Viral Pathogenesis: Virus Interaction with the cell and the whole organisms

Patterns of Infection

Studying the biology of an infected cell is a useful first step in understanding what kind of pathology the virus will cause in the host.

Some virus infections kill the cell rapidly, producing a burst of new particles (**cytopathic viruses**), while other infections result in the release of virus particles without causing immediate host cell death (**noncytopathic viruses**).

Alternatively, some infections neither kill the cell, nor produce any progeny, but rather remain dormant or become **abortive infections** .
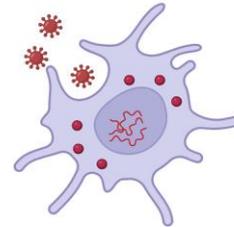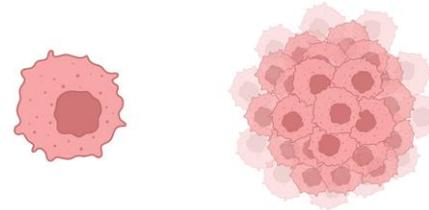
Patterns of Infection

Patterns of infection:

Cytopathic
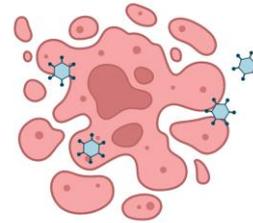
Non Cytopathic

Abortive

Effects of the virus on cells

Cytopathic effect (CPE): morphological alterations of infected cells that occur both in the host and in cell cultures in vitro; these are characteristic of different virus groups and are useful in diagnostic laboratories.

Many viruses also have the ability to inhibit the synthesis of cellular macromolecules (shut-off), and this prevents the cell from repairing the damage caused by the virus.

Effects of the virus on cells

The cytopathic effect can be represented by:

a rounding of the cells followed by lysis

formation of giant multinucleated cells (syncytia) due to the presence of virus-specific fusion proteins (e.g., paramyxoviruses, herpesviruses, some retroviruses)

formation of aggregates of viral components and/or altered cellular structures in the nucleus or cytoplasm of the cell (inclusions).

Cytopathic effect CPE
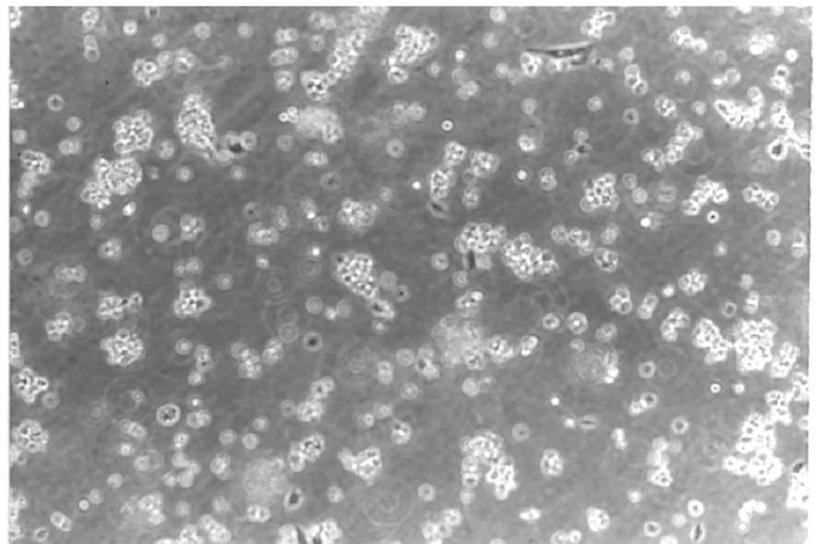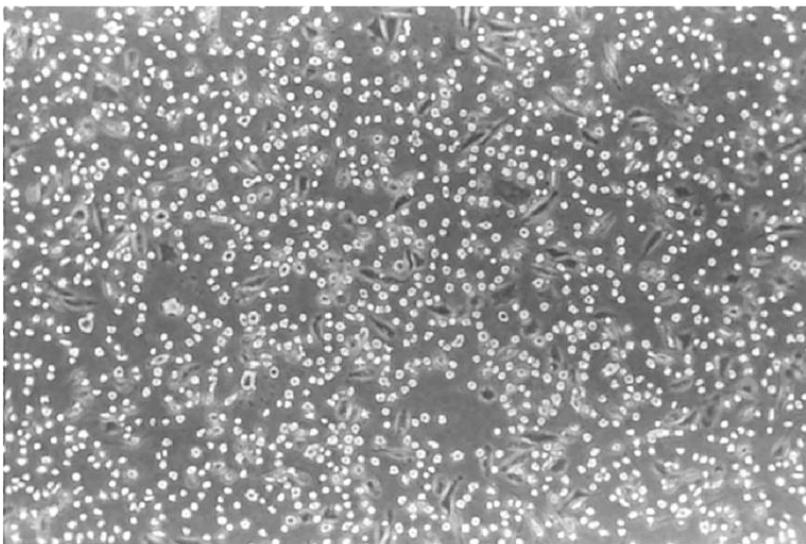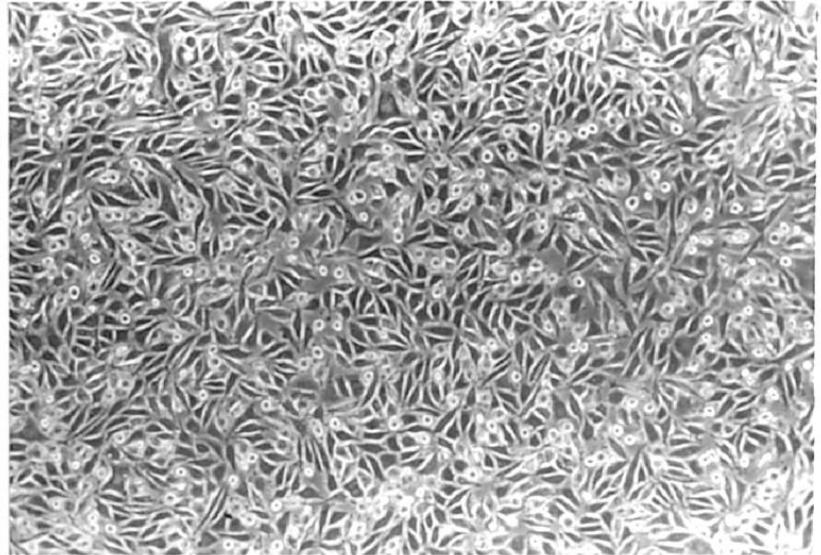
CPE

In alcuni casi si osserva fusione tra le cellule con formazione di sincizi (frecce),

La formazione di sincizi facilita la transmissione del virus alle cellule circostanti.
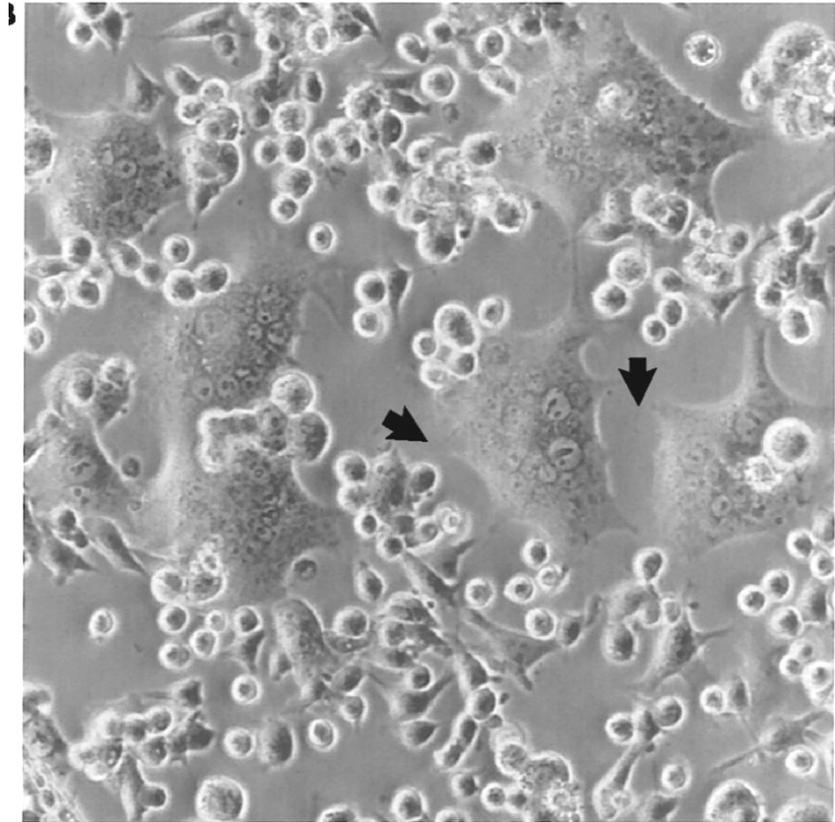
proteine virali ad attività fusogena

pH-indipendente (es: paramyxovirus)

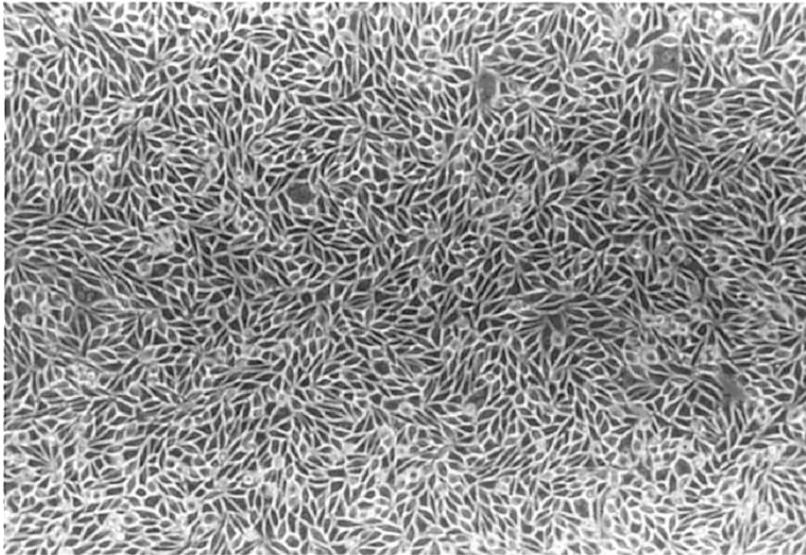# Effects of the virus on cells

The penetration of the virus into the cell generates a new entity: the infected cell.

Cellular infection can lead to three distinct outcomes (which depend on the type of virus and the type of cell):

- death of the cell (cytocidal infections) due to a lytic event

- latent or chronic infection

- transformation

Possible fates of a virus infected cell
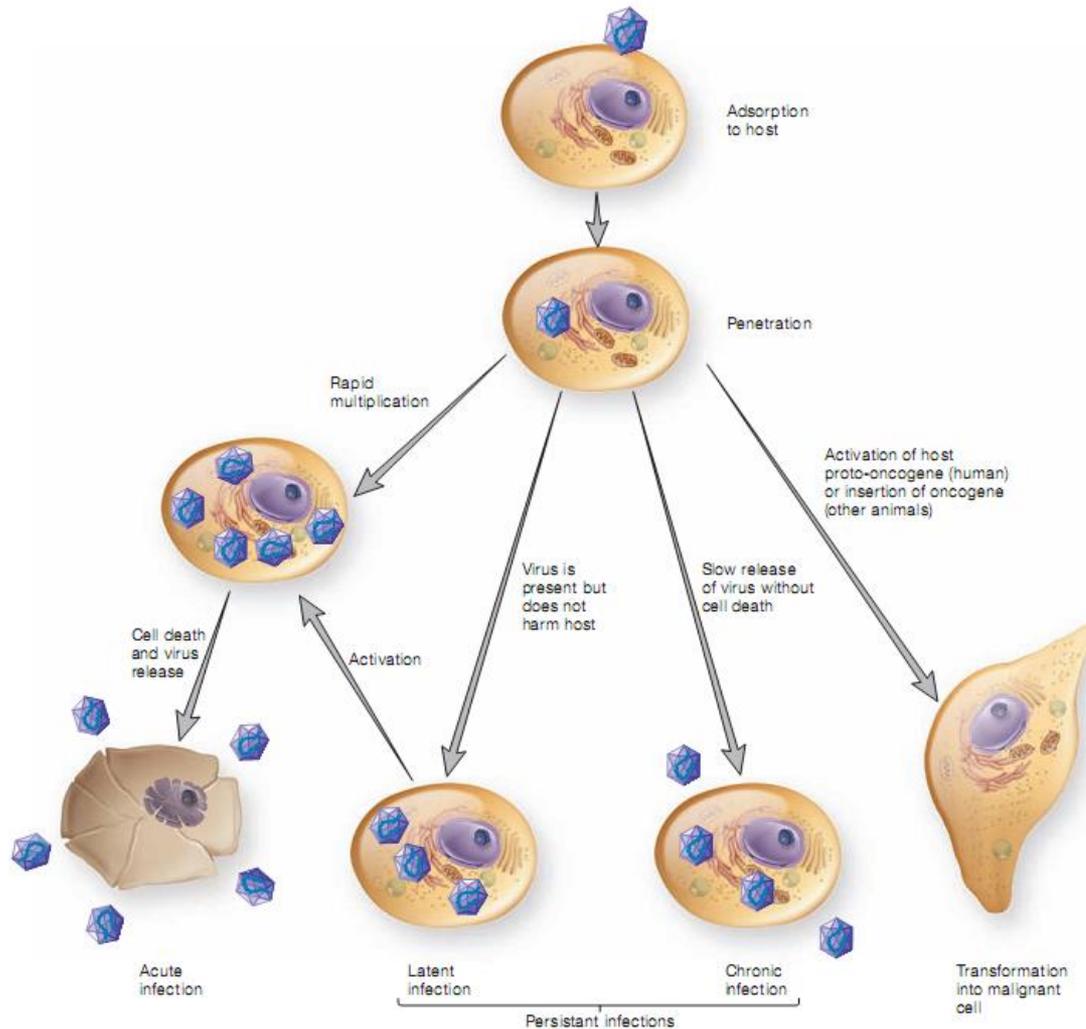
Is the disease the outcome of all viral infections?

Virus specific disease outcomes are driven by differences in viral replication cycles, modes of transmission, tissue tropism, interactions with the host immune response etc.

