Influenza Virus Not cRAFTy Enough to Dodge Viperin

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Interferons elicit antiviral responses by inducing the expression of a large number of host cell genes. In this issue of *Cell Host & Microbe*, Wang and colleagues report that the interferon-inducible protein viperin inhibits influenza A virus release by impairing the formation of cholesterol-enriched plasma membrane microdomains, or lipid rafts. Viperin appears to disrupt lipid rafts by suppressing the activity of farnesyl diphosphate synthase, a key enzyme in isoprenoid biosynthesis.

Lipid rafts are membrane microdomains, present predominantly in the plasma membrane, that are highly enriched in cholesterol and sphingolipids containing saturated acyl chains (Hancock, 2006). An important property of lipid rafts is that they preferentially incorporate some proteins while excluding others, and the association of proteins with rafts is regulated by posttranslational modifications and a wide array of protein-protein interactions. Lipid rafts are dynamic structures that can undergo changes in size and composition in response to a variety of stimuli. Rafts have been proposed to serve as concentration platforms that promote specific, regulated, protein-protein interactions. Although the function, and indeed the very existence, of lipid rafts continues to be controversial, several independent experimental approaches have validated their importance in numerous biological processes (Hancock, 2006).

Recent progress in understanding virus replication at the molecular level has revealed that a number of enveloped viruses associate with lipid rafts upon their entry into, or exit from, their host cells (Ono and Freed, 2005). Notable examples include the retroviruses (e.g., HIV-1), paramyxoviruses (e.g., Sendai and measles), filoviruses (e.g., Ebola), and orthomyxoviruses (e.g., influenza A). The two major spike glycoproteins of influenza A, hemagglutinin (HA) and neuraminidase (NA), are raft associated, and the matrix protein (M1), which is largely responsible for organizing the virus assembly process, also associates with rafts when coexpressed with HA and NA (Figure 1) (Schmitt and Lamb, 2005). Lipid composition and membrane fluidity analyses of influenza virus particles have demonstrated properties consistent with assembly taking place in lipid raft microdomains (Scheiffele et al., 1999).

Interferons (IFNs) are cytokines that are produced by cells in response to viral infections. These molecules bind broadly expressed receptors and induce the expression of IFN-regulated genes through the activation of JAK tyrosine kinases and STAT transcription factors (Platanias, 2005). Hundreds of genes are regulated by IFNs and their products mount a vigorous antiviral response. In most cases, the mechanism by which IFN-inducible genes restrict viral replication remains to be defined.

In the current issue of *Cell Host & Microbe*, Wang and colleagues document that the IFN-inducible protein viperin (for *v*irus *i*nhibitory *p*rotein, *e*ndoplasmic *r*eticulum-associated, *in*terferon inducible) impairs the release of influenza virus, thereby markedly disrupting its replication (Wang et al., 2007). Previous studies showed that viperin expression is strongly induced by both type I and type II IFNs and is also activated by a number of viral infections (Chin and Cresswell, 2001). The concept that viperin acts as an IFN-inducible antiviral factor is further supported by the observation that ectopic expression of viperin potently inhibits the replication of human cytomegalovirus (Chin and Cresswell, 2001).

A striking aspect of the Wang et al. study is that expression of viperin inhibits the release of influenza virus particles from the plasma membrane by disrupting lipid rafts. Evidence that viperin expression disperses lipid rafts includes the observation that the influenza virus HA, which is associated with raft microdomains and is therefore typically insoluble in Triton X-100 at 4°C (Schmitt and Lamb, 2005), is extracted in low concentrations of Triton X-100 in viperin-expressing cells. Furthermore, coclustering of HA and the raft-associated protein placental alkaline phosphatase with the raft-specific sphingolipid GM1 is diminished by viperin expression. Finally, viperin expression results in an increase in membrane fluidity and correspondingly elevated mobility of HA, consistent with disruption of the normally rigid lipid raft microenvironment. An important control for these experiments was provided by parallel analysis of the vesicular stomatitis virus (VSV), whose replication is generally thought to be raft independent and whose behavior was not affected by viperin expression.

