Human Cytomegalovirus Directly Induces the Antiviral Protein Viperin to Enhance Infectivity

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Viperin is an interferon-inducible protein that is directly induced in cells by human cytomegalovirus (HCMV) infection. Why HCMV would induce viperin, which has antiviral activity, is unknown. We show that HCMV-induced viperin disrupts cellular metabolism to enhance the infectious process. Viperin interaction with the viral protein vMIA resulted in viperin relocalization from the endoplasmic reticulum to the mitochondria. There, viperin interacted with the mitochondrial trifunctional protein that mediates β-oxidation of fatty acids to generate adenosine triphosphate (ATP). This interaction with viperin, but not with a mutant lacking the viperin iron-sulfur cluster–binding motif, reduced cellular ATP generation, which resulted in actin cytoskeleton disruption and enhancement of infection. This function of viperin, which was previously attributed to vMIA, suggests that HCMV has coopted viperin to facilitate the infectious process.

The importance of the type 1 interferon (IFN) pathway in directing the antiviral response is well established (1, 2). Although many proteins are induced by IFN stimulation or viral infection (1, 2), the functions of most of them remain unexplored. Moreover, despite the antiviral effects of IFN-inducible proteins, some viruses directly induce them for reasons that are largely not well understood. Viperin is an IFN-inducible iron-sulfur (Fe-S) cluster–binding antiviral protein (3–5). It is induced in various cell types by both type I and II IFNs, polyinosinic-polycytidylic acid, double-stranded RNA (dsRNA), viral DNA, and lipopolysaccharide and by infection with many viruses, including human cytomegaloviruses (HCMV) (5–13). Although viperin inhibits HCMV infection when preexpressed in cells (5), the virus directly induces viperin expression independently of IFN production (13). This suggests that viperin induction might be advantageous to the virus.

As an initial approach to the potential mechanism, we investigated the intracellular distribution of viperin during HCMV infection (14). We previously showed that viperin is redistributed from the endoplasmic reticulum (ER) through the Golgi to the virus assembly compartment (AC) upon HCMV infection and suggested that this reflected a strategy to evade the antiviral effects of ER-localized viperin (5). Consistent with previous studies, IFN-induced viperin localized to the ER (fig. S1A). We were surprised, however, to observe that viperin induced in human foreskin fibroblasts (HFFs) by HCMV infection could be detected in association with mitochondria at 1 day post infection (dpi) (fig. S1B) and at the AC at 3 to 4 dpi (fig. S1C). The viral mitochondrial inhibitor of apoptosis (vMIA) is an HCMV-encoded protein known to traffic to mitochondria (15–18), which suggested a possible mechanism for mitochondrial targeting of vMIA during HCMV infection. We observed that viperin colocalized with vMIA to the mitochondria at 1 dpi (Fig. 1A). At 3 dpi viperin was redistributed to the AC, although vMIA remained associated with the mitochondria (Fig. 1A). Coimmunoprecipitation experiments showed that endogenous viperin and vMIA can interact with each other in virally infected cells (Fig. 1A).

