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SENSORS FOR REPLICATING VIRUSES AND INNATE IMMUNITY

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Abstract Recent studies show the involvement of cytoplasmic RNA helicase family, RIG-I, MDA5 and LGP2 in antiviral innate immune responses. RIG-I and MDA5 are primarily responsible for the detection of viral infection and triggering activation cascade for type I interferon genes in many cell types. RIG-I consists of N-terminal CAspase Recruitment Domain (CARD) and a domain with signatures of DExD/H box helicase (helicase domain). Functional analyses revealed that the helicase domain detects viral RNA and CARD triggers the activation of downstream signaling cascade, including activation of transcription factors, NF-κB, IRF-3 and IRF-7. RIG-I binds to double stranded (ds)RNA, however it does not simply function as a binding receptor for dsRNA, since RIG-I with disrupted ATP binding site is incapable of signaling. A model is proposed that in the absence of dsRNA, RIG-I forms "closed" conformation and upon binding to dsRNA, it conforms into "open" structure exposing CARD. We produced recombinant RIG-I protein using Baculo virus system and purified it to homogeneity. Biochemical properties, including dsRNA binding activity, ATPase activity and helicase activity, of recombinant RIG-I were investigated. The results suggested that RIG-I requires certain structure of ligand RNA that is specific to viral (or non-self) origin. Furthermore, we found evidence that RIG-I conforms a certain structure upon binding to dsRNA in the presence of ATP. These results were consistent with the above model for activation of RIG-I. Furthermore, we observed that RIG-I forms oligomers in virus-infected cells and artificial oligomerization of RIG-I CARD mimics virus-induced signaling, resulting in the activation of interferon and other cytokine genes. These results highlight how viral replication in cytoplasm is detected by RIG-I helicase and switch on signal cascades for initial antiviral responses.

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Higher organisms including humans are equipped to combat viruses using two kinds of immune responses: innate and adaptive immunity. Unlike adaptive immunity, which is characterized by its specificity and memory, innate immunity is provoked early in infection and is critical as an initial response. The type I interferon (IFN) system plays a major role in antiviral innate immunity[1]. Upon viral infection, type I IFN is secreted in body fluid and expands IFN response signals, resulting in the activation of various enzymes that prevent viral replication. In addition to antiviral activity, type I IFN has been known to exert various biological effects

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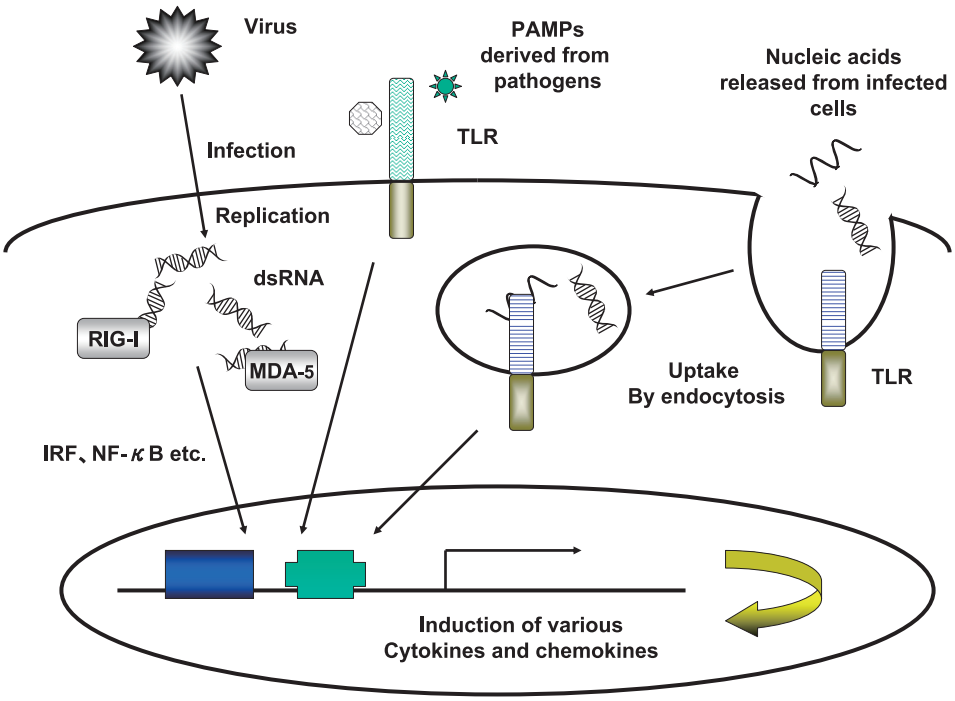

