Viral strategies to control cellular translation

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Initiation of eukaryotic translation

[Diagram showing the initiation of translation with labels for Cycloheximide and GMPPNP]
Picornavirus genomes fail to meet the inclusion criteria for 40S ribosomal subunit scanning from the 5' end of the mRNA on four counts:

1) they are uncapped mRNAs,
2) contains highly structured 5'UTRs
3) The 5’UTR are approximately 750 nt. long,
4) which harbour about 10 cryptic AUG codons
Viral and cellular IRES

Table 2. Internal ribosome entry sites in viral genomes

<table>
<thead>
<tr>
<th>Virus</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poliovirus</td>
<td>Pelletier and Sonenberg 1988</td>
</tr>
<tr>
<td>Rhinovirus</td>
<td>Borman and Jackson 1992</td>
</tr>
<tr>
<td>Encephalomyocarditis virus</td>
<td>Jang et al. 1988</td>
</tr>
<tr>
<td>Foot-and-mouth disease virus</td>
<td>Kuhn et al. 1990</td>
</tr>
<tr>
<td>Hepatitis C virus</td>
<td>Tsukiyama-Kohara et al. 1992</td>
</tr>
<tr>
<td>Bovine viral diarrhea virus</td>
<td>Poole et al. 1995</td>
</tr>
<tr>
<td>Friend murine leukemia virus gag mRNA</td>
<td>Berlioz and Darlix 1995</td>
</tr>
<tr>
<td>Moloney murine leukemia virus gag mRNA</td>
<td>Vagner et al. 1995b</td>
</tr>
<tr>
<td>Rous sarcoma virus</td>
<td>Delhaear and Darlix 2000</td>
</tr>
<tr>
<td>Human immunodeficiency virus env mRNA</td>
<td>Buck et al. 2001</td>
</tr>
<tr>
<td><em>Plautia stali</em> intestine virus</td>
<td>Sasaki and Nakashima 1999</td>
</tr>
<tr>
<td><em>Rhopalosiphum padi</em> virus</td>
<td>Domier et al. 2000</td>
</tr>
<tr>
<td>Cricket paralysis virus</td>
<td>Wilson et al. 2000b</td>
</tr>
<tr>
<td>Kaposi's sarcoma-associated herpesvirus</td>
<td>Grundhoff and Ganem 2001;</td>
</tr>
<tr>
<td></td>
<td>Bielecki and Talbot 2001</td>
</tr>
</tbody>
</table>

