Lost in Translation

Lecture 10
Biology W3310/4310
Virology
Spring 2016

Translation is that which transforms everything so that nothing changes.
—GÜNTER GRASS
• Most eukaryotic mRNAs except organelle mRNAs and certain viral mRNAs

• 5'-7-methylguanosine (m7G) joined to second nucleotide of mRNA by 5'-5' phosphodiester linkage

• Directs pre-mRNAs to processing and transport pathways, regulates mRNA turnover, required for efficient translation by 5'-end dependent mechanism
5′-untranslated region
- 3 – >1,000 nt in length, typically 50 – 70 nt
- Often contains RNA secondary structures; must be unwound to allow passage of ribosome
- Length and secondary structure influence translation efficiency

3′-untranslated region
- Can regulate translation initiation, translation efficiency, mRNA stability
- poly(A) tail, necessary for efficient translation
Translational machinery

- Initiation proteins (eIF)
- Elongation proteins (eEF)
- Termination proteins (eRF)
5’-end dependent initiation
5’-end dependent initiation

5’-end-dependent initiation

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Juxtaposition of mRNA ends

Pea enation mosaic virus
Barley yellow dwarf virus

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Which statement about the 5’-cap on mRNA is incorrect?

1. It consists of m7G joined to second nucleotide of mRNA by an unusual 5′-5′ phosphodiester linkage
2. It is present on most cellular mRNAs
3. It is required for efficient translation by 5′-end dependent initiation
4. It binds the cap-binding protein eIF4E
5. It is found on mRNA but not pre-mRNA
Other mechanisms for decoding have been discovered in virus-infected cells.
Ribosome shunting

-80 kcal/mole
Ribosome shunting

- 35s mRNAs of plant caulimoviruses
- Late adenovirus mRNAs
- P/C mRNA of Sendai virus

Shunting is predicted to decrease dependence of mRNAs for eIF4F during initiation by reducing the need for mRNA unwinding.
Internal initiation

Viral (+) strand genome

UTR

VPg

5'

AₙAₐ₉₃'

UTR

Translation, processing

Capsid

Proteases and RNA synthesis

P1

P2

P3

VP0

VP3

VP1

2A

2B

2C

VP4

VP2

VPg

2A_pro

3C_pro

3D_pol

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IRES = internal ribosome entry site
No IRES

AUG   UAA

No protein

IRES

AUG   UAA

Translation
A Type 1 IRES

B Type 2 IRES

C Type 3 IRES

D Type 4 IRES

eIF4G footprint

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5′-end-dependent initiation

all eIFs

Type 1 or 2 IRES

all eIFs except eIF4E

Hepatitis C virus IRES

eIF2, eIF3
What do ribosome shunting and internal ribosome initiation have in common?

1. Cap recruitment of 40S subunit
2. Both involve RNA secondary structures
3. Ribosome scanning through the entire 5’-UTR
4. Both require cap-binding protein eIF4E
5. All of the above
Aminoacyl
Peptidyl
Exit
Methionine-independent initiation

Cricket paralysis virus

- Can assemble 80S ribosomes without any eIFs or Met-tRNAi
- RNA mimics tRNAi
Methionine-independent initiation

Turnip yellow mosaic virus

- Can assemble 80S ribosomes without any eIFs or Met-tRNAi
- RNA mimics tRNAi
Eukaryotic mRNA (monocistronic)

UTR  Open reading frame  UTR

5' C  AUG  Stop  A_n A_O H^3'

Bacterial and archaeal mRNA (polycistronic)

UTR  ORF1  ORF2  ORF3  UTR

5' AUG  Stop  AUG  Stop  AUG  Stop  3'

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Maximizing coding capacity of the viral genome

- Polyprotein (*Picornaviridae, Flaviviridae, Togaviridae, Arenaviridae, Bunyaviridae, Retroviridae*)
- Subgenomic mRNAs (*Rhabdoviridae, Paramyxoviridae, Togaviridae*)
- Segmented genome (*Orthomyxoviridae, Reoviridae*)
- RNA Splicing (*Orthomyxoviridae, Adenoviridae, Polyomaviridae*)
- Internal initiation (IRES) (*Picornaviridae, Flaviviridae*)
- Leaky scanning (*Retroviridae, Paramyxoviridae*)
- Re-initiation of translation (*Orthomyxoviridae, Herpesviridae*)
- Suppression of termination (*Retroviridae, Togaviridae*)
- Ribosomal frameshifting (*Retroviridae*)
Polyprotein synthesis