Figure 1 Recognition of PAMPs by TLR and RIG-I family helicases. Transmembrane receptor TLR is expressed on the plasma or endosomal membranes and senses extracellular PAMPs. RIG-I family helicases detect dsRNA in the cytoplasm. Activation of these receptors transduces signals resulting in overlapping, but in a different set of target genes including cytokines and chemokines.

such as cell cycle regulation, differentiation and immune modulation. Furthermore, innate immune responses lead to the activation of specific cells with antigen-presenting functions to facilitate the initiation of adaptive immunity.

The triggering of the IFN system is the activation of IFN genes. Since the initial discovery of type I IFN, the activation mechanism of the type I IFN gene has been a major focus of biologists. Although several double-stranded (ds) RNA binding proteins such as PKR have been attributed to the detection of replicating viral RNA, gene knockout studies do not support its role. Recent functional analyses of TLR revealed that TLRs function as pathogen receptors including those of viral origin[2]. TLR3 has been identified as a receptor for exogenous dsRNA: however TLR3-deficient cells activate type I IFN genes normally, suggesting the existence of other receptor (s). Our expression cloning identified an RNA helicase as an essential receptor for virus-derived dsRNA (Figure 1)[3]. We describe in this article the recently identified function of the RIG-I family of RNA helicases in innate immune reactions to infecting viruses.

Cytoplasmic receptor, RIG-I helicase family

Since dsRNA such as polyI:polyC is known to induce IFN synthesis, it is generally accepted that dsRNA is the major viral product responsible for the activation of innate immune responses. TLR3 was first shown to confer responsiveness to exogenously added polyI: polyC in HEC293T cells, and is thus hypothesized to function as a physiological sensor for replicating viruses. However, TLR-3-deficient cells are normally responsive to viral infection or poly I: poly C transfection, suggesting an alternative cytoplasmic sensor.

