The Interferon System

Takashi Fujita
Institute for Virus Research
Kyoto University
Days after influenza virus infection

- Fever
- Neutralizing antibody
- Nasal wash
- Interferon
- Virus
- Neutralizing antibody
- Serum
- Days
Infectious Diseases

Human (Animal) \(\xrightarrow{\text{Pathogen}}\) Human (Animal)

- Bacteria
- Parasites
- Viruses
- others
Virions

Vaccinia

Herpes

Adeno

Papilloma

Reo

Sindbis

Polio

Influenza

[Image description: Electron micrographs of viruses including Vaccinia, Herpes, Adeno, Papilloma, Reo, Sindbis, Polio, and Influenza.]
VIRAL ENTRY

1. Poliovirus
1a. Poliovirus
2. Newcastle Disease Virus
3. Influenza Virus
4. HIV
5. Vaccinia Virus
6. Adenovirus
7. Herpes Virus

outside cytoplasm nucleus

Fields VIROLOGY
VIRAL EXIT

Fields VIROLOGY
Interfering Factor (1954)

Alick Isaacs

Interferon (1957)

Jean Lindenmann
REPLICATION OF PICORNAVIRUS

DSRNA (Replicative Intermediate)

DSRNA (Replicative Form)

Fields VIROLOGY
EMCV: Non-enveloped, +strand RNA virus
CELLS INFECTED WITH RABIES VIRUS

VIRION OF RABIES VIRUS
Vesicular Stomatitis Virus

Antiviral Effect of Interferon

1000 U/ml Interferon-β

Vesicular Stomatitis Virus
How Interferon Works?
Action of Interferon

Interferon$

JAK$

STAT$

ISGs: Interferon Stimulated Genes

Antiviral State
Viruses

Antiviral Proteins
- PKR
- OAS
- Tetherin
- Etc.

Antiviral State

1. Penetration
2. Uncoating
3. Transcription
4. Translation
5. Assembly
6. Release of Progeny Viruses
The PKR System

IFNs → PKR

Virus Replication

Inhibition of Viral Protein Synthesis

dsRNA → PKR

dsRNA

eIF2

Active eIF2

ATP

Inactive eIF2
The 2'-5'A/RNase L System
Tetherin/BST-2/CD137
How Interferon is Produced?
Production of Interferon

VIRUS

Viral Replication
Viral RNA

Viral RNA Sensors

IRF-3, IRF-7
NF-κB

Interferon
Regulatory Sequences for IFN and IFN-inducible genes

IFN genes

IFN-β gene:

\[-110\]
\[-100\]
\[-90\]
\[-80\]
\[-70\]
\[-60\]

```
AAAATGTAAATGACATAGGAAAAACTGAAAGGGAGAAAGTGAAAGTGGAATCGCCTCGAAT
```

IFN-α1 gene:

```
GAGTGCATGAGAAACACAGAAATGGAAAGTGCCAGAAA
```

IFN-inducible genes

2’-5’AS: AGGAAA–CGAAACCA
PKR: GGGAAAACGAAACAG
GBP-1: ATGAAACTGAAAGTA
ISG15: GGGAAAACGAAACTG

Studies in late 1980’s
# Interferon Regulatory Factors (IRFs)

