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"TORCing" Neutrophil Chemotaxis

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During cell migration, chemoattractant-induced signaling pathways determine the direction of movement by controlling the spatiotemporal dynamics of cytoskeletal components. In this issue of *Developmental Cell*, Liu et al. report that the target of rapamycin complex 2 (TORC2) controls cell polarity and chemotaxis through regulation of both F-actin and myosin II in migrating neutrophils.

Chemotaxis, the ability of cells to directionally migrate in response to external cues (chemoattractants), is central to many normal cell functions that include the migration of cells in embryonic development, immune responses, and wound healing. Great strides have been made in our understanding of directed cell migration during the past decade, revealing unexpected complexity in the molecular mechanisms underlying the ability of cells to perform chemotaxis. The work by Liu et al. (2010) presented in this issue of *Developmental Cell* adds to the mix by identifying a new pathway through which the target of rapamycin complex 2 (TORC2) and cAMP control cell polarity and motility during neutrophil chemotaxis.

Upon detection of a chemoattractant, amoeboid cells, such as neutrophils and *Dictyostelium* cells, reorganize their cytoskeleton and rapidly polarize in the direction of the chemotattractant source. Localized polymerization of F-actin at the front of the cell and assembly of myosin II in actomyosin filaments at the sides and rear together provide protrusive and contractile forces that drive cell motility. For G protein-coupled receptor (GPCR)-driven chemotaxis in both neutrophils and *Dictyostelium*, "frontness" signaling pathways include the activation of PI3K and production of PIP3 at the side of the cell with the highest chemoattractant concentration, leading to the local recruitment and activation of PIP3-dependent guanine exchange factors for the small GTPase Rac (RacGEFs) that activate Rac. Active Rac then drives localized polymerization of F-actin, and this "frontness" signaling pathway is amplified through positive feedback loops involving the actin cytoskeleton. In neutrophils, the "backness" signaling pathway involves activation of the small GTPase RhoA, which promotes actomyosin assembly through activation of the RhoA-dependent kinase ROCK. ROCK in turn phosphorylates myosin light chain (MLC) and thereby activates myosin II to promote contraction of the trailing edge. These "frontness" and "backness" signals appear to be mediated through distinct heterotrimeric G proteins: Gi mediates "frontness" signaling to the actin cytoskeleton, while G12/13 mediates "backness" signaling to promote myosin II assembly (Wang, 2009 and references therein).

The study by Liu et al. (2010) now reveals that TORC2 plays a critical role in neutrophil chemotaxis by regulating both F-actin and myosin II, probably via two independent pathways (Figure 1). Regulation of actin dynamics is a widely conserved and highly studied function of TORC2 (Cybulski and Hall, 2009). In migrating *Dictyostelium* cells, TORC2 controls both cell motility and the relay of the chemoattractant signal through regulation of F-actin polymerization and activation of adenylyl cyclase, respectively, by modulating the activity of Akt/PKB and the PKB-related kinase PKBR1 (Lee et al., 2005; Kamimura et al., 2008; Charest et al., 2010; Cai et al., 2010). Using an shRNA-mediated knockdown (KD) of the TORC2-specific component Rictor, causing loss of TORC2 function, Liu et al. (2010) show that, in chemotaxing neutrophils, TORC2 is required for cell polarity and the spatial regulation of F-actin, but not its polymerization. Although chemoattractant-induced F-actin polymerization is intact in Rictor KD cells, these cells fail to restrict F-actin to the side of the cell closest to the chemoattractant source. Liu et al. (2010) also found that, as in *Dictyostelium* cells, TORC2 signaling promotes chemoattractant-induced production of cAMP. In neutrophils, they show this activity is specifically mediated through adenylyl cyclase 9 (AC9). The same group previously reported that stimulation of human neutrophils by the chemoattractant and GPCR ligand fMLP leads to Gi-mediated activation of AC9, identifying a noncanonical pathway by which GPCRs can stimulate cAMP production (Mahadeo et al., 2007). Although the mechanism by which TORC2 activates AC9 remains to be determined, Liu et al. (2010) demonstrate that it requires the TORC2 substrate PKC and not Akt/PKB. Interestingly, while cAMP production is completely inhibited in AC9 KD cells or upon treatment of wild-type cells with a PKC inhibitor, the chemotaxis defects of these cells are not as severe as those seen in Rictor KD cells. Unlike Rictor KD cells, cells lacking AC9 or PKC activity show normal localization of F-actin and, thus, can establish polarity along the chemoattractant gradient, but have severe defects in the ability to retract the cell's posterior, suggesting that PKC and AC9 control posterior myosin II function. Overexpression of AC9, which results in elevated and unregulated cAMP accumulation, causes a similar chemotaxis defect, suggesting that a cycling of cAMP levels during chemotaxis is required for posterior retraction.

Liu et al. (2010) then further showed that TORC2-mediated and AC9-dependent cAMP production plays an important role in modulating the RhoA-ROCK-myosin pathway during chemotaxis. They demonstrate that decreased cAMP production because of AC9 KD leads to increased RhoA activity, resulting in increased ROCK-mediated phosphorylation of MLC and enhanced "back" activity.

