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SPOTLIGHT

A Leep1 into migration and macropinocytosis

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Actin organization underpins conserved functions at the leading edge of cells. In this issue, Yang et al. (2021. *J. Cell Biol.* https://doi.org/10.1083/jcb.202010096) characterize Leep1 as a bi-functional regulator of migration and macropinocytosis through PIP_3 and the Scar/WAVE complex.

Asymmetry at the molecular and structural levels is a key feature, conserved through evolution, for unicellular and multicellular organisms to perform essential cellular functions. Cell migration is a clear example where asymmetry plays an essential role by ensuring a polarized morphology with structurally and biochemically specialized leading and trailing edges, each mediating different roles in motility (1). *Dictyostelium* is a valuable model for dissecting the molecular mechanisms of cell polarity. At the leading edge of cells, actin-rich pseudopods are extended for cell motility and actin frames the formation of dorsal ruffles, which form macropinocytic cups to uptake extracellular fluid and nutrients in large (>0.2-µm diameter) vacuolar macropinosomes. There is keen interest in macropinocytosis as an essential pathway for migration and immune surveillance in innate immune cells and for nutrient acquisition and enhanced survival in cancer cells (2, 3). In this issue, Yang et al. describe how Leep1, a novel polarity protein in *Dictyostelium*, fine-tunes actin dynamics to support macropinocytosis and cell motility through its interaction at the interface of membrane PIP_3 and the Scar/WASP-family verprolin-homologous protein (WAVE) complex (4).

First, the authors conducted a proteomic screen to identify polarity regulators among peripheral membrane proteins recruited in response to cAMP, a chemoattractant for *Dictyostelium*. Their screen relied on comparison with the translocation dynamic of the Pleckstrin homology (PH) domain of the cytosolic regulator of adenylyl cyclase (PHcrac), which relocates to the plasma membrane and binds $PI3,4P_2/PIP_3$ upon stimulation with cAMP. Among the leading edge proteins, the researchers identified known chemotactic regulators such as ForG, and also uncharacterized proteins, including Leep1, which they named after "Leading edge enriched protein 1." They confirmed that Leep1 redistributed to the cell periphery in response to cAMP and folic acid gradients. Leep1 was strongly associated with macropinocytic cups, and it was recruited, albeit infrequently, to pseudopods in randomly migrating cells. Importantly, Leep1 colocalized with PHcrac in macropinocytic cups but was excluded from membranes associated with the PI phosphatase Pten. This led them to analyze Leep1 domains, and they found leucine-rich repeats (LRR) in a mid-section and a PH domain-like fold at the N terminus. Using truncation mutants, the authors established that the N terminus of Leep1 mediated membrane binding and localization to macropinocytic cups. They further confirmed that PIP_3 is required for Leep1 membrane binding by using lipid dot blots to assess in vitro lipid binding and PI3K inhibitors to manipulate phospholipid dynamics.

Although Leep1 associated prominently with newly forming macropinocytic cup membranes, up to the point of cup closure, Leep1 knockouts in *Dictyostelium* presented no overt phenotypes and no dire loss of uptake or migration. In a closer examination, however, Yang et al. found that Leep1+ knockout mutants presented a ∼35% reduction in the rate of macropinocytosis, as measured by dextran uptake (4). This impaired macropinocytosis was related to the formation of shallower cups that failed to close or that formed smaller vesicles in comparison to the wild-type strain. Such reduction in the number of closed macropinocytic cups and deficient nutrient intake translated into an increased culture time for the mutants growing in liquid medium. Interestingly, the loss of Leep1 did not affect the size of ruffles nor block cell chemotaxis but decreased pseudopod splitting; these results imply that, although dispensable for directed cell migration, Leep1 is needed for fine-tuning pseudopod dynamics during movement.

Following overexpression of Leep1 in *Dictyostelium*, there was a dramatic appearance of spiky filopodia all over cells. Only Leep1 mutants with an intact C terminus and membrane binding domain produced abundant filopodia and, simultaneously, these cells generated smaller and shallower macropinocytic cups and failed to internalize dextran efficiently. The researchers observed a stark difference in actin-based projections on cells with different levels of Leep1, but how this resulted in a trade-off between filopodia and macropinocytic cups is a query that they did not explore further. It would be interesting to examine such a rheostat effect on F-actin in mammalian cells, where ruffle-associated filopodia can coexist with and contribute to macropinocytosis (5, 6). To study the pathways and binding partners through which Leep1 could modulate

