HSV1 Pulls the Deamidation tRIGger

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Viruses have evolved a remarkable array of strategies to escape the host's innate immune responses. In this issue of *Cell Host & Microbe*, Zhao et al. (2016b) reveal a viral strategy to inactivate RIG-I signaling that relies on deamidation of RIG-I.

The innate immune response represents an essential step in antiviral defense. This response starts with the specific detection of viral nucleic acids by dedicated pattern recognition receptors (PRRs), such as retinoic acid-inducible gene I (RIG-I, DDX58), the prototypical member of the cytosolic PRRs of the RIG-I like receptor (RLR) family. RLRs belong to the superfamily 2 (SF2) RNA helicases and contain a central DExD/H box catalytic core made of two RecA-like domains, Hel1 and Hel2, forming a cleft that binds and hydrolyzes ATP. In addition, RLRs possess a specific conserved insertion domain, HEL2i, that orchestrates dsRNA binding. Each RLR also contains a related C-terminal domain (CTD) that mediates specific recognition of viral RNAs. In RIG-I, the CTD specifically recognizes 5′pp(p)-blunt-ended dsRNA, a cytoplasmic pathogen-associated molecular pattern (PAMP) that is thought to be present in many RNA virus infections. RIG-I can also respond to cytoplasmic DNA, after it has been transcribed into 5′ pppRNA by cytoplasmic RNA polymerase III (Ablasser et al., 2009). In addition, RIG-I contains tandem caspase activation and recruitment domains (CARDs) that mediate downstream signaling to the mitochondrial adaptor protein MAVS through CARD-CARD interactions (Figure 1, left panel) (Luo et al., 2013).

In its resting state, RIG-I is in an autorepressed conformation, its CARD domains bound to the helicase domain, while its CTD is available to scan the environment for traces of PAMPs. Upon binding viral RNA, the CTD caps the blunt dsRNA end, making contacts with the 5′ppp and the terminal nucleotides while the helicase domain wraps around the dsRNA. The RNA-bound complex is now competent for ATP binding and hydrolysis. Conformational change occurs, leading to the release of the CARDs and their polyubiquitin-dependent oligomerization. The oligomeric complex then interacts with MAVS, the mitochondrial downstream effector, imprinting its oligomerization on MAVS, which leads to the activation of the IRF3/7 and NF-kB pathways and the production of various antiviral cytokines (Figure 1, left panel) (for review, see Kolakofsky et al., 2012).

Faced with this potent antiviral system, viruses have evolved various strategies to escape and/or counteract these defenses. Herpesviruses manipulate metabolic pathways due in part to their capacity to hijack cellular components of these pathways. The Feng group has previously shown that a tegument protein of some gamma-herpesviruses (γHV) has the remarkable property of inducing RIG-I activation independently of nucleic acid PAMPs or ATP binding and hydrolysis. This "illegitimate" activation of RIG-I is the result of an amidotransferase-mediated deamidation of two asparagine in the Hel1 domain and one glutamine in the first CARD domain by the viral tegument protein vGAT, a homolog of the cellular phosphoribosyl-formylglycinamidine synthetase (PFAS). vGAT itself is enzymatically inactive but acts by recruiting the cellular PFAS, forming a complex that binds and deamidates RIG-I. At first glance, this constitutive activation of RIG-I may appear counterproductive for the virus, but it has several major advantages. First, it allows the activation of the NF-kB pathway that is required for optimal virus replication, and at the same time this complex inhibits the IRF3/7 pathway, preventing the production of antiviral cytokines. Finally, these modifications prevent any further RIG-I activation by bona fide RNA PAMPs, thereby sterilizing this signaling pathway (Dong et al., 2012) (Figure 1, central panel).

In this issue, Zhao et al. (2016b) show that HSV1 (an alpha-herpesvirus, αHV), also induces RIG-I deamidation, but in this case the deamidation inhibits RIG-I activation in response to well-defined agonists (certain Sendai virus stocks and low-molecular-weight poly[I:C]) (Zhao et al., 2016b). Using co-immunoprecipitation to screen for viral proteins that bind RIG-I, they identified a late structural tegument protein, UL37. UL37 has no sequence homology with vGAT, but like vGAT, it induces the NF-kB pathway needed for optimal viral replication. In this case, the induction is due to the direct interaction of UL37 with the NF-kB-signaling adaptor TRAF6 (Figure 1, right panel) (Liu et al., 2008). UL37 also induces the deamidation of two asparagines to aspartic acid in the Hel2i domain of RIG-I (N495D and N549D) that are involved in dsRNA binding. RIG-I mutants with aspartates at these positions (RIG-I-DD) cannot be activated, and a specific inhibitor of deamidation prevents virus-induced RIG-I inhibition. In contrast to γ-vGAT, which needs a cellular protein co-factor, deamidase activity of UL37 was found to be associated with the purified viral protein itself. The two deamidated asparagines of RIG-I lie in a critical region of the helicase domain that is involved in dsRNA binding, and RIG-I-DD is specifically impaired in binding 5′ppp-dsRNA in EMSA studies with purified RIG-I. As a consequence, RIG-I-DD cannot hydrolyze ATP or be activated. Single mutants show that only D549 is impaired in dsRNA binding, whereas both are equally impaired in ATPase activity. Structural data show that N549 can make two hydrogen bonds with T504 of the α-helical RNA-binding domain. Deamidation of N549, as well as its conversion to alanine,

