BioMol Concepts, Vol. 3 (2012), pp. 255–266 • Copyright © by Walter de Gruyter • Berlin • Boston. DOI 10.1515/bmc-2011-0057

Review

Viperin: a radical response to viral infection

Kaitlin S. Duschene and Joan B. Broderick*

Department of Chemistry and Biochemistry, Montana State University, Bozeman, MT 59715, USA

*Corresponding author
e-mail: jbroderick@chemistry.montana.edu

Abstract

One of the first lines of defense of the host immune response to infection is upregulation of interferons, which play a vital role in triggering the early nonspecific antiviral state of the host. Interferons prompt the generation of numerous downstream products, known as interferon-stimulated genes (ISGs). One such ISG found to be either directly induced by type I, II, and III interferons or indirectly through viral infection is the 'virus inhibitory protein, endoplasmic reticulum-associated, interferon-inducible' protein, or viperin. Not only is viperin capable of combating a wide array of viral infections but its upregulation is also observed in the presence of endotoxins, various bacterial infections, or even in response to other immune stimuli, such as atherosclerotic lesions. Recent advances in the understanding of possible mechanisms of action of viperin involve, but are perhaps not limited to, interaction with farnesyl pyrophosphate synthase and disruption of lipid raft domains to prevent viral bud release, inhibition of hepatitis C virus secretory proteins, and coordination to lipid droplets and inhibition of viral replication. Unexpectedly, new insight into the human cytomegalovirus induction of this antiviral protein demonstrates that mitochondrial viperin plays a necessary and beneficial role for viral propagation.

Keywords: immune response; interferon; radical SAM enzyme; viperin; viral infection.

Introduction

Interferons (IFNs) are cell-signaling proteins produced by many cells in response to viral infection and are responsible for the regulation of hundreds of genes whose products are vital in the host viral response mechanism. One such protein product triggered by both type I (α/β) and II (γ) IFNs is viperin, also termed RSAD2 or Cig5 in humans (1, 2). In addition to regulation by IFNs, recent studies show that viperin has wide-ranging antiviral activity against dsRNA and DNA viruses, as well as microbial infection. Such viperin-inducible RNA and DNA viruses include the human cytomegalovirus (HCMV), vesicular stomatitis virus (VSV), pseudorabies virus (PrV), yellow fever virus, hepatitis C virus (HCV), Japanese encephalitis virus (JEV), Sindbis (SIN) virus, rhinovirus, and dengue virus (1, 3–8).

Regulation of viperin gene expression occurs through both IFN-dependent and -independent pathways (see Figure 1) (3, 9, 10). IFN-dependent induction as noted with the PrV, Sendai virus, and SIN virus, as well as lipopolysaccharides (LPSs), occurs through activation of Toll-like receptor 3, which is the receptor for virus-derived dsRNA, that then mediates the expression of necessary transcription factors, including IFN-regulatory factor-3 and -7 (IRF-3 and IRF-7), NF-κB, and c-Jun/ATF-2 (3, 6, 9–12). Procession along the type I IFN pathway triggers Jak-Stat pathway activation leading to IFN-stimulated gene factor-3 (ISGF3) complex formation, involving the interaction of phosphorylated STAT1, STAT2, and IRF-9. The ISGF3 complex then binds to IFN-stimulated response elements (ISREs) along IFN-stimulated gene (ISG) promoters resulting in viperin gene expression (13). IFN-independent mechanistic pathways of viperin induction have been observed for both the VSV and the JEV, which directly regulate expression through transcription factors IRF-1 (for VSV) or IRF-3 and AP-1 (for JEV) (3, 6).

Despite its central role in the host defense mechanism, little is known regarding the specific function of viperin and how its activity mediates the antiviral response. One potential function for viperin observed in influenza A virus-infected cells shows that the interaction of viperin with farnesyl pyrophosphate synthase (FPPS), an enzyme essential for isoprenoid biosynthesis and engaged in lipid raft coordination, led to disruption of the lipid raft microdomains and inhibition of viral bud release from the cell membrane (14). Moreover, viperin has been shown to prevent soluble protein secretion from the endoplasmic reticulum (ER) as well as cause distortion of the ER morphology, thereby possibly affecting transport of components critical to viral complex formation and replication (15). Recent insight into the structural and biochemical characteristics has classified viperin as a radical *S*-adenosyl-L-methionine (SAM) enzyme, as well as established the N-terminal α-helix as necessary and sufficient in localizing the protein to the cytosolic face of both the ER and lipid droplets while the C-terminal region associates with virally encoded proteins at the replication complex (RC) site resulting in abrogation of viral replication (15–18).

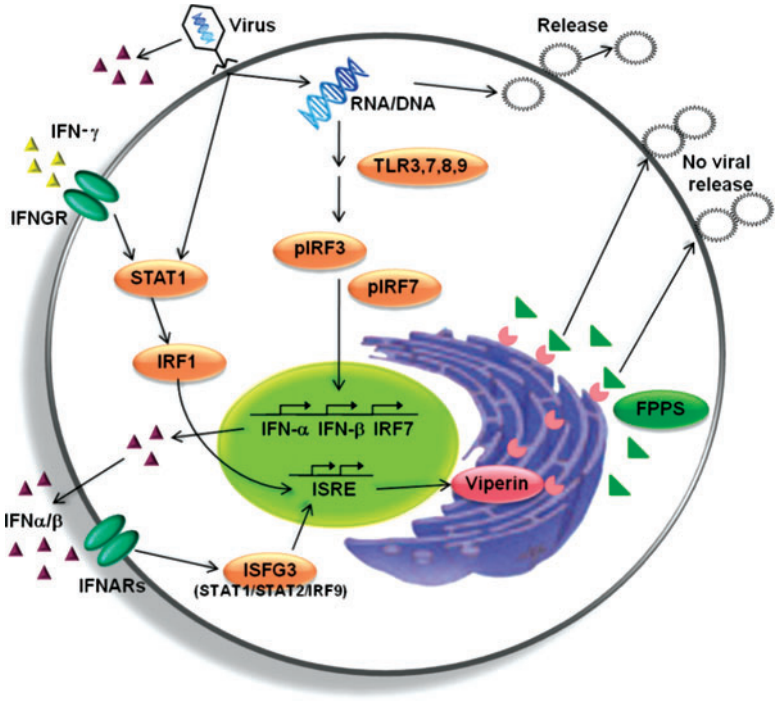

Figure 1 Schematic drawing of a host immune response to viral infection.

IFN-dependent vs. IFN-independent upregulation of viperin

Evidence for upregulation of a common gene was reported in a number of studies in which the identities of both the virus and the host cell varied, and both IFN-dependent and IFN-independent pathways were implicated. Using differential display analysis, Zhu et al. (1) found that HCMV, a betaherpesvirus, upregulated several IFN-stimulated mRNAs in human fibroblasts. Six of 26 differentially expressed mRNAs were transcribed from previously unreported partial cDNA sequences, including the *c*ytomegalovirus *i*nduced *g*ene number 5 or *cig5*. Soon after the discovery of the *cig5* gene, Boudinot and coworkers (19) observed a new fish gene that was upregulated in rainbow trout leukocytes by infection with a fish rhabdovirus, the viral hemorrhagic septicemia virus (VHSV). Like *cig5*, this gene, termed *vig1* for *V*HSV-*i*nduced *g*ene number 1, was capable of being directly (i.e., IFN-independently) induced by a virus as well as indirectly through an IFN-α-dependent pathway (1, 19).

In 2000, Grewal et al. (2) identified a rat cDNA, known as *best5*, whose mRNA was stimulated by both type I (α,β) and type II (γ) IFNs in osteoblasts during osteoblast differentiation and bone formation. The similarities found among *cig5*, *vig1*, and *best5* suggested a new family of IFN-stimulated genes in which all demonstrate a conserved response by the host to viral infections. Boudinot and coworkers (3) discovered a mouse homologue, *mvig*, that was directly induced by the rhabdovirus VSV, yet was indirectly modulated by secretion of type I IFNs by the alphaherpesvirus PrV in mouse dendritic cells. Boudinot and coworkers (3) also found that in the presence of LPS, a strong bacterial activator of dendritic cells, *mvig* was highly induced as early as 2 h post activation. Stimulation of *mvig* in response to LPS activation demonstrates that this class of ISGs responds to pathogenic bacterial infections as well as viral infection.

Chin and Cresswell (20) came upon the identification of an IFN-γ-responsive gene while studying the IFN pathway of human macrophages through representational differential analysis. Sequence alignments of this full-length gene matched it with that of two gene fragments, named *cig5* and *cig33*, that had been previously identified by Zhu et al. (1). *cig5* corresponded to the 5′ region and *cig33* to the 3′ region of the full-length gene, hereby termed *cig5*. While IFN-γ induction of *cig5* was primarily observed in human macrophages, regulation by both IFN-α and -β was widespread among a wide array of cell types (20). Supplementary analysis indicated that although HCMV directly stimulates Cig5 expression, other mechanisms may exist to counteract the host immune response; recent findings shed light onto this subject and will be discussed later in this review (20–23).

