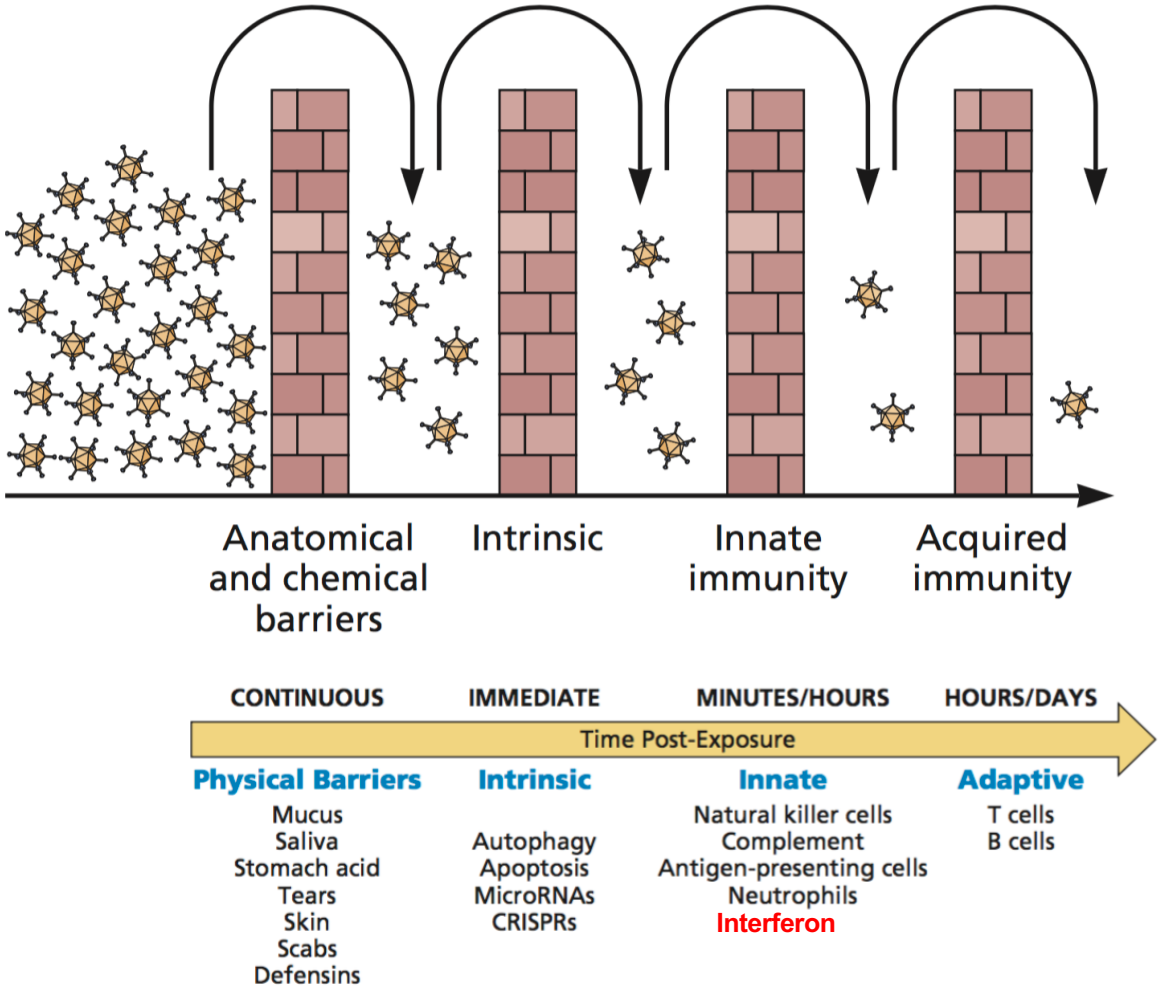
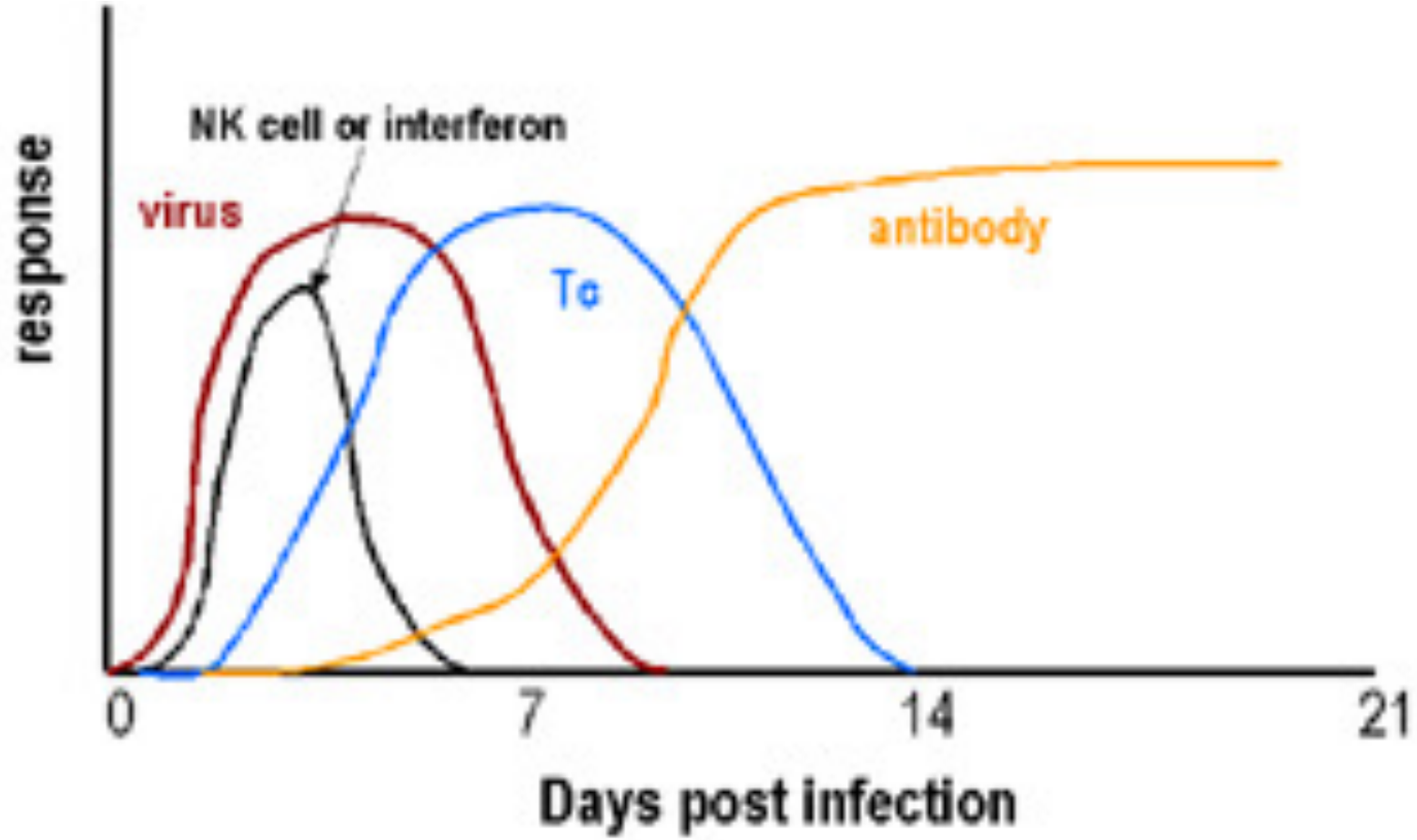
Interferon and the anti-viral state

Host defenses

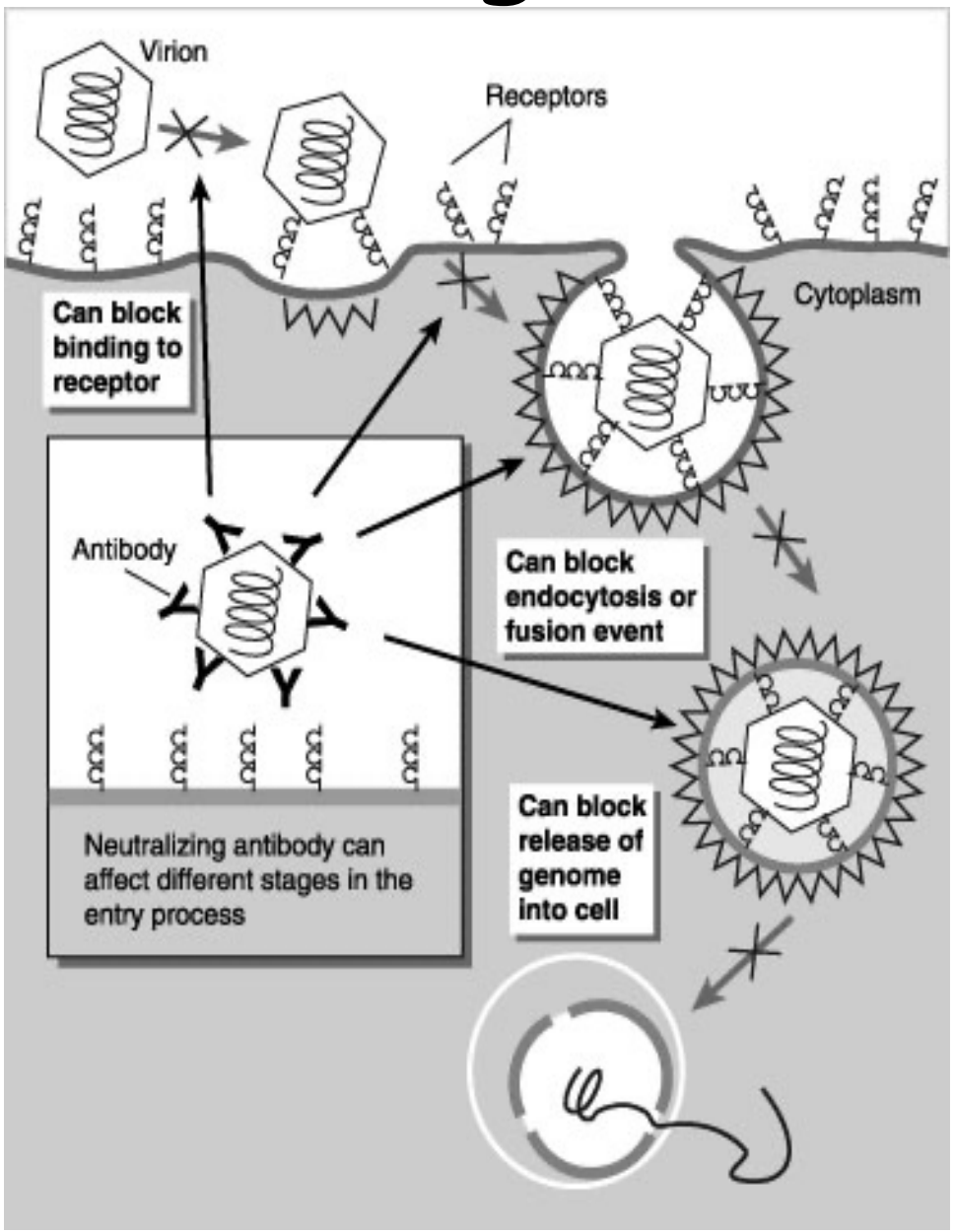


Principles of Virology, ASM Press



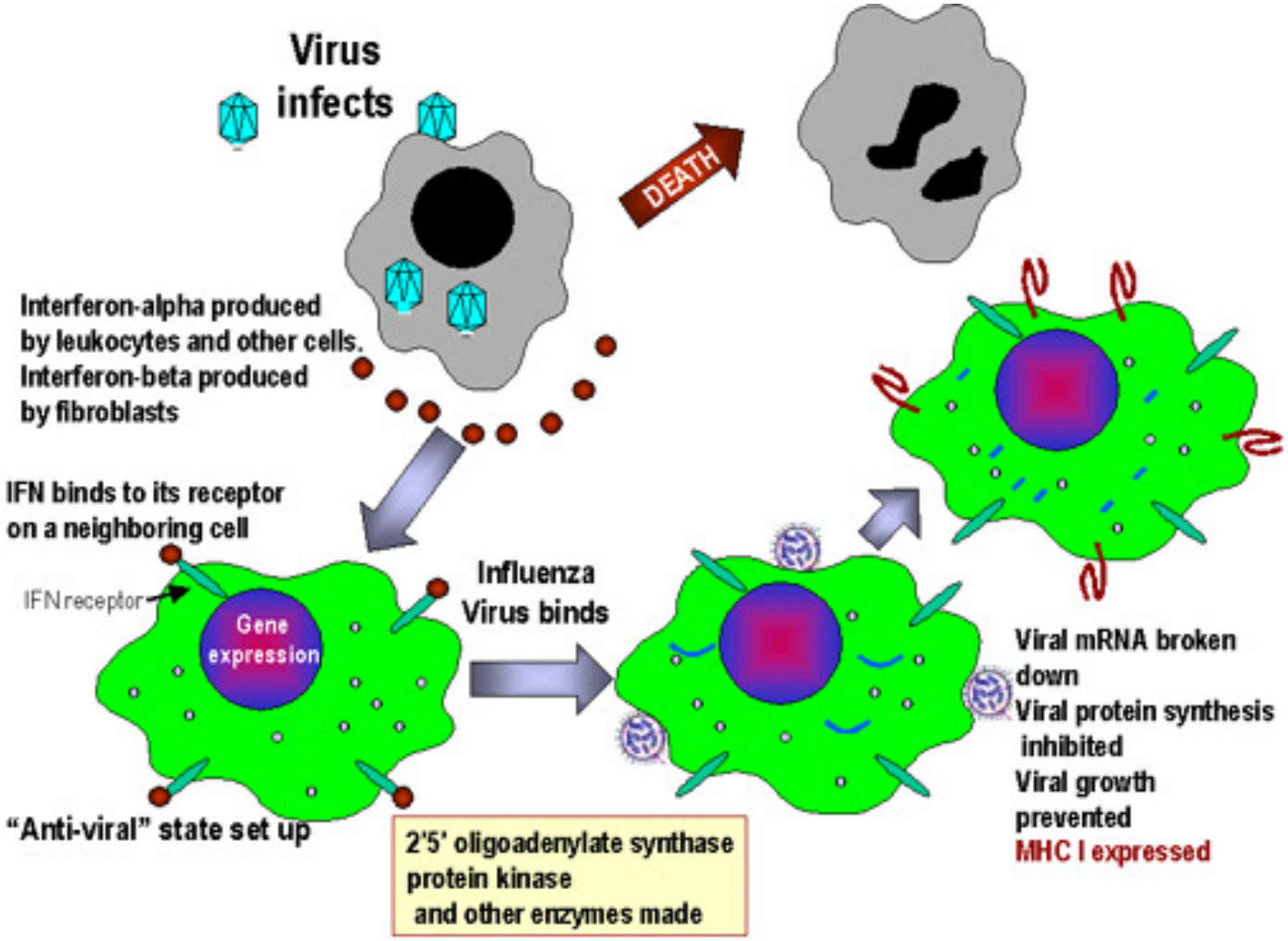
Typical response to an acute virus infection