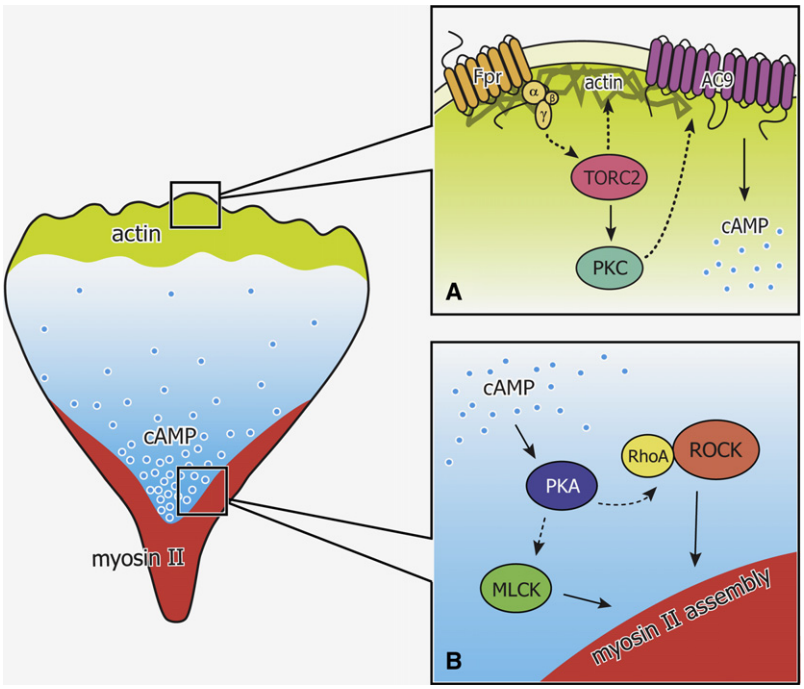

Figure 1. TORC2 Controls Neutrophil Polarity and Chemotaxis by Regulating F-Actin Polarity and Myosin II Assembly

(A) TORC2 is presumably activated at the front of the cells, in a chemoattractant- and Gi-dependent manner, controlling the polarity of F-actin and locally activating PKC. In turn, PKC promotes the activation of AC9 and cAMP production.

(B) Through unknown mechanisms, the chemoattractant-stimulated, TORC2/PKC-dependent cAMP production oscillates and becomes enriched at the posterior of cells, where, most likely through PKA acting on RhoA and/or MLCK, it regulates the inhibition of myosin II assembly. Fpr, Formyl peptide receptor. Figure designed and drawn by Jessica Chang.

Furthermore, in AC9 KD cells, phosphorylated MLC, a marker for assembled myosin II, extends to the front of the cells instead of being restricted to the posterior and sides. Using an in vivo FRET biosensor for cAMP, they also show that cAMP levels in normal cells are lowest in extending pseudopodia, while the back of cells displayed much higher cAMP levels that fluctuate dynamically during migration. As proposed by the authors, these observations suggest that TORC2-dependent, transient cAMP accumulations control the cyclical phosphorylation-dephosphorylation of myosin II that occurs during neutrophil chemotaxis and is required for periodic back contraction and relaxation. The mechanism by which cAMP is enriched at the posterior of migrating neutrophils is unknown but most likely does not involve localized activation of AC9 because the cyclase is uniformly distributed along the plasma membrane of migrating cells and TORC2 is believed to be activated at the leading edge in a Gi-dependent manner. It is also not clear what controls the periodic accumulation of cAMP, but cAMP production and degradation do nevertheless undergo tightly controlled spatiotemporal regulation during neutrophil chemotaxis and are crucial for efficient migration. The inhibitory effect of TORC2-cAMP signaling on the "backness" RhoA-myosin II pathway most likely occurs through PKA-mediated inhibition of one or more of the components in the pathway. As discussed by Liu et al. (2010), PKA can phosphorylate and negatively regulate RhoA, as well as several RhoGEFs, but could alternatively exert its inhibitory effect through phosphorylation-mediated inhibition of MLC kinase, which also controls myosin II phosphorylation (Liu et al., 2010 and references therein).

The studies by Liu et al. indicate that TORC2 plays a key role by integrating chemotaxis signaling to mediate cell polarization. As discussed above, previous evidence suggested that "frontness" and "backness" signals are mutually exclusive and inhibit each other, underlying the self-polarizing ability of amoeboid cells (Wang, 2009 and references therein). However, the work by Liu et al. (2010) argues that Gi, a regulator of "frontness," controls cell polarity through TORC2 by regulating localized F-actin polymerization at the front while inhibiting myosin II assembly through stimulation of cAMP production at the posterior. Interestingly, Rac1 signaling has a similar effect in human neutrophil chemotaxis, in which Rac1 was found to be essential for RhoA and myosin II activation at the posterior, while stimulating F-actin polymerization at the leading edge (Pestonjamasp et al., 2006). Thus, a key question that remains unanswered is how TORC2 mediates F-actin polymerization, which Liu et al. show is not controlled by TORC2 activation of Akt.

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