HCMV-infected fibroblast cells were studied to determine whether Cig5 was capable of inhibiting viral production, namely by tracking expression of structural proteins, including IE1, IE2, pp65, gB, and pp28, necessary for viral assembly and maturation (24–30). Although synthesis of the immediate-early proteins IE1 and IE2 remained steady, significant reduction of the early-late (pp65), late (gB), and true late (pp28) gene products was observed (20). As these structural proteins are essential for HCMV replication, it appeared that Cig5 was functioning as an antiviral agent by inhibiting the proper synthesis and/or action of a necessary component in the late

stages of viral propagation. In turn, Chin and Cresswell (20) named the Cig5 protein viperin (for *v*irus inhibitory protein, endoplasmic reticulum-associated, interferon-inducible). The 'ER-associated' portion of the name will be further discussed later in this review.

In vivo and *in vitro* experiments investigating viperin expression in response to HCV infection found *cig5* to be a type I ISG, on the basis of the heightened levels of viperin mRNA and protein expression in Huh-7 cells treated with IFN-α, which led to a decline in HCV RNA levels by ~50% (5). These results were further substantiated by studies of the human immunodeficiency virus type 1 (HIV-1) replication in human astrocytes, cells necessary for maintaining the homeostatic environment of the central nervous system. These studies demonstrated that the dsRNA analog polyriboinosinic polyribocytidylic acid (pIC), known to act as an immunostimulant by simulating viral infections, activated the antiviral response mechanism including increased expression of viperin (9, 31–33). Suppression of viperin expression in the presence of pIC was achieved through preincubation with neutralizing antibodies to IFN-β, which again suggests that *cig5* is most likely a type I ISG dependent on IFN mediation.

Probing the promoter binding domain of viperin

Additional studies probing the direct vs. indirect molecular mechanisms of the host defense response focused on virus-induced viperin expression in wild-type macrophages and cells deficient in either IRF-3 or the type I IFN heterodimeric receptor complex (IFNα/βR) (10). The absence of the IRF-3 or IFNα/βR in macrophages respectively impaired or completely abolished viperin production even in the presence of either LPS or pIC, signifying a dependence on type I IFN activation. Moreover, IRF-3 knockout macrophages induced viperin in response to Sendai virus infection, whereas the IFNα/βR knockout cells were incapable of viperin induction, thereby demonstrating that viperin regulation proceeds through an IRF-3-independent yet IFN-dependent pathway. Use of a transcription factor-binding site prediction program to map the viperin promoter identified IRF binding sites (IRF1-3) and ISREs, capable of binding either IRFs or the ISGF3 (Figure 2) (13, 34). Studies of this promoter region using DNA binding assays identified ISGF3 as the key factor in modulating viperin expression through binding to the ISREs, whereas binding of IRF-3 led to minimal promoter activity. Contrary to the previous findings, Grandvaux et al. (34) found that cells constitutively expressing active IRF-3 acted in an IFN-independent manner through binding of ISREs. Furthermore, Severa et al. (10) found that the positive regulatory domain I-binding factor 1 (PRDI-BF1 or BLIMP1) competitively inhibits both virus-induced and/or IFN-β-induced viperin expression by binding to the IRF-2 and IRF-3/ISRE sites of the viperin promoter region, thereby blocking binding of ISGF3. Perhaps the negative feedback of PRDI-BF1 serves to keep IFN activity in check, whereas uncontrolled it could lead to symptoms akin to an autoimmune disorder (35).

Elucidating the immune response pathway for viperin regulation led researchers to study the differential effects of the JEV and the SIN virus on viperin expression (6). *cig5* RNA levels increased in both JEV- and SIN-infected cells. Through reliance on IFN activation, SIN triggered viperin expression by way of ISGF3 complex binding to the ISRE of the viperin promoter region. Conversely, JEV works independently of the IFN pathway through activation of the cellular transcription factors AP-1 and IRF-3, both shown to initiate expression by binding to and stimulation of the ISRE, further substantiating earlier work by Chan et al. (6) and Grandvaux et al. (34). Additionally, JEV possesses a viral evasion mechanism in response to viperin activity by downregulating protein expression through ubiquitination and proteosomal degradation thereby making it difficult for infected cells to clear the virus (6).

Another transcription factor, IRF-1, stimulated in response to both VSV infection and IFN-γ induction, regulated viperin expression proceeding through both IFN-dependent and -independent manners (see Figure 1) (10, 36, 37). The IFN-dependent pathway proceeds through IFN-β upregulation due to viral infection, followed by the induction of ISGs through activation of the ISGF3 complex. Along this pathway, ISGs induce IRF-1, leading to the eventual expression of viperin. The IFN-independent pathway may be initiated

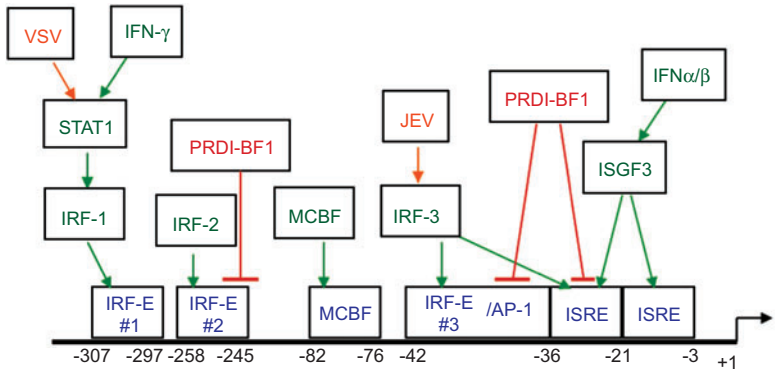

Figure 2 Viperin promoter binding region. Human viperin promoter region and motifs displayed as the number of nucleotides relative to the transcription initiation site (GenBank accession number NM_080657).
The first nucleotide of viperin mRNA is positioned as +1. Green arrows indicate activation; red dashes indicate inhibition.

through viral infection and/or IFN-γ production, either of which are capable of activating STAT1, resulting in IRF-1 stimulation then viperin upregulation (36, 38, 39). VSV effectively shuts down the immune response mechanism occurring through type I IFN signaling by blocking IFN-β production. As a result, the IFN-independent pathway functions to circumvent the ability of the virus to cut off the dependent pathway.

Interesting results by Saitoh et al. (40) add a new layer of complexity to the cellular antiviral response; not only do IFNs play an integral role in viperin expression but their results also show that viperin is actually capable of governing IFN levels through a positive feedback loop. In plasmacytoid dendritic cells (pDCs), viperin interaction with IRF7 as well as various signaling proteins, including IRAK1 and TRAF6, occurs on the surface of lipid droplets. This complex formation leads to IRF7 activation, which is then delivered to the nucleus for induction of type I IFNs. Viperin-deficient pDCs were incapable of IFN induction as were pDCs with altered lipid bodies, therefore suggesting that viperin along with its localization to lipid droplets are critical for IFN regulation (40).

Is viperin control exclusively linked to viral infection and IFN induction?

Not only is viperin activity widespread among viruses but studies have also demonstrated its presence in a number of other biological arenas, as noted earlier by the stimulation of *mvig* in response to bacterial activators, LPSs (3). Research by Olofsson et al. (41) aimed at identifying causes for the onset of vascular inflammation leading to the development of atherosclerosis. Such factors influencing vascular inflammation appear to include oxidized lipoproteins, bacteria, and viruses, including CMV (42–45). While investigating the vascular inflammatory process, gene array analysis found that *cig5* was highly induced by bacterial LPSs. Further analysis determined that LPSs, CMV, and IFN-γ all successfully induced viperin in human endothelial and smooth muscle cells and also by LPS in normal human arteries. Upregulation of viperin may be necessary for a localized response against the CMV pathogen. Viperin mRNA and protein levels were found to be elevated in human arteries with atherosclerotic lesions but not in healthy arteries, thereby possibly linking the antiviral response to CMV with the immune response due to atherosclerotic lesions.

