functionally important members will therefore remain a major challenge in epithelial cell biology.

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The Immunological Synapse and Actin Assembly: A Regulatory Role for PKCθ

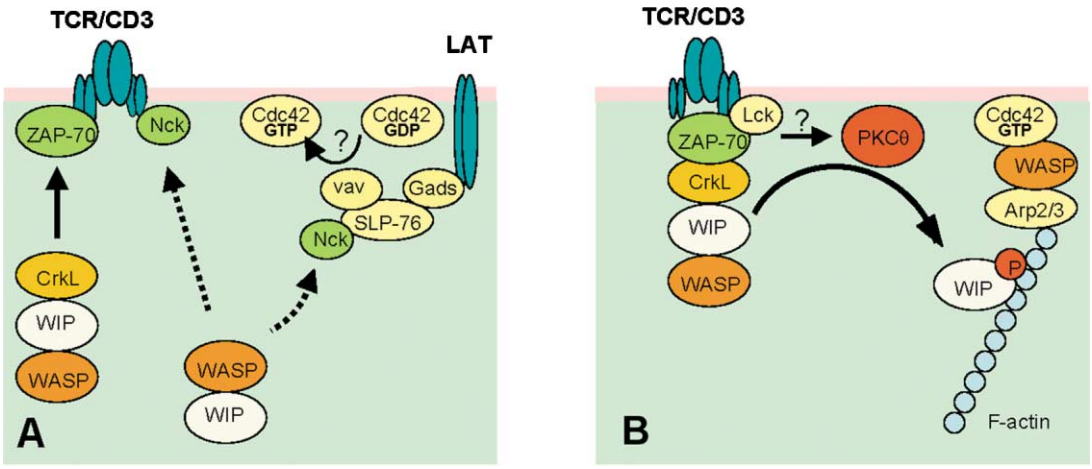
Engagement of the T cell receptor leads to the accumulation of filamentous actin, which is necessary for the formation of the immunological synapse and subsequent T cell activation. In the December issue of *Molecular Cell*, Sasahara et al. provide new insights into the link between the T cell receptor and actin assembly in the immunological synapse, and reveal a critical regulatory role for PKCθ in this process.

When a mature T cell encounters an antigen-presenting cell (APC), the sequential engagement of adhesion receptors and of the T cell receptor (TCR) with MHC-peptide complexes on the surface of the APC initiates the formation of a specialized cell-cell junction that is called the immunological synapse (IS) or the supramolecular activation cluster (SMAC). The actin cytoskeleton plays a critical role in this process, both for the molecular segregation of the T cell surface receptors and for the spatial organization of intracellular signaling components (Dustin and Cooper, 2000). The actin cytoskeleton may also be involved in the recruitment and enrichment, within the IS, of lipid rafts, specialized membrane microdomains enriched in saturated lipids and cholesterol that are important for T cell activation (Viola, 2001).

The Wiskott-Aldrich syndrome protein (WASP) plays an essential role in controlling TCR-induced actin nucleation and polymerization, and more generally in the regulation of cytoskeletal rearrangements important for hematopoietic cell function (Thrasher, 2002). Mutations in the WASP-encoding gene result in Wiskott-Aldrich syndrome, an X-linked recessive disease characterized by immune disregulation and platelet disorders (Thrasher, 2002). Several recent studies have addressed the molecular mechanism by which TCR triggering activates WASP. WASP recruitment to the IS is dependent on its proline-rich domain, suggesting that WASP is binding to a membrane-proximal SH3-containing protein (Cannon et al., 2001). Upon plasma membrane recruitment, WASP is thought to be activated by binding to Cdc42-GTP. This interaction most likely induces a conformational change in WASP that allows its C-terminal acidic domain to bind and activate the Arp2/3 complex of actin nucleating proteins (Thrasher, 2002). To prevent actin polymerization in the absence of TCR engagement, WASP activity must be tightly controlled. WASP interacting protein, WIP, controls actin polymerization in two ways: it keeps WASP in an inactive conformation that prevents it from binding to Arp2/3, but it also binds and stabilizes actin filaments (Martinez-Quiles et al., 2001).

