The Infected Cell

Lecture 11
Biology 4310
Virology
Spring 2021

He hath eaten me out of house and home.
—WILLIAM SHAKESPEARE
King Henry IV, Part II
The Infected Cell

- So far we have focused on viral gene expression, genome reproduction, and assembly of virus particles
- These depend on host metabolic, biosynthetic, signaling and trafficking systems
- An integrated description of the impact of virus infection on the host cell
The Infected Cell

- Signal transduction
- Gene expression
- Metabolism
- Remodeling of cellular organelles
Signal transduction

- Cells must sense their environment and respond appropriately
- Signal transduction pathways govern every aspect of cell physiology and conduct
- Virus infections can change signaling to promote reproduction

Pi3k-Akt-mTor signaling

Green arrows: Stimulate
Red arrows: Inhibit
Signaling via Pi3k facilitates virus entry
Common activation of Pi3k-mTor relay

A

Hepatitis C virus
NS5A
p85
p110
PI3k
NSP1
Rotavirus

E4 Orf1
Ad5

Cell survival

Transcription/replication of HBV genome

AKT
Apoptosis

mTOR
Translation

B

HHV8

PI3k
p85
p110

HBV X

↑ cyclin D and proliferation

AKT
Apoptosis

Autophagy

mTOR
Translation
Viral RNA blocks Akt activation to induce apoptosis

sfRNA needed for formation of plaques and pathogenicity in mice

sfRNA inactivates Akt, promotes apoptosis (concentration of anti-apoptotic protein Bcl-2 is reduced)
Inhibition of cellular gene expression

Poliovirus infection also inhibits pol I, pol III

Reduce competition of cellular with viral mRNAs for translation machinery
Inhibition of cellular pre-mRNA processing by viral proteins
Regulation of mRNA turnover

- Cell mRNA degradation proteins removed or relocalized in virus infected cells
- Pan, Dcp1a, Xrn1 degraded in poliovirus infected cells
Viral proteins initiate mRNA degradation
Which of the following is a consequence of viral proteins modifying signal transduction pathways to promote replication?

A. Poliovirus inhibition of transcription by RNA pol II
B. Herpes simplex virus protein blocking pre-mRNA splicing
C. Disruption of actin filaments to allow endocytosis
D. Initiation of mRNA degradation by viral proteins
Viral inhibition of cell translation

(A) Graph showing the rate of protein synthesis over time postinfection for uninfected and poliovirus-infected cells.

(B) Image of a gel showing various proteins labeled 3CD, 1CD, 2BC, VP0, 2C, and VP3 at different time points postinfection.
Modulation of cap recognition

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

**De phosphorylation of 4E-bp1**
- **Poliovirus**
- **Encephalomyocarditis virus**

**5'-end-dependent initiation**
- **elf4E**
- **elf4G**
- **elf4A**
- **elf3**
- **40S**
- AUG
- UAA
Internal initiation

5'-end-dependent initiation

eIF4E

Type 1 or 2 IRES

eIF4E

Hepatitis C virus IRES

eIF4E

all eIFs

all eIFs except eIF4E

eIF2, eIF3

Internal initiation
Regulation of translation initiation

Diagram showing the regulation of translation initiation with the involvement of eIF2, eIF2B, and eIF3, along with the activation process mediated by activators such as ER stress, PERK, GCN2, PKR, and RNA deprivation. The diagram illustrates the ternary complex formation and the inhibition of translation initiation due to free eIF2B decline.
Activation of Pkr

PKR activation by PACT

PKR activation by dsRNA

(dsRNA) + (dsRBM1, dsRBM2)

Active PKR

(dsRBM1, dsRBM2, dsRBM3)
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

inactive eIF2α subunit

no phosphorylation of eIF2α subunit

active protein synthesis

protein synthesis inhibited
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 σ3
- Vaccinia virus E3L

**Antagonists of RNA:**
- Adenovirus VA RNA 1
- 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.

A. GDP, eIF2alpha  
B. dsRNA, eIF2alpha  
C. dsRNA, eIF2B  
D. ssRNA, eIF2alpha  
E. None of the above
Production of large quantities of virus particles places high demands on host cell biosynthetic systems

- Nucleotides, amino acids, fatty acids
- Energy is needed! 4 ATP to make a single peptide bond
- Virus infection impacts cell metabolism
Increased glycolysis in virus infected cells

Low pH = production of lactic acid, product of glycolysis

See Rhinoviruses have a sweet tooth
http://www.virology.ws/2018/08/23/rhinoviruses-have-a-sweet-tooth/
Glucose metabolism

- Glucose is major breakdown product of dietary carbohydrate
- Converted to pyruvate, yields 2 ATP and 2 NADH
- Intermediates allow more ATP synthesis
- Perturbed in virus infected cells
Two herpesviruses have different effects on glycolysis

Hypothesis: HSV has shorter reproduction cycle, need more nucleotide precursors to synthesize viral DNAs
Fatty acid synthesis

- Acetyl-CoA cannot cross mitochondrial membrane
- Most citrate in HCMV infected cells leave mitochondria for fatty acid synthesis
- Inhibition of malonyl-CoA or FAS reduces yield of HCMV particles
- Activity of another member of this shuttle is increased in cells infected with VSV
Virus-induced changes in glucose metabolism and human disease

Two hepatotropic viruses

- Liver site of glycogen and *de novo* glucose synthesis
- HBV infection associated with development of type 2 diabetes*; infection stimulates levels of enzymes involved in glucose synthesis
- T2D also associated with HCV infection, which causes reduced glucose uptake and increase glucogenesis

*insulin deficiency/resistance
Citric acid (TCA) cycle

- Central hub of carbon metabolism
- Yields precursors for biosynthesis of many compounds
- Virus infections impact cycle (prevents halting of TCA cycle)
Lipid metabolism

- Triacylglycerols are primary energy source (oxidation) and store in most organisms
- Membrane synthesis
- Lipid metabolism modulated in virus infected cells: oxidation, synthesis
HCMV infection induces synthesis of very long chain fatty acids for assembly

- HCMV infection increases carbon flux from glucose to acetyl-CoA
- Not only increased FA production but very long chain FA found in viral membrane - required for infectivity
- Increased availability of activators for transcription of genes for lipid synthesis
Increased synthesis and accumulation of fatty acids in HCV infected cells

**A**

- **FOXO1**
  - Insulin
  - Exit from nucleus
  - G6P, PCK2

**B**

Mock

Infected

Human hepatocytes

Net effect: accumulation of lipid droplets

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Go to:

b.socrative.com/login/student
room number: virus

How might virus infection lead to increased levels of ATP?

A. Stimulation of glucose uptake  
B. Increased glycolysis  
C. Increased oxidation of fatty acids  
D. Increased utilization of glutamine  
E. All of the above
Remodeling of cellular organelles: Co-option of cytoplasmic membranes in PV infected cell

Increased import of fatty acids into poliovirus-infected cells
HCV replication and assembly compartments

A Replication

- dsRNA
- Positive-strand viral RNA
- Negative-strand viral RNA
- HCV NS3
- HCV NS3-NS4A
- HCV NS5A
- HCV NS5B
- HCV NS4B

B Assembly

- dsRNA
- Viral envelope proteins
- Capsid protein
- NS5B
- NS2
- p7

Lipid droplet

ER

Cytosol

Nucleus

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Glycolytic pyruvate kinase recruited into viral replication complex to generate ATP for RNA synthesis

Tomato bushy stunt virus
Next time: Infection basics