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SPOTLIGHT

Kindlin-3 stokes the life span of podosomes

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Macrophages and other cell types can form podosomes, highly dynamic adhesive structures that mediate the interaction of these cells with the surrounding matrix. In this issue, Klapproth et al. (2019. *J. Cell Biol.* https://doi.org/10.1083/jcb.201903109) find that kindlin-3 regulates podosome stability by recruiting leupaxin, with concomitant effects on PTP-PEST phosphatase activity and paxillin phosphorylation.

Kindlin-3 belongs to the kindlin family of focal adhesion molecules that contain FERM (fragment 4.1, ezrin, radixin, and moesin) domains. Like other members of the family, kindlin-3 mediates interactions between membrane receptor integrins and the actin cytoskeleton. Kindlin-3 itself localizes to both focal adhesions and the podosomes, and acts as an activator of integrins together with talin, regulating cell growth and migration. Kindlin-3 is expressed only in hematopoietic cells (1) but can also function in a variety of cell types when it is expressed ectopically. However, how kindlin-3 contributes to the assembly and stability of adhesion complexes and integrin-mediated signaling in hematopoietic cells remains unknown. In this issue, Klapproth et al. set out to investigate the role of kindlin-3 in the regulation of podosome formation and stability (2).

Cell–matrix contacts include focal complexes, focal adhesions, fibrillar adhesions, podosomes, invadopodia, and reticular adhesions (3). Podosomes, which Klapproth et al. focus on in their study (2), are adhesion structures that are morphologically distinct from focal adhesions but share almost the same molecular components (4). A podosome is composed of two parts: a dense actin core, which contains actin-regulatory proteins, and a ring of signaling and adaptor proteins embedded in an actin cloud, which surrounds the actin core and is connected to the extracellular matrix via integrins (5). Compared with focal adhesions, podosomes are more dynamic and are involved in extracellular matrix degradation and cell invasion (6).

The paxillin gene family of adapter proteins, which also includes leupaxin and Hic-5 (7), contain N-terminal LD 1–5 domains that can target to podosomes and C-terminal LIM 1–4 domains that target these proteins to focal adhesions via the LIM2 and LIM3 domains (8). Leupaxin is primarily expressed in hematopoietic cells and shares a similar domain structure with paxillin in the four-leucine–rich LIM 1–4 region. Klapproth et al. identify leupaxin as a new kindlin-3 interactor by yeast two-hybrid screening (2), and this interaction was subsequently confirmed in macrophages isolated from Flag–kindlin-3 knockin mice, i.e., cells capable of forming numerous and dynamic podosomes. Interestingly, Klapproth et al. found that leupaxin could be recruited to podosomes in a kindlin-3–dependent manner whereas paxillin recruitment is kindlin-3 independent.

Klapproth et al. found that kindlin-3–mediated leupaxin recruitment into podosomes results in reduced phosphorylation of paxillin and increased podosome stability (2). So what is the underlying mechanism behind this? First, the authors thought that low kindlin-3 expression may affect leupaxin localization in podosomes, and they found greatly reduced leupaxin levels in podosomes of $K3^{n/-}$ cells, whereas paxillin levels remain unchanged, indicating that it is leupaxin but not its paralog paxillin that requires kindlin-3 for podosomal recruitment. The authors also observed a reduction of total cellular leupaxin levels in $K3^{n/-}$ cells, which suggests that the interaction with kindlin-3 stabilizes cellular leupaxin.