**Fig. 1.** The HCMV vMIA protein targets viperin to mitochondria. (A) HFF cells were infected with HCMV at a multiplicity of infection (MOI) of 2 for 1 or 3 days. Cells were stained with antibodies against vMIA (4B6-B) and viperin (MaP.VIP), and MitoTracker Red. A representative image from two individual experiments is shown. (B) HFF cells were infected with HCMV at a MOI of 2 for 1 day or treated with IFN-α (1000 U/ml) for 16 hours. Cells were labeled with [35S]methionine-cysteine for 2 hours and lysed in detergent. The immunoprecipitation was performed with normal mouse serum (NMS), antibody against Myc (9E10), or MaP.VIP antibody (top and middle). The precipitates were SDS stripped, and released material was reimmunoprecipitated with MaP.VIP (top) or an antibody against vMIA (DC35) (middle). The reciprocal immunoprecipitation was performed with normal rabbit serum (NRS), hemagglutinin (HA)—specific antibody or DC35 antibody, and the stripped immunoprecipitates were reimmunoprecipitated with MaP.VIP (bottom). Coimmunoprecipitated vMIA or viperin were detected after SDS polyacrylamide gel electrophoresis (SDS-PAGE). (C) HFF cells transiently expressing vMIA and/or vMIA-Myc were examined by immunoen. The sections were stained first with antibodies against viperin and/or Myc (9E10) and then with a secondary antibody, 5- or 10-nm gold conjugate, respectively. A representative field is shown (Nu, nucleus; Mito, mitochondria). The arrows indicate gold-labeled viperin and vMIA-Myc. A representative image from two individual experiments is shown. Scale bars, 10 μm (A) and 200 nm (C).
infected cells (Fig. 1B). The viperin interaction with vMIA was confirmed in transiently expressing cells by cross-linking followed by solubilization and immunoprecipitation (fig. S2A) and by fluorescent protein-fragment complementation analysis (PCA) (fig. S2, B to E). Analysis by immunoelectron microscopy (immuno-EM) (Fig. 1C) or confocal immunofluorescence microscopy (fig. S2F) also confirmed that the vMIA interaction directly induced viperin targeting to mitochondria.

vMIA has been reported to be responsible for the disruption of the actin cytoskeleton observed after HCMV infection that is believed to facilitate infection (19–23). The vMIA-induced redistribution of viperin to mitochondria suggested that this effect might be indirect. To examine this question, we used murine embryonic fibroblasts (MEFs) from viperin-depleted (knockout, KO) mice or COS-7 cells, in which endogenous viperin is not induced by double-stranded DNA transfection as it is with HFFs (fig. S3, A and B). The redistribution of viperin by vMIA was also seen in cotransfected MEFs (fig. S3C). We monitored alterations of the actin cytoskeleton in cells transiently expressing vMIA and/or viperin by staining with phalloidin (Fig. 2 and fig. S4). Consistent with previous reports (19, 20), cytoskeletal organization was markedly disrupted, with loss of stress fibers, in wild-type (WT) MEFs expressing vMIA alone, in which endogenous viperin is induced by the transfection protocol (Fig. 2A). However, the actin cytoskeleton was indistinguishable from that of nontransfected cells when vMIA was expressed in viperin KO MEFs, where no endogenous viperin is induced (Fig. 2A). When viperin and vMIA were coexpressed in viperin KO MEFs, the actin cytoskeleton was dramatically disrupted (Fig. 2B). This suggests that viperin is required for the vMIA-induced disruption of the actin cytoskeleton. The actin cytoskeleton was maintained in all cell types expressing viperin alone (fig. S4A). The same patterns of cytoskeletal disruption were also observed in COS-7 cells (fig. S4B). We quantified the actin stress fibers by measuring the phalloidin intensity in three-dimensional images of cells transiently expressing vMIA and/or viperin (fig. S4C). In comparison

**Fig. 2. Mitochondrial viperin is responsible for disrupting the actin cytoskeleton.** (A and B) WT or viperin KO MEFs were transiently transfected with plasmids expressing vMIA and/or viperin. Cells were stained with antibodies against vMIA (4B6-B) and/or viperin (MaP.VIP), and Alexa Fluor 546 phalloidin was used to visualize F-actin. The arrows indicate transfected cells and the arrowheads, nontransfected cells. A representative image from three individual experiments is shown. (C) Actin stress fibers were quantified in MEFs expressing vMIA and/or viperin. The mean values from transfected cells were normalized to those (set as 100) from nontransfected cells on the same image. Data are presented as means of three independent experiments (n = 6 to 7) ± SEM. *P < 0.001. (D) The N-terminal 42 residue α-helical region of viperin WT or viperin (DCA) was replaced by a mitochondrial localization sequence (MLS, amino acids 2 to 34) of vMIA. The MLS was directly fused to enhanced green fluorescent protein (EGFP) as a negative control. Viperin KO MEFs transiently expressing the indicated chimeric viperin proteins were stained with MaP.VIP antibody and Alexa Fluor 546 phalloidin. The arrows indicate transfected cells and the arrowheads, nontransfected cells. A representative image from three individual experiments is shown. (E) Actin stress fibers were quantified as described above. Data are presented as means of three independent experiments (n = 5 to 8) ± SEM. *P < 0.001. Scale bars, 20 μm (A, B, and D).
with nontransfected cells, the phalloidin intensities in WT MEFs expressing vMIA alone, or both WT and viperin KO MEFs coexpressing vMIA and vMIA, were reduced by about 50 to 60%, whereas the intensity in viperin KO MEFs expressing vMIA alone was not reduced (Fig. 2C). These results suggest that viperin targeting to mitochondria is required for the disruption of the actin cytoskeleton.

