Interferons, Cytokines & Viruses 7/1/13

Michael McGarvey

What are Cytokines?

Signalling Molecules

Polypeptides

~ 30 kd

Soluble (most)

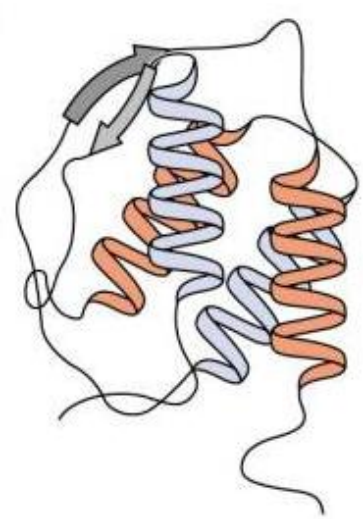
Similar structure to polypeptide hormones

Cytokine Structures

α-Helical

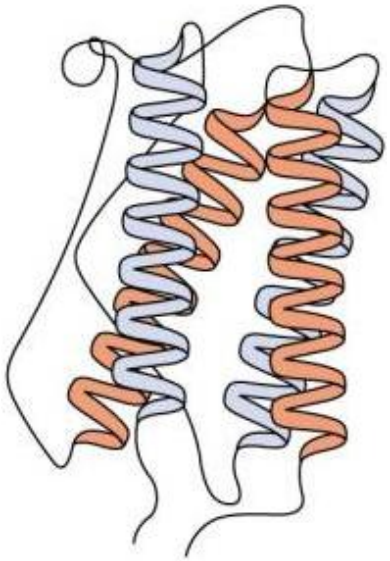
β-Sheet

Short α-helices ca 15aa

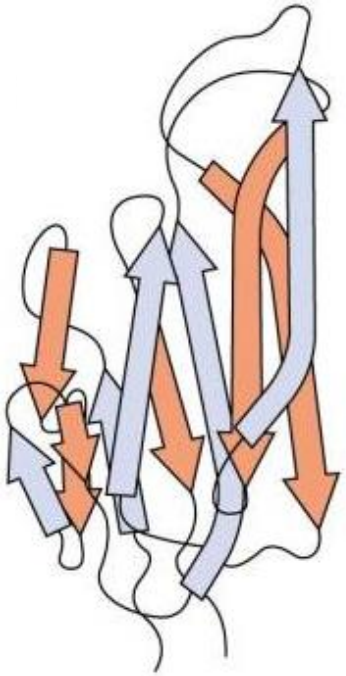


IFN-γ
IL-2
IL-9

Long α-helices ca 25aa



IFN-α/β
IL-6
IL-12



TNF-α

How Do They Work?

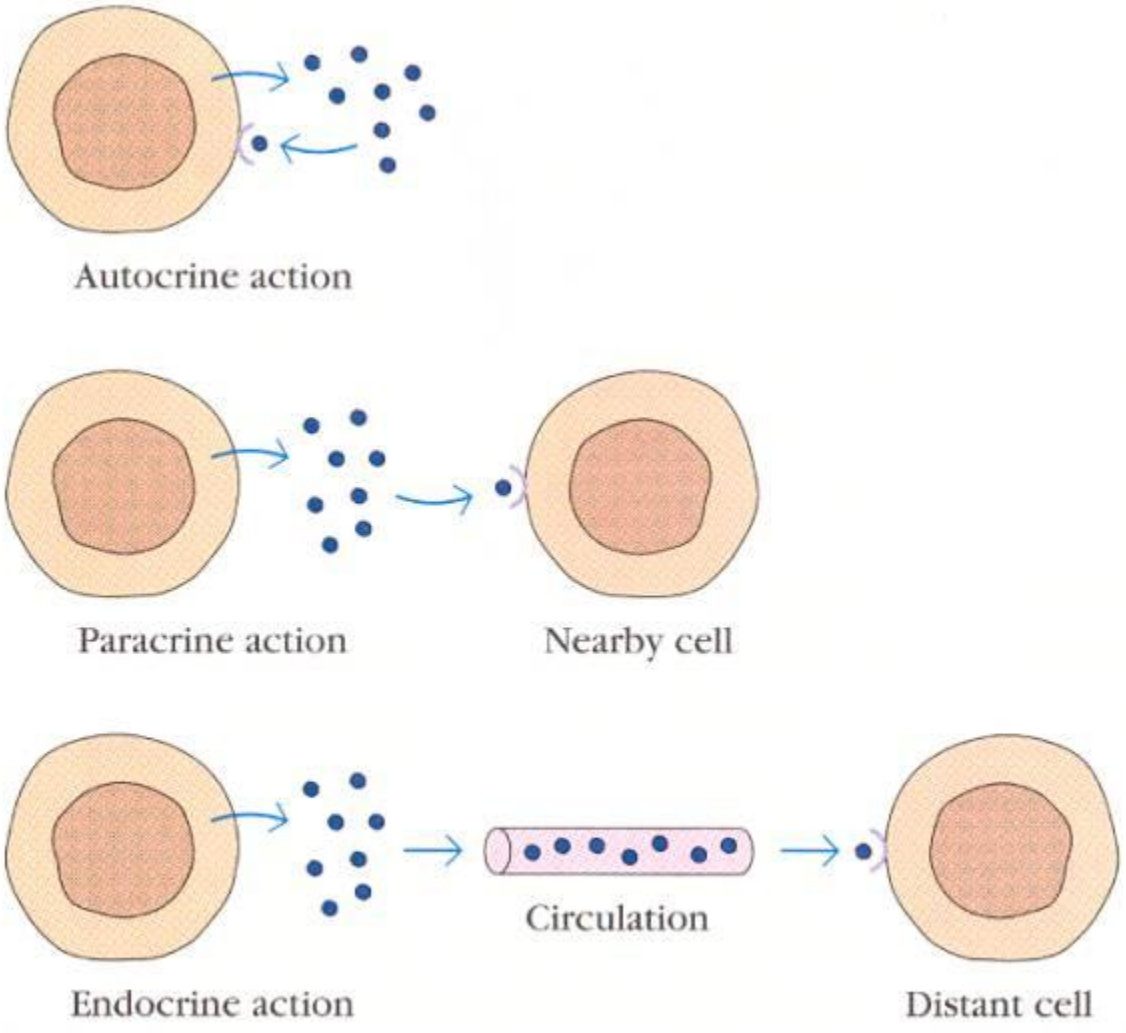
Receptors at cell surface

Activate intracellular signalling pathways

Expression induced (by microbial infection)

Overlapping functions

Cytokine Effects: Distance



Nomenclature

Interferons	**Type I**	**(IFN α, β, κ, ω)**
	Type II	**(IFN γ)**
	Type III	**(IFN-λ1, λ2, λ3)**
Interleukins		**(IL-1, IL-2, ...IL-36?)**
Tumour Necrosis Factors		**(TNFα, TNFβ... FASL, TRAIL)**

Classification

1. Initial and Innate Cytokines

Classification

1. Initial and Innate Cytokines

2. Adaptive Cytokines

3. Chemokines

4. Haematopoietic Growth Factors

Initial and Innate Cytokines

IFN α/β

Two IFNβ, multiple (~21) IFNα genes

Made by most cells – response to viral infection

Stimulate specific expression of Interferon Specific Genes ("ISGs")

100s of ISGs

Initial and Innate Cytokines

IFN α/β

Other activities:

- **Activates NK cell cytotoxic activity**
- **Enhances MHC I antigen presentation**
- **Facilitation of T-cell IFNγ responses**

Initial and Innate Cytokines

TNF-α (Proinflammatory)

- **Upregulation of MHC class I**
- **Immunoregulatory, antiviral pathways activated**
- **Stimulates cell proliferation**
- **Anti-apoptotic factors**
- **Stimulates liver cells → produce C-reactive protein**

Adaptive Cytokines

Produced mainly or exclusively by T-cells

IL-2

T-cells express IL-2R
Autocrine growth factor
T-cell proliferation
Induces IFNγ expression

IFNγ

Enhanced antigen processing and MHC presentation
Switching of immunoglobulin classes
Stimulates nitric oxide synthase (iNOS)

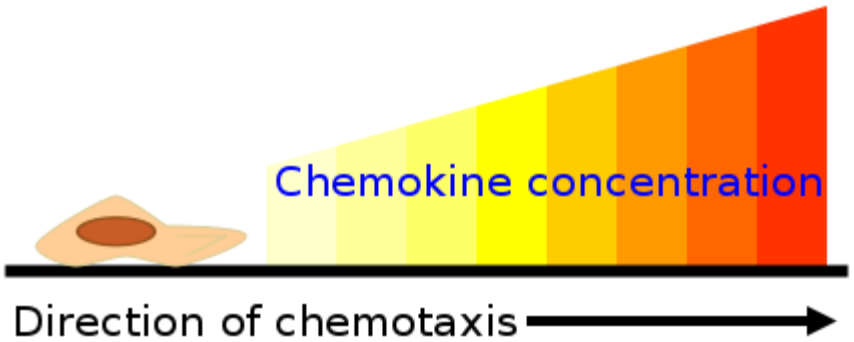
Chemokines

Chemotactic Cytokines = Chemokines

8 – 12 kd in size

Four Families

C, CC, C-X-C & C-X3-C



Chemokines

CCL3 (Macrophage Inflammatory Protein)