Neutralizing antibodies



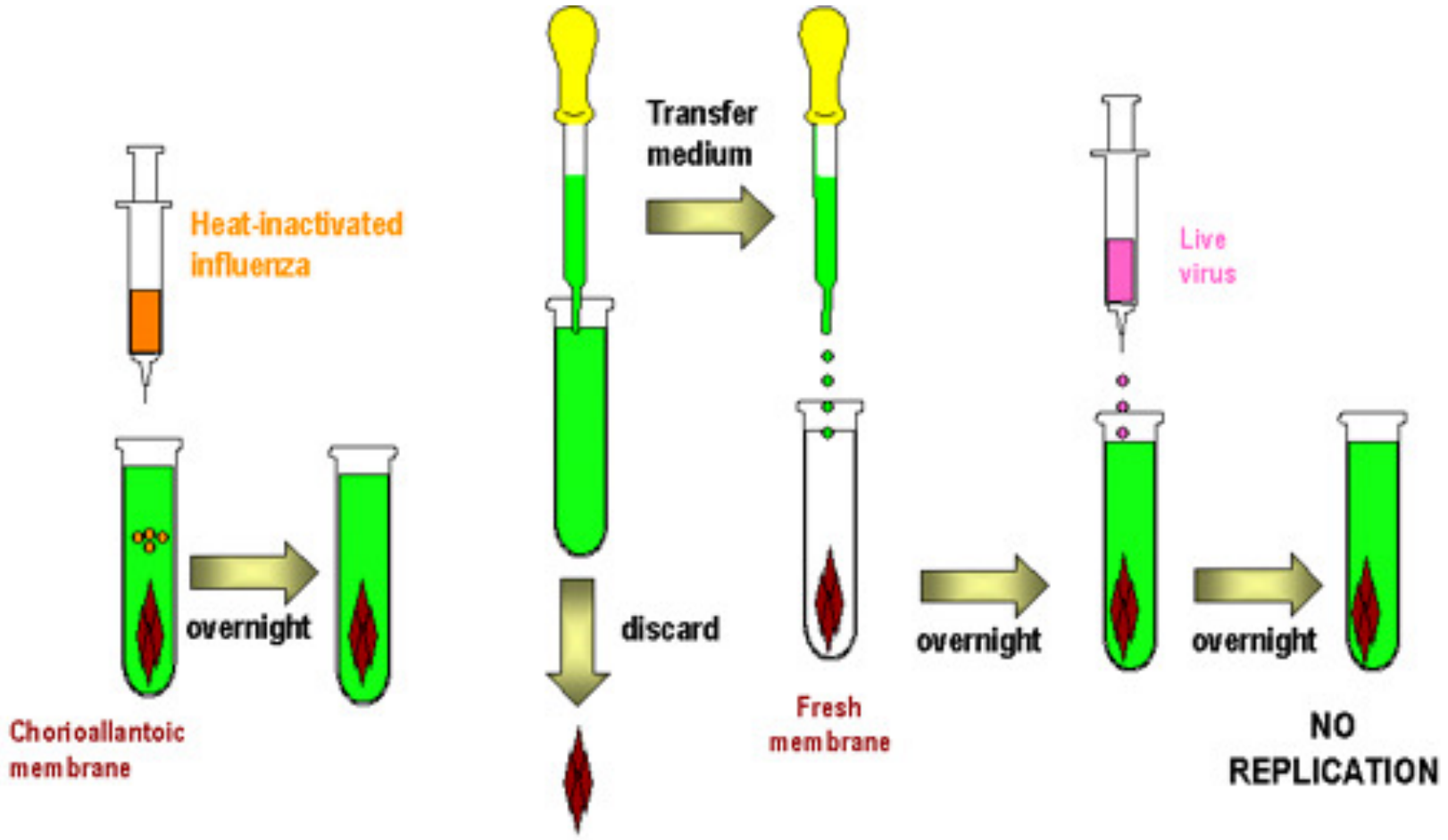
From Wagner and Hewlett *Basic virology* (2003) Blackwell Science Press

Interferon


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
Influenza
Virus binds
2'5' oligoadenylate synthase
protein kinase
and other enzymes made
Viral mRNA broken
down
Viral protein synthesis
inhibited
Viral growth
prevented
MHC I expressed

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



The Discovery of Interferon

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

Types and Properties of Interferon

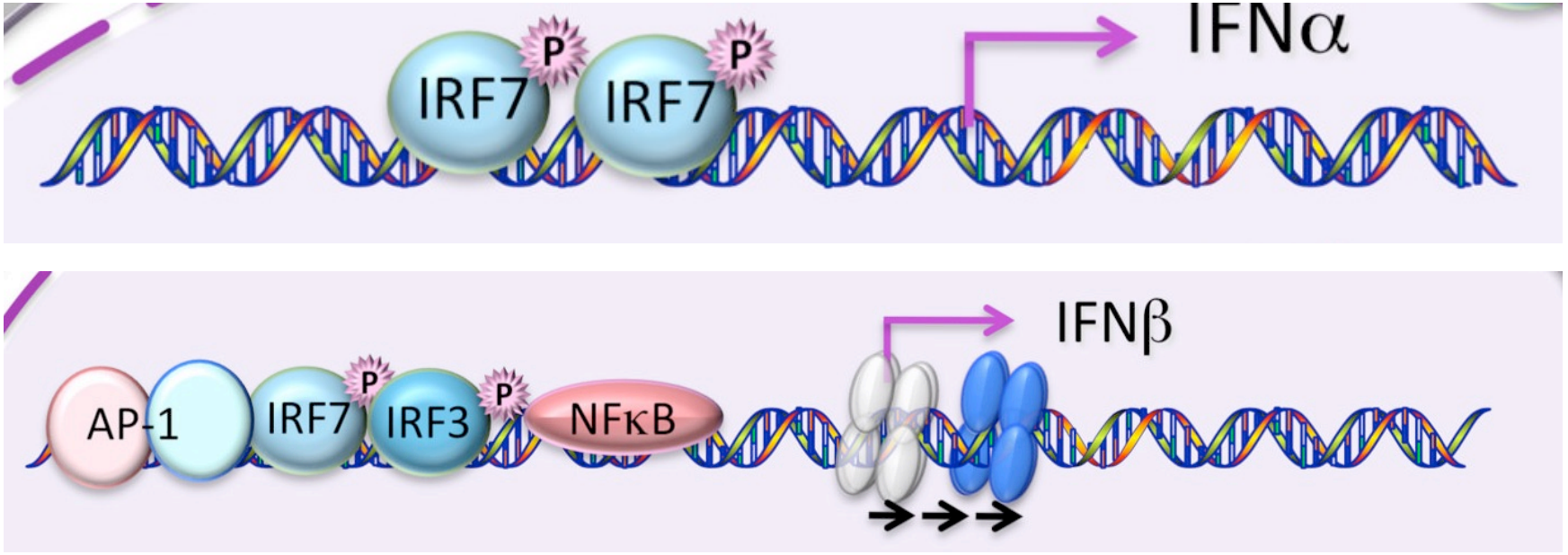
Property	Alpha	Beta	Lambda	Gamma
	Leukocyte Type I	Fibroblast Type I	Type III	Immune IFN Type II
Inducers	Viruses (RNA>DNA) dsRNA	Viruses(RNA> DNA) dsRNA	Viruses(RNA> DNA) dsRNA	Antigens, Mitogens
Principal source	Leukocytes	Fibroblasts	Any cell	Lymphocytes

Interferon Induction

- **interferon-alfa, interferon-beta and interferon-lambda**
  - -Induced by
    - -Viral infections (mainly RNA viruses)
    - -dsRNA
    - -Some bacterial components

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

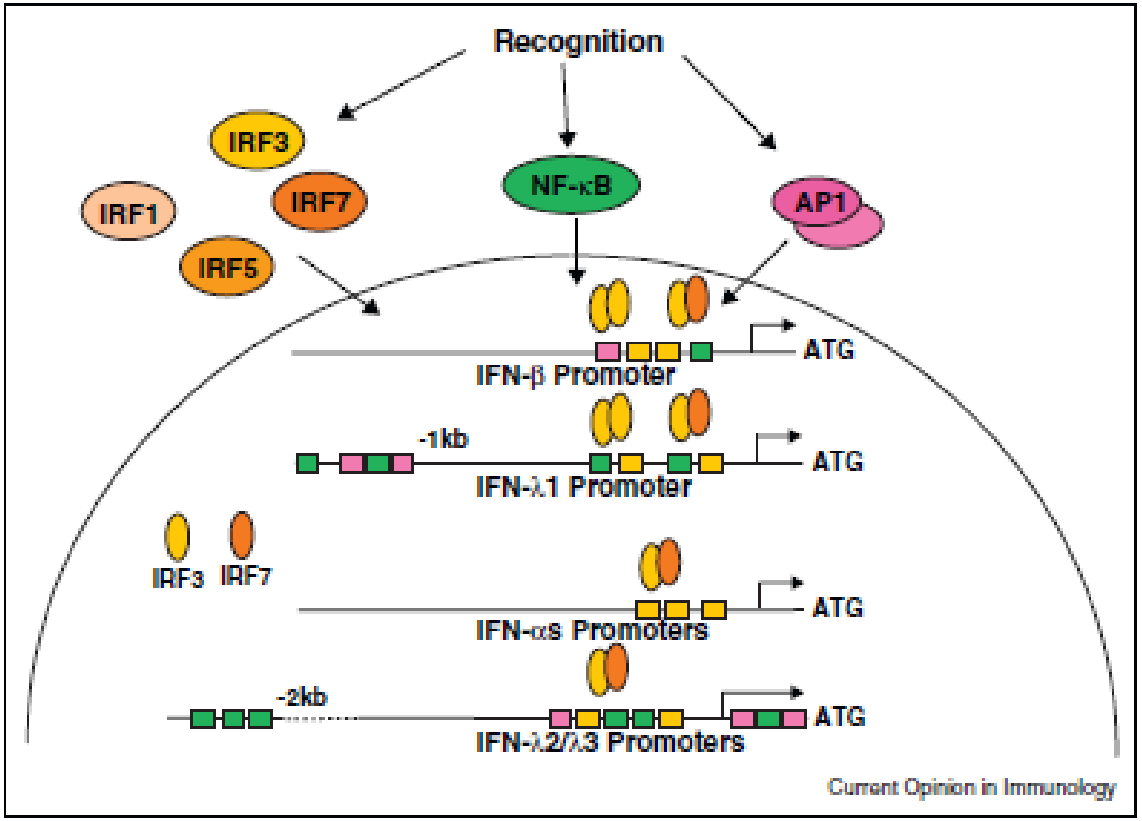
Induction of transcription of Type I-INFs



IFN-β gene transcriptional regulation is controlled by either IRF3 or IRF7, whereas IFN-α genes, are more dependent on IRF7.
This observation is important because IRF3 is constitutively and ubiquitously expressed in cells and, when activated upon viral entry, induces expression of the IFN-β

Curr Opin Virol. 2011, 1: 476–486

Induction of transcription of Type I and Type III-INFs



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. This observation is important because 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.

Interferon Induction

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

Interferon Induction

Three PRR families have been identified as nucleic acid-sensing PRRs:

Endosomal Toll-like receptors (TLRs)

Cytoplasmic retinoic acid inducible gene (RIG-I)-like receptors (RLRs)

Cytoplasmic DNA sensor molecules (mostly uncharacterised)

RIG-1=retinoic acid inducible gene

Interferon Induction

Recognition of foreign nucleic acids by PRRs

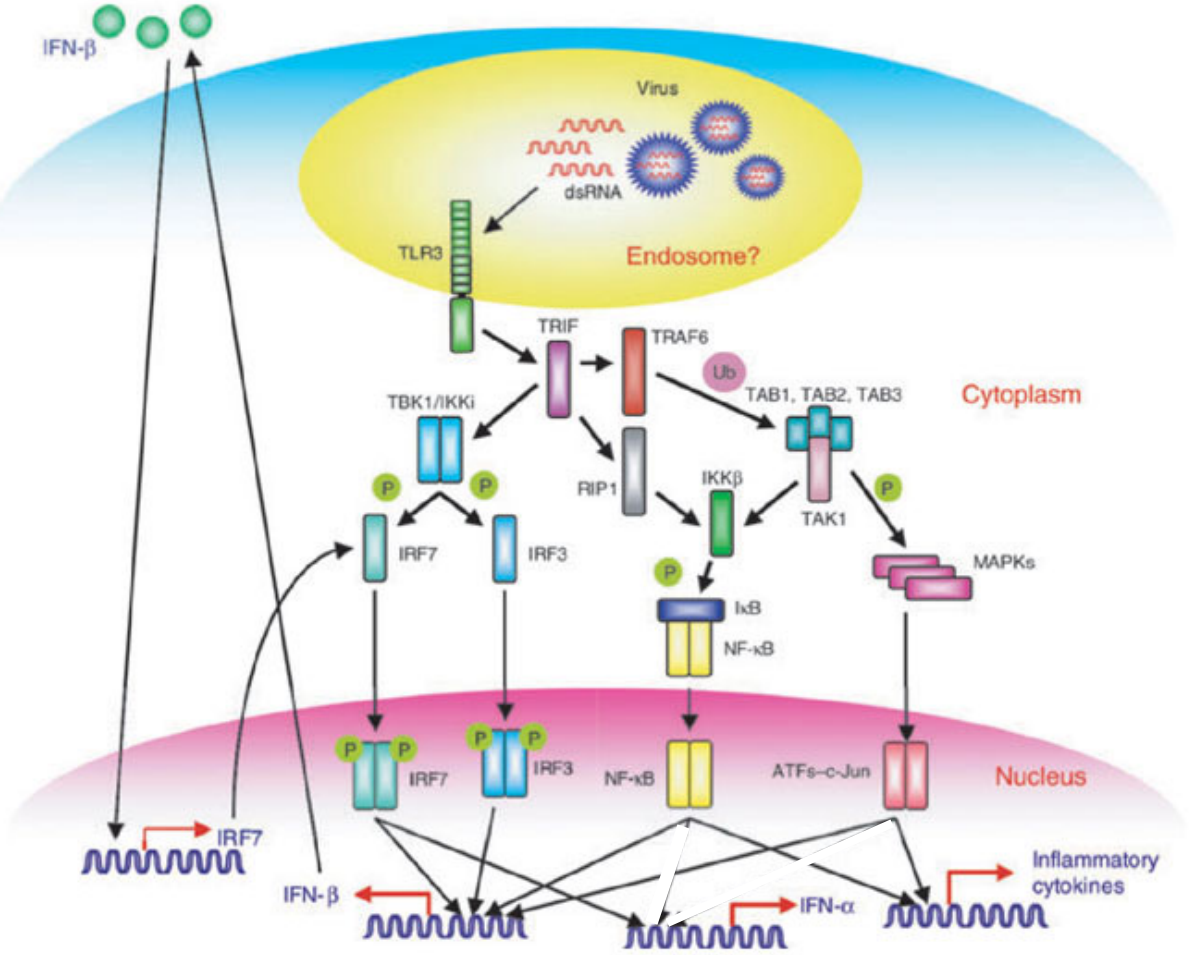
PRRs		Viral nucleic acids
TLRs	TLR3	dsRNA
	TLR7/8	ssRNA
	TLR9	CpG DNA
RLRs	RIG-I	5′ppp-dsRNA (short, panhandle)
	MDA5	dsRNA (long)
DNA sensor	DAI	dsDNA