Further investigation into the immune response of viperin regulation due to bacterial infection used the fish viperin gene, *SoVip*, from red drum, *Sciaenops ocellatus* (46). Interestingly, *in vivo* challenges of *S. ocellatus* with pIC and a fish pathogen, *Edwardsiella tarda*, enhanced mRNA levels of *SoVip* in the liver, whereas the fish pathogens *Listonella anguillarum* and *Streptococcus iniae* led to down-regulation. Similar regulation patterns were observed *in vitro* in primary hepatocytes. While it is still unknown why various pathogens differentially regulate viperin in *S. ocellatus*, perhaps there is a correlation between viperin control and the mechanism by which pathogens can bind to and infect a cell. Continued studies are necessary to explain regulation at the transcriptional level; however, it appears that viperin is not only valued for its antiviral activity but also for its antibacterial activity.

Viperin has also been implicated in pregnancy. Microarray analysis identified *cig5* as a gene induced by the type I IFN, IFN-τ, during early pregnancy from the conceptus in ovine and bovine endometria (47). The role of viperin in this case may be to assist in establishing an antiviral state in the uterus during pregnancy as the conceptus would not have a fully developed immune system to ward off potential infection (47).

Results from studies investigating viperin expression due to acute and chronic lymphocytic choriomeningitis virus infection identified viperin as an effective indicator of an IFN response in infected cells, such as lymphocytes, neutrophils, macrophages, and dendritic cells (48). Using plaque assays, flow cytometry, and cell sorting techniques, Hinson et al. concluded that the kinetics response of viperin in certain types of infected leukocytes mimicked the IFN-α response levels, making it possible to use viperin expression to indicate a type I IFN response due to acute or chronic viral infection. In addition, immunoelectron microscopy results demonstrated viperin localized to lipid-droplet-like organelles in neutrophils, which are principally involved in immune responses to bacterial and parasitic infections, and could therefore act to protect neutrophils as well as other cell types by suppressing bacterial replication from these lipid droplets (48–50).

Structural and biochemical characterization of viperin

Viperin appears to be composed of three domains: the N-terminal domain (Cig5, amino acids ~1–76), a radical SAM domain (Cig5, amino acids ~77–209), and a C-terminal domain (Cig5, amino acids ~210–361) (51, 52). The amino acid sequence of human viperin (hViperin or Cig5) is highly conserved among mammals and lower vertebrates with the exception of the N-terminal region. Sequence alignments of full-length human viperin show 83% identity with *Mus musculus* (Vig1, mouse), 82% with *Rattus norvegicus* (Best5, rat), 83% with *Bos taurus* (bovine), 97% with *Pongo abelii* (Sumatran orangutan), 83% with *Sus scrofa* (CIG6/IRG6, pig), and 79% with *Oncorhynchus mykiss* (Vig1, rainbow trout) (Figure 3). All sequences with the exception of trout (and other lower vertebrates not shown in this figure) contain a leucine zipper motif in the N-terminal region, which is generally found to be involved in protein-protein/DNA interactions. When the N-terminal 1–70 amino acids were excluded from sequence alignments, the homologies between viperin and the other species increase to 92%, 91%, 92%, 97%, 91%, and 79% for mouse (72–362), rat (70–360), bovine (73–363), orangutan (71–361), pig (72–362), and trout (58–348), respectively.

Boudinot et al. (19) and Frey et al. (53) originally suggested viperin to be a radical SAM enzyme on the basis of the presence of four conserved motifs identified in Vig1 and Cig5 that are known to be common among many other members of the radical SAM family of enzymes (Figure 3).

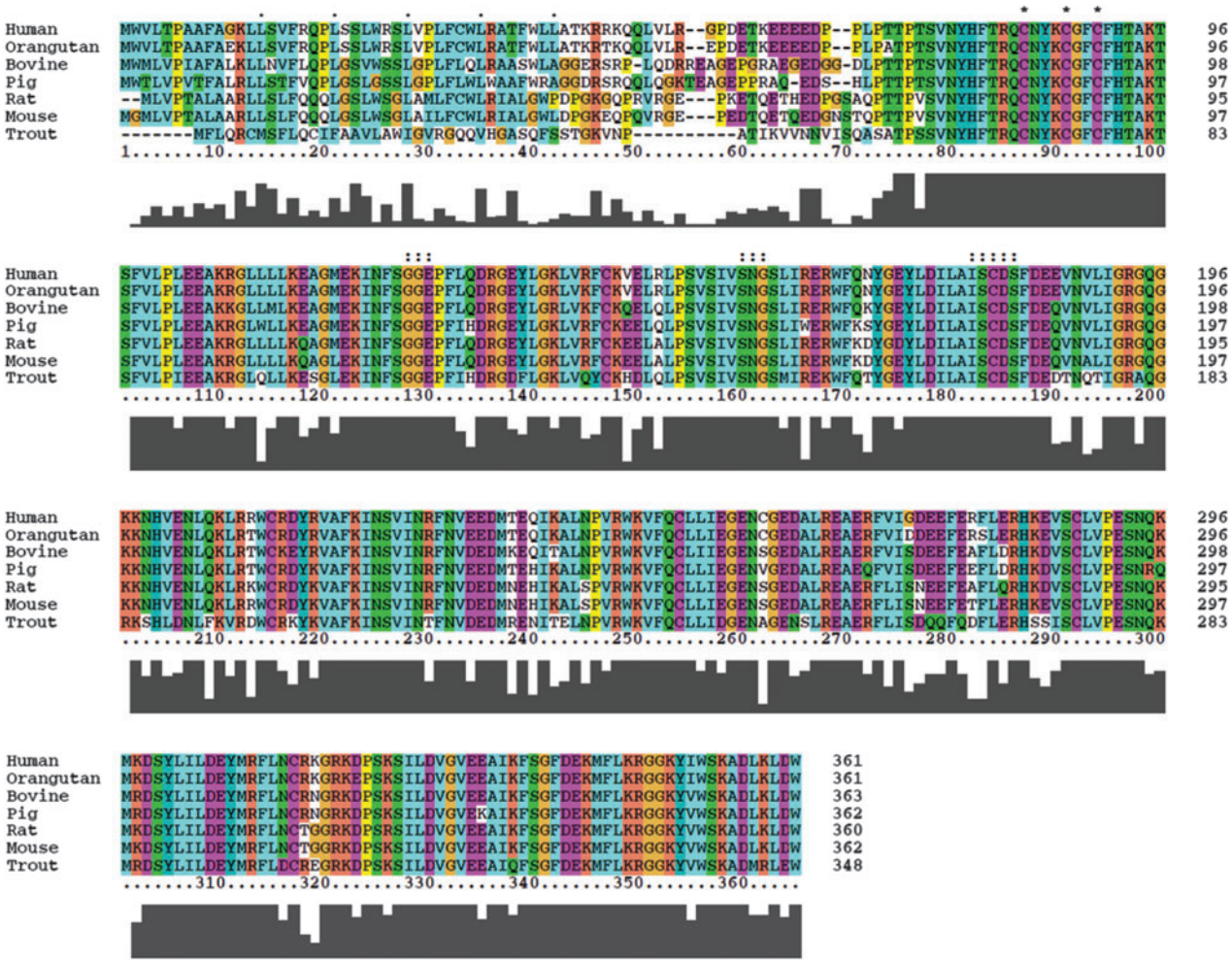

Figure 3 Sequence alignments of the amino acids of viperin from seven animal species, performed with ClustalX2.
The UniProtKB accession numbers are as follows: *Homo sapiens* Q8WXG1, *Pongo abelii* Q5RCW8, *Bos taurus* Q2HJF9, *Sus scrofa* Q9MZU4, *Rattus norvegicus* O70600, *Mus musculus* Q8CBB9, and *Oncorhynchus mykiss* O93384. The putative leucine zipper domain is denoted with periods (.); the CX3CX2C motif is denoted with asterisks (*); and the conserved GGE, SNG, and ISCDS radical SAM motifs are denoted with colons (:).

The CX_3CX_2C motif is important for binding three irons of a [4Fe-4S] cluster, while the GGE, SNG, and ISCDS motifs all appear to function in the binding of SAM, a molecule that coordinates to the unique iron of the Fe-S cluster and serves either as a substrate or cofactor (Figures 3 and 4) (53–55). In the characterized radical SAM enzymes, the [4Fe-4S] cluster is necessary for the reductive cleavage of SAM to generate methionine and a 5′ deoxyadenosyl radical (5′-dAdo·) that is then able to perform a variety of radical-mediated enzymatic reactions, such as sulfur insertions, hydrogen abstractions, rearrangements, methylation reactions, DNA repair, and cofactor biosynthesis (53, 56, 57). Homology models were generated using the Phyre server program to compare the sequence of hViperin with other proteins with known crystal structures (58). Results from the homology search provided several representative three-dimensional structures for hViperin in which the most complete structures were observed in comparison with MoaA, HydE, and PFL-AE, all of which are established radical SAM enzymes (Figure 5). From these comparative structures, it appears that viperin possesses a partial $(\beta/\alpha)_6$ triosephosphateisomerase (TIM) barrel, a characteristic feature of all radical SAM enzymes that plays a vital role in sealing off the active site for radical chemistry (59). The homology models also display a disordered region at the N-terminus, which in comparison with other radical SAMs, such as BioB and HydE, may serve as a flexible ‘lid loop’ that can undergo conformational changes upon substrate binding to further protect the active sites during catalysis (59).