**DNA Binding Domain**

<table>
<thead>
<tr>
<th>IRF</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRF-1</td>
<td>Activator, Ubiquitous</td>
</tr>
<tr>
<td></td>
<td>--- Immunomodulation, Tumor suppressor-like</td>
</tr>
<tr>
<td>IRF-2</td>
<td>Repressor, Ubiquitous</td>
</tr>
<tr>
<td></td>
<td>--- Immunomodulation, Oncogenic function</td>
</tr>
<tr>
<td>IRF-4 (Pip/LSIRF)</td>
<td>Activator, Lymphoid specific</td>
</tr>
<tr>
<td></td>
<td>--- Lymphocyte development, Oncogenic function</td>
</tr>
<tr>
<td>IRF-8 (ICSBP)</td>
<td>Repressor/Activator, Lymphoid specific</td>
</tr>
<tr>
<td></td>
<td>--- Lymphocyte development, Tumor suppressor-like</td>
</tr>
<tr>
<td>IRF-9 (ISGF3γ)</td>
<td>Activator (DNA binding subunit of ISGF3), Ubiquitous</td>
</tr>
<tr>
<td></td>
<td>--- IFN-signaling</td>
</tr>
<tr>
<td>IRF-3</td>
<td>Activator, Ubiquitous</td>
</tr>
<tr>
<td></td>
<td>--- Induction of IFN-α/β genes</td>
</tr>
<tr>
<td>IRF-7</td>
<td>Activator, Ubiquitous, IFN-inducible</td>
</tr>
<tr>
<td></td>
<td>--- Induction of IFN-α/β genes</td>
</tr>
<tr>
<td>IRF-5</td>
<td>Activator?, IFN-inducible?</td>
</tr>
<tr>
<td>IRF-6</td>
<td>Repressor?</td>
</tr>
</tbody>
</table>

Studies in 1990’s
Ectopic Expression of IRF-3 Augments IFN-α, -β Promoter Activity

![Graph showing luciferase activity](chart.png)
DNA binding Complex Induced by Virus Infection

L929 (HA-IRF-3) F5 (HA-IRF-3) cells

Control Anti IRF-3 Anti HA Anti p300 Anti CBP Anti p300+CBP

NDV

VA-IRF ISGF3

F5 (HA-IRF-3) cells

Control Anti IRF-3 Anti p300 Anti CBP Anti p300+CBP

HEC-1 (IFNR-)
Time course of polyI:C-induced Nuclear Translocation of Endogenous IRF-3 and NF-κB in HeLa Cells
IFN gene regulation by IRF-3

Signal

IRF-3 <cytoplasm> P P

p300/CBP IFN genes

mock NDV

vector IRF-3 - + - + :NDV:

Western - + dimer monomer

Native/Western

IRF-3
Crystal Structure of IRF-3

Graduate School of Pharmaceutical Sciences
Hokkaido University
& CREST/JST, Kawaguchi

Kiyohiro Takahasi
Nobuo N. Suzuki
Masataka Horiuchi
Hiroaki Terasawa
& Fuyuhiko Inagaki
Potential phosphorylation sites in IRF-3 regulatory domain

WT: LVEMARVGGASSLENTVDLHISNSHPLSLTSQYKAYLQDLVEGMDFGQPGES

386A: LVEMARVGGASSALEASENTVDLHISNSHPLSLTSQYKAYLQDLVEGMDFGQPGES

→ No activation

5D: LVEMARVGGASSSLENTVDLHIDNDHPLDLDDQYKAYLQDLVEGMDFGQPGES

→ Constitutive active

5A: LVEMARVGGASSSLENTVDLHIANAHPLALAAADQYKAYLQDLVEGMDFGQPGES

→ Virus-inducible activation
Purification of recombinant IRF-3 from *E. Coli*

(A) Purification of recombinant IRF-3 from *E. Coli*.

(B) SDS-PAGE gel showing the purification of recombinant IRF-3. Molecular weights are indicated in kDa.

- **1.** Protein loaded without IPTG
- **2.** Protein loaded with IPTG
- **3.** Protein loaded with IPTG and GroE
- **4.** Protein loaded with IPTG and Trx

**Notes:**
- S: Soluble fraction
- I: Insoluble fraction

**CBB staining**

**αHis Western blot**
Quasi dimer in the crystal
Close-up of SRR-loop: HLR-pocket interaction

Mutations of SRR-loop and HLR-pocket
Activation of IRF-3 and Smad at Low Resolution
$\text{TBK-1, IKK-i(ε) } \rightarrow \text{ p-IRF-3, p-IRF-7}$

$\text{IKK(α, β, γ) } \rightarrow \text{ p-IκB}$
Regulation of Antiviral Innate Immunity by RIG-I-Like Receptors

Takashi Fujita
Institute for Virus Research
Kyoto University
Virus- and IFN-induced Signals

Virus

dsRNA

IFN Receptor

RIG-I

IFN-α genes

IFN-β gene

IRF-3

IFN inducible genes

Antivirus activities

cytoplasm

nuclear
RIG-I-Like Receptor (RLR)

RNA Binding Domain

Helicase Domain: Conformational Change?

