How cells use sophisticated signaling mechanisms to control production of interferon

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The immune system has a delicate balance to maintain. When certain infected cells detect an invader, they use a molecule called interferon to rally the body's defenses. The immune system responds to this rallying cry by immediately boosting its general antiviral defenses and simultaneously initiating a more specialized secondary response. But interferon production must be finely tuned: Too much can provoke immune cells to attack the body's own cells indiscriminately.

Type I interferon plays such an important role in immune defense that the body has three known pathways to trigger its production in response to microbial infection. New research by Howard Hughes Medical Institute (HHMI) scientists has found that all three pathways use a common mechanism to communicate with the protein that switches on type I interferon-producing genes.

"Induction of type I interferons has to be tightly regulated, because overproduction can lead to autoimmune diseases like lupus," says Zhijian 'James' Chen, an HHMI investigator at the University of Texas Southwestern Medical Center who led the research, which was published January 30, 2015, in the journal *Science*. Indeed, mutations in many of the genes involved in these interferon-inducing pathways have been linked to autoimmune diseases in humans. Better understanding of the signaling cascade that triggers interferon production could help scientists develop improved treatments for these diseases in the future, Chen says.

Chen explains that each of the three type I interferon-triggering pathways recognizes a particular signal of infection. Invading viruses and bacteria often deliver and replicate their genetic material in the main compartment of the cell known as the cytoplasm, where host DNA is not normally found. A sensor protein called RIG-I detects viral RNA in this cytosolic compartment, which usually indicates the presence of an RNA virus. Cytosolic DNA, which can be introduced by a variety of microbes, including bacteria, DNA viruses, and retroviruses, is detected by a sensor called cGAS, which Chen's lab discovered in 2012. Nucleic acids in membrane-bound compartments called endosomes also indicate viral infection, and are detected by sensors called Toll-like receptors.

Each of these three receptors cooperates with its own adaptor protein to relay its message that an invader is present and interferon is needed. Toll-like receptors work with an adaptor called TRIF, the cGAS receptor works with the adaptor protein STING, and the RIG-I receptor pairs with an adaptor that Chen's lab discovered in 2005 called MAVS.

Chen says scientists had suspected that all three adaptor proteins relayed their message by activating the same protein, an enzyme called TBK1 that is essential for the induction of type 1 interferons. But TBK1 can be activated by other proteins without triggering interferon production. Chen and his colleagues suspected there must be something else going on.

Siqi Liu, a graduate student in Chen's lab, made a key observation: a site in the MAVS protein that becomes tagged with a chemical group called a phosphate (when the adaptor is stimulated) resides in a tiny segment of the protein that strongly resembles a segment of the STING protein. The motif, which contains three identical amino acids in a five amino acid stretch, is so short that it would have been very difficult to find through computational analysis. "If you don't know what you're looking for, it's very hard to find," Chen says.

"But Siqi actually recognized it with her eyes."

Once they had identified the motif, the researchers searched for it in other proteins. Sure enough, MAVS and STING proteins from humans and other mammals that they analyzed all contained the motif. It was also present in TRIF, the adaptor protein that works with Toll-like receptors, as well as in IRF3, the transcription factor that turns on interferon production.

In a series of biochemical experiments, the scientists showed that when the infection-detecting proteins RIG-1 and cGAS signal to their respective adaptor proteins, MAVS and STING, the shared motif becomes phosphorylated. This summons IRF3 to the adaptor protein so that it can be activated. "Basically, phosphorylation of the adaptor protein provides the license for IRF3 to be activated by TBK1," Chen explains.

The biochemical experiments were conducted in a test tube, which allowed the researchers to examine the proteins' interactions in fine detail. To test whether those interactions actually affected cells' ability to respond to infection, they conducted a new set of experiments in which they manipulated proteins from the signaling pathways inside cells, depleting them with genetic tools or replacing them with mutated versions. Their findings supported their model not only for the MAVS and STING pathways, but also for the Toll-like receptor/TRIF pathway, Chen says.

"We have provided a mechanism that explains how this key transcription factor is activated by three distinct pathways known to induce type I interferons," Chen says. Now, he says, his team plans to examine that mechanism in more detail, with further biochemical analyses and structural studies. Chen is also interested in developing small molecules that interfere with the interactions his team has uncovered. "If we can generate inhibitors of these pathways, these molecules might be used as therapeutic agents to treat autoimmune diseases in the future," he says.

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