DAI=DNA-dependent activator of IRFs

Toll-Like Receptors

- TLRs are a family of single-transmembrane proteins expressed predominantly in immune cells, such as macrophages and DCs.
- Particularly, pDCs, also known as interferon-producing cells, are a restricted subset of DCs with a plasmacytoid morphology that are specialized in secreting copious type I interferon, particularly IFN-α, after stimulation with viral nucleic acids

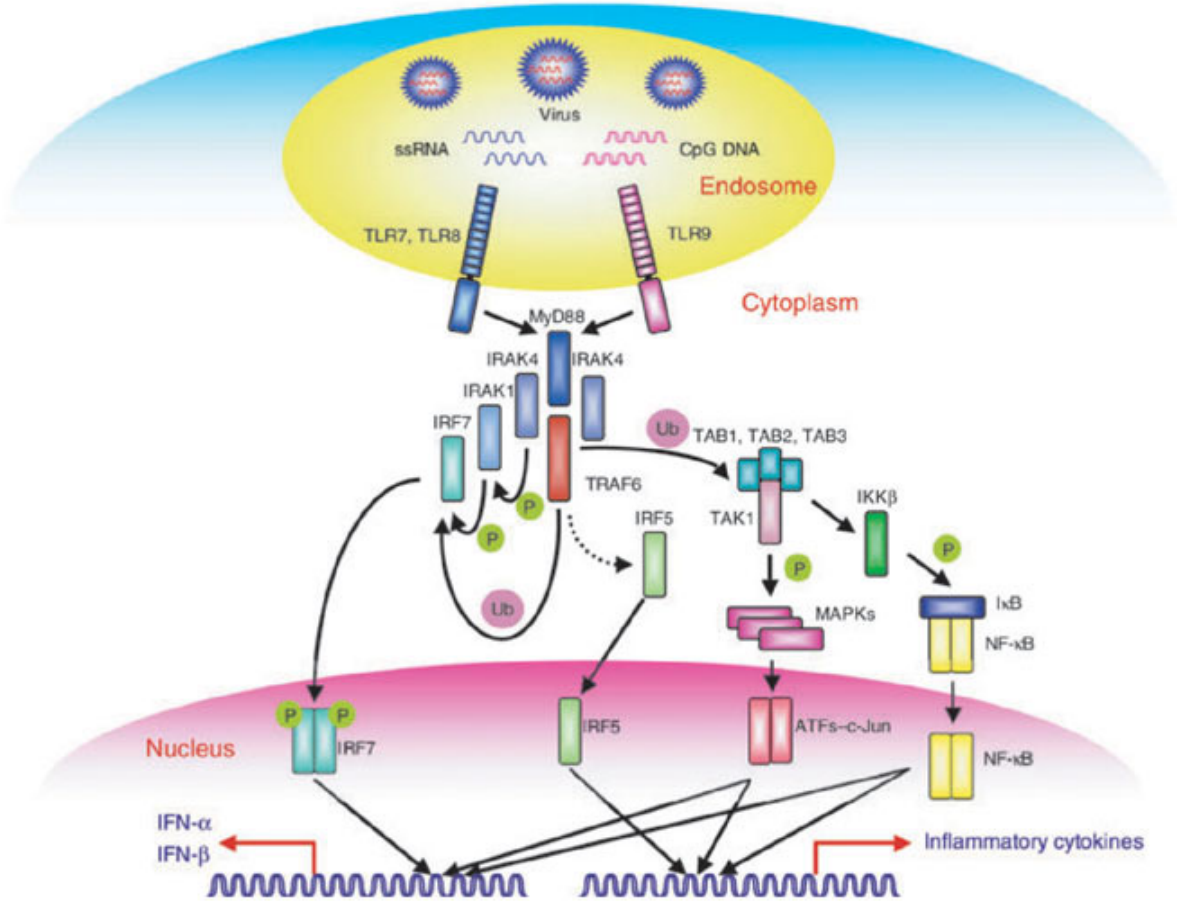
Interferon Induction by dsRNA binding to TLR3



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.

Modified from Nature Immunology 7, 131 - 137 (2006)

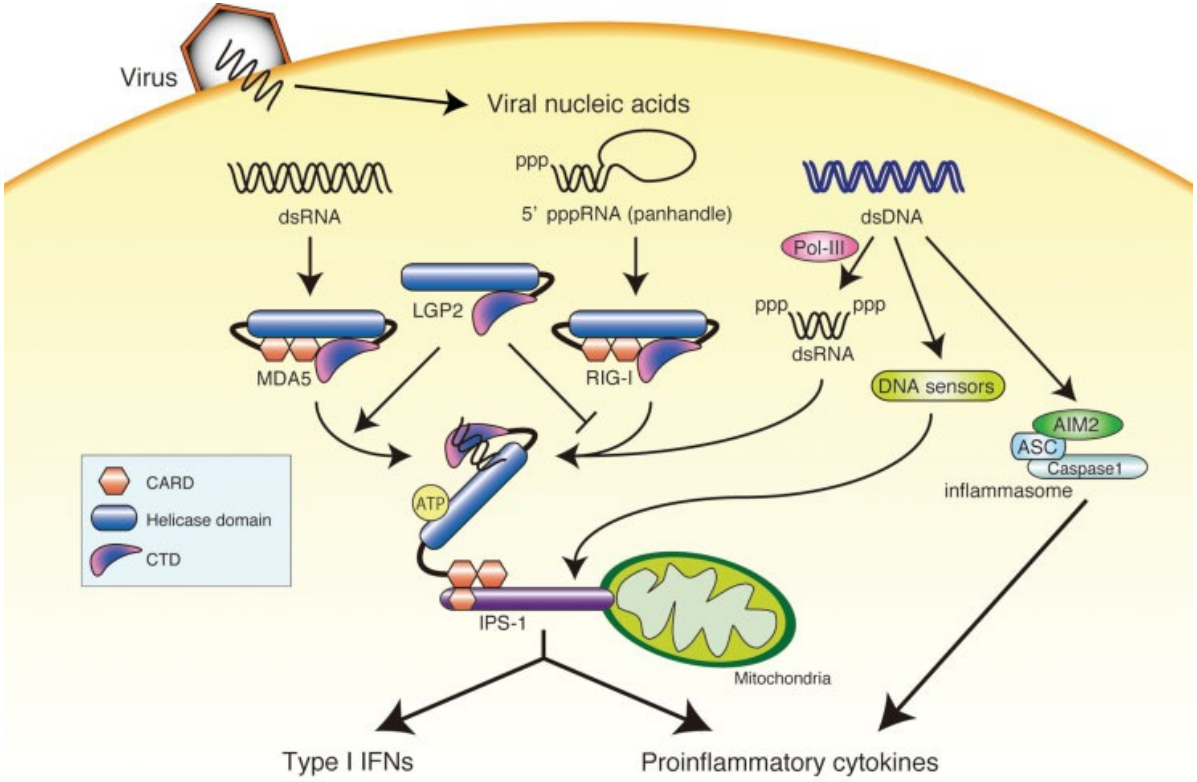
Interferon Induction by ssRNA and CpG DNA



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.

Nature Immunology 7, 131 - 137 (2006)

Cytoplasmic sensors for viral nucleic acids.



IPS-1: IFN β-promoter stimulator

DAI=DNA-dependent activator of IRFs

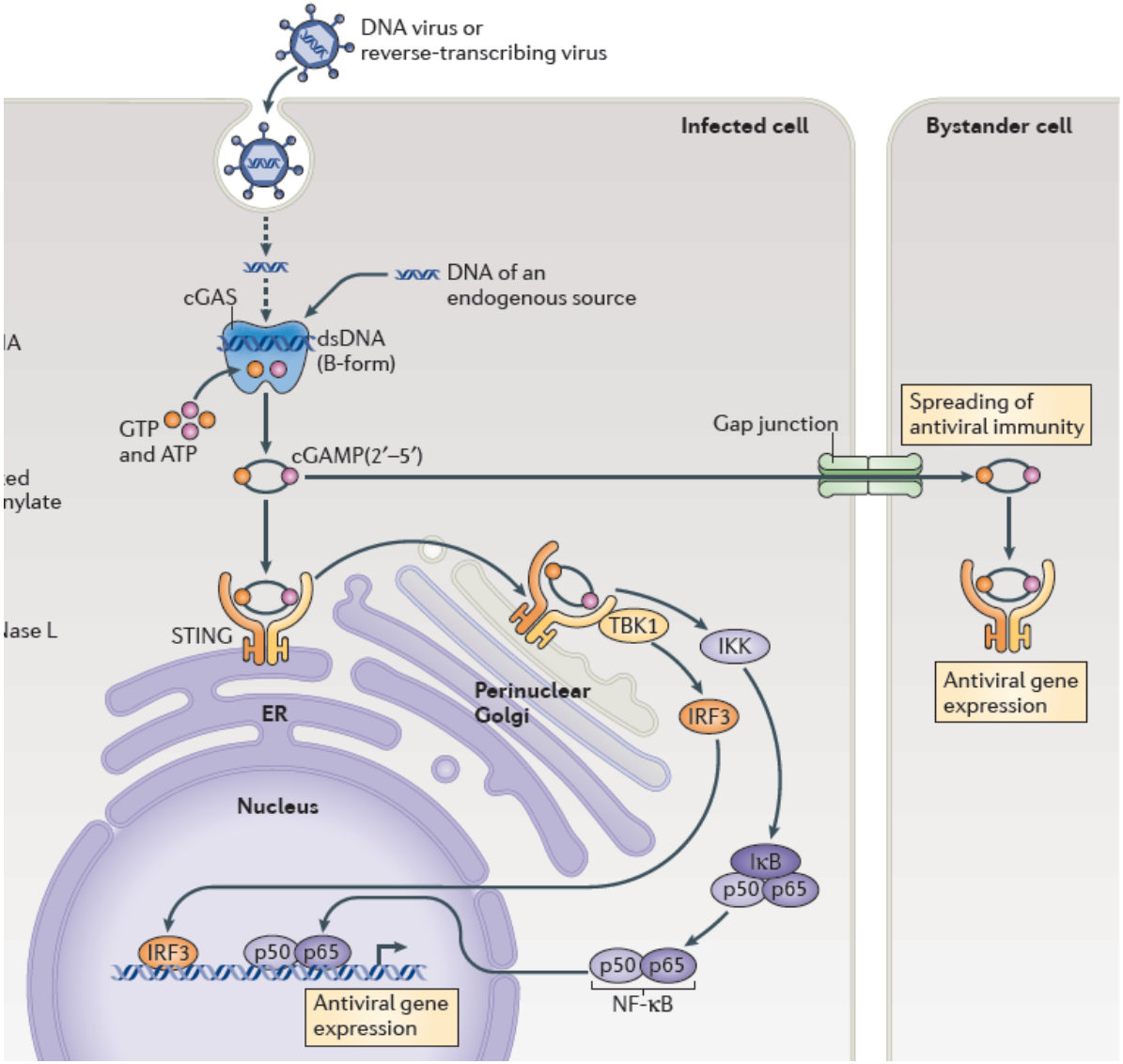
Cytoplasmic sensors for viral nucleic acids. RIG-I and MDA5 recognise viral 5'-triphosphate-containing RNA with panhandle structure and long dsRNA, respectively. On the other hand, DNA sensors are thought to recognise cytoplasmic viral dsDNA, and activate the IPS-1-dependent signalling. Some cytoplasmic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

Rev. Med. Virol. 2010; 20: 4–22.

DNA sensors

cGAS - OAS homologue cyclic GMP–AMP (cGAMP) synthase.

Wu, J. et al. Cyclic GMP-AMP is an endogenous second messenger in innate immune signaling by cytosolic DNA. Science 339, 826–830 (2013).
Sun, L., Wu, J., Du, F., Chen, X. & Chen, Z. J.Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. Science 339, 786–791 (2013).



(cGAS) is activated by cytosolic dsDNA to synthesize the non-canonical cyclic dinucleotide (CDN) cGAMP(2′–5′) as its second messenger molecule (using the substrates ATP and GTP). cGAMP(2′–5′) binds to and activates the endoplasmic reticulum (ER)-resident receptor stimulator of interferon genes (STING), which subsequently translocates to a perinuclear Golgi compartment where it obtains its signalling-competent state. This results in the activation of transcription factors that initiate antiviral and pro-inflammatory gene expression. At the same time, cGAMP(2′–5′) can also diffuse through gap junctions to initiate antiviral activity in bystander cells.

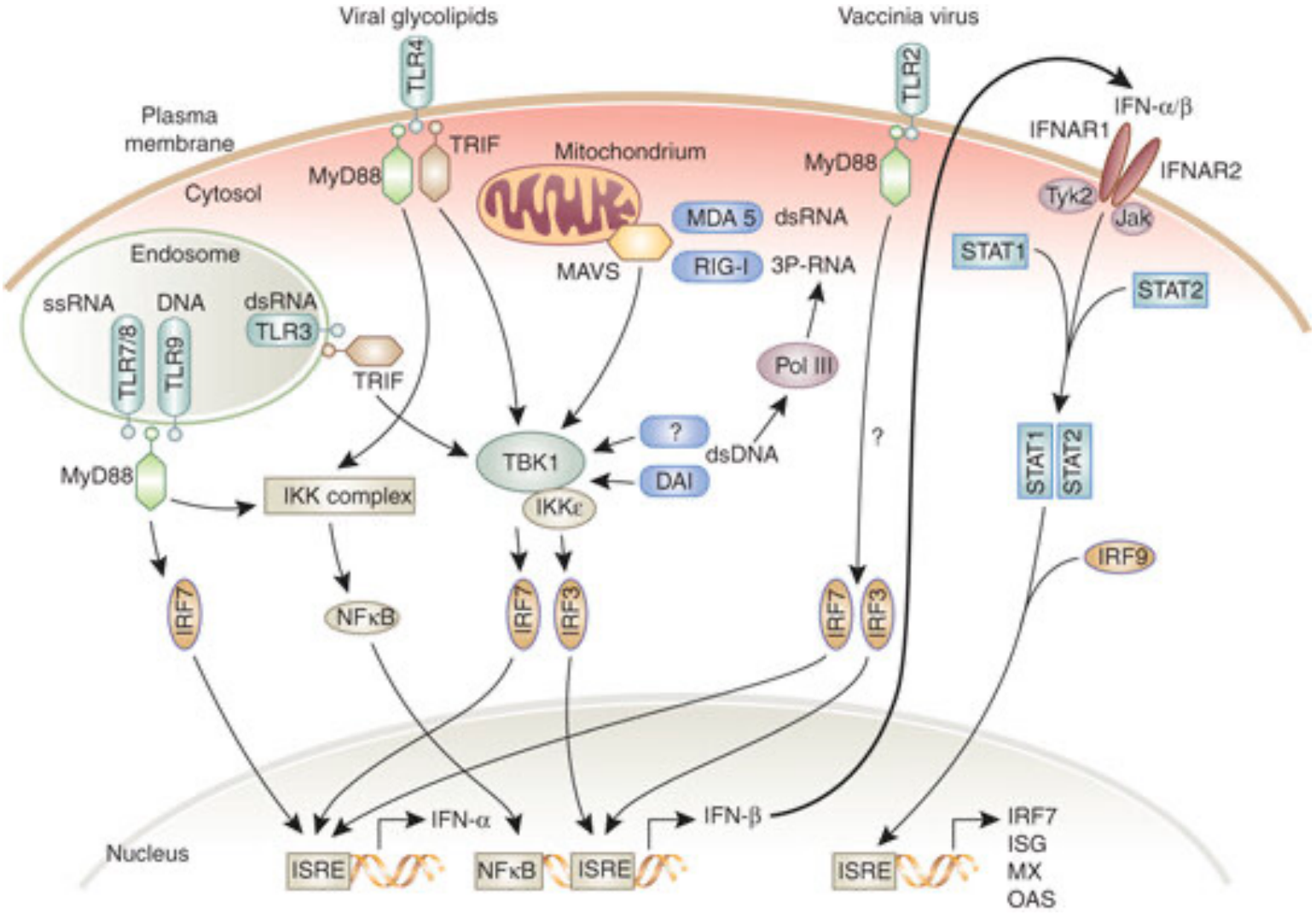
Molecular mechanisms and cellular functions of cGAS–STING signalling