Signaling Domain

Conserved helicase motifs

CARD

RNA Binding Domain

K270: ATP Binding

TAS

T55I

23%

35%

41%

31%

RIG-I:

MDA5:

LGP2:

1

925

1025

678
RIG-I Binds to dsRNA but not ssRNA or dsDNA
Activation of RLR: Exposure of CARD

1. CTD
2. CARD
3. OFF

1. CTD
2. CARD
3. OFF

1. CTD
2. CARD
3. ON

CARD
Analysis of RIG-I-/- and MDA5-/- mice


IFN gene induction by MEFs from KO mice
JEV challenge to KO mice
EMCV challenge to KO mice
# Differential Functions of RIG-I Family Helicases

<table>
<thead>
<tr>
<th>Helicase</th>
<th>Positive Regulator</th>
<th>Viruses</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIG-I</td>
<td></td>
<td>NDV, Sendai virus, Influenza virus, VSV, JEV, WN, etc.</td>
</tr>
<tr>
<td>MDA5</td>
<td></td>
<td>Picorna viruses, WN, etc.</td>
</tr>
<tr>
<td>LGP2</td>
<td></td>
<td>EMCV, VSV</td>
</tr>
</tbody>
</table>
A

IFN-β (ng/ml)

WT RIG-I -/- MDA5 -/-

0 1 2

0 8

B

IFN-β (ng/ml)

mock 0 60 180

RNaseIII treatment (min)

WT RIG-I -/- MDA5 -/-

* Not detected

C

Relative induction (%)

0 1 5 10 30 60 180

RNaseIII treatment (min)

WT MDA5 -/- RIG-I -/-

* * * *

D

IFN-β (ng/ml)

RNaseA Bal31 Hydrolysis

0 2 4 6 8

RNaseIII treatment (min)

WT RIG-I -/- MDA5 -/-

Kato, H. et al, JEM, 2008
RNA structure RLR

<table>
<thead>
<tr>
<th>RNA</th>
<th>Structure</th>
<th>RLR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short poly I:C</td>
<td>pp p p p p p p</td>
<td>RIG-I</td>
</tr>
<tr>
<td>(~300 bp)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&gt; 4 kbp)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>in vitro T7 transcript</td>
<td>p p p p p (polyU, UC)n</td>
<td>RIG-I</td>
</tr>
<tr>
<td>(with copyback)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV RNA</td>
<td>p p p p p (polyU, UC)n</td>
<td>RIG-I</td>
</tr>
<tr>
<td>RNaseL cleavage product</td>
<td>p p p p p (polyU, UC)n</td>
<td>RIG-I</td>
</tr>
<tr>
<td>(RNA of host/virus)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viral genomic RNA</td>
<td>p p p p p (polyU, UC)n</td>
<td>RIG-I</td>
</tr>
<tr>
<td>(with panhandle structure)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RNA of DI particle</td>
<td>p p p p p (polyU, UC)n</td>
<td>Unidentified</td>
</tr>
<tr>
<td>(copyback)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High molecular weight RNA</td>
<td>p p p p p (polyU, UC)n</td>
<td>MDA5</td>
</tr>
<tr>
<td>(RNA web)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
RNA Binding Domain

Helicase Domain: Conformational Change?