What is the connection between viperin and lipid rafts? Through yeast two-hybrid screening, Wang et al. identified farnesyl diphosphate synthase (FPPS) as a viperin-interacting protein. FPPS is an enzyme that plays an important role in isoprenoid biosynthesis by catalyzing the formation of farnesyl diphosphate (FPP), a precursor for several classes of essential metabolites including sterols, dolichols, carotenoids, and ubiquinones

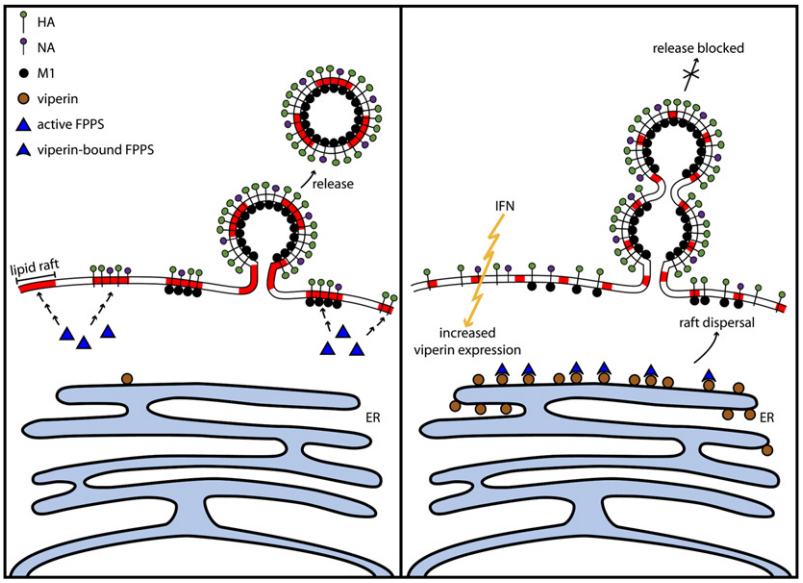

Figure 1. Influenza A Virus Release Is Inhibited Following Binding of FPPS by the IFN-Induced Protein Viperin

In cells not treated with IFN (left side), lipid rafts promote influenza budding. IFN treatment (right side) induces the expression of viperin, which binds to FPPS, subsequently leading to dispersal of lipid rafts in the plasma membrane and a block in virus budding and release.

(Szkopinska and Plochocka, 2005). FPP also serves as a substrate for protein farnesylation and geranylgeranylation. Wang et al. convincingly show, by coimmunoprecipitation analysis, that endogenous FPPS and viperin interact in vivo, perhaps leading to an inhibition of FPPS enzymatic activity. Conversely, overexpression of FPPS reverses the effects of viperin expression and restores influenza virus production. Likewise, loading up viperin-expressing cells with exogenous cholesterol decreases the mobility of HA in the plasma membrane. Finally, siRNA-mediated knockdown of FPPS reduces influenza virus production, further strengthening the link between FPPS disruption and impaired virus release.

A notable feature of viperin-expressing cells is the production of aberrant stalk-like or elongated structures in which multiple influenza particles are connected to each other by a thin membrane tether (Figure 1). Intriguingly, similar ''daisy-chain'' structures are also induced by mutations in HA and NA that prevent their association with lipid rafts (Jin et al., 1997). These morphological observations suggest that assembly of influenza A particles takes place more-or-less normally in viperin-expressing cells but that virus particle release is inhibited. This apparent release block contrasts with the findings obtained for HIV-1, for which raft disruption by cholesterol depletion inhibits the association of the major structural protein (Gag) with membrane and impairs the assembly process itself (Ono et al., 2007). In this regard, it will be of great interest to determine whether viperin expression disrupts particle production for other viruses, like HIV-1, that assemble in lipid rafts, and if so, at what step of the assembly/release process the block is imposed. Such studies are likely to provide new insights into the role of lipid rafts in enveloped virus replication.

It remains to be determined which of the many pathways FPPS regulates is responsible for its function in the IFN-mediated antiviral response. Wang and coworkers were not able to detect an effect of viperin expression on protein isoprenylation, nor did they observe any viperin-induced changes in cholesterol biosynthesis. Examination of FPPS mutants may help illuminate how this enzyme promotes influenza release.

A requirement for lipid rafts in enveloped virus replication is well established, and the antiviral activity of IFN-inducible genes has been the focus of much research. The significance of the Wang et al. study is that it connects these two areas of investigation by identifying FPPS, an enzyme involved in cholesterol biosynthesis, as a target of the IFN-inducible gene viperin. In doing so, the study opens up new possibilities for antiviral intervention focused on enzymes like FPPS that function in pathways leading to lipid raft formation. In this context, it is interesting to note that FPPS is the target of nitrogen-containing bisphosphonates, a class of drugs already in clinical development for the treatment of bone disorders and neoplasias.

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