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SPOTLIGHT

Phollow the phosphoinositol: Actin dynamics at the B cell immune synapse

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Actin remodeling promotes B cell activation by enabling B cell antigen receptor clustering in the immune synapse. In the current issue of *JCB*, Droubi et al. (2022. *J. Cell Biol.* https://doi.org/10.1083/jcb.202112018) find that this process is initiated by the lipid phosphatase INPP5B, which shapes synaptic actin architecture by locally depleting phosphatidylinositol 4,5 bisphosphate.

Leukocytes rely on stereotyped interfaces called immune synapses to regulate signal transduction, establish cell polarity, and internalize and release biomolecules. In B cells, synapses are essential for the recognition and uptake of viral and bacterial antigens (1). Synapse formation also drives B cell differentiation into antibody producing plasma cells or memory B cells, and it plays a critical role in somatic hypermutation, the process by which B cells generate mature, high affinity antibodies.

Synapse formation in B cells is induced by the recognition of multimeric or surface bound antigen by the B cell receptor (BCR), a transmembrane form of the B cell's own immunoglobulin (2). This drives myriad intracellular signaling events that dramatically alter B cell physiology and promote differentiation. A key step in this process is the formation of BCR microclusters in the plasma membrane, which facilitates signal amplification by concentrating receptors and associated kinases. In their resting state, BCRs are embedded in the cortical filamentous (F)-actin cytoskeleton, which constrains their mobility (3). Receptor engagement triggers transient F-actin disassembly, enabling the coalescence of BCRs into microclusters (4). Despite intensive research, the mechanisms controlling this cytoskeletally gated remodeling process remain poorly understood.

In this issue of *JCB*, Droubi et al. (5) identify the inositol 5-phosphatase INPP5B as a critical regulator of BCR-induced F-actin disassembly. To study the function of this enzyme in the DT40 B cell line, the authors implemented an inducible degradation system in which they inserted an auxin degron tag in the INPP5B gene. The resulting B cells could then be depleted of INPP5B by 1 h of auxin treatment. This elegant approach enabled the authors to drill down on the acute functions of INPP5B while mitigating potential artifacts arising from the comparison of genetically modified cells with their parental lines. In this manner, they were able to demonstrate that INPP5B is required for BCR clustering, B cell spreading on antigen-coated surfaces, and downstream signaling.

INPP5B hydrolyzes phosphatidyl inositol 4,5 phosphate (PIP_2), a signaling lipid that interacts directly with several critical cytoskeletal regulators (6). Using a protein biosensor for PIP_2, Droubi et al. (5) found that this lipid strongly accumulated in the immune synapse at early timepoints. As the synapse spread, however, PIP_2 was depleted from plasma membrane regions containing nascent microclusters of BCR. Importantly, BCR clustering was also associated with local depletion of F-actin, implying that PIP_2 controls BCR dynamics through the cytoskeleton. Consistent with this interpretation, the redistribution of both PIP_2 and F-actin was dramatically suppressed in INPP5B-deficient cells, leading to defective BCR clustering and attenuated synapse growth. These results strongly suggest that INPP5B-mediated PIP_2 hydrolysis promotes BCR cluster formation by inducing the depletion of synaptic F-actin.

Given the close spatiotemporal correlation between INPP5B-induced PIP_2 hydrolysis and F-actin depletion, Droubi et al. (5) next examined PIP_2-regulated proteins with established roles in cortical F-actin dynamics. Cofilin contributes to F-actin turnover by severing existing filaments (7), whereas ezrin stabilizes interactions between the plasma membrane and the cortex by physically coupling F-actin to PIP_2 and to transmembrane proteins like CD44 (8). Both molecules are regulated by phosphorylation, which inhibits the F-actin severing activity of cofilin while promoting the crosslinking function of ezrin. Intriguingly, both proteins were highly phosphorylated in INPP5B deficient cells, indicative of reduced F-actin turnover and increased cytoskeletal stability. Hence, it seems likely that INPP5B-

