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Balance of Power in Host-Virus Arms Races

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Summary

The sensing of viral RNA by the host innate immune system is mediated by RIG-I and its partner PACT. In this issue Luthra et al. (2013) show that Ebola virus VP35 protein counteracts the action of PACT at the price of compromising its own function in viral replication.

Host cells combat viral infection by mobilizing an innate immune response mediated by antiviral cytokines such as type I interferons. This response can be triggered by the recognition of invading viral RNA by prototypic cytoplasmic sensor RIG-I together with its partners and activators such as PACT (Kok et al., 2011). PACT and its homolog TRBP are multifunctional double-stranded RNA (dsRNA)-binding proteins that also function in RNA silencing and the regulation of protein kinase R (PKR) activity. To what extent PACT participates in antiviral response in physiological context and whether viruses have evolved countermeasures to inhibit the activity of PACT remain open questions in the field. In this issue, Basler and associates found several missing pieces in the puzzle. They showed that Sendai virus was unable to induce interferons in *Pact*-deficient mouse embryonic fibroblasts. They also provided the first evidence for the counteraction of PACT by Ebola virus-encoded interferon-antagonizing protein VP35. Furthermore, their characterization of the mutual antagonism between PACT and VP35 revealed a balance of power in the host-Ebola virus interaction (Luthra et al., 2013). According to their model, whereas VP35 counteracts PACT in the activation of type I interferon production, PACT fights back by impeding the function of VP35 in viral RNA synthesis and replication (Figure 1).

Ebola virus is a highly lethal pathogen. Evasion of innate immunity is a major mechanism by which Ebola virus causes severe disease in human. VP35 is a prominent virulence factor that is both an essential cofactor of viral polymerase and an interferon antagonist that contributes to immune evasion. Targeting of PACT by VP35 uncovers a new viral countermeasure in the battle against host innate antiviral response. Basler and associates demonstrated that VP35 suppresses PACT-induced activation of RIG-I by preventing PACT from interacting with RIG-I (Luthra et al., 2013). Since VP35 is structurally and functionally related to other viral interferon-antagonizing and dsRNA-binding proteins such as influenza A virus NS1, herpes simplex virus type 1 Us11 and vaccinia virus E3L, it will be of great interest to see whether PACT targeting would be a general mechanism of immune evasion adopted by multiple pathogenic viruses. Theoretically these viral proteins

might operate in the same way as VP35 if they could also bind PACT. Indeed, both influenza NS1 and herpes Us11 are known to interact with PACT (Li et al., 2006; Peters et al., 2002), raising the possibility that they might also prevent PACT from interacting with and activating RIG-I. On the other hand, although NS1 is not a component of viral polymerase, it is also recruited to the polymerase complex through an interaction with NP (Robb et al., 2011). Interestingly, PACT binds PA subunit of viral polymerase and exhibits antiviral activity by perturbing polymerase-mediated transcriptional activity (Tofforeau et al., 2011). Thus, the mutual antagonism between VP35 and PACT might represent a recurrent theme in host-virus interaction. With slight variations the theme would also be seen in the context of other viruses including influenza. In this scenario, PACT could execute its antiviral activity through at least two mechanisms: activation of RIG-I-dependent production of type I interferons and perturbation of viral polymerase-mediated viral RNA synthesis and replication. In this regard, further investigations are required to clarify the biological significance of NS1-PACT and PACT-PA interactions in suppressing influenza virus replication and shaping innate immune response.

VP35 was demonstrated to suppress PACT activity by preventing its interaction with RIG-I (Luthra et al., 2013). Elucidating how PACT activates RIG-I remains the next challenge in the field. RIG-I is strikingly similar to Dicer that also bears a DEAD box RNA helicase domain. Human Dicer has two dsRNA-binding partners TRBP and PACT (Kok et al., 2007). The identification of the role of PACT in the activation of RIG-I was initially prompted by the activation of Dicer by TRBP and PACT (Kok et al., 2011). Findings in the study of how TRBP and PACT activate Dicer would be very instructive in the analysis of PACT activation of RIG-I. Several recent studies have shed light on the mechanistic details of the roles of TRBP and PACT in Dicer function. Interestingly, TRBP and PACT display an ATP-independent and RNA length-dependent diffusion activity exclusively on dsRNA (Koh et al., 2013). TRBP and its homologs in fruit flies are capable of altering Dicer cleavage rate and site in microRNA (miRNA) processing, leading to the production of miRNAs of distinct target specificities

(Fukunaga et al., 2012). In addition, TRBP and PACT exert differential effects on Dicer and Ago2 in the processing of miRNAs and short interfering RNAs (siRNAs). They cooperate to determine substrate selection, guide strand selection and cleavage specificity (Noland and Doudna, 2013). In comparison with the influence of TRBP and PACT on Dicer, it will not be surprising if PACT could function to concentrate and select RNA ligands for RIG-I. PACT might also tip the balance towards an RNA-induced conformational change of RIG-I leading to its activation. It will be of particularly great interest to see whether PACT would be capable of altering the enzymatic and biological activity of RIG-I impinging on partner selection and activation of downstream effectors.

