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## Unscrambling B-cell signals

*RIKEN researchers uncover new roles for old players in a complex web of cross-talking proteins important in immunity and cancer.*

Coordination of cell functions is a bewilderingly complex process. In the latest issue of *The Journal of Experimental Medicine*, scientists led by Tomohiro Kurosaki at RIKEN's Research Center for Allergy and Immunology report findings that improve our understanding of a key communication network within immune cells.

B cells are the key immune system agents that create antibodies. One way that B cells can respond to external stimuli is through a surface protein called the B cell receptor (BCR). When a specific antigen (a molecule that stimulates an immune response) docks in the BCR, sparking a signal transduction event, in which a cascade of biochemical reactions among enzymes occurs that regulates immune responses, inflammation and cell survival.

One such signal transduction pathway involves the Nuclear factor- $\kappa$ B (NF $\kappa$ B) and inhibitor of Nuclear factor- $\kappa$ Bkinase (IKK) families of proteins, which are major players in cell signaling. As DNA-binding proteins regulating gene expression, the ubiquitous NF $\kappa$ B proteins activate a large number of genes that eventually lead to complex physiological and biochemical events, dramatically amplifying the original signal. When NF $\kappa$ B-signaling goes awry, various immune disorders and cancers may ensue. Hence, the intricate molecular mechanisms by which BCR engagement is coupled to NF $\kappa$ B activation have been subjected to intense scrutiny.

In all non-stimulated cells, NF $\kappa$ Bs are kept in check by being bound to IKK proteins and sequestered outside the nucleus, away from the genes. When activated by various stimuli, the IKKs are modified by phosphorylation - the addition of phosphate groups, by enzymes called kinases, to the side arms of amino acids, the building blocks of proteins, which leads to their degradation by the cell's house-keeping processes. The liberated NF $\kappa$ Bs can then enter the nucleus to regulate many genes.

Clearly, IKK - a complex of at least three protein kinases, IKK $\alpha$ , IKK $\beta$  and IKK $\gamma$  - represents a major control point, one affected by various adaptor proteins. These

include CARMA1, a scaffolding protein that brings together Bcl10, a bridge-like protein, and an effector protein, MALT1, into an assemblage (CBM complex) working in concert to stimulate the IKK complex. For B-cells, PKC $\beta$ , another kinase, acts upstream of the CBM complex to activate IKK, but the exact molecular mechanisms involved, including how CARMA1 becomes phosphorylated, and thus activated, to mobilize the assembly of the CBM and IKK complexes, remain undefined. The RIKEN researchers focused on clarifying how this crucial piece fits into the intricate NF $\kappa$ B/IKK puzzle.

Kurosaki and colleagues used special chicken cells expressing BCR to explore the specific regions of the CARMA1 protein important for IKK activation. These experiments hinged on engineered antibodies that identify specifically phosphorylated amino acids, allowing the scientists to eventually pinpoint critical CARMA1 amino acids that are phosphorylated during BCR engagement. By systematically deleting from these chicken cells the activities of several suspected kinases, the researchers concluded that PKC $\beta$  phosphorylated one of these CARMA1 amino acids, Ser668, whereas IKK $\beta$  was responsible for phosphorylating another amino acid, Ser578.

To confirm these results, the RIKEN group tested whether the CARMA1-directed enzymatic deficiencies in these chicken cells could be rescued by adding external, active, sources of these specific enzymes into the cells. As expected, phosphorylation of Ser668 was restored by active PKC $\beta$ , while re-introduced, active, IKK $\beta$  phosphorylated Ser578. IKK $\beta$ 's role upstream of the IKK complex is particularly intriguing. A biochemical examination verified that CBM complex assembly is dependent on Ser578 phosphorylation. These findings were further supported with the significant inhibition of CBM assembly observed in cells containing a CARMA1 mutant harboring a different amino acid in the Ser578 position.

The RIKEN scientists theorize that IKK $\beta$  plays more complex feedback roles in NF $\kappa$ B-signaling than previously believed. A deeper understanding of these critical switches will shape the development of therapeutic agents for autoimmune disorders and cancers.

**Original work:**

Shinohara, H., Maeda, S., Watarai, H., Kurosaki, T. I $\kappa$ B kinase  $\beta$ -induced phosphorylation of CARMA1 contributes to CARMA1-Bcl10-MALT1 complex formation in B cells. *The Journal of Experimental Medicine*, published online on December 17,

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