Interdisciplinary Research Unveils How SID-1 Recognizes dsRNA and Initiates Systemic RNAi

RNA interference (RNAi) is a fascinating biological process in worms, plants, fungi, and metazoans that has been used as a valuable tool in studying gene functions and as therapeutics. In *Caenorhabditis elegans*, the multipass transmembrane protein, systemic RNA interference defective protein 1 (SID-1), has an indispensable role in the uptake and spreading of double-stranded RNA (dsRNA) between cells and tissues, resulting in systemic RNAi. In addition, two human SID-1 homologs, SID1 transmembrane family member 1 (SIDT1) and SIDT2, were suggested to be involved in RNA transport.

However, the underlying molecular mechanisms of how SID-1 specifically distinguishes dsRNA from single-stranded RNA (ssRNA) and DNA and facilitates subsequent dsRNA transport between cells remain unknown. The answers to those questions would be important for understanding systemic RNAi and aiding in RNA-related applications. Recently, Dr. ZHANG Jiangtao from Prof. JIANG Daohua's group at the Institute of Physics (IOP), Chinese Academy of Sciences (CAS), by combining cryo-EM, *in vitro*, and *in vivo* experiments, demonstrated how SID-1 specifically recognizes dsRNA and provided important insights into the internalization of dsRNA by SID-1.

For over two decades, SID-1 was thought to act as a dsRNA channel. Here, the researchers solved high-resolution cryo-EM structures of SID-1 and the human SID-1 homologs SIDT1 and SIDT2 (Figure 1a), which revealed the conserved architecture of *C. elegans* and human SID-1 homologs. The SID-1 homologs are organized in a homo-dimeric fashion. Surprisingly, the SID-1 dimer does not show any obvious pore within the transmembrane domain (TMD), suggesting that

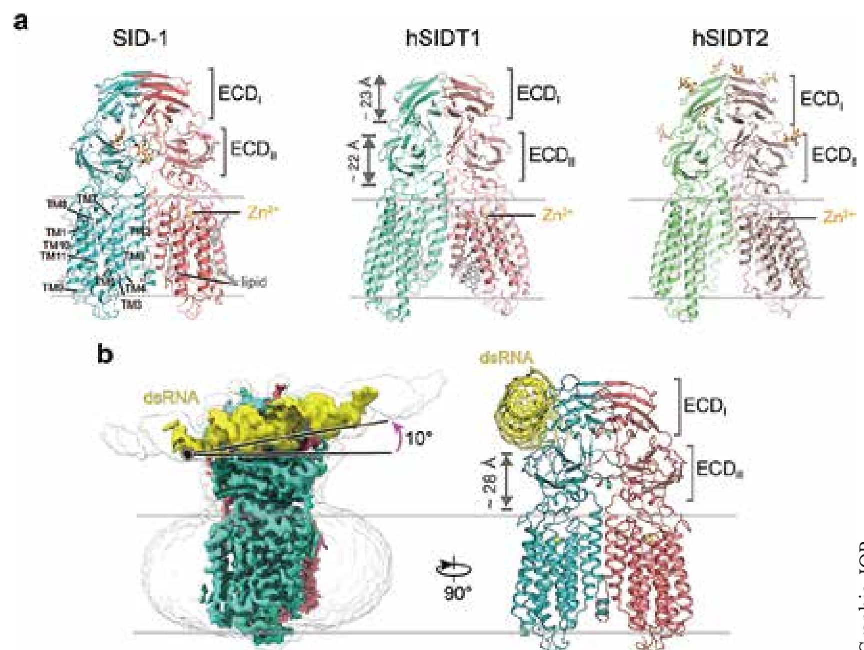

Figure 1: Cryo-EM structures of SID-1 homologs (a) and the SID-1/dsRNA complex (b).

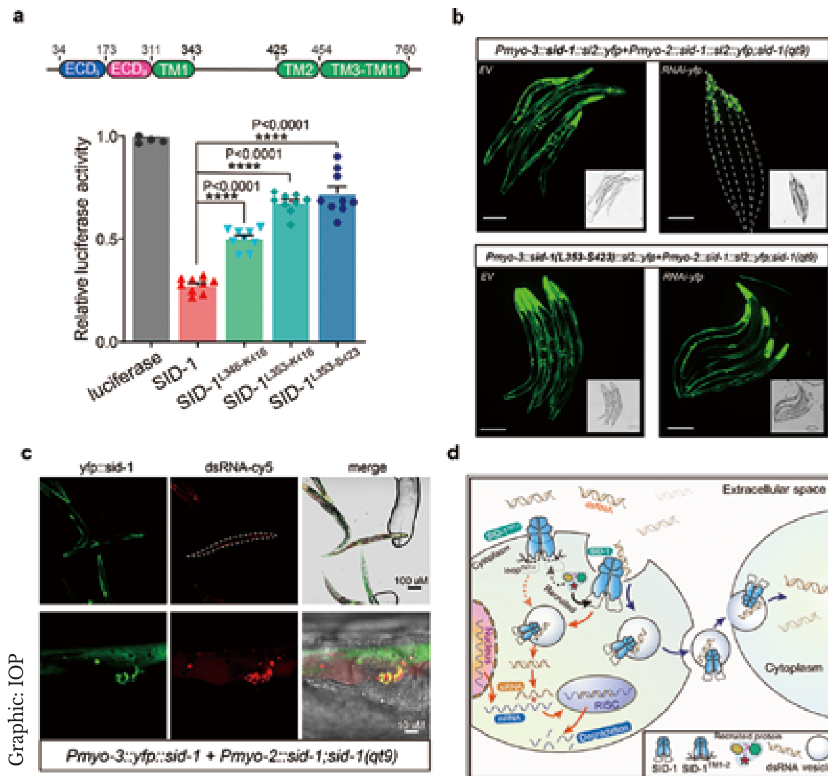

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Figure 2: a. dsRNA uptake in S2 cells of SID-1^{TM1-2} mutants; b. Defects in systemic RNAi of SID-1^{TM1-2}; c. The co-localization of SID-1 and dsRNA; d. The proposed model of the internalization of dsRNA by SID-1.

SID-1 may not function as a dsRNA channel. MST binding assays confirmed that SID-1 can potently and specifically bind to dsRNA but not dsDNA.

Subsequently, the researchers obtained the cryo-EM structure of the SID-1–dsRNA complex (Figure 1b), demonstrating the detailed dsRNA binding mode and the molecular determinants for how SID-1 distinguishes dsRNA from ssRNA and DNA. Interestingly, such determinants are not present in human SIDT1 or SIDT2. The structural findings were supported by mutagenesis studies using MST binding assays, dsRNA uptake in S2 cells, and *in vivo* systemic RNAi assays.

Finally, the researchers showed that the removal of the long intracellular loop transmembrane helices 1 and 2 did not affect SID-1 dimerization, cell localization, or dsRNA binding, but it significantly impaired dsRNA uptake in S2 cells (Figure 2a) and systemic RNAi in *C. elegans* (Figure 2b). Moreover, co-localization revealed that SID-1 and dsRNA co-locate in vesicle-like subcellular organelles (Figure 2c). Based on these results, the researchers proposed that SID-1 functions as a dsRNA receptor and facilitates subsequent dsRNA internalization via recruiting endocytic related proteins using the long loop (Figure 2d).

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