Both VP35 and PACT are pleiotropic proteins that engage multiple partners. How they coordinate the interaction with different partners spatially and temporally within the infected cells remains to be clarified. Rescue experiments conducted with *Pact*-deficient cells lent strong support to the notion that VP35 suppression of interferon production is mediated through PACT (Luthra et al., 2013). However, it will still be of importance to explore whether the interaction with PACT is also critical in the suppression of PKR and Dicer activity by VP35. In addition, further investigations are required to determine whether some of the partners of VP35 and PACT form multi-subunit complexes or are mutually exclusive as in the case of RIG-I and VP35 in the interaction with PACT. Mass spectroscopic analysis of subcellular proteomes and interactomes derived from cytoplasmic, nuclear and mitochondrial fractions of infected cells collected at different phases of infection would reveal key information concerning the dynamic interaction of VP35 and PACT with their partners. Determination of the affinity of binding between different partners using biochemical and biophysical methods might also shed light on what binding partners of VP35 and PACT would prevail in the competition. A detailed analysis of the results from biochemical fractionation and protein dynamics will provide important clues to the VP35 and PACT interactome. For example, analysis of protein complex formation would suggest new directions to investigate whether and how VP35 and PACT partners and homologs such as

PKR, TRBP, Dicer, MDA5 and LGP2 might impact on the mutual antagonism of VP35 and PACT. On the other hand, in one simple model to explain mutual antagonism of VP35 and PACT, the VP35-PACT complex is sequestered in a subcellular compartment or is more prone to ubiquitination and proteosome-dependent degradation, preventing their interaction with respective partners such as viral polymerase and RIG-I. Further analysis of the fate of VP35-PACT in infected cells is therefore warranted. Getting to the bottom of all these questions will substantially advance our understanding of host-virus interaction and innate antiviral response.

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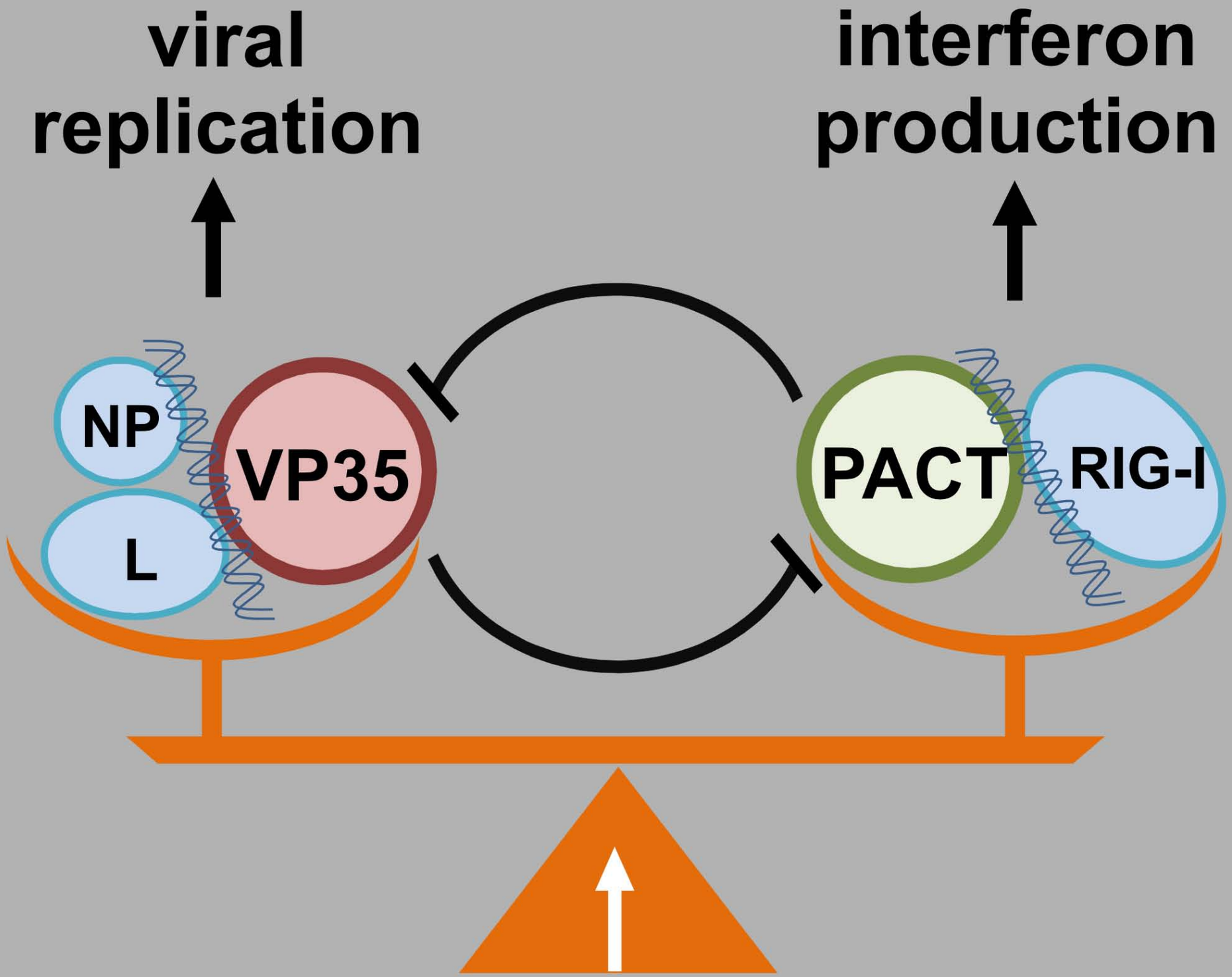
Figure Legend

Figure 1. Striking a Balance in Host-Ebola Virus Interaction

Ebola virus VP35 is a dsRNA-binding protein and an essential cofactor of the viral polymerase complex which also contains NP and L proteins. In this context VP35 functions to facilitate viral RNA synthesis and replication. On the other hand, VP35 is an interferon-antagonizing protein that counteracts the activity of PACT and RIG-I in the activation of interferon production. However, Ebola virus has to pay the price for the suppression of PACT by VP35, the activity of which in viral replication is inhibited by PACT in return.


viral replication
interferon production
NP
VP35
L
PACT
RIG-I