Moriah Beck, Associate Professor, Wichita State University

Title: New actin branching mechanism facilitated by palladin

Abstract:
Palladin is a recently discovered actin binding protein that plays a key role in both normal cell migration and invasive cell motility, yet its precise function in organizing the actin cytoskeleton is unknown. The majority of this previous research has focused on the scaffolding activity of palladin, whereas our results here add a new dimension to the relationship between palladin and actin by highlighting the direct role in actin assembly. Here we show that the C-terminal immunoglobulin-like domain of palladin, which is directly responsible for actin binding and bundling, also stimulates actin polymerization *in vitro*. Palladin eliminates the lag phase that is characteristic of the slow nucleation step of actin polymerization and dramatically reduced depolymerization. Using *Listeria* infections we show that palladin is co-opted by the bacteria during cellular entry and intracellular motility. Depletion of palladin caused shorter and misshapen comet tails, and when actin- or VASP-binding mutants were overexpressed, comet tails disintegrated or became thinner. We also show that *Listeria* comet tail-based motility could be initiated and maintained in the presence of an Arp2/3 inhibitor and in Arp2/3 complex depleted cells when levels of palladin were increased. Using purified protein components we demonstrate that *Listeria* actin clouds and comet tails can be generated by palladin in the absence of the Arp2/3 complex. Collectively, our results demonstrate that palladin stimulates actin polymerization and indicate that palladin is part of an actin organization and nucleation complex that can functionally replace the Arp2/3 complex in actin-rich structures.

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