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RESEARCH HIGHLIGHT

A phosphoinositide kinase triggers migrasome formation

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Migrating cells leave behind them a trace of large vesicles known as migrasomes, which are formed from retraction fibers by mechanisms that are incompletely understood. Now, Ding et al. show that the phosphoinositide PIP2 orchestrates clustering of integrin molecules at sites of migrasome formation, suggesting that the phosphoinositide kinase that produces PIP2 is the trigger of migrasome biogenesis.

Migrasomes are newly discovered organelles that consist of large vesicles formed from retraction fibers at the trailing end of migrating cells. They play important physiological roles in intercellular signaling, intercellular transfer of proteins and mRNAs, and shedding of damaged mitochondria, with consequences for development and disease.[1]

Although migrasomes are characterized by their high content of tetraspanin proteins,[2] their biogenesis has remained enigmatic until now. A clue has been provided by the detection of α5-integrin molecules at sites of retraction fibers where migrasomes are formed.[3] Integrins are heterodimeric transmembrane proteins that connect cells to the extracellular matrix (ECM),[4] and integrins might thus form contacts between migrasomes and the ECM. Such contacts could potentially contribute to migrasome biogenesis, but evidence for this has been lacking, and it has not been understood how integrins are recruited to specific sites on retraction fibers.

Another molecule enriched on migrasomes is the lipid, phosphatidylinositol 4,5-*bis*phosphate, abbreviated as PIP2.[1] PIP2 belongs to a group of lipids known as phosphoinositides, which are phosphorylated derivatives of the abundant phospholipid, phosphatidylinositol. Each of the seven phosphoinositides has different cellular functions, which is defined by their ability to recruit or activate specific protein effectors.[5] Using a probe for PIP2 in live-cell microscopy, Ding et al.[6] noticed that PIP2 emerged on migrasomes slightly prior to α5-integrin. This made them hypothesize that PIP2 might play a role in migrasome biogenesis.

To test their hypothesis, the authors first investigated how PIP2 emerges at migrasomes. PIP2 can be formed by several phosphoinositide kinases, but Ding et al. found that PIP2 at migrasomes is generated by the phosphoinositide 5-kinase PIP5K1A, which phosphorylates phosphatidylinositol 4-phosphate into PIP2. PIP5K1A has previously been shown to control molecular processes involving the plasma membrane, including regulation of actin-dependent plasma membrane dynamics.[5] PIP5K1A was indeed found to localize to migrasomes, and drug-induced inhibition of PIP5K1A prevented migrasome biogenesis. The essential role of PIP5K1A in migrasome formation was confirmed by the observation that migrasomes failed to form in PIP5K1A knockout cells, in which retraction fiber formation and cell migration were unaffected, and could be rescued by transfection with wild-type but not kinase-deficient PIP5K1A.

Like other phosphoinositides, PIP2 is relatively short-lived in membranes. It can be dephoshorylated by phosphoinositide phosphatases or hydrolyzed by phospholipase C (PLC) enzymes, which convert PIP2 into diacylglycerol and inositol 1,4,5-*tris*phosphate.[5] Ding et al. found that the PLC enzyme PLCD3 was present on migrasomes, and knockout of PLCD3 caused enhanced migrasome formation, consistent with a role for PLCD3 in negative control of PIP2-dependent migrasome formation. This was supported by the finding that reintroduction of PLCD3 into the knockout cells reverted migrasome formation back to normal.

The essential role of PIP2 in migrasome formation raised the question of which protein effector(s) it controls to define the sites of migrasome biogenesis. By comparing lists of known PIP2-interacting proteins with lists of migrasome proteins identified by proteomic analyses, Ding et al. compiled a list of 23 candidate PIP2-binding proteins on migrasomes, some of which were confirmed experimentally to localize to migrasomes.

One of these stood out as particularly interesting, namely the small GTPase Rab35. Rab GTPases control specific membrane dynamic processes, including organelle biogenesis,[7] and Ding et al. therefore investigated the possibility that Rab35 mediates migrasome formation. Using fluorescently tagged Rab35 in conjunction with live-cell imaging, the authors observed that Rab35 was initially diffusely distributed along retraction fibers, then concentrated at branch points, and eventually intense Rab35 foci enlarged and grew into migrasomes. Importantly, the localization of Rab35 to migrasomes was prevented by knockout or drug-induced inhibition of PIP5K1A, indicating that Rab35 is recruited to migrasome formation sites by PIP2. Mechanistically, this could be explained by an interaction of a patch of positively charged amino acids at the C-terminus of Rab35 with the negatively charged headgroup of PIP2, and replacement of the basic amino acid residues with neutral ones prevented recruitment.

Rab GTPases function as molecular switches that are active in their GTP-bound form, which promotes effector binding, whereas they are inactive in their GDP-bound form.[7] Knockout of Rab35 strongly inhibited migrasome formation, and this could be rescued by wild-type Rab35 and a constitutively active (GTPase-defective) mutant, but not a dominant-negative (GDP-bound) mutant or a mutant unable to interact with PIP2. This indicates that Rab35 mediates migrasome biogenesis downstream of PIP2.