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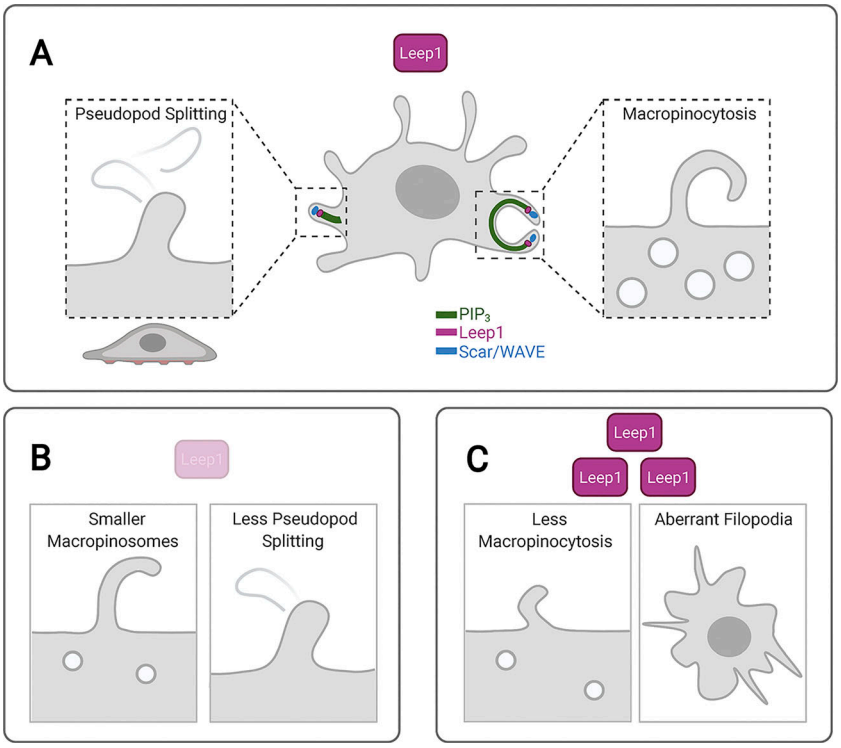

Figure 1. **PIP_3-recruited Leep1 negatively regulates the Scar/WAVE complex to modulate pseudopod and macropinosome dynamics. (A)** Leep1 is enriched at the rims of macropinocytic cups, where it supports macropinosome formation, and is periodically in pseudopods, where it regulates pseudopod splitting. **(B)** Loss of Leep1 produces smaller macropinosomes and reduces pseudopod splitting. **(C)** Overexpression of Leep1 leads to unconstrained filopodia formation and inefficient macropinocytosis.

actin-based protrusions, they performed immunoprecipitations and mass spectrometry. In formaldehyde-cross-linked samples, they found Leep1 engaged in weak or transient interactions with the actin nucleating complex Scar/WAVE through its PirA and NapA components. Interestingly, another earlier screening approach had revealed Leep1 as an uncharacterized binding partner of NapA (7). Moreover, the Scar/WAVE complex is of high interest in this context given its known association with macropinosomes, filopodia, and pseudopods (8, 9). Scar/WAVE disruption in *Dictyostelium* reduces macropinocytosis and increases filopodia formation, similarly to overexpression of Leep1. The highly dynamic and transient interaction of PIP_3-recruited Leep1 corresponded here to its adjacent (but not overlapping) recruitment of Scar/WAVE on the membrane (Fig. 1). Indeed, this mirrors the previous observation that, although high concentrations of PIP_3 recruit Scar/WAVE complex to the cell periphery, they do not overlap in the membrane in *Dictyostelium* (8). Yang et al. have now identified Leep1 as the link that promotes the spatially distinct recruitment of Scar/WAVE by PIP_3 to the leading edge(4). The authors explored this link in detail and found that deletion of the C terminus, but not the N terminus, in Leep1 mutants abolished Scar/WAVE recruitment. C-terminal mutants also failed to promote filopodia formation when overexpressed and could not rescue the macropinocytosis defect in Leep1 knockouts. Therefore, the N terminus of Leep1 is needed to position it on the membrane where its C terminus can interact with Scar/WAVE to either make filopodia or macropinosomes. An intriguing observation was that PirA and Leep1 were alternately recruited for moving and retraction or splitting of pseudopods, respectively. Overall, the actions of Leep1 are consistent with its role as a negative regulator of Scar/WAVE.

This exciting study introduces Leep1 as a novel lipid-recruited actin regulator on macropinosomes and pseudopods, where it joins a throng of diverse actin modulators on these domains. The relatively mild phenotype of Leep1 knockouts implies there are actin regulators with complementary or compensatory roles, and other proteins in this context have recently come to light through studies in *Dictyostelium*. CYFIP-related Rac interactor is a Rac1 binding partner, and while it is not recruited by PIP_3, it is also a negative regulator of Scar/WAVE that regulates actin for leading edge extension and retraction during migration (7). The recently described BAR domain–containing protein RGBARG (RCC1, RhoGEF, BAR, and RasGAP-containing protein; 10) coordinates lipid and actin dynamics from sites at the rim of macropinocytic and phagocytic cups in *Dictyostelium*. RGBARG coordinates Rac1 activation at the tip of cups to drive actin extension while suppressing Ras in the interior to shape cups. How Leep1 intersects with these and other disparate players is yet to be reconciled into mechanistic models that explain the spatio-temporal coordination of PIP_3, Scar/WAVE, and Rac1 pathways that generate distinct actin-mediated protrusions. While a degree of similarity with conserved capping protein, Arp2/3 and myosin-I protein has been noted for Leep1, future studies will help clarify whether Leep1 has a conserved mammalian equivalent with similar cellular roles at the leading edge. Overall, the study by Yang et al. represents an important leap toward understanding fundamental regulators of the complex interactions coordinating actin-based macropinocytosis and migration.

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