Feature Review

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Prion-like polymerization as a signaling mechanism

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The innate immune system uses pattern recognition receptors such as RIG-I and NLRP3 to sense pathogen invasion and other danger signals. Activation of these receptors induces robust signal transduction cascades that trigger the production of cytokines important for host protection. MAVS and ASC are essential adaptor proteins downstream of RIG-I and NLRP3, respectively, and both contain N-terminal domains belonging to the death domain superfamily. Recent studies suggest that both MAVS and ASC form functional prion-like fibers through their respective death domains to propagate downstream signaling. Here, we review these findings, and in this context discuss the emerging concept of prion-like polymerization in signal transduction. We further examine the potential benefits of this signaling strategy, including signal amplification, host evolutionary advantage, and molecular memory.

Beneficial prions

Two decades ago, the discovery of the first prion, the mammalian prion protein (PrP), elucidated the mechanistic basis of a group of infectious neurodegenerative diseases – the misfolding of PrP from a soluble α-helical conformation (PrP^{C}) to β-sheet rich aggregates (PrP^{SC}) that bind and convert other soluble PrP^{C} into PrP^{SC} [1]. Following this initial discovery, the characterization of other prion-like proteins ensued, and prions are now well accepted as proteins that adopt distinct conformations, at least one of which is self-propagating and heritable upon cell division [2,3]. In yeast and fungi, over a dozen prion determinants have been described, some as potential adaptive mechanisms of phenotypic diversification during environmental fluctuations [4–6]. Two notable examples include the essential yeast translation termination factor SUP35 and the fungal protein HET-s. SUP35 contains a prion domain (NM) at its N terminus followed by an enzymatic domain (SUP35C) that carries out its translation termination function. Following prion conversion, SUP35 forms large, insoluble aggregates that compromise its enzymatic activity, resulting in stop codon read-through and translation of novel RNAs that may be beneficial during stress. HET-s prion, by contrast, mediates cell fusion-induced cell death by converting its allelic variant, HET-S protein (big-S), into a membrane permeabilizing toxin that kills the fused cell; this has been suggested to be a possible mechanism of self-defense [7].

In higher eukaryotes, functional prions have been described in *Aplysia* and *Drosophila*, where members of the CPEB protein family undergo prion conversion that facilitates memory formation [8,9]. In mammals, however, prion-like proteins have been, until recently, invariably associated with diseases of protein aggregation [10–12]. Biochemical, structural, and genetic studies have now revealed that the adaptor proteins MAVS and ASC propagate respective downstream signals through prion conversion, and that this signal amplification strategy is crucial for the initiation of the innate immune response to pathogens detected by the RIG-I and inflammasome pathways. These findings suggest a beneficial role for prions in mammals and highlight a highly conserved function for prion proteins in host defense. We discuss these recent findings here and propose that prion-like polymerization is a conserved mechanism of signal transduction that mediates host defense. We first review evidence leading to the identification of prion-like properties in MAVS and ASC. We then discuss the significance of these properties in signal transduction and their broad conservation in light of homology with a cell death pathway in fungi. We conclude by examining the advantages of signaling through prion-like polymerization and highlight important outstanding questions in the field.

Identification of MAVS as a functional mammalian prion

RIG-I-like receptors (RLRs), which include RIG-I, MDA5, and LGP2, survey the cytoplasm and are essential for the detection of RNA viruses [13]. RIG-I specifically binds viral RNAs bearing 5′ triphosphates (5′ppp) or diphosphates (5′pp), which distinguish them from cellular RNAs that normally contain a 5′-cap or other modifications [14–16]. Our group has estimated that as few as 20 5′ppp–RNA molecules can trigger a robust antiviral response through RIG-I, suggesting an ultrasensitive mechanism of signal transduction [17].

RIG-I contains N-terminal tandem caspase activation and recruitment domains (CARDs) [RIG-I(N)], a middle helicase domain, and a C-terminal domain (CTD). Following activation by 5′ppp–RNA, RIG-I oligomerizes through the binding of RIG-I(N) to lysine 63 (K63)-linked polyubiquitin chains that are not anchored to any cellular proteins [17,18]. Oligomerized RIG-I then activates the adaptor

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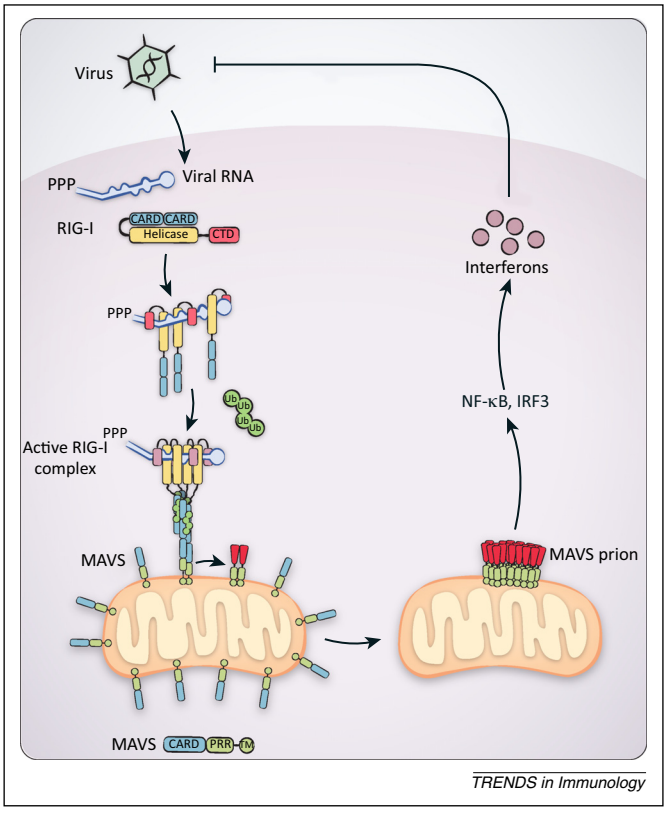

Figure 1. Model of RIG-I-dependent MAVS activation. Following virus infection, 5′ppp–RNA is generated in the cytoplasm and recognized by RIG-I, bringing multiple RIG-I proteins into proximity and releasing its N-terminal tandem CARDs from autoinhibition. Free RIG-I CARDs can then bind to unanchored K63-linked polyubiquitin, which induces the formation of an active RIG-I tetramer. The active RIG-I tetramer then interacts with the mitochondrial adaptor protein MAVS, through CARD–CARD interactions, and converts MAVS into its prion form. The initial MAVS prion seeds then rapidly convert other native MAVS proteins into the filamentous prion form, eventually leading to the activation of NF-κB and IRF3 for antiviral interferon production. Abbreviations: 5′ppp, 5′ triphosphates; CARD, caspase activation and recruitment domain; K63, lysine 63; NF-κB, nuclear factor-κB; IRF3, interferon regulatory factor 3.

protein MAVS. MAVS is a mitochondrial tail anchored protein and also contains an N-terminal CARD (MAVS^{CARD}), which is essential for MAVS activation through interactions with RIG-I CARDs [19]. Activated MAVS then signals downstream to the kinases IKK and TBK1, which activate transcription factors, nuclear factor-κB (NF-κB) and interferon regulatory factor 3 (IRF3), respectively, to induce antiviral cytokine expression [20] (Figure 1).

RIG-I and MAVS belong to the death domain (DD) superfamily of proteins, which play diverse and important roles in immune, inflammatory, and cell death signaling [21]. Members of the DD superfamily share a common six α-helical structural fold and contain characteristic domains (CARD, DED, DD, and PYRIN), which further distinguish these proteins into subfamilies. The DDs largely function through homotypic interactions. However, while some DD-containing proteins signal through limited oligomers, others have been observed to form much larger polymers [22–24].

MAVS^{CARD} is a bona fide prion domain

Studies on the RIG-I signaling pathway led to the identification of the adaptor protein MAVS (also known as IPS-1, VISA, and CARDIF) [19,25–27]. In addition to its unique mitochondrial localization, MAVS was also found to be resistant to detergent extraction after viral infection, suggesting active MAVS underwent significant biochemical changes [19]. Indeed, subsequent analyses revealed that virus infection led to the aggregation of MAVS into very large particles that were resistant to 2% SDS, as revealed by semi-denaturing detergent agarose gel electrophoresis (SDD-AGE), a commonly used assay to detect prion particles [28]. Surprisingly, recombinant MAVS proteins formed prion-like fibers with a protease resistant core composed of MAVS^{CARD}. Cell free assays revealed that substoichiometric amounts of MAVS^{CARD} fibers (which lack the signaling domain) were capable of converting endogenous full-length MAVS into SDS-resistant polymers; importantly, these newly converted polymers were functional in triggering downstream signaling [28]. Subsequent super-resolution imaging of virus-infected cells revealed rod-shaped MAVS clusters on the mitochondria, providing evidence for MAVS fiber formation in cells [29]. From these results, we proposed that MAVS propagates antiviral response through functional prion conversion.

To test if MAVS had other prion-like properties, we took a genetic approach using the well-established yeast SUP35-based prion assay, where a candidate prion domain is tested for its ability to functionally replace SUP35^{NM}, a *bona fide* yeast prion domain [30]. In yeast strains expressing MAVS^{CARD}–SUP35C in place of endogenous SUP35 (NM–SUP35C), MAVS^{CARD} faithfully reconstituted the key functions and properties of the SUP35 prion domain [31]. As with all prions, transient overexpression of MAVS^{CARD} greatly enhanced the frequency of MAVS^{CARD} polymerization, resulting in phenotypes that were stably inherited over numerous cell divisions. The MAVS^{CARD} prion state, [*MAVS^{CARD}*+], was also dominant, cytoplasmically inherited, and dependent on the MAVS^{CARD} protein. As with all other prion domains, MAVS^{CARD} also exhibited a two-step nucleation and polymerization-dependent prion conversion. Thus, from these biochemical and genetic studies, MAVS harbors key hallmarks of prions [28,31].

RIG-I mediated MAVS polymerization

RIG-I(N) directly nucleates MAVS prion conversion

Key to fully comprehending the mechanisms mediating MAVS activation is understanding how active RIG-I leads to MAVS prion conversion. In yeast, which lack a RIG-I-like pathway, the expression of RIG-I(N) is sufficient to induce MAVS^{CARD}–SUP35C prion conversion, suggesting a direct interaction between the CARDs of RIG-I and MAVS for signal transduction [31]. Point mutations in MAVS^{CARD} that abolished RIG-I-dependent antiviral signaling and MAVS polymerization in mammalian cells also abrogated RIG-I(N)-induced MAVS^{CARD} prion conversion in yeast, indicating that the yeast assay faithfully recapitulated RIG-I-dependent MAVS activation and that MAVS prion conversion is necessary for signaling [20,31].

K63 polyubiquitin-mediated oligomerization activates RIG-I

While the fully active RIG-I complex awaits structural elucidation, accumulating evidence suggests that unlike the previously proposed monomer or dimer [32], the