To identify viperin elements involved in the disruption of the actin cytoskeleton, two mutated constructs were used. In the first, residues 1 to 42 were deleted [viperin (ΔN)] to eliminate ER association (24), and in the second, two cysteine residues (88 and 91) were mutated to alanine to eliminate Fe-S cluster association [viperin (DCA)]. When viperin (ΔN) and vMIA or vMIA and vMIA were coexpressed in viperin KO MEFs, viperin (ΔN) was distributed throughout the cytoplasm, whereas viperin (DCA) colocalized with vMIA in mitochondria (fig. S5A). The lack of vMIA interaction with viperin (ΔN) and the interaction of vMIA with viperin (DCA) were confirmed by PCA analysis (fig. S5, B and C). When we examined the cells coexpressing these proteins, however, we observed that the actin cytoskeleton was maintained (fig. S5, D and E). These data indicated a requirement for both mitochondrial localization and Fe-S cluster binding for the viperin-mediated effects.

To determine whether vMIA played any additional role in the process, we generated chimeric WT and Fe-S cluster–binding mutant viperin constructs in which the N-terminal amphipathic α-helix, responsible for ER and lipid droplet association (24, 25), was replaced by the mitochondrial localization sequence (MLS) of vMIA (fig. S6, A to C). We also generated a fusion construct with the MLS linked to green fluorescent protein (GFP) directly. We then examined the status of the actin cytoskeleton in viperin KO MEFs or COS-7 cells expressing each chimeric protein (Fig. 2, D and E, and fig. S6D). Although the actin cytoskeleton was disrupted in cells expressing MLS-viperin, it was disrupted by 60 to 70% in cells expressing MLS-viperin, which indicated that directly targeted viperin mediates actin cytoskeleton disruption independently of vMIA. Similar results were produced in viperin KO MEFs expressing a chimeric vMIA protein in which the N-terminal amphipathic α helix was replaced by the MLS of Tom70, a host cellular mitochondrial protein (fig. S7); this finding excluded the possibility that the MLS from vMIA was responsible for the effect. The actin cytoskeleton was maintained in cells expressing MLS-viperin (DCA), again showing that Fe-S cluster binding is necessary for cytoskeletal disruption (Fig. 2, D and E, and fig. S6D).

Previous studies have shown that the actin cytoskeleton is disrupted by both ATP depletion and ischemia (26–28) and possibly by vMIA-mediated inhibition of mitochondrial ATP generation (19). To determine whether the reduction of ATP generation is mediated by vMIA or by associated viperin, we measured intracellular ATP levels in viperin KO MEFs expressing vMIA-Myc, WT viperin, or viperin mutants (Fig. 3A). ATP levels were reduced only in cells expressing MLS-viperin, by ~50%, which indicates that mitochondrionally targeted, Fe-S cluster–binding vMIA is responsible for the decrease (Fig. 3A).

A proteomics analysis of viperin-interacting proteins suggested that the ATP reduction induced by targeting viperin to mitochondria could involve a viperin interaction with the β subunit of the mitochondrial trifunctional protein (TFP) (fig. S8A). TFP is a multienzyme complex, composed of four α subunits (HADHA) and four β subunits (HADHB), that catalyzes the last three steps of fatty acid β-oxidation, a major mechanism of cellular ATP production (29, 30). We confirmed the interaction of HADHB with vMIA by co-immunoprecipitation and by chemical cross-linking followed by solubilization and immunoprecipitation (Fig. 3B and fig. S8, B and C).

