INNATE IMMUNITY

Triggering RIG-I

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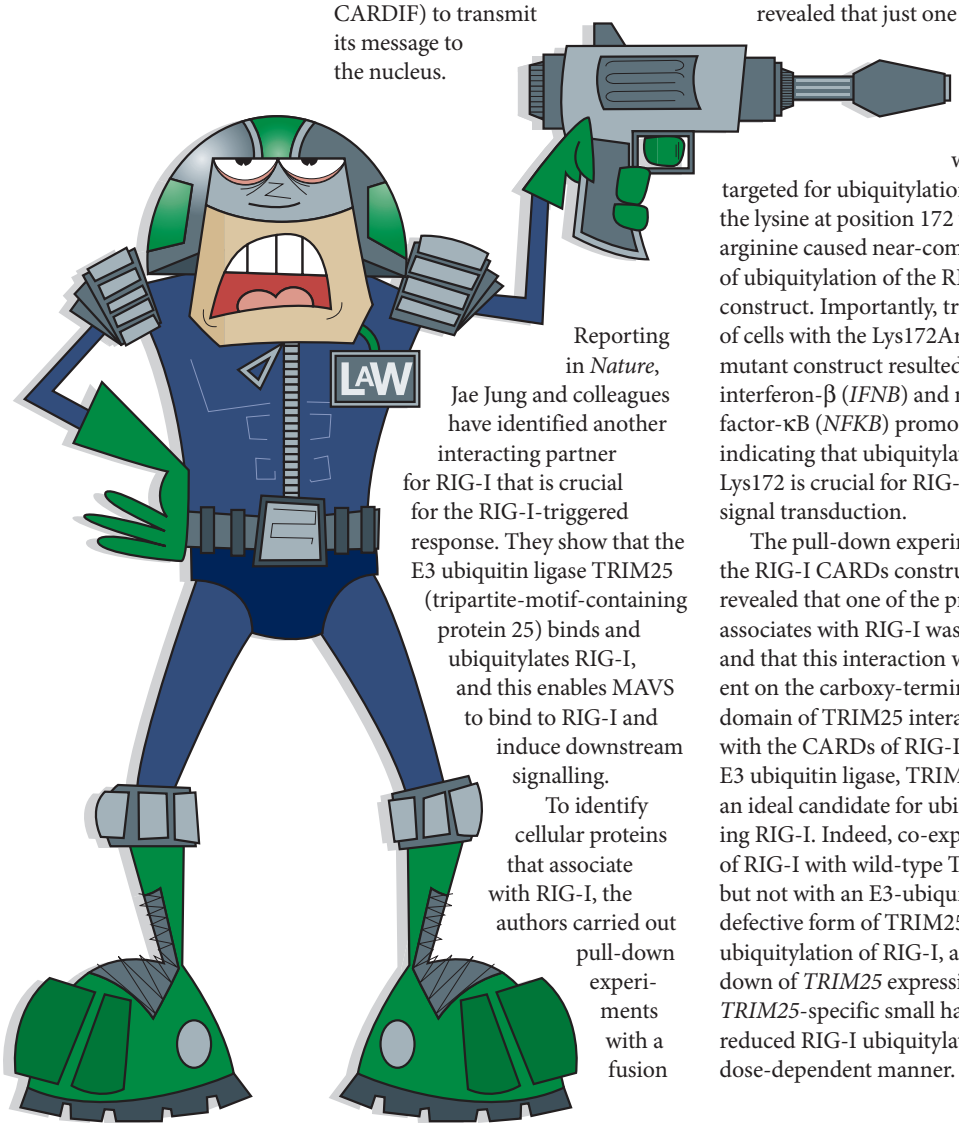
When viral RNA enters the cytosol, a recently identified cytosolic receptor known as retinoic-acid-inducible gene I (RIG-I) is on hand to detect it and trigger a type I interferon (IFN)-mediated response that protects the host against viral infection. RIG-I is known to partner with MAVS (mitochondrial antiviral signalling protein; also known as VISA, IPS1 and CARDIF) to transmit its message to the nucleus.



Reporting in *Nature*, Jae Jung and colleagues have identified another interacting partner for RIG-I that is crucial for the RIG-I-triggered response. They show that the E3 ubiquitin ligase TRIM25 (tripartite-motif-containing protein 25) binds and ubiquitylates RIG-I, and this enables MAVS to bind to RIG-I and induce downstream signalling.

To identify cellular proteins that associate with RIG-I, the authors carried out pull-down experiments with a fusion construct of the two CARDs (caspase-recruitment domains) of RIG-I tagged with GST (glutathione S-transferase). They first noticed that the RIG-I fusion construct was present in various ubiquitylated forms, and that the level of ubiquitylation increased on infection of transfected cells with Sendai virus. Site-directed mutagenesis studies revealed that just one of the 18 lysine residues of the RIG-I CARDs was mainly targeted for ubiquitylation. Replacing the lysine at position 172 with an arginine caused near-complete loss of ubiquitylation of the RIG-I fusion construct. Importantly, transfection of cells with the Lys172Arg RIG-I mutant construct resulted in reduced interferon-β (*IFNB*) and nuclear factor-κB (*NFKB*) promoter activity, indicating that ubiquitylation of Lys172 is crucial for RIG-I-induced signal transduction.

The pull-down experiments with the RIG-I CARDs construct also revealed that one of the proteins that associates with RIG-I was TRIM25, and that this interaction was dependent on the carboxy-terminal SPRY domain of TRIM25 interacting with the CARDs of RIG-I. Being an E3 ubiquitin ligase, TRIM25 seemed an ideal candidate for ubiquitylating RIG-I. Indeed, co-expression of RIG-I with wild-type TRIM25, but not with an E3-ubiquitin-ligase-defective form of TRIM25, increased ubiquitylation of RIG-I, and knockdown of *TRIM25* expression using *TRIM25*-specific small hairpin RNA reduced RIG-I ubiquitylation in a dose-dependent manner. Moreover, the level of TRIM25-mediated ubiquitylation of RIG-I correlated with the level of *IFNB* and *NFKB* promoter activity.

Further studies confirmed that TRIM25 targeted Lys172 of RIG-I for ubiquitylation, as the Lys172Arg RIG-I construct was minimally ubiquitylated when co-expressed with TRIM25 and a Lys172-only RIG-I construct (which contains five Lys→Arg substitutions but has Lys172 intact) underwent robust ubiquitylation. Transfection of RIG-I-deficient fibroblasts with this Lys172-only RIG-I construct was also capable of inducing IFNβ production in response to viral infection. The importance of Lys172 ubiquitylation in RIG-I signal transduction was further highlighted by the observation that MAVS only efficiently associated with RIG-I constructs that contained this lysine residue.

Last, the authors confirmed a crucial role for TRIM25-mediated ubiquitylation of RIG-I in the induction of antiviral responses by showing that *Trim25*$^{-/-}$ mouse embryonic fibroblasts produced lower amounts of IFNβ in response to infection and supported 100-fold higher viral replication than wild-type and *Trim25*$^{+/-}$ fibroblasts.

This study reveals a crucial role for TRIM25-mediated ubiquitylation of RIG-I in facilitating the interaction of MAVS with RIG-I, which ultimately leads to the induction of antiviral immunity.

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ORIGINAL RESEARCH PAPER Gack, M. U. *et al.* TRIM25 RING-finger E3 ubiquitin ligase is essential for RIG-I-mediated antiviral activity. *Nature* 28 March 2007 (doi:10.1038/nature05732)