which start codon was used. First she examined sequences around functional start codons to see if their surrounding nucleotides might influenced usage.

	Position on mRNA							
Nucleotide	-4	-3	-2	-1	1	2	3	4
A	20	80	30	25	100	0	0	25
G	10	13	10	15	0	0	100	60
C	60	5	35	60	0	0	0	2
U	10	2	25	10	0	100	0	10
Consensus	**c**	**A**	**x**	**c**	**A**	**U**	**G**	**G/a**

Kozak Nucleic Acid Research 1981

Testing the Rule

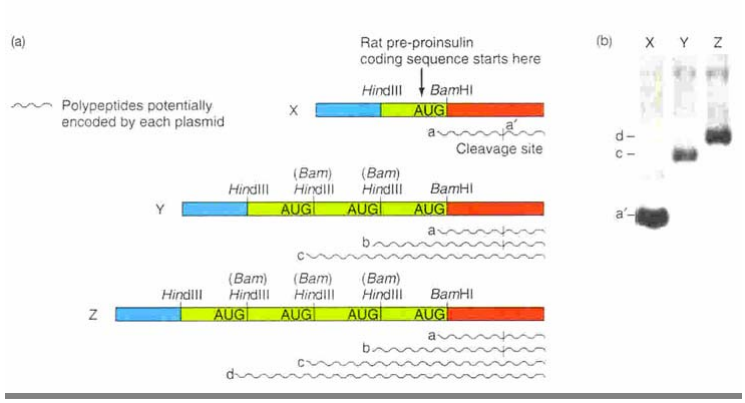


Kozak used mutant start codon for proinsulin gene to test importance of consensus sequence in start codon usage

Kozak Cell 1986

Ribosome Scanning

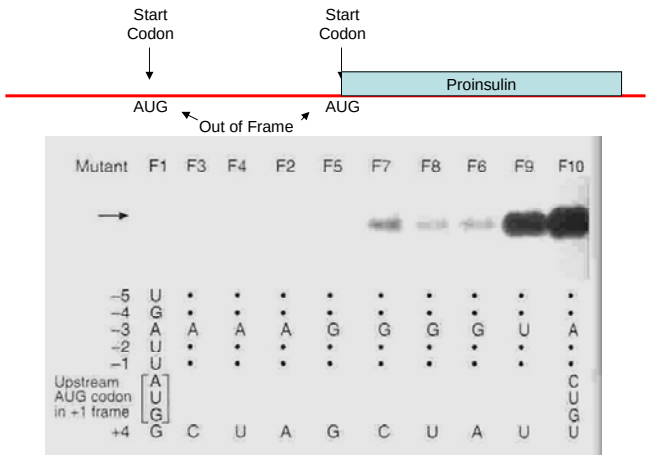
Does ribosome preferentially use first start codon?



Kozak constructed artificial versions of proinsulin gene with multiple start codons.