Karl- Peter Hopfner 1,2 ✉ and Veit Hornung 1,2 ✉

NAture Reviews | MOLECULAR CELL BIOLOGy volume 21 | September 2020

The cGAS–STING signalling axis, comprising the synthase for the second messenger cyclic GMP–AMP (cGAS) and the cyclic GMP–AMP receptor stimulator of interferon genes (STING), detects pathogenic DNA to trigger an innate immune reaction involving a strong type I interferon response against microbial infections. Notably however, besides sensing microbial DNA , the DNA sensor cGAS can also be activated by endogenous DNA , including extranuclear chromatin resulting from genotoxic stress and DNA released from mitochondria, placing cGAS–STING as an important axis in autoimmunity , sterile inflammatory responses and cellular senescence. Initial models assumed that co- localization of cGAS and DNA in the cytosol defines the specificity of the pathway for non- self, but recent work revealed that cGAS is also present in the nucleus and at the plasma membrane, and such subcellular compartmentalization was linked to signalling specificity of cGAS. Further confounding the simple view of cGAS–STING signalling as a response mechanism to infectious agents, both cGAS and STING were shown to have additional functions, independent of interferon response. These involve non- catalytic roles of cGAS in regulating DNA repair and signalling via STING to NF- κB and MAPK as well as STING-mediated induction of autophagy and lysosome- dependent cell death. We have also learnt that cGAS dimers can multimerize and undergo liquid–liquid phase separation to form biomolecular condensates that could importantly regulate cGAS activation. Here, we review the molecular mechanisms and cellular functions underlying cGAS–STING activation and signalling, particularly highlighting the newly emerging diversity of this signalling pathway and discussing how the specificity towards normal, damage-induced and infection- associated DNA could be achieved.

Afferent and efferent pathways of IFN signaling

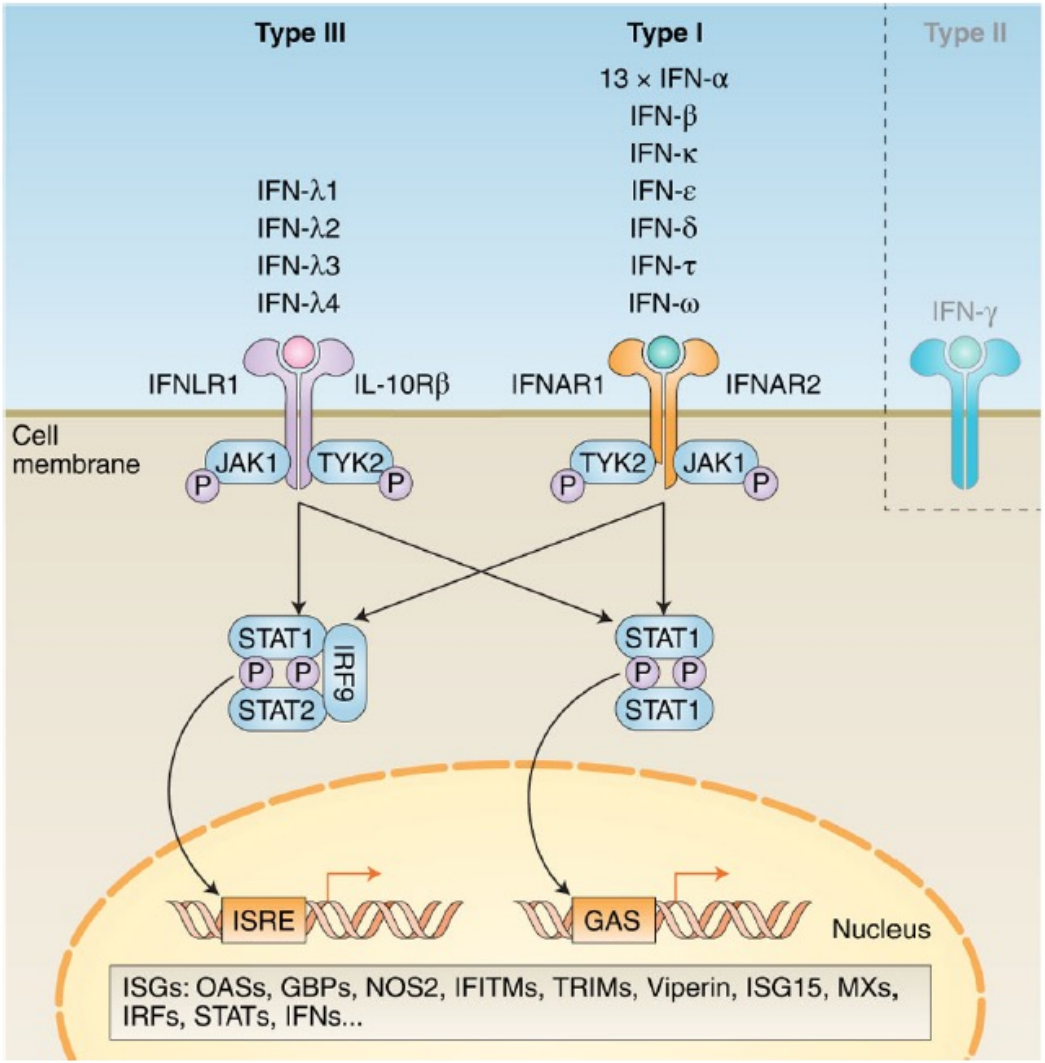


Kidney International (2010) 77, 848–854

Afferent and efferent pathways of IFN signaling. Induction and function of the IFN-a and IFN-b system are regulated by various pathogen recognition receptors. Activating TLR7 and TLR9 by ssRNA and viral (and bacterial) DNA in intracellular endosomes lead to the activation of interferon regulatory factor (IRF) 7 in an MyD88-dependent manner. Viral dsRNA stimulation of TLR3 results in the activation of IRF3 and IRF7 through TRIF. TLR4 signals the binding of endotoxin or viral proteins from the cell surface. Other viral surface molecules signal through TLR2. The RIG-I-like helicases RIG-I and MDA5 are cytosolic sensors for viral RNA. RIG-I interacts with ssRNA and dsRNA containing uncapped 50-triphosphate ends. MDA5 is activated by dsRNA in the cytosol. The helicases associate with the mitochondrial-associated adaptor protein (MAVS), which interacts with the kinases TBK1 and IKKe, leading to the phosphorylation of IRF3 and IRF7. Viral dsDNA leading to type I interferon production through TBK1 and IRF3 is mediated by DAI and other so-far-unknown cytosolic DNA sensors. In human cells, RNA-polymerase III can convert cytosolic dsDNA into 50-triphosphate RNA, which induces type I through RIG-I. Phosphorylation of IRF3 and IRF7 leads to homodimerization and translocation into the nucleus, resulting in the activation of the transcription of IFN-a genes. Activation of the IKK complex through MyD88 induces NF-kB activity, which induces IFN-b gene transcription together with the IRF3/IRF7 homodimers. Type I IFN can act in a paracrine and in an autocrine manner. IFN-a and IFN-b are recognized by the common receptor IFNAR1/2, resulting in the activation of the JAK/STAT1 pathway. The IFN-dependent induction of IRF7 further autoamplifies IFN signaling and the expression of additional interferon-stimulated genes (ISGs), including 20–50-oligoadenylate synthetases (OAS) and myxovirus resistance gene (MX). DAI, DNA-dependent activator of IFN-regulatory factor; dsRNA, double-stranded RNA; IFN, interferon; IFNAR; type I IFN receptor; JAK, Janus-activated kinase; MDA5, melanoma differentiation antigen 5; NFkB, nuclear factor-kB; ssRNA, single-stranded RNA; RIG-I, retinoic acid inducible gene-I; TLR, Toll-like receptor; TRIF, Toll/interleukin (IL)-1 receptor domain-containing adaptor-protein inducing interferon beta.

Interferon receptor signalling

Within type I and III, there are multiple IFNs; within type II, there is only a single IFN. Each type has a distinct heterodimeric cell surface receptor: 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. P, phosphate; OASs, oligoadenylate synthases; GBPs, guanylate-binding proteins; NOS2, nitric oxide synthase 2; IFITMs, IFN-induced transmembrane proteins; TRIMs, tripartate motif proteins

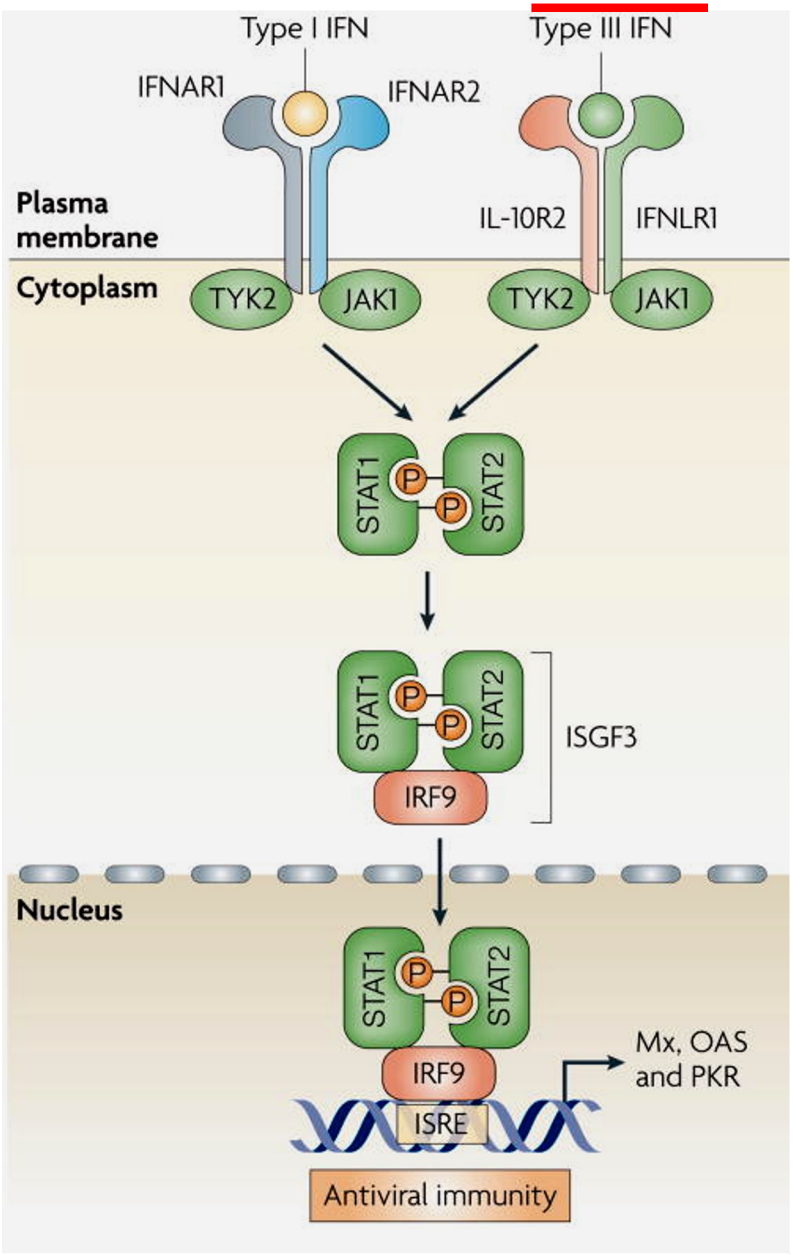


Nature Microbiology | VOL 914 4 | JUNE 2019 | 914–924 | www.nature.com/naturemicrobiology

Interferon receptor signalling

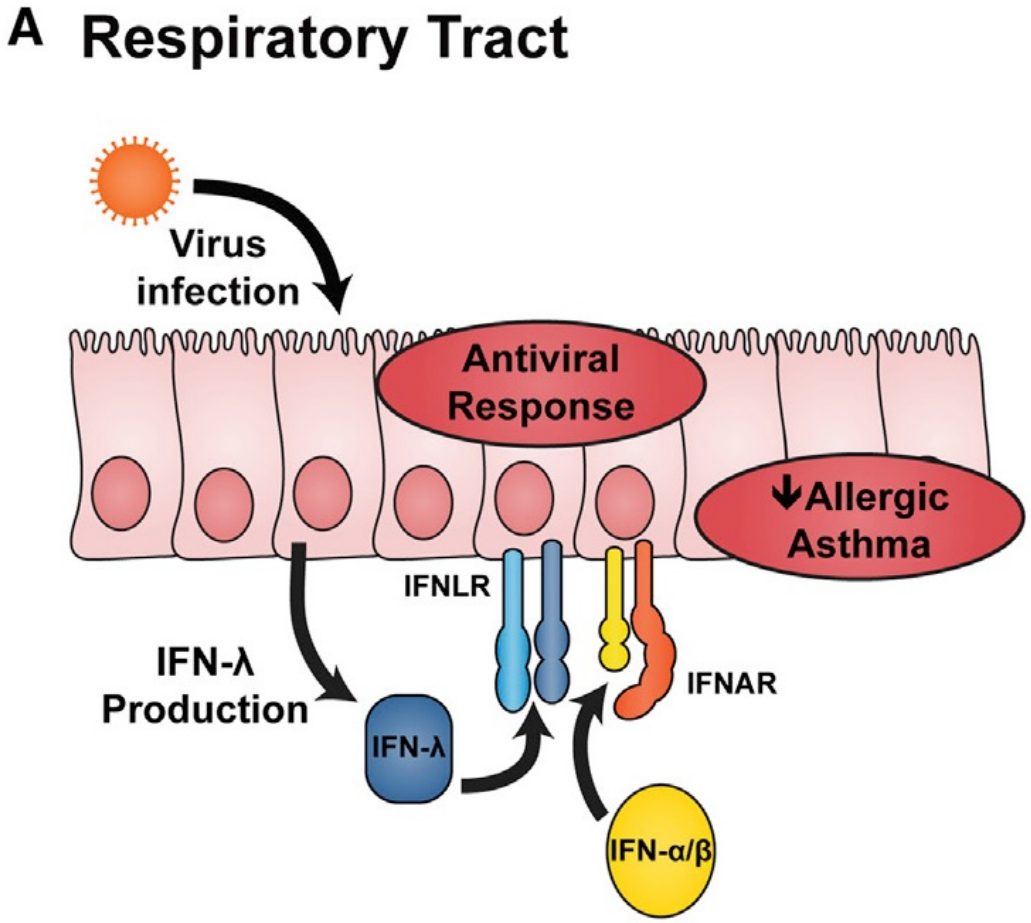
Type III IFN or IFN-lambda (IFN-λ)

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.



