Phosphorylation and Dephosphorylation of the RIG-I-like Receptors: A Safety Latch on a Fateful Pathway

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Activation of antiviral responses by RNA sensors RIG-I and MDA5 must be stringently controlled. In this issue of *Immunity*, Wies et al. (2013) show that a requirement for activation-induced dephosphorylation of these proteins reinforces this restriction.

Cells sense viral RNA by one of two structurally related sensor proteins of the RIG-I-like receptor (RLR) family—RIG-I and MDA5.

The best-studied signaling functions of the RLRs are those initiated by their induced binding to MAVS (also known as IPS-1, CARDIF, or VISA), an adaptor protein anchored to the outer membrane of the mitochondrion. Once activated, MAVS initiates a number of antiviral signaling pathways, most notably those of transcription factors IRF3 (via protein kinases TBK1 and IKKε) and NF-κB.

To allow effective sparking of this antiviral response even by small numbers of viral particles, MAVS activation unfolds explosively. Self-association of just a few MAVS molecules, once bound with RLRs, triggers an avalanche of their aggregation and hence also their massive activation (Hou et al., 2011).

This vehement initiation of cellular changes would cause great harm to the host if it were induced unnecessarily. Therefore, besides including the core MAVS components that mediate its signaling activity, this signaling complex contains accessory proteins that restrain its function (Ramos and Gale, 2011). The study by Wies et al. (2013) in this issue of *Immunity* describes a mechanism that affects the functioning of the MAVS complex in this way. The mechanism, rather than operating after MAVS is activated by RLRs, exerts its effects on the RLRs themselves.

Signaling by both RIG-I and MDA5 is triggered by their binding to MAVS through two caspase activation and recruitment domains (CARDs) located in tandem at their N termini. In addition, these molecules contain a central DExD/H-box helicase domain and a C-terminal domain (CTD). Some mechanisms that restrict the function of these RLRs upstream of MAVS activation have already been described. First, their structure dictates their folding into an autoinhibited form. In the absence of stimulation, accessibility of the CARDs is restricted by constitutive binding of one of them to the DExD/H-box. This state is altered upon infection. Binding of viral nucleic acid, first to the CTD and then to the DExD/H-box domain, releases the CARDs via a process facilitated by ATPase activity of the DExD/H-box domain (Kolakofsky et al., 2012). Their release is a necessary condition for enabling the CARDs to activate MAVS. However, it does not suffice for the purpose; another structural change is still needed and is imposed by the binding of K63-linked polyubiquitin chains to specific sites in the RLRs. This binding dictates clustering of RIG-I and MDA5 into oligomeric structures; it perhaps also causes conformational changes in these proteins, as a result of which they acquire the ability to activate MAVS (Jiang et al., 2012). In the case of RIG-I, the activating polyubiquitin chains are generated by the actions of two E3 ubiquitin ligases, TRIM25 and RNF135 (Riplet). TRIM25 is recruited to the CARDs upon their exposure, and it then links a polyubiquitin chain to a specific lysine residue in the RIG-I CARDs and possibly also generates free polyubiquitin chains. RNF135 binds to the CTD. The mechanism of generation of the polyubiquitin chains that are required for activation of MDA5 is unknown.

The findings presented by Wies et al. (2013) in the present issue, as well as in other publications by this group (Nistal-Villán et al., 2010; Gack et al., 2010), reveal that activation of MAVS by the RLRs is further restricted by the need for another covalent modification. Certain serine and threonine residues in the CARDs of RIG-I and MDA5 were previously found to be constitutively phosphorylated prior to stimulation. For the RLRs to be activated, these phosphate groups must be removed. The dephosphorylation is mediated by two specific protein phosphatases, PP1α and PP1γ (but not PP1β), which are recruited to the sensor molecules after RNA binds to them.

The data suggest that the dephosphorylation occurs as a consequence of exposure of the CARDs upon viral RNA binding and precedes the binding of TRIM25 to RIG-I. In an independent study, inhibitory phosphorylation that is reversed by phosphatases upon viral infection has also been shown to occur in the CTD of RIG-I (Sun et al., 2011).