Structural characterization was carried out on full-length viperin and various dissected fragments of the protein using nuclear magnetic resonance and circular dichroism (CD) spectroscopy. Fragments 71–361, 81–361, and 214–361 were expressible but only found in inclusion bodies. Fragments 43–361 and 45–361 were also expressible; however, unfortunately, fragment 43–361 was only partially soluble, whereas greater solubility was achieved for fragment 45–361 (60).

Figure 4 Site-differentiated CX_3CX_2C cluster.
(A) Conserved CX_3CX_2C motifs derived from viperin (*Homo sapiens*), cofactor for molybdopterin biosynthesis MoaA (*Escherichia coli*), cofactor for FeMo biosynthesis NifB (*Rhizobium leguminosarum*), pyruvate formate lyase activating enzyme PFLAE (*E. coli*), lysine 2,3-aminomutase LAM (*Bacillus subtilis*), and (B) iron-sulfur cluster bound to CX_3CX_2C motif in PFL-AE (3CB8).

Reconstitution of viperin$_{45–361}$ enhanced both the thermal stability, from 30°C to 45°C before observed precipitation, as well as the order of the secondary structure as compared with the as-isolated fragment. Analysis of the C-terminal region, residues 214–361, using far-UV CD indicated the presence of ~24% α-helix, ~16% β-strand/turn, and ~60% random coil. Also of interest, viperin$_{214–361}$ experienced high levels of precipitation in salt-containing buffers (even as low as 12 mM NaCl), yet completely solubilized in salt-free water. If viperin, like other radical SAM proteins, adopts a partial or full TIM barrel structure, then deletion of the N-terminal 213 amino acids may lead to the insolubility of viperin$_{214–361}$ due to loss of necessary interactions for proper tertiary packing and exposure of the hydrophobic side chains to surrounding solvent (54, 60). Loss of the N-terminal 1–44 amino acids led to increased solubility and reconstitution of viperin$_{45–361}$ improved the stability of the protein, whereas analysis of viperin$_{214–361}$, which is composed of mainly unordered secondary structures, was highly precipitous in solvent and presumably requires the presence of the TIM barrel structure for its stability.

Suggestions that viperin is a radical SAM enzyme were ultimately confirmed by Duschene and Broderick (16) after anaerobic reconstitution of the iron-sulfur cluster provided spectroscopic data consistent with other known radical SAM proteins. Reconstitution of the N-terminally truncated protein (viperin$_{43–361}$) with iron and sulfide resulted in protein with a dark brown color and an enhanced amount of iron (3.7±0.1 mol Fe/mol viperin). UV-Vis analysis of the reconstituted protein displayed a sharp feature at ~315 nm and broader feature between ~370–450 nm, both of which are consistent with proteins known to contain $[4Fe\text{-}4S]^{2+}$ clusters (16, 61–63). Further spectroscopic characterization using electron paramagnetic resonance (EPR) provided evidence that viperin$_{43–361}$ does indeed bind an iron-sulfur cluster; reconstituted viperin$_{43–361}$ resulted in a nearly isotropic signal produced by a $[3Fe\text{-}4S]^{1+}$ cluster, while photoreduction of the protein resulted in loss of the $[3Fe\text{-}4S]^{1+}$ signal and emergence of a nearly axial signal attributable to $[4Fe\text{-}4S]^{1+}$ clusters (Figure 6C). Addition of SAM to the photoreduced protein led to alteration of the EPR signal, which has been noted with other radical SAM proteins signifying SAM coordination to the $[4Fe\text{-}4S]^{1+}$ cluster (64, 65).

The demonstrated ability of this reconstituted viperin to reductively cleave SAM to methionine and 5′-dAdo provided additional confirmation that viperin is a radical SAM enzyme (Figure 6A,B). The reductive cleavage of SAM is a characteristic reaction of radical SAM enzymes, whereby the reduced $[4Fe\text{-}4S]^{1+}$ cluster on the enzyme injects an electron into SAM, thus promoting cleavage to produce methionine and a 5′-dAdo radical. When substrate is present, the 5′-dAdo radical abstracts a hydrogen atom from the substrate, which then undergoes further transformation (53, 54). In some instances, these enzymes have been shown to carry out uncoupled SAM cleavage, such that in the absence of substrate the enzyme still cleaves SAM (59). As the substrate of viperin remains unknown, high-performance liquid chromatography (HPLC) analysis was used to test for the presence of 5′-dAdo that may result from uncoupled cleavage of SAM by photoreduced viperin. In the absence of viperin, SAM remained the major observable peak; however, when reduced viperin was added to the reaction mixture, two new peaks appeared where the major peak corresponded to 5′-dAdo and the minor peak corresponded to *S*-(5′-deoxyadenosyl)-L-homocysteine (SAH), a degradation product of SAM in the reaction, as confirmed by electrospray-ionization mass spectroscopy.

Potential modes of action

Although for many years now, researchers have shown that viperin is capable of hindering the viral replication process either through viral-mediated direct or indirect pathways, it has only been in the past few years that researchers are beginning to understand the mechanisms through which viperin acts as an antagonist to viral propagation. In 2007, Wang and colleagues (14) showed that viperin inhibited the influenza A virus by impairing the release of viral buds from the plasma membrane (Figure 7). This group used thin-section electron

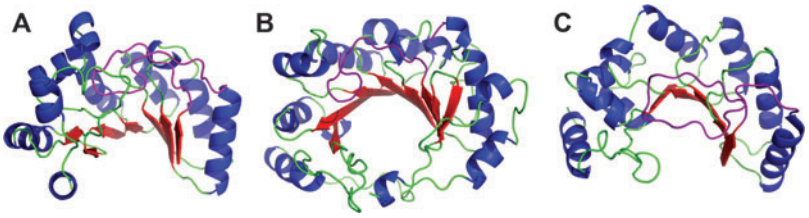

Figure 5 Homology models of hViperin as compared with three other radical SAM enzymes of highest homology.
(A) HydE; (B) MoaA; (C) PFL-AE. Blue, helix; red, β-sheet; green, random coil; purple, disordered region, potential 'lid loop'.

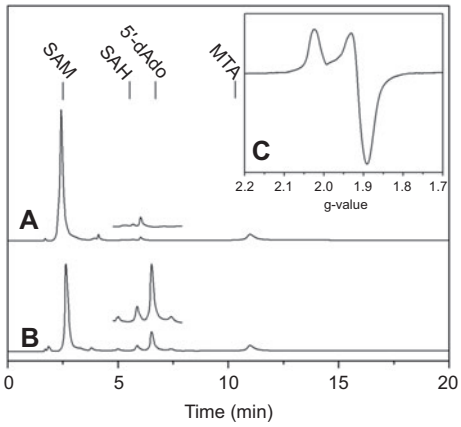

Figure 6 HPLC analysis of SAM cleavage assay (16).
(A) Control assay without viperin. The elution times of standard solutions are as follows: *S*-adenosylmethionine (SAM) at 2.5 min, *S*-(5′-adenosyl)-L-homocysteine (SAH) at 5.5 min, 5′-deoxyadenosine (5′-dAdo) at 6.7 min, and methylthioadenosine (MTA) at 10.3 min. (B) Assay containing viperin. Inset: expanded region between 5 and 8 min highlighting the appearance of 5′-dAdo. (C) EPR spectrum at 12K of reduced viperin showing the presence of a $[4Fe\text{-}4S]^{+}$.

microscopy to observe that upon induction of viperin through doxycycline treatment, the viral buds on the plasma membrane of cells possessed an abnormally large amount of distinct stalk-like or 'daisy-chain' structures vs. the untreated cells with ordinary buds that appeared ready to be 'pinched off' for viral release, suggesting that viperin does not affect initial bud formation but late-stage virion release. The influenza virus budding stage is triggered by the accumulation of lipid rafts, which are proposed to be involved in the intake and outtake of viral particles (66, 67). It was determined that viperin disrupts lipid raft microdomains, increasing the lateral mobility of associated transmembrane glycoproteins (14, 68). In contrast to influenza A, VSV is generally considered to bud from the plasma membrane through a raft-independent mechanism. Although VSV infection can lead to viperin upregulation (3), Wang et al. found that viperin was incapable of inhibiting the viral replication of VSV, further supporting the notion that viperin inhibition of viral replication proceeds through disruption of lipid rafts. Other viruses known to propagate using lipid rafts include the Sendai virus, Ebola, and HIV-1 (69); it remains unclear at this time as to whether viperin inhibition of viral replication occurs through a similar mechanism as that observed with influenza A.