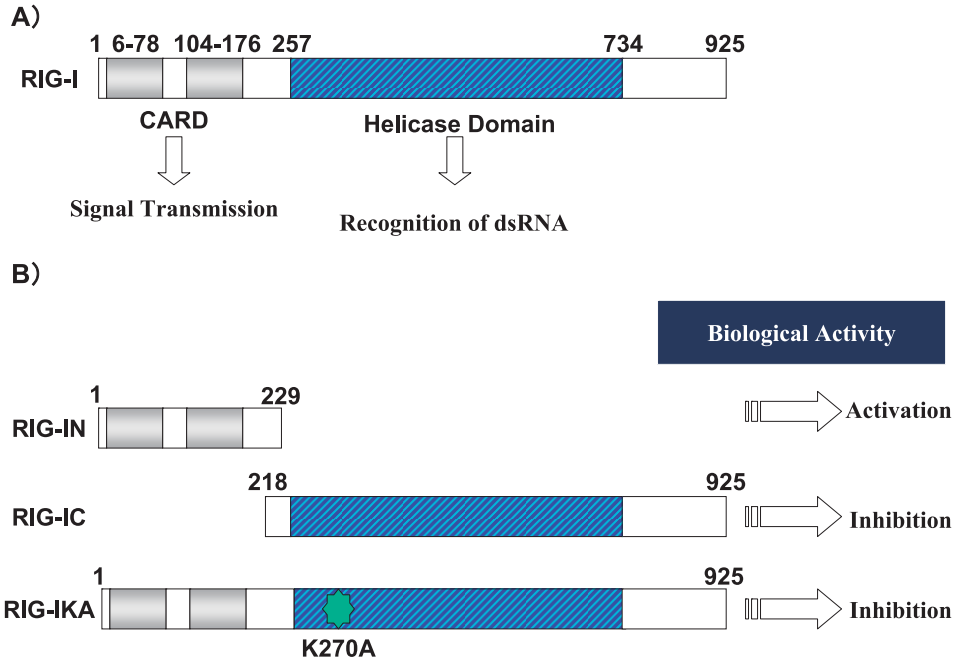

Figure 2 Structure-function relationship of RIG-I. A: Structure of RIG-I. B: Biological acitivity of RIG-I mutants.

To identify this hypothetical sensor, we screened an expression library for enhanced virus-induced activation of the reporter construct. One clone encoded partial cDNA for human RIG-I[3]. RIG-I is a putative RNA helicase containing two repeats of CARD at the N-terminal region and a DExH/D box helicase homology region at its C-terminal region (Figure 2).

RIG-I exhibits specific binding activity to dsRNA. Overexpression of RIG-I in cultured cells did not significantly activate the IFN promoter: however, overexpression of the N-terminal region containing two CARD repeats alone constitutively activated the IFN promoter. This suggests that CARD is essential and sufficient for signaling, and is under negative regulation by the C-terminal region. Full-length RIG-I is accumulated as an inactive form: however, it can be activated by viral infection or transfection of dsRNA. This supports the speculation that inhibition of CARD by the C-terminal region is reversed by dsRNA. Interestingly, RIG-I that lacks CARD acts as a dominant-negative inhibitor of virus-induced activation of IFN-β promoter. Furthermore, K270A mutation, which disrupts the ATP binding motif within the conserved helicase domain, also functions as a dominant inhibitor. These observations suggest that, in addition to dsRNA binding, ATP hydrolysis is necessary for the induced unmasking of CARD.

In the human genome database there are genes encoding RIG-I-related helicases, named MDA5 and LGP2. MDA5 exhibits a similar domain structure to RIG-I, characteristic of two repeat CARD and the helicase domain (Figure 3) [4].

The third helicase LGP2 lacks CARD. Functional analyses of these helicases using cell culture revealed that MDA5 functions as a positive signaling regulator, similar to RIG-I. Recent gene disruption of RIG-I and MDA5 revealed that these helicases detect different viruses[5]. MDA5 is essential to detect picorna virus infection and RIG-I is in charge of other virus types tested. Interestingly, this virus specificity likely reflects different RNA species

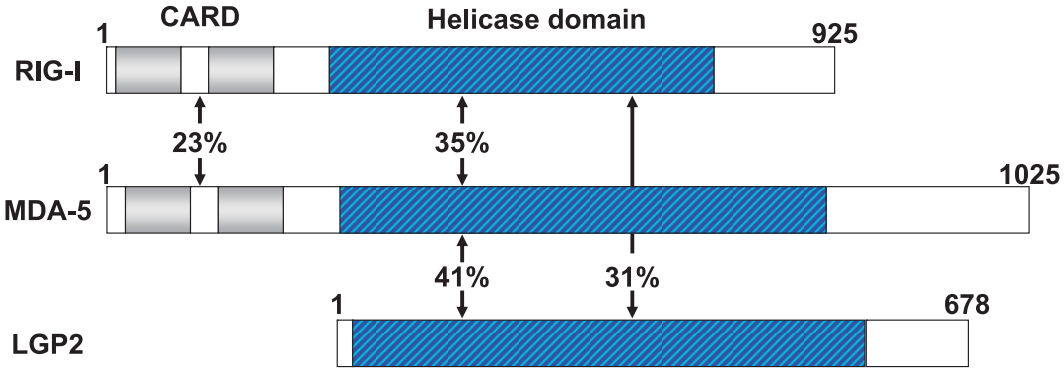

Figure 3 Structure of RIG-I family helicases. Human and mouse RIG-I family consists of RIG-I, MDA5 and LGP2. Percentage indicates amino acid identitiy between corresponding domains.

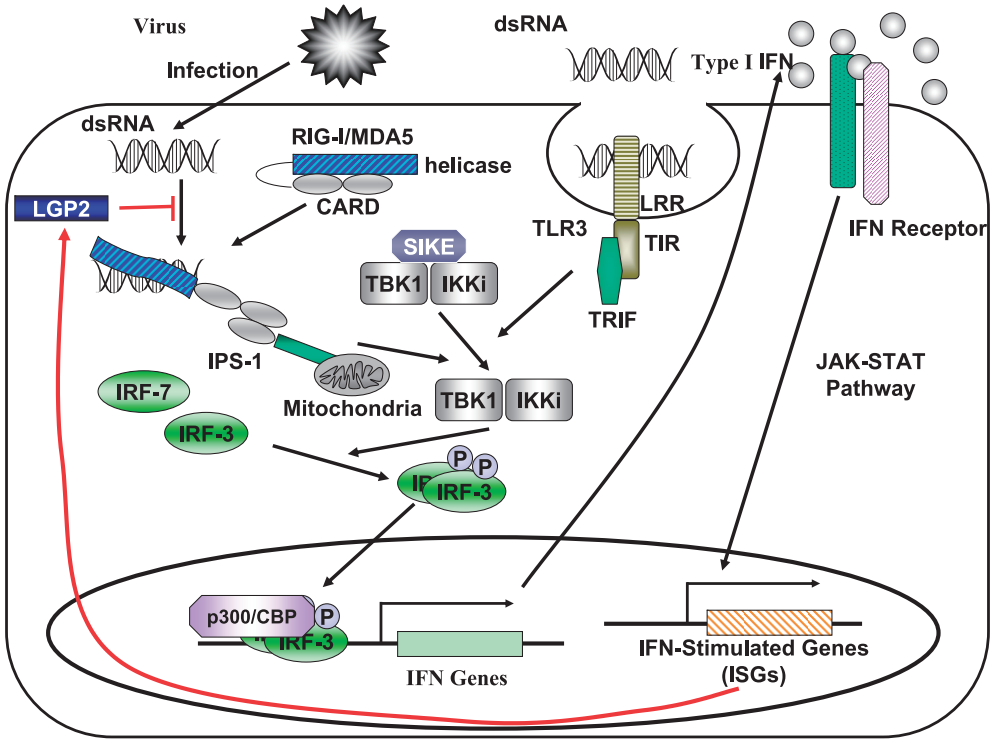

Figure 4 Signaling cascade induced by dsRNA. TLR3 recognizes extracellular dsRNA in endosomes. Upon dsRNA binding, the cytoplasmic domain of TLR3 transmits a signal to an adaptor, TRIF. Cytoplasmic dsRNA is recognized by RIG-I and MDA5. CARD of these helicases interacts with an adaptor, IPS-1, which localizes on the outer membrane of mitochondria. Signals mediated by TRIF and IPS-1 activates common protein kinases TBK-1 and IKK-I, resulting in phosphorylation-mediated activation of transcription factor IRF-3. IRF-3 as a complex with coactivator CBP or p300 activates target genes including type I IFN genes. Secreted IFN activates secondary signals through IFN receptor and JAK-STAT pathway to activate ISGs.

generated by respective viruses. At present, the chemical basis of this difference is not known. Functional analyses of LGP2 in cell culture revealed that LGP dominantly inhibits the virus-induced activation of IFN genes. Since LGP2 is transcriptionally induced by autocrine IFN, its function as a feedback negative regulator is suggested[4].

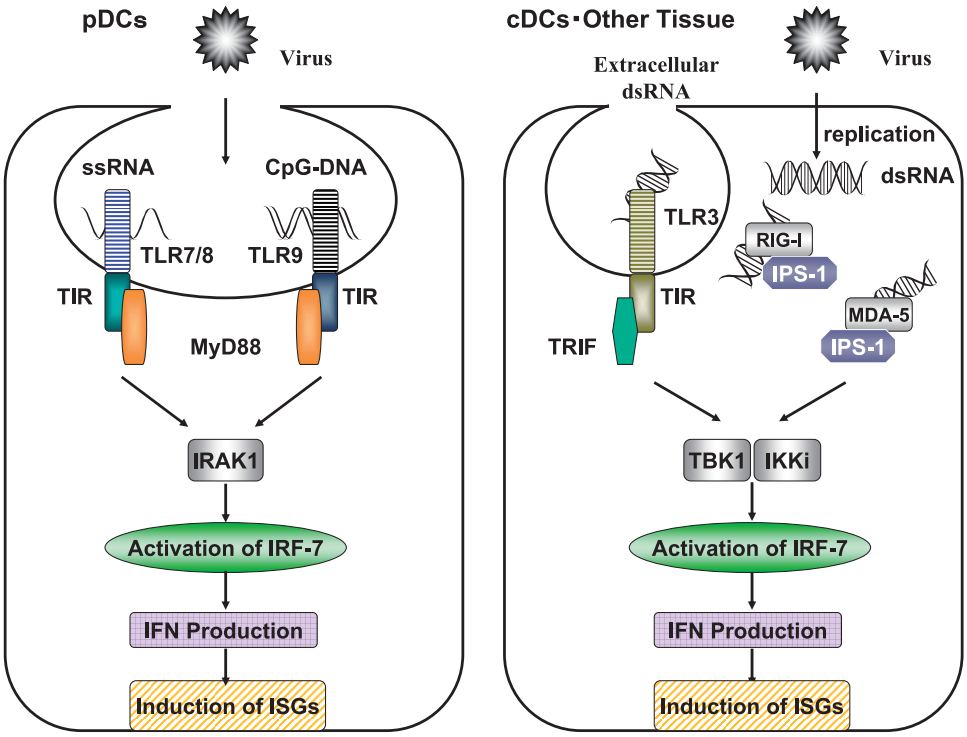

Figure 5 Tissue-specific signaling cascade for IFN gene activation. Virus infection triggers a distinct signaling cascade in pDCs and other cell types including cDCs. IFN induction in pDC is dependent on MyD88, IRAK1 and IRF-7, whereas these adaptors are dispensable in other cell types.

Signaling cascades of antiviral innate responses

A comparison of signaling cascades initiated by the detection of dsRNA by TLR3 and RIG-I/MDA5 is illustrated in Figure 4.

TLR3 activation by dsRNA occurs in the endosome and the signal is transmitted through TRIF, TBK-1/IKKi kinases. The latter kinases are responsible for specific phosphorylation and activation. It was shown that TBK-1/IKK-I kinases are under negative regulation by SIKE. RIG-I/MDA5 activates a novel adaptor IPS-1[7,8] (MAVS, Cardif, VISA) containing a single copy of CARD. Interestingly, IPS-1 is anchored on the outer membrane of mitochondria via its C-terminal transmembrane domain. Although mitochondria association is critical for signaling, its mechanism is elusive. IPS-1 apparently activates the IRF-3 kinases TBK-1/IKK-i[9]. SIKE is implicated for negative regulation of TBK-1/IKK-i. Thus, RIG-I/MDA5 activate a distinct signaling cascade from TLR3 and the signal is converged at TBK-1/IKK-i[10].

As mentioned earlier, TLR7/8 and TLR9 detect distinct viral PAMPs and activate signaling cascades, MyD88, IRAK1 and IRF-7. So far, this signaling has been showed to be specific to pDCs, which are responsible for the production of high levels of serum IFN-γ (Figure 5)[5,6].

In other cell types, including cDCs, viral infection mainly activates the RIG-I/MDA5 signaling cascade as demonstrated by using knockout mice.

Multiple receptors detect viral replication and result in the activation of several common and unique signaling cascades. This multiplicity likely reflects the viral strategy to evade host

responses during the long history of host-virus evolution. All replication-competent viruses may retain several inhibitors against host responses, such as V protein of paramyxo viruses[6] and NS3/4A of HCV. Identification of these viral inhibitors would help to counteract viral strategy and develop new therapies against viral infection.

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