Table 3. Internal ribosome entry sites in cellular mRNAs

<table>
<thead>
<tr>
<th>Gene product</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth factors</td>
<td></td>
</tr>
<tr>
<td>Fibroblast growth factor 2</td>
<td>Vagner et al. 1995a</td>
</tr>
<tr>
<td>(FGF2)</td>
<td></td>
</tr>
<tr>
<td>Plasmid-derived growth factor B (PDGF-B) (transcription factor)</td>
<td>Bernsche et al. 1997</td>
</tr>
<tr>
<td>Vascular endothelial growth factor (VEGF)</td>
<td>Akin et al. 1998; Stein et al. 1998; Hass et al. 1998</td>
</tr>
<tr>
<td>Cyclin A1</td>
<td>Johannes et al. 1999</td>
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<tr>
<td>Transcription factors</td>
<td></td>
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<tr>
<td>Ultradselective</td>
<td>Ye et al. 1997</td>
</tr>
<tr>
<td>MYT2</td>
<td>Kim et al. 1998</td>
</tr>
<tr>
<td>NF-kB derepressing factor NF1</td>
<td>Omary et al. 2000</td>
</tr>
<tr>
<td>ANL1/RUNX1</td>
<td>Panor et al. 2000</td>
</tr>
<tr>
<td>Grx homodomain protein</td>
<td>Chappell et al. 2000a</td>
</tr>
<tr>
<td>Oncogenes</td>
<td></td>
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<tr>
<td>v-myc</td>
<td>Nakano et al. 1997; Someley et al. 1988</td>
</tr>
<tr>
<td>Fos-1</td>
<td>Johannes et al. 1999</td>
</tr>
<tr>
<td>Protein kinase P38, ras</td>
<td>Cornish et al. 2000</td>
</tr>
<tr>
<td>Cytochrome oxidases</td>
<td></td>
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<tr>
<td>Cat-1</td>
<td>Fernandez et al. 2001</td>
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<tr>
<td>Nuclear factor NF-1</td>
<td></td>
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<td>Translation factors</td>
<td></td>
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<tr>
<td>Lengthy initiation factor 4G</td>
<td>Lautz and Overbaugh 2000</td>
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<tr>
<td>(NFAT-4G)</td>
<td>Gun and Roberts 1995</td>
</tr>
<tr>
<td>Death-associated protein 5</td>
<td>Johannes and Sarnow 1998</td>
</tr>
<tr>
<td>(DAP5)</td>
<td>Helsinki-Korhonen et al. 2000</td>
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<tr>
<td>Activators of apoptosis</td>
<td></td>
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<tr>
<td>Apoptotic process</td>
<td>Coldwell et al. 2000</td>
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<tr>
<td>Necrotic factor Apep-1</td>
<td></td>
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<tr>
<td>Dendritically localized proteins</td>
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<tr>
<td>Activity-regulated cytoskeletal protein (ARC)</td>
<td>Pinksuff et al. 2001</td>
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<tr>
<td>α subunit of calcium-calmodulin-dependent kinase II (CaMKII)</td>
<td>Pinksuff et al. 2001</td>
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<td>Microtubule-associated protein 2 (MAP2)</td>
<td>Pinksuff et al. 2001</td>
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<tr>
<td>Neuregulin (RCS)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td></td>
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<tr>
<td>Immunoglobulin heavy chain binding protein (RIP)</td>
<td>Maciejak and Sarnow 1991</td>
</tr>
<tr>
<td>Larvaglutigen</td>
<td>Carter and Sarnow 2000</td>
</tr>
<tr>
<td>g subunit of mitochondrial H^+ -ATP synthase</td>
<td>Izquierdo et al. 2000</td>
</tr>
<tr>
<td>Ornithine dehydrogenase</td>
<td>Pyrona et al. 2000</td>
</tr>
</tbody>
</table>
Gold standard measure of IRES activity

Adapted from Hellen and Pestova, 1999.
Picornaviruses

Type I → Enteroviruses/Rhinoviruses

Type II → Cardiovirus/Apthtoviruses

Type II → Hepatitis A virus
Special structural characteristics of cricket paralysis-like virus RNAs. (a) In contrast to picornaviruses, the parts of the RNA coding for non-structural and structural proteins are separated into separate open reading frames, ORF1 and ORF2, respectively. (b) It has been proposed that the intergenic internal ribosome entry site (IRES) region interacts with the 40S ribosomal subunit in such a way as to mimic an occupied P-site. The 3' stem-loop structure is thought to form a pseudoknot, together with the overlined nucleotides just upstream of the CAA codon used to initiate translation of the Plautia stali intestine virus capsid protein.
A cell cycle-dependent internal ribosome entry site

Pyronnet et al., 2000, *Mol Cell*
List of IRES-trans-acting-factors (ITAFs)

Functional data
*PCBP1 and PCBP2 → Poliovirus 5'UTR

*PTB → binds to EMCV, FMDV, hRhinovirus, HAV, HCV IRES -Apaf-1 and

*unr → binds to human rhinovirus IRES

*La → poliovirus, HCV and coxsackievirus B3, HIV-1 leader, Human T-lymphotropic virus type I - XIAP and BIP and IRESs.

*ITAF$_{45}$ → also known as murine proliferation-associated protein (*Mpp1*) binds to IRES FMDV

Without functional data

*hnRNPL → binds to HCV IRES

*GADPH → HAV IRES

*Nucleolin → Poliovirus IRES
Control of translation in HeLa cells infected with Poliovirus

![Graph showing rate of protein synthesis over time post-infection](image-url)
Host Cell Shutoff

1. tRNA Cleavage (*T-even phages*)

2. eIF4F Complex Formation
   a) eIF4G Cleavage (*Poliovirus, FMDV*).
   b) eIF4E Dephosphorylation (*Influenza virus, VSV*).
   c) 4E-BPs Dephosphorylation (*VSV, EMCV*).