Another important observation by Wang et al. was that viperin decreases the activity of FPPS, an enzyme involved in the isoprenoid biosynthesis pathway and known to catalyze the condensation reaction of dimethylallyl pyrophosphate with two molecules of isopentenyl diphosphate to generate farnesyl pyrophosphate (14, 70). Further analysis suggested that blocking FPPS activity led to greater lateral mobility of membrane-associated glycoproteins and, in turn, increased membrane fluidity. However, these results were not attributable to an

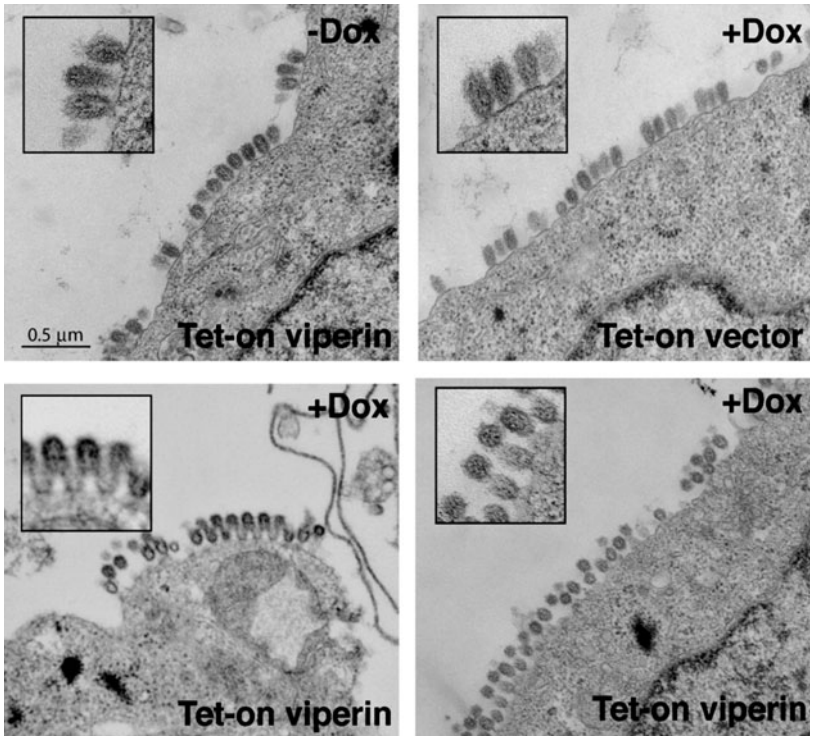

Figure 7 Viperin expression in response to 14 h of influenza A infection inhibits release of viral buds from cell membrane (14).
Top left: untreated viperin-inducible cells showing formation of viral buds; top right: pretreated doxycycline control cells with viral buds forming on cell membrane; bottom left: pretreated doxycycline viperin-inducible cells with stalk-like viral bud structures; bottom right: pretreated doxycycline viperin-inducible cells with daisy chain-like viral bud structures.

overall decrease in protein isoprenylation, and coexpression of FPPS sufficiently reversed the viperin-induced inhibition of viral propagation (14). These findings represent a significant step in determining a mode of action for viperin by identifying a potential target of the host immune response, FPPS.

While it has been mentioned that viperin is an HCMV-inducible protein, this brings into question why a virus would directly generate a protein, such as viperin, which is known to generally inhibit the infectious process. From their research, Seo and coworkers (71) provide a pathway of action in which viperin actually serves an advantageous role for HCMV replication rather than an inhibitory one. While observing intracellular distribution of viperin post HCMV infection, viperin was found to colocalize with the viral mitochondrial inhibitor of apoptosis (vMIA), an HCMV-encoded protein active in the mitochondria (72–75). Proteomics analysis along with immunoprecipitation demonstrated that once transported to the mitochondria, viperin interacts with the β-subunit of the mitochondrial trifunctional protein (HADHB), an enzyme vital to the generation of cellular ATP that catalyzes the final three steps of fatty acid β-oxidation (76, 77). Interaction of HADHB with mitochondrial viperin resulted in inhibition of mitochondrial trifunctional protein activity as measured through reduced fatty acid β-oxidation production, lowered cellular ATP levels, and reduced NADH/NAD^+ ratios (71). These effects, while determined to be dependent on the presence of vMIA and the iron-sulfur cluster in viperin, ultimately resulted in disruption of the actin cytoskeleton, which facilitates HCMV infection. Seemingly, HCMV uses vMIA to target viperin to the mitochondria where viperin can then exert dramatic effects on the cellular bioenergetics, leading to ATP levels sufficiently low enough to induce cytoskeletal disorder. In the presence of vMIA to inhibit apoptosis, HCMV can effectively circumvent the host's immune response of programmed cell death, therefore enabling viral replication (71).

Immunofluorescence studies of IFN-α-treated fibroblasts showed that viperin colocalizes with the ER-bound protein, calnexin (20). Researchers initially theorized that viperin associates with the ER by means of the N-terminal leucine zipper motif (19, 20). This idea was further substantiated in 2009 by Hinson and Cresswell (15) who demonstrated that the amphipathic N-terminal α-helical domain of viperin is responsible for cytosolic ER localization. Additional studies showed viperin-expressing cells with an altered morphology where the smooth ER took on a crystalloid ('lattice-like pattern of hexagonally packed tubules'; ref. 15) structure, which has been proposed to result from dimerization of ER membrane-bound proteins and further propagated through curvature of the ER membrane due to interaction with the amphipathic N-terminal region (see ref. 16, Figure 2A) (15, 78–85). Results from immunoprecipitation experiments with two differentially tagged viperins showed that viperin is capable of dimerization or multimerization regardless of whether the N-terminal α-helical region is present in the protein. Additional domain characterization of viperin found that the N-terminal α-helical region alone was capable of inhibiting soluble protein secretion from the ER as well as transportation of proteins from the ER to Golgi apparatus, thereby representing a potential mode of action leading to the ultimate inhibition of HCV replication by disrupting the viral RC (15). The necessity of the N- or C-terminal regions for the antiviral activity of viperin is still a matter of debate according to several reports. Early mutational studies by Wang and coworkers (14) observed that the absence of the N-terminus severely hindered anti-HCV activity, a result that was reproduced by Helbig et al. (17) for both the N- and C-terminal regions. However, another study found the N-terminus unnecessary for antiviral activity (18). Furthermore, contrasting results among mutational studies of the conserved radical SAM motifs dispute the necessity of these motifs as possessing any significant impact on HCV replication (14, 17). These conflicting results are possibly attributable to various cell lines and/or levels of expression used in each study (18).

As stated earlier, not all viruses bud from lipid rafts; therefore, the question remains how viperin inhibits replication in these cases. In the case of HCV, viral replication occurs in the cytosol and uses an ER-localized RC composed of cellular membranes, nonstructural and cellular proteins, and replicating RNA. After maturation of the HCV core, the protein along with the nonstructural proteins and the RC localize to nearby lipid droplets, intracellular sites useful for lipid storage and transfer as well as protein storage and degradation (86–89). Certain nonstructural proteins, such as NS5A, possess N-terminal amphipathic α-helical regions that, like viperin, target the membranes of the ER and lipid droplets (17, 88–92). The C-terminal region of viperin associated with the nonstructural protein NS5A at the lipid droplet interface as well as with VAP-A (human vesicle-associated membrane protein-associate protein subtype A) and NS5A at the RC site near the ER membrane (17). VAP-A is a proviral agent required for genomic RNA replication, which associates with NS5A at the RC (93). Viperin expression not only downregulated NS5A levels but also interfered with RNA replication perhaps through interaction with VAP-A (17, 18). A potential mechanism of action could be through C-terminal domain interactions between viperin and NS5A and/or VAP-A. While complexation of the C-terminal domains of VAP-A and NS5A anchors the nonstructural protein to the ER membrane, competitive binding oviperin with VAP-A could disrupt formation of the RC. Preventing vital interactions between VAP-A and NS5A could sufficiently destabilize NS5A interactions with cellular membranes, thereby inhibiting viral RNA replication (17, 18). In summary, these studies provide functionality for the N- and C-terminal domains in which the N-terminus is vital for protein folding, membrane association, cellular localization, and protein secretion, whereas the C-terminus appears to be involved in the recognition and/or binding of substrates and other cofactors, resulting in the eventual inhibition of viral replication (54, 59, 94).