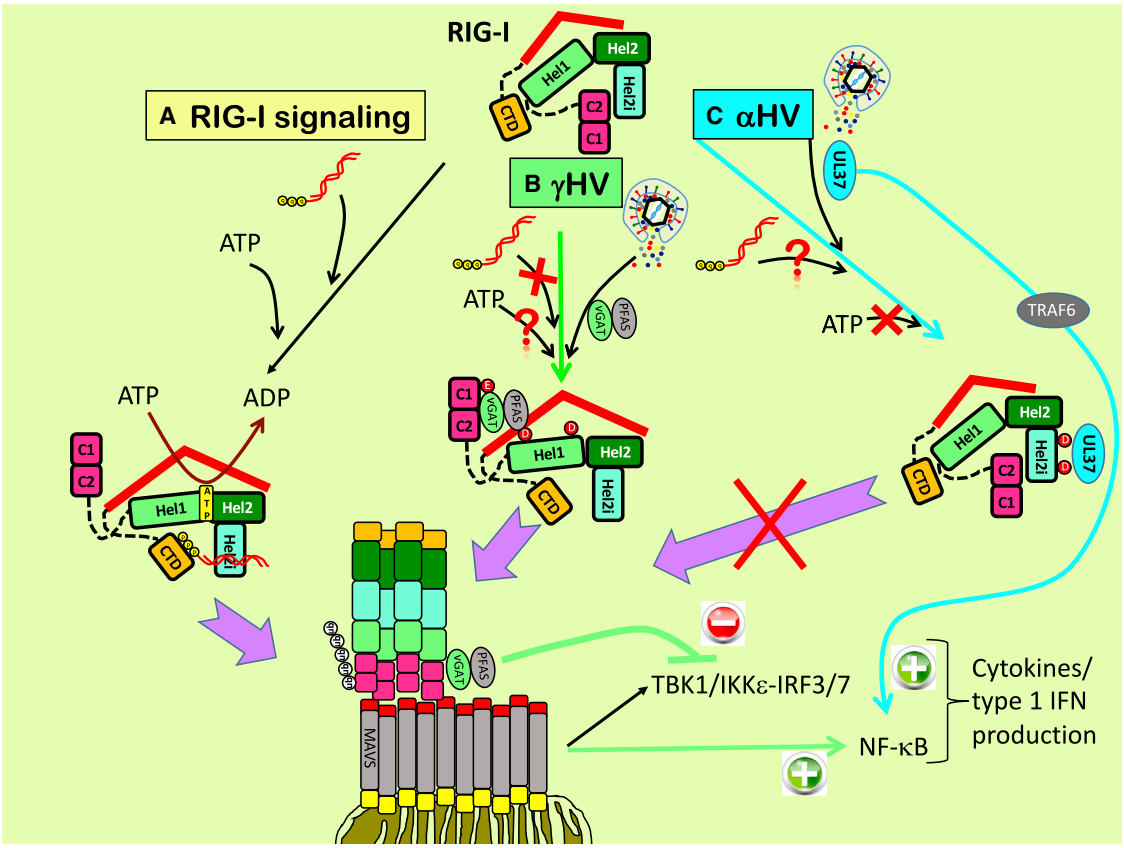

Figure 1. Deamidation in RIG-I Signaling

(A) Upon binding of viral RNA, the CTD caps the blunt end, making contacts with the 5′ppp and the terminal nucleotides, while the helicase domain wraps around the dsRNA. The RNA-bound complex is now competent for ATP binding and hydrolysis, which in turn leads to conformational changes that release the CARDs, permitting polyubiquitin-dependent oligomerization. The oligomeric complex then interacts with MAVS, the mitochondrial downstream effector, imprinting its oligomerization on the mitochondrial platform and leading to the activation of the IRF3/7 and NF-kB pathways and the production of various antiviral cytokines.
(B) The γHV tegument protein vGAT recruits the enzymatically active cellular PFAS to induce deamidation of RIG-I, leading to constitutive activation of RIG-I independent of dsRNA binding and ATP hydrolysis. While activating the NF-kB pathway that allows for optimal virus replication, vGAT simultaneously prevents IRF activation, thus preventing an immune response (green arrows).
(C) The αHV tegument protein UL37 has the dual capacity to interact with TRAF6 to activate the NF-kB pathway and to directly induce RIG-I deamidation, preventing stable dsRNA binding, ATP binding and hydrolysis, and RIG-I signaling (blue arrows).

prevents hydrogen bond formation and RIG-I activation. Replacing N549 with glutamine retains the capacity to form these hydrogen bonds, and in contrast to vGAT, UL37-induced RIG-I deamidation is restricted to asparagines. Mutant RIG-I in which N495 and N549 are replaced by glutamine (RIG-I-QQ) is resistant to deamidation, and RIG-I activation in response to HSV-1 infection and appropriate RIG-I agonists is fully restored. Known protein deamidases contain a catalytic cysteine, and systematic cysteine mutation of UL37 identified C819 and C850 as critical for this activity. This maps the deamidase domain to the C-terminal part of UL37. Further analyses pinpointed the catalytic cysteine to C819. A recombinant HSV1 with a mutated UL37-C819S restores RIG-I activation. The authors conclude that UL37 is critical for HSV-1 to evade immune response via deamidation of RIG-I (Figure 1, right panel).