Although actin remodeling is important for T cell activation, the exact nature of the link between the TCR and the actin cytoskeleton is not known. In the December issue of *Molecular Cell*, Sasahara and colleagues now identify CrkL as a protein that could make the link between the TCR and the WIP-WASP complex (Sasahara et al., 2002). It is well established that TCR engagement leads to the rapid tyrosine phosphorylation of the cytoplasmic portions of the TCR-associated CD3 chains, and the subsequent recruitment of ZAP-70, a tyrosine kinase that plays a central role in controlling downstream TCR signaling events. The molecular link between tyrosine-phosphorylated ZAP-70 and the WIP-WASP complex, the authors reasoned, was likely to be made by an SH2- and SH3-containing adaptor protein such as Crk or the Crk-like protein, CrkL. Consistent with this idea, tyrosine-phosphorylated ZAP-70 was found to bind to the recombinant SH2 domain of CrkL, and CrkL's SH3 domain, in turn, bound (constitutively) to a region within WIP containing two typical PxLPxK/R consensus SH3 binding sites. By coimmunoprecipitation experiments from lysates of unstimulated versus activated T cells, the authors go on to demonstrate TCR triggering-dependent recruitment, via CrkL, of a preformed CrkL-WIP-WASP complex to ZAP-70. Moreover, WIP and WASP were found to translocate to lipid rafts and to enrich in the IS in a ZAP-70- and CrkL-dependent manner.

Recruitment of the WIP-WASP complex via ZAP-70 and CrkL is an attractive possibility, but it may not be



Model for Recruitment and Activation of WASP by the T Cell Receptor

(A) Upon TCR engagement, a cytoplasmic CrkL-WIP-WASP complex is recruited to the TCR via phosphorylated ZAP-70. Alternative pathways for WASP recruitment, via Nck-SLP-76-Gads-LAT, or directly via CD3ε-bound Nck, may contribute.

(B) PKCθ-dependent phosphorylation of WIP induces the dissociation of WASP, allowing its activation by Cdc42-GTP and F-actin stabilization by WIP.

the only way to recruit WASP to the IS (see Figure, panel A). Another SH2- and SH3-containing adaptor protein, Nck, binds to WASP via one of its SH3 domains, and to SLP-76 via its SH2 domain, and it has been proposed that a complex composed of the SLP-76-associated protein, SLAP/Fyb, SLP-76, Nck, and WASP, is recruited to the IS via the lipid raft-associated protein LAT (Krause et al., 2000). Sasahara et al. indeed observe a reduction in the amounts of WASP and F-actin accumulated at the T cell contact zone in SLP-76-deficient T cells, consistent with a role for SLP-76 in WASP recruitment to the IS (Sasahara et al., 2002). Yet another possibility is given by the recent observation that Nck can bind to the TCR-associated CD3ε chain upon TCR ligation (Gil et al., 2002), thereby allowing direct Nck-mediated recruitment of WASP to the TCR-CD3 complex. The relative contributions of each of these pathways to WASP recruitment are unclear, and should be assessed in future studies. Mutation of the CrkL-SH2 binding site in ZAP-70, for example, would be predicted to impair CrkL-mediated WIP-WASP recruitment and F-actin polymerization. On the other hand, a mutated form of Nck, deficient in WASP binding, should be useful in addressing the relative contribution of Nck to WASP recruitment.

In the absence of TCR triggering, WASP binding to WIP prevents spontaneous actin nucleation (Martinez-Quiles et al., 2001). So how is this inhibitory interaction released upon T cell activation? Sasahara et al. suggest that PKCθ, a serine/threonine kinase critically involved in T cell activation, may control this step. PKCθ translocates to the IS and is enriched in lipid rafts, but little is known about its in vivo substrates (Arendt et al., 2002). A key observation of this study is that WIP has a consensus PKC phosphorylation motif within its WASP binding region. Through a series of elegant biochemical studies, the authors show that PKCθ-dependent phosphorylation of this site negatively regulates WIP binding to WASP. Consistent with these findings, a point mutation of the WIP phosphorylation site showed markedly reduced WASP binding. Moreover, T cells deficient in PKCθ had a severely impaired F-actin increase upon TCR engagement, lending strong support to the notion that PKCθ-dependent phosphorylation of WIP is critical for its dissociation from WASP (see Figure, panel B). Interestingly, PKCθ has also been reported to phosphorylate moesin, a member of the ERM family of actin cytoskeletal-membrane linkage proteins that play a regulatory role in the exclusion of CD43 from the T cell/APC contact area (Shaw, 2001). Regulation of the actin cytoskeleton by phosphorylation of F-actin binding proteins may therefore prove to be a general principle of PKCθ action.

The highly T lymphocyte-specific expression of PKCθ and its critical role in actin polymerization and T cell activation make this protein kinase a very attractive target for the development of specific kinase inhibitors with immunomodulatory potential.

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Flies kNOw How to Signal

A recent study has discovered a surprising role for nitric oxide in the *Drosophila* immune response. NO-mediated signaling was implicated in the communication between the site of a localized infection and the major immune organ of the fly, the fat body.