Activation of the RLRs thus apparently occurs through the sequential release of a series of inhibitory latches, each imposed by a different set of molecules: a conformational change dictated by the binding of viral RNA to the RLRs; binding of phosphatase to specific sites on RLRs exposed by this conformational change and dephosphorylation of specific residues in them; and activation of E3 ligases and binding of K63-linked polyubiquitin chains to the dephosphorylated receptors, resulting in the assembly of RLR molecules to a multimolecular form capable of activating MAVS (Figure 1).
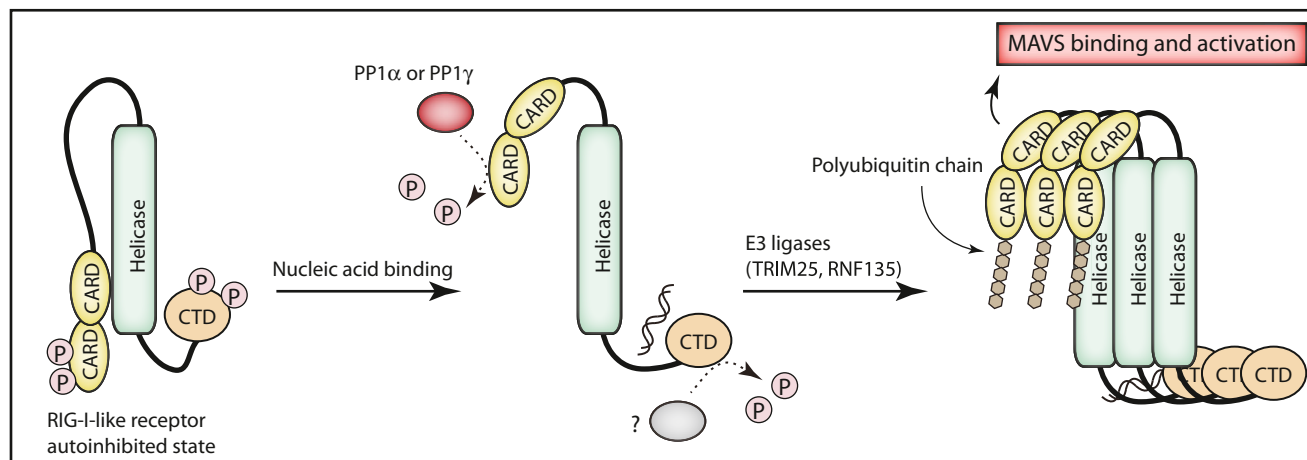
Figure 1. Activation of the RIG-I-like Receptors Necessitates Their Dephosphorylation

Activation of RIG-I and MDA5 occurs through a sequentially dependent series of conformational changes and covalent modifications. In the nonstimulated state, one of the two CARDs in these RLRs is bound to the DExD/H-box helicase domain, thus maintaining these RIG-I and MDA5 molecules in an autoinhibited form. Several serine and threonine residues in the molecules are constitutively phosphorylated. Binding of viral nucleic acid releases the CARDs in a process facilitated by ATPase activity of the DExD/H-box domain. This conformational change provides protein phosphatases with access to distinct binding sites in the RLRs. Dephosphorylation in the CARD is mediated by PP1α and PP1γ. The identity of the phosphatases affecting the CTD is unknown. Dephosphorylation of the RLRs allows activation of E3 ubiquitin ligases, which generate K63-linked polyubiquitin chains. In the case of RIG-I, the E3 ligases are TRIM25, which binds to the dephosphorylated CARDs, and RNF135 (Riplet), which binds to the CTD. The E3 enzymes affecting MDA5 are still unknown. Binding of polyubiquitin chains to the dephosphorylated RLRs induces their oligomerization and binding to the adaptor protein MAVS. This binding triggers activation of MAVS molecules, leading to an antiviral state and inflammation.

Does the regulation of RLR function through phosphorylation and dephosphorylation indeed serve as a safeguard against excessive responses and as a mode of adjustment to need? According to the data of Wies et al. (2013), recruitment of phosphatase(s) to the RLRs occurs passively, merely as a consequence of exposure of their binding sites on the CARDs of the RLRs. It is not clear, however, whether the phosphatase availability or activity (or both) depends on other signals or whether their availability and function can be modulated by regulatory proteins. There is also no information on whether the activities of the protein kinases responsible for phosphorylation of the RLRs are subject to modulation (in the case of RIG-I, PKC-α and PKC-β phosphorylate the regulatory residues in the CARD [Maharaj et al., 2012] and casein kinase II phosphorylates those in the CTD [Sun et al., 2011]).

For gaining a better understanding of the implications of this regulation of RLR function, research will be needed to further clarify the regulation of the protein kinases and phosphatases affecting the RLRs and to reveal what additional proteins participate in this regulation. Also needed is more information about the conformational changes imposed on the RLRs by their phosphorylation and about the full range of functional consequences of such phosphorylation.

Detection of foreign nucleic acids in the cytoplasm by specific sensor proteins and activation of defense mechanisms by these proteins are among the principal modes of protection against infection. The discovery that phosphorylation and dephosphorylation of specific residues in such sensors play decisive roles in their activation provides an exciting point of view of their function, and further exploration is warranted.

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