Due to differences in host genetics, host immune status, viral dose, or route of inoculation, infection with the same virus may result in varied disease outcomes in different individuals

Viral Infections

Some viruses can replicate without causing apparent symptoms = asymptomatic infections

Other viruses can lead to diseases ranging from paucisymptomatic to mild-moderate symptoms, critical illness, multiorgan failure, and lethality

Determinants of Viral Disease

Direct effect of the virus:
cytopathogenic attitude (cell lysis)
transformation

Indirect effect:
Immunopathology (excessive immune response, inflammation, cytokine storm, immunocomplexes, lesions caused by T cell responses)

Determinants of Viral Disease

- cytopathogenic attitude of the virus

- immunopathology

- initial inoculum of the virus

- compromised host, competence of the immune system

- host genetic background

- age

- previous exposure to the virus (immunity)

Viral Pathogenesis

Viral, host, and environmental factors may contribute to shaping infection outcome
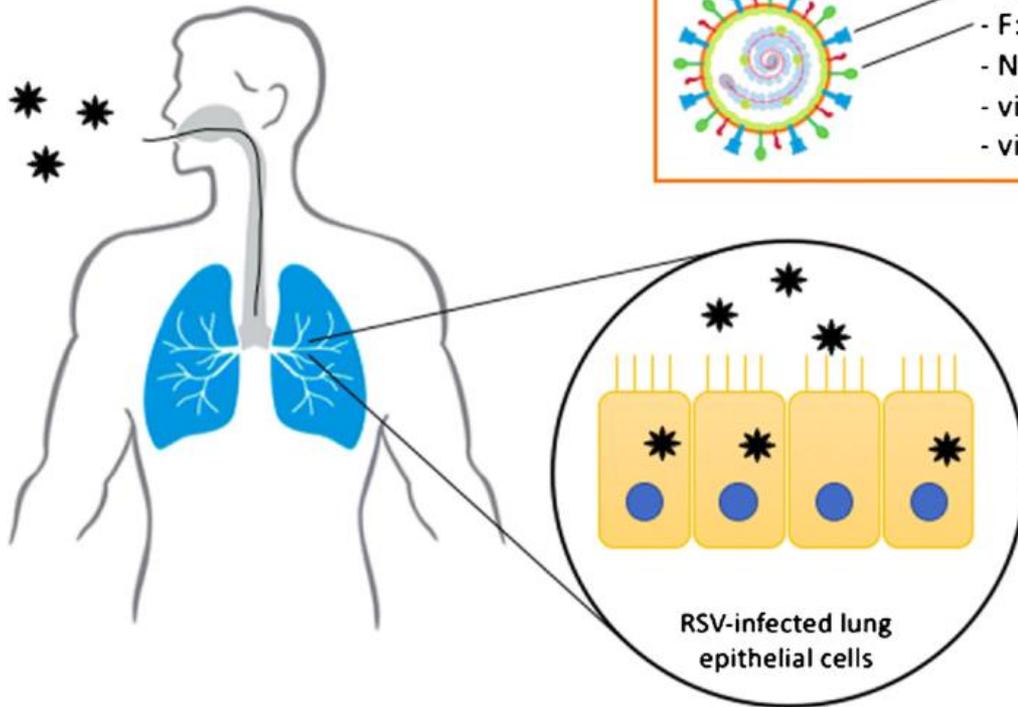
Viral factors:
- G: glycosylation; sG neutralizing antibodies avoidance
- F: conformational change; infectivity and protection
- NS1 and NS2: IFN type I inhibition
- viral strains and isolates
- viral load

Host factors:
- gender: male
- lung and heart anomalies
- low vitamin D level
- inadequate immune system
- genetic polymorphism

Environmental factors:
- exposure to smoke
- air pollution
- winter season

RSV-infected lung epithelial cells

Tabella 37.4 Progressione clinica delle infezioni virali.

Infezioni virali clinicamente manifeste	Infezioni virali asintomatiche
1. Il virus è direttamente citopatico e distrugge nella sua replicazione le cellule infettate	1. Nessun danno al tessuto infettato da parte della replicazione virale
2. Il virus, ancorché non direttamente citopatico, induce nella sua replicazione una risposta citotossica verso le cellule infettate	2. Il tessuto infettato non ha un ruolo funzionale di rilievo
3. Il virus non è citopatico, ma nel corso dell'infezione fa acquisire alla cellula infettata, attraverso diversi meccanismi, le caratteristiche di "cellula trasformata"	3. Il danno prodotto dall'infezione è al di sotto della soglia di funzionalità del tessuto infettato
	4. Il tessuto è rapidamente riparato successivamente al danno prodotto dalla replica virale

Patterns of infection and disease progression

Acute
- Rhinovirus
- Rotavirus
- Influenza virus

Acute infection ➜ Recovery (or death)

Chronic Infection
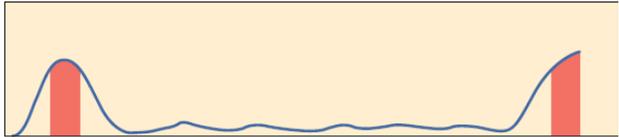
Latent infection
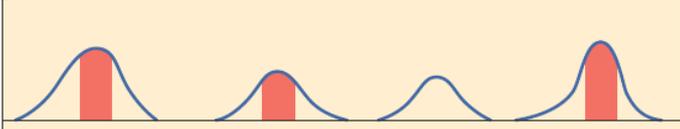
Persistent infections

Infezioni acute non persistenti

La maggior parte delle infezioni virali si estingue con la guarigione e la scomparsa del virus dall'organismo

Il sistema immunitario, una volta attivato, è in grado di eliminare il virus con efficacia

Il virus non è più rilevabile, se non in caso di eventuale re-infezione.

Immunità efficace e spesso duratura

Talvolta continua più a lungo la replicazione nei «siti di immunoprivilegio»

Infezioni persistenti

In altri casi l'infezione, dopo la fase acuta può diventare persistente, e **durare anche per tutta la vita**.

Il sistema immunitario non è in grado di eradicare l'infezione
- Cronica
- Latente
- A lento decorso (es. Encefalite subacuta scelrosante da morbillo)

Infezioni croniche

Sono caratterizzate dalla <u>continua presenza del virus</u> che continua a essere prodotto, anche dopo la fase acuta, in genere a livelli molto più bassi e per lungo tempo, **compatibili con la sopravvivenza dell'ospite.**

Esempi classici: **virus dell'epatite C, HIV, HBV**

I danni per l'ospite sono tipicamente da accumulo (immunodeplezione, autoimmunità, infiammazione cronica, oncogenesi)

Latenza Virologica:

- Il genoma del virus permane sotto forma di **acido nucleico integrato nel genoma cellulare** o è presente in **forma episomica**.

- L'espressione genica è drasticamente ridotta e **non si ha la produzione di particelle virali complete**

- Esempio cardine: Herpesviridae

- In condizioni di **immuno-soppressione**, il virus può "risvegliarsi" e iniziare un nuovo ciclo di replicazione produttiva (recidiva), che di norma si conclude a sua volta con una ulteriore fase di latenza.

Table 5.2 Some persistent viral infections of humans

Virus	Site(s) of persistence	Consequence(s)
Adenovirus	Adenoids, tonsils, lymphocytes	None known
Epstein-Barr virus	B cells, nasopharyngeal epithelia	Burkitt's lymphoma, Hodgkin's disease
Human cytomegalovirus	Kidneys, salivary gland, lymphocytes,[a] macrophages,[a] stem cells,[a] stromal cells[a]	Pneumonia, retinitis
Hepatitis B virus	Liver, lymphocytes	Cirrhosis, hepatocellular carcinoma
Hepatitis C virus	Liver	Cirrhosis, hepatocellular carcinoma
Human immunodeficiency virus	CD4+ T cells, macrophages, microglia	AIDS
Herpes simplex virus types 1 and 2	Sensory and autonomic ganglia	Cold sore, genital herpes
Human T lymphotropic virus types 1 and 2	T cells	Leukemia, brain infections
Papillomavirus	Skin, epithelial cells	Papillomas, carcinomas
Polyomavirus BK	Kidneys	Hemorrhagic cystitis
Polyomavirus JC	Kidneys, central nervous system	Progressive multifocal leukoencephalopathy
Measles virus	Central nervous system	Subacute sclerosing panencephalitis, measles inclusion body encephalitis
Rubella virus	Central nervous system	Progressive rubella panencephalitis
Varicella-zoster virus	Sensory ganglia	Zoster (shingles), postherpetic neuralgia

[a]Proposed but not certain.

No single mechanism is responsible for establishing a persistent viral infection. However, when viral cytopathic effects are minimized, and host defenses are suppressed, a persistent infection is likely.

The pattern of infection and the cell fate may vary for the same virus depending on the infected cell (i.e. apoptosis or IFN response could be blocked in certain tissues by viral functions not in other tissues... Persistence vs non persistence)
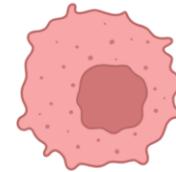
Viral Pathogenesis

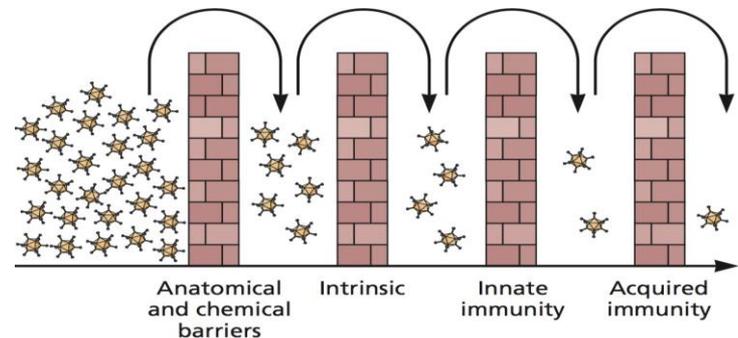Three main requirements for viral infection

Infectious dose (enough virus to sustain replication)

Cells accessible, susceptible, permissive

Local antiviral defense overcome

Anatomical and chemical barriers | Intrinsic | Innate immunity | Acquired immunity

Viral tissue tropism and host range

Viral tropism is defined as the capacity of a virus to infect specific cells, tissues, and species. It depends on both viral and cellular characteristics—with the presence of the cognate receptors for viral attachment molecules being of undeniable importance

Receptor/co-receptor Interactions:
HIV gp120 interactions with CD4 and subsequent viral tropism for these cells, directly contributes to disease pathogenesis
Moreover, HIV co-receptor interaction further illustrates the importance in driving viral pathogenesis. Interaction with CCR5 or CXCR4 leads to viral fusion. A small subset of individuals has a nonfunctional form of CCR5 (CCR5Δ32), and these individuals are highly resistant to HIV infection

Zoonotic viruses: for the successful transition from the natural host to humans a zoonotic virus must either interact with receptors conserved between species or mutate (eg. Avian influenza, SARS-CoV)

Viral tropism
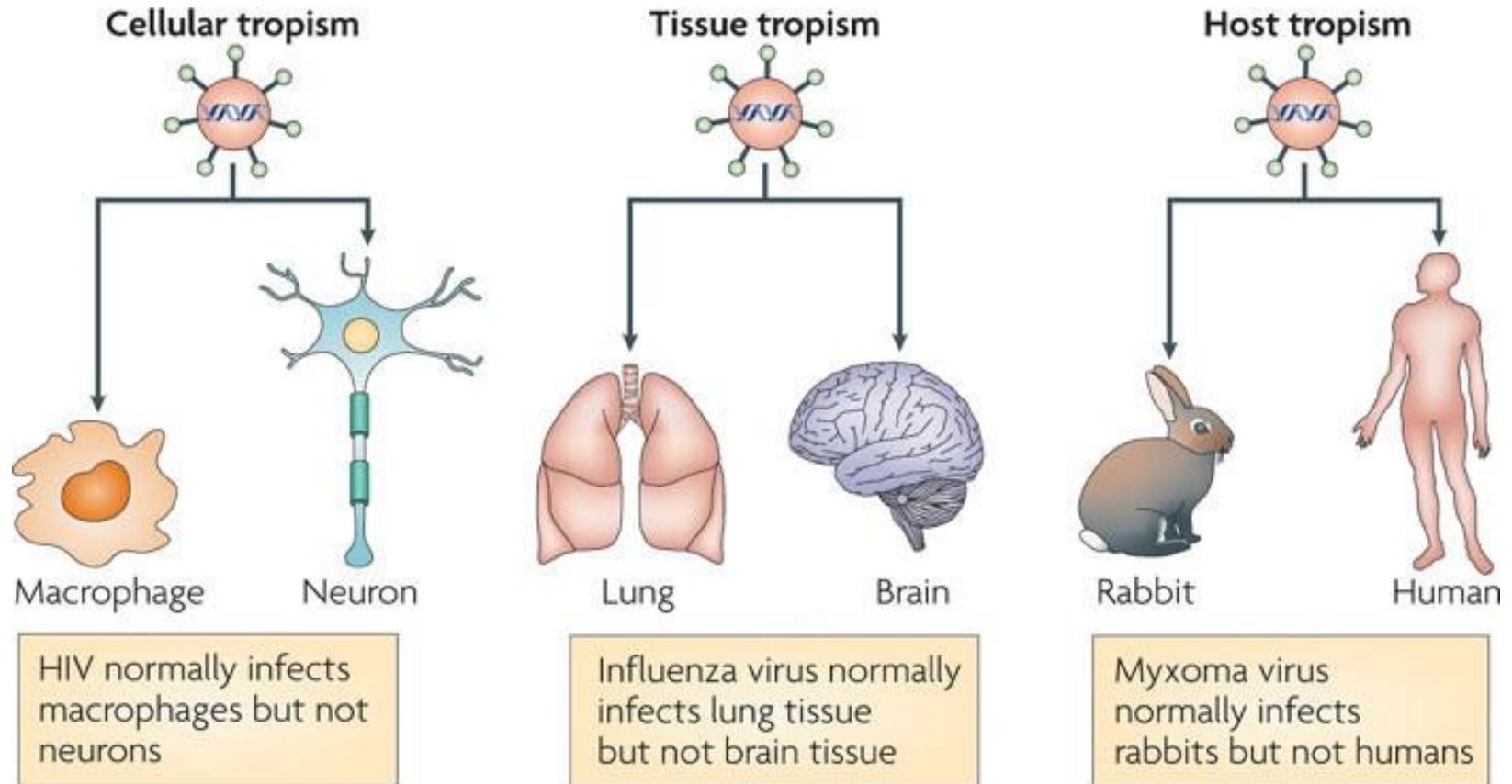
McFadden, G., *et al. Nat Rev Immunol* **9**, 645–655 (2009).

Viral tropism is a determinant of the resulting pathology

Meningitis/ Encephalitis
- Rabies
- JC virus
- LCM virus
- Measles
- Arbovirus

Parotitis
- Mumps virus

Eye infections
- Herpes simplex virus
- Adenovirus
- Cytomegalovirus

Common cold
- Rhinoviruses
- Parainfluenza virus
- Respiratory syncytial virus

Pharyngitis
- Cytomegalo virus
- Adenovirus
- Epstein- Barr virus

Pneumonia
- Influenza virus
- Parainfluenza
- Respiratory syncytial virus
- Adenovirus
- Coronavirus

Cardiovascular
- Coxsackie B virus

Hepatitis
- Hepatitis virus type A,B,C,D and E

Pancreatitis
- Coxsackie B virus

Gastroenteritis
- Adenovirus
- Rotavirus
- Norovirus
- Astrovirus
- Coronavirus

Skin infection
- Small pox
- Rubella
- Measles
- Human papilloma virus
- Human herpes virus 6
- Varicella zoster virus

Sexually transmitted diseases
- Herpes simples type 2
- Human papillomavirus
- HIV

Host defenses

Anatomical and chemical barriers

Intrinsic

Innate immunity

Acquired immunity

Skin, mucus, tears, low pH, surface cleansing

Host defenses

CONTINUOUS	IMMEDIATE	MINUTES/HOURS	HOURS/DAYS

Time Post-Exposure

Physical Barriers	**Intrinsic**	**Innate**	**Adaptive**
Mucus		Natural killer cells	T cells
Saliva	Autophagy	Complement	B cells
Stomach acid	Apoptosis	Antigen-presenting cells	
Tears	MicroRNAs	Neutrophils	
Skin	CRISPRs	**Interferon**	
Scabs			
Defensins			

Host defenses against viral infections

1. Physical barriers

2. Chemical barriers

3. Intrinsic cellular defenses

4. Innate soluble immune response: interferons, cytokines, inflammation, fever, complement

5. Innate cellular immune response: DC, macrophages, NK…

6. Adaptive humoral immune response

7. Adaptive cellular immune response

Host defenses against viral infections

PRINCIPLES *The early host response: cell-autonomous and innate immunity*

- The immediate response to an infection is based on two coupled processes: detection and alarm.