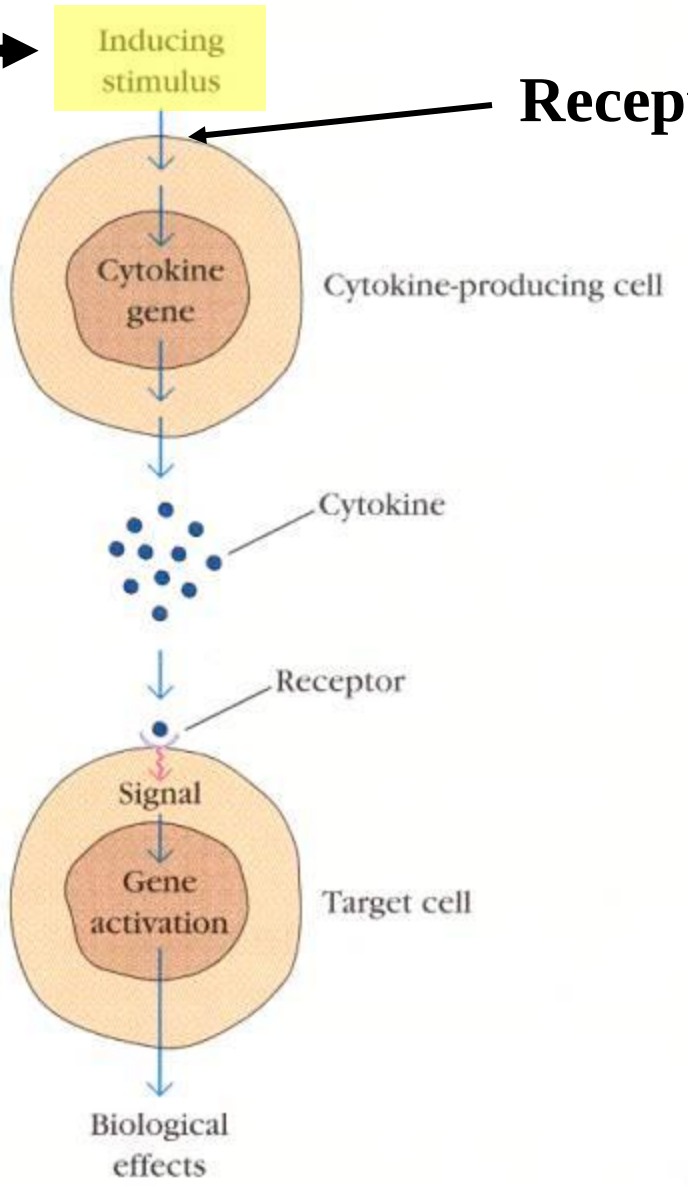
- **Induce the synthesis and release of other pro-inflammatory cytokines IL-1, IL-6 & TNF-α from fibroblasts and macrophages**
- **Migration of protective NK cells into CMV infected liver (innate)**
- **Tissue inflammation – influenza, HSV, Coxsackie infections**

Cytokines Induced During Virus Infections

Virus	Cytokine Expression	
	Initial and Innate	Adaptive
Lymphocytic choriomeningitis virus	**IFN-α/β**	**IFN–γ, IL-2** IL-4, TGF-β
Murine cytomegalovirus	**IFN-α/β** TNF-α, IL1α, IL-6 IL-12, IFN-γ	**IFN-γ, IL-2**
Herpes Simplex Virus type 1	**IFN-α/β** TNF-α, IL1α, IL-6	**IFN–γ, IL-2** IL-4, IL-5
Influenza A	**IFN-α/β** TNF-α, IL18, IL-12, IFN-γ	**IFN-γ, IL-2** IL-4, IL-5, IL-10
HCV	**IFN-α/β** TNF-α, IL-15, IL-28	**IFN-γ, IL-12** IL-10, TGF-β

Induction and Function of Cytokines

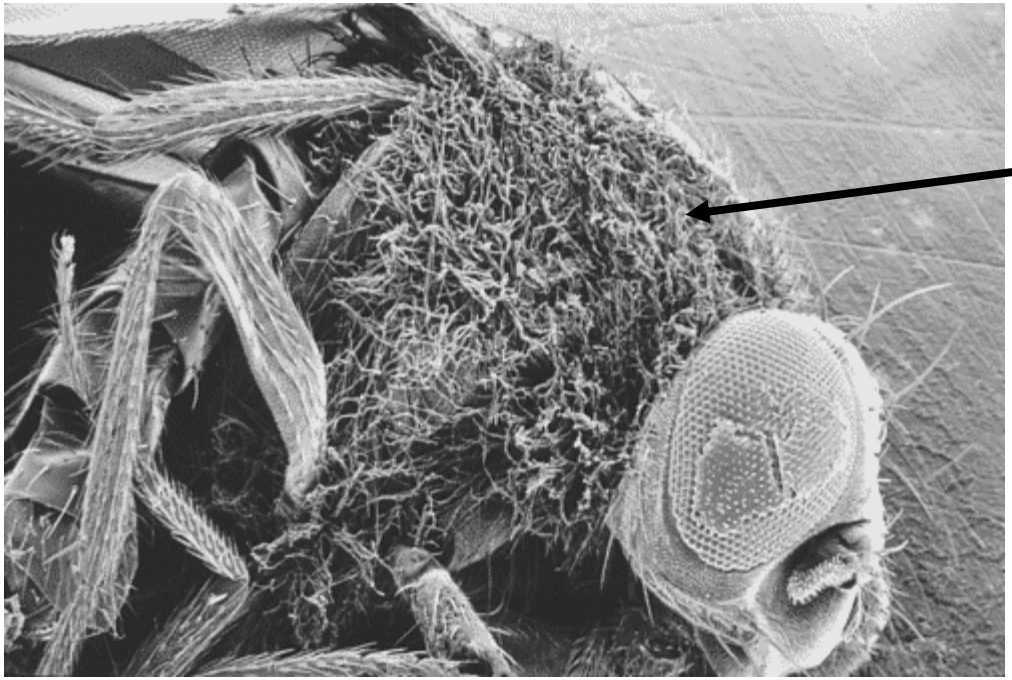
What induces cytokine production?



Toll-Like Receptors

Toll protein (*Drosophila*)

- **membrane protein**
- **embryonic development**
- **adult immunity**



SEM of Drosophila

Toll-Like Receptors

Toll protein (*Drosophila*)

- membrane protein
- embryonic development
- adult immunity
- Intracellular domain similar to human IL-1R

Toll-like receptor (Human)

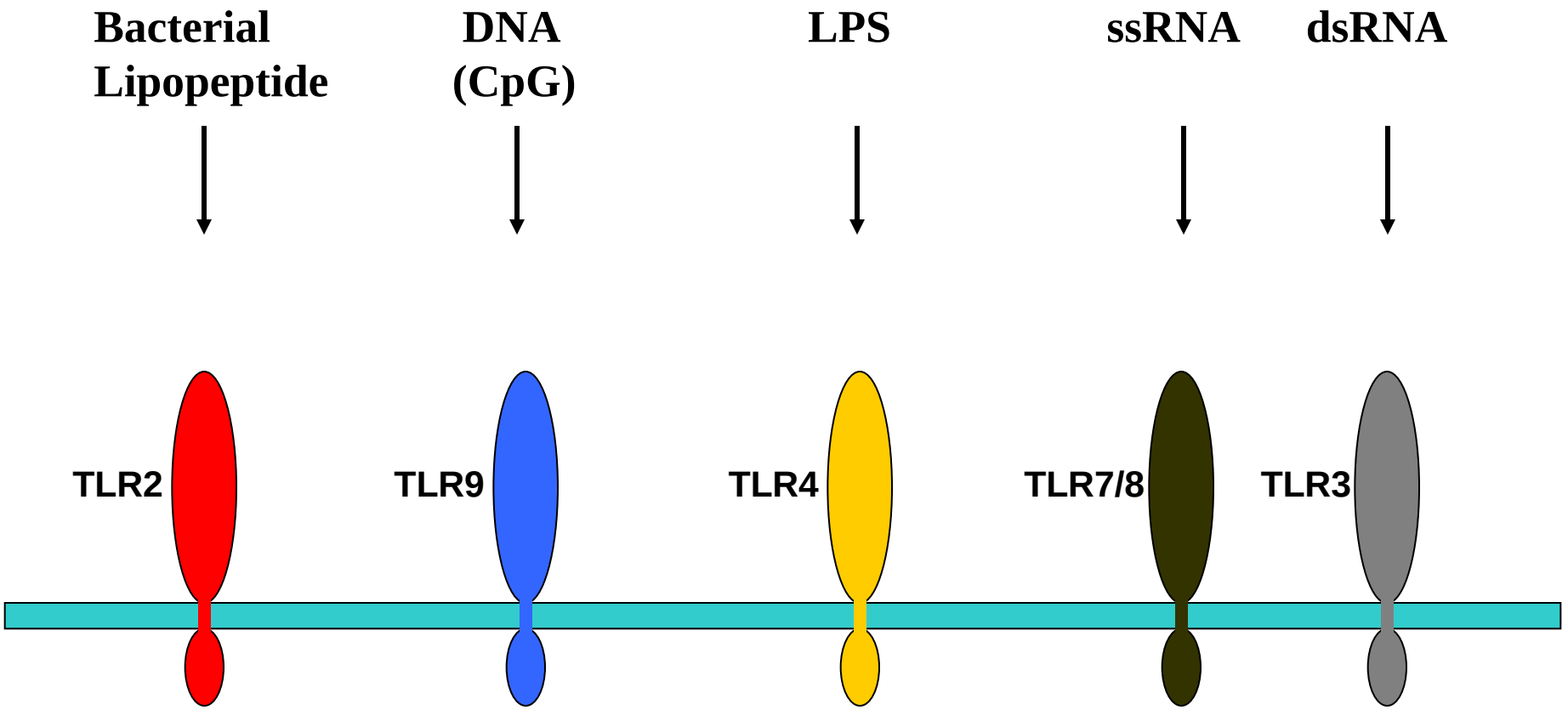
– Homologous genes to Toll

- currently 13 TLRs
- pattern recognition molecules
- Recognise microbial component
- Major part of Innate Immune responses

