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NEWS AND VIEWS

Chemotaxis: new role for Ras revealed

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A recent study of chemotaxis revealed a new role for the proto-oncogene Ras in the social ameba *Dictyostelium discoideum*. Chemotaxis, the directional movement of cells toward chemokines and other chemoattractants, plays critical roles in diverse physiological processes, such as mobilization of immune cells to fight invading microorganisms, targeting of metastatic cancer cells to specific tissues, and guidance of sperm cells to ova during fertilization. This work, published in the July 26 issue of *The Journal of Cell Biology*, was conducted in Dr. Devreotes' lab at John Hopkins University and Dr. Parent's lab at National Cancer Institute. This research team demonstrated that RasC functions as an upstream regulator of TORC2 and thereby governs the effects of TORC2-PKB signaling on the cytoskeleton and cell migration.

Many of the core components of the underlying chemotaxis signaling network have been elucidated in *D. discoideum*. Chemoattractants are sensed by G-protein-coupled receptors (GPCRs), which leads to the activation heterotrimeric G-proteins, small Ras-like G-proteins, and phosphoinsositide 3-kinase (PI3K), resulting in the generation of phosphatidylinositol-(3,4,5)-trisphosphate (PIP_3). This phospholipid, in turn, prompts the membrane translocation of proteins containing pleckstrin homology (PH) domains, such as cytosolic regulator of adenylyl cyclase (CRAC) and protein kinase B (PKB), which regulate the cytoskeleton rearrangements during chemotaxis. Importantly, many of these components and their activation are highly localized to the leading edge of cells undergoing chemotaxis, assuring that cytoskeletal changes needed for directional movement are spatially restricted (reviewed in Jin et al., 2009).

Although it has been well established that the PIP_3 pathway plays an important role in the regulation of chemotaxis, additional pathways that act in parallel with the PIP_3 pathway have recently been revealed. For instance, phospholipase A2 (PLA2) was reported to mediate chemotaxis in parallel with PIP_3 pathway (Chen et al., 2007). In addition, a PIP_3-*independent* pathway in which PKB is activated by TORC2 (target of rapamycin complex 2) was found to regulate chemotaxis (Lee et al., 2005; Kamimura et al., 2008). However, the mechanism by which TORC2 is regulated in chemotaxis was poorly understood prior to the publication of the recent report by Cai et al. (2010).

D. discoideum possesses two PKB homologs, namely PKBA, which contains a PH domain and is dynamically recruited to the plasma membrane by PIP_3, and PKBR1, which is tethered to the plasma membrane via N-terminal myristoylation. In their previous work, the authors discovered that both PKBA and PKBR1 are activated by TORC2-mediated phosphorylation of their hydrophobic motifs (HMs) (Kamimura et al., 2008) and phosphoinositide-dependent kinase (PDK)-mediated phosphorylation of their activation loops (ALs) (Kamimura and Devreotes, 2010). In the present study, Cai and colleagues investigated whether Ras family proteins activate TORC2 and, if so, what are the effects of this Ras-TORC2 pathway on chemotactic responsiveness.

In order to examine whether Ras proteins are required for TORC2-mediated activation of PKB, the scientists first determined the PKB activity in different Ras knock-out cells. They found that phosphorylation of the HM of PKBR1, the ALs of both PKBR1 and PKBA, and many PKB substrates were significantly reduced in *rasC*$^-$ but not *rasG*$^-$ cells relative to wild-type cells. To further explore rasC's role in PKB activation, the authors examined the consequences of expressing activated RasC ($RasC^{Q62L}$) and found that it dramatically prolonged the phosphorylation kinetics of the PKBR1 and multiple PKB substrates, suggesting that RasC is indeed involved in regulating the PKB pathway. In addition, $RasC^{Q62L}$ expression also prolonged actin polymerization and impaired chemotaxis in wild-type cells, effects that were suppressed in cells lacking Pianissimo (*piaA*$^-$), an essential component of TORC2. Taken together these findings

represent the first genetic evidence that RasC functions upstream of TORC2 to regulate PKBR1 and chemotaxis.

The finding that RasC is required for the activation of the PKB pathway *in vivo* encouraged the scientists to further elucidate the function of RasC in TORC2-PKB pathway activation *in vitro*. They demonstrated that PKB phosphorylation can be reconstituted by mixing immunopurified TORC2 with membranes containing $RasC^{Q62L}$ (but not those containing inactive wild-type RasC). In addition, the authors found that TORC2 specifically co-immunoprecipitates with activated RasC but not inactive RasC, suggesting that RasC and TORC2 physically interact in a regulated fashion, further supporting the conclusion that activated RasC stimulates TORC2 activity.

In summary, this study by Cai and colleagues reveals a novel RasC-TORC2-PKB signaling pathway with important roles in chemotaxis and suggests that RasC interacts directly with TORC2. Presumably RasC interacts with the Ras-binding domain of Rip3, a component of *D. discoideum* TORC2 and an ortholog of mammalian TORC2 subunit mSin1. These findings are complemented by a recent report from Charest et al. (2010) demonstrating the existence of a novel RasGEF-containing complex that translocates to the plasma membrane upon chemoattractant stimulation, promotes activation of RasC, and is the target of PKB-mediated negative feedback that apparently terminates RasC activation. Together, these studies provide novel mechanistic insights into the activation of TORC2 and its regulation in chemotaxis. The findings that RasC activation dictates the kinetics of downstream signaling events and the demonstration of PKB-mediate feedback regulation of RasC suggest that RasC is a key regulatory node in the chemotaxis signaling network and may be critical for the exquisite temporal and spatial regulation of the cytoskeleton that occurs in chemotaxis.

TORC2 is also known to mediate cell proliferation and survival and thus is being aggressively pursued as a target for novel cancer therapeutics. However, prior to this study, little was known about how TORC2 is activated. Therefore, it seems likely that the regulatory information about the RasC and TORC2 discovered here in *D. discoideum* will lead to new therapeutic strategies for treatment of inflammatory disorders and possibly also cancer.

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