Kozak Cell 1983

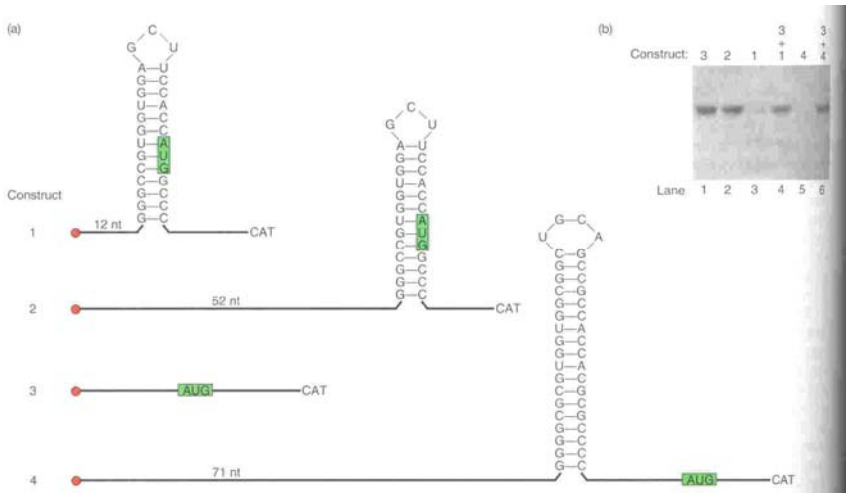
Early AUG's



Mutations in first start codon

Kozak Cell 1986

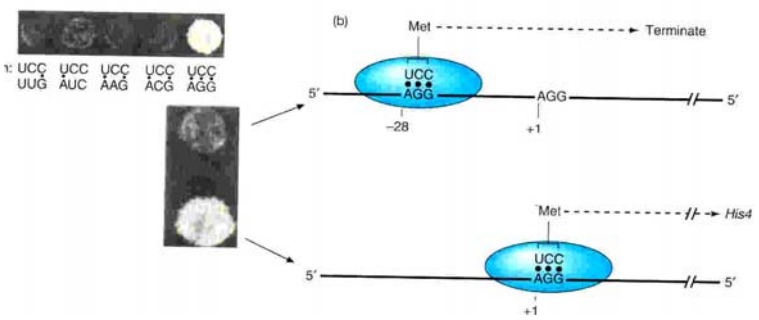
Effect of Secondary Structure



Kozak Mol Cell Biol 1989

Role of tRNAi

Thomas Donahue

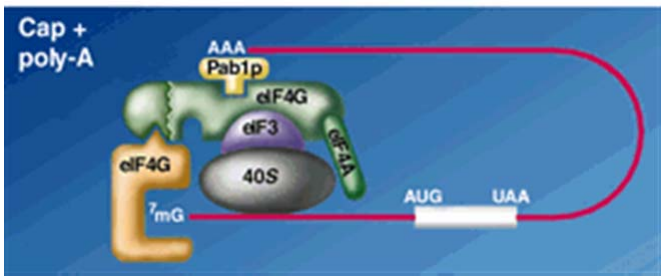


Cigan et al Science 1988

Role of polyA tail and initiation

Daniel Gallie

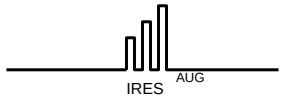
mRNA	Luciferase mRNA half-life (min)	Luciferase Activity (Protein)
Uncapped		
polyA-	31	2941
polyA+	44	4480
Capped		
polyA-	53	62595
polyA+	100	1331917



Alternative Modes of Initiation

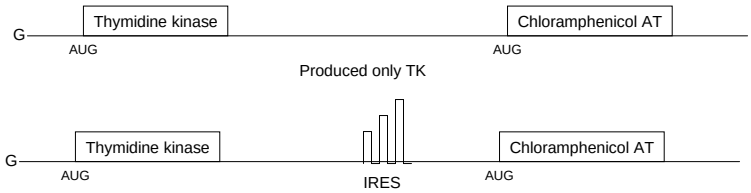
1. Internal Ribosome Entry Site (IRES)
2. Ribosomal Shunting
3. Re-initiation from microcistrons

Internal Ribosome Initiation Sites (IRES)



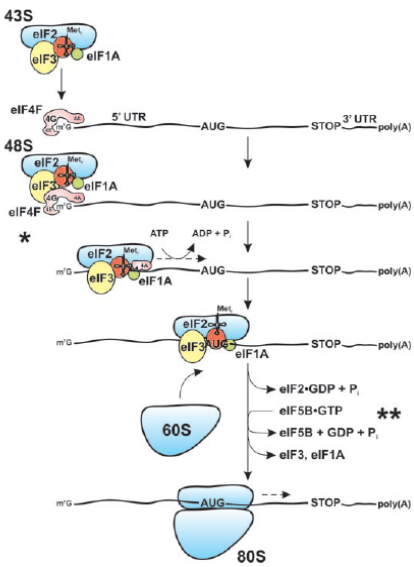
Many viral mRNA lack CAP but have region of elaborate secondary structure Before start codon – both seem to preclude CAP depedent scanning

