

APRIL 16, 2010

An Immune Response in a Test Tube

A molecule best known for fighting off cellular clutter is now recognized as an important defender against another cellular threat: viruses. New research from HHMI investigator Zhijian Chen shows that ubiquitin, which helps cells identify unwanted proteins so that they can be removed, is also a vital component of the innate immune system.

The body's first response to infection rallies elements of the innate immune system, which responds to infection uniformly and with limited specificity. Innate immune components provide a general defense, employing molecular mechanisms much like those of antibiotics or broad-spectrum antiviral drugs. In vertebrates, this early response is bolstered by the more targeted adaptive immune system, which kicks in later. Although innate immunity is evolutionarily ancient – present not just in animals, but also in plants, fungi, and other primitive organisms -- biologists are only beginning to discover how it works.

In the April 16, 2010, issue of the journal *Cell*, Chen, and his colleagues report that they have reconstructed in a test tube an important pathway the innate immune system uses to fend off viruses, and used their model to begin understanding how this natural defense system works.

"This work uncovers a fundamental mechanism of immune defense against many forms of viral infection – influenza, hepatitis, SARS, West Nile, and others."

- Zhijian "James" Chen

According to Chen, this is the first time an immune pathway that has been created in a test tube to mimic all of the events that occur between the detection of a microbial molecule – viral RNA – and the activation of a transcription factor – IRF3. As such, he says, it will be a powerful tool for dissecting out exactly how the innate immune system responds to viruses, he says.

Already, the research provides surprising evidence that ubiquitin -- a protein best known for its role in ridding cells of corrupt or unneeded proteins -- plays a key role in defending cells against invading viruses. "This work uncovers a fundamental mechanism of immune defense against many forms of viral infection -- influenza, hepatitis, SARS, West Nile, and others," Chen said. "We believe this mechanism likely operates in most human cells."

For decades, ubiquitin was thought to have only one function—tagging other proteins for destruction by the cell's proteasome, a structure that acts as a garbage disposal for unwanted proteins. But work from several groups, including Chen's lab at the University of Texas Southwestern Medical Center, is making it clear that evolution has put the small, seemingly ubiquitous molecule to work in other capacities. They have shown that its activity is central to both branches of the immune system, and there are also hints that ubiquitin may contribute to cell growth and cancer progression.

The first indication that ubiquitin could do more than tag unwanted proteins came in 2000 with Chen's discovery that it can switch on a major immunity and cell-growth signaling system known as the NF-kappa-B pathway. A single ubiquitin molecule is not enough to switch on the pathway, but Chen and his colleagues found that when several ubiquitin molecules join forces, linking to a target protein and to each other via a specific site known as K63 (an amino acid called lysine, the 63rd amino acid in the ubiquitin protein), they can activate NF-kappa-B. This mechanism for adding a chain of ubiquitins to a protein is called K63 polyubiquitination, and is distinct from the way in which a cell builds the ubiquitin chain that flags unwanted proteins for destruction.

In the current study, Chen's group set out to determine how ubiquitin contributes to the innate immune system's antiviral defenses. Their focus was on an immune protein called RIG-I, which binds to the RNA of viruses that have invaded cells. When bound to RNA, RIG-I triggers a cascade of signals that activate the NF-kappa-B pathway, as well as an antiviral protein called IRF3, which increases the production of immune-stimulating interferon proteins.

To tease out the role of each of the components of the RIG-1 pathway, Chen's team reconstructed that pathway inside a test tube. Coordinating a rapid immune response to a viral invader requires the cooperation of many cellular components, all of which had to go into the test tube. The team began building their test-tube immune system by adding RIG-I, the intracellular fluid known as cytosol, and the power-producing organelles called mitochondria. All of these components help alert the innate immune system that a virus is present. These alone, however, were not enough to switch on IRF3 to summon the innate immune system to action.

Since previous research had suggested that RIG-I might also need K63 polyubiquitination to become active, Chen's team added in ubiquitin and the enzymes that could construct the K63 chains. These molecules, they discovered, were key to setting off the events needed to fight a virus.

"These K63 polyubiquitin chains are present in human cells, and likely all mammalian cells, and are extremely potent in activating RIG-I in this antiviral pathway," Chen said.

Chen's team found that free-floating K63 chains made of several ubiquitin proteins could bind tightly to a portion of RIG-I known as the CARD domain, most likely after a viral RNA has bound to a different domain and thereby made it more accessible. Chen says the ubiquitin chains activate viral-RNA-bound RIG-I so potently that in a cell, only few would be needed to trigger RIG-I signaling.

Scientists have long thought that ubiquitin signaling occurs when a ubiquitin chain is built one molecule at a time, beginning with the attachment of a single ubiquitin molecule to a target protein. So the activation of RIG-I by free K63 polyubiquitin chains is a bit surprising--but it's not the first time Chen's group has observed this strategy phenomenon. In work published in *Nature* last year, Chen and his colleagues reported that free-floating K63 ubiquitin chains activate the NF-kappa-B pathway. "So now we have two examples showing that these free polyubiquitin chains can activate signaling cascades," said Chen. "We think that they represent a new class of intracellular signaling molecules."

Chen's team plans further experiments with the reconstituted RIG-I pathway. "With this system, we'd like to go downstream in the signaling cascade and try to understand the mechanisms of activation at each stage," he said. "So there's still a lot of work left to do."