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Peroxisomes Are Signaling Platforms for Antiviral Innate Immunity

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SUMMARY

Peroxisomes have long been established to play a central role in regulating various metabolic activities in mammalian cells. These organelles act in concert with mitochondria to control the metabolism of lipids and reactive oxygen species. However, while mitochondria have emerged as an important site of antiviral signal transduction, a role for peroxisomes in immune defense is unknown. Here, we report that the RIG-I-like receptor (RLR) adaptor protein MAVS is located on peroxisomes and mitochondria. We find that peroxisomal and mitochondrial MAVS act sequentially to create an antiviral cellular state. Upon viral infection, peroxisomal MAVS induces the rapid interferon-independent expression of defense factors that provide short-term protection, whereas mitochondrial MAVS activates an interferon-dependent signaling pathway with delayed kinetics, which amplifies and stabilizes the antiviral response. The interferon regulatory factor IRF1 plays a crucial role in regulating MAVS-dependent signaling from peroxisomes. These results establish that peroxisomes are an important site of antiviral signal transduction.

INTRODUCTION

A fundamental feature of eukaryotic cells is the use of membrane-bound organelles to compartmentalize activities and serve as scaffolds for signal transduction. The best-characterized signaling pathways involve membrane-bound receptors that respond to extracellular or lumenal stimuli. In these instances, the spatial separation of an extracellular stimulus from the cytosol mandates the use of organelles as signaling platforms, as transmembrane receptors must transmit information across a lipid bilayer. However, an important gap exists in our knowledge of how stimuli from the cytosol are able to initiate specific signaling events.

How common is the use of organelles in signal transduction from cytosolic receptors? An example of this situation can be found in the study of virus-host interactions. The ability to detect cytosolic viruses depends on the RIG-I-like receptor (RLR) family of proteins, which are soluble RNA helicases that detect viruses containing RNA (and in some cases DNA) genomes (Ablasser et al., 2009; Chiu et al., 2009; Yoneyama et al., 2004). The best characterized RLRs, RIG-I and MDA-5, detect 5′-triphosphate-containing short double-stranded RNA (dsRNA) and long dsRNA, respectively (Kato et al., 2008; Pichlmair et al., 2006). RLRs can either detect viral RNA directly or after RNA polymerase III-mediated transcription of microbial DNA (Ablasser et al., 2009; Chiu et al., 2009; Kato et al., 2008). Mice deficient in either of these RLRs are sensitive to different classes of viruses, underscoring both their specificity of action and their importance in immune defense (Gitlin et al., 2006; Kato et al., 2006).

Although RIG-I and MDA-5 have specificities for different ligands, both induce a common signaling pathway that triggers the expression of type I interferons (IFNs) and IFN-stimulated genes (ISGs). Many ISGs function as direct antiviral effectors, acting to prevent viral genome replication, viral particle assembly, or virion release from infected cells. Generally, it is thought that RLRs induce the expression of IFNs that act in both autocrine and paracrine manners to amplify ISG expression. However, ISGs can also be induced directly upon viral infection, without the need for IFN signaling (Collins et al., 2004; Mossman et al., 2001). At the receptor-proximal level, RLR-dependent responses are regulated by the adaptor protein MAVS (also called IPS-1, Cardif, or VISA) (Nakhaei et al., 2009). Upon viral detection, MAVS binds to RLRs and promotes the activation of NF-κB, AP-1, and various interferon regulatory factors (IRFs), which act to induce ISGs and create an antiviral state in the cell. Although much has been learned about the genetics of RLR signaling, less is known about where within

the cell signal transduction occurs. Identifying the sites of RLR signal transduction is critical to understanding how antiviral networks are integrated into the general cellular infrastructure within which they operate.

The first clue that cytosolic RLR signaling may occur from organelles came from studies of the MAVS adaptor. MAVS contains a C-terminal transmembrane domain that anchors it to the mitochondrial outer membrane (Seth et al., 2005). It is from this location that MAVS is thought to engage active RLRs and induce signal transduction. Whether mitochondria are the only organelles that promote RLR-mediated signaling has not been addressed.

Mitochondria have long been appreciated to have an intimate functional relationship with peroxisomes (Hettema and Motley, 2009). Both are membrane-bound organelles found in mammalian cells and are involved in the metabolism of lipids and reactive oxygen species. However, while mitochondria are well-established sites of both antiviral signaling and antiviral apoptosis, peroxisomes are thought to function solely as metabolic organelles.

Recently, several mitochondrial proteins have been found to reside also on peroxisomes. Included in this group are the outer membrane proteins Fis1 and Mff, which regulate the morphology of both organelles (Gandre-Babbe and van der Bliek, 2008; Koch et al., 2005). Interestingly, Fis1, Mff, and MAVS all have similar domain structures: each contains an N-terminal effector domain and a C-terminal localization motif, which consists of a transmembrane domain and a short lumenal tail containing basic amino acids. That other so-called ''tail-anchored'' mitochondrial outer membrane proteins operate from peroxisomes raised the possibility that MAVS also functions from these organelles.