3. PABP inactivation (*Poliovirus, Reovirus*)

4. eIF2 Phosphorylation (*VSV, SV, EMCV*)

5. Interferon system
   a) Activation of PKR and Rnase L
eIF4F Cap-Binding Protein Complex: A Regulator of Virus Infection Efficiency?
eIF4F cap-binding protein complex
Initiation of eukaryotic translation
Translation Initiation
eIF4G inactivation
Cleavage of eIF4G by Picornaviruses
Inhibition of Host Cell Protein Synthesis after Poliovirus Infection: Correlation with the Cleavage of eIF4GI and eIF4GII

4A3 is required for NMD but not for bulk translation.
eIF4GI/II are required for poliovirus-IRES-dependent translation

Costa-Mattioli and Sonenberg, Unpublished data

Dominant negative mutants of mammalian translation initiation factor eIF-4A define a critical role for eIF-4F in cap-dependent and cap-independent initiation of translation.

Pause A, Methot N, Svitkin Y, Merrick WC, Sonenberg N.
Department of Biochemistry, McGill University, Montreal, Quebec, Canada.


Canonical eukaryotic initiation factors determine initiation of translation by internal ribosomal entry.

Pestova TV, Hellen CU, Shatsky IN.
Department of Microbiology and Immunology, Morse Institute for Molecular Genetics, State University of New York Health Science Center at Brooklyn, 11203-2098, USA.
Costa-Mattioli and Sonenberg, *Unpublished data*
eIF4E phosphorylation
eIF4E is phosphorylated on Ser209 by Mnk1/2

growth factors
hormones
mitogens
cytokines
RAS
stress
RAF
MEK
P38 MAPK
ERK

eIF4E
Mnk1/2

P

eIF4G
Adenovirus reduces eIF4E phosphorylation

Cuesta et al., 2000

Feigenblum and Schneider, 1993; Zhan et al., 1994
VSV reduces eIF4E but not MNK1 phosphorylation

Connor and Lyles; 2002
Mnk2 and Mnk1 Are Essential for Constitutive and Inducible Phosphorylation of Eukaryotic Initiation Factor 4E but Not for Cell Growth or Development

Takeshi Ueda,1† Rie Watanabe-Fukunaga,1,2† Hidehiro Fukuyama,1,3 Shigekazu Nagata,1,2,3 and Rikiro Fukunaga1,2,3*
Phosphorylation of eIF4E by Mnk-1 enhances HSV-1 translation and replication in quiescent cells
4E-BPs phosphorylation
The 4E-BPs regulate eIF4F formation

- 4E-BPs
- eIF4F complex
- eIF4E
- eIF4G
- eIF4A
- 4E-BP
- eIF4E

Regulators:
- Hormones
- Growth factors
- Mitogens
- Cytokines
- GPCR agonists
- Adenovirus
- Serum starvation
- Amino acid deprivation
- EMCV
- Poliovirus
- Environmental stress
Adenovirus inactivates the translation inhibitors 4E-BP1 and 4E-BP2
Adenovirus inactivates the translation inhibitors 4E-BP1 and 4E-BP2

Gingras et al., 1997
Phosphorylation of 4E-BP1 decreases in parallel with the inhibition of host synthesis in EMCV-infected cells

Gingras et al., 1996
Signaling pathway to 4E-BPs phosphorylation

growth factors

wortmannin  \[\rightarrow\]  PI3K

Akt/PKB

rapamycin  \[\rightarrow\]  FRAP/mTOR

4E-BP1
Rapamycin enhances EMCV translation

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
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<tbody>
<tr>
<td>Mock</td>
<td>EMCV</td>
<td>EMCV + rapa</td>
<td>Mock</td>
<td>EMCV</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
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<table>
<thead>
<tr>
<th></th>
<th>P1</th>
<th>3D</th>
<th>VP0</th>
<th>2C</th>
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<tbody>
<tr>
<td>VP1</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>VP3</td>
<td></td>
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</tr>
<tr>
<td>2A</td>
<td></td>
<td></td>
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</table>