- Microbes contain unique components, including certain carbohydrates, nucleic acids, and proteins, that are recognized by cellular pattern recognition receptors present either on the cell surface or in the cytoplasm.

- Binding of a particular ligand to a pattern recognition receptor initiates a signal transduction cascade that results in activation of cytoplasmic transcription regulatory proteins such as Nf-κb and interferon regulatory factors.

- Apoptosis is a normal biological process that can be induced by the biochemical alterations initiated by virus infection.

- Most cells synthesize interferon when infected, and the released interferon inhibits reproduction of a wide spectrum of viruses.

- Phagocytes gather information and initiate the host immune response by taking up cellular debris and extracellular proteins released from dying or apoptosing cells.

- Mechanisms to limit viral reproduction that do not result in the death of the infected cell include autophagy, epigenetic silencing, RNA interference, cytosine deamination, and Trim protein interference.

- Infected cells, sentinel phagocytes, and cellular components of the innate and adaptive immune response secrete many different proteins that can result in activation and recruitment of immune cells, induction of signaling pathways, tissue damage, and fever.

- The innate immune response is crucial in antiviral defense because it can be activated quickly, functioning within minutes to hours of infection.

Intrinsic and innate immunity

- Viruses replicate very rapidly and would quickly overwhelm a host organism if it were undefended.

- The adaptive immune response is tailored to pathogen, is restricted to animals, and takes several days to gather momentum.

- In that period, processes of innate and intrinsic immunity slow down and contain a virus so that the host can gain ascendancy over it.

  - *Intrinsic:* Always present in the uninfected cell, sometimes specific against certain viruses: apoptosis, autophagy, antiviral restriction proteins

  - *Innate immune system*: Induced by infection (cytokines and cells)

Viral pathogenesis:
Intrinsic resistance to viruses in vertebrates

Host restriction factors
Apoptosis
Autophagy

Host restriction factors (HRFs)

HRFs are critical components of the early antiviral response. These cellular proteins inhibit viral replication and spread by impeding essential steps in the viral life cycle, such as viral entry, genome transcription and replication, protein translation, viral particle assembly, and release

Resistance factor	Virus targeted	Action
APOBEC3G	HIV	Causes genome hypermutation
IFITM3	Influenza A	Interferes with attachment or entry
MxA	Influenza A; other viruses	Binds nucleoprotein complexes
Tetherin	HIV, other retroviruses; paramyxo, filo, rhabdo and arenaviruses; KSHV	Reduces particle release

Host restriction factors (HRFs)

Table 1 Intrinsic antiviral factors

Name	Target virus	Key role(s)
APOBEC3G	HIV-1, SIV, EIAV, MLV, foamy virus, hepatitis B virus	Edit C to U in HIV DNA (negative strand); inhibit reverse transcription and integration
TRIM5α	HIV-1, MLV	Block uncoating of the incoming virion; promote innate immune signaling by sensing retroviral capsid
Tetherin	HIV-1, MLV, HTLV-1, Ebola virus, KSHV	Block release of enveloped viruses
SAMHD1	HIV-1	Inhibit HIV replication in myeloid cells, probably by regulating cellular dNTP supply
TREX1	HIV-1	Remove cytosolic nonproductive reverse-transcribed DNA; inhibit innate immune responses to HIV-1
IFITM family	Influenza virus, Dengue virus, West Nile virus	Block cytosolic entry
IFIT family	Influenza virus	Recognize 5′ triphosphate and the lack of 2′-O-methylation in viral RNA and inhibit translation
MxA	Influenza virus, other RNA viruses	Block transcription
RNase L	Many RNA viruses	Cleave single-stranded RNA in U-rich sequences; activate antiviral innate immunity
PKR	Many RNA viruses	Inhibit virus translation by protein phosphorylation; promote innate immune signaling

EIAV, equine infectious anemia virus; HTLV-1, human T lymphotropic virus type 1; KHSV, Kaposi's sarcoma–associated herpesvirus.
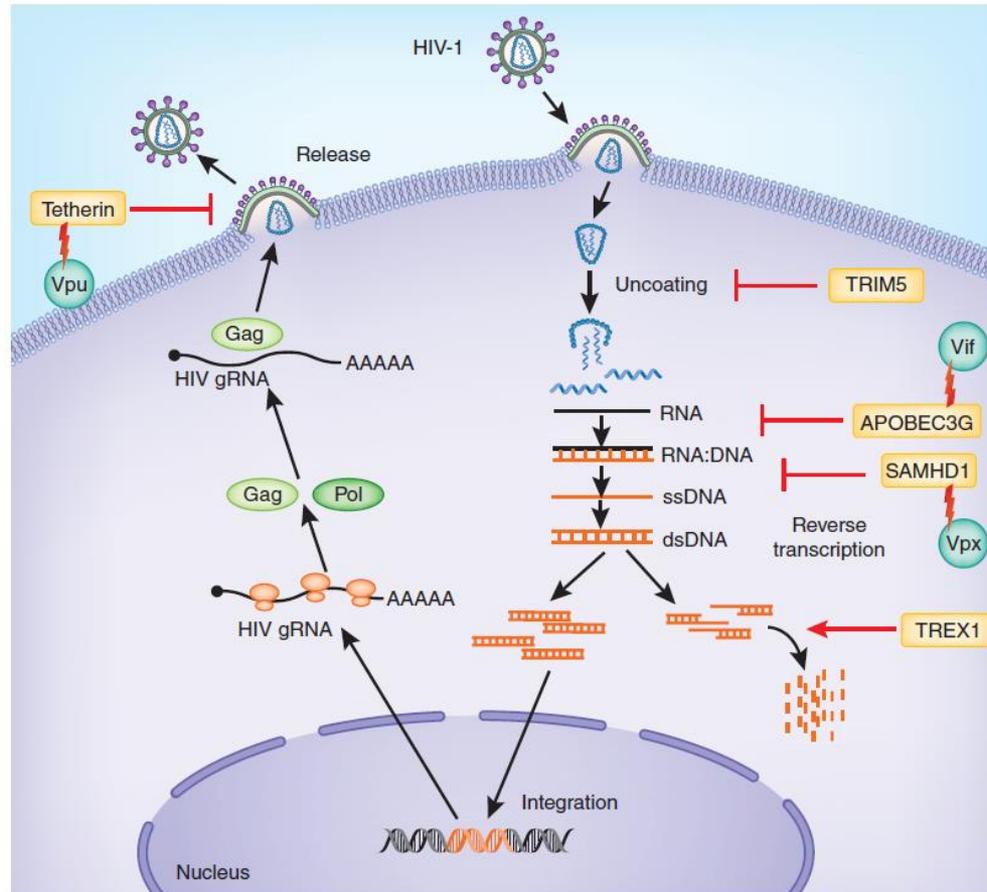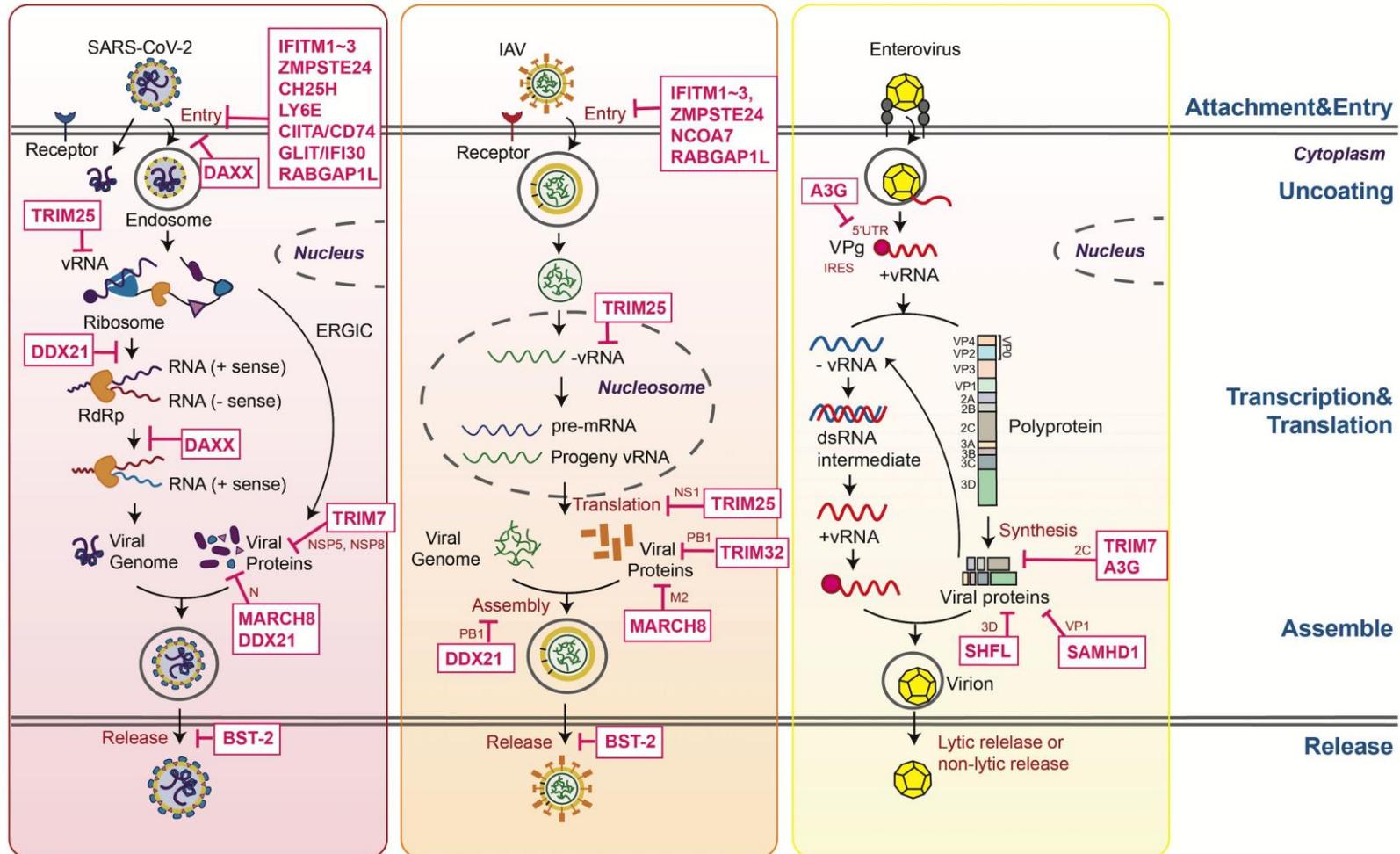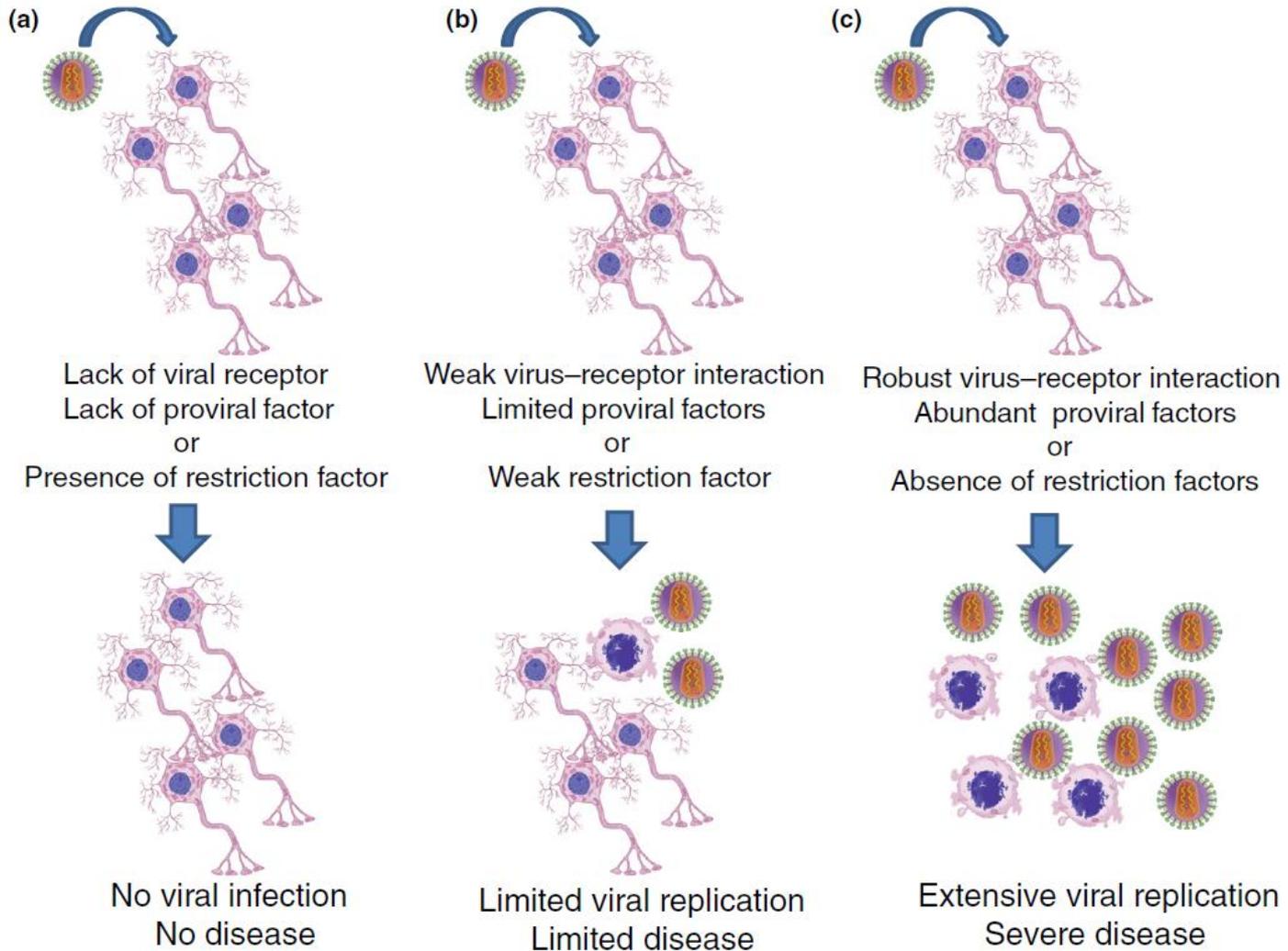
Host restriction factors (HRFs)

Figure 2 Intrinsic antiviral factors against HIV-1. Many steps of the HIV-1 life cycle are targeted by intrinsic antiviral factors such as TRIM5α, APOBEC3G, tetherin and SAMHD1. HIV-1 has evolved strategies to counteract these intrinsic antiviral factors, through accessory proteins such as Vif, Vpu and Vpx or other unknown mechanisms that are now under investigation. gRNA, genomic RNA; ssDNA, single-stranded DNA; dsDNA, double-stranded DNA.

These HRFs are classified into two types: interferon-stimulated genes (ISGs) and non-interferon-stimulated genes (non-ISGs). Unlike ISGs, these non-ISGs are constitutively expressed in cells and are not induced by interferons.