Immune activation in insects has been the focus of intense research over the last few years, powered by molecular and genetic techniques available in *Drosophila*. These studies have produced the broad outlines of the intracellular signaling pathways responsible for immune activation and have highlighted the similarities and differences between insect and mammalian innate immune signaling systems (Silverman and Maniatis, 2001). In *Drosophila*, infection stimulates the production of very high levels of several potent antimicrobial peptides. Production of these peptides is primarily controlled by transcriptional regulation, via NF-κB transcription factors. Antimicrobial peptide genes are expressed in the fat body (the insect liver analog) and in circulating macrophage-like blood cells, known as hemocytes. Two signaling pathways that control antimicrobial regulation have been characterized. The IMD pathway is stimulated by Gram-negative bacterial infection and leads to the activation of the NF-κB homolog Relish and production of antibacterial peptides. In contrast, the Toll pathway responds to Gram-positive bacterial and fungal infections and activates the NF-κB factors Dif and Dorsal, which control expression of the antifungal peptide Drosomycin (Silverman and Maniatis, 2001). Although many of the components of these two signaling pathways have been identified by genetic and molecular techniques, the mechanisms that transmit a signal from the site of a local infection to the major immune organ, the fat body, remain unclear. One possibility is that insects have "sentinel" cells, throughout the periphery, that produce signaling molecules (like cytokines) that activate immune-inducible gene expression in the fat body.

Until recently, technical considerations have prevented the analysis of signaling from local infection sites to the fat body. Most infection models in *Drosophila* rely on systemically infecting the animal (larvae or adult) with high levels of pathogenic microbes by injection. However, two natural infection models have been established, one with a bacterial pathogen (*Erwinia caratova caratova*) that uses an oral route for infection (Basset et al., 2000) and another with a fungal pathogen (*Beauveria bassiana*) whose germinating spores can penetrate the exoskeleton (Lemaitre et al., 1997). With these pathogens, signaling from a localized infection to the fat body can be examined.

In a recent paper, Foley and O'Farrell used the *Erwinia* natural infection model to characterize the role of nitric oxide (NO) in the *Drosophila* immune response. They set out to study the microbicidal activity of nitric oxide (NO) in flies but instead found that NO plays an essential signaling role in the insect immune response (Foley and O'Farrell, 2003). In mammalian immunity, NO is best known for its antimicrobial activity within phagocytic cells. NO and its derivatives are thought to directly kill microorganisms by nitrosylation, nitration, and oxidation of essential microbial components (DNA, lipids, and proteins) (Bogdan, 2001). However, NO can also act as a signaling molecule in the immune system as well as in the vasculature and nervous system. NO transduces signals by S-nitrosylation or metal-nitrosylation of target proteins. For example, in the endothelium, NO causes blood vessel relaxation by metal-nitrosylation and activation of soluble guanyl cyclase (sGC). In the immune system, NO signaling mechanisms are not entirely clear, but NO has been shown to inhibit TNFα-induced NF-κB activation, and nitrosylation of caspases is known to inhibit FAS-induced caspase activity (Mannick and Schonhoff, 2002). Also, in *Drosophila*, NO has been implicated in signal transduction in CNS development and in the response to hypoxia. In both cases, NO-mediated signaling is thought to activate the *Drosophila* sGC (Gibbs et al., 2001; Wingrove and O'Farrell, 1999).

Exploiting the *Erwinia* natural infection model and pharmacological agents that modulate NO levels, Foley and O'Farrell implicate NO in signaling from the site of infection (the gut) to the major immune organ (the fat body) of the fly. Pharmacologic inhibition of NO production causes a decrease in host survival upon infection and dramatically reduces the level of antimicrobial peptide gene expression in the fat body and hemocytes. Pharmacologic production of NO has the opposite effect, causing a robust activation of antimicrobial gene expression. Activation of antimicrobial peptide gene expression by exogenous NO donors requires IMD, while immune induction by IMD overexpression occurs even in the presence of the NO inhibitor. Thus, NO probably functions by activating the IMD signaling pathway.

However, NO is not likely to act directly on fat body cells to activate the IMD pathway. NO-mediated activation of the IMD pathway is impaired in a mutant (*domino*) that lacks hemocytes. This observation suggests that NO might be involved in signaling from the site of infection (the gut) to the circulating hemocytes, which, in turn, would activate immune-inducible gene expression in the fat body. Consistent with this idea, NOS activity in the gut of infected animals is substantially elevated. Although the results with the *domino* mutant must be interpreted with caution, because this is a pleiotropic mutant with defects in many cell types, Foley and O'Farrell do demonstrate that some immune responses are intact in *domino* mutant fat bodies.

Surprisingly, Foley and O'Farrell found that, while NO inhibitors dramatically reduce the survival of naturally