Beretta et al., 1996
Rapamycin and Wortmannin rescue an EMCV defective mutant

Svitkin et al., 1998
Eukaryotic Translation Initiation Factor 4E Availability Controls the Switch between Cap-Dependent and Internal Ribosomal Entry Site-Mediated Translation†

Yuri V. Svitkin,1 Barbara Herdy,1 Mauro Costa-Mattioli,1 Anne-Claude Gingras,1‡ Brian Raught,1‡ and Nahum Sonenberg1,2*

Department of Biochemistry1 and McGill Cancer Center,2 McGill University, Montreal, Quebec H3G 1Y6, Canada

Received 6 June 2005/Returned for modification 5 July 2005/Accepted 13 September 2005
4E-BP1 and eIF4E Oppositely Regulate EMCV RNA Translation and Replication in Untreated Extract
4E-BP1 and eIF4E oppositely regulate EMCV synthesis in an untreated Krebs-2 cell extract
4E-BP1 and eIF4E Regulate EMCV RNA Translation in A Nuclease-Treated Extract Only in the Presence of Competing Cellular mRNAs
eIF4E Knockdown Stimulates EMCV Infection
Characterization of eIF4E-Knockdown Cells

A

B

control

eIF4E

80S polysomes

OD254

Sedimentation
eIF4E Knockdown Stimulates PV Infection

A

<table>
<thead>
<tr>
<th>sRNA</th>
<th>control</th>
<th>eIF4E</th>
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</thead>
<tbody>
<tr>
<td>Time (h) 2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

B

C

D

![Graph showing PFU/ml (10^6) for control and eIF4E siRNA conditions.](image)
eIF4E Knock Down Stimulates EMCV Infection

siRNA
not infected
EMCV (h p.I.)

\( kD \)
170
130
100
72
55
42
33
24
17
11

\[ \text{eIF4E}_{331} \]

\[ \text{Control} \]

\[ \text{WB: } \beta\text{-actin} \]

\[ \text{WB: } \text{eIF4E} \]

\[ \text{\( ^{35} \text{S-Met} \)} \]
eIF4E Knock Down Enhances EMCV Yields (Plaque Assay)

<table>
<thead>
<tr>
<th>siRNA</th>
<th>eIF4E</th>
<th>control</th>
</tr>
</thead>
<tbody>
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</table>

Mock 6 hours

4 hours post infection

6 hours post infection
eIF4E Knock Down Stimulates Poliovirus Infection

siRNA not infected Poliovirus (h p.l.)

<table>
<thead>
<tr>
<th>siRNA</th>
<th>Control</th>
<th>eIF4E331</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 3 4 5 6</td>
<td>2 3 4 5 6</td>
</tr>
</tbody>
</table>

WB: β−actin

WB: eIF4E

WB: 35S-Met
A model for regulation of EMCV replication by 4E-BPs
Picornaviruses

IRES type I and II require all the canonical initiation factors but not eIF4E. What about IRES type III?
VSV reduces 4E-BP1 phosphorylation

Connor and Lyles, 2002
PABP
Closed loop model of translation initiation complex
Closed Loop Model
eIF2alpha kinases

YEAST-General Control Pathway

GCN2 → eIF2 → eIF2 P → General Translation → GCN4

MAMMALIAN Stress-Responsive Pathway

eIF2-α Kinases → eIF2 → eIF2 P → ATF4
PKR
Dey et al., 2005
Overexpression of PKR render cells resistant to EMCV infection (Meurs et al., 1992).

Reduction of PKR convert EMCV infection into a permanent infection (Yeung et al., 1999).
PKR−/− MEFs are more permissive for VSV infection than are control MEFs.
EMCV titers in brain and serum of treated and untreated mice determined 72 h after infection.