A
Viral (+) strand genome

5′ VPG
UTR

Translation/processing

A

P1

P2

P3

VP0
VP3
VP1
2A
2B
2C
P3

VP4
VP2

2A\text{pro}

3C\text{pro}

B
Viral (+) strand genome

5′
UTR

Translation/processing

UTR

Host signal peptidase

Viral serine protease (NS3)

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Leaky scanning

- C proteins
- P 568 aa
- A 
- A OH
- AUG 104
- P 568 aa
- C' 215 aa
- ACG 81
- C 204 aa
- AUG 114
- Y1 181 aa
- AUG 183
- Y2 175 aa
- AUG 201

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Suppression of termination

eRF1 and eRF3 recognize all 3 stop codons (UAA, UAG, UGA)

Stop codons may be recognized by charged tRNA - misreading, or charged suppressor tRNA (e.g. selenocysteine for UGA)
Suppression of termination

A

UAGGGAGGUCAG CAGGAUAACCCUCAAAAGUCGGG

5' C G A A G C

Gag Pol

B

nsP1 nsP2 nsP3 nsP4

nsP2 proteinase

P123

P1234

nsP2 proteinase

Readthrough translation

Translation

Nonstructural ORF

Structural ORF

AₜA₀₃'
Ribosomal frameshifting

mRNA

5' → C → 3'

Gag

Pol

Protein

Gag

N

C

Gag-Pol

N

C
Compared with a polycistronic mRNA, a monocistronic mRNA:

1. Does not require an AUG start codon
2. Does not bind the 40S ribosomal subunit
3. Has only one open reading frame
4. Is only found in viruses and bacteria
5. All of the above
Regulation of translation in virus-infected cells

- Initiation
- Elongation
- Termination
The diagram illustrates the process of translation initiation and the role of eIF2α kinases in the context of ER stress. The activators of eIF2α kinases include:

- ER stress
- aa deprivation
- dsRNA

These activators lead to the phosphorylation of eIF2α, which in turn inhibits the ternary complex formation, thereby declining free eIF2B and inhibiting translation initiation.

Key components include:
- eIF2: eukaryotic initiation factor 2
- eIF3: eukaryotic initiation factor 3
- eIF1: eukaryotic initiation factor 1
- eIF1A: eukaryotic initiation factor 1A
- GTP: guanosine triphosphate
- GDP: guanosine diphosphate
- 40S: 40S ribosomal subunit
- Met: methionine codon
- AUG: start codon

The diagram highlights the complex interplay between these factors and the regulatory mechanisms governing translation initiation.
PKR and cellular antiviral response

- PKR induced and activated by virus infection
- Leads to inhibition of host translation, apoptosis
- Different viral mechanisms have evolved to inactivate the PKR pathway
Adenovirus VA RNA I prevents activation of PKR

VA RNA I

PKR inactive

dsRNA

eIF2α subunit phosphorylation

inhibited

protein synthesis

active protein synthesis

no phosphorylation of eIF2α subunit

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Viral proteins and RNAs that counter inactivation of eIF2

- Vaccinia virus K3L (pseudosubstrate)
- Herpes simplex virus γ34.5 (phosphatase regulatory subunit)
- Herpes simplex virus gB
- Vaccinia virus K3L
- Hepatitis C virus E2

**dsRNA-binding proteins:**
- Herpes simplex virus US11
- Influenza virus NS1
- Reovirus α3
- Vaccinia virus E3L

**Antagonists of RNA:**
- Adenovirus VA RNA I
- Epstein-Barr virus EBER

**Antagonists of protein:**
- Vaccinia virus K3L
- Sendai virus C
- Hepatitis virus NS5A
- Adenovirus E1b55K, E4Orf6
PKR is an interferon-induced enzyme that is activated by ____, leading to phosphorylation of ____ and inhibition of translation.

1. GDP, eIF2alpha
2. dsRNA, eIF2alpha
3. dsRNA, eIF2B
4. ssRNA, eIF2alpha
5. None of the above
Modulation of cap recognition

- elf4E dephosphorylation
  - Adenovirus
  - Influenza virus

- Poliovirus 2A
  - Foot-and-mouth disease virus L

- Dephosphorylation of 4E-bp1
  - Poliovirus
  - Encephalomyocarditis virus

5'-end-dependent initiation

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Stress granules