Sonenberg dicistronic test

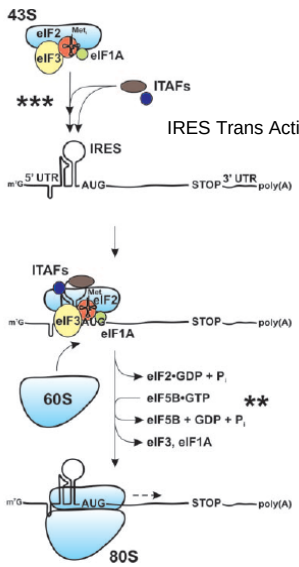


Pelletier and Sonenberg Nature 1988

CAP dependent Ribosome Scanning



Internal Ribosome Entry Site

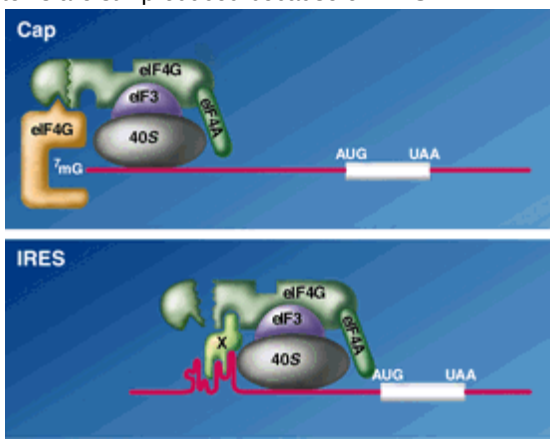


IRES Trans Acting Factors

Komar and Hatzoglou JBC 2005

Viral IRES

Some viruses interfere in host protein production by interrupting CAP dependent Initiation – the picornavirus has a protease that cleaves N terminal domain from eIF4G so that it can no longer interact with eIF4E. Host translation is reduced but the virus proteins are still produced because of IRES

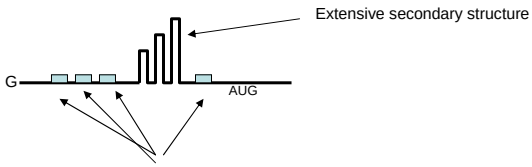


Hentz Science 1997

Cellular IRES

- IRES have been identified in cellular genes maybe as many as 5%.
- Many of these genes containing IRES are expressed during stressful conditions (e.g. heat shock)
- The cell represses CAP dependent initiation during stress conditions.
- mRNA with IRES are still expressed even during the stressful condition.

Ribosome Shunting

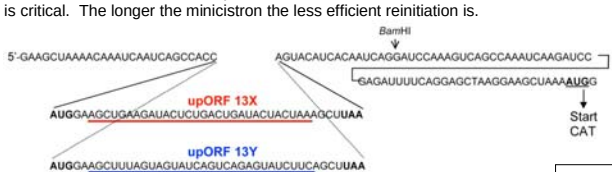


Mutliple sites with complementary sequence to 18S rRNA

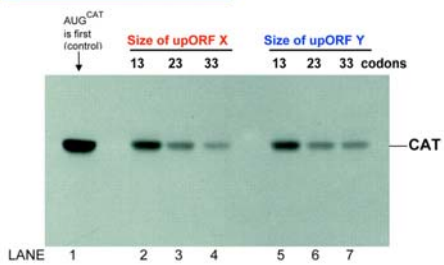
- Ribosome binds mRNA at CAP
- Ribosome scans to 18S sites
- Ribosome that bypasses or “shunts” regions of mRNA and re-engages downstream elements of mRNA.
- Believed to allow polycistronic mRNA in some viruses

Re-initiation from minicistrons

Some mRNA has short “mini-cistrons” proceeding the functional open reading frame. Ribosome apparently initiate translation on minicistron, terminate at stop codon and reinitiate at second start codon. The short size of the minicistron is critical. The longer the minicistron the less efficient reinitiation is.



How would you test influence of intergenic region between cistrons?

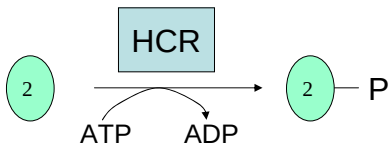


Kozak Nucleic Acid Research 2001

Regulation of Translation

- Global Regulation of Translation
  - Heme Regulation
  - Insulin Regulation
- Gene Specific Regulation of Translation
  - 5' UTR sequences and mRNA binding proteins
  - MicroRNA's (miRNA)