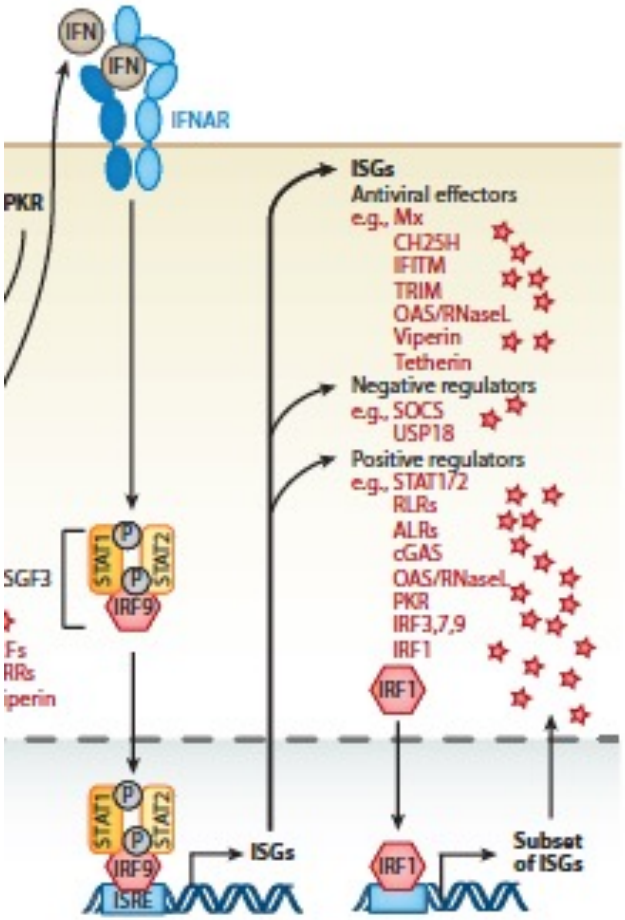
*nature reviews | **immunology***

Interferon receptor signalling



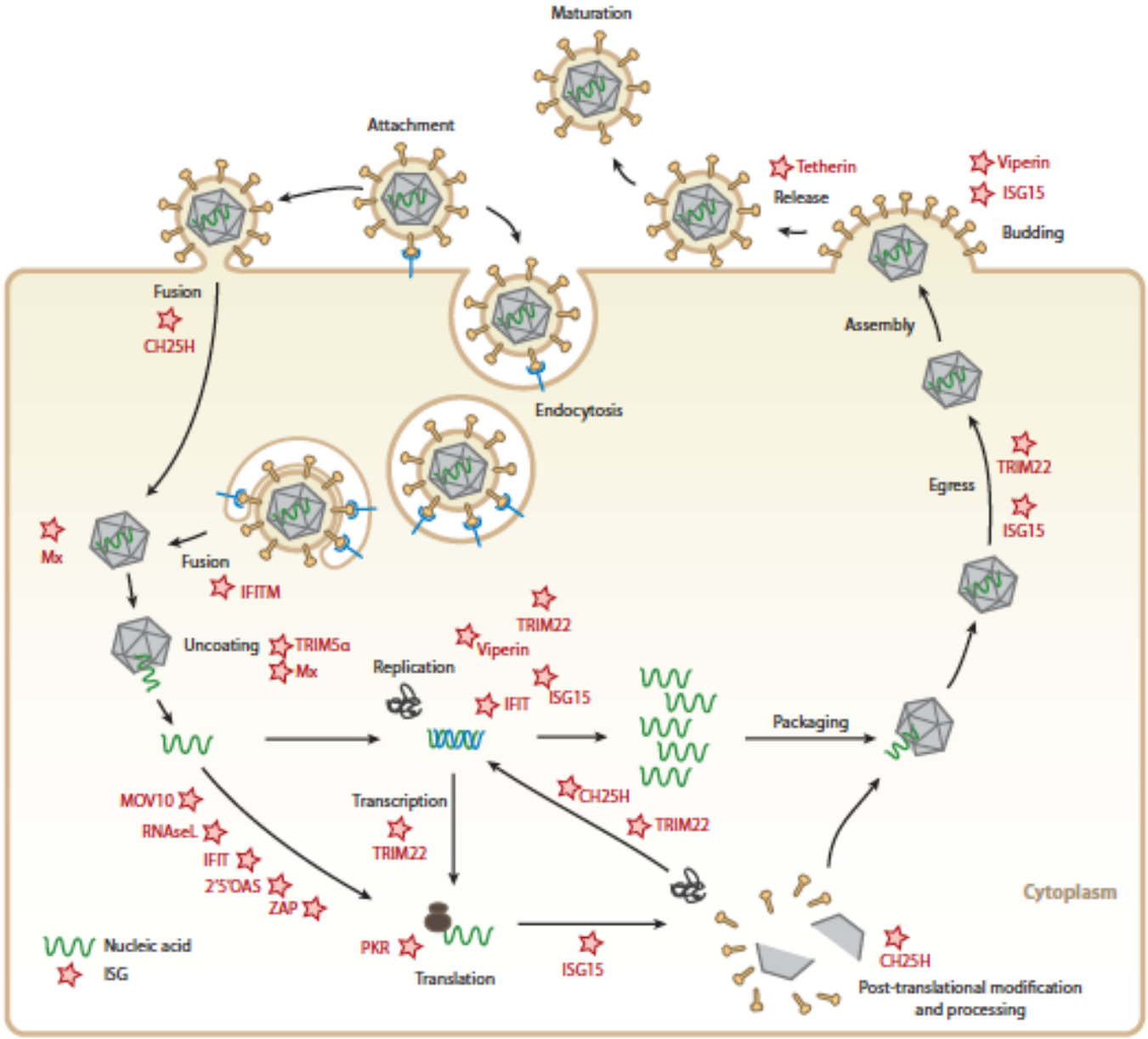
Epithelial responses to viral infection in the lung. When respiratory viruses infect airway epithelia, these cells can establish an antiviral state through the autocrine and paracrine action of type I or type III IFNs. Respiratory epithelial cells can respond to both IFN-λ and IFN-α/β to activate an antiviral response. However, IFN–λ is a dominant IFN produced after viral infection in the respiratory tract, which also express the IFN-λ receptor. In cases where this response is dominated by IFN-λ, the epithelial antiviral state can be established without systemic immune cell activation.

Immunity 43, July 21, 2015 Elsevier Inc.



IFN induces gene expression via the JAK-STAT pathway, resulting in expression of a large spectrum of ISGs that can be divided into antiviral effectors and negative or positive regulators of IFN signaling. Many ISGs control viral, bacterial, and parasite infection by directly targeting pathways and functions required during pathogen life cycles. Upregulation of chemokines and chemokine receptors enables cell-to-cell communication, whereas negative regulators of signaling help resolve the IFN-induced state and facilitate the return to cellular homeostasis. Additional ISGs encode for proapoptotic proteins, leading to cell death under certain conditions. A special case of positive regulators is **IRF1**, which upon expression directly translocates to the nucleus to enhance expression of a subset of ISGs.

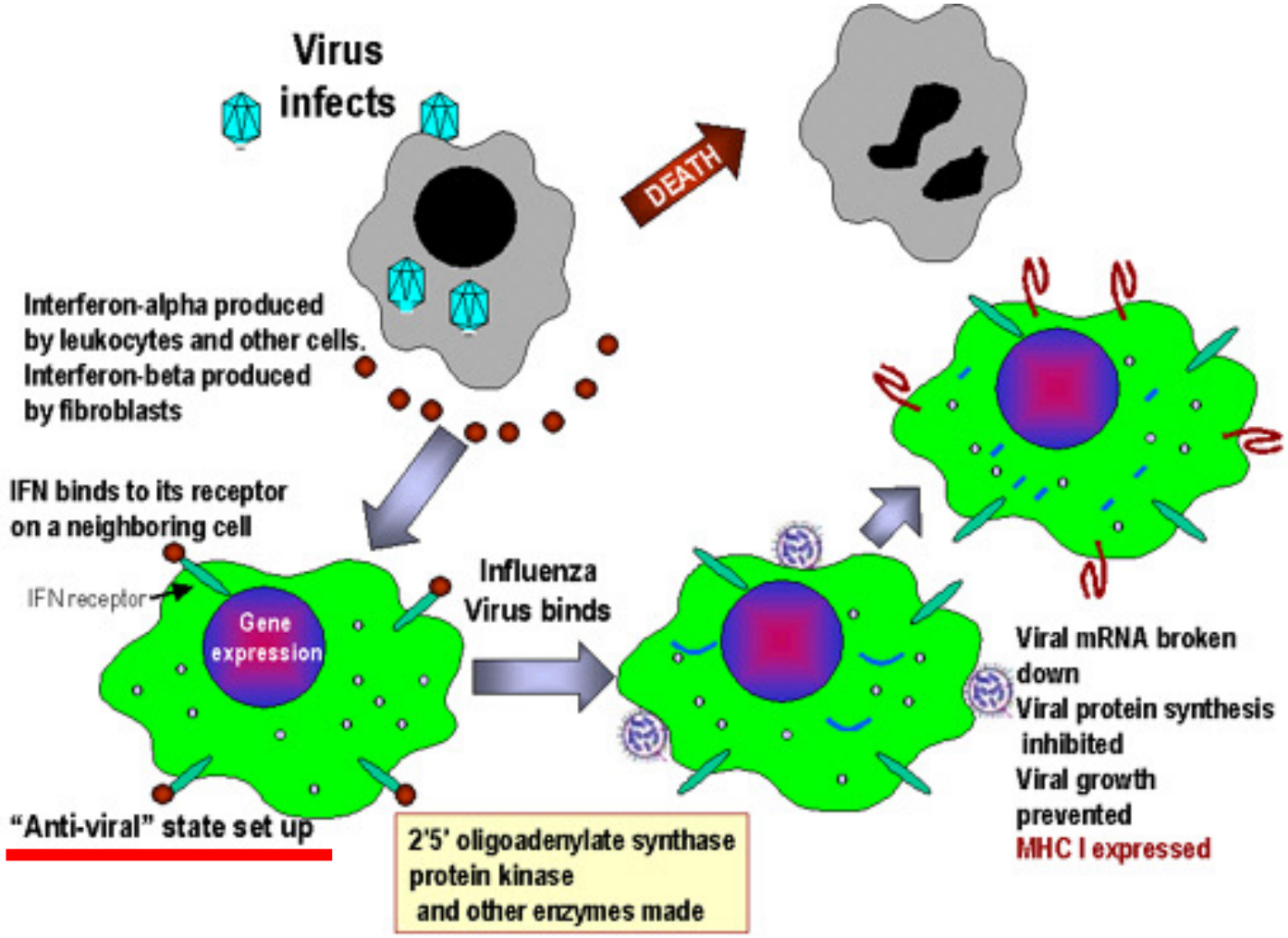
Targets for interferon (IFN)-stimulated proteins within viral life cycles



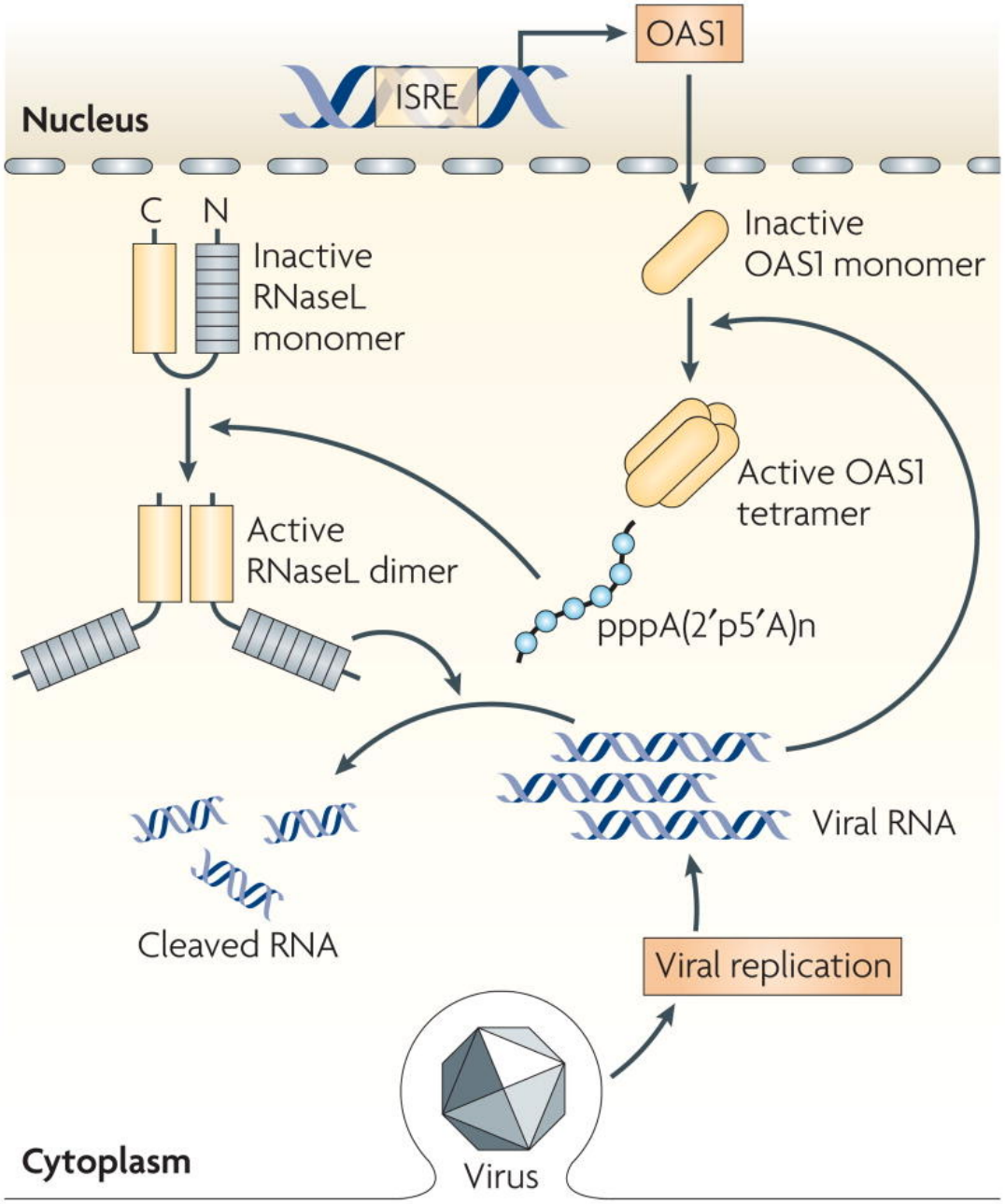
Annu. Rev. Immunol. 2014. 32:513–45

Targets for interferon (IFN)-stimulated proteins within viral life cycles. IFN-stimulated gene (ISG) products (stars) interfere with different stages of different viral life cycles. Cholesterol-25-hydroxylase (CH25H) affects viruses early, presumably at the host-membrane fusion event; at protein maturation of viral structural proteins by prenylation; and at protein maturation of viral replication enzymes. IFN-induced transmembrane (IFITM) protein members inhibit endocytic-fusion events of a broad spectrum of viruses. Tripartite motif protein 5!(TRIM5!) inhibits human immunodeficiency virus 1 (HIV-1) uncoating of the viral RNA. The myxoma resistance protein 1 (Mx1) inhibits a wide range of viruses by blocking endocytic traffic of incoming virus particles and uncoating of ribonucleocapsids. Some ISGs inhibit viruses by degrading viral RNA and/or blocking translation of viral mRNAs, such as 2!,5!-oligoadenylate synthetase (OAS) and latent ribonuclease L (RNase L), protein kinase R (PKR), Moloney leukemia virus 10 homolog (MOV10), and zinc-finger antiviral protein (ZAP). IFN-induced proteins with tetratricopeptide repeats (IFIT) inhibit protein translation and have been implicated in viral RNA degradation as well. TRIM22 inhibits viral transcription, replication, or trafficking of viral proteins to the plasma membrane. ISG15 can inhibit viral translation, replication, or egress. Viperin has been shown to inhibit viral replication or virus budding at the plasma membrane. Finally, tetherin traps otherwise mature virus particles on the plasma membrane and thus inhibits viral release, exerting its effect broadly on many enveloped viruses.

