Minireview

New tricks for old NODs

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Published: 25 April 2008

*Genome **Biology*** 2008, **9:**217 (doi:10.1186/gb-2008-9-4-217)

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2008/9/4/217

Abstract

Recent work has identified the human NOD-like receptor NLRX1 as a negative regulator of intracellular signaling leading to type I interferon production. Here we discuss these findings and the questions and implications they raise regarding the function of NOD-like receptors in the antiviral response.

Upon infection with a pathogen, the host cell must recognize its presence, communicate this to neighboring cells and tissues and initiate a biological response to limit the spread of infection and clear the pathogen. Recognition of invading microbes proceeds via specialized intracellular and extracellular proteins termed pattern recognition receptors (PRRs), which recognize conserved molecular motifs found on pathogens, known as pathogen-associated molecular patterns (PAMPs). Recognition of PAMPs by PRRs leads to the activation of downstream transcription factors, resulting in induction of programs of host defense gene expression designed to effect immunity to the pathogen. In the innate immune response to viruses, the genes activated include those for the type I interferons - the primary cytokines mediating the innate response to viral infection. In mammals, these comprise IFNβ, 13 IFNαs and the more recently discovered IFNω. Type I interferons signal via the IFNα/β receptor to induce further sets of genes that regulate cellular metabolic processes, intracellular nutrient availability, apoptotic responses and direct elimination of the pathogen [1].

The recognition of single-stranded RNA viruses in the intracellular space is based on the processing of their genomes by one of at least two cellular RNA helicases - RIG-I/DDX58 and MDA5/Helicard [2,3]. This processing generates a conformational change in the helicases, allowing their twin caspase-recruitment domains (CARDs) to interact directly with a single amino-terminal CARD in the adaptor protein MAVS (also known as IPS-1, VISA or Cardif), which is anchored to the outer mitochondrial membrane [4-7]. MAVS complexes with the adaptor protein TRAF3, recruiting the scaffold protein TANK and the IκB kinases (IKKs) TANK-binding kinase 1 (TBK1) and IKKε, which activate the transcription factor IRF3. IRF3 activation leads to the transcriptional activation of a number of antiviral genes, including that for IFNβ (Figure 1) [8-11]. MAVS also acts as a bifurcation point for a second signaling pathway that can be triggered by RIG-I and some other PRRs. In this pathway the transcription factor NF-κB is activated, resulting in the activation of NF-κB-responsive genes (Figure 1) [4-7,10,12].

In a paper recently published in *Nature*, Moore *et al.* [13] have shown that these MAVS-mediated pathways can be inhibited by the action of an intracellular NOD-like receptor (NLR), the protein NLRX1, indicating that members of this ancient family of pathogen sensors can evolve to acquire new regulatory roles in mammalian host defense.

NOD-like receptors and the antiviral response

The NLR proteins generally act as intracellular sensors of infection, analogous to the cell-surface Toll-like receptors (TLRs), and their role in responses to bacterial and viral pathogens is of considerable current interest. These proteins are components of an evolutionarily ancient