Global Regulation
Heme-Control Repressor

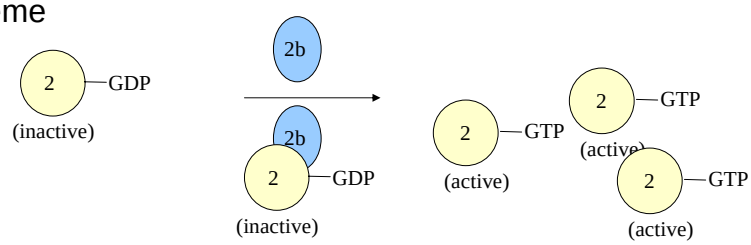


High Heme Conditions
Heme inhibits HCR protein
eIF2 is not phosphorylated
eIF2 functions as previously described

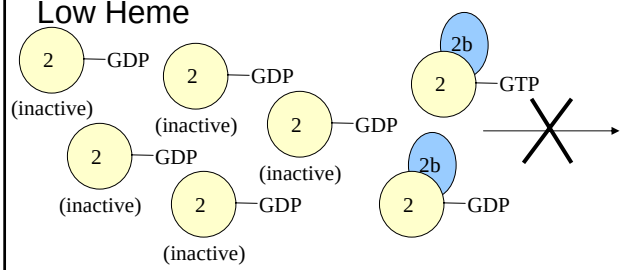
Low Heme
In absence of Heme HCR Protein become active kinase
HCR phosphorylates eIF2
Phosphorylated eIF2 has increase affinity for eIF2b which fails to release eIF2 after nucleotide exchange.
This effectively reduces pool of eIF2b available to reactivate eIF2

HCR results in depletion of pool of free eIF2b

High Heme



Low Heme

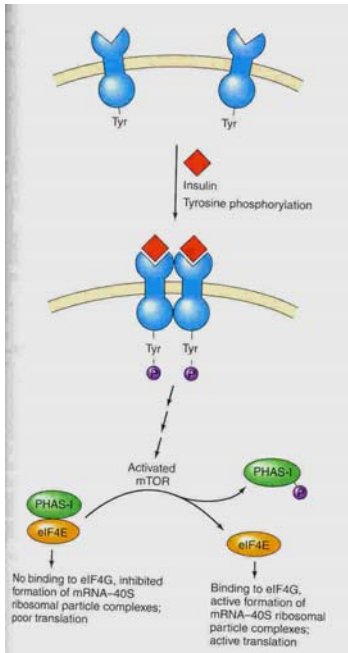


No Free Active eIF2-GTP

Global Regulation Translation Other eIF2b Kinases

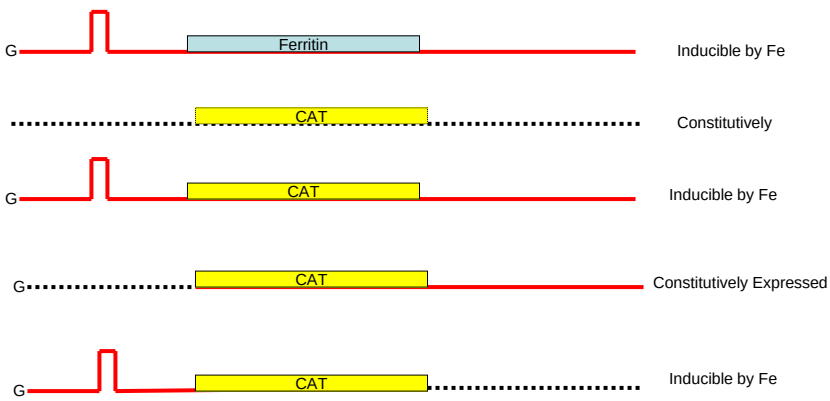
- Double Stranded RNA activated inhibitor of protein synthesis (DAI). Phosphorylates eIF2 in the presences of double stranded RNA. Protects cells from viral infection.
- GCN2 Kinase of yeast phosphorylates eIF2 under conditions of amino acid starvation.