(a) Lack of viral receptor / Lack of proviral factor / or / Presence of restriction factor → No viral infection / No disease

(b) Weak virus–receptor interaction / Limited proviral factors / or / Weak restriction factor → Limited viral replication / Limited disease

(c) Robust virus–receptor interaction / Abundant proviral factors / or / Absence of restriction factors → Extensive viral replication / Severe disease

Though receptor interactions represent a crucial component of virus–host interactions and viral pathogenesis, a number of other factors can also determine which tissues a virus infects.

'Proviral' factors are host molecules that promote efficient viral replication.
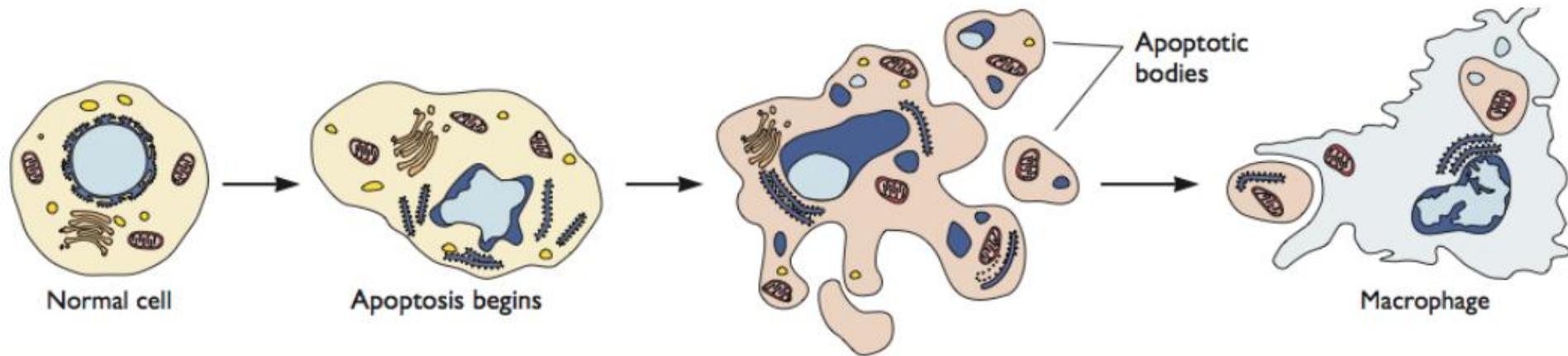
Apoptosis:
Intrinsic resistance to viruses in vertebrates
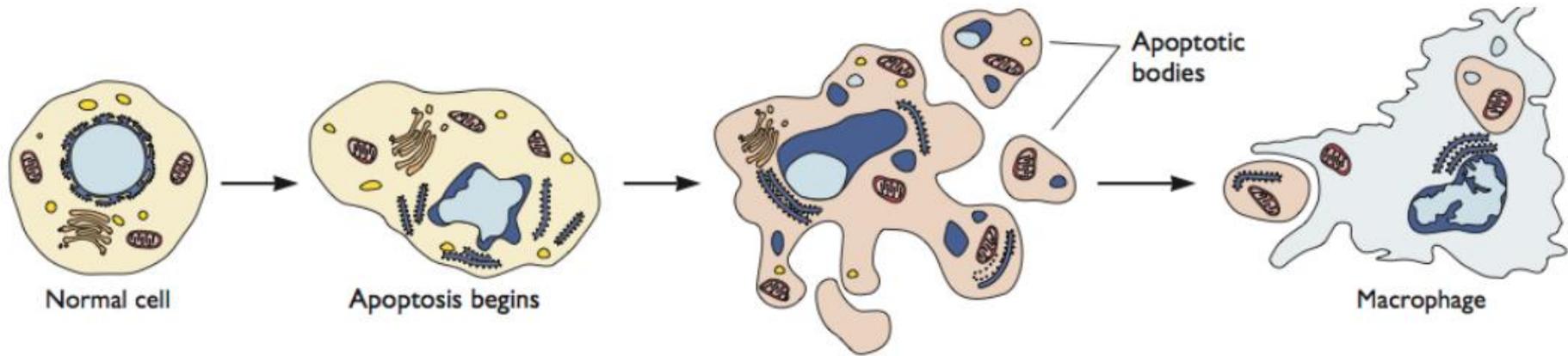
The death of an infected cell may help the host because it interrupts the replication cycle and releases the pathogen for killing by immune cells. However, viruses can induce cell death to facilitate their spread or killing the infected immune cells or inhibit these programmed death pathways to hide in the host cells from the immune cells

Apoptosis:
Intrinsic resistance to viruses in vertebrates

Cellular Target	Virus	Gene	Function
Bcl-2	Adenovirus	E1B 19K	Bcl-2 homolog
	Epstein-Barr virus	LMP-1	Increases synthesis of Bcl-2; mimics CD40/Tnf receptor signaling
Caspases	Adenovirus	14.7K	Inactivates caspase-8
Cell cycle	Hepatitis B virus	pX	Blocks p53-mediated apoptosis
	Human papillomavirus	E6	Targets p53 degradation
	Simian virus 40	Large T	Binds and inactivates p53
Fas/Tnf receptors	Adenovirus	E3 10.4/14.5K	Internalizes Fas
	Cowpox	CrmB	Neutralizes Tnf and LT-α
	Myxoma virus	MT-2	Secreted Tnf receptor homolog
vFLIPs; DED box-containing proteins	Human herpesvirus 8	K13	Blocks activation of caspases by death receptors
Oxidative stress	Molluscum contagiosum virus	MC066L	Inhibits UV- and peroxide-induced apoptosis; homologous to human glutathione peroxidase
Caspase 8 activation, Bax localization	Human Cytomegalovirus	UL36, UL37x1	Inhibit extrinsic and mitochondrial apoptosis

AUTOPHAGY

- **Self-degradative process** that is important for balancing sources of energy at critical times in development and in response to nutrient stress

- **Housekeeping role** in removing misfolded or aggregated proteins, clearing damaged organelles, such as mitochondria, endoplasmic reticulum and peroxisomes, as well as eliminating intracellular pathogens.

- Generally thought of as a **survival mechanism**

- **May promote cell death under stress** (e.g. viral infection, cancer)

Autophagy-virus interplay

Autophagy has a role as a defensive system against viral infections

- Degradation of viral components and newly forming virus particles (**xenophagy**)
- Cooperates with innate immunity

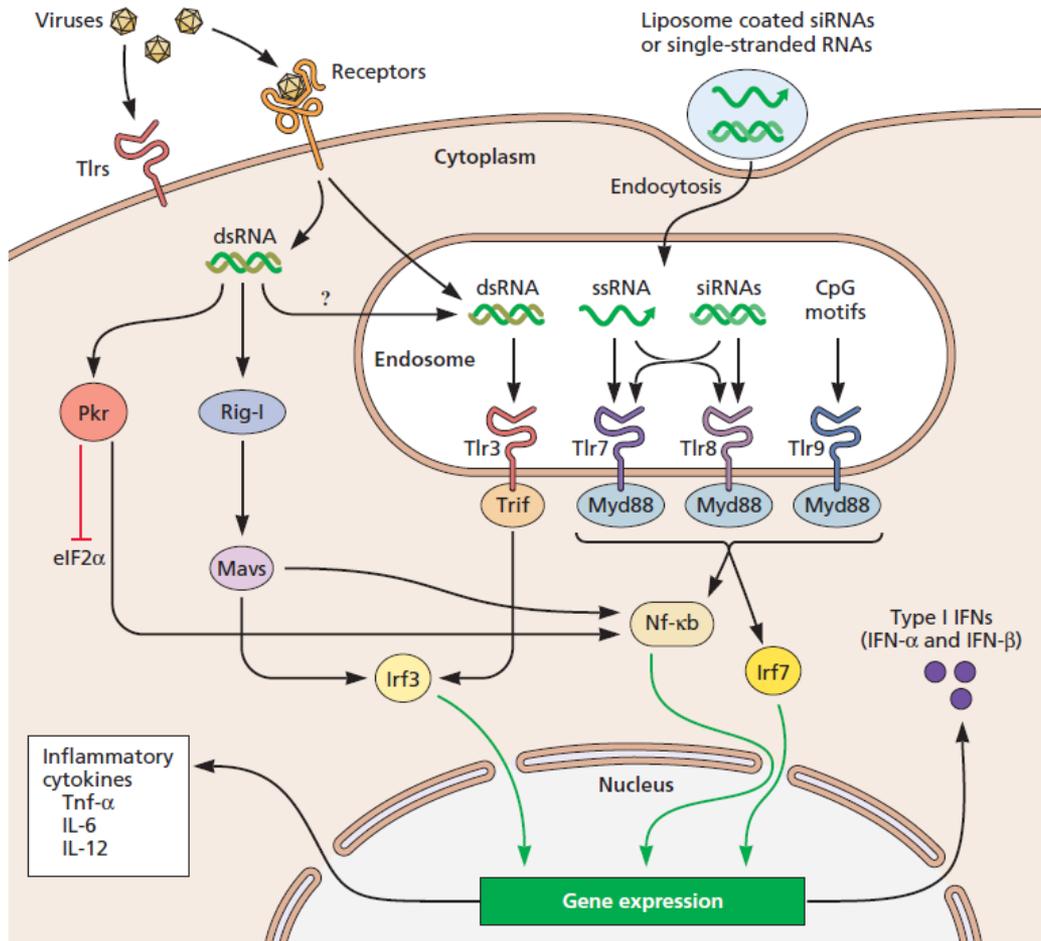**Viruses interact variably with the autophagy mechanisms**

- Virulence factors may **block** autophagy
- Some viruses **exploit** autophagy for their replication or to egress from cells without lysis (establishment of chronic productive infection)
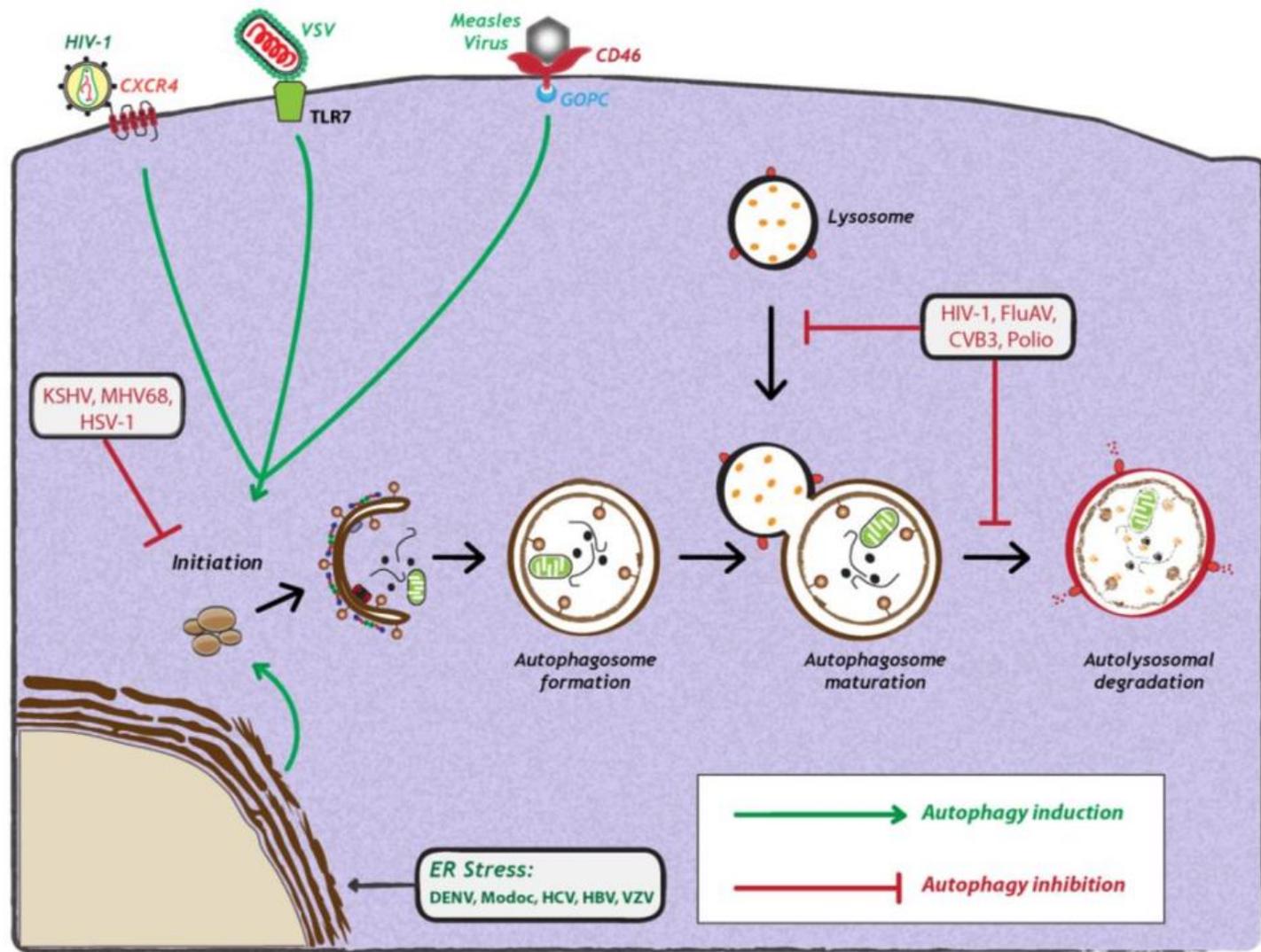
Viral manipulation of autophagy. Viruses can either induce (green arrows) or inhibit the autophagy pathway (red studded lines). Viral induction of autophagy can be achieved during viral entry via interaction with cell surface receptors, via interaction with stress sensors or during viral replication. Viruses also inhibit autophagy at early or late stages of the pathway, during initiation or maturation, respectively.

Innate antiviral response

- PRRs – Pattern-recognition receptors
- PAMPs – Pathogen-associated molecular patterns
- Major viral PAMPs are viral nucleic acids

Figure 3.2 Recognition of foreign nucleic acids in mammalian cells. The Tlrs, Rig-I, and Pkr all contribute to detection of microbe-associated molecular patterns including ssRNA, dsRNA, RNA nucleotides, siRNAs, and unmethylated CpG-containing oligonucleotides. As the receptor's cognate nucleic acid is bound on the cell surface, in the cytoplasm, or in the lumen of endosomes, signal transduction leads to activation of Nf-κb, Irf3, or Irf7 to induce expression of inflammatory cytokines and IFN-α/β. Important cytoplasmic proteins in the signal transduction cascade, including Trif and Myd88, bind the cytoplasmic tails of endosomal Tlr proteins after they have engaged their cognate ligand. Viral RNA and DNA may be exposed in the lumen of endosomes after degradation or uncoating events. Pkr is autophosphorylated when dsRNA is bound, leading to phosphorylation of its substrates. One such substrate is the α subunit of eukaryotic translation initiation factor 2 (eIF2α). Phosphorylation of this protein blocks protein synthesis.