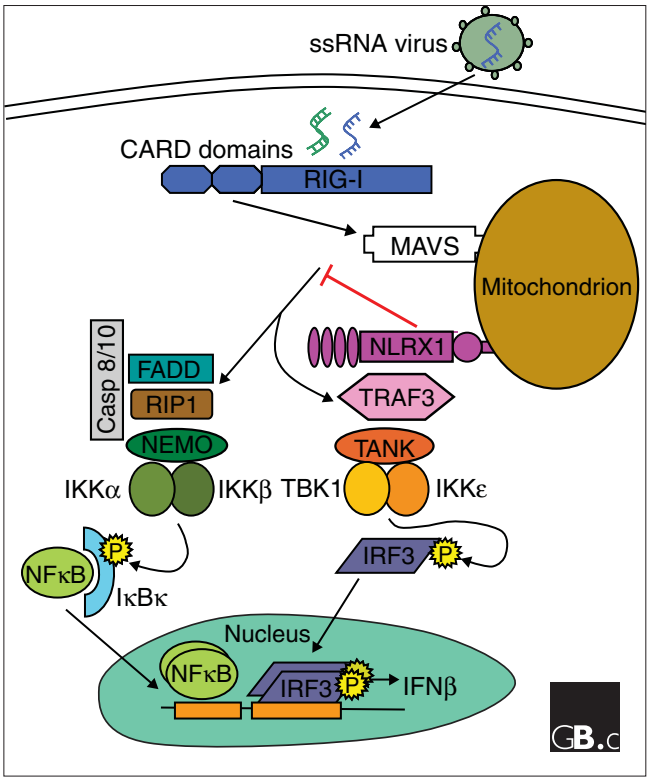

Figure 1
Activation of the transcription factors IRF3 and NF-κB in response to infection with a single-stranded RNA virus. On viral infection, RIG-I activated by viral RNA interacts with the adaptor protein MAVS, which represents a bifurcation point for the activation of IRF3 and NF-κB via activation of distinct IKK family members. Activation of NF-κB involves phosphorylation of its cytoplasmic inhibitor IκBκ, which tags that protein for destruction with the consequent release of NF-κB. IRF3 and NF-κB in turn activate a number of genes important in the antiviral response, including that for IFNβ. NLRX1 has been recently shown to inhibit this pathway, possibly by blocking the interaction of RIG-I with MAVS.

immune mechanism that appears to have evolved before the divergence of the plant and animal kingdoms - in plants, NLRs function as sensors of infection or physiological 'danger' signals that trigger cell-death processes to limit the spread of disease [14]. NLRs contain a central nucleotide-binding domain (NBD) and a series of leucine-rich repeats (LRRs), the latter appearing to constitute a regulatory sensor region that enables activation of the protein [15]. Most NLRs also contain an effector domain such as a CARD or pyrin domain, with which activated NLRs can interact with proteins such as the CARD- and pyrin-containing adaptor protein ASC, which links pyrin-containing NLRs with the CARD domain of the protease caspase-1 [15,16]. Whereas their plant-based relatives primarily mediate cell-death processes, some mammalian NLRs have been suggested to regulate genetic responses directly, as in the case of NOD1 and NOD2, or indirectly by mediating the proteolytic activation of cytokines that in turn activate pathways leading to expression of host-defense genes [17].

Of the latter NLRs, one of the best characterized in responses to viral infection is NLRP3/NALP3/CIAS, which mediates caspase-1 activation via aggregation with ASC and caspase-1 into 'inflammasomes'. These inflammasomes mediate the autoproteolytic cleavage of caspase-1 into its active form, which in turn cleaves the pro-inflammatory cytokines IL-1β and IL-18, enabling them to be secreted [16]. The demonstration that NALP3 is involved in caspase-1 activation and the secretion of IL-1β and IL-18 in macrophages in response to RNA and DNA viruses helped to clarify the role of NLRs in antiviral responses [18,19]. These findings suggested that in mammals NLR proteins retained their classical role as soluble activators of caspases in response to viral infection, much as they do in plants. But was it possible that NLRs could also have a quite different role in regulating host-defense pathways?

The recent study by Moore *et al.* [13] suggests that old NODs can indeed learn new tricks. These authors used bioinformatics approaches to predict a mitochondrial localization for NLRX1 (also known as CLR11.3 or NOD9), one of 22 NLRs found in humans. After verifying its localization in the outer mitochondrial membrane, the group assessed whether NLRX1 might be involved in MAVS-mediated antiviral responses, given that MAVS is also anchored on the mitochondrial surface. Indeed, their biochemical data suggest that the NBD of NLRX1 interacts with the CARD domain of MAVS, even in the absence of viral infection. Interestingly, they found that NLRX1 overexpression seems to strongly repress MAVS or RIG-I-driven IFNβ and NFκB reporter activity and IRF3 dimerization. Furthermore, the authors show that knockdown of NLRX1 by small interfering RNAs leads to increased interferon production in response to MAVS overexpression or viral infection. Taken collectively, their data suggest that NLRX1 attenuates MAVS-mediated activation of NFκB and IRF3, possibly by interfering with the interaction of RIG-I with MAVS. These findings suggest that NLRX1 functions to negatively regulate interferon responses activated via RIG-I, highlighting the malleability of the evolutionarily ancient NLR family in its capacity to carry out numerous immunological functions in distinct cellular compartments.

Further questions about NLRX1

This study leaves a number of interesting questions still open. In particular, the precise mechanism by which NLRX1 inhibits MAVS-mediated signaling is not clear. The data of Moore *et al.* [13] suggest that MAVS and NLRX1 may interact constitutively, and that NLRX1 can inhibit the interaction between RIG-I and MAVS. While this suggests that NLRX1 interferes with the interaction between RIG-I and MAVS, it follows that this interference must be overcome to allow for proper interferon signaling. Perhaps activated RIG-I has a higher affinity for the CARD domain of MAVS than does NLRX1, thus titrating out the NLRX1-MAVS

interaction and allowing interferon signaling. Alternatively, the LRR domain of NLRX1 might pick up 'danger' signals generated by viral infection in a fashion similar to NALP3, thus releasing inhibition by making the NLRX1-MAVS interaction less favorable. Furthermore, as NLRX1 can inhibit interferon signaling induced by overexpressed MAVS in the absence of virus, the role of NLRX1 in blocking the interaction between MAVS and downstream interferon signaling components should be addressed.

An interesting, although elusive, aspect of the MAVS-NLRX1 story is the function of mitochondrial localization for these proteins. In both cases, loss of mitochondrial localization as a result of experimental manipulation or cleavage from the membrane by viral proteases, as in the case of deactivation of MAVS by the hepatitis C virus protease NS3/4A, completely destroys the function of these proteins [5,6,13]. It may be, as Moore *et al.* [13] suggest, that the mitochondrion provides a useful platform on which sufficient concentrations of signaling elements can be marshaled to effect downstream signaling processes. Given the key role of mitochondria in apoptotic and metabolic functions and the intimate relationship of these processes with viral infection, it is no small leap to reason that MAVS and NLRX1 may serve as an interface between them. In addition, as with MAVS, cleavage of NLRX1 from the mitochondrial surface by endogenous or viral proteases might serve as a mechanism for damping NLRX1-mediated inhibition of interferon production.

It was previously shown by the same group that Monarch-1/NLRP12, a soluble NLR family member, can inhibit activation of the noncanonical NF-κB pathway in response to CD40 stimulation [15,20]. Thus, Monarch-1, and now NLRX1, represent what is probably a recent evolutionary retooling of some NLRs from inflammatory or cell-death mediators to checkpoint proteins designed to regulate immunological signaling processes. Given the fact that NLRs essentially act as molecular switches in response to stimuli sensed via their LRRs, it seems logical that they might be adapted to act as negative regulators that can be inducibly released or activated in the appropriate conditions. Indeed, the concept of such switches is recapitulated in many other biological systems: the Ras family of GTPases is but one example.

A persistent question and the genesis of significant debate within the innate immunity field is the mechanism by which these NLR switches are activated. Taking a precedent from the study of Toll-like receptors, some of whose LRR domains have been shown to physically interact with ligands, the conventional wisdom has been that NLRs also respond to specific PAMPs. Indeed, NOD1 and NOD2 have been shown to respond via their LRRs to bacterial peptidoglycans, although convincing biochemical evidence showing a direct interaction is lacking [16,17]. However, several studies showing that NALP3-mediated inflammasome formation is induced by a wide range of stimuli, from uric acid crystals to double-stranded RNA to ionophore stimulation, has thrown this conventional wisdom into disfavor [18,19,21-23]. The prevailing alternative hypothesis is that NLRs respond to nonspecific cellular perturbations or danger signals rather than discrete ligands. Thus, it will be important to determine what, if any, signal might be sensed via the LRRs of NLRX1. Given that NALP3 also responds to viral infection, it will be interesting to determine whether these two NLRs might respond to the same signal upon viral infection.

It is clear that there are numerous unanswered questions on the biology of NLRX1 in the interferon response as well as on the biology of NLRs in general. Although the interferon response might be considered an evolutionary contemporary of NLRs, the findings of Moore *et al.* [13] clearly suggest that the members of this family of proteins, and NLRX1 in particular, have evolved to play significant roles in directly regulating pathways that control more modern biological functions.

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