ARTICLE ADDENDUM

Syndapin bridges the membrane-cytoskeleton divide during furrow extension

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ABSTRACT

BAR domain proteins can regulate ‘membrane reservoirs’ that provide surface area and buffer membrane tension. Syndapin is an F-BAR and SH3 domain containing protein involved in cytoskeletal remodelling and endocytosis. The Syndapin F-BAR domain is uniquely versatile compared to others in the family and can bend phospholipid membranes into tubules of various diameters and directly bind actin. The Syndapin SH3 domain can also interact with actin remodelling proteins and modulate cytoskeletal contractility. Pseudocleavage furrow extension in the syncytial division cycles of Drosophila embryos requires the homeostatic control of conserved processes that control plasma membrane tension and actin contractility. We find that Syndapin plays an important role in promoting pseudocleavage furrow extension. We propose a model involving roles for Syndapin in membrane dynamics and direct or indirect effect on the cytoskeleton to explain how it affects pseudocleavage furrow growth, independent of its role in endocytosis.

KEYWORDS

actin; Drosophila; F-BAR; furrow extension; metaphase furrow; plasma membrane tension; syncytium; syndapin

Plasma membrane extension is a hallmark of cytokinesis in eukaryotic cells and in pseudocleavage furrow (PF) formation during metaphase of Drosophila syncytial cycles. PF extension, like any cellular membrane protrusion is achieved by 2 homeostatically regulated processes: membrane addition by trafficking and increased actin contractility to provide the force for furrow extension. Here, we discuss possible mechanisms by which an F-BAR domain protein, Syndapin orchestrates membrane and cytoskeletal dynamics during PF extension downstream of furrow initiation proteins during the Drosophila syncytial division cycles. Syndapins belong to the conserved F-BAR subfamily of BAR domain proteins that bend membranes by scaffolding and/or insertion into one membrane leaflet. They bind to membranes via their F-BAR domain and interact with proteins like N-WASp and Dynamin for actin assembly and endocytosis via their SH3 domain. Unlike other F-BAR proteins like CIP4 or FCHO1 that generate tubes of low curvature (~100 nm), Syndapins can stabilize tubules of various diameters (~10–100 nm) and facilitate membrane constriction in vitro giving them a unique membrane remodelling ability. This could allow Syndapins to recruit to and regulate vesicle formation during endocytosis, cilia length in hair cells, dendritic spines, filopodia, lamellipodia and the cytokinetic furrow.

PFs form between adjacent syncytial spindles unlike conventional cytokinetic furrows which form at the central spindle region. The interphase apical membrane is lined by a mesh of cortical actin in a cap over each nucleus. The actin caps expand during prophase meet to form furrows in metaphase (Fig. 1). The protein Scrambled is important for recruitment of actin nucleator Arp2/3 at the margin of the caps to drive their expansion. Scrambled and Arp2/3 mutant embryos result in smaller caps and fail to form PFs. RacGAP50C and RhoGEF2 overlap with astral microtubules at the cap periphery and initiate PF formation. RhoGEF2 is specific for syncytial division cycles and its overexpression generates ectopic furrows but it does not have a role in conventional cytokinesis like RhoGEF Pebble. Activated Rho1 is present in an apical ring during interphase that extends basally (at future sites of PF ingestion) in metaphase. We find that Syndapin mutant embryos have shorter PF length. RhoGEF2 localization is unaffected upon Syndapin depletion; also overexpression of Syndapin does not generate ectopic furrows, thus, suggesting

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that it has a role in PF extension. Our data along with previous studies show that in the absence of formin Diaphanous (Dia), Anillin and septins, PF extension fails (Fig. 1). Syndapin mutant embryos affect the distribution of Dia and the septin, Peanut. Most remarkably, overexpression of Syndapin in RhoGEF2 mutant embryos reversed the loss of Dia, Anillin and Peanut, thus showing that Syndapin presence is at least partly responsible for their distribution on the PF membrane. Not surprisingly, the overexpression of Syndapin reversed the PF extension defect in RhoGEF2 mutant embryos. Syndapin may also interact with N-WASp in PFs and regulate Arp2/3-based actin polymerization, thus, also affecting PF extension, but this function remains to be ascertained.