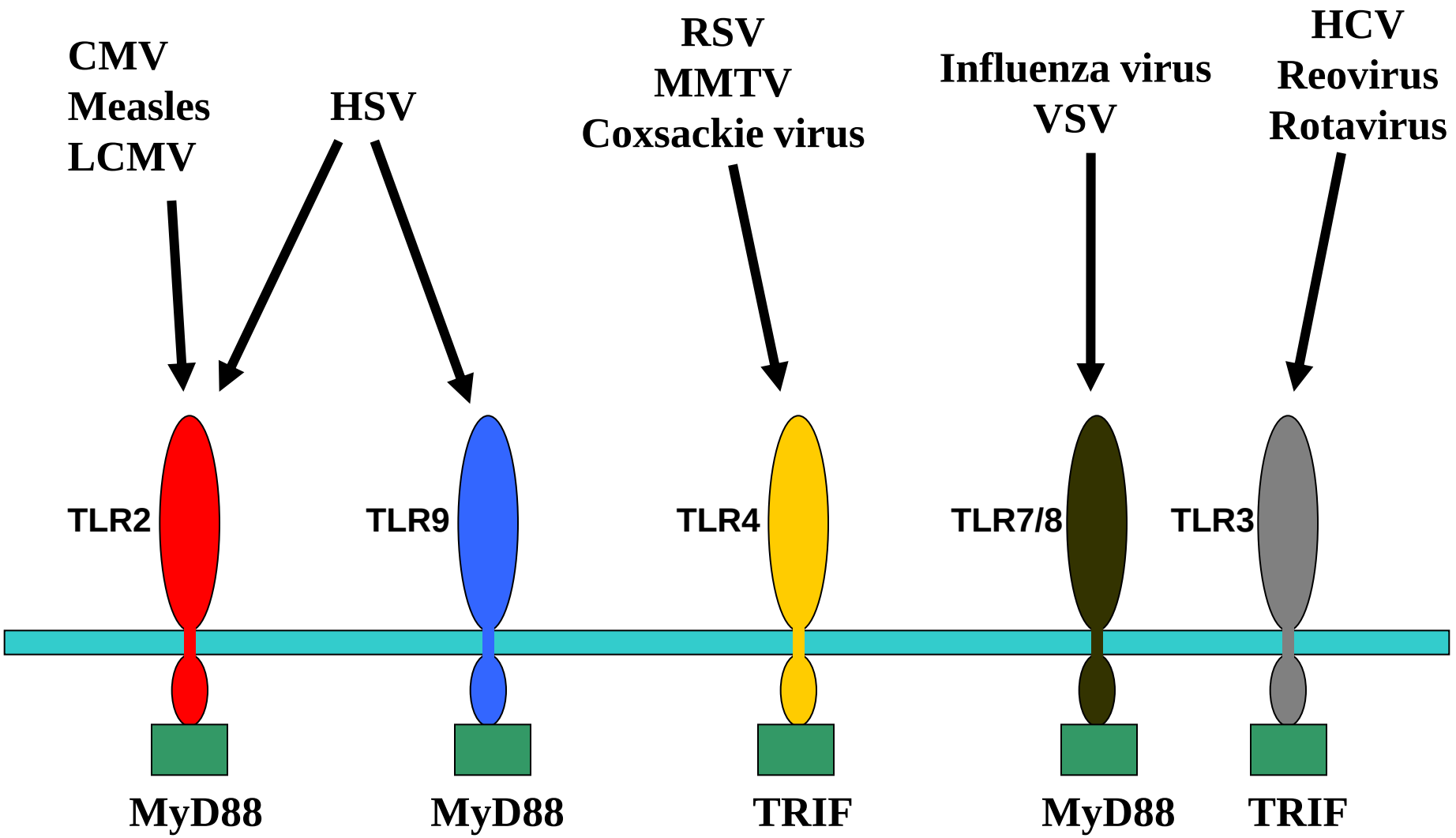
Toll-Like Receptors

Pattern Recognition

Pathogen Associated Molecular Patterns (PAMPS)

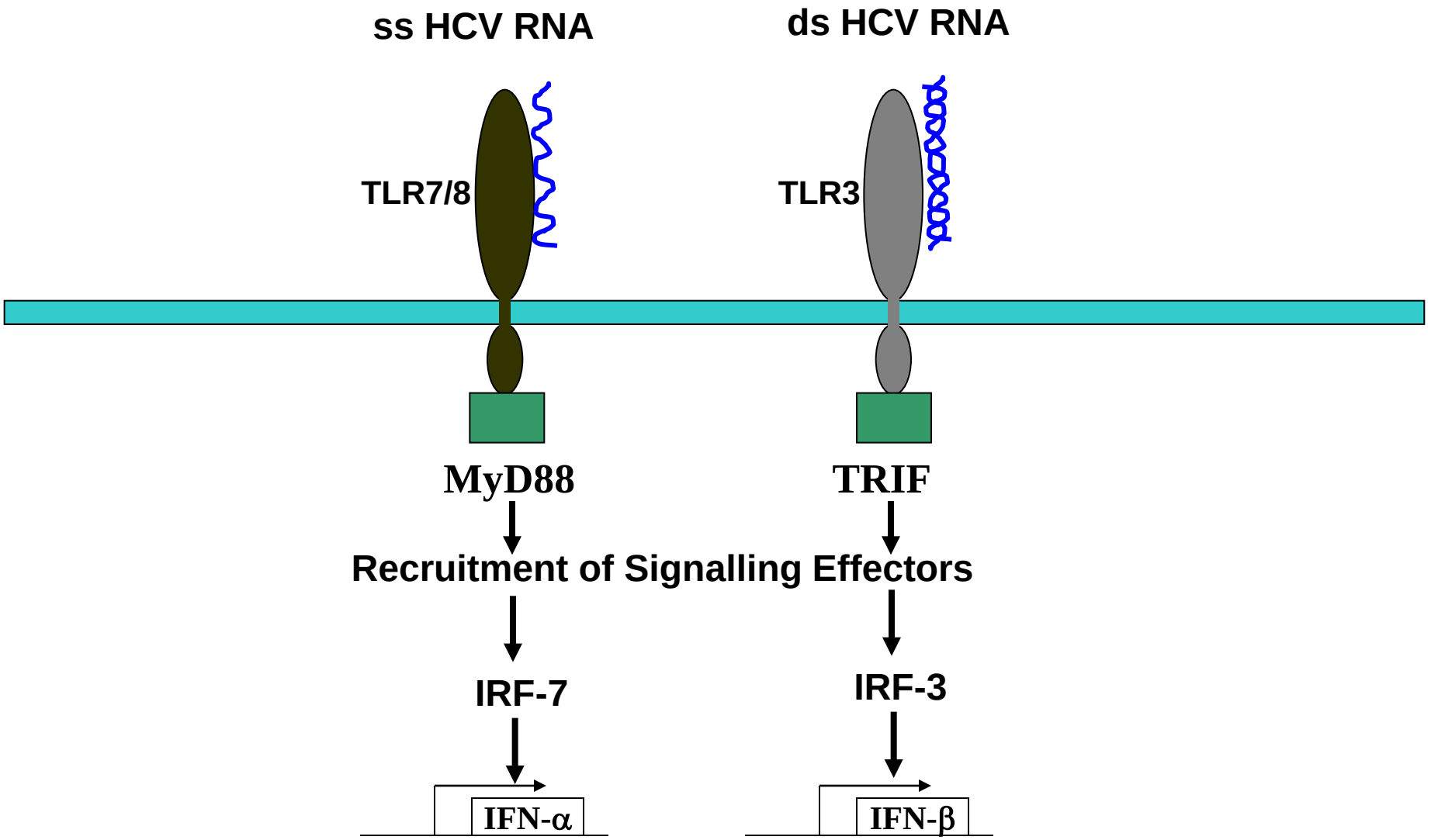


Toll-Like Receptors: Virus Recognition



Adaptor Proteins

Toll-Like Receptors: Virus Recognition



RIG-I Like Receptors (RLRs)

Cytoplasmic pattern recognition receptors

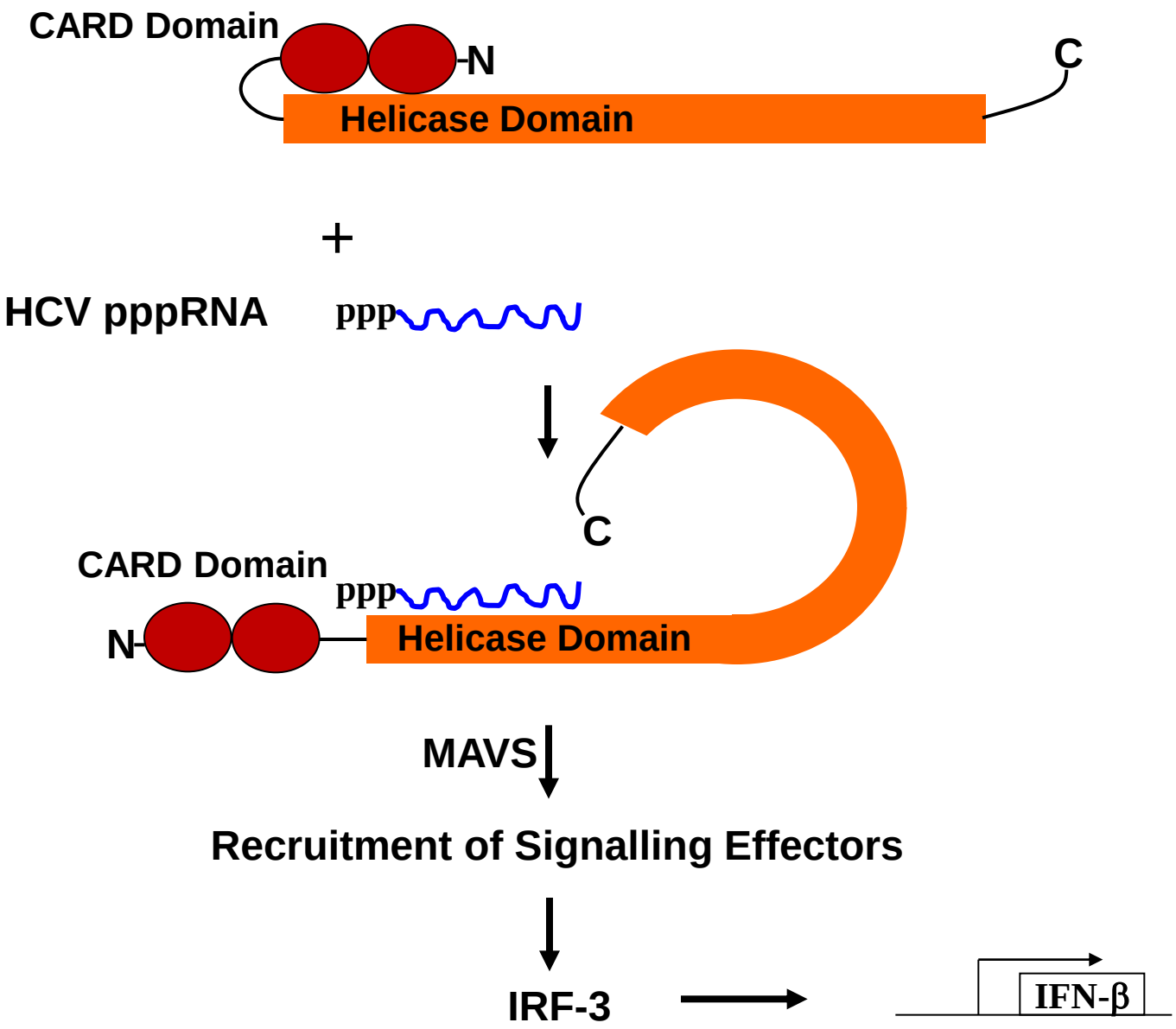
Three related proteins:

- **RIG-I (retinoic acid-inducible gene 1)**
- **MDA5 (Melanoma Differentiation-Associated protein 5)**
- **LGP2 (Laboratory of Genetics and Physiology 2)**

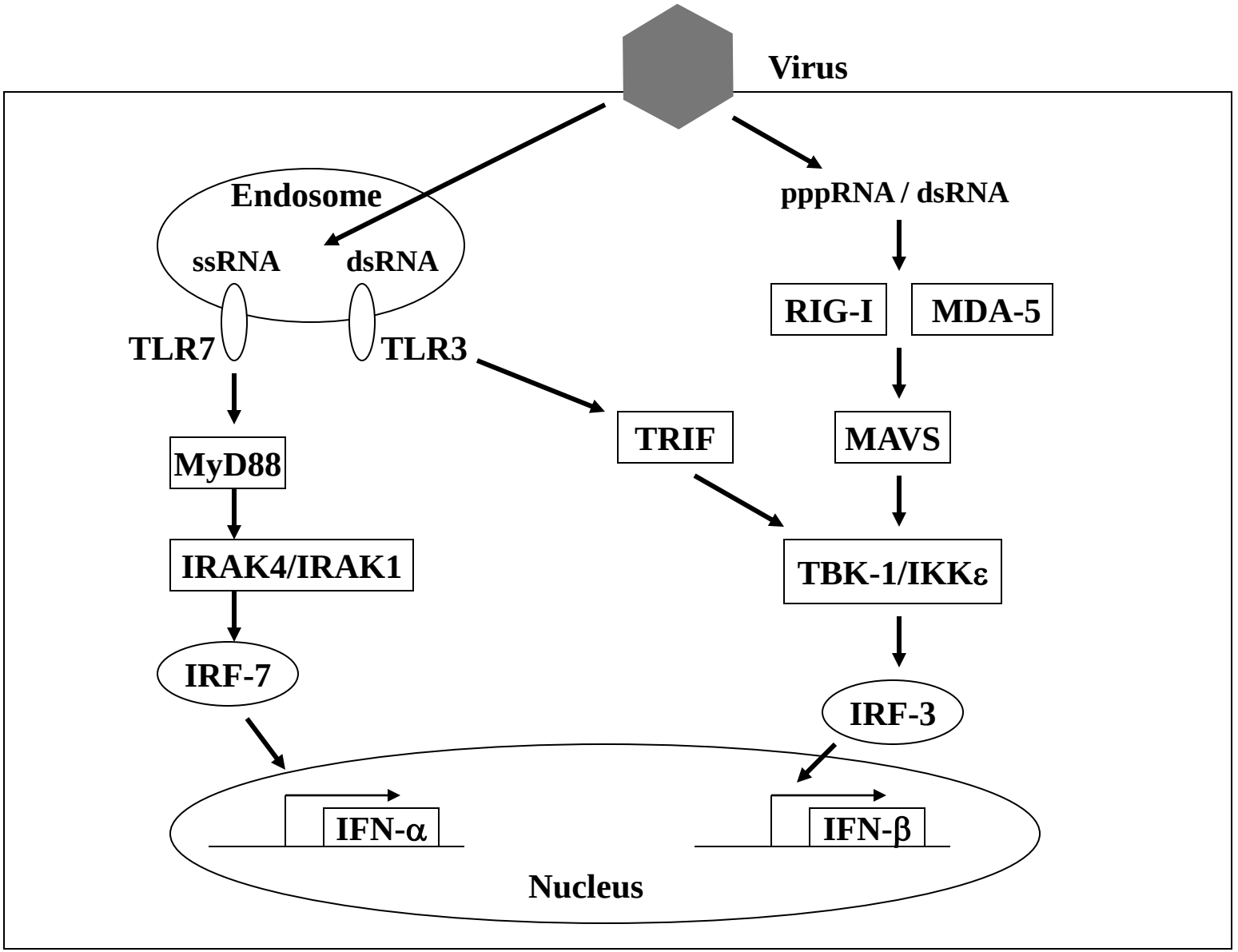
Structure of RLRs:

- **RNA helicase-DEAD box motifs**
- **Caspase recruitment domain (CARD)**

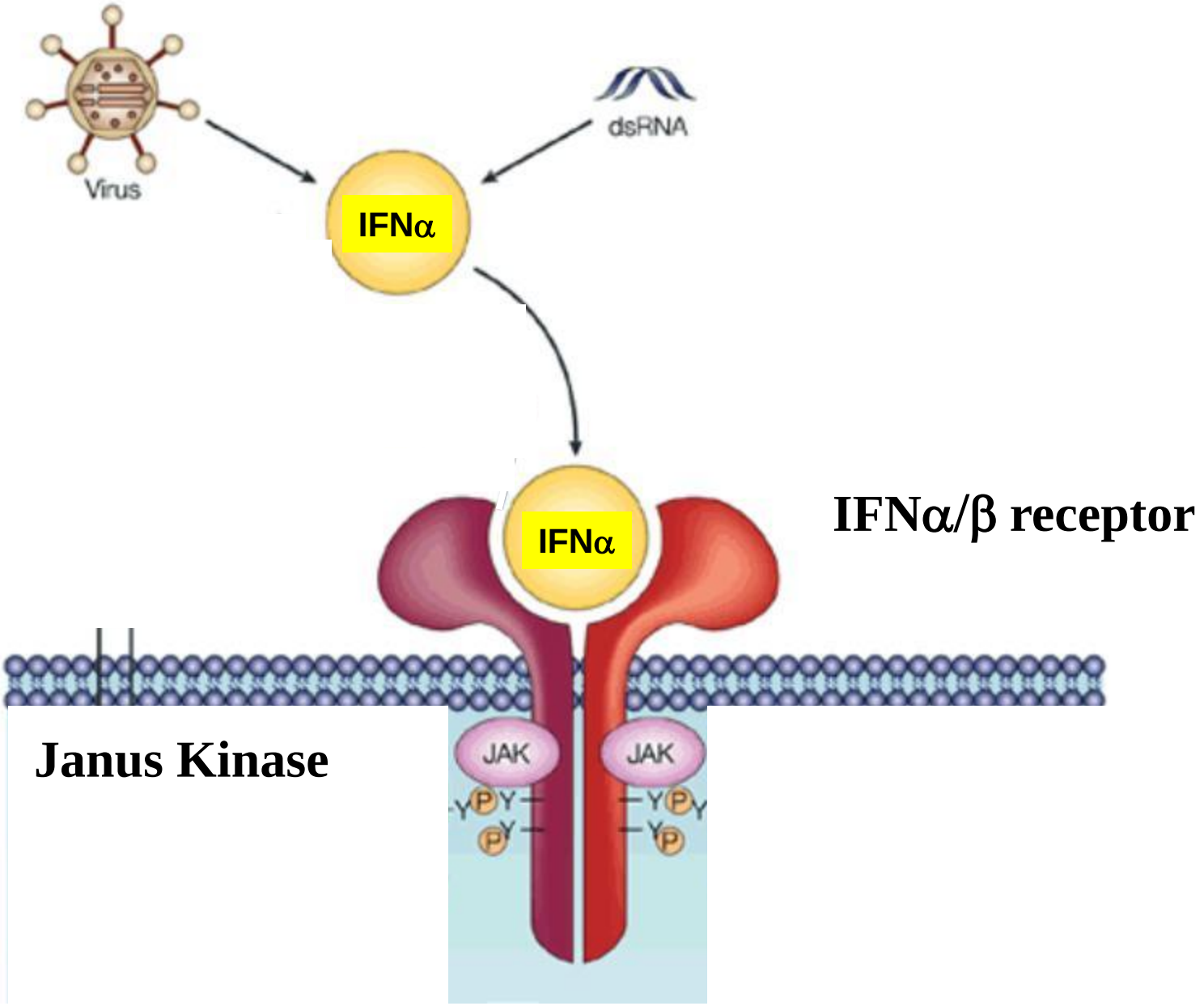
RIG-I Triggering of IRF-3

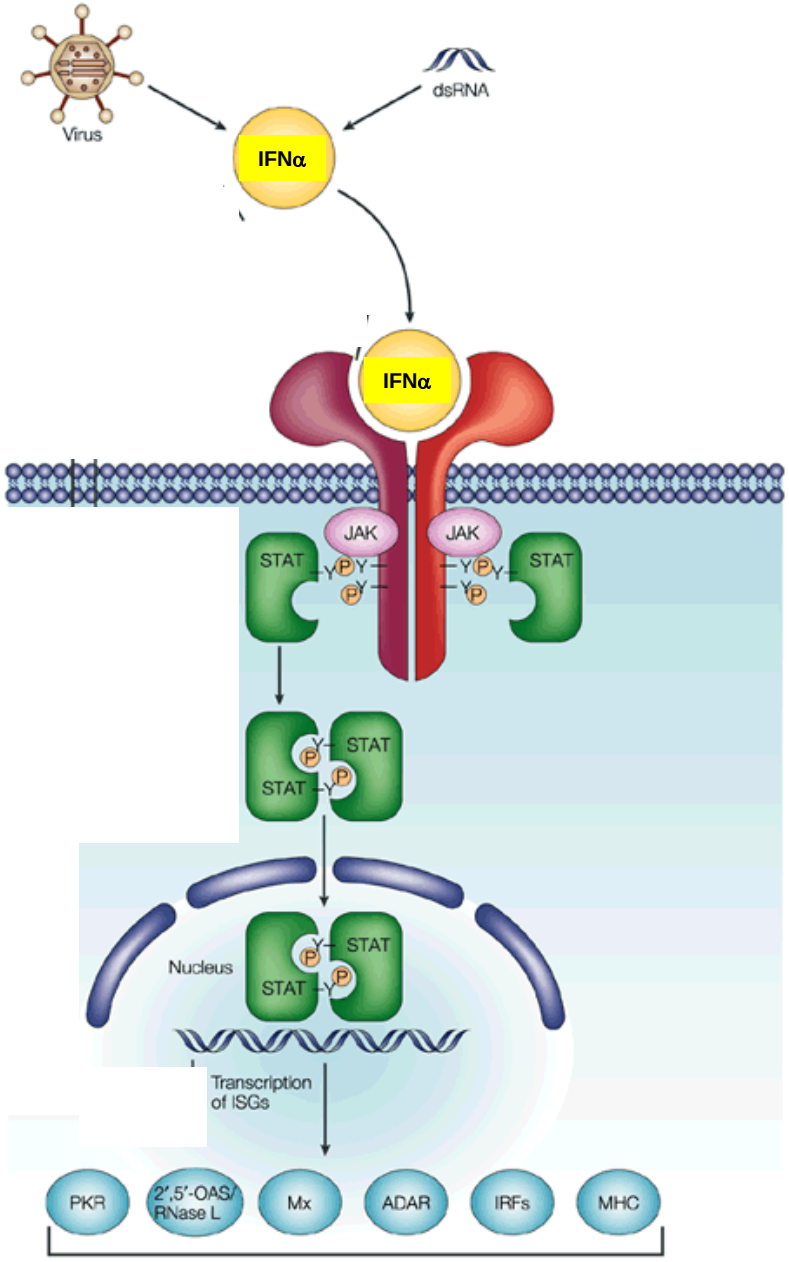


Interferon Responses to HCV Infection



Interferon Stimulation: JAK/ STAT Pathway





Antiviral, antiproliferative and immunoregulatory responses

Activation of JAK and STAT by Various Cytokines

Cytokine	Jak	STAT
IFN-γ	Jak1, Jak2	1
IFN-α/β	Tyk2, Jak1	1, 2, 3, (4)
IL-2	Jak1, Jak3	(1), 3, 5
IL-6	Tyk2, Jak1, Jak2	1, 3
IL-12	Tyk2, Jak2	3, 4

Mechanisms Of Interferon Action:

2’- 5’ oligoadenylate sythetases	**2-5 (A)**
Protein Kinase R	**PKR**
Adenosine Deaminase	**ADAR**
Mx Proteins	**MxA**

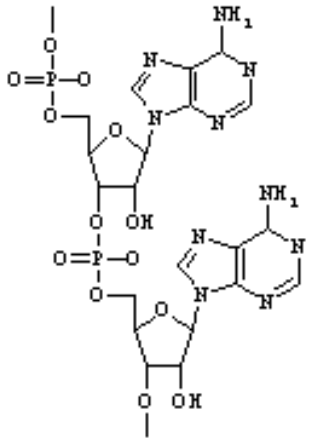
Others (many)
ISG56 Gene Family, IRFs, P200, MHC, iNOS, ...

Mechanisms Of Interferon Action: 2-5 (A)

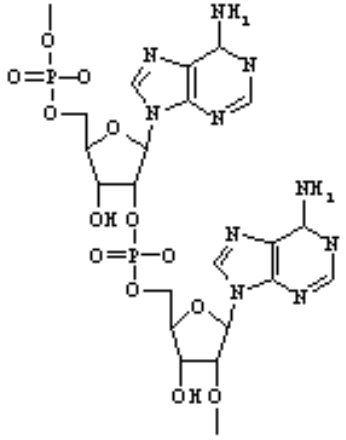
2-5 (A) Synthetases (family of enzymes)

Stimulated by dsRNA binding

Synthesize 2-5 (A) from ATP

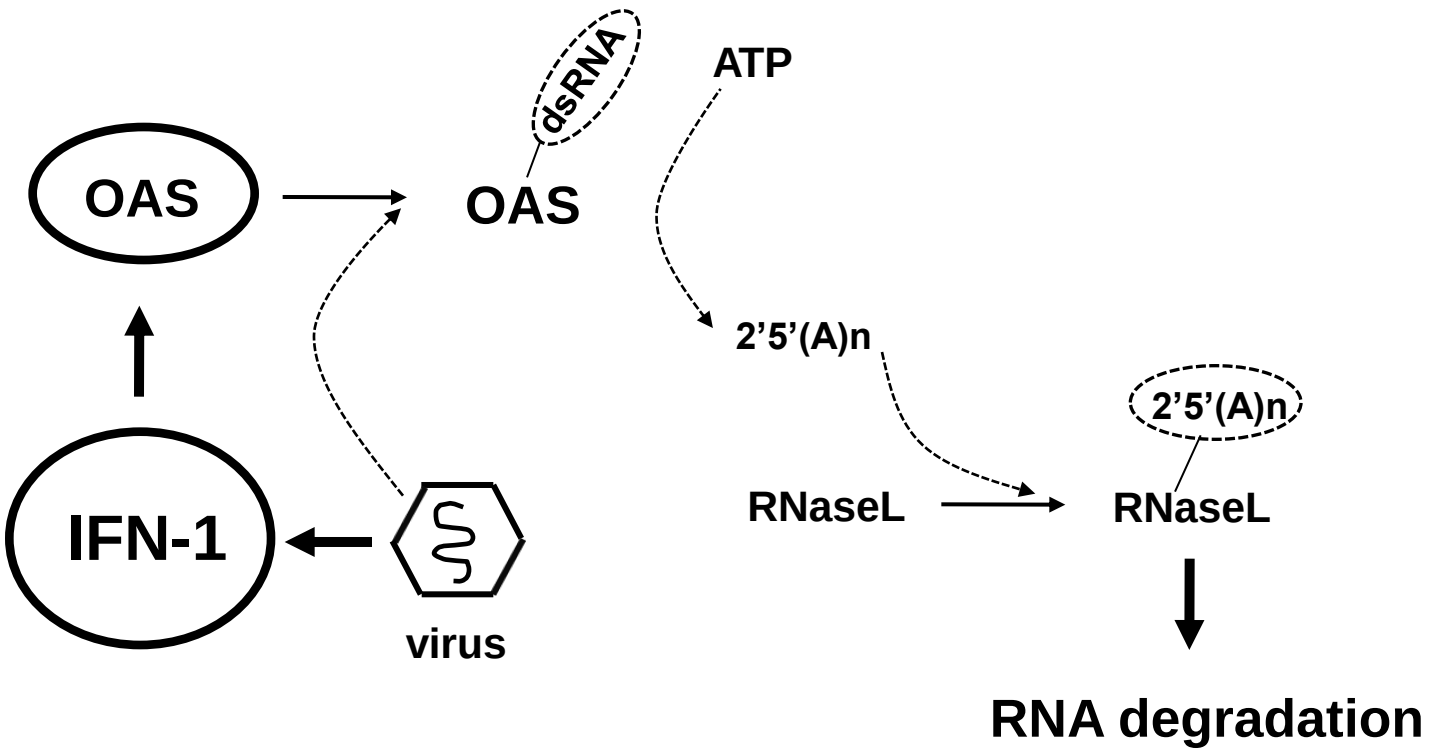


3-5 (Normal) RNA linkage



2-5 (A) Linkage

Mechanisms Of Interferon Action: 2-5 (A)



Mechanisms Of Interferon Action: PKR

PKR inactive in uninfected cells

Binds dsRNA

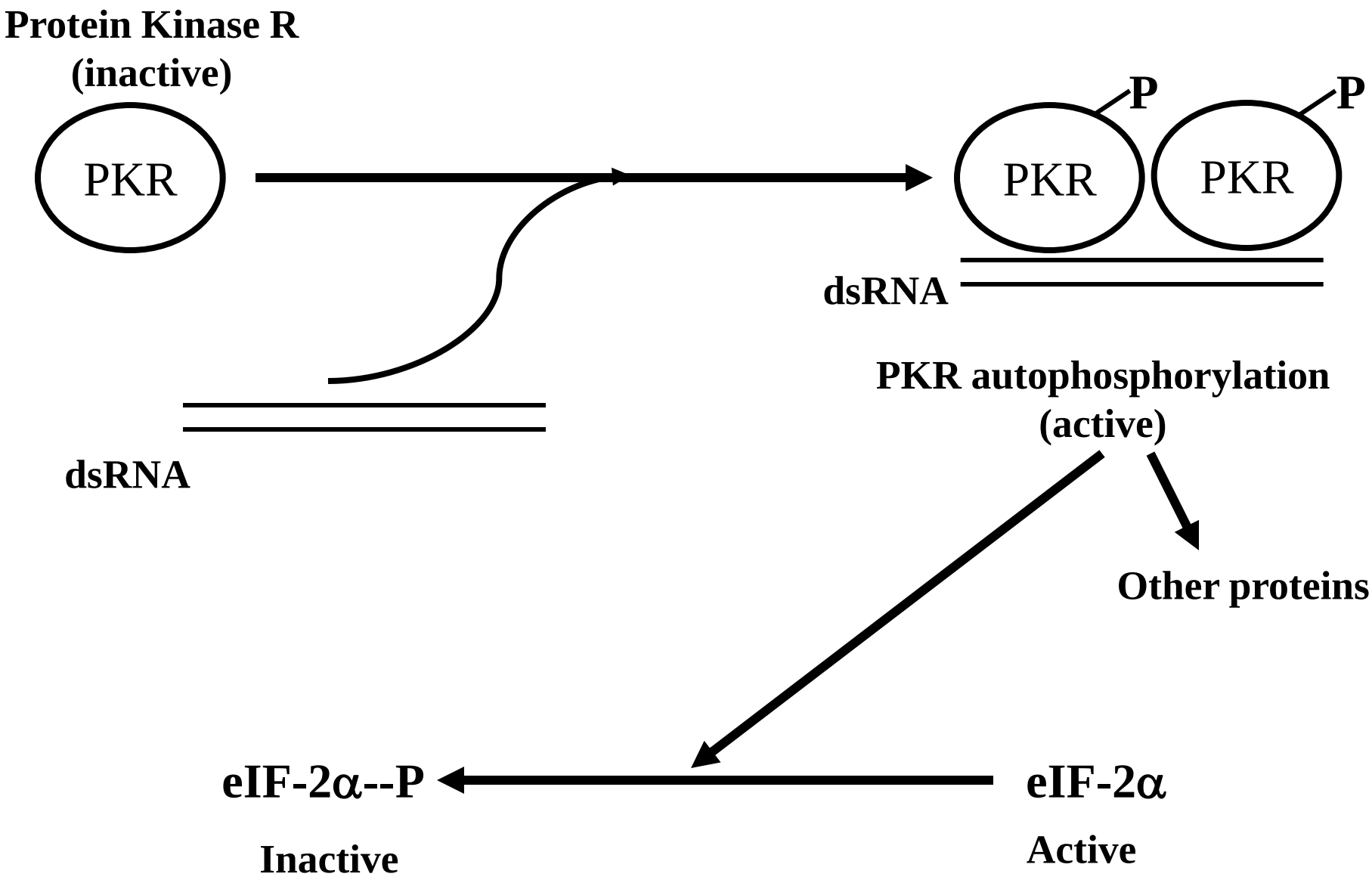
Autophosphorylates

Inactivates eIF-2

Inhibits translation

Virus inhibitors – adenovirus: VA RNA,
- influenza virus: P58IPK (cellular protein)