Expert opinion

Viperin appears to be a central and highly conserved protein in the host cell response to viral infection. That this conserved protein uses radical SAM chemistry to carry out its function is

intriguing and provides a basis for speculation on the specific function of viperin. As a member of the radical SAM superfamily, viperin is expected to carry out the characteristic type of chemical reaction observed in the superfamily. Although the substrates, and thus the ultimate chemical transformation catalyzed, vary widely among superfamily members, there are unifying mechanistic features observed for these enzymes that provide clues to the function of viperin. Central to these unifying features is the production of a 5′-deoxyadenosyl radical by the reductive cleavage of SAM; in all characterized radical SAM enzymes, this radical intermediate abstracts a hydrogen atom from substrate to initiate subsequent reactions. Such subsequent reactions can include sulfur insertion, dehydration, rearrangement, thiomethylation, carbon-carbon bond cleavage, and potentially other reactions that can be initiated by H atom abstraction. It therefore follows that the antiviral activity of viperin is mediated by a hydrogen atom abstraction; however, the major question as to the identity of the substrate of viperin remains. Cresswell and coworkers have provided numerous experimental results that may provide clues to this central question. The ability of viperin to alter ER membrane curvature not only inhibits secretion of soluble HCV-encoded proteins but could also potentially affect formation of or interactions with lipid droplets from the ER, thereby inhibiting HCV replication (15, 92). Additionally, competitive inhibition for binding of nonstructural protein NS5A to VAP-A provides functionality for the C-terminus of viperin as well as a mode of action for HCV inhibition (17, 18). Or, perhaps, viperin is modifying lipids residing within lipid droplets (i.e., through interaction with FPPS, an enzyme involved in cholesterol biosynthesis, or various isoprenoid precursors) that may be essential for HCV replication (15, 92). Viperin interaction with FPPS, as has been observed for influenza virus, leads to perturbations in lipid raft domains, thus ultimately affecting viral replication, budding, and release. Conceivably, viperin carries out radical SAM chemistry either on another protein, such as FPPS, or perhaps on a cholesterol precursor, thereby altering the consistency and functionality of lipids, lipid droplets, and/or lipid raft domains (14, 95, 96).

It is also of interest to note that the mechanism of viperin expression appears to vary with the type of viral infection, with expression occurring in some cases through IFN-dependent pathways and other times through IFN-independent pathways. This variation allows viperin a multitude of pathways to circumvent the innate ability of a single virus to overcome an antiviral response of the host immune system.

Acknowledgments

Research on viperin and radical SAM enzymes in the Broderick laboratory is supported by the National Institutes of Health (GM54608 to JBB). The authors are grateful to Dr. Peter Cresswell for generously allowing us to borrow previously published work.

Conflict of interest statement

K.D. and J.B. have no conflicts of interest to disclose.

References

1. Zhu H, Cong JP, Shenk T. Use of differential display analysis to assess the effect of human cytomegalovirus infection on the accumulation of cellular RNAs: induction of interferon-responsive RNAs. Proc Natl Acad Sci USA 1997; 94: 13985–90.
2. Grewal TS, Genever PG, Brabbs AC, Birch M, Skerry TM. Best5: a novel interferon-inducible gene expressed during bone formation. FASEB J 2000; 14: 523–31.
3. Boudinot P, Riffault S, Salhi S, Carrat C, Sedlik C, Mahmoudi N, Charley B, Benmansour A. Vesicular stomatitis virus and pseudorabies virus induce a vig1/cig5 homologue in mouse dendritic cells via different pathways. J Gen Virol 2000; 81: 2675–82.
4. Khaiboullina SF, Rizvanov AA, Holbrook MR, St Jeor S. Yellow fever virus strains Asibi and 17D-204 infect human umbilical cord endothelial cells and induce novel changes in gene expression. Virology 2005; 342: 167–76.
5. Helbig KJ, Lau DT, Semendric L, Harley HA, Beard MR. Analysis of ISG expression in chronic hepatitis C identifies viperin as a potential antiviral effector. Hepatology 2005; 42: 702–10.
6. Chan YL, Chang TH, Liao CL, Lin YL. The cellular antiviral protein viperin is attenuated by proteasome-mediated protein degradation in Japanese encephalitis virus-infected cells. J Virol 2008; 82: 10455–64.
7. Proud D, Turner RB, Winther B, Wiehler S, Tiesman JP, Reichling TD, Juhlin KD, Fulmer AW, Ho BY, Walanski AA, Poore CL, Mizoguchi H, Jump L, Moore ML, Zukowski CK, Clymer JW. Gene expression profiles during in vivo human rhinovirus infection insights into the host response. Am J Respir Crit Care Med 2008; 178: 962–8.
8. Fink J, Gu F, Ling L, Tolfvenstam T, Olfat F, Chin KC, Aw P, George J, Kuznetsov VA, Schreiber M, Vasudevan SG, Hibberd ML. Host gene expression profiling of dengue virus infection in cell lines and patients. PLoS Neglect Trop Dis 2007; 1: e86.
9. Rivieccio MA, Suh HS, Zhao Y, Zhao ML, Chin KC, Lee SC, Brosnan CF. TLR3 ligation activates an antiviral response in human fetal astrocytes: a role for viperin/cig5. J Immunol 2006; 177: 4735–41.
10. Severa M, Coccia EM, Fitzgerald KA. Toll-like receptor-dependent and -independent viperin gene expression and counter-regulation by PRDI-binding factor-1/BLIMP1. J Biol Chem 2006; 281: 26188–95.
11. Suh HS, Zhao ML, Rivieccio M, Choi S, Connolly E, Zhao Y, Takikawa O, Brosnan CF, Lee SC. Astrocyte indoleamine 2,3-dioxygenase is induced by the TLR3 ligand poly(I:C): mechanism of induction and role in antiviral response. J Virol 2007; 81: 9838–50.
12. Borden EC, Sen GC, Uze G, Silverman RH, Ransohoff RM, Foster GR, Stark GR. Interferons at age 50: past, current and future impact on biomedicine. Nat Rev Drug Discov 2007; 6: 975–90.
13. Nakaya T, Sato M, Hata N, Asagiri M, Suemori H, Noguchi S, Tanaka N, Taniguchi T. Gene induction pathways mediated by distinct IRFs during viral infection. Biochem Biophys Res Commun 2001; 283: 1150–6.
14. Wang X, Hinson ER, Cresswell P. The interferon-inducible protein viperin inhibits influenza virus release by perturbing lipid rafts. Cell Host Microbe 2007; 2: 96–105.
15. Hinson ER, Cresswell P. The N-terminal amphipathic α-helix of viperin mediates localization to the cytosolic face of the endoplasmic reticulum and inhibits protein secretion. J Biol Chem 2009; 284: 4705–12.