Retinoic acid-inducible gene I
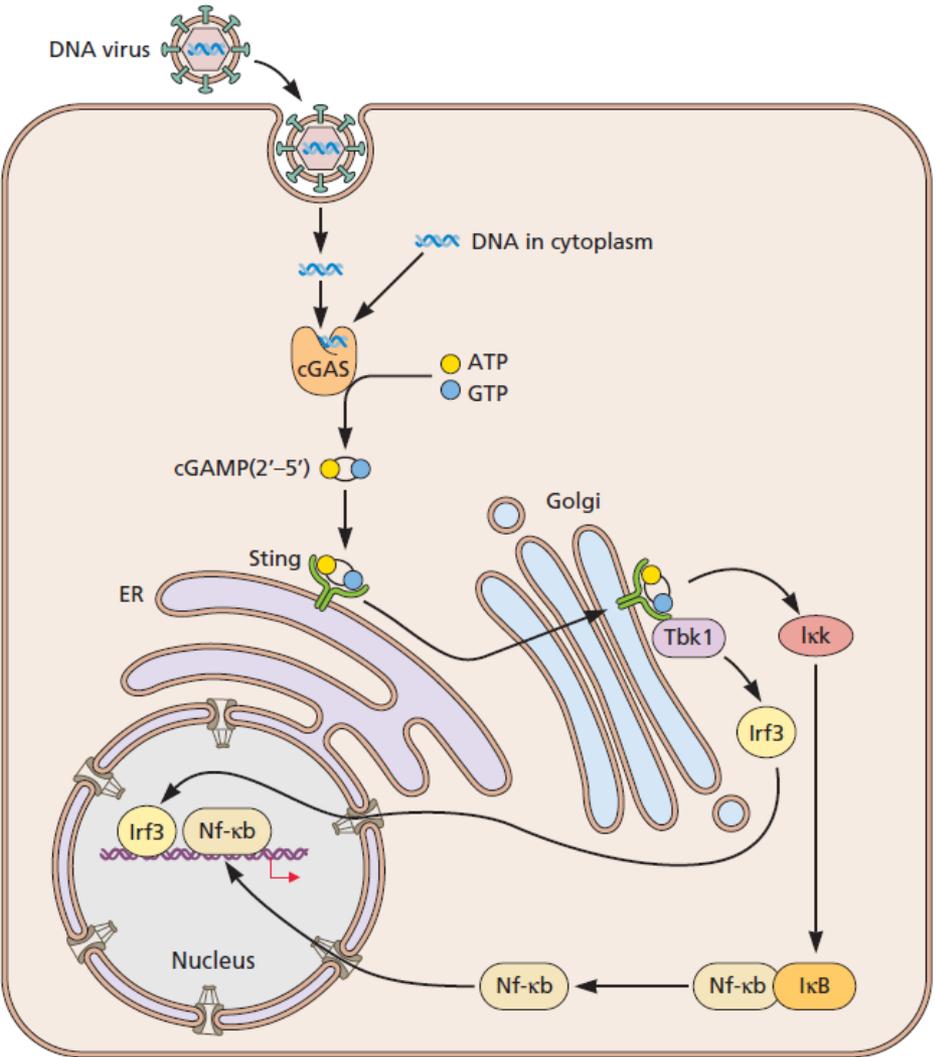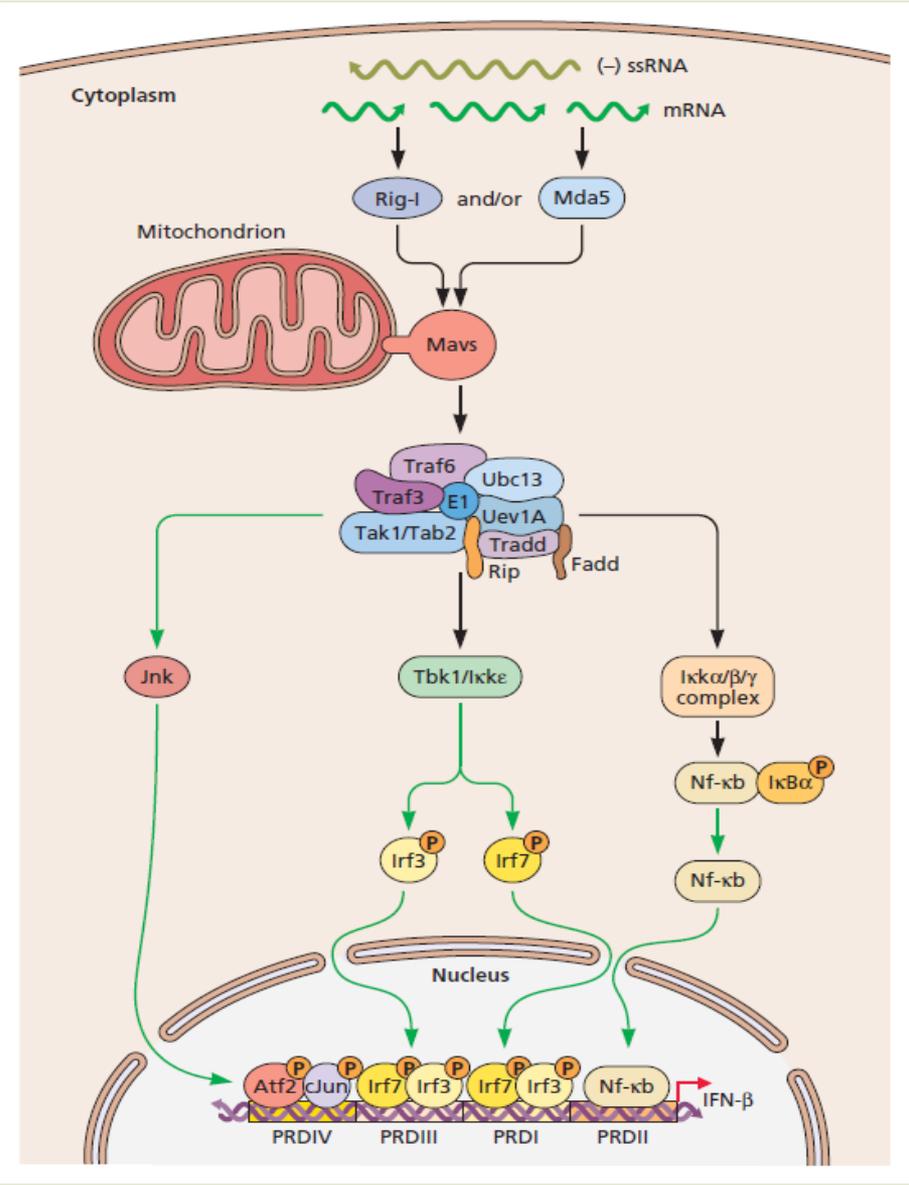Melanoma differentiation-associated protein 5

Figure 3.3 The cGAS/Sting axis in innate immunity. dsDNA in the cytoplasm (either from microbes with DNA genomes or from the cell nucleus) is detected by cyclic GMP-AMP (cGAMP) synthase (cGAS), which is activated to synthesize the cyclic dinucleotide (CDN) cGAMP(2′-5′) as its second messenger molecule (using the substrates ATP and GTP). cGAMP(2′-5′) then binds and activates the endoplasmic reticulum (ER)-resident receptor stimulator of interferon genes (Sting). Activated Sting then translocates to a perinuclear Golgi compartment, where it binds to TANK-binding kinase 1 to activate Irf3 and to induce Nf-κb activation.

Box 13.2

Pattern recognition receptors that respond to virus infection[*]

Receptor[†]	Pathogen pattern recognized	Examples of viruses affected[‡]
TLR2 (PM)	Envelope fusion proteins	Class 1: CMV, HSV, VZV
		Class 5: LCMV, measles, VSV
TLR3 (endo)	dsRNA	Class 1: EBV
		Class 3: Reovirus
		Class 4: EMCV, West Nile virus
		Class 5: RSV
TLR4 (PM)	Envelope fusion proteins	Class 5: Ebola virus, RSV
		Class 6: MMTV
TLR7/8 (endo)	GU-rich ssRNA	Class 4: Coxsackie B, Sendai virus
		Class 5: influenza A virus, VSV
		Class 6: HIV1
TLR9 (endo)	DNA with unmethylated CpG	Class 1: CMV, HAdV, HSV
RIG-I (RLR; cyto)	RNA with 5' triphosphate; short dsRNA	Class 1: EBV
		Class 3: Reovirus
		Class 4: flaviviruses
		Class 5: orthomyxo, paramyxo and rhabdoviruses
Mda5 (RLR; cyto)	Long dsRNA	Class 1: vaccinia
		Class 5: PIV5 (was SV5), other paramyxoviruses
IFI16 (cyto)	dsDNA	Class 1: HSV, KSHV
AIM2 (cyto)	dsDNA	Class 1: vaccinia
DDX41 (cyto)	dsDNA	Class 1: HAdV, HSV
DDX1/3/21 DHX9/36 (cyto)	dsRNA	Class 3: Reovirus
		Class 5: influenza A virus, VSV
DHX9/36 (cyto)	CpG dsDNA	Class 1: HSV
PolIII (cyto)	AT-rich dsDNA	Class 1: EBV, HAdV, HSV
DAI (cyto)	dsDNA	Class 1: HSV
cGAS (cyto)	dsDNA	Class 1: HSV; vaccinia

[*] The fact that a virus is not listed for a given receptor does not mean that the receptor is necessarily irrelevant for that virus.

[†] Receptor abbreviations: cGAS – cyclic GMP-AMP synthetase; cyto – cytoplasmic; DDX/DHX – DEAD/DEAH box helicase; endo – endocytic compartment; PM – plasma membrane; PolIII –RNA polymerase III; RLR – RIG-I-like receptor; TLR – Toll-like receptor.

[‡] Virus abbreviations: CMV – cytomegalovirus; EBV – Epstein Barr virus; EMCV – encephalomyocarditis virus; HAdV – human adenovirus; HIV1 – human immunodeficiency virus 1; HSV – herpes simplex virus; KSHV – Kaposi's sarcoma herpesvirus; LCMV – lymphocytic choriomeningitis virus; MMTV – mouse mammary tumour virus; PIV5 – parainfluenzavirus 5; RSV – respiratory syncytial virus; SV5 – simian virus 5; VSV – vesicular stomatitis virus; VZV – varicella-zoster virus.
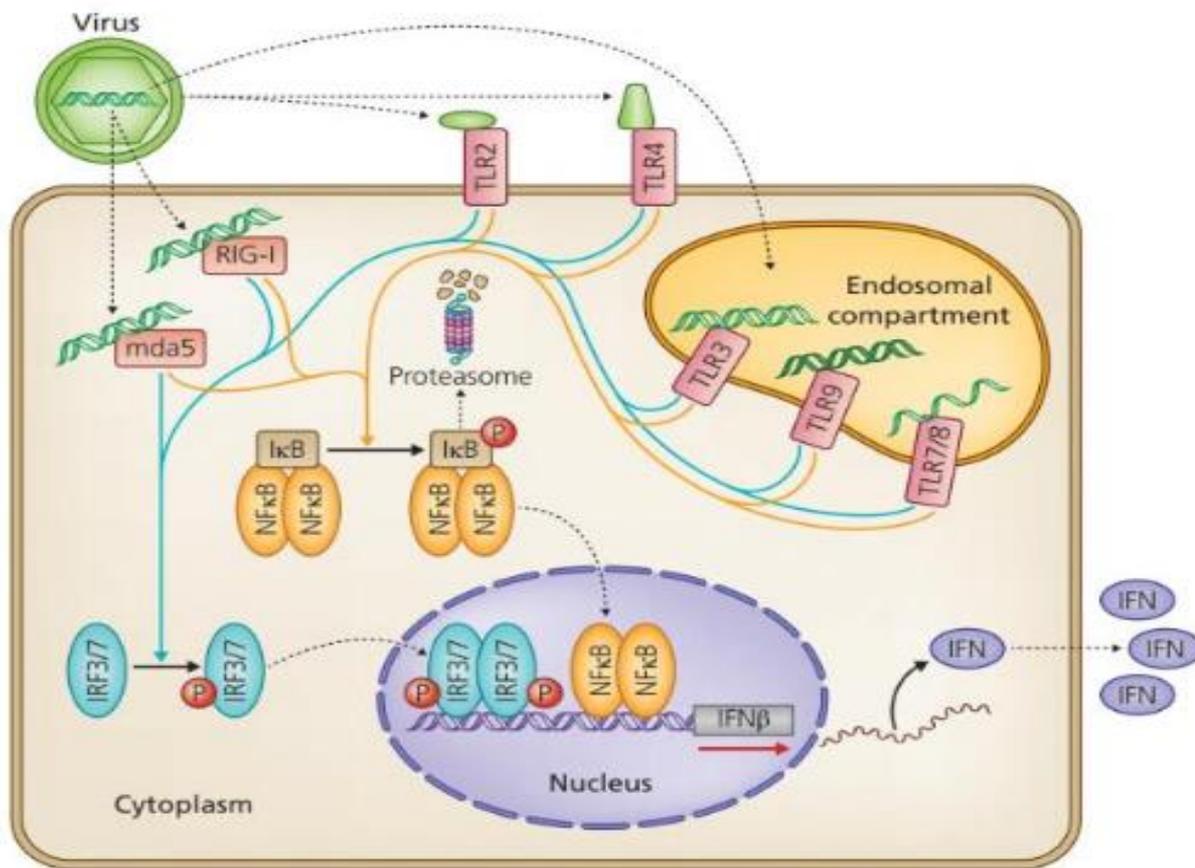
Fig. 13.1 Detection of virus by pattern recognition receptors (PRR). Infection is sensed by a combination of plasma membrane, endosomal and cytoplasmic PRRs of which the most significant for virus infection are TLR2, 3, 4, 7/8, 9, RIG-I and mda5. Cytoplasmic DNA receptors (not shown) are also important for DNA virus recognition. Each virus will be recognized by one or more PRR. Activated PRRs recruit specific adaptor molecules and, via signalling cascades, cause the phosphorylation of IRF3 or IRF7 (blue arrows) and IκB (orange arrows). Phospho-IRF3/7 forms homo- or hetero-dimers which bind target promoters in the nucleus, including the one controlling IFNβ gene expression. Phospho-IκB is a target for ubiquitin-mediated degradation, releasing active NFκB to bind target promoters. IRF3/7 and NFκB, with ATF/c-jun (not shown) activate IFNβ gene expression. Movement of factors is indicated by black dotted arrows. Note that TLR7/8 and 9 activate IRF7, not IRF3, and operate principally in plasmacytoid dendritic cells; most cell types do not express IRF7 prior to stimulation.