To assess whether the vMIA interaction with HADHB affects cellular bioenergetics, we measured fatty acid oxidation levels, ADP/ATP ratios, and the ratios of the reduced form of nicotinamide adenine dinucleotide (NADH)/nicotinamide adenine dinucleotide (NADH/NAD+) (29, 30). We confirmed the interaction of HADHB with vMIA by co-immunoprecipitation and by chemical cross-linking followed by solubilization and immunoprecipitation (Fig. 3B and fig. S8, B and C).

To confirm that vMIA interaction with HADHB is responsible for the metabolic effects observed, we adopted a genetic approach, measuring cellular ATP levels in patient-derived TFP-deficient human fibroblasts transiently expressing vMIA-Myc, WT vMIA, or vMIA mutants, with or without GFP tags (Fig. 3, C and D). In WT HFF cells expressing vMIA-Myc (which induces

**Fig. 3.** Viperin interaction with mitochondrial TFP mediates reduction of cellular ATP levels. (A) Cellular ATP levels were measured in vMIA KO MEFs transiently expressing the indicated proteins. The control value from Myc alone was set as 100%. Data are presented as means of three independent experiments ± SEM. *P < 0.001. (B) Endogenous vMIA interaction with the β subunit (HADHB) of TFP. HFF cells were infected with HCMV at a MOI of 2 for 1 day. Cells were lysed and immunoprecipitated with normal mouse serum (NMS) or antibodies against Myc (9E10) or viperin (MaPVIP), and Western blots were probed using antibody against HADHB. (C and D) Cellular ATP levels were measured from WT (C) or TFP-deficient HFF cells [HADHA(−) or HADHB(−)] (D) transiently expressing the indicated proteins. Data are presented as means of at least two independent experiments ± SEM. *P < 0.001.
endogenous viperin), MLS-viperin and MLS-viperin-GFP, the ATP levels were reduced to 50 to 70% of control levels (Fig. 3C). In HADHA-deficient or HADHB-deficient fibroblasts, however, ATP levels were unaffected (Fig. 3D), as was the actin cytoskeleton (fig. S8, G and H). Taken together, the data indicate that interaction of the mitochondrial target viperin with HADHB reduces TFP activity, which lowers cellular ATP levels and disrupts the actin cytoskeleton.

Finally, to assess whether viperin induced by HCMV infection also mediates actin cytoskeleton disruption, induction in HFFs [HFtelo cells expressing telomerase (31)] was suppressed by stably expressing two different short hairpin RNAs (shRNAs) (Fig. 4A). We then examined the actin cytoskeleton in viperin knockdown (KD) HFtelo cells after infection (Fig. 4A). In contrast to cells expressing no shRNA or control luciferase shRNA, in viperin KD cells the pattern of actin stress fibers was indistinguishable from that in non-infected cells (Fig. 4A), as were the ATP levels (Fig. 4B). We then asked if, as expected (21, 22), eliminating stress fiber disruption affected HCMV replication. We determined the kinetics of replication for viruses recovered from viperin KD cells by single-step growth assays. Although HCMV production was unaffected at 3 days post infection, after 6 days it was reduced by more than 10-fold in the viperin KD cells compared with control shRNA-expressing cells (Fig. 4C). To exclude the possibility that viperin shRNA expression induced IFN- or dsRNA-dependent expression of other IFN-stimulated genes (ISGs) that might inhibit HCMV replication, we examined the expression of a variety of ISGs and found no induction (fig. S9, A and B). As a control for the specificity of the shRNA effects, we generated a recombinant HCMV (HCMV.mVIP) in which the loci US7-US16, nonessential for in vitro replication, were replaced by mouse viperin-GFP under an inducible promoter such that expression could be enhanced by the addition of doxycycline (fig. S9C). Expression of mouse viperin-GFP, not susceptible to the effects of the human-specific shRNAs, by HCMV.mVIP rescued the WT HCMV phenotype in viperin shRNA-expressing cells, both in terms of viral replication (Fig. 4D) and actin stress-fiber disruption (fig. S9D).