Thus, two herpesviruses employ a tegument protein to activate the NF-kB pathway and inhibit the IRF arm of RIG-I signaling via deamidation of RIG-I, but with opposite strategies: αHV inhibits RIG-I, while γHV induces its constitutive activation. For γHV, deamidation of residues in the Hel1 domain that are involved in ATP binding and hydrolysis leads to constitutive activation of RIG-I. It is pretty clear that there is no binding to pathogenic RNAs and no ATP hydrolysis in this context. The question remains whether ATP binding is also impaired, since it has been shown that binding without hydrolysis may lead to inappropriate RIG-I activation (Lässig et al., 2015). In contrast, Zhao et al. (2016b) show that αHV-induced deamidation of residues in the Hel2i domain, involved in duplex RNA binding, leads to the inability to activate RIG-I. In this case, it is pretty clear that both ATP binding and hydrolysis are impaired. The defect in 5′ppp-dsRNA binding that is thought to initiate the immune response, via the high affinity of the CTD for the 5′ppp blunt moiety of the dsRNA, is more surprising. One possible explanation is that the Hel2i domain is absolutely required to stabilize the dsRNA/RIG-I complex prior to ATP binding. If the RNA structure does not conform to the expected pattern of a pathogenic RNA (e.g., a double-stranded structure of a given length) and cannot be properly engaged by the helicase domain, there is no ATP binding, and the

RNA may be immediately released. This binding instability might be important for RIG-I to discriminate between self and non-self RNAs and to prevent an unwanted immune response. In this context, deamidation of crucial residues in the Hel2i domain could prevent stable binding to pathogenic dsRNA, leading to the release of this RNA prior to ATP binding and activation of RIG-I.

Finally, this work highlights the role of enzymatic deamidation in innate signaling. Several bacterial proteins were previously shown to employ a deamidase activity toward various signaling molecules, to escape immune responses. The Feng group's studies show that viruses also use this strategy (Zhao et al., 2016a). The finding that two different herpesviruses appear to use amidotransferase activity to subvert RIG-I-mediated innate immunity suggests that deamidation is likely a common mechanism that cells use to modify an activity in response to other events. Why herpesviruses appear to have chosen this particular form of protein modification for subverting RIG-I is an enigma. The question remains whether cells also use the amidotransferase reaction to regulate innate signaling. In any event, how these herpesvirus-induced deamidations modify RIG-I activation adds new insight into the mechanism of action of this important PRR.

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HIV Latency TORn Down

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Combination therapy for HIV infection is effective at controlling disease but fails to eradicate the virus because a persistent reservoir of cells harbors latent HIV DNA. In this issue of *Cell Host & Microbe*, Besnard et al. (2016) show that the mTOR kinase is essential to reactivate HIV from latency.

Progress in HIV therapy over the last two decades has been equally exciting and frustrating. Exciting, because the development of over 30 different drugs, which are carefully chosen in combination antiretroviral therapy (cART), has transformed a lethal and devastating disease into a manageable, chronic condition that can be controlled over decades. Victory over the virus, however, is only apparent; this is where frustration comes in: if an infected patient, even after years of successful suppression of viral replication, stops therapy, viral replication and thus overall viral load bounce back. It is an unfortunate matter of fact that there is still no sterilizing cure for HIV infection. Decades of research on the molecular reasons underlying the failure of currently available drugs to elicit a cure have come to a clear conclusion: these agents target various steps in the replicative cycle of the virus but are not effective against the integrated, silent viral DNA. HIV thus persists in treated patients due to the existence of a reservoir of transcriptionally silent, integrated viral DNA in resting, memory CD4+ T cells (Ruelas and Greene, 2013).

Understanding why, after integration into the host-cell genome, the HIV DNA becomes silent and how it gets reactivated has been a matter for intense investigation over the last 20 years. Taking advantage of an elegant, cell-based functional screening approach, Verdin and colleagues now demonstrate that the integrity and function of protein complexes formed by the mammalian (or mechanistic) target of rapamycin (mTOR) kinase are essential to reactivate HIV-1 from latency (Besnard et al., 2016). The authors took advantage of a previously developed collection of short-hairpin RNAs (shRNAs) targeting all human genes with a complexity of 25 different shRNAs per gene (Bassik et al., 2013). Pools of lentiviral vectors expressing this ultracomplex shRNA library were used to infect a cell line in which the HIV DNA, modified to express the green fluorescent protein, was latently integrated into the host cell genome. Searching for shRNAs that prevented reactivation from latency upon cell stimulation was a means to functionally identify cellular genes that are relevant in this process. Besides identifying some transcriptional activators and repressors that are well