Paxillin phosphorylation at tyrosine 31 (Y31) and Y118 is known to promote adhesion turnover, podosome disassembly, and reorganization, and leupaxin is known to suppress the tyrosine phosphorylation of paxillin in focal adhesions (9). Therefore, Klapproth et al. (2) hypothesized that podosome lifetime may be linked to kindlin-3–dependent recruitment of leupaxin and the subsequent regulation of paxillin phosphorylation at Y31 and Y118. They measured the individual podosome lifetime in leupaxin-null and control RAW macrophage-like cells expressing lifeact-GFP by live cell imaging and the results showed that 50% of the podosomes in control cells last 250 s or longer, whereas the cumulative time for podosome distribution in leupaxin-null cells was reduced to ∼180 s (Fig. 1 A). These data suggested that the leupaxin–paxillin interaction controls podosome lifetime. To examine whether this reduction of podosome lifetime is due to elevated paxillin phosphorylation on Y31 and Y118, they transfected control and leupaxin-null RAW cells with mCherry-tagged wild type or paxillin-2YF, a non-phosphorylatable paxillin mutant. They found an extension of the podosomal lifetime of paxillin-2YF–expressing wild-type and leupaxin-null RAW cells compared with cells transfected with wild-type paxillin (Fig. 1 B). These data suggest that paxillin phosphorylation on Y31 and Y118 accounts for the reduction of podosome lifetime in the absence of leupaxin. Paxillin phosphorylation on Y31 and Y118 can be removed by PTP-PEST, a tyrosine phosphatase. The researchers demonstrated that this removal by PTP-PEST requires kindlin-3–mediated recruitment of

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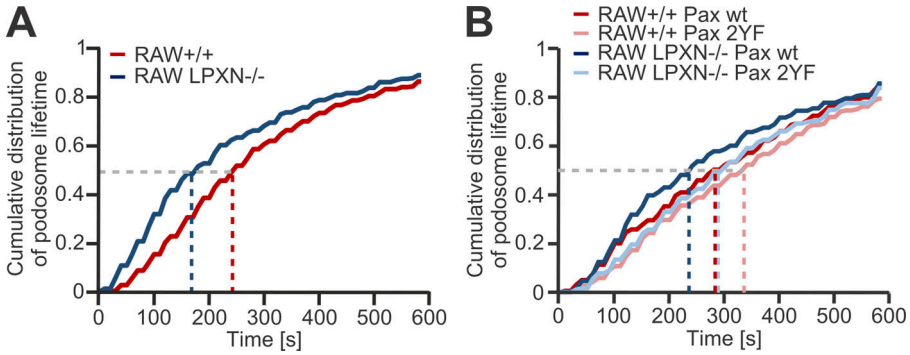

Figure 1. **Loss of leupaxin promotes podosome turnover, and paxillin phosphorylation accelerates podosome turnover. (A)** Lifetimes of single podosomes present at time point 0 were analyzed from time-lapse movies by measuring the time until they disappeared. The cumulative distribution of these measurements is shown. 10–30 podosomes were measured per cell. **(B)** Podosome lifetime was assessed as described in A and plotted as a cumulative distribution. 10–30 podosomes were measured per cell. This figure was reproduced from Fig. 4, D and E, in Klapproth et al. (2).

leupaxin into podosomes, and without leupaxin, PTP-PEST can bind to phosphorpaxillin but does not dephosphorylate it.

Who arrives first at podosomes—paxillin or kindlin-3? The researchers saw that paxillin is already in podosomes before kindlin-3 shows up, and paxillin recruitment to podosomes does not require kindlin-3. Interestingly, paxillin is dispensable for podosome formation, but podosomal organization and stability depend on paxillin. However, kindlin-3 is needed for recruitment of leupaxin to podosomes and is also required to cluster integrins to induce integrin-mediated signaling within podosomes (10). Therefore, paxillin family proteins are not crucial for podosome formation but for mediating integrin signaling and controlling the stability of these adhesion structures.

Based on the investigation done by Klapproth et al. in this study and previous reports by others, we now know that kindlin-3 is not required for the initial formation of podosomes but is essential for the activation of integrin signaling that promotes their longer-term assembly. Kindlin-3 stabilizes and prolongs the lifetime of podosomes through recruitment of leupaxin and inhibition of paxillin phosphorylation, which raises several new questions for future investigation. How is paxillin phosphorylation regulated by leupaxin? How is the tyrosine phosphatase activity of PTP-PEST controlled by leupaxin? Is leupaxin stabilized by kindlin-3 in the cytoplasm or in podosomes, and what mechanism underlies this stabilization? We look forward to new insights into how this kindlin-3–leupaxin–paxillin signaling axis controls these dynamic adhesive structures.

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