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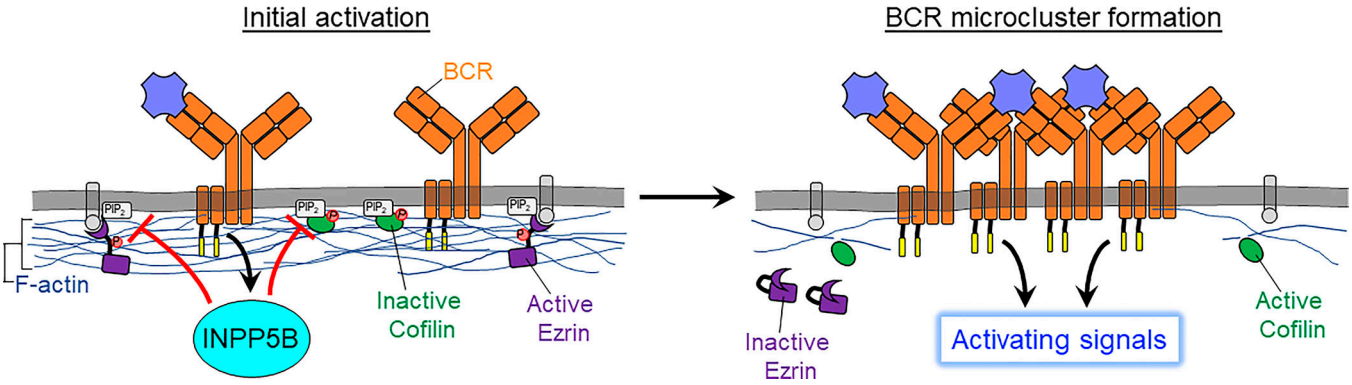

Figure 1. **Schematic diagram of INPP3B dependent BCR clustering.** Initial antigen recognition by the BCR activates INPP5B, which hydrolyzes PIP_2 in the plasma membrane. This promotes local F-actin disassembly via regulation of cofilin and ezrin, enabling BCRs to coalesce into microclusters.

induced cytoskeletal remodeling in B cells involves the downregulation of cofilin and the concomitant upregulation of ezrin (Fig. 1).

This study is important because it advances a compelling model for the initiation of BCR clustering at the immune synapse. It also establishes INPP5B as a novel potential drug target for treating B cell leukemia, many forms of which are associated with dysregulated BCR signaling. Several open questions remain, however. Perhaps the most obvious of these is how INPP5B might be targeted to the synapse for local PIP_2 depletion. Outside of its phosphatase core, INPP5B contains several domains that could control its subcellular localization. In evaluating the contribution of each module, future investigators could potentially exploit the observation made by Droubi et al. (5) that the closely related phosphatase OCRL plays no role in BCR clustering. One could imagine employing a series of INPP5B-OCRL chimeras to identify critical functional determinants.

It will also be important to determine the precise role of INPP5B signaling in the context of other established lipid second messenger pathways. Most relevant to the present study is prior work demonstrating that phospholipase C-γ2 (PLC-γ2), which converts PIP_2 to diacylglycerol, also promotes BCR clustering at the immune synapse (9). That both INPP5B and PLC-γ2 are required for proper synapse assembly implies that these enzymes' functions are nonredundant. Droubi et al. (5) propose a temporal model in which INPP5B-mediated PIP_2 hydrolysis induces initial BCR microcluster formation, while PLC-γ2, which is recruited directly to the microclusters, promotes the growth and maintenance of these structures. It is also possible that the two enzymes confer distinct functionalities by virtue of their products, phosphatidylinositol 4-phosphate and diacylglycerol, which could influence synapse maturation in different ways.

Finally, the extent to which INPP5B regulates the subsequent steps of antigen extraction and presentation remains to be determined. It is thought that cortical F-actin remodeling around BCR clusters lays the groundwork for their internalization, which is a complex biomechanical process involving the regulated assembly of uptake machinery and the strategic exertion of interfacial pulling forces (10, 11). B cells target lysosomes to the synapse as well, which release degradative enzymes that facilitate antigen extraction (12). The role(s) of lipid second messengers in this coordinated endo- and exocytosis are poorly understood, but will likely be critical given the complex choreography of cytoskeletal and membrane components that occurs.

Dissecting these processes in the context of B cell antigen uptake could potentially contribute to our understanding of other immune cell interactions as well. For example, polarized membrane dynamics are critical for cytotoxic T lymphocytes and natural killer cells, which form immune synapses with infected or transformed target cells and then kill them by releasing toxic perforin and granzyme into the interface. Cytotoxic secretion occurs at specific synaptic domains defined by the local depletion of F-actin and the engagement of mechanically active integrins (13, 14). It will be interesting to see if local lipid micropatterning, of the sort characterized by Droubi et al. (5), plays a role in this process and in other critical immune cell functions that require localized membrane dynamics. The tools and concepts featured in the present study could in this way contribute to our understanding of not only how synapses are formed but also how they mediate subsequent effector responses.

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