Signaling Domain

Conserved helicase motifs

Repression Domain

RNA Binding Domain

RIG-I Like Receptors (RLR)

CARD

K270: ATP Binding

T55I

TAS

T55I

RIG-I

MDA5

LGP2

Repression Domain

Conserved helicase motifs

Repression Domain

Helicase Domain: Conformational Change?
RIG-I functions as double-stranded RNA sensor/signaling switch
Intracellular localization of RIG-I and antiviral innate immunity

Viral Transcription
- Viral RNA
  - (dsRNA)
  - Viral Translation
    - Viral Protein
  - Virion Packaging

RIG-I
REPLICATION OF INFLUENZA VIRUS

1. Adsorption
2. Endocytosis
3. Fusion and uncoating
4. Low pH
5. mRNA
6. Translation
7. Nucleocapsid
8. Packaging
Non-structural Protein 1: Strong Antagonist of Interferon System
Genomic RNA of Influenza A virus

“Panhandle” Structure
*Natural Ligand for RIG-I*
Stress Signals (Virus Infection, heat shock etc.)

- PKR (dsRNA)
- GCN2 (aa. Starvation)
- HRI (oxidation)
- PERK (ER stress)

- eIF2α Phosphorylation (Ser51)

- Formation of Stress Granules
  - RLR
  - TIAR
  - TIA1
  - eIF3
  - G3BP
  - HuR
  - PABP
Oxidative Stress Induces SG but not Interferon
Virus-induced SG contains viral RNA (anti virus SG)
Colocalization of MDA5, SG markers and viral dsRNA
Influenza genomic RNA

NUCLEUS

CYTOPLASM

NS1
PKR

R LR

TIAR
Formation of Stress Granules
TIA1
eIF3

G3BP
HuR

PABP

RLR activation
eIF2α Phosphorylation (Ser51)
Virus infection and

*Stress Granule*
Different Patterns of SG formation
By viral infection
**Different Patterns of SG formation**

*By viral infection*
SG Induction and Viral Replication

*Picornaviridae*
Minutes after EMCV infection
EGFP-G3BP

Q325E cleavage resistant
Minutes after EMCV infection

GFP-G3BP Q325E
G3BP is cleaved by EMCV infection

G3BP is cleaved by 3C protease
G3BP Q325E inhibits EMCV replication

G3BP knockdown enhances EMCV replication
G3BP Q325E enhanced IFN and cytokine gene activation
Viral Inhibition of SG Formation

Influenza A virus: NS1
West Nile, Dengue virus: targets TIA/TIAR
Polio virus, EMCV: 3C protease
Sendai virus: C protein, trailer RNA
Measles virus: C protein
Semliki Forest Virus: unknown
Junin virus: nucleoprotein, glycoprotein precursor
Rota virus: NSP3
Vaccinia virus: E3L
Viral Inhibitors of RLR Signaling

SG

dsRNA

PKR

G3BP

EMCV 3C

Polio V 3C

TMEV Leader

Influenza NS1

HCV NS3/4A

IPS-1/MAVS/VISA/Cardif

TBK-1

SFTSV NS

IRF-3
Mitochondrion and Antiviral Responses

Onoguchi K. et al., PLoS Pathogens 2010
Redistribution of IPS-1 by Sendai Virus infection
Mitochondrial redistribution is induced by different viruses
Confocal

High resolution

+poly I:C
Re-distribution of IPS-1 on mitochondrion
MFN1 is necessary for IPS-1 redistribution (NDV)
MFN 1 positively regulates IFN production induced by virus or 5’pppRNA
Critical role of MFN1 but not MFN2 in IFN gene activation (KO MEFs: obtained from David Chan’s lab)
Summary

Type I and III interferon genes

Mitofusin 1

Inflammatory cytokine genes

Mitochondrion

PKR

avSG

NFκB

IRFs

IPS-1

TRAFs

TBK-1
Mitochondrion

Energy Production

Regulation of Cell Death

Antiviral Immunity

Platform for interactions between signaling adaptors

Regulation of IPS-1 distribution by fusion/fission