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ANTIVIRAL IMMUNITY

Speed and endurance required

New research shows that the innate immune response to cytosolic viruses through retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) must be both rapid and sustained and that these properties are mediated by different signalling pathways. Sustained antiviral signalling at mitochondrial membranes is supported and preceded by the rapid response of peroxisome-based antiviral pathways.

Responses to viral double-stranded RNA through the RLR family members RIG-I and melanoma differentiation-associated gene 5 (MDA5) are regulated by the adaptor protein mitochondrial antiviral signalling protein (MAVS; also known as CARDIF, VISA or IPS1), which is anchored to the mitochondrial membrane. Given the close metabolic links between mitochondria and peroxisomes — which are found in nearly all eukaryotic cells and are required for fatty acid metabolism — and the fact that several mitochondrial proteins have been found on peroxisomes, the authors set out to determine if MAVS also localizes to peroxisomes.

In mouse embryonic fibroblasts (MEFs) stained with peroxisome- and mitochondria-specific markers, MAVS protein was detected on both mitochondria and peroxisomes. After biochemical fractionation of human hepatocytes, MAVS was detected in both peroxisome- and mitochondria-containing fractions. Peroxisomal MAVS was not just 'en route' to mitochondria — in human fibroblasts lacking peroxisomes, MAVS was still delivered to the mitochondria. So, in human and mouse cells, peroxisomes are a bona fide reservoir of MAVS.

To compare the mitochondrial and peroxisomal functions of MAVS, MAVS-deficient MEFs were transfected with MAVS constructs targeted to different subcellular locations: peroxisomes only, mitochondria only, or peroxisomes and mitochondria. After reovirus infection, which triggers signalling through RIG-I and MDA5, cells with peroxisomal MAVS (peroxisomes only or peroxisomes and mitochondria) induced expression of the antiviral protein viperin within 4 hours of infection, whereas cells with mitochondria-only MAVS had delayed viperin induction. In cells with peroxisome-only MAVS, viperin expression was transient. This indicates that although peroxisomal MAVS is required for the rapid induction of antiviral effectors, mitochondrial MAVS is required for a sustained response.

The expression of antiviral proteins such as viperin can be induced directly or through the actions of type I interferons (IFNs). All cells expressing mitochondrial MAVS produced type I IFNs after reovirus infection, but cells with peroxisome-only MAVS did not. When the expression or function of IFNs was inhibited, viperin expression after reovirus infection was decreased in cells with mitochondrial MAVS but not in cells with peroxisome-only MAVS. Therefore, the rapid expression of viperin induced by peroxisomal MAVS occurs directly through an IFN-independent pathway, which is then followed and enhanced by an IFN-dependent feedforward loop mediated by mitochondrial MAVS.

Genome-wide expression analysis showed that peroxisomal and mitochondrial MAVS induce distinct transcriptional responses. Cells expressing MAVS in only peroxisomes or mitochondria restricted reovirus replication to a similar extent as did wild-type cells for the first 24 hours after infection but by 72 hours had viral titres similar to MAVS-deficient cells, which shows that MAVS responses from both organelles are required for maximal antiviral activity.

The authors suggest that, as well as inducing a rapid response to all viruses, peroxisomal MAVS might be particularly important to combat viruses that interfere with host type I IFN production, such as vesicular stomatitis virus (VSV). For example, cells expressing mitochondria-only MAVS, and therefore lacking the IFN-independent peroxisomal MAVS pathway, were as susceptible as MAVS-deficient cells to VSV infection.

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ORIGINAL RESEARCH PAPER Dixit, E. *et al.* Peroxisomes are signaling platforms for antiviral innate immunity. *Cell* **141**, 668–681 (2010)