HCMV specifically targets viperin to mitochondria and uses its ability to inhibit TFP-mediated ATP generation to disrupt the actin cytoskeleton, which facilitates viral replication. These effects, previously attributed to vMIA, result from the ability of vMIA to recruit induced endogenous viperin to mitochondria. Although mitochondrial viperin reduced ATP levels, it did not induce apoptosis in transiently transfected cells when we used terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick end labeling (TUNEL) assays (fig. S10), which suggested that HCMV uses viperin to reduce ATP levels sufficiently to induce actin cytoskeleton disruption but not apoptosis. Although the precise mechanism by which viperin exerts its effect on TFP function remains unclear, immunofluorescence showed that viperin and TFP are both detectable at the outer and inner mitochondrial membranes (fig. S11). Viperin interaction with TFP on the outer membrane may inhibit internalization and maturation of TFP, or viperin association with TFP on the inner membrane could directly inhibit its enzymatic activity.

We have observed a shift to glycolysis, which reduces the pH of the culture medium (fig. S12), when viperin is overexpressed in cells; this finding implies that TFP inhibition can occur with WT viperin. Presumably, this effect is exacerbated when viperin is driven to mitochondria by vMIA interaction or by providing it with an MLS. Cells may normally regulate a viperin mitochondrial function that is exploited by HCMV to inhibit

![Figure 4](https://www.sciencemag.org)
Deciphering the Rhizosphere Microbiome for Disease-Suppressive Bacteria

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Disease-suppressive soils are exceptional ecosystems in which crop plants suffer less from specific soil-borne pathogens than expected owing to the activities of other soil microorganisms. For most disease-suppressive soils, the microbes and mechanisms involved in pathogen control are unknown. By coupling PhyloChip-based metagenomics of the rhizosphere microbiome with culture-dependent functional analyses, we identified key bacterial taxa and genes involved in suppression of a fungal root pathogen. More than 33,000 bacterial and archaeal species were cultured, and known mechanisms of pathogen suppression were unravelled. Our data indicate that upon attack by a fungal root pathogen, plants can exploit microbial consortia from soil for protection against infections.

Similar to other eukaryotes, plants and their microbiomes can be viewed as “superorganisms” in which the plant relies, in part, on the soil microbiota for specific functions and traits. In return, plants exude up to 21% of their photosynthetically fixed carbon in the root-soil interface (1), i.e., the rhizosphere, thereby feeding the microbial communities and influencing their activity and diversity. For decades, studies about the interplay between plants and rhizosphere microorganisms have focused on pathogens, symbiotic rhizobia, and mycorrhizal fungi, yet there is evidence that other groups of soil microorganisms can affect plant growth and health (2). It even has been postulated that plants actively recruit beneficial soil microorganisms in their rhizospheres to counteract pathogen assault (3). One well-known phenomenon is the occurrence of disease-suppressive soils, a property conferred by the resident microbiota via as yet unknown mechanisms (4, 5). Hence, the aim of this study is to decipher the rhizosphere microbiome to identify such disease-suppressive microbes and to unravel the mechanisms by which they protect plants against root diseases.

We used a high-density 16S ribosomal DNA (rDNA) oligonucleotide microarray, referred to as the PhyloChip (6, 7), to identify key bacterial and archaeal community members in the rhizosphere of plants grown in a disease-suppressive soil. We subsequently targeted and isolated specific bacterial taxa to elucidate the biosynthetic genes and pathways underlying pathogen control.

**References and Notes**

14. Materials and methods are available as supporting material on Science Online.

**Supporting Online Material**

www.sciencemag.org/cgi/content/full/science.1202007/DC1

Materials and Methods
Figs. S1 to S12

References

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**Fig. 1. (A) Effect of R. solani infection on growth of sugar beet seedlings in disease-suppressive (S) and disease-conductive (C) soils. (B) Percentage (mean ± SEM, N = 4) of seedlings with damping-off symptoms in suppressive soil (S), conductive soil (C), conductive soil amended with 10% (w/w) of suppressive soil (CS), or suppressive soil heat-treated at 50°C (S50) or 80°C (S80). Different letters above the bars indicate statistically significant differences (P < 0.05, Student-Newman-Keuls).**