Interferon-dependent anti-viral response

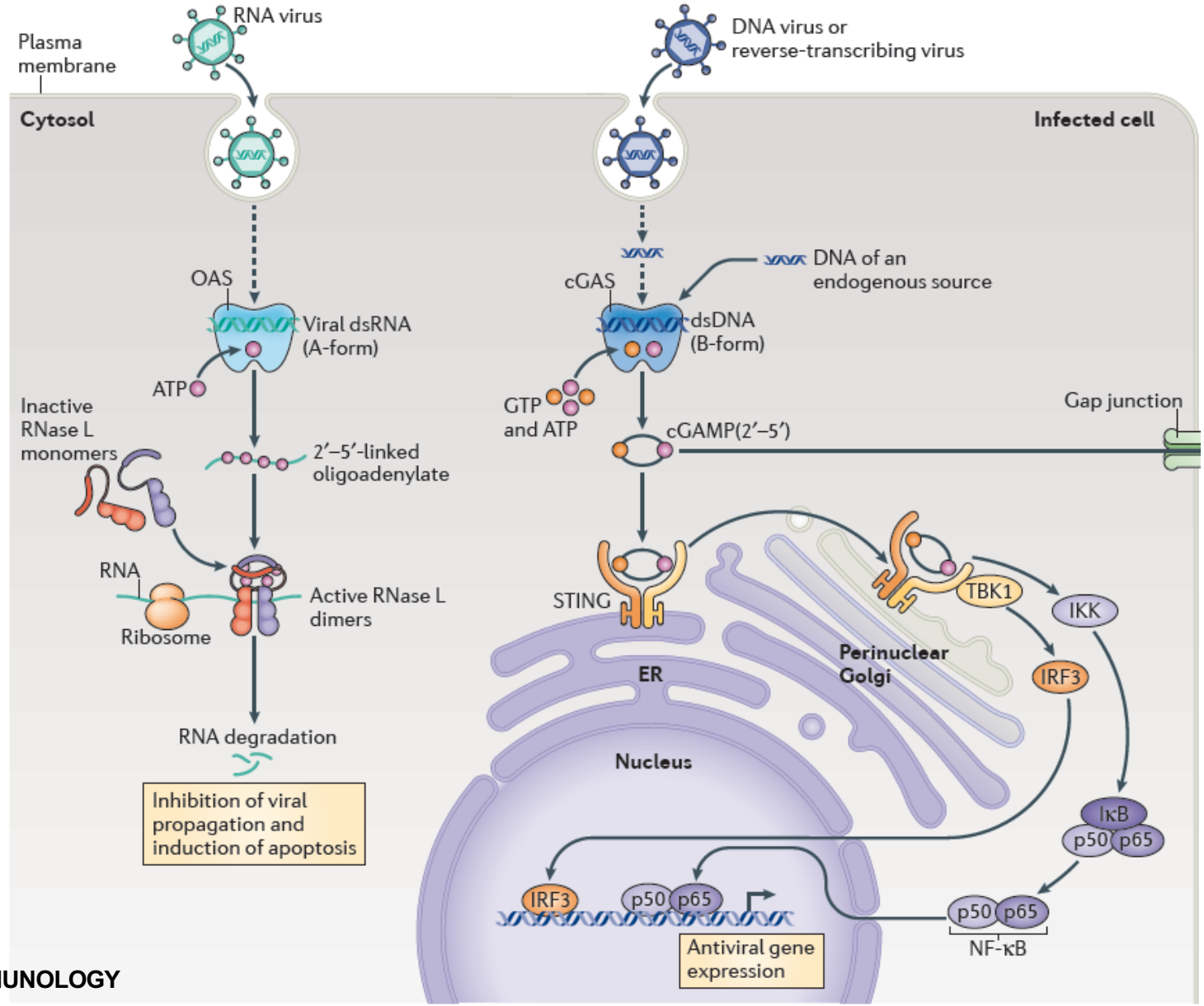


The OAS-RNaseL antiviral pathway

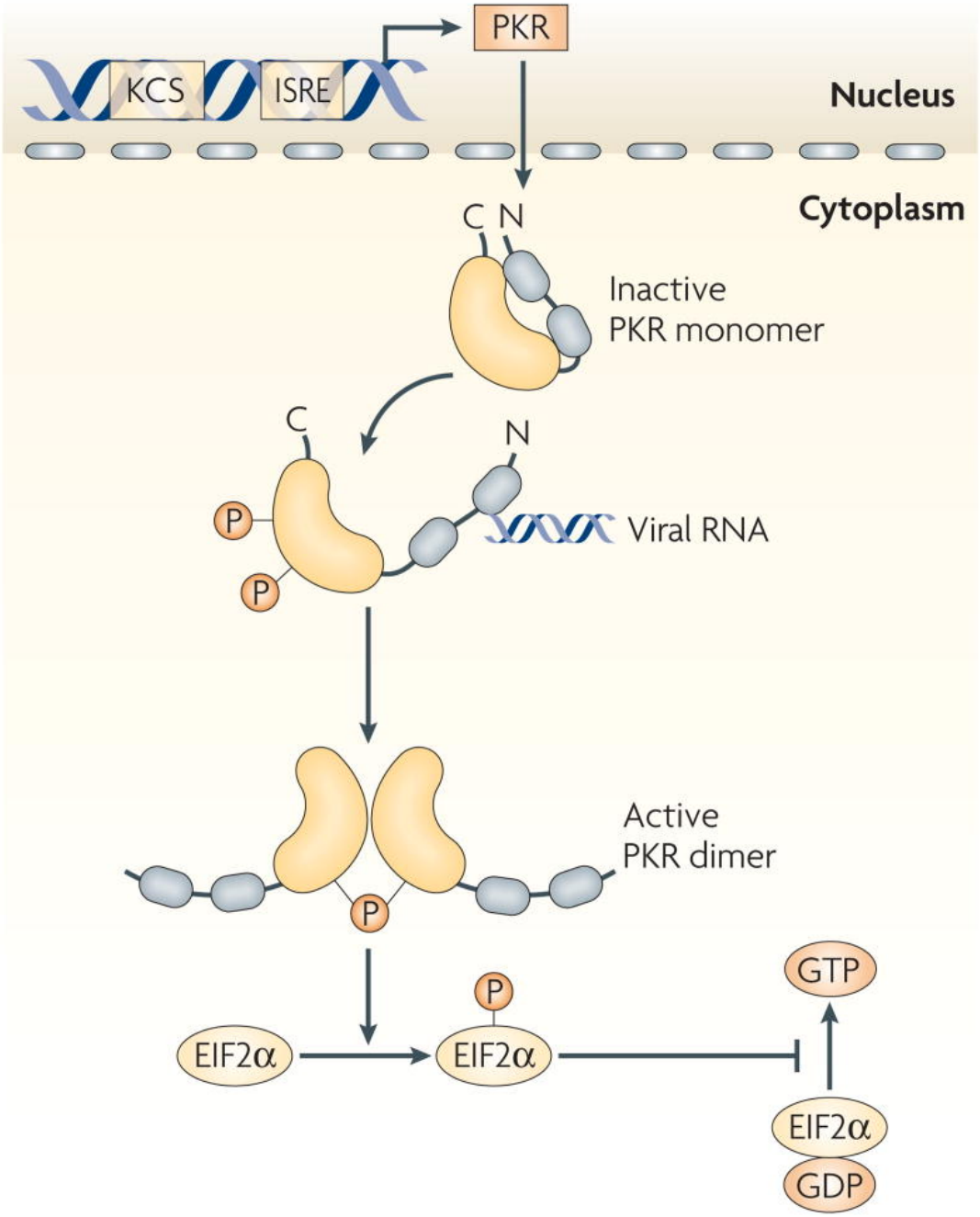


*nature reviews | **immunology***

Comparison of the OAS1–RNase L and cGAS–STING axes in innate immune signalling and antiviral defence.



Mechanism of action of PKR



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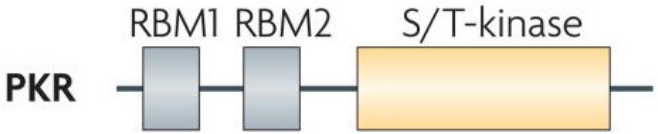
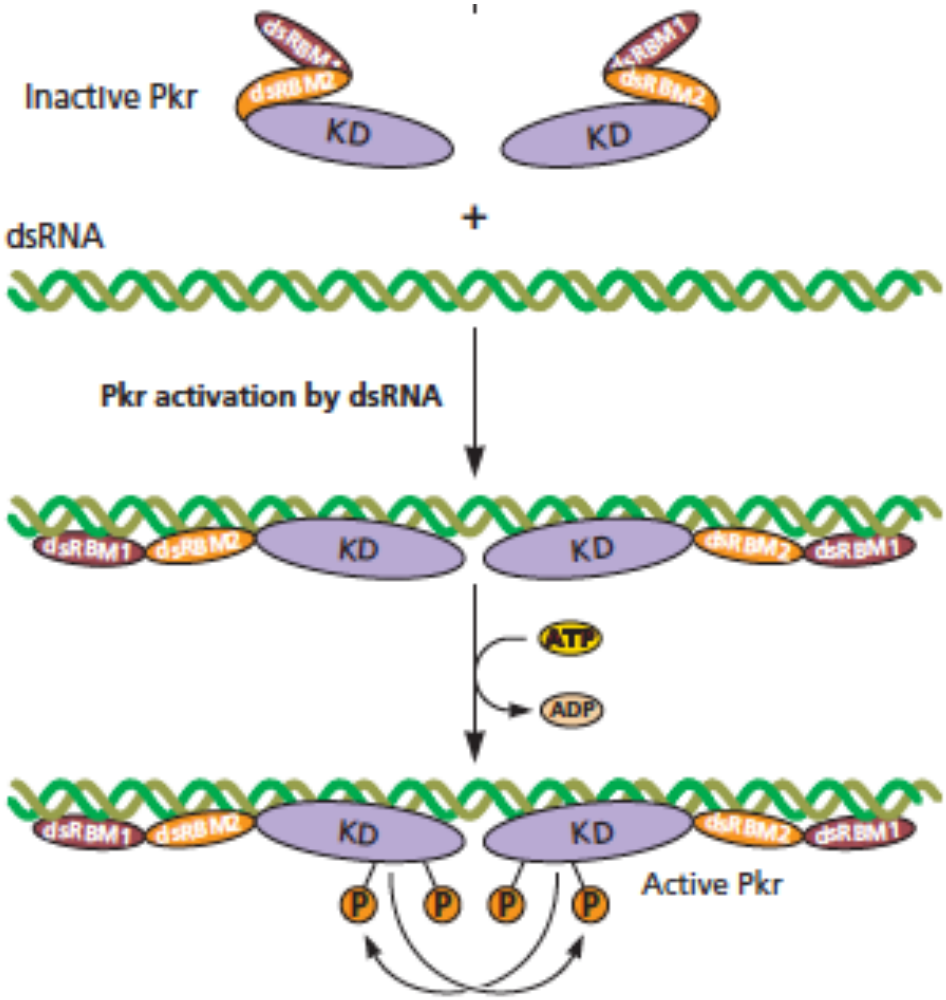
PKR activation mechanism



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.