16. Duschene KS, Broderick JB. The antiviral protein viperin is a radical SAM enzyme. FEBS Lett 2010; 584: 1263–7.
17. Helbig KJ, Eyre NS, Yip E, Narayana S, Li K, Fiches G, McCartney EM, Jangra RK, Lemon SM, Beard MR. The antiviral protein viperin inhibits hepatitis C virus replication via interaction with nonstructural protein 5A. Hepatology 2011; 54: 1506–17.
18. Wang S, Wu X, Pan T, Song W, Wang Y, Zhang F, Yuan Z. Viperin inhibits hepatitis C virus replication by interfering with the binding of NS5A to host protein hVAP-33. J Gen Virol 2011; vir.0.033860-0v1–vir.0.033860-0.
19. Boudinot P, Massin P, Blanco M, Riffault S, Benmansour A. vig-1, a new fish gene induced by the rhabdovirus glycoprotein, has a virus-induced homologue in humans and shares conserved motifs with the MoaA family. J Virol 1999; 73: 1846–52.
20. Chin KC, Cresswell P. Viperin (cig5), an IFN-inducible antiviral protein directly induced by human cytomegalovirus. Proc Natl Acad Sci USA 2001; 98: 15125–30.
21. Samuel CE. Antiviral actions of interferons. Clin Microbiol Rev 2001; 14: 778–809.
22. Stark GR, Kerr IM, Williams BR, Silverman RH, Schreiber RD. How cells respond to interferons. Annu Rev Biochem 1998; 67: 227–64.
23. Weber F, Kochs G, Haller O. Inverse interference: how viruses fight the interferon system. Viral Immunol 2004; 17: 498–515.
24. Wathen MW, Thomsen DR, Stinski MF. Temporal regulation of human cytomegalo-virus transcription at immediate early and early times after infection. J Virol 1981; 38: 446–59.
25. Wathen MW, Stinski MF. Temporal patterns of human cytomegalovirus transcription – mapping the viral RNAs synthesized at immediate early, early, and late times after infection. J Virol 1982; 41: 462–77.
26. Silva MC, Yu QC, Enquist L, Shenk T. Human cytomegalovirus UL99-encoded pp28 is required for the cytoplasmic envelopment of tegument-associated capsids. J Virol 2003; 77: 10594–605.
27. Schmolke S, Drescher P, Jahn G, Plachter B. Nuclear targeting of the tegument protein Pp65 (Ul83) of human cytomegalovirus – an unusual bipartite nuclear-localization signal functions with other portions of the protein to mediate its efficient nuclear transport. J Virol 1995; 69: 1071–8.
28. Abate DA, Watanabe S, Mocarski ES. Major human cytomegalovirus structural protein pp65 (ppUL83) prevents interferon response factor 3 activation in the interferon response. J Virol 2004; 78: 10995–1006.
29. Homman-Loudiyi M, Hultenby K, Britt W, Soderberg-Naucler C. Envelopment of human cytomegalovirus occurs by budding into Golgi-derived vacuole compartments positive for gB, Rab 3, trans-Golgi network 46, and mannosidase II. J Virol 2003; 77: 3191–203.
30. Sanchez V, Greis KD, Sztul E, Britt WJ. Accumulation of virion tegument and envelope proteins in a stable cytoplasmic compartment during human cytomegalovirus replication: characterization of a potential site of virus assembly. J Virol 2000; 74: 975–86.
31. Verdijk RM, Mutis T, Esendam B, Kamp J, Melief CJM, Brand A, Goulmy E. Polyriboinosinic polyribocytidylic acid [poly(I:C)] induces stable maturation of functionally active human dendritic cells. J Immunol 1999; 163: 57–61.
32. Akiyama Y, Stevenson GW, Schlick E, Matsushima K, Miller PJ, Stevenson HC. Differential ability of human-blood monocyte subsets to release various cytokines. J Leukoc Biol 1985; 37: 519–30.
33. Manetti R, Annunziato F, Tomasevic L, Giannò V, Parronchi P, Romagnani S, Maggi E. Polyinosinic acid-polycytidylic acid promotes T-helper type-1 specific immune-responses by stimulating macrophage production of interferon-α and interleukin-12. Eur J Immunol 1995; 25: 2656–60.
34. Grandvaux N, Servant MJ, tenOever B, Sen GC, Balachandran S, Barber GN, Lin R, Hiscott J. Transcriptional profiling of interferon regulatory factor 3 target genes: direct involvement in the regulation of interferon-stimulated genes. J Virol 2002; 76: 5532–9.
35. Kunzi MS, Pitha PM. Interferon targeted genes in host defense. Autoimmunity 2003; 36: 457–61.
36. Stirnweiss A, Ksienzyk A, Klages K, Rand U, Grashoff M, Hauser H, Kröger A. IFN regulatory factor-1 bypasses IFN-mediated antiviral effects through viperin gene induction. J Immunol 2010; 184: 5179–85.
37. Sun BJ, Nie P. Molecular cloning of the viperin gene and its promoter region from the mandarin fish Siniperca chuatsi. Vet Immunol Immunopathol 2004; 101: 161–70.
38. Kroger A, Koster M, Schroeder K, Hauser H, Mueller PP. Activities of IRF-1. J Interferon Cytokine Res 2002; 22: 5–14.
39. Pine R, Canova A, Schindler C. Tyrosine-phosphorylated P91 binds to a single-element in the Isgf2/Irf-1 promoter to mediate induction by IFN-α and IFN-γ, and is likely to autoregulate the P91 gene. EMBO J 1994; 13: 158–67.
40. Saitoh T, Satoh T, Yamamoto N, Uematsu S, Takeuchi O, Kawai T, Akira S. Antiviral protein Viperin promotes Toll-like receptor 7- and Toll-like receptor 9-mediated type I interferon production in plasmacytoid dendritic cells. Immunity 2011; 34: 352–63.
41. Olofsson PS, Jatta K, Wågstäter D, Gredmark S, Hedin U, Paulsson-Berne G, Söderberg-Nauclér C, Hansson GK, Sirsjö A. The antiviral cytomegalovirus inducible gene 5/viperin is expressed in atherosclerosis and regulated by proinflammatory agents. Arterioscler Thromb Vasc Biol 2005; 25: E113–6.
42. Streblow DN, Orloff SL, Nelson JA. Do pathogens accelerate atherosclerosis? J Nutr 2001; 131: 2798S–804S.
43. Hendrix MGR, Salimans MMM, Vanboven CPA, Bruggeman CA. High prevalence of latently present cytomegalovirus in arterial-walls of patients suffering from grade-III atherosclerosis. Am J Pathol 1990; 136: 23–8.
44. Nicholson AC, Hajjar DP. Herpesviruses in atherosclerosis and thrombosis – etiologic agents or ubiquitous bystanders. Arterioscler Thromb Vasc Biol 1998; 18: 339–48.
45. Hunter GC, Henderson AM, Westerband A, Kobayashi H, Suzuki F, Yan ZQ, Sirsjo A, Putnam CW, Hansson GK. The contribution of inducible nitric oxide and cytomegalovirus to the stability of complex carotid plaque. J Vasc Surg 1999; 30: 36–49.
46. Dang W, Zhang M, Hu YH, Sun L. Differential regulation of Sciaenops ocellatus viperin expression by intracellular and extracellular bacterial pathogens. Fish Shellfish Immunol 2010; 29: 264–70.
47. Song G, Bazer FW, Spencer TE. Pregnancy and interferon tau regulate RSAD2 and IFIH1 expression in the ovine uterus. Reproduction 2007; 133: 285–95.
48. Hinson ER, Joshi NS, Chen JH, Rahner C, Jung YW, Wang X, Kaech SM, Cresswell P. Viperin is highly induced in neutrophils and macrophages during acute and chronic lymphocytic choriomeningitis virus infection. J Immunol 2010; 184: 5723–31.
49. Nathan C. Neutrophils and immunity: challenges and opportunities. Nat Rev Immunol 2006; 6: 173–82.
50. Segal AW. How neutrophils kill microbes. Ann Rev Immunol 2005; 23: 197–223.
51. Jiang D, Guo H, Xu C, Chang J, Gu B, Wang L, Block TM, Guo J-T. Identification of three interferon-inducible cellular enzymes