Global Regulation Translation Insulin and Growth Factors



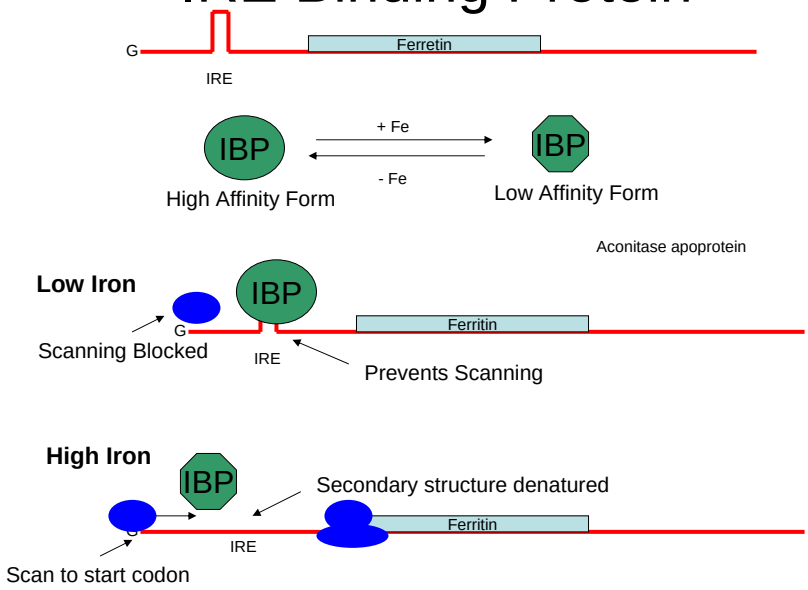
Gene Specific Regulation5' UTR sequences

Ferritin – An iron storage protein that is induced by high levels of iron. Ferritin mRNA is always present at low levels but is not translated when iron concentrations are low.

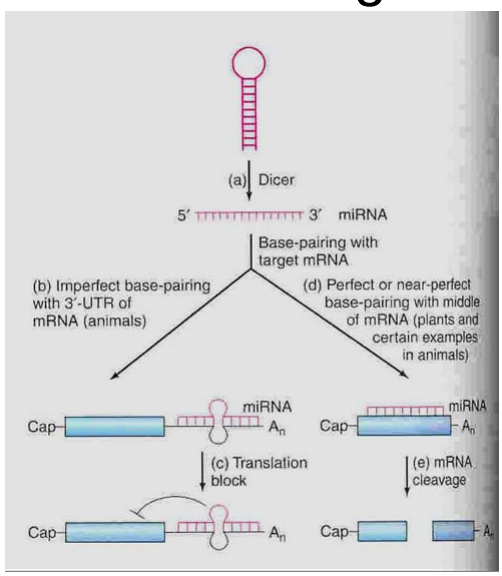


Aziz and Munro PNAS 1987

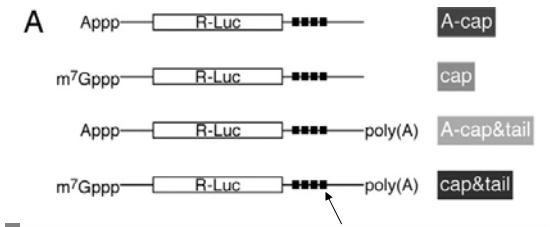
IRE Binding Protein

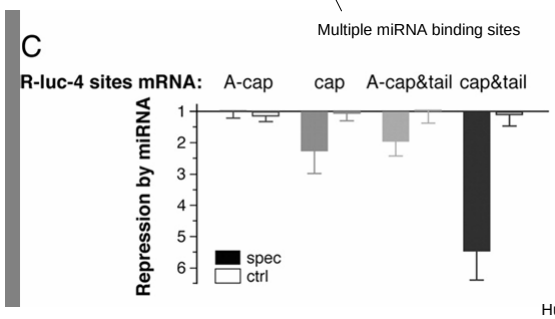


microRNA Regulation



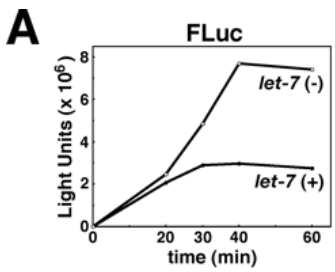
miRNA translation repression requires both a CAP and polyA tail

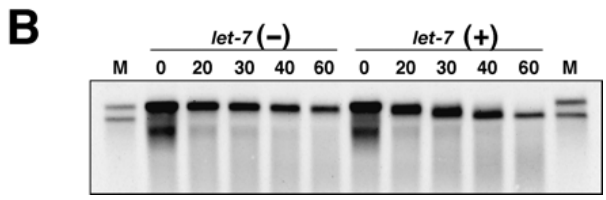




Humphreys et al PNAS 2005

miRNA and PolyA tail length





Wakiyama et al Genes and Dev 2007