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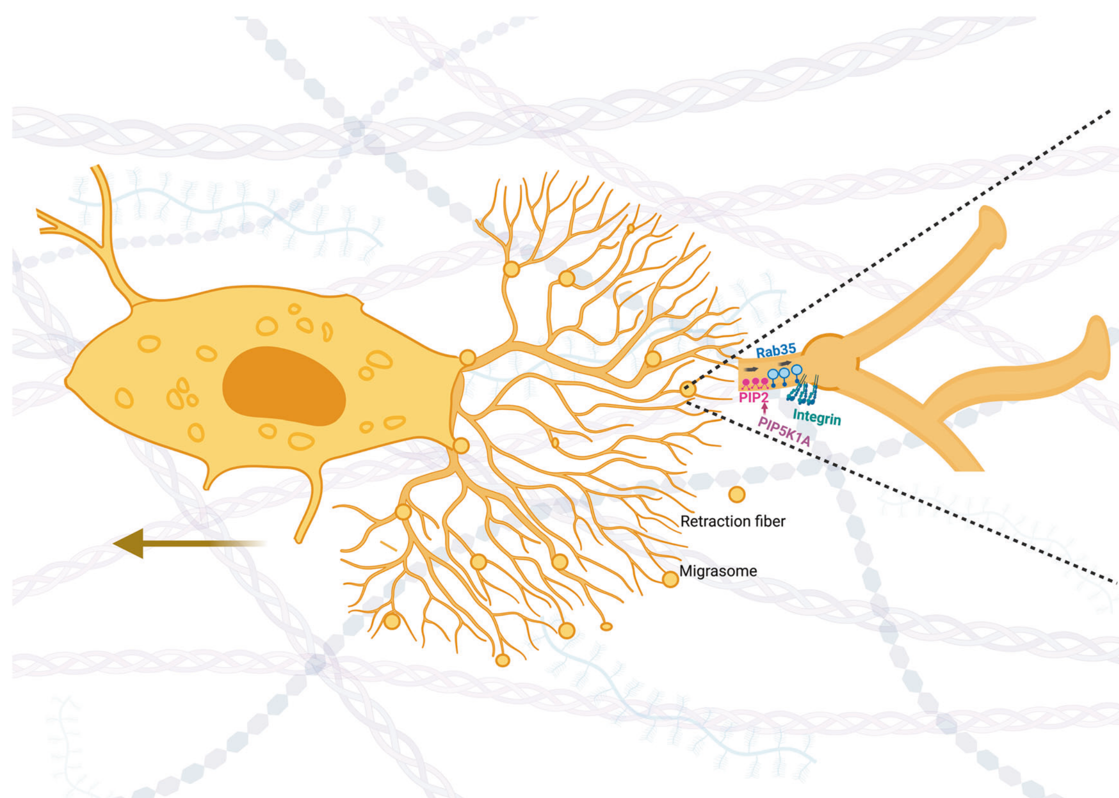

Fig. 1 PIP5K1A produces PIP2 at specific sites along retraction fibers of migrating cells. This causes recruitment of Rab35, which in turn recruits integrin molecules. Integrin clustering causes localized interactions with the ECM and marks the sites of migrasome biogenesis. Figure generated with BioRender.com.

How might Rab35 control migrasome formation? Rab35 is known to control integrin trafficking,[8] and this fact, combined with the knowledge that integrins appear at sites of migrasome formation, made Ding et al. hypothesize that Rab35 could control migrasome biogenesis via integrins. To test this hypothesis, the authors monitored the localization of fluorescently tagged α5-integrin during migrasome formation in cells growing on fibronectin, an ECM ligand for α5β1-integrin dimers. In wild-type cells, α5-integrin localized strongly to migrasomes, whereas knockout of Rab35 caused an even distribution of α5-integrin along retraction fibers.

A membrane-proximal amino acid sequence motif in α5-integrin, previously found to mediate its interaction with Rab21, was required for targeting α5-integrin to sites of migrasome formation. Treating cells with a membrane-permeant peptide containing this motif abolished integrin targeting and migrasome formation, indicating that Rab35-mediated integrin recruitment is a key step in migrasome formation. Ding et al. were unable to demonstrate a direct interaction between Rab35 and α5-integrin. This could either reflect difficulties in detecting weak interactions with a membrane protein, or the interaction between the two proteins could be indirect.

In any case, the results from Ding et al. convincingly demonstrate how a phosphoinositide kinase orchestrates migrasome formation (Fig. 1). PIP2 production by PIP5K1A determines the sites along retraction fibers that will develop into migrasomes. Rab35 is recruited by PIP2, and α5-integrin (most likely as dimer with β1-integrin) is recruited by Rab35. This causes clustering of integrin molecules that initiates migrasome formation.

As all novel discoveries, the recent results also raise some new questions. Most importantly, how is PIP5K1A recruited and activated to determine the sites of nascent migrasomes? Further studies are needed to reveal whether this is due to specific biochemical properties of the retraction fiber membrane, or biophysical cues such as membrane curvature or interactions with the ECM. Other questions concern how Rab35 is activated and how integrin clustering results in migrasome biogenesis.

The new results not only shed light on the mechanisms of migrasome formation but also provide tools for how to manipulate migrasome formation for future physiological studies. Drug-induced inhibition of PIP5K1A could be one way to suppress migrasome formation in vivo, and Ding et al. already demonstrated that this inhibitor as well as the membrane-permeant peptide that disrupts Rab35–integrin interaction prevent migrasome formation in zebrafish embryos. This gives great prospects for a further understanding of the importance of migrasomes in biology.

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ADDITIONAL INFORMATION

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