IFN mediated innate immunity
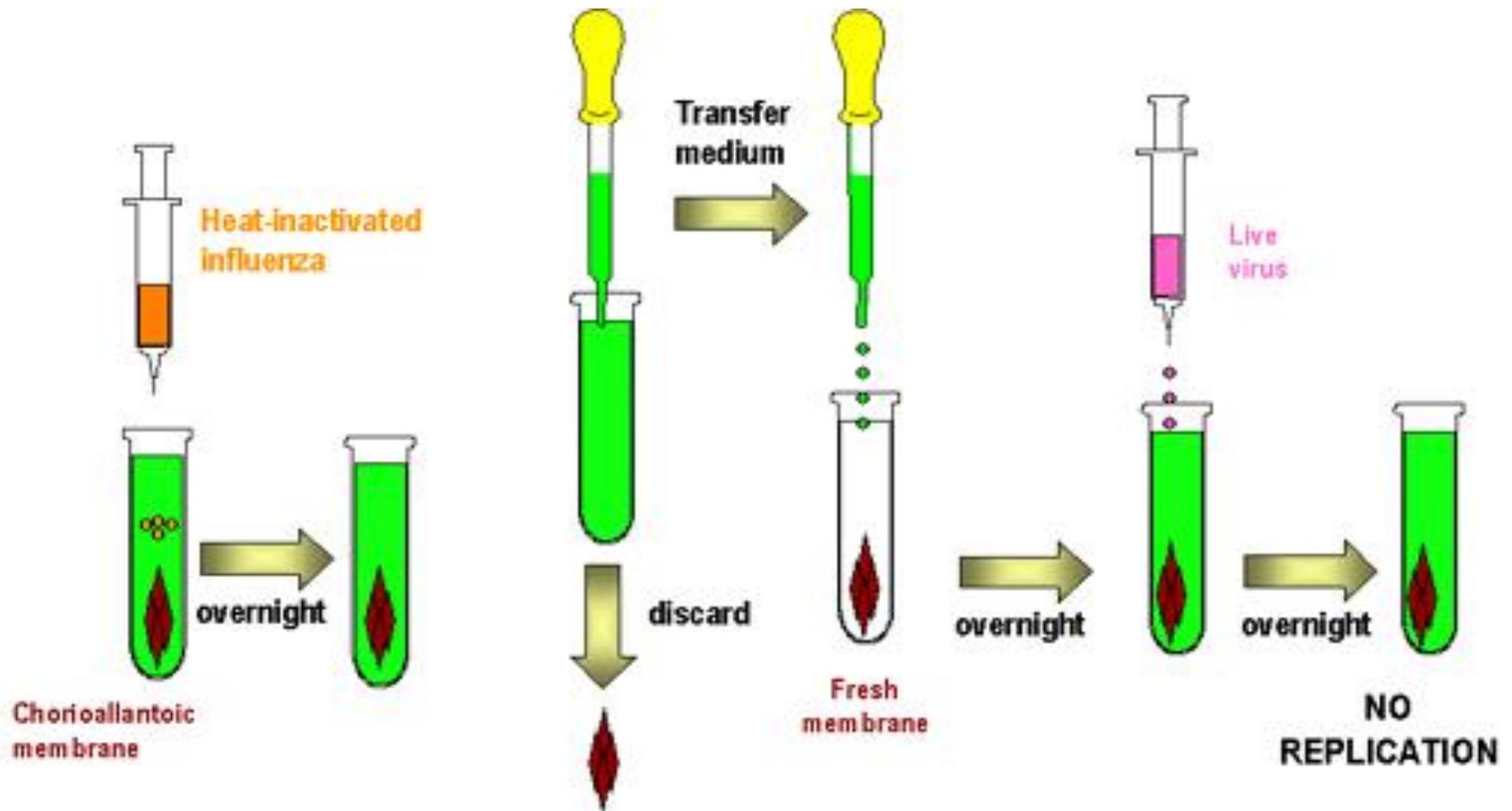
The Discovery of Interferon

From Isaacs and Lindenmann, Proc. Roy Soc B, 1957

The IFN system can be dangerous

- IFN induces the expression of many deleterious gene products – most of our cells have IFN receptors

- IFNs have dramatic physiological consequences: fever, chills, nausea, malaise

- *Every viral infection results in IFN production*, one reason why 'flu-like' symptoms are so common

IFN mediated immunity

Type	Subtype	Receptor
Type I	INF-α INF-β INF-ε INF-κ INF-ω	IFNAR1、IFNAR2
Type II	INF-γ	IFNGR1、IFNGR2
Type III	INF-λ	IFNLR1、IFNLR2

Interferon Induction

- **interferon-alfa, interferon-beta and interferon-lambda**
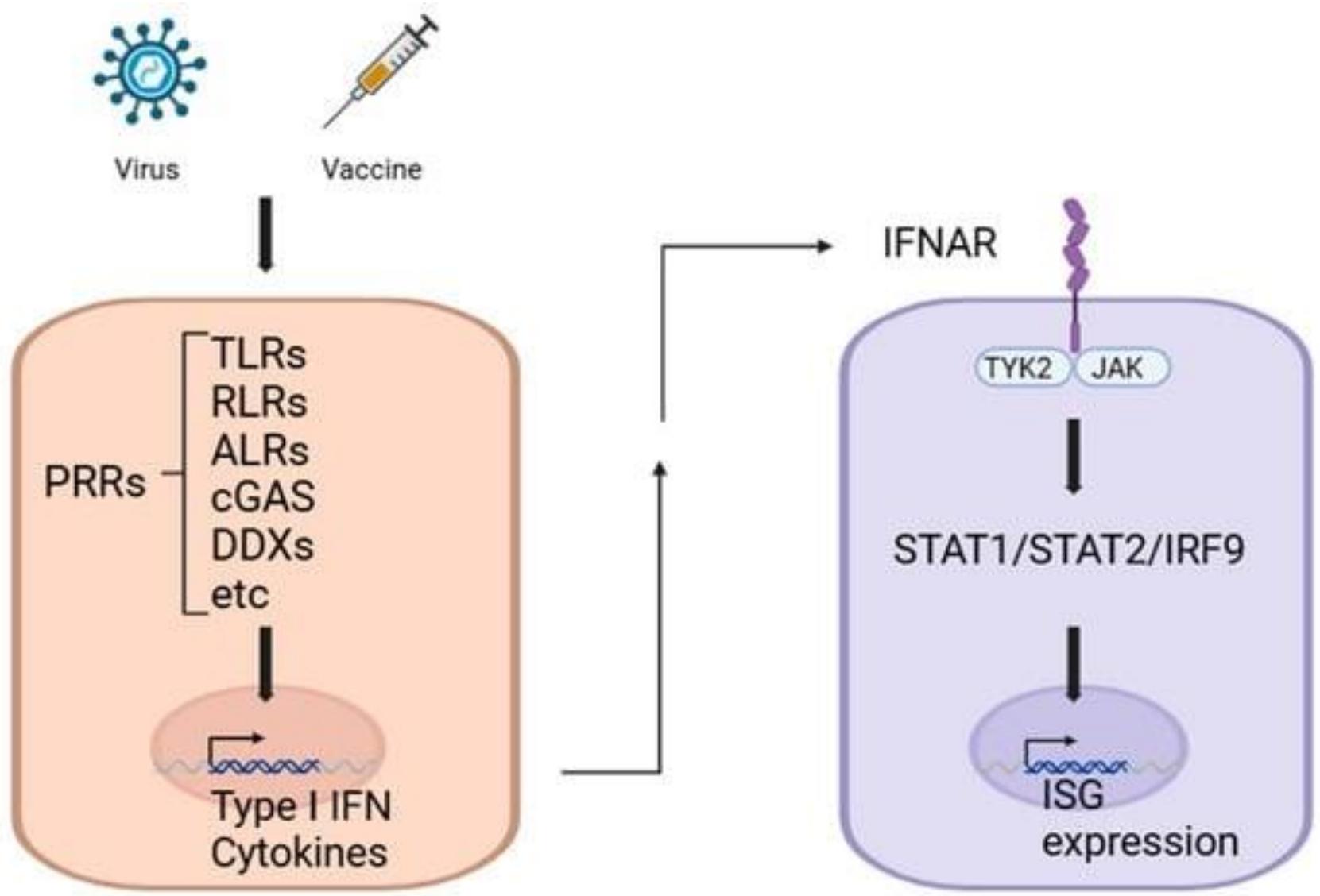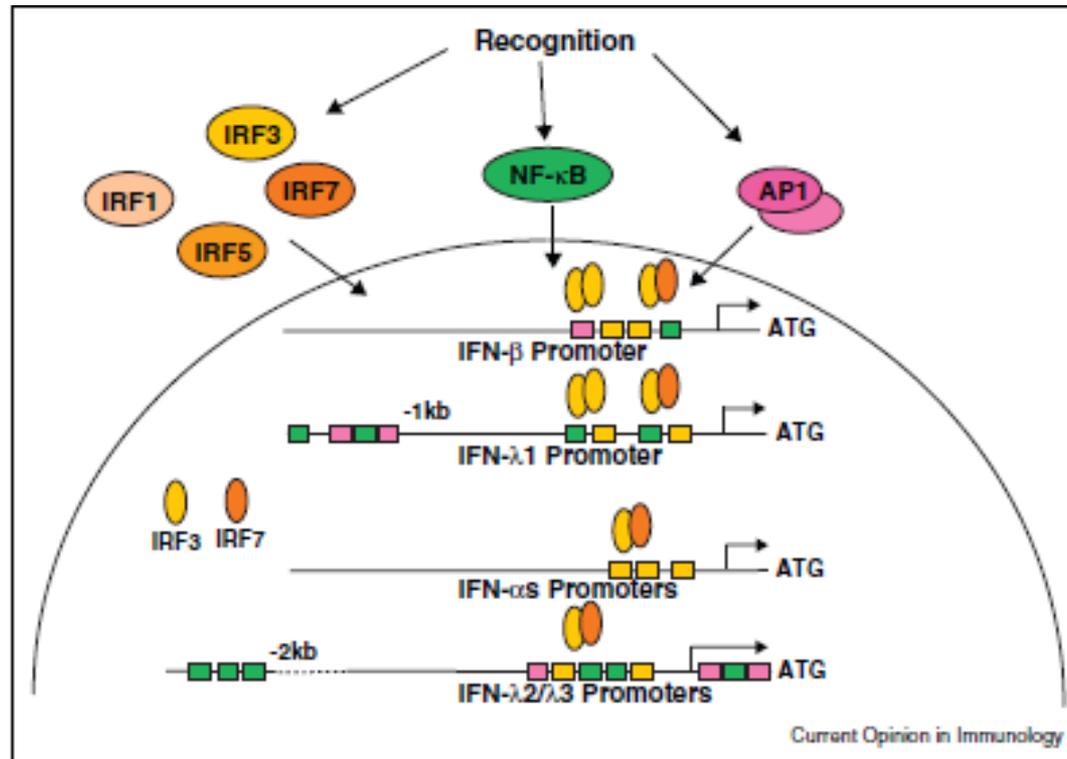 - Induced by
    - Viral infections
    - dsRNA
    - Some bacterial components

- **interferon-gamma**
  - antigens, mitogens

Virus

Vaccine

PRRs { TLRs
RLRs
ALRs
cGAS
DDXs
etc

Type I IFN
Cytokines

IFNAR

TYK2   JAK

STAT1/STAT2/IRF9

ISG
expression

# Induction of transcription of Type I and Type III-IFNs

Human IFNλ1 and IFN-β genes have similar transcriptional regulation that is controlled by either IRF3 or IRF7, whereas IFN-λ2/ 3 genes, like most IFN-α genes, are more dependent on IRF7.
IRF3 is constitutively and ubiquitously expressed in cells and, **when activated upon viral entry, upregulates expression of the IFN-β and IFN-λ1 genes**. By contrast, **IFN-α and IFN-λ2/3 genes are unresponsive to IRF3 alone and require IRF7 that is not constitutively expressed in most cell types but is induced in response to IFNs.** In humans, both IFN-β and IFN-λ1 can prime cells for virus-induced IFN-α and IFN-λ2/3 production by upregulating IRF7 expression. Similar to IFN-β, the IFN-λ1 gene represents an early response gene, whereas IFN-λ2/3 are likely to be expressed similar to IFN-αs, with delayed kinetics.

IFN Induction by dsRNA binding to TLR3
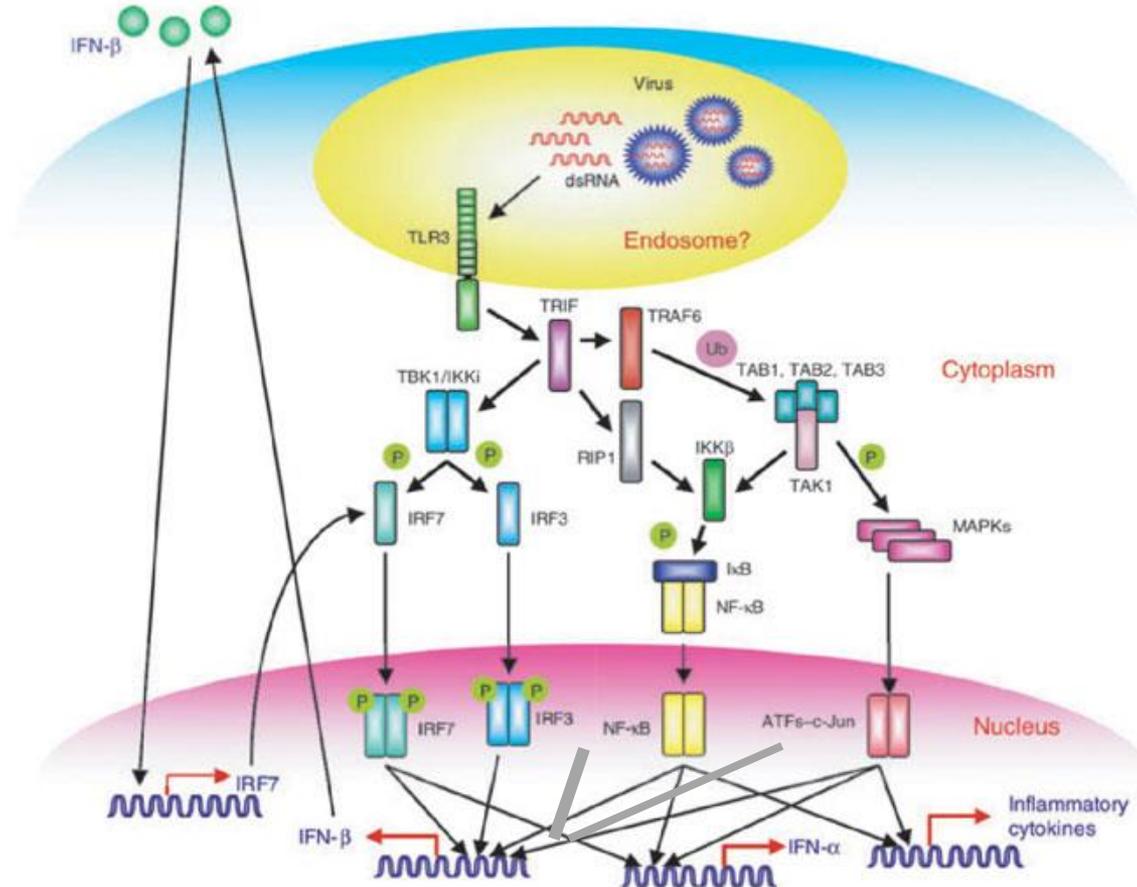
After recognizing dsRNA, TLR3 transmits signals through TRIF, which interacts with TBK1, RIP1 and TRAF6. TBK1, together with IKKi, phosphorylates (P) IRF3, allowing IRF3 to translocate into the nucleus and activate type I and III interferon promoters, particularly the IFN-β and IFN-λ1 promoters. Secreted IFNs stimulate expression of IRF7, which induces also IFN-α and the other λ IFNs.