that inhibit the replication of hepatitis C virus. J Virol 2008; 82: 1665–78.
52. Weizhang J, Xiuxia Z, Rong H, Qionglin G. Cloning and characterization of interferon stimulated genes Viperin and ISG15, and their promoters from snakehead Channa argus. Progr Nat Sci 2007; 17: 1425–35.
53. Frey PA, Hegeman AD, Ruzicka FJ. The radical SAM superfamily. Crit Rev Biochem Mol Biol 2008; 43: 63–88.
54. Nicolet Y, Drennan CL. AdoMet radical proteins – from structure to evolution – alignment of divergent protein sequences reveals strong secondary structure element conservation. Nucleic Acids Res 2004; 32: 4015–25.
55. Vey JL, Yang J, Li M, Broderick WE, Broderick JB, Drennan CL. Structural basis for glycyl radical formation by pyruvate formate-lyase activating enzyme. Proc Natl Acad Sci USA 2008; 105: 16137–41.
56. Sofia HJ, Chen G, Hetzler BG, Reyes-Spindola JF, Miller NE. Radical SAM, a novel protein superfamily linking unresolved steps in familiar biosynthetic pathways with radical mechanisms: functional characterization using new analysis and information visualization methods. Nucleic Acids Res 2001; 29: 1097–106.
57. Fontecave M, Mulliez E, Ollagnier-de-Choudens S. Adenosylmethionine as a source of 5′-deoxyadenosyl radicals. Curr Opin Chem Biol 2001; 5: 506–11.
58. Kelley LA, Sternberg MJE. Protein structure prediction on the Web: a case study using the Phyre server. Nat Protoc 2009; 4: 363–71.
59. Duschene KS, Veneziano SE, Silver SC, Broderick JB. Control of radical chemistry in the AdoMet radical enzymes. Curr Opin Chem Biol 2009; 13: 74–83.
60. Shaveta G, Shi JH, Chow VTK, Song JX. Structural characterization reveals that viperin is a radical S-adenosyl-L-methionine (SAM) enzyme. Biochem Biophys Res Commun 2010; 391: 1390–5.
61. Hagen KS, Watson AD, Holm RH. Synthetic routes to Fe2S2, Fe3S4, Fe4S4, and Fe6S9 clusters from the common precursor [Fe(Sc2H5)4]2- – structures and properties of [Fe3S4(Sr)4]3- and [Fe6S9(Sc2H5)2]4-, examples of the newest types of Fe-S-Sr clusters. J Am Chem Soc 1983; 105: 3905–13.
62. Kulzer R, Pils T, Kappl R, Huttermann J, Knappe J. Reconstitution and characterization of the polynuclear iron-sulfur cluster in pyruvate formate-lyase-activating enzyme – molecular properties of the holoenzyme form. J Biol Chem 1998; 273: 4897–903.
63. Ugulava NB, Frederick KK, Jarrett JT. Control of adenosylmethionine-dependent radical generation in biotin synthase: a kinetic and thermodynamic analysis of substrate binding to active and inactive forms of BioB. Biochemistry 2003; 42: 2708–19.
64. Liu A, Graslund A. Electron paramagnetic resonance evidence for a novel interconversion of [3Fe-4S](+) and [4Fe-4S](+) clusters with endogenous iron and sulfide in anaerobic ribonucleotide reductase activase in vitro. J Biol Chem 2000; 275: 12367–73.
65. Walsby CJ, Ortillo D, Yang J, Nnyepi MR, Broderick WE, Hoffman BM, Broderick JB. Spectroscopic approaches to elucidating novel iron-sulfur chemistry in the "Radical-SAM" protein superfamily. Inorg Chem 2005; 44: 727–41.
66. Leser GP, Lamb RA. Influenza virus assembly and budding in raft-derived microdomains: a quantitative analysis of the surface distribution of HA, NA and M2 proteins. Virology 2005; 342: 215–27.
67. Scheiffele P, Rietveld A, Wilk T, Simons K. Influenza viruses select ordered lipid domains during budding from the plasma membrane. J Biol Chem 1999; 274: 2038–44.
68. Shvartsman DE, Kotler M, Tall RD, Roth MG, Henis YI. Differently anchored influenza hemagglutinin mutants display distinct interaction dynamics with mutual rafts. J Cell Biol 2003; 163: 879–88.
69. Ono A, Freed EO. Role of lipid rafts in virus replication. Adv Virus Res 2005; 64: 311–58.
70. Ashby MN, Edwards PA. Identification and regulation of a rat-liver cDNA-encoding farnesyl pyrophosphate synthetase. J Biol Chem 1989; 264: 635–40.
71. Seo JY, Yaneva R, Hinson ER, Cresswell P. Human cytomegalovirus directly induces the antiviral protein viperin to enhance infectivity. Science 2011; 332: 1093–7.
72. Al-Barazi HO, Colberg-Poley AM. The human cytomegalovirus UL37 immediate-early regulatory protein is an integral membrane N-glycoprotein which traffics through the endoplasmic reticulum and Golgi apparatus. J Virol 1996; 70: 7198–208.
73. Colberg-Poley AM, Patel MB, Erezo DP, Slater JE. Human cytomegalovirus UL37 immediate-early regulatory proteins traffic through the secretory apparatus and to mitochondria. J Gen Virol 2000; 81: 1779–89.
74. Goldmacher VS. vMIA, a viral inhibitor of apoptosis targeting mitochondria. Biochimie 2002; 84: 177–85.
75. Goldmacher VS, Bartle LM, Skaletskaya A, Dionne CA, Kedersha NL, Vater CA, Han JW, Lutz RJ, Watanabe S, Cahir McFarland ED, Kieff ED, Mocarski ES, Chittenden T. A cytomegalovirus-encoded mitochondria-localized inhibitor of apoptosis structurally unrelated to Bcl-2. Proc Natl Acad Sci USA 1999; 96: 12536–41.
76. Bartlett K, Eaton S. Mitochondrial β-oxidation. Eur J Biochem 2004; 271: 462–9.
77. Eaton S, Bursby T, Middleton B, Pourfarzam M, Mills K, Johnson AW, Bartlett K. The mitochondrial trifunctional protein: centre of a β-oxidation metabolon? Biochem Soc Trans 2000; 28: 177–82.
78. Fukuda M, Yamamoto A, Mikoshiba K. Formation of crystalloid endoplasmic reticulum induced by expression of synaptotagmin lacking the conserved WHXL motif in the C terminus – structural importance of the WHXL motif in the C2B domain. J Biol Chem 2001; 276: 41112–9.
79. Yamamoto A, Masaki R, Tashiro Y. Formation of crystalloid endoplasmic reticulum in COS cells upon overexpression of microsomal aldehyde dehydrogenase by cDNA transfection. J Cell Sci 1996; 109: 1727–38.
80. Snapp EL, Hegde RS, Francolini M, Lombardo F, Colombo S, Pedrazzini E, Borgese N, Lippincott-Schwartz J. Formation of stacked ER cisternae by low affinity protein interactions. J Cell Biol 2003; 163: 257–69.
81. Antonny B. Membrane deformation by protein coats. Curr Opin Cell Biol 2006; 18: 386–94.
82. McMahon HT, Gallop JL. Membrane curvature and mechanisms of dynamic cell membrane remodelling. Nature 2005; 438: 590–6.
83. Zimmerberg J, Kozlov MM. How proteins produce cellular membrane curvature. Nat Rev Mol Cell Biol 2006; 7: 9–19.
84. Drin G, Casella JF, Gautier R, Boehmer T, Schwartz TU, Antonny B. A general amphipathic α-helical motif for sensing membrane curvature. Nat Struct Mol Biol 2007; 14: 138–46.
85. Drin G, Antonny B. Amphipathic helices and membrane curvature. FEBS Lett 2010; 584: 1840–7.
86. Barba G, Harper F, Harada T, Kohara M, Goulinet S, Matsuura Y, Eder G, Schaff Zs, Chapman MJ, Miyamura T, Bréchot C. Hepatitis C virus core protein shows a cytoplasmic localization and associates to cellular lipid storage droplets. Proc Natl Acad Sci USA 1997; 94: 1200–5.
87. Miyanari Y, Atsuzawa K, Usuda N, Watashi K, Hishiki T, Zayas M, Bartenschlager R, Wakita T, Hijikata M, Shimotohno K. The

lipid droplet is an important organelle for hepatitis C virus production. Nat Cell Biol 2007; 9: 1089–97.
88. Guo Y, Cordes KR, Farese RV Jr, Walther TC. Lipid droplets at a glance. J Cell Sci 2009; 122: 749–52.
89. Elazar M, Cheong KH, Liu P, Greenberg HB, Rice CM, Glenn JS. Amphipathic helix-dependent localization of NS5A mediates hepatitis C virus RNA replication. J Virol 2003; 77: 6055–61.
90. Brass V, Bieck E, Montserret R, Wölk B, Hellings JA, Blum HE, Penin F, Moradpour D. An amino-terminal amphipathic α-helix mediates membrane association of the hepatitis C virus nonstructural protein 5A. J Biol Chem 2002; 277: 8130–9.
91. Elazar M, Liu P, Rice CM, Glenn JS. An N-terminal amphipathic helix in hepatitis C virus (HCV) NS4B mediates membrane association, correct localization of replication complex proteins, and HCV RNA replication. J Virol 2004; 78: 11393–400.
92. Hinson ER, Cresswell P. The antiviral protein, viperin, localizes to lipid droplets via its N-terminal amphipathic α-helix. Proc Natl Acad Sci USA 2009; 106: 20452–7.
93. Gao L, Aizaki H, He JW, Lai MM. Interactions between viral nonstructural proteins and host protein hVAP-33 mediate the formation of hepatitis C virus RNA replication complex on lipid raft. J Virol 2004; 78: 3480–8.
94. Layer G, Heinz DW, Jahn D, Schubert WD. Structure and function of radical SAM enzymes. Curr Opin Chem Biol 2004; 8: 468–76.
95. Mackenzie J. Wrapping things up about virus RNA replication. Traffic 2005; 6: 967–77.
96. Miller S, Krijnse-Locker J. Modification of intracellular membrane structures for virus replication. Nat Rev Microbiol 2008; 6: 363–74.

Received October 19, 2011; accepted February 23, 2012

Kaitlin S. Duschene was born in 1978. She received her BS and MS degrees in biochemistry from Furman University where she studied the effects of potential anticancer drugs synthesized in the lab of Dr. Moses Lee in both *in vitro* and *in vivo* models. Prior to coming to Montana State University she studied the activity of HIF1a in prostate cancer in the lab of Dr. Jonathan Simons, president of Winship Cancer Institute, Emory University. She is currently working under Dr. Joan Broderick where her research interests are iron-sulfur clusters involved in mammalian immune response mechanisms.

Joan B. Broderick was born in 1965. She received a Bachelor of Science in Chemistry from Washington State University and a PhD from Northwestern University, where she was a National Science Foundation Graduate Fellow. She was an American Cancer Society postdoctoral fellow at MIT before joining the faculty at Amherst College as Assistant Professor in 1993. She moved to Michigan State University in 1998 and to Montana State University in 2005, where she is currently Professor of Chemistry and Biochemistry. Her research interests are in mechanistic bioinorganic chemistry, with a particular focus on enzymes utilizing iron-sulfur clusters to catalyze radical reactions.