

Scientists Identify New Regulatory Mechanism for Critical Protein Signaling Domain

By Eric Sauter

In a study with far-reaching implications, scientists at The Scripps Research Institute and other institutions have for the first time identified a new *in vivo* regulatory mechanism for the PH Domain, a component of many proteins that allows them to move from a cell's interior to the cell membrane in response to stimulation of cell surface receptors. The findings offer a promising avenue for the development of novel therapies for immunodeficiency or autoimmune diseases.

The study is being published in *Science Express*, an advanced online edition of the journal *Science*, on April 5. It will appear in the print version of *Science* later this spring.

In findings the authors called "unexpected and striking," the study found that a new regulating messenger IP_4 , a small soluble molecule, augments the binding of three different PH domain proteins to one of the most commonly recognized membrane lipids, PIP_3 . The study also showed that inhibiting production of IP_4 can result in reduced protein binding to membranes and reduced activation of key signaling molecules in developing T cells, leading to a block of T cell maturation and to severe immunodeficiency in animal models.

"This study changes how we think about the T cell receptor signaling process and a well established general signaling mechanism, protein recruitment to membranes through PH domains," said Karsten Sauer, a Scripps Research scientist who led the study. "For the first time, we have clearly established a new way through which the PH domain can be positively regulated *in vivo*— IP_4 augments the binding of PIP_3 to these domains. In fact, it resembles PIP_3 . Until our study, the widely accepted idea was that recruitment of PIP_3 binding PH domains to the membrane was primarily regulated by the supply and turnover of PIP_3 . The second completely new finding is that PH domain proteins can form aggregates through their PH domains. PH domain aggregation may enhance the membrane binding process."

Sauer suggests models for how IP_4 might augment the binding of PIP_3 . In one, IP_4 binds to a PH domain, changing its structure to one with a high affinity for IP_4 and PIP_3 , a process commonly known as the "induced fit" model. In the second model, the PH domain pre-exists as an aggregate; IP_4 binding to one subunit forces changes in the other subunit(s) so that they bind PIP_3 with greater ease. This process is commonly known as an allosteric or cooperative mechanism.

Sauer offers a helpful analogy for the newly uncovered mechanism: " IP_4 acts very like a clever engineer. If you were trying to dock your ship at a space station but found that the docking stations weren't fully compatible, the station would send over an engineer called IP_4 and he would reconfigure your dock to fit the space station."

Repercussions for T Cell Development

Signaling from T cell receptors triggers the generation of PIP_3 and IP_4 , Sauer explained, leading to the recruitment of proteins to the cell membrane from the cytosol, the internal part of the cell. This receptor signal is part of the mechanisms by which T cells, key players of the immune system, protect us against attack by



"This study changes how we think about the T cell receptor signaling process and a well established general signaling mechanism," said Scripps Research Assistant Professor Karsten Sauer. Photo by Kevin Fung.

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A Place Called Discovery

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pathogens such as viruses. However, defective T cell development can lead to a number of immune diseases including allergies like asthma and autoimmune diseases like rheumatoid arthritis, while severely reduced T cell development or function can lead to immunodeficiency diseases such as AIDS.

Using a mouse mutant lacking *ItpkB*, the enzyme that produces IP_4 , Sauer and his colleagues showed that without the enzyme, T cells could not develop to maturity.

Because T cell development is regulated by receptor signaling, Sauer said, if the T cell doesn't signal properly, it is normally killed through programmed cell death or other mechanisms. In immature T cells—called double positive cells—if the signal is correct, the cells undergo positive selection in the thymus and are allowed to develop into mature CD4 or CD8 single positive (SP) T cells. Damaged or nonfunctional cells are eliminated.

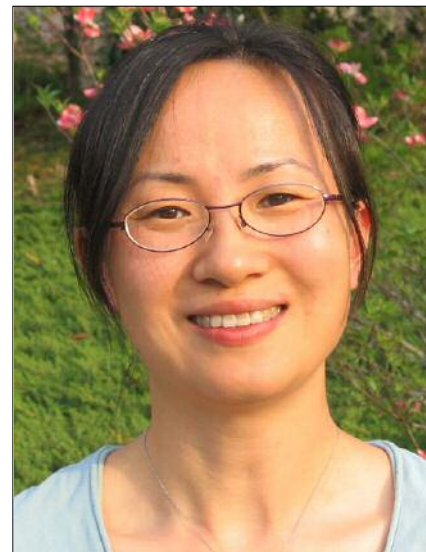
" IP_4 is generated after T cell receptor stimulation," he said. "We found that if IP_4 cannot be made due to lack of *ItpkB*, important signaling molecules including the protein tyrosine kinase *Itk* can not be properly recruited to the cell membrane. *Itk* is a key activator of another enzyme, *PLC γ 1*, in T cells. *PLC γ 1* is important for signaling in many cells, because it generates the secondary messenger molecules IP_3 and DAG (diacylglycerol). We found that *ItpkB* deficient double positive cells have reduced *PLC γ 1* activity and cannot make normal amounts of DAG. Without the IP_4 or DAG messengers, which are essential for positive selection of T cells, these *ItpkB*-deficient T cells cannot develop into mature, functional cells."

Positive selection of T cells is disrupted by this lack of IP_4 and DAG in the mutant mice, the study noted. Quite remarkably, the authors could rescue several aspects of positive selection by treating *ItpkB* deficient cells with a DAG analog, PMA. DAG is well known for helping to mediate T cell receptor signaling by activating the Ras/Erk pathway, a key signal pathway whose misregulation is often involved in cancer. It is still too early to tell if IP_4 plays a broader role in Ras regulation in other cells, Sauer said.

"This is something we stumbled into—the possibility of regulating the Ras pathway with IP_4 at the level of DAG production through *PLC γ 1*," he said. "In the immature, double positive T cells, this circuitry is essential for positive selection. If you prevent IP_4 production, you can never get positive selection—unless you provide a DAG analog like PMA—and that is a very provocative discovery."

Other authors of the study, "Positive Regulation of *Itk* PH Domain Function by Soluble IP_4 ," are first author Yina H. Huang of The Scripps Research Institute; and co-authors Juris A. Grasis and Constantine D. Tsoukas of San Diego State University; Andrew T. Miller, Stephen Soonthornvacharin and Michael P. Cooke of the Genomics Institute of the Novartis Research Foundation; and Ruo Xu and Amy H. Andreotti of Iowa State University.

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Research Associate Yina Huang was first author of the study, which was published in *Science Express*.

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