Team learns how cellular protein detects viruses and sparks immune response

February 19 2009

A study led by researchers at the University of Illinois reveals how a cellular protein recognizes an invading virus and alerts the body to the infection.

The research, described this week in the journal *Science* and led by Illinois physics professor and Howard Hughes Medical Institute investigator Taekjip Ha, settles a debate over how the protein, RIG-I (pronounced rig-EYE), is able to distinguish between viral RNA and self (or cellular) RNA.

"RIG-I is the first molecule in the immune response to detect viral RNA," said Sua Myong, lead author on the study and a professor at the U. of I.'s Institute for Genomic Biology. Unlike most other proteins known to detect viral infections only in specialized immune cells, RIG-I is active in every cell type in the body, she said.

The RIG-I protein has two major parts: caspase-recruitment domains (CARDs) and an ATPase domain that consumes ATP, the cellular fuel molecule.

Previous studies had shown that the CARD domains actually inhibit the activity of RIG-I when no virus is present, but are vital to sounding the alarm and triggering an immune response once a certain type of virus has been detected.

Other studies had found that RIG-I recognizes an important feature of

viral RNAs that is missing from most human RNAs. This feature, a "triphosphate" tag at a particular end, the "five-prime" (5') end, of viral RNA, is a viral fingerprint that tells RIG-I that something is amiss. Detection of this tag starts a cascade of reactions that allows RIG-I to broadcast a message to other cellular components, and ultimately to other cells.

The researchers also knew that RIG-I was usually active in the presence of
double-stranded RNA, not the single-stranded RNA found in most animal cells.

Earlier research had also shown that the central ATPase domain is critical to the function of the molecule. A single mutation in this region shuts down its activity altogether.

"We knew that the CARD domain was responsible for transmitting the antiviral signaling," Myong said. "And we knew how the 5'-triphosphate tag is detected. But a big question remained about the ATPase domain: It was using ATP to do something - but what?"

To solve that mystery, the researchers used a technique termed "protein-induced fluorescent enhancement." This method makes use of a fluorescent dye that, when attached to a specific region of a molecule such as RNA, glows with more or less intensity depending on its proximity to a protein that is interacting with that molecule.

Using this technique, the researchers found that the RIG-I protein moves back and forth (translocates) selectively on double-stranded RNA, and that this activity is greatly stimulated in the presence of 5'-triphosphate.

By requiring both the 5'-triphosphate and the double-stranded RNA for it to function, the RIG-I protein is able to very accurately detect a viral

invader, said Ha.

Most cellular RNAs have their triphosphate tails bobbed, capped or otherwise modified before circulating in the cytosol of the cell, he said. "So this is one powerful way of distinguishing viral RNA from cellular RNA."

Prior to this study, researchers did not know if RIG-I sensed both the double-stranded RNA and the 5'-triphosphate separately, or in an integrated manner, said Myong.

"Our work bridges the gap," she said. "We show that it does both in an integrated manner."

Source: University of Illinois at Urbana-Champaign