IFN Induction by ssRNA and CpG DNA
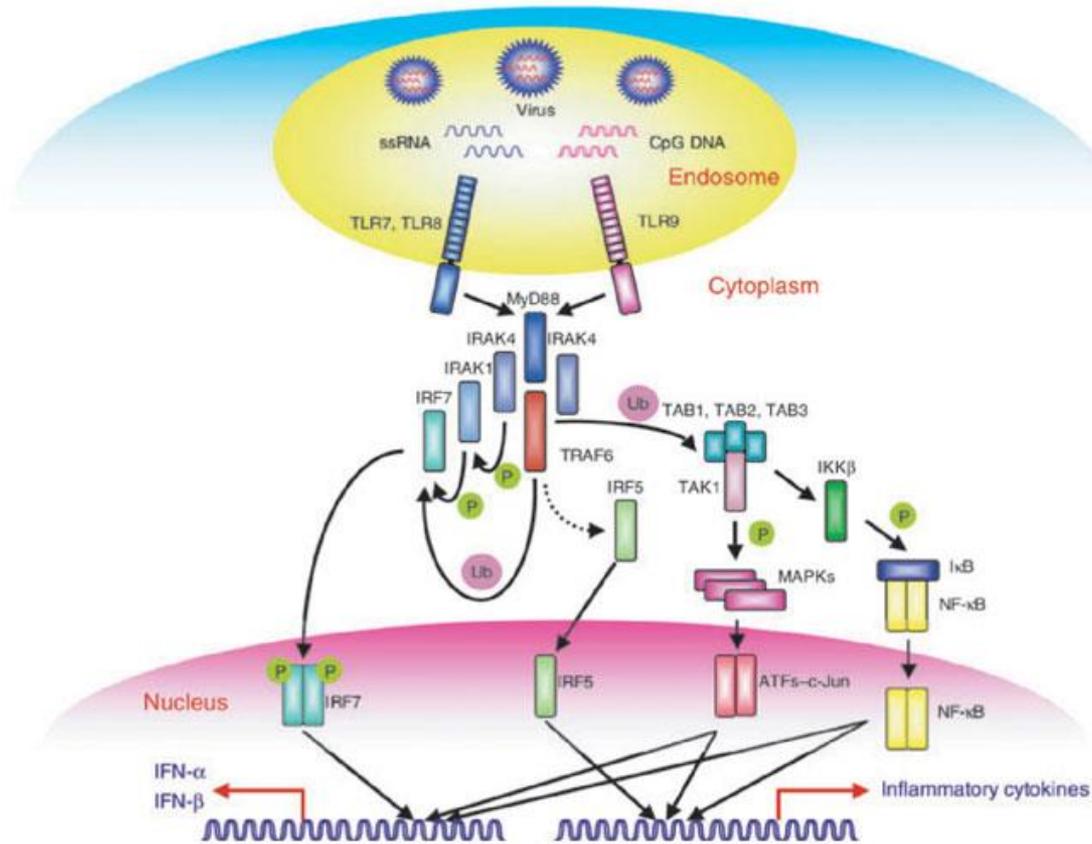
TLR7/8 and TLR9 recognise ssRNA and CpG DNA, respectively, utilise MyD88 as adaptor and specifically activate IRF-7 in addition to NF-kB and ATF2/c-jun.

Cytoplasmic sensors for viral nucleic acids.
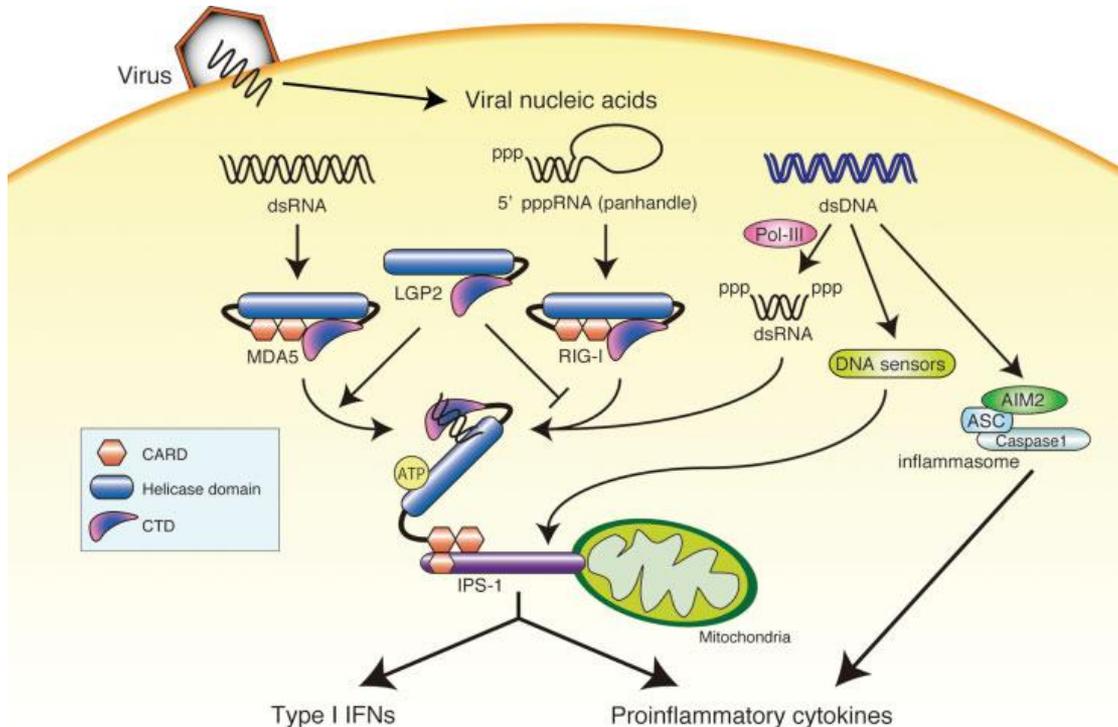
IPS-1: IFN β-promoter stimulator

DAI=DNA-dependent activator of IRFs

Cytoplasmic sensors for viral nucleic acids. RIG-I and MDA5 recognise viral 5'-triphosphate-containing RNA with panhandle structure and long dsRNA, respectively. On the other hand, DNA sensors are thought to recognise cytoplasmic viral dsDNA, and activate the IPS-1-dependent signalling. Some cytoplasmic dsDNAs, such as poly(dA:dT), are transcribed by Pol-III, and the resultant dsRNA is recognised by RIG-I.
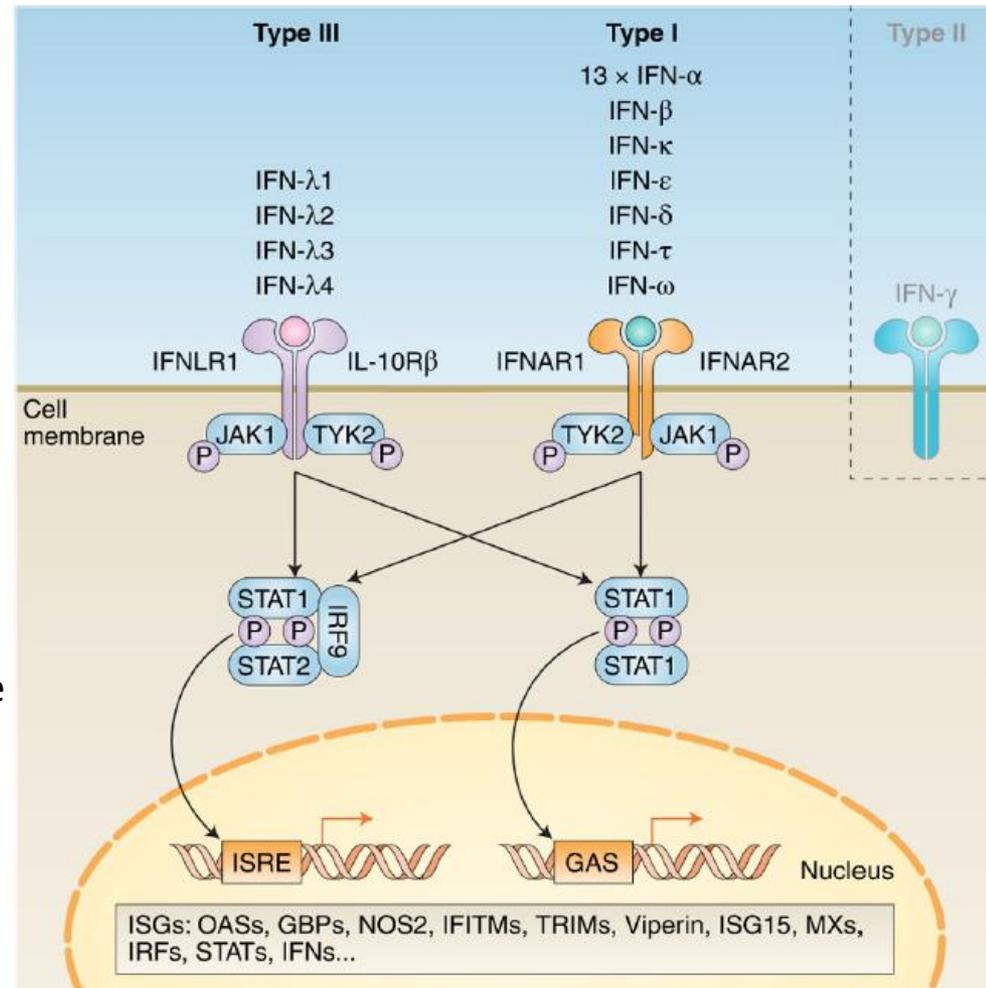
Interferon receptor signalling

type I IFNs bind to the IFNAR receptor complex (composed of IFNAR1 and IFNAR2); type III IFNs bind to the IFNLR receptor complex (composed of INFLR1 and IL-10Rβ).

Binding of an IFN to either receptor complex results in cross-phosphorylation of JAK1 and TYK2 on the cytoplasmic domains of the receptor subunits.

This triggers phosphorylation of STAT1 and STAT2. Following phosphorylation, these STATs form various complexes that translocate into the nucleus, where they bind IFN-stimulated response elements (ISREs) or gamma-activated sequences (GASs) on the promoters of ISGs.

Binding to these promoter elements results in the transcription of hundreds of genes involved in antiviral response, including ISGs, IFNs, IRFs and STATs

Interferon receptor signalling

Type III IFN or IFN-lambda (IFN-λ)

In humans, the type III IFN family consists of four members: IFN-λ1 (IL-29), IFN-λ2 (IL28A), IFN-λ3 (IL-28B) and IFN-λ4. IFN-λ receptors are largely restricted to cells of epithelial origin.
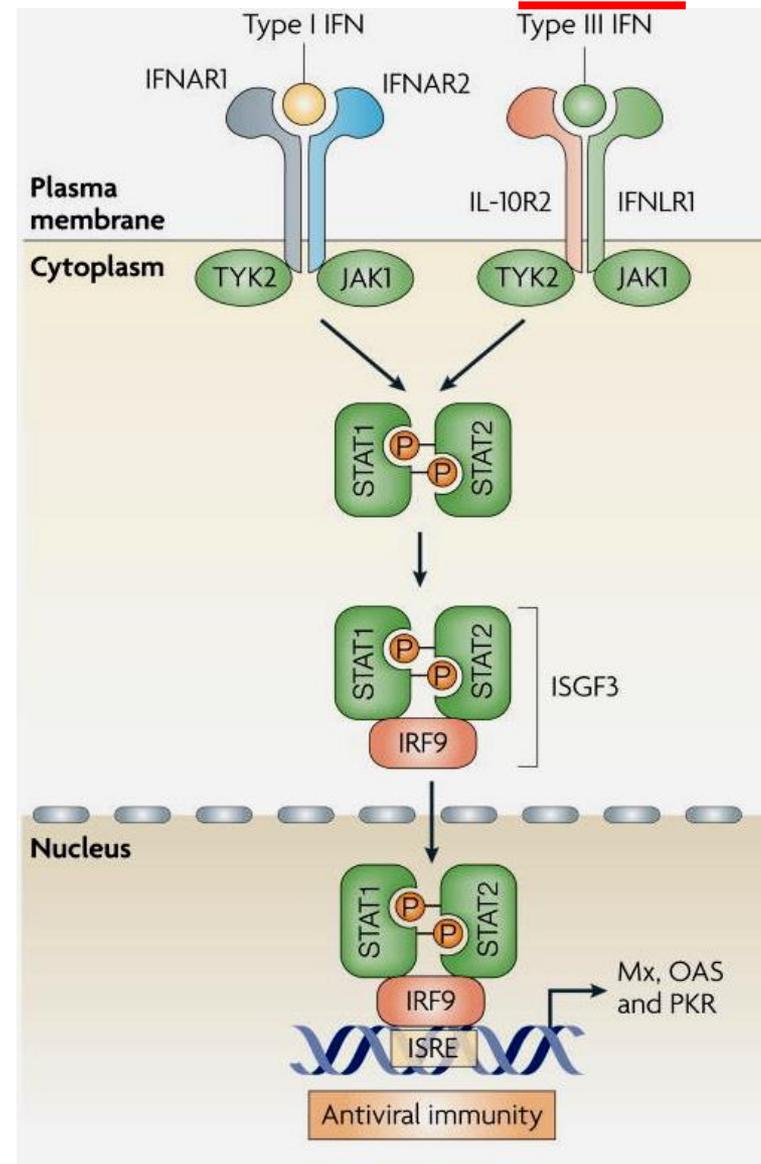
IFN Action Produces an Antiviral State

Interferon: effectors

RNase L and 2'-5'-oligo(A) synthetase.

Another well-studied antiviral response induced by IFN is mediated by two enzymes and dsRNA.

RNase L can degrade most cellular and viral RNA species. Its concentration increases 10- to 1,000-fold after IFN treatment, but the protein remains inactive unless a second enzyme is synthesized.

This enzyme, 2'-5'-oligo(A) synthetase, makes oligomers of adenylic acid, but only when triggered by dsRNA. These unusual nucleotide oligomers then activate RNase L, which in turn begins to degrade all host and viral mRNA.

We now know from studies of mouse mutants defective in RNase L that this enzyme is important for the IFN-b response to viral infection.

RNA fragments produced by RNAse L have double-stranded regions that allow them to be identified by RNAse L and Mda5, enhancing the production of IFN-b.