Mechanisms Of Interferon Action: PKR

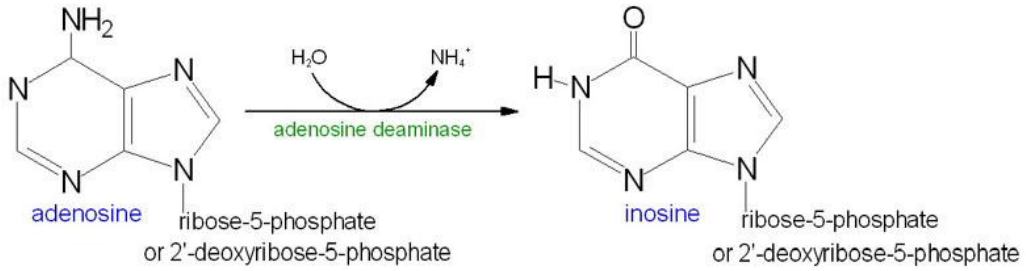


Mechanisms Of Interferon Action: ADAR

Adenosine deaminase

dsRNA is substrate

Catalyses deamination of adenosine to inosine (in viral RNA)

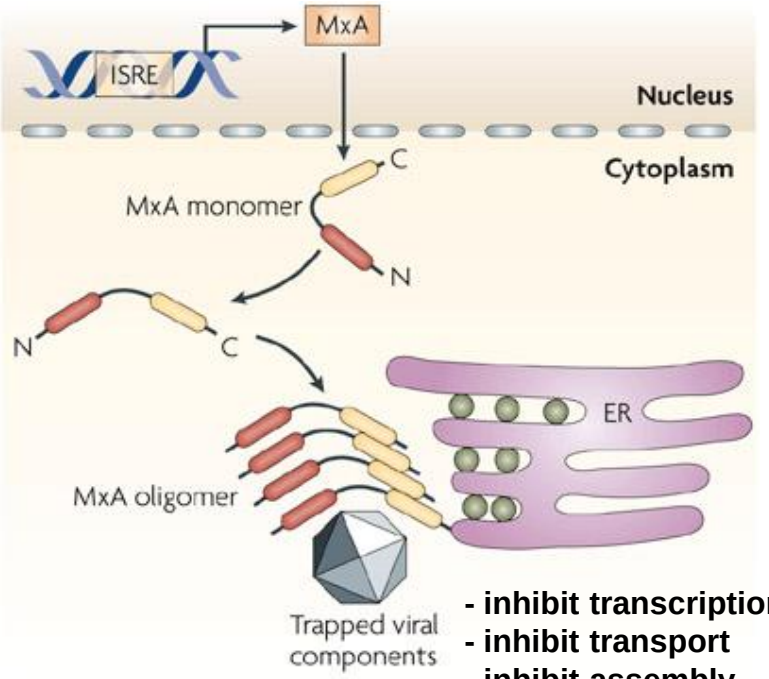


RNA "editing" changes protein coding capacity
- **site specific amino acid substitutions**

Mechanisms Of Interferon Action: Mx Proteins

MxA and MxB GTPases induced by type I IFNs

Involved in endocytosis and vesicular transport

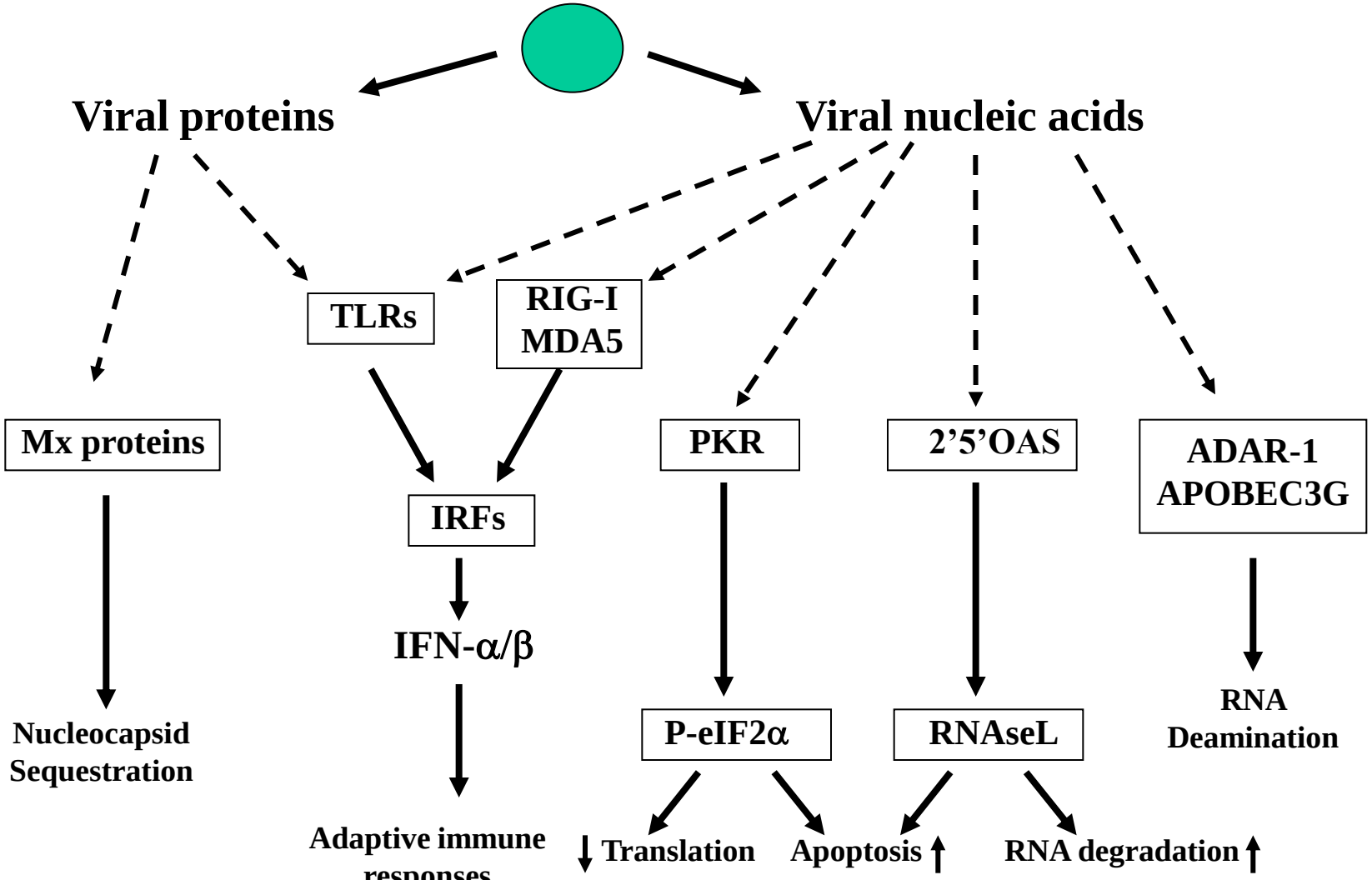


- inhibit transcription
- inhibit transport
- inhibit assembly

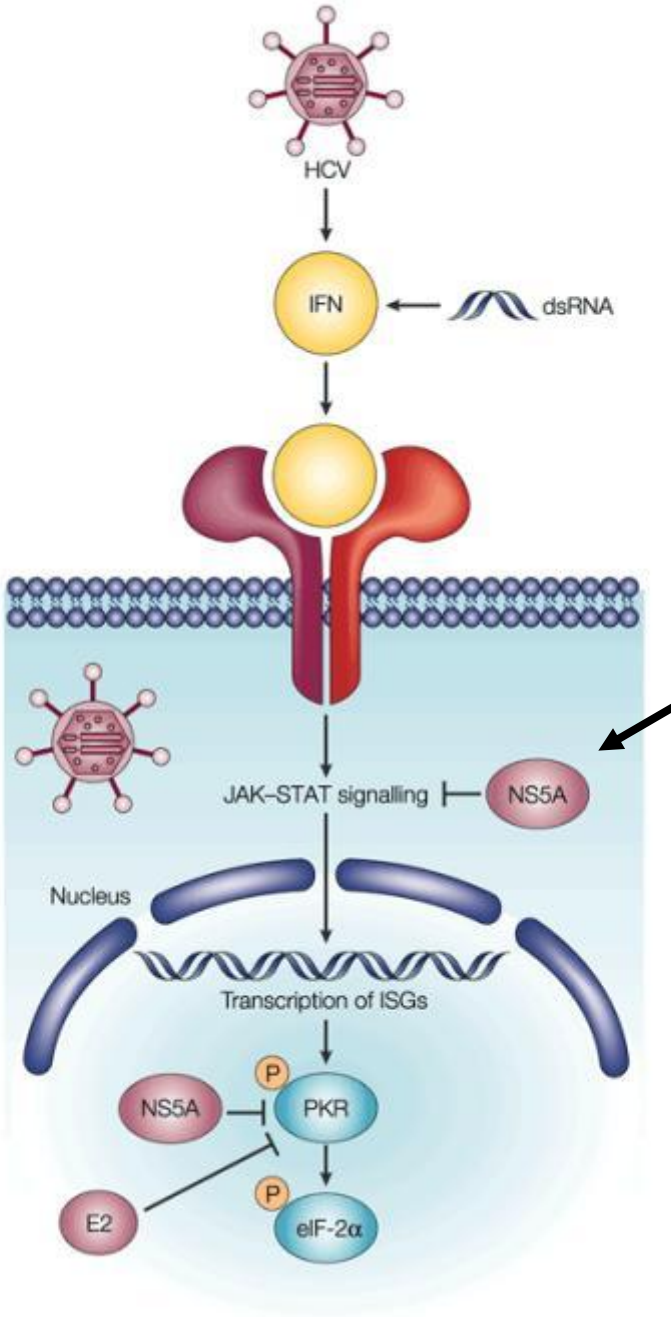
Originally found to block influenza replication

