The difficulty lies, not in the new ideas, but in escaping old ones...

-- JOHN MAYNARD KEYNES
5’-cap structure

- Found on 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

Eukaryotic 80S ribosome

- 18S rRNA (30 proteins)
- 28S, 5.8S, and 5S rRNAs (50 proteins)

• Initiation proteins (eIF)
• Elongation proteins (eEF)
• Termination proteins (eRF)
5'-end dependent initiation
**eIF4G**

N-terminal domain

C-terminal domain

N

Pabp binding

eIF4E binding

eIF3 binding

eIF4A binding

Mnk1 binding

Cleavage with 2A protease

L protease

---

**5'-end-dependent initiation**

eIF4E

eIF4G

eIF4A

5' C

eIF3

40S

AUG UAA

---

**Juxtaposition of mRNA ends**

Pabp

AAAAAAAAA

eIF4E

eIF4G

eIF4A

5' C

eIF3

40S

AUG UAA

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

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

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Ribosome shunting

- 35s mRNAs of plant pararetroviruses
- 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

VPg

5'

UTR

AₙAₖOH₃'
IRES = internal ribosome entry site
The diagram illustrates the role of an Internal Ribosome Entry Site (IRES) in viral RNA translation. In the presence of an IRES (left), translation is initiated at the AUG codon following the UAA codon, resulting in the formation of a secondary structure that facilitates translation initiation. In the absence of an IRES (right), translation cannot proceed from the AUG codon following the UAA codon, and no protein is produced.
5'-end-dependent initiation

Type I or II IRES

Hepatitis C virus IRES

all eIFs

all eIFs except eIF4E

eIF2, eIF3

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Go to:

m.socrative.com
room number: virus

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
Methionine-independent initiation

- Can assemble 80S ribosomes without any eIFs or Met-tRNAi
- RNA mimics tRNAi
RNA genome of cricket paralysis virus

Binds 40S ribosome independent of initiation proteins

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Eukaryotic mRNA (monocistronic)

UTR → Open reading frame → UTR

5′ → AUG → Stop → 3′

A_nA_{OH}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

B
Viral (+) strand genome

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Leaky scanning

AUG 104
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)

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Suppression of termination

A

UAGGGAGGUCAG CAGGAUAACCUCAAAGUCGGGGGG

Gag Pol

B

nsP1 nsP2 nsP3 nsP4

nsP2 proteinase

P123 P1234

nsP2 proteinase

Readthrough translation

Translation

Nonstructural ORF Structural ORF

5' A n A O H3'
Ribosomal frameshifting

mRNA

5' | mRNA | Gag | Pol |
3'

Protein

Gag
N C

Gag-Pol
N C

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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
Translation initiation

**Activator**
- ER stress
- aa deprivation
- dsRNA
- Perk
- Gen2
- Pkr

**eIF2α kinases**

ATP

ADP

GDP

GDP

Free eIF2B declines, and translation initiation is inhibited
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
# Targets of viral inhibitors of eIF2α phosphorylation

<table>
<thead>
<tr>
<th>Target</th>
<th>Virus</th>
<th>Inhibitor</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>dsRNA</td>
<td>Herpes simplex virus</td>
<td>Us11</td>
<td>Binds and sequesters dsRNA</td>
</tr>
<tr>
<td></td>
<td>Influenza virus</td>
<td>NS1</td>
<td>Binds and sequesters dsRNA</td>
</tr>
<tr>
<td></td>
<td>Reovirus</td>
<td>σ3</td>
<td>Binds and sequesters dsRNA</td>
</tr>
<tr>
<td></td>
<td>Vaccinia virus</td>
<td>E3L</td>
<td>Binds and sequesters dsRNA</td>
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<td>Pkr</td>
<td>Adenovirus</td>
<td>VAI RNA</td>
<td>Blocks activation by dsRNA</td>
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<tr>
<td></td>
<td>Epstein-Barr virus</td>
<td>EBER</td>
<td>Blocks activation by dsRNA</td>
</tr>
<tr>
<td></td>
<td>Human immunodeficiency virus type 1</td>
<td>TAR RNA</td>
<td>Blocks activation by dsRNA</td>
</tr>
<tr>
<td></td>
<td>Herpes simplex virus</td>
<td>Us11</td>
<td>Binds Pkr</td>
</tr>
<tr>
<td></td>
<td>Kaposi sarcoma herpesvirus</td>
<td>vIRF-2</td>
<td>Binds Pkr</td>
</tr>
<tr>
<td></td>
<td>Baculovirus</td>
<td>PK2</td>
<td>Inhibits dimerization</td>
</tr>
<tr>
<td></td>
<td>Hepatitis C virus</td>
<td>NS5A</td>
<td>Inhibits dimerization</td>
</tr>
<tr>
<td></td>
<td>Human immunodeficiency virus type 1</td>
<td>Tat</td>
<td>Reduces Pkr expression</td>
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<tr>
<td>eIF2α</td>
<td>Hepatitis C virus</td>
<td>E2</td>
<td>Pseudosubstrate, blocks Pkr-eIF2α interaction</td>
</tr>
<tr>
<td></td>
<td>Human immunodeficiency virus type 1</td>
<td>Tat</td>
<td>Pseudosubstrate, blocks Pkr-eIF2α interaction</td>
</tr>
<tr>
<td></td>
<td>Vaccinia virus</td>
<td>K3L</td>
<td>Pseudosubstrate, blocks Pkr-eIF2α interaction</td>
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<tr>
<td>Phosphatase</td>
<td>Herpes simplex virus</td>
<td>γ34.5</td>
<td>Binds phosphatase, directs to eIF2α</td>
</tr>
<tr>
<td></td>
<td>Simian virus 40</td>
<td>T antigen</td>
<td>Downstream of eIF2α?</td>
</tr>
<tr>
<td>Pact</td>
<td>Herpes simplex virus</td>
<td>Us11</td>
<td>Binds Pact</td>
</tr>
</tbody>
</table>

*RBM, RNA-binding motif.*
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
microRNA (miRNA)

• Small non-coding RNAs encoded within the viral or cellular genome
• Transcribed as 60-70 nt pri-miRNA by either pol II or pol III
• Processed by the cell to ~22 nt
• Control gene expression post-transcriptionally via either repression of translation or mRNA degradation
Viruses and cellular miRNAs

- miRNAs target viral or cellular genes needed for viral replication
- miR-141 induced during enterovirus infection inhibits translation of eIF4E mRNA
Modulation of cap recognition

Poliovirus 2A Foot-and-mouth disease virus L

4E-bp1

Dephosphorylation of 4E-bp1 Poliovirus Encephalomyocarditis virus

5'-end-dependent initiation

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