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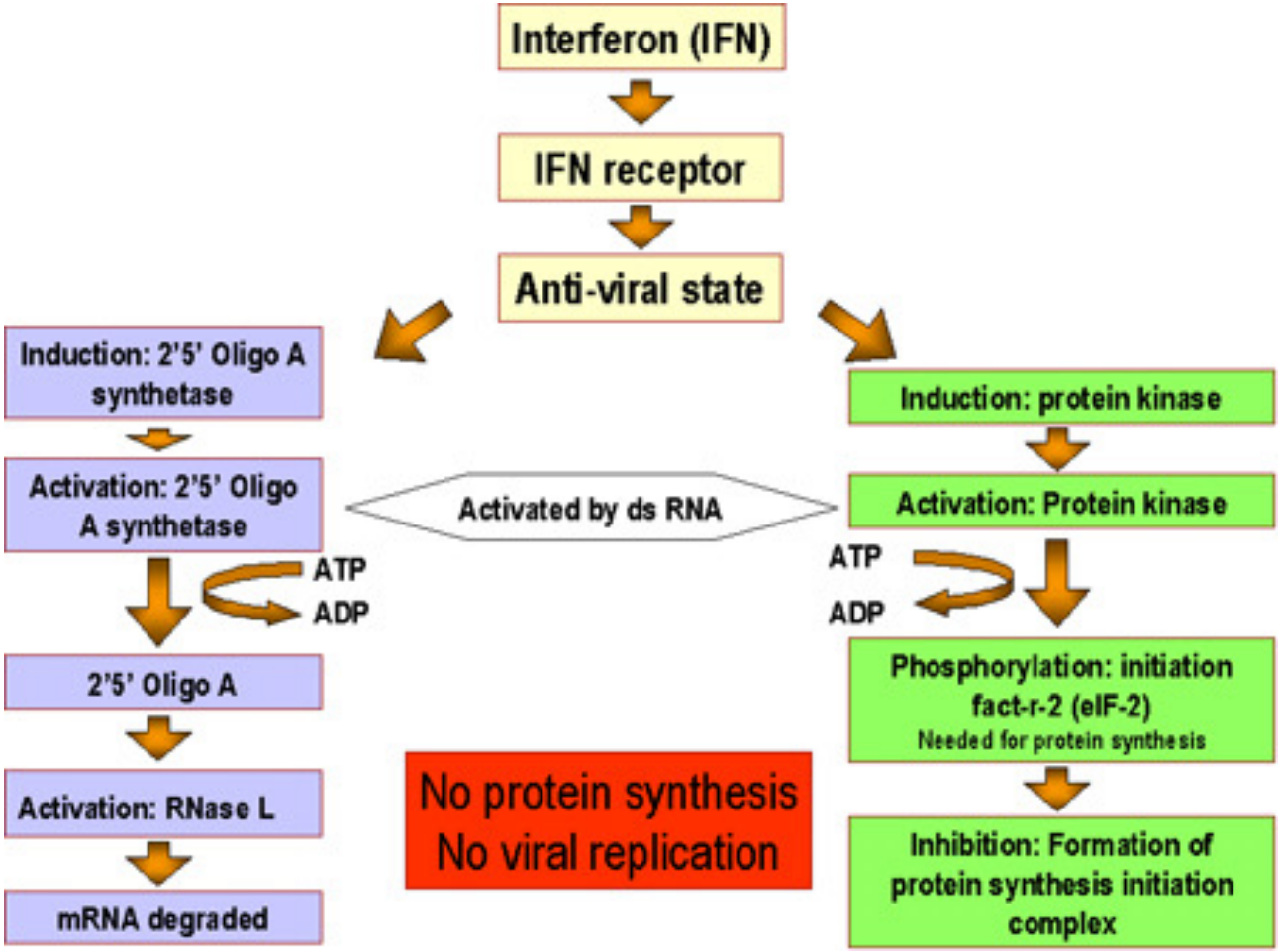
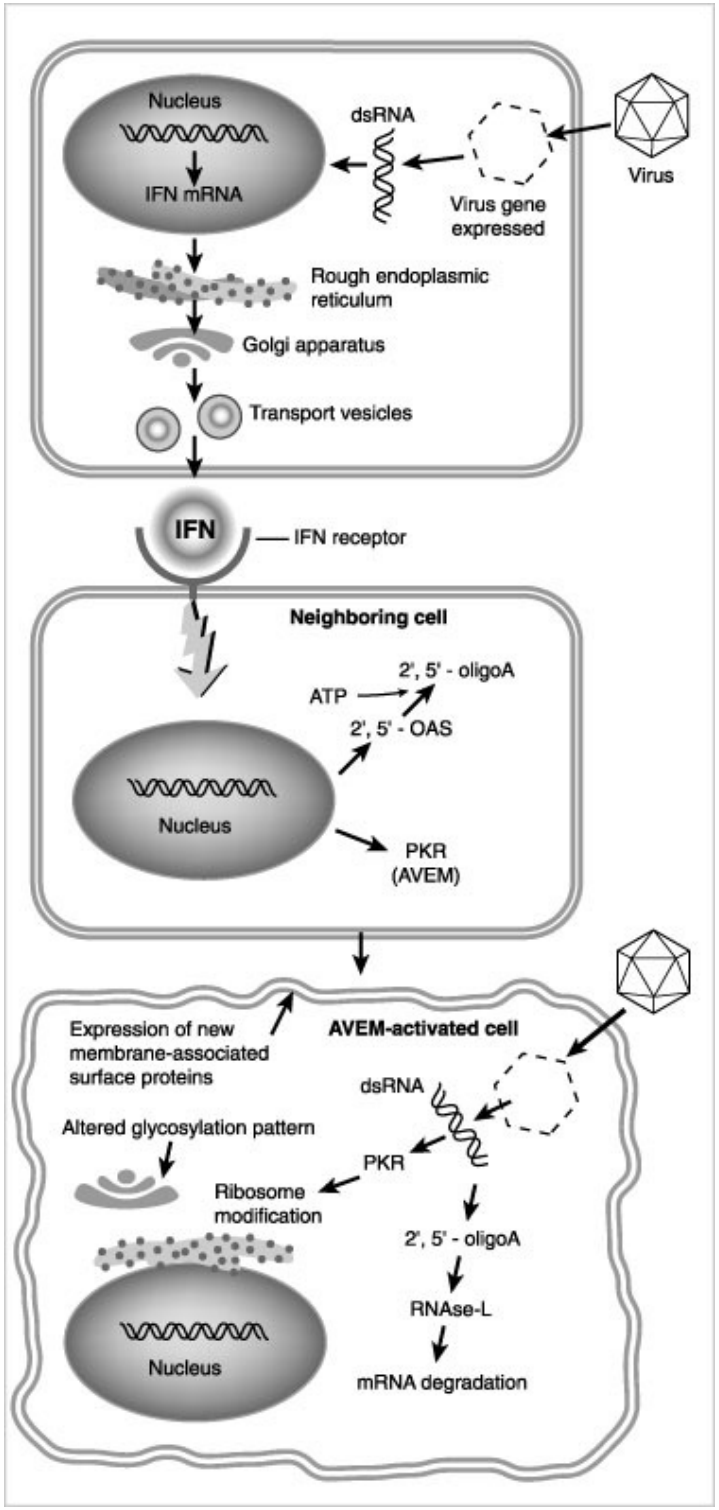
From Flint et al. *Principles of Virology* (2015), ASM Press

Interferon: effectors



Interferon: antiviral activity

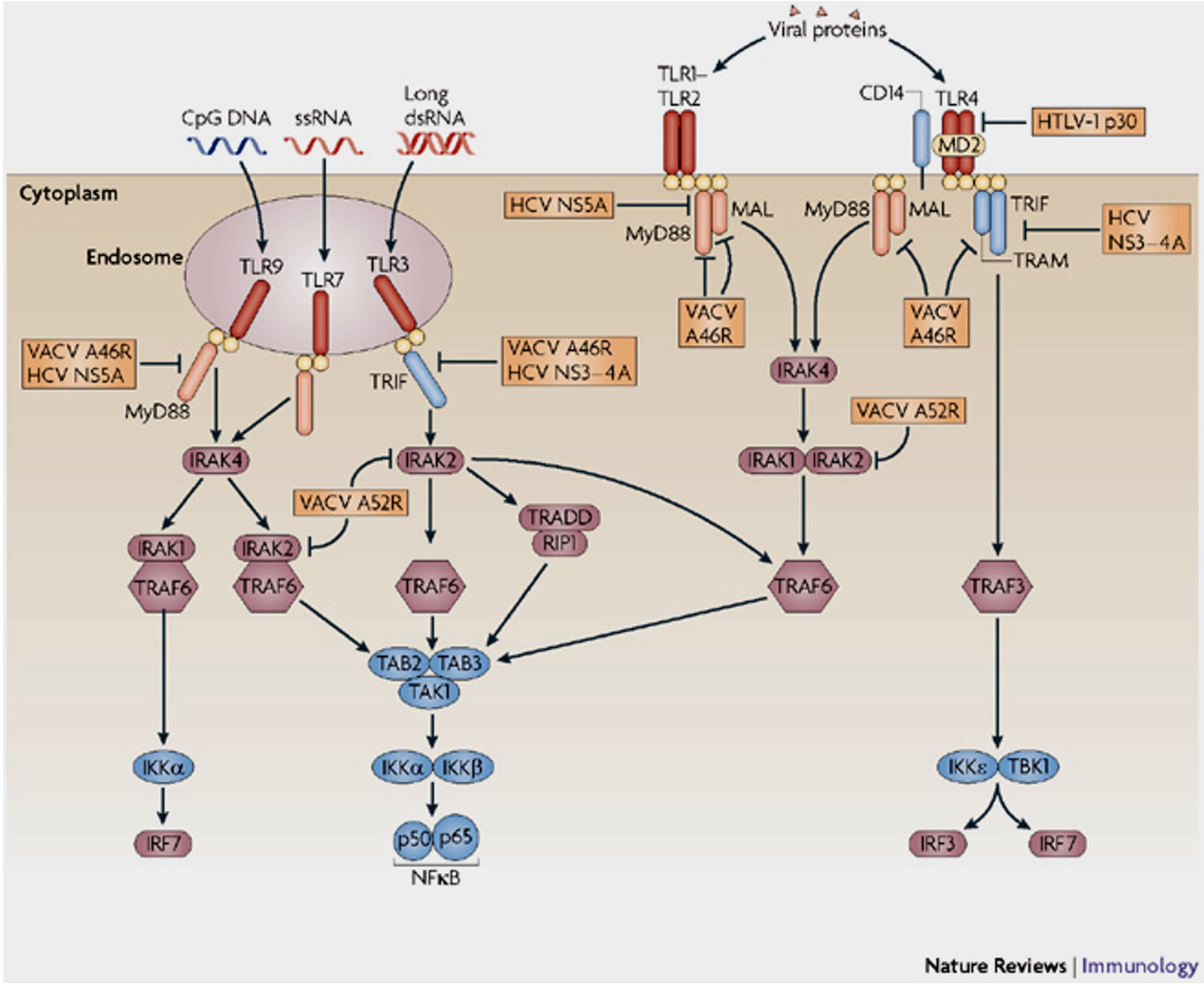


From Wagner and Hewlett "Basic Virology" (2004)-Blackwell

Protein	Virus	Assigned functions in evading or subverting PRR signalling pathways	Refs
VP35	Ebola virus	Sequesters viral dsRNA	112
		Inhibits IRF3 activation downstream of IPS1	112
NS3–4A	Hepatitis C virus	Degrades TRIF to inhibit TLR3 signalling	35
		Cleaves IPS1 from its mitochondrial tether to disable RLR signalling pathways	48,69,70
		Inhibits IRF3 phosphorylation by disrupting the TBK1–IRF3 interaction	75
NS5A	Hepatitis C virus	Inhibits PKR through direct binding	113
		Inhibits OAS through direct binding	114
		Inhibits TLR signalling by binding MyD88	36
E3L	Vaccinia virus	Sequesters viral dsRNA	115
		Inhibits PKR through direct binding	116
		Prevents DAI from interacting with DNA	5
		Binds to and disables ISG15	93
A52R	Vaccinia virus	Inhibits TLR-induced NFκB activation by binding IRAK2	38
		Enhances TLR-induced IL-10 production by binding TRAF6	106
NS1	Influenza A virus	Sequesters viral dsRNA	117
		Binds to RIG-I and suppresses RIG-I signalling	51

DAI, DNA-dependent activator of IRFs; ds, double stranded; IFN, interferon; IL-10, interleukin-10; IL-1R, IL-1 receptor; IPS1, *IFNB*-promoter stimulator 1; IRAK2, IL-1R-associated kinase 2; IRF3, IFN-regulatory factor 3; ISG15, IFN-stimulated protein of 15 kDa; MyD88, myeloid differentiation primary-response gene 88; NFκB, nuclear factor-κB; NS1, nonstructural protein 1; OAS, 2′, 5′-oligoadenylate synthetase; PKR, IFN-inducible dsRNA-dependent protein kinase; PPR, pattern-recognition receptor; RLR, retinoic-acid-inducible gene I (RIG-I)-like receptor; TANK, TRAF-family-member-associated NFκB activator; TBK1, TANK-binding kinase 1; TLR, Toll-like receptor; TRAF6, TNFR-associated factor 6; TRIF, TIR-domain-containing adaptor protein inducing IFNβ.

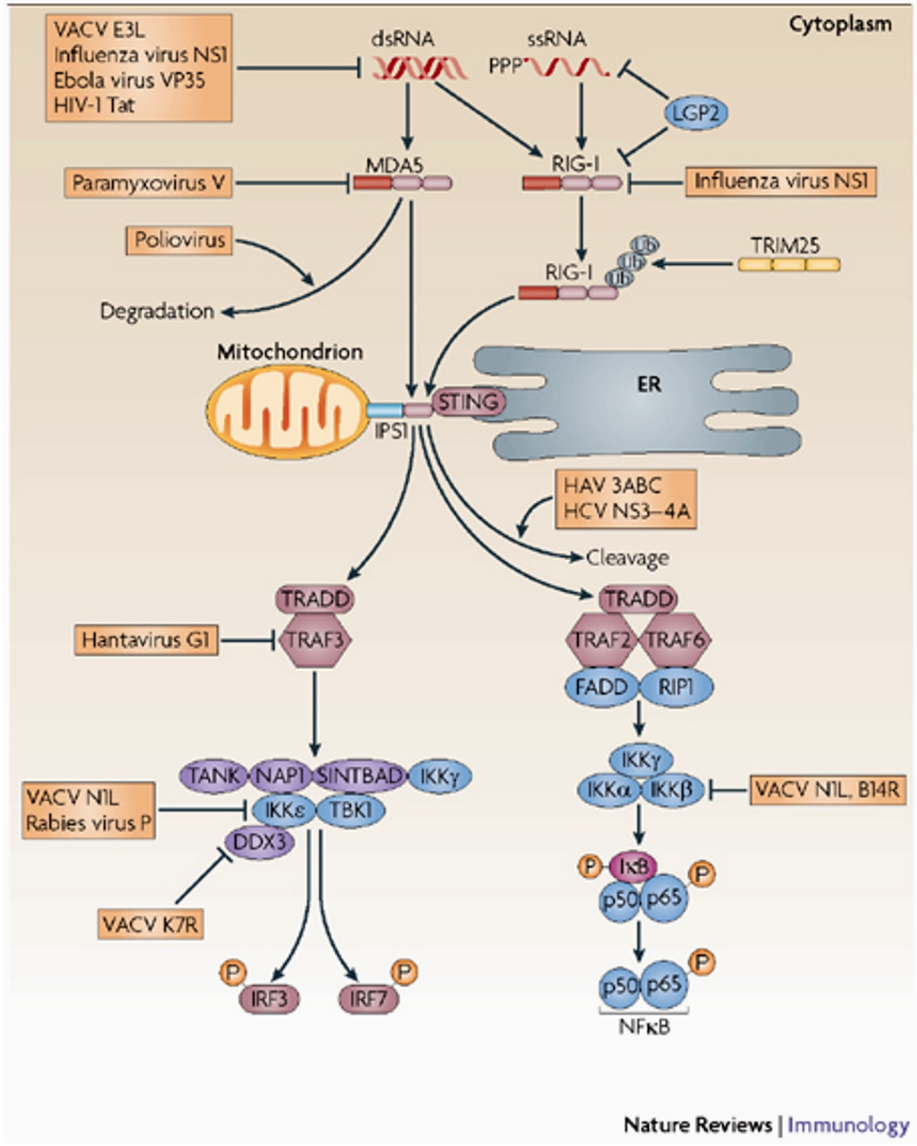
Viral evasion of Toll-like receptor signalling



