A Prion-like Trigger of Antiviral Signaling

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DOI 10.1016/j.cell.2011.07.018

The MAVS protein plays a critical role in the assembly of an antiviral signaling complex on mitochondrial membranes. Hou et al. (2011) now report that virus infection induces a conformational change in MAVS, leading to the prion-like formation of functional self-aggregates that provide a sensitive trigger for antiviral signaling.

Type I interferons (IFN α and β) play an essential role in the cellular antiviral response by coordinating the innate and adaptive immune systems. Intracellular viral RNA produced during virus infection is detected by the RNA helicases RIG-I or MDA5 (Skaug et al., 2009). RNA binding induces a conformational change in these helicases, exposing N-terminal tandem caspase activation and recruitment domains (CARDs) that bind to the CARD domain in the adaptor protein MAVS, which is located primarily on the outer membrane of mitochondria (Seth et al., 2005). MAVS associates with and signals through adaptor proteins that activate TBK1 and IκB kinases (IKK), which in turn activate the transcription factors IRF3/7 and NFκB, respectively. These transcription factors translocate to the nucleus and coordinately induce the expression of IFN α and β and many other antiviral genes. Many of the components of this signaling cascade have been identified, and their role within the pathway determined. However, the biochemical mechanisms involved in signaling complex assembly and the activation of downstream signaling pathways are poorly understood (Skaug et al., 2009). In this issue of *Cell*, Hou et al. (2011) exploit a robust and faithful in vitro system for antiviral signaling (Zeng et al., 2009) to uncover a remarkable prion-like mechanism for signaling complex assembly.

Previous studies showed that activation of RIG-I not only requires the binding of double-stranded viral RNA, but also binding of the CARD domain of RIG-I to unanchored polyubiquitin chains of lysine-63 (K63) linkage (Zeng et al., 2010). The Chen lab was the first to show that covalently bound K63 ubiquitin functions to mediate interactions between adaptor proteins in the activation of IKK kinases (Skaug et al., 2009). The RIG-I/ubiquitin study reveals that short unanchored K63 ubiquitin chains are high-affinity ligands that bind and activate RIG-I, thus expanding the role of K63 ubiquitin in signal transduction (Zeng et al., 2010).

Further exploitation of this system for antiviral signaling now reveals an additional surprise—that virus infection induces the formation of detergent-resistant, well-ordered, high-molecular weight aggregates of the MAVS protein, and these aggregates are potent activators of IRF3 (Hou et al., 2011). Recombinant MAVS protein can form similar functional aggregates in vitro. These aggregates appear as amyloid fibril structures in the electron microscope and are partially resistant to proteinase digestion. Furthermore, preformed MAVS aggregates can efficiently induce the endogenous MAVS to form functional aggregates on the mitochondria, suggesting that they are prion-like. Additional experiments revealed that the CARD domain of MAVS is both necessary and sufficient for the formation of MAVS fibrils in vitro. Strikingly, in the presence of K63 ubiquitin chains and viral RNA, RIG-I promotes the conversion of monomeric MAVS proteins on the mitochondrial membrane to functional aggregates that trigger downstream signaling. A model for MAVS activation based on the findings by Hou et al. is illustrated in Figure 1.

Prions are characterized by the pathogenic properties of the so-called prion protein PrP, associated with diseases such as Creutzfeldt-Jakob disease in humans, and with the self-propagation of multimers with properties of an amyloid (Colby and Prusiner, 2011). This mechanistic feature of prion aggregation has also been observed with certain yeast proteins and with the neuronal *Aplysia* CPEB protein, all of which bear a glutamine/asparagine Q-/N-rich domain (Si et al., 2003). In the latter case, the neurotransmitter serotonin controls a prion-like switch that results in a self-perpetuating form that is proposed to mediate the long-term maintenance of synaptic plasticity and memory storage (Si et al., 2003, 2010). It is important to note that neither CPEB nor MAVS display pathology or transmission through generations. Si et al. have proposed a new class of proteins "that utilize a self-perpetuating multimeric state to create a self-sustaining altered activity state in the cell in response to specific stimuli" (Si et al., 2010). The MAVS protein fits this definition well, and the mechanistic benefit is a high level of sensitivity to virus infection. Remarkably, fewer than 20 molecules of viral RNA and K63 ubiquitin chains are sufficient to activate IRF3 (Zeng et al., 2010). A key unanswered question relates to the reversibility of MAVS conformational change. A "memory" of this change would not be a desired property, as one may imagine this potentially leading to uncontrolled antiviral signaling and associated pathology.