Syndapin mutant embryos do not completely abolish PFs; therefore it is possible that other F-BAR proteins also influence PF extension. Overexpression of F-BAR protein Cip4, an activator of Arp2/3 nucleator complex, phenocopies Dia loss-of-function phenotypes. Cip4 functions synergistically with a related F-BAR protein Nostrin and their depletion forms tubular vesicles at adherens junctions. However, their role in PF formation remains to be investigated.

Polarized membrane insertion via exocytosis requires WASp-mediated activation of Arp2/3 for release of secretory cargo. RalA is a small-GTPase that functions through the exocyst complex to control directed endocytosis and vesicle recycling by Rab11 are implicated in PF formation during the syncytial cycles. Rab11 recruits Nuf and this, in turn, affects RhoGEF2 recruitment and Rho1 activation. However, we find that overexpression of Syndapin does not rescue the endocytic defects seen in RhoGEF2 mutant embryos, but reverses the PF extension defects. Hence, even though Syndapin mutant embryos are defective in endocytosis, PF extension does not depend upon Syndapin mediated endocytosis. Interestingly, we find that enabling slow actin polymerization by cytochalasinD treatment in Syndapin-depleted embryos can rescue the PF extension phenotype. The mammalian Syndapin-2 F-BAR domain (and not other F-BAR proteins like CIP4 or FCHO2) directly binds and slows down depolymerization rates of actin filaments. Syndapin could either directly bind actin and modulate its polymerization or indirectly affect actin-remodeling proteins. During furrow extension, plus ends of actin filaments are oriented toward the plasma membrane (few nm apart) so that a single monomer is added every time the filament tip and membrane separate from each other by simple brownian motion. There exists a competition, albeit weak, between negatively charged liposomes and F-actin for interaction with Syndapin’s F-BAR domain, hence, Syndapin could shuttle between membrane and actin-bound states to coordinate PF extension.

Figure 1. The syncytial Drosophila nuclear cycle proceeds from interphase to prophase during which the apical caps expand and meet to form PFs. PF initiation involves recruitment of RhoGEF2 to the membrane and subsequent activation of Rho1 by RhoGEF2. From prophase to metaphase, PF extension occurs during which microvilli-like protrusions on the apical surface could flatten to supply membrane to the PF as it extends. Syndapin may associate with microvilli and regulate their length. Rho1 activates Diaphanous for actin assembly that drives PF extension. RacGAP50C recruits Anillin which interacts with Peanut and Syndapin which, in turn, interact with actin and regulate actin dynamics and PF extension.
membrane addition\textsuperscript{28} and embryos mutant for RalA contain shorter furrows and have defective actin polymerization.\textsuperscript{29} Since embryos mutant for Syndapin that have short furrows and a defect in actin polymerization, it is possible that RalA and the exocyst complex will not function efficiently in membrane addition.

Cortical tension plays a role in the extent of furrow progression.\textsuperscript{30} Assembly/disassembly of BAR proteins depends on plasma membrane tension and can, therefore, buffer membrane tension during mechanical stress.\textsuperscript{31} Since Syndapin-2 associates with proteins that regulate microvillar length,\textsuperscript{32} it is possible that other dynamic actin protrusions like microvilli present on the apical surface of \textit{Drosophila} syncytial embryos may serve as membrane reservoirs for the extension of the PF, and Syndapin could mediate this release. Concurrent with this model, we find that Syndapin localizes to the apical microvillar membrane in interphase and is enriched on the lateral membrane during metaphase. Loss of Syndapin, Peanut and Dia from the PF shows loose membrane in the furrow, probably due to reduced plasma membrane tension and results in defects in furrow elongation.\textsuperscript{16}

To summarize, we propose a model (Fig. 1) that encompasses Syndapin’s