Viral evasion of Toll-like receptor signalling

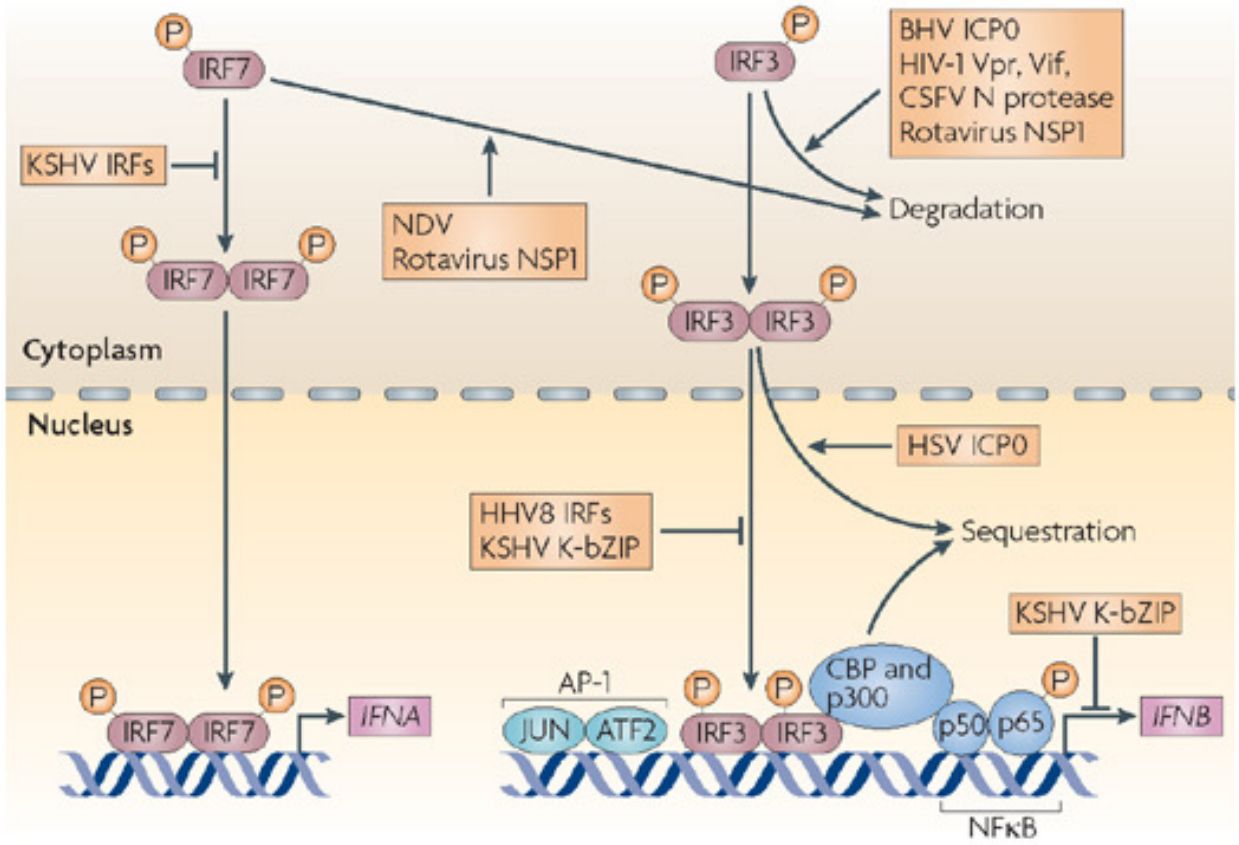
Following activation, Toll-like receptors (TLRs) recruit the adaptor proteins myeloid differentiation primary-response gene 88 (MyD88), TIR-domain-containing adaptor protein inducing IFN (TRIF), MyD88-adaptor-like (MAL) and TRIF-related adaptor protein (TRAM), as indicated. These then initiate signalling cascades involving IL-1R-associated kinase (IRAK) and TNFR-associated factor (TRAF) proteins, which finally converge at the activation of the I B kinase (IKK) family members IKK , IKK , IKK and TBK1 (TANK-binding kinase 1). The vaccinia virus (VACV) protein A46R sequesters all these adaptor proteins, whereas the hepatitis C virus (HCV) protein NS5A selectively binds MyD88 and the HCV NS3–4A protease cleaves TRIF. Human T-cell leukaemia virus type 1 (HTLV-1) protein p30 acts even further upstream by reducing the expression of TLR4. VACV A52R binds to and inhibits IRAK2, thereby affecting several TLR pathways that lead to nuclear factor- B (NF B) activation. dsRNA, double-stranded RNA; IFN, interferon; IL-1R, interleukin-1 receptor; IRF, IFN-regulatory factor; I B, inhibitor of NF B; MD2, myeloid differentiation protein 2; ssRNA, single-stranded RNA; RIP1, receptor-interacting protein 1; TAB, TAK1-binding protein; TAK1, transforming-growth-factor- -activated kinase 1; TANK, TRAF-family-member-associated NF B activator; TIR, TLR/IL-1R; TNFR, tumour-necrosis factor receptor; TRADD, TNFR-associated via death domain.

Viral evasion of retinoic-acid-inducible-gene-I-like receptor signalling



RIG-I (retinoic-acid-inducible gene I) and MDA5 (melanoma differentiation-associated gene 5), termed RIG-I-like receptors (RLRs), are activated by cytoplasmic RNA during viral infection. Both signal using ***IFNB***-promoter stimulator 1 (IPS1), which is tethered to the mitochondrial membrane. When IPS1 is engaged by RLRs, it recruits downstream signalling complexes that lead to the activation of the IFN-regulatory factors (IRFs) and nuclear factor- B (NF B). In addition, signalling through RIG-I requires the adaptor STING (stimulator of IFN genes), which resides in the endoplasmic reticulum (ER). RLR signalling is inhibited by viral proteins that either bind RIG-I, MDA5 or IPS1 directly or cause their degradation. The I B kinase (IKK) family members are also a common target for viral proteins. DDX3, DEAD-box protein 3; dsRNA, double-stranded RNA; FADD, FAS-associated via death domain; HAV, hepatitis A virus; HCV, hepatitis C virus; IFN, interferon; I B, inhibitor of NF B; LGP2, laboratory of genetics and physiology 2; NAP1, NF B-activating kinase-associated protein 1; NS1, nonstructural protein 1; PPP, 5' triphosphate; RIP1, receptor-interacting protein 1; SINTBAD, similar to NAP1 TBK1 adaptor; ssRNA, single-stranded RNA; TANK, TRAF-family-member-associated NF B activator; TBK1, TANK-binding kinase 1; TNFR, tumour-necrosis factor receptor; TRADD, TNFR-associated via death domain; TRAF, TNFR-associated factor; TRIM25, tripartite motif-containing 25; ub, ubiquitin; VACV, vaccinia virus.

Inhibition of interferon-regulatory factor 3 (IRF3) and IRF7 by viral proteins.



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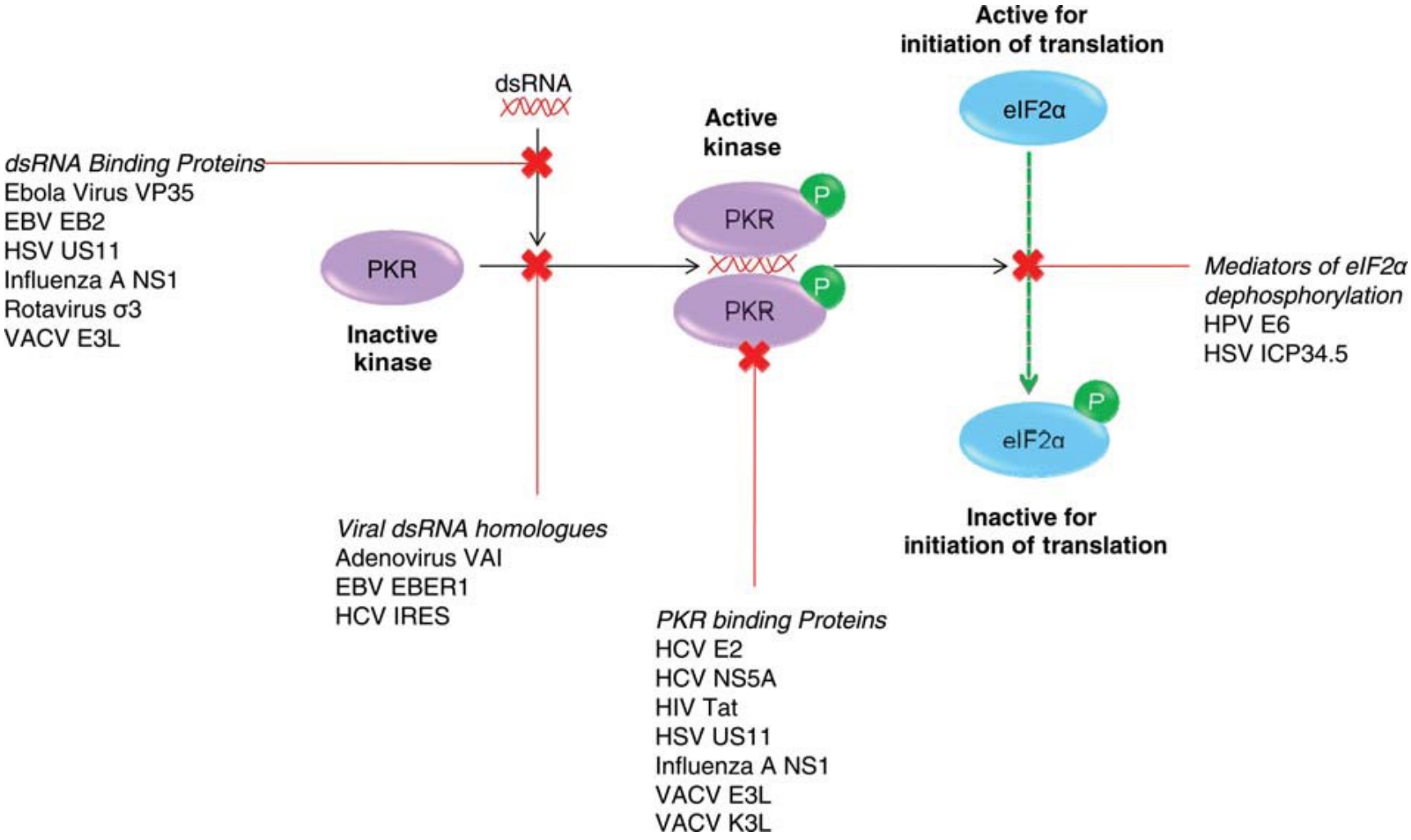
Inhibition of interferon-regulatory factor 3 (iRF3) and iRF7 by viral proteins. IRF3 and IRF7 are activated by phosphorylation, they then homodimerize and translocate to the nucleus, where they interact with their co-activators CREB-binding protein (CBP) and p300 and induce the expression of genes such as interferon-α (*IFNA*) and *IFNB*. Viruses inhibit IRFs by inducing their degradation, sequestering them or competing with them for binding to promoter sequences. AP1, activator protein 1; ATF2, activating transcription factor 2; BHV, bovine herpesvirus; CREB, cyclic-AMPresponsive- element-binding protein; CSFV, classical swine fever virus; HHV, human herpesvirus; HSV, herpes simplex virus; ICP0, infected cell protein 0; NDV, Newcastle disease virus; NFκB, nuclear factor-κB; NSP1, non-structural protein 1; KSHV, Kaposi's sarcoma-associated herpesvirus.

Viral evasion of interferon stimulated genes



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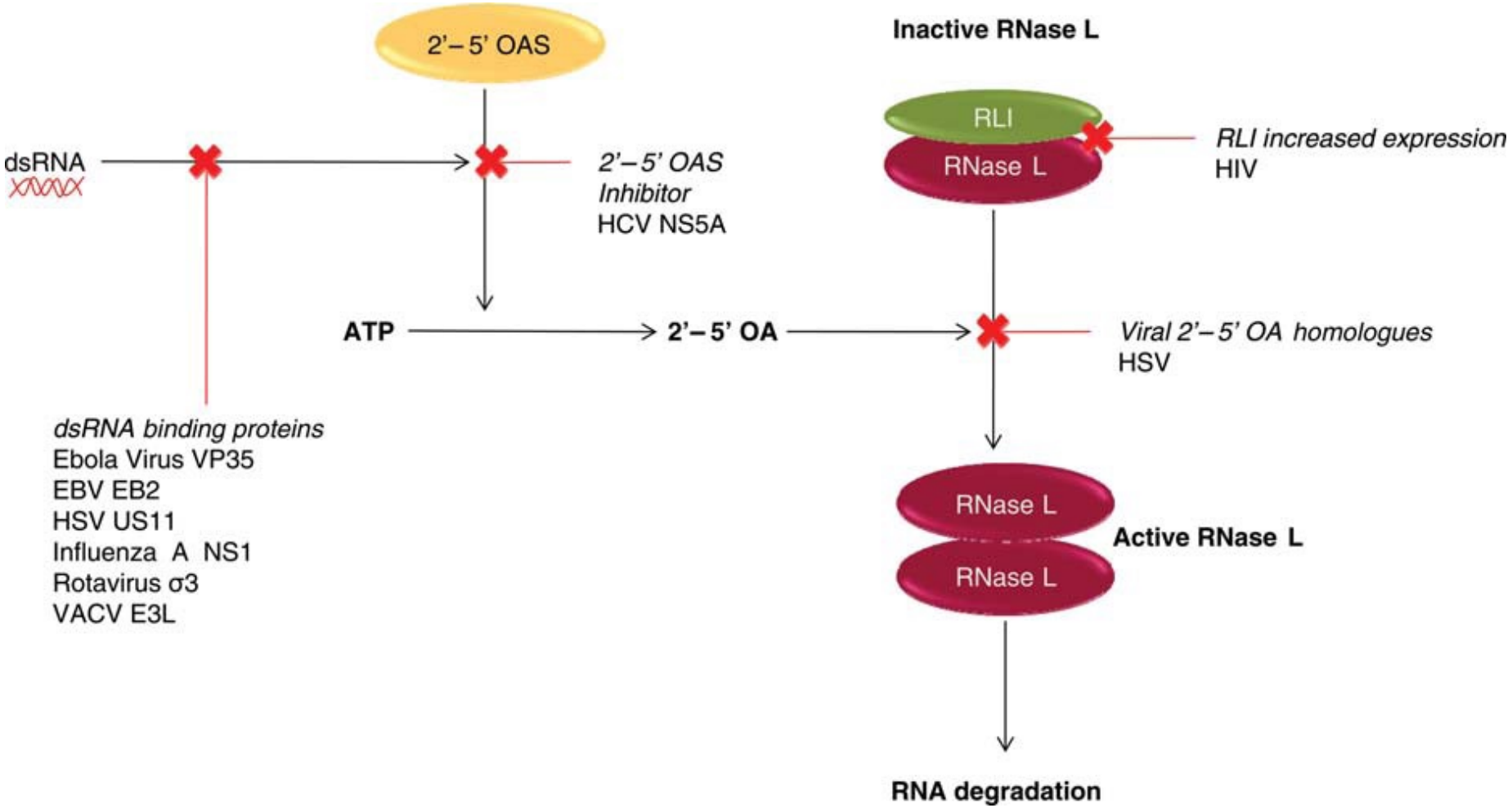
Viral evasion of interferon stimulated genes



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