Yang et al., 1995
TABLE 1. PKR$^{-/-}$ or PKR$^{+/+}$ mice infected i.n. with various doses of VSV$^a$

<table>
<thead>
<tr>
<th>Genetic background</th>
<th>Strain</th>
<th>i.n. dose (PFU)</th>
<th>No. of mice surviving at day 5/total no. of mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>PKR$^{+/+}$</td>
<td>BALB/c</td>
<td>$5 \times 10^4$</td>
<td>5/5</td>
</tr>
<tr>
<td></td>
<td>CD1</td>
<td>$5 \times 10^4$</td>
<td>5/5</td>
</tr>
<tr>
<td></td>
<td>BALB/c × 129</td>
<td>$5 \times 10^4$</td>
<td>5/5</td>
</tr>
<tr>
<td>PKR$^{-/-}$</td>
<td>BALB/c × 129</td>
<td>$5 \times 10^4$</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$5 \times 10^3$</td>
<td>0/4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$5 \times 10^2$</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$5 \times 10^1$</td>
<td>0/3</td>
</tr>
</tbody>
</table>

Stojdl et al., 2000
PERK
Baltzis et al., 2004
Baltzis et al., 2004
Activation of PKR → 1) induction of interferon response through the IRF-1, NF-KB, 2) Inhibition of protein synthesis, 3) induction of apoptosis.
GCN2
A NEW ROLE OF THE eIF2α KINASE, GCN2, IN THE CELLULAR RESPONSE TO VIRAL INFECTION.

Juan José Berlanga, Iván Ventoso, Luis Carrasco, César de Haro

Centro de Biología Molecular “Severo Ochoa” (CSIC-UAM)
TRANSLATIONAL CONTROL BY THE eIF2alpha KINASES

Iniciación de la traducción
CONTROL TRADUCCIONAL DE LA EXPRESIÓN DE GCN4 EN S. cerevisiae Y ATF4 EN MAMÍFeros

Respuesta a estrés en mamíferos

- Señales de estrés
  - eIF2α (fosforilación)
    - ATF4 (traducción)
      - Genes de respuesta a estrés (transcripción)

Respuesta a estrés en levaduras

- Escasez de nutrientes
  - eIF2α (fosforilación)
    - GCN4 (traducción)
      - Enzimas biosintéticas (transcripción)
The genomic RNA of Sindbis virus stimulates phosphorylation of eIF2α through GCN2
LA ACTIVACIÓN DE GCN2 POR EL RNA DEL VIRUS SINDBIS IMPLICA DOS REGIONES NO ADYACENTES DEL EXTREMO 5' DEL RNA VIRAL Y EL DOMINIO HIS-RS DE GCN2
CINÉTICA DE SÍNTESIS DE PROTEÍNAS VIRALES EN FIBROBLASTOS EMBRIONARIOS OBTENIDOS DE RATONES CONTROL Y GCN2−/−
SÍNTESIS DE PROTEÍNAS VIRALES Y EFECTO CITOPÁTICO EN FIBROBLASTOS EMBRIONARIOS OBTENIDOS DE RATONES CONTROL, GCN2-/- Y PKR-/-
Efecto de la sobreexpresión de GCN2 en la replicación y en la síntesis de proteínas virales

Producción viral en un ciclo de infección

<table>
<thead>
<tr>
<th>Clon 3T3</th>
<th>EMCV</th>
<th>SFV</th>
<th>SV</th>
<th>VSV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>7.16 ± 0.35</td>
<td>9.02 ± 0.35</td>
<td>8.20 ± 0.15</td>
<td>7.74 ± 0.12</td>
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<tr>
<td>GCN2-K618R</td>
<td>7.36 ± 0.20</td>
<td>9.13 ± 0.48</td>
<td>8.30 ± 0.35</td>
<td>7.40 ± 0.02</td>
</tr>
<tr>
<td>GCN2-WT</td>
<td>7.10 ± 0.43</td>
<td>8.00 ± 0.42</td>
<td>6.10 ± 0.21</td>
<td>6.00 ± 0.01</td>
</tr>
</tbody>
</table>