We have discovered that MAVS does indeed reside on peroxisomes and can induce antiviral signaling from this organelle. Our work supports a model whereby peroxisomal MAVS induces the immediate expression of antiviral factors that function to contain a nascent infection. Long-term containment of the infection, however, requires the function of mitochondrial MAVS as well. These data demonstrate that peroxisomes are not simply metabolic organelles, but rather serve as critical subcellular hubs that promote MAVS-dependent antiviral immunity.

RESULTS

MAVS Is Located on Both Mitochondria and Peroxisomes

MAVS has a similar domain organization to other tail-anchored membrane proteins that function from mitochondria and peroxisomes (Gandre-Babbe and van der Bliek, 2008; Koch et al., 2005). We therefore sought to determine whether MAVS also resides on peroxisomes. The subcellular localization of MAVS was examined in mouse embryonic fibroblasts (MEFs) whose peroxisomes were marked by a DsRed allele containing a type 1 peroxisomal targeting signal (PTS1). In addition to staining structures that appeared to be mitochondria, MAVS was detected on PTS1-positive peroxisomes scattered throughout the cell (Figure 1A). A similar staining pattern was seen for Mff (Figure 1A), which functions from both peroxisomes and mitochondria (Gandre-Babbe and van der Bliek, 2008). In contrast, the Toll-like receptor (TLR) adaptor protein TIRAP (Fitzgerald et al., 2001; Horng et al., 2001) was not detected on peroxisomes (Figure 1A). To confirm that the peroxisomal staining was distinct from mitochondria, we also stained cells with MitoTracker. Although no costaining was detected between PTS1 and MitoTracker, MAVS was detected on both PTS1-positive peroxisomes and MitoTracker-positive mitochondria (Figure 1B). Similar results were obtained when epitope-tagged MAVS in murine macrophages (Figure S1 available online) or endogenous MAVS in human hepatocytes were examined (Figure 1C). As an independent means of assessing MAVS localization, hepatocytes were biochemically fractionated to separate peroxisomes and mitochondria, which were respectively distinguished by Pex14 and mtHSP70 (Figure 1D). Both MAVS and Fis1 (a protein that occupies both organelles [Koch et al., 2005]) were detected in fractions containing either peroxisomes or mitochondria. Collectively, on the basis of studies in both human and mouse cells, these data establish that peroxisomes are a bona fide reservoir of the RLR adaptor protein MAVS.

One possible reason MAVS is present on peroxisomes is that newly synthesized MAVS might first pass through peroxisomes en route to mitochondria. To address this possibility, we used human fibroblasts from a patient lacking a functional Pex19 protein. Pex19 controls peroxisome biogenesis, and thus Pex19-deficient cells contain no peroxisomes or peroxisomal remnant structures (Matsuzono et al., 1999; Sacksteder et al., 2000). Notably, MAVS was delivered to mitochondria in Pex19-deficient cells (Figure 1E), indicating that the pathway to mitochondria does not require a peroxisomal intermediate. Moreover, MAVS localized to both peroxisomes and mitochondria in Pex19-deficient cells that expressed Pex19 after transient transfection or retroviral gene transfer (Figure 1E). It is therefore unlikely that localization of MAVS to peroxisomes is the result of a biosynthetic pathway for delivering outer membrane proteins to mitochondria.

A Systematic Strategy to Separate Functions of Peroxisomal and Mitochondrial MAVS

Our finding that MAVS is located on peroxisomes raised the possibility that these organelles serve as a site of antiviral signal transduction. We first considered using Pex19-deficient cells to address sufficiency of mitochondrial MAVS in antiviral signaling, but since peroxisomes are required for biochemical processes that occur in mitochondria, Pex19-deficient cells have profound defects in mitochondrial function (Wanders, 2004). We therefore used the alternative approach of genetically separating the putative mitochondrial and peroxisomal functions of MAVS. This was accomplished by replacing the previously defined MAVS localization motif (Seth et al., 2005) with a set of domains that instead direct the protein to a single compartment (Figure 2A). Using the localization motif of the peroxin Pex13 (Fransen et al., 2001), we created a protein called MAVS-Pex. By deleting the MAVS localization motif, we also created a cytosolic allele (MAVS-Cyto) (Seth et al., 2005). Because the fidelity of mitochondrial sorting signals is not always transferrable to other proteins (Ingelmo-Torres et al., 2009), we lastly created two different alleles of MAVS containing a sorting signal derived from two proteins residing on the mitochondrial outer membrane protein, either OMP25 or Fis1 (Koch et al., 2005; Nemoto and De Camilli, 1999).