Interferon: effectors

dsRNA-activated protein kinase. Viral and cellular protein synthesis in infected cells is often stopped abruptly. In many cases, this phenomenon, mediated by a cellular dsRNA-activated protein kinase (Pkr), is lethal to both the virus and the infected cell

Establishment of the Pkr-mediated antiviral state is a two-step process, in which IFN promotes the increased production and accumulation of an inactive protein that can become activated only when it encounters double-stranded viral RNA

Active Pkr phosphorylates the alpha subunit of the eIF2 translation initiation protein (eIF2a), rendering it incapable of supporting protein synthesis in the cell (consequences are cell death or autophagy)
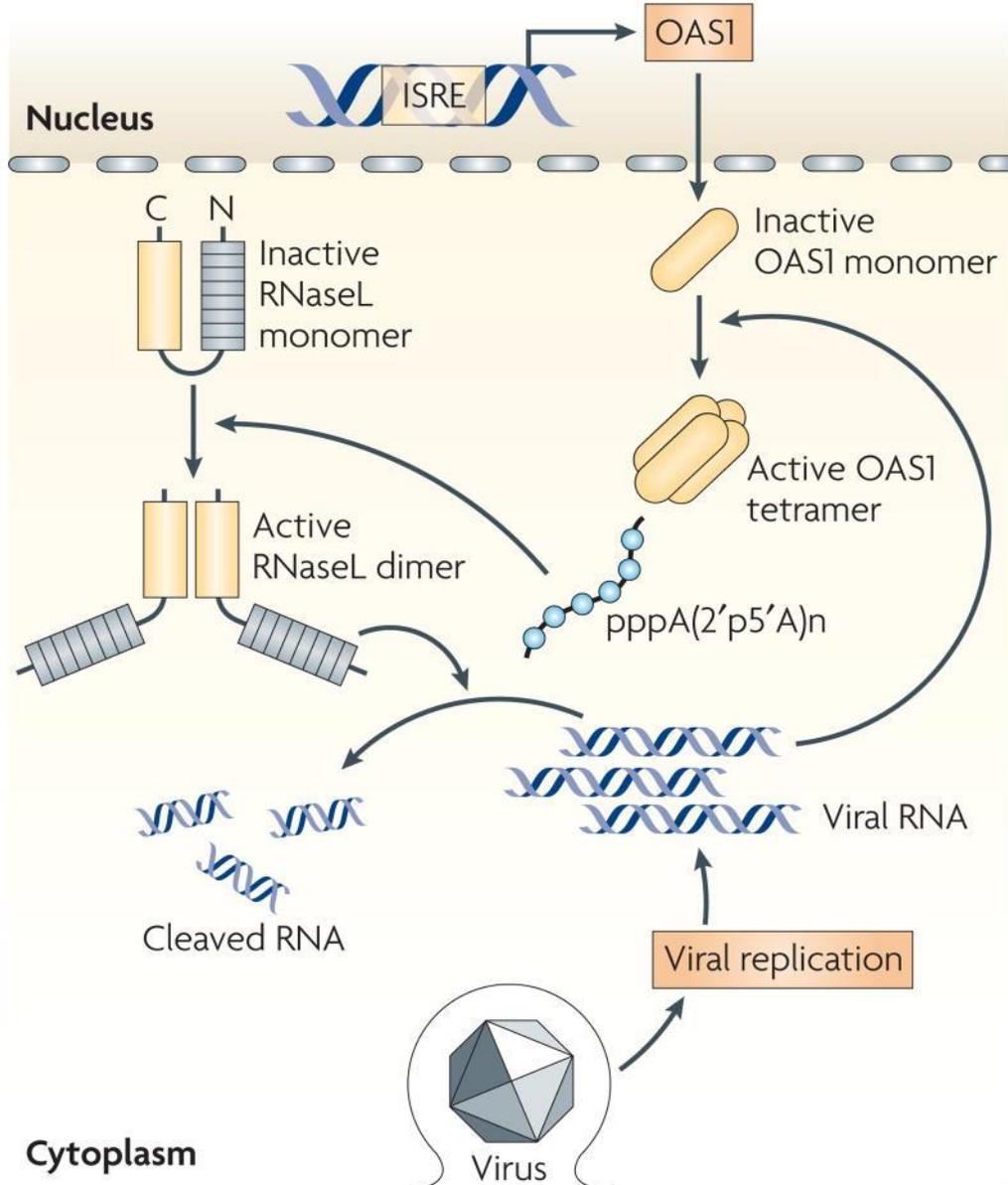
Many viral genomes encode proteins that can block the lethal actions of Pkr
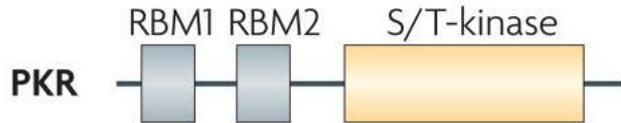
Interferon: effectors

IFN Action Produces an Antiviral State
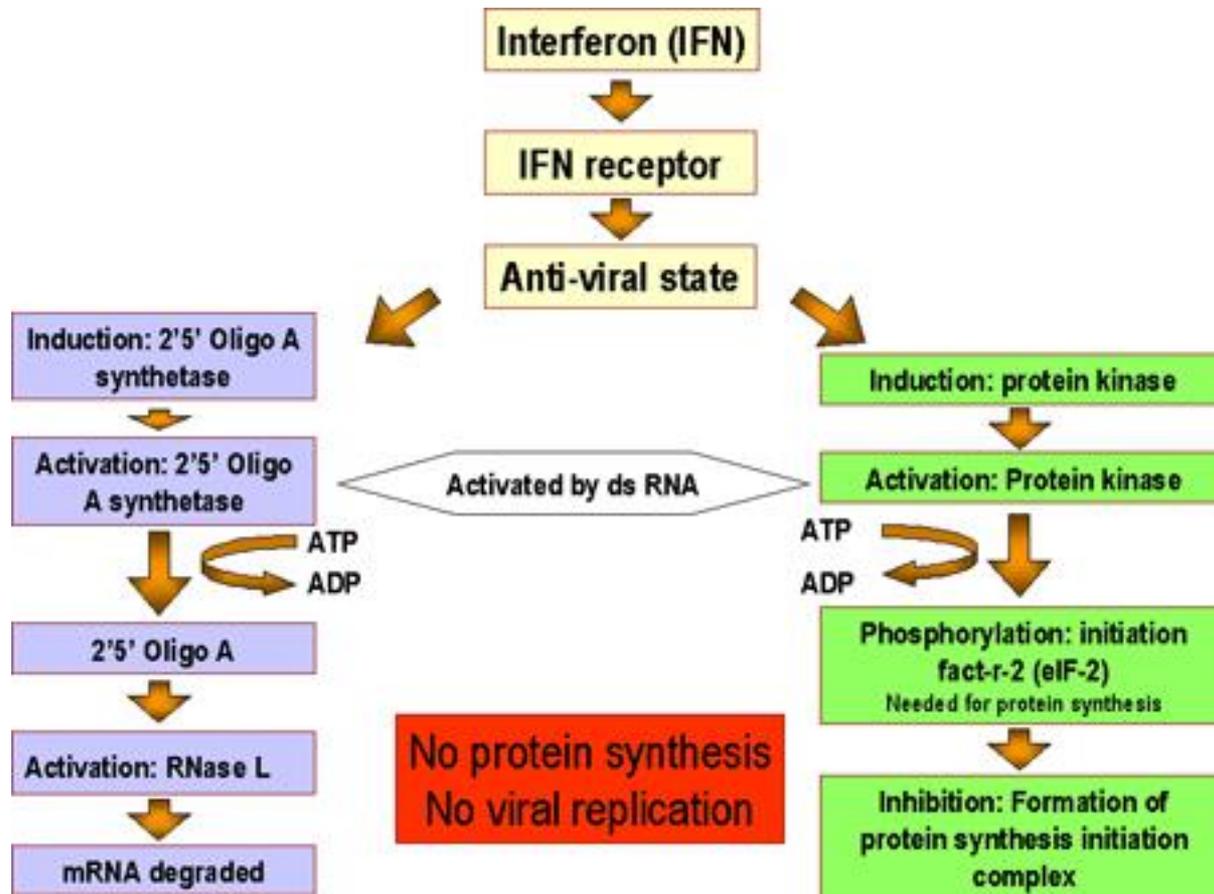
The OAS-RNaseL antiviral pathway

PKR activation mechanism

RNA-binding motif (RBM), which together constitute the N-terminal RNA-binding domain (RBD).
When two or more molecules of inactive Pkr bind to one dsRNA molecule, cross-phosphorylation occurs because of the physical proximity of the molecules. Phosphorylation is thought to cause a conformational change in the kinase domain (KD) to allow phosphorylation of other substrates, including eIF2.

From Flint et al. *Principles of Virology* (2015), ASM Press
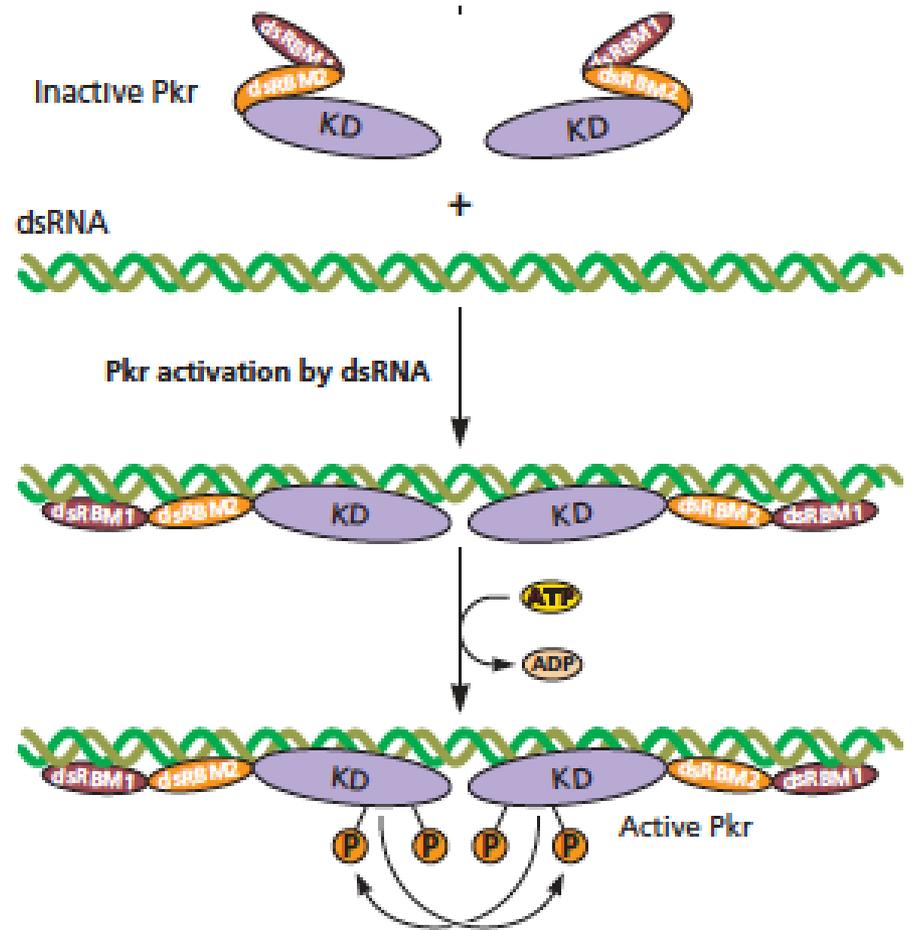
Targets for interferon (IFN)-stimulated proteins within viral life cycles

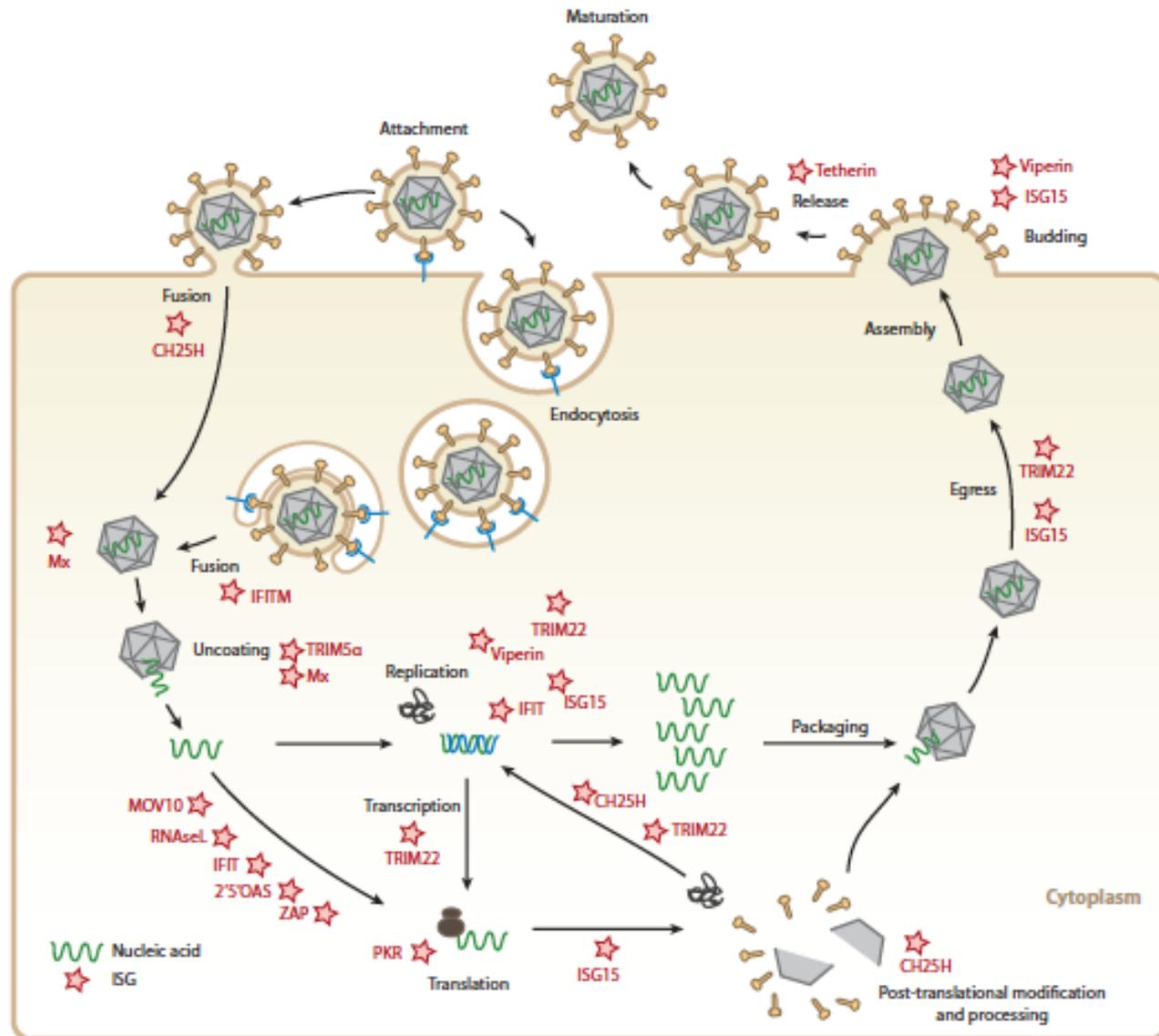**Table 3.5** Some viral modulators of the interferon response[a]

Type of modulation	Representative viruses	Viral protein, if known	Mechanism of action
Inhibition of IFN synthesis	Epstein-Barr virus	Bcrf1	IL-10 homolog, inhibits production of IFN-γ
	Vaccinia virus	A18R	Regulates dsRNA production
	Foot-and-mouth disease virus	L	Host protein synthesis block
IFN receptor decoys	Vaccinia virus	B18R	Soluble IFN-α/β decoy receptor
Inhibition of IFN signaling	Adenovirus	E1A	Decreases quantity of Stat1 and P48, blocks Isgf3 formation, interferes with Stat1 and Cbp/p300 interactions
	Vaccinia virus	VH1	Viral phosphatase reverses Stat1 activation
	Human papillomavirus 16	E7	Binds p48
	Hepatitis C virus	NS5a	Blocks formation of Isgf3 and Stat dimers
	Nipah virus	V protein	Prevents Stat1 and Stat2 activation and nuclear accumulation
Block function of IFN-induced proteins	Adenovirus	VA-RNA I	Binds dsRNA, blocks Pkr
	Herpes simplex virus 1	US11	Blocks Pkr activation
		ICP34.5	Redirects protein phosphatase 1α to dephosphorylate eIF2α, reverses Pkr action
	Vaccinia virus	E3L	Binds dsRNA and blocks Pkr
		K3L	Pkr pseudosubstrate, decoy
	Human immunodeficiency virus type 1	TAR RNA	Blocks activation of Pkr
		Tat	Pkr decoy
	Hepatitis B virus	Capsid protein	Inhibits MxA
	Influenza virus	NS1	Binds dsRNA and Pkr, blocks action of Isg15
	Reovirus	σ3	Binds dsRNA, inhibits Pkr and 2'-5'-oligo(A) synthase

Interferon-dependent anti-viral response

Virus infects

DEATH

Interferon-alpha produced by leukocytes and other cells. Interferon-beta produced by fibroblasts

IFN binds to its receptor on a neighboring cell

IFN receptor

Gene expression

"Anti-viral" state set up

2'5' oligoadenylate synthase protein kinase and other enzymes made

Influenza Virus binds

Viral mRNA broken down
Viral protein synthesis inhibited
Viral growth prevented
MHC I expressed