Now shown to inhibit many viruses

Innate Responses to Virus Infection

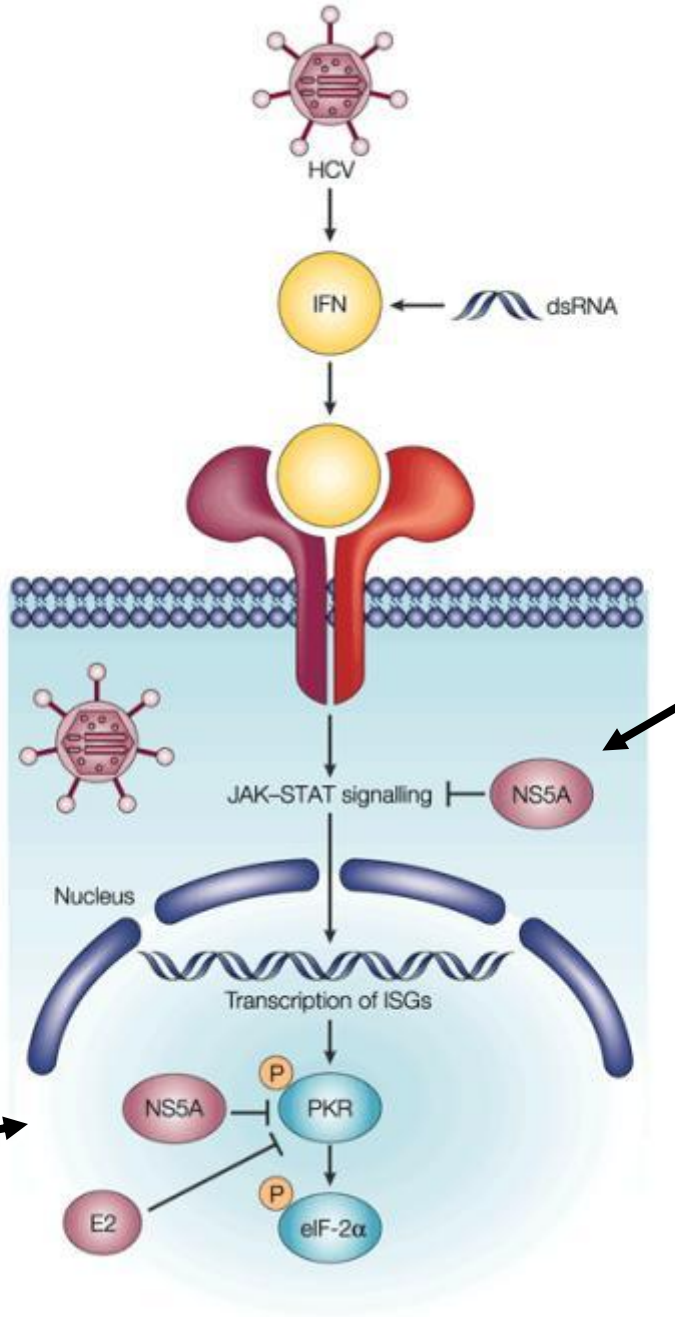


HCV



NS5A can block JAK-STAT signalling

HCV



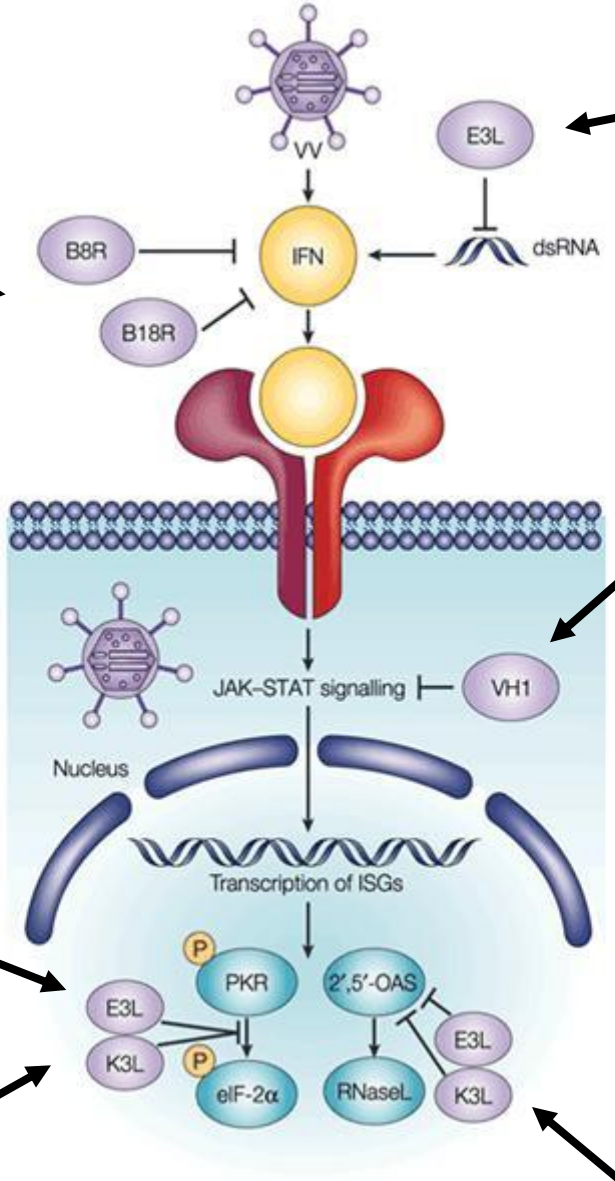
NS5A can block JAK-STAT signalling

NS5A and E2 can block PKR kinase activity

Poxvirus

E3L binds dsRNA and reduces INF responses

B8R and B18R are soluble IFN receptors that block IFN binding to cell



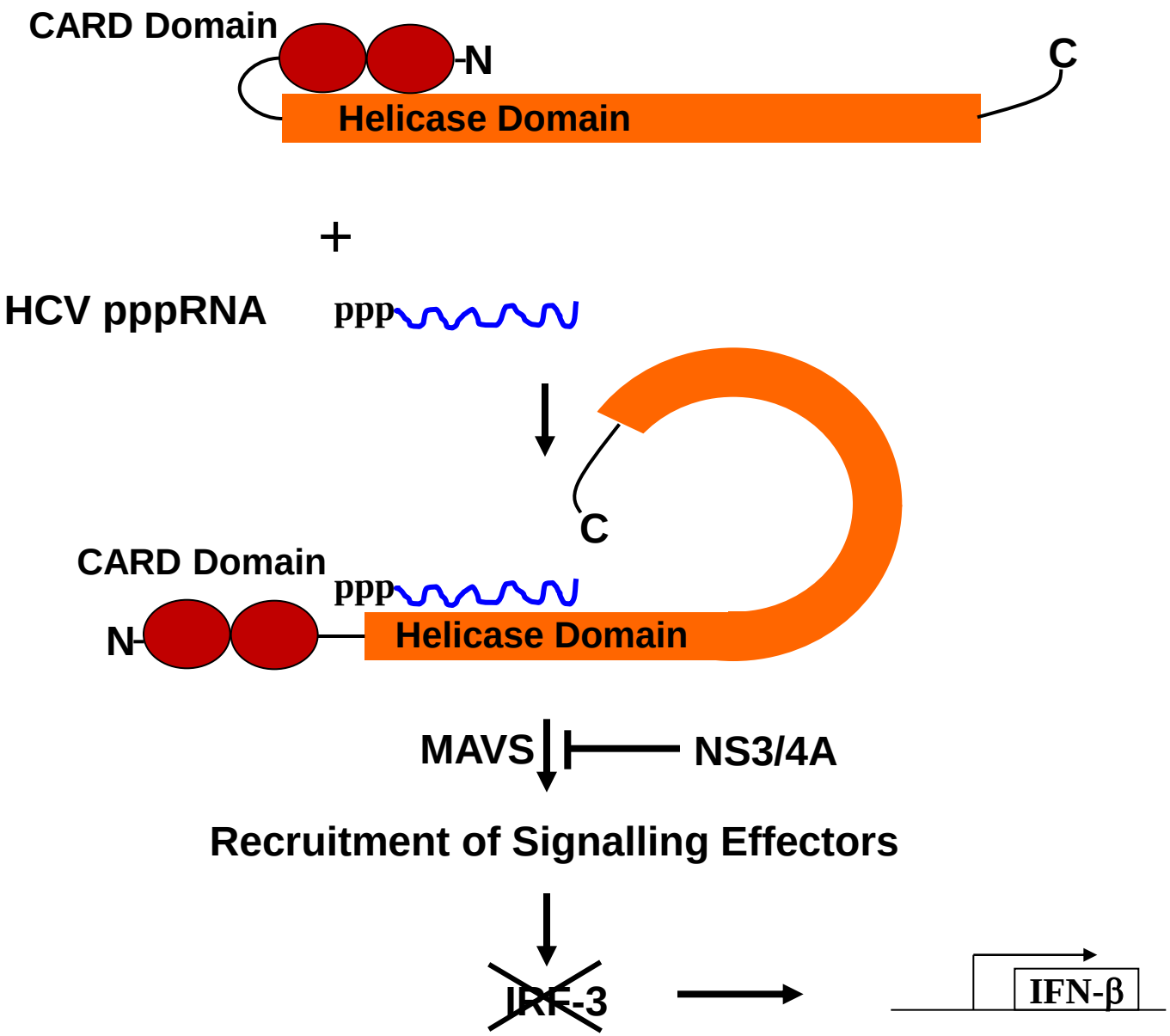
Vaccinia phosphatase VH1 dephosphorylates STATs blocking their signals

E3L blocks PKR activation

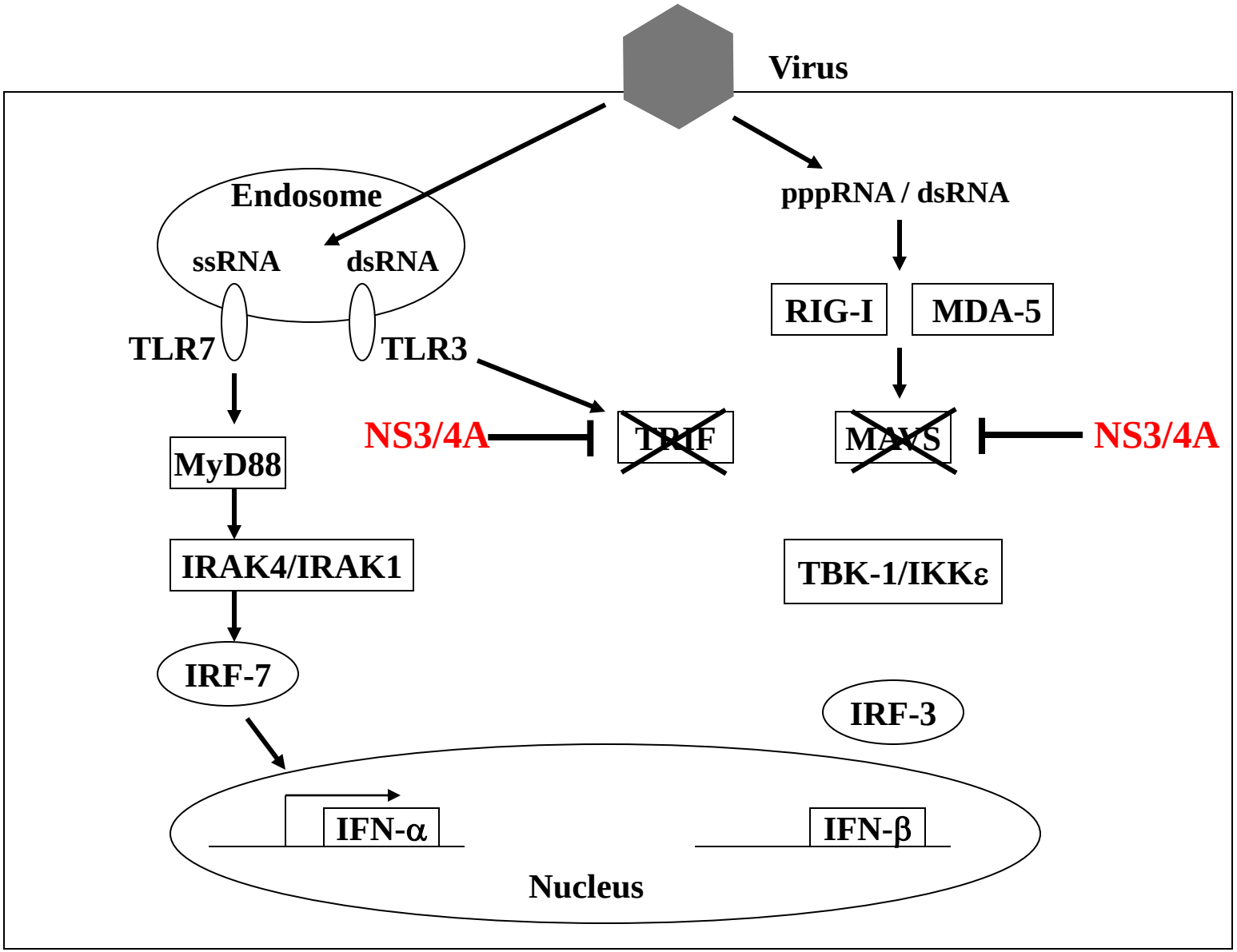
K3L (an eIF-2α homologue) blocks PKR action

E3L and K3L blocks 2’5’-OAS

RIG-I Triggering of IRF-3



Interferon Responses to HCV Infection - NS3/4A Inhibition



Suppressors of Cytokine Signalling (SOCS)

Induced by cytokines

Negative regulators of cytokine activity (negative feedback mechanism)

Induced by different viruses (e.g. HIV-1, HCV, HBV, HSV-1 RSV)

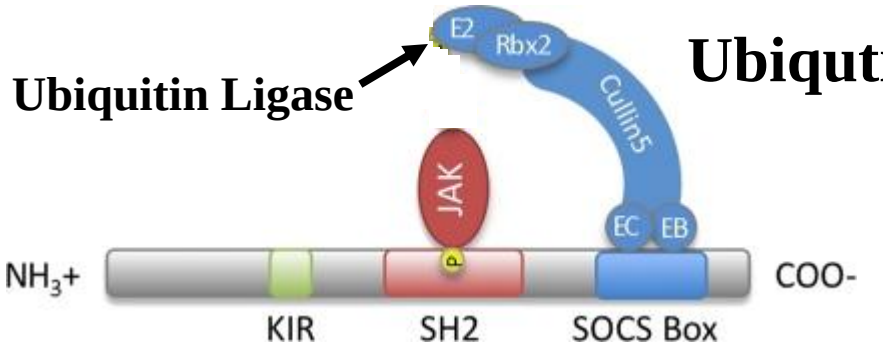
Viruses can exploit to reduce antiviral responses

SOCS Degradation of JAK/STAT Proteins

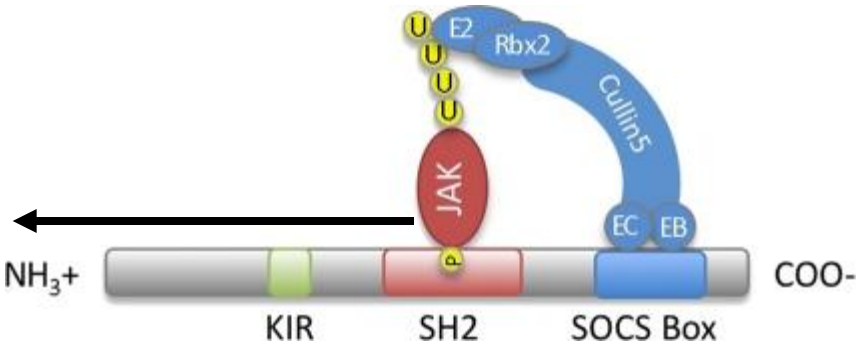
SOCS protein

Ubiquitin Ligase

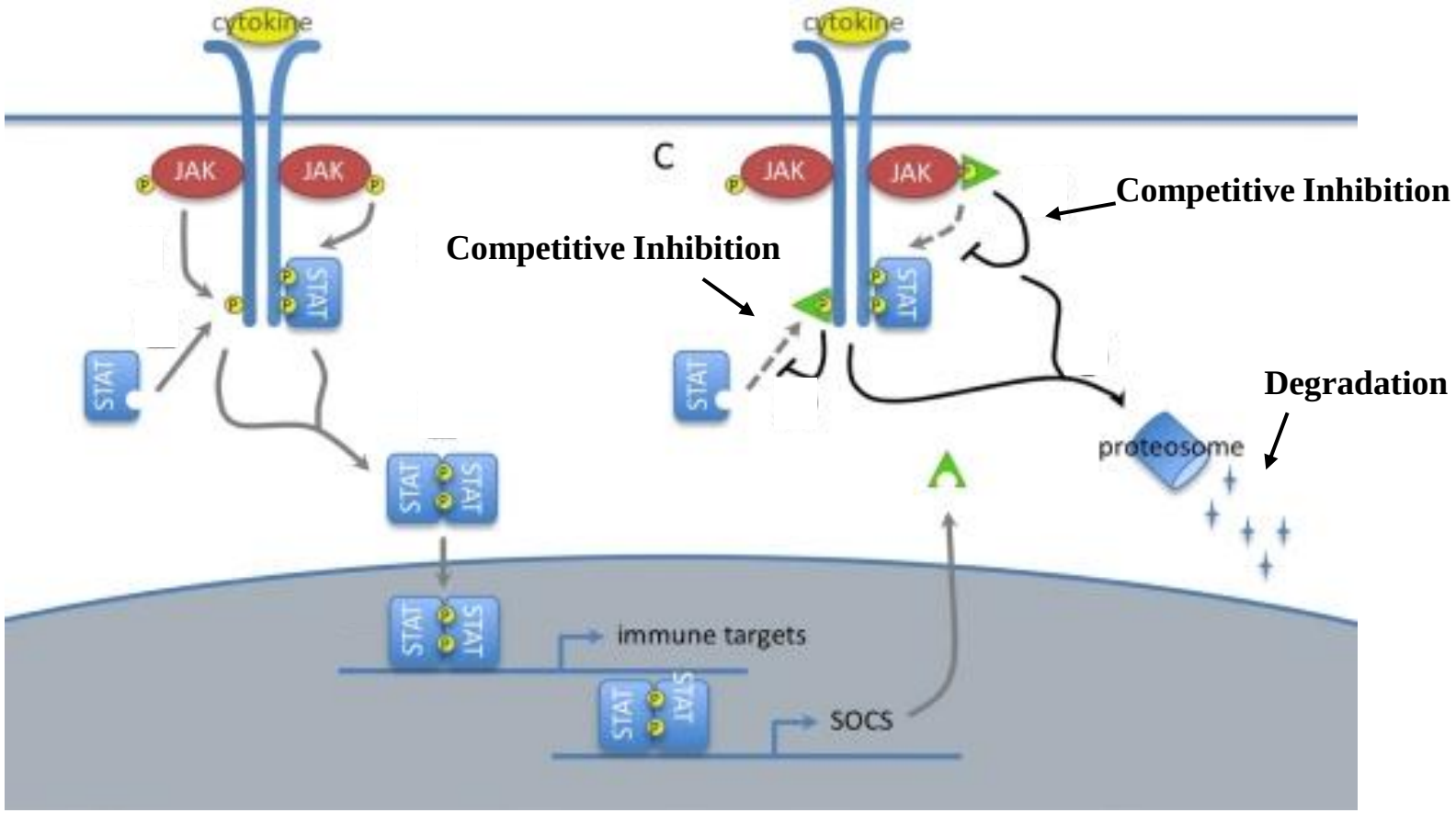
Ubiqutination complex



Proteosome



SOCS Inhibition of JAK/STAT Pathway



Antiviral Therapy and SOCS

HCV core protein induces SOCS3

- Increases virus replication

HCV can lead to glucose intolerance

- SOCS3 mediates ubiquitination of insulin receptor

HCV genotype 1 patients

- Most resistant to INF-α therapy
- have higher levels of SOCS3

Suppression of SOCS

- Synthetic SOCS siRNAs
- Mimic peptide of phosphorylated JAKS activation loop

References

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