It is important to note that there are no known amino acid sequence similarities

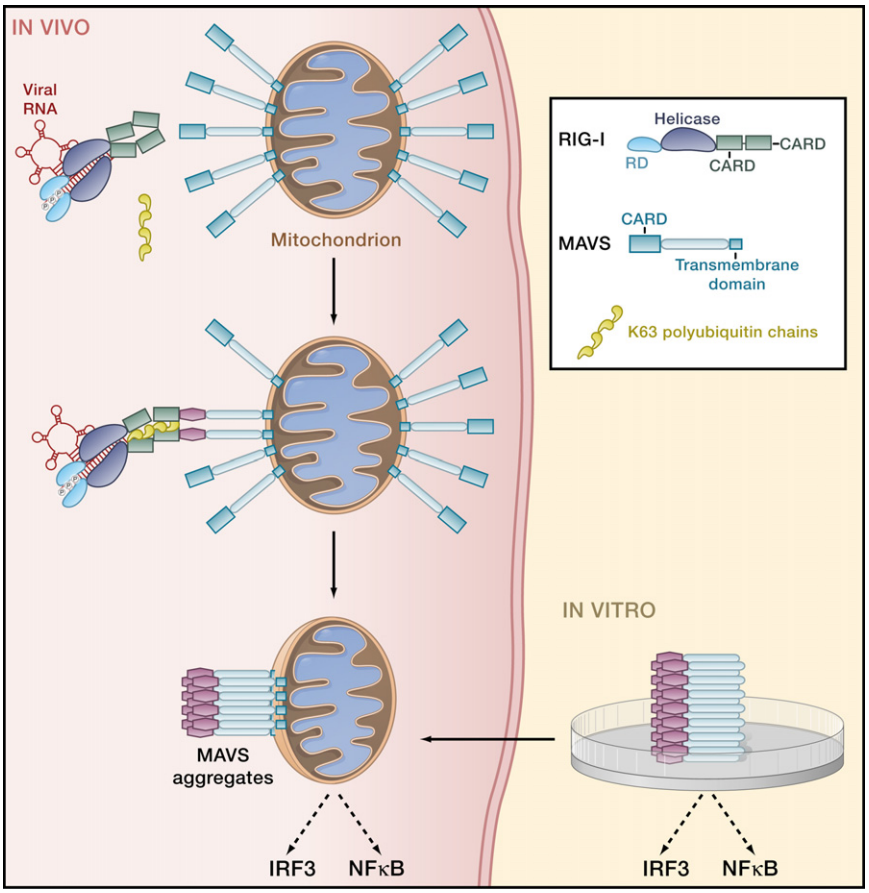

Figure 1. MAVS Forms Prion-like Aggregates to Activate Innate Immunity

Virus infection activates the RIG-I/MAVS pathway. Viral RNA binds to the C-terminal regulatory domain (RD) of RIG-I and induces a conformational change and dimerization. The central helicase domain is required for this process. The CARD domain of the RIG-I dimer then binds unanchored lysine-63 (K63) polyubiquitin chains and activates MAVS on the mitochondria membrane through the MAVS CARD domain. This interaction triggers a conformational switch of MAVS (changed color and shape of MAVS CARD in the diagram), which leads to the formation of MAVS aggregates on the mitochondrial membrane. MAVS aggregates are composed of MAVS polymers and potently activate downstream signaling cascade. Critical transcription factors IRF3 and NFκB are subsequently activated to induce the expression of interferon and other antiviral genes. MAVS aggregates behave like prions: they are partially resistant to detergent extraction and proteinase digestion and can efficiently self-propagate. The CARD domain of MAVS directly mediates the self-aggregation, and the transmembrane domain (TM) greatly facilitates MAVS aggregate formation on the mitochondria in vivo (left). Functional prion-like MAVS polymers can also form by self-aggregation in vitro in the absence of the TM domain (right). MAVS polymers formed in vitro can potently induce endogenous MAVS to form functional aggregates on the mitochondria from uninfected cells. This property closely resembles that of infectious prions.

between the MAVS polymers and the glutamine/asparagine Q-/N-rich prions or polyQ proteins. However, hints of a possible alternative aggregate structure are provided by a recent structural study of Q-/N-rich and polyQ peptides showing that they form α-helical coiled coils in vitro and assemble into multimers (Fiumara et al., 2010). Coiled coils (CCs) are known to mediate protein-protein interactions and oligomerization. A key role for this structure in prion and polyQ expansion diseases was provided by studies showing that mutations that disrupt coiled coils suppress aggregation and activity, whereas mutations that enhance coiled coils promote aggregation. Fiumara et al. (2010) have proposed that CCs may either be intermediate structures in the transition from helical domains to β sheets or they may be self-sufficient mediators of the prion switch and amyloidogenesis. As they point out, this mechanism may have important implications for the distinction between pathogenic and functional prions. Whereas the toxicity of pathogenic prions and amyloids relies on a misfolding into β sheets, nonpathogenic and functional Q-/N-rich prions may use CC-based conformational changes for switching physiological activity on and off in a regulated and persistent manner (Fiumara et al., 2010). Whether this notion applies to proteins lacking Q-/N-rich sequences, such as MAVS, remains to be established.

The structure of the MAVS CARD domain has been solved and is characterized by a six-helical bundle arrangement (Potter et al., 2008). The amino acid sequences of the RIG-I/MDA5 and MAVS CARD domains are only distantly related; neither have obvious Q/N regions, but both are essential for antiviral immunity. Interactions between the CARD domains of RIG-I/MDA5 and MAVS promote the formation and spreading of MAVS aggregates, which robustly activates downstream signaling. The MAVS aggregates do not appear to be in the form of a β-pleated sheet characteristic of some prions based on the lack of staining with Congo red (Hou et al., 2011). However, an interesting speculation is that the helical bundles of the CARD domain unfold into extended helices that then interact as intermolecular coiled coils.

Understanding the structural transition of the MAVS CARD domains from the monomer to the aggregated polymer may provide insights into general mechanisms for ordered aggregation-dependent functional transitions. It seems likely that the elegant mechanism for signaling complex assembly proposed by Hou et al. (2011) did not occur once in nature and will soon be revealed in other signaling pathways.

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Regulatory Revolution: Evolving the "Anti-LacI" Repressor

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DOI 10.1016/j.cell.2011.07.015

Much of adaptation is based upon changes in gene expression, but the emergence of new regulatory logic has not been observed directly. Now, Poelwijk et al. report evolving the *lac* repressor (LacI) to reverse its regulatory logic, resulting in an "anti-LacI" that represses transcription when bound to its "inducer."

From the origin of new body-plans (Carroll, 2008) to beneficial changes in beak morphology (Mallarino et al., 2011), the evolution of gene regulation is critical in adaptation over many timescales. Even when populations of microbes adapt in the laboratory, promoters and regulatory proteins are disproportionately recovered as targets of beneficial mutations (Barrick et al., 2009). The selective advantage of experimentally evolved changes in expression has typically arisen from altered levels of the affected gene products, but what about the evolution of new regulatory logic itself?

Most laboratory experiments use a constant environment for selection, and thus, developing new responses to changes in the environment tend not to be particularly advantageous. Furthermore, it is unclear how many mutations, perhaps acting in concert, are needed to change the qualitative properties of a gene regulatory system. In this issue, Poelwijk et al. present a tour de force study in which they use an elegant synthetic system to apply alternating selective pressures on a genetic module of *Escherichia coli* (Poelwijk et al., 2011). In the end, the authors coerce the *E. coli* lactose repressor (encoded by *lacI*) to have exactly the opposite logic as it does naturally.

The *E. coli* lactose repressor, encoded by the *lacI* gene, is one of the best understood transcriptional regulators. It binds the *lac* operator and represses transcriptional initiation in the absence of its inducer such as allolactose or its analog isopropyl β-D-1-thiogalactopyranoside (IPTG), but once LacI binds IPTG, the repressor releases from the operator (Figure 1A).

In order to select for the opposite response, that is repression in the presence of IPTG and lack thereof in its absence, Poelwijk et al. construct an operon with the *lac* operator controlling two genes that permit expression to be rewarded (by resistance to the antibiotic chloramphenicol) or punished (by sensitivity to sucrose) depending upon which chemical is added to the medium. Poelwijk et al. also include in the operon the reporter gene *lacZ*, which encodes β-galactosidase, to quantify total expression of the operon.

From here, the strategy is simple. First, iteratively introduce a hearty dose of variation specifically in *lacI* by PCR mutagenesis. Next, select upon this variation by alternating periods of growth in medium supplemented with sucrose and IPTG with growth in medium with chloramphenicol and not IPTG (i.e., to select for the opposite logic). Then, repeat.

What are the chances that LacI would readily reverse its response to inducer and become the "anti-LacI"? After the first round of variation and selection, the results did not seem promising. In fact, the evolution appears to reach a roadblock. LacI variants that abandon the wild-type logic (which led to maximal punishment emerged) exhibit no logic at all; they simply allow constitutive expression of the downstream genes, completely independent of IPTG (Figure 1A). After the next round, however, Poelwijk and colleagues find variants that perform the desired inverse logic, and these variants dominate after the final, third round. The authors then carefully analyze the genetic basis of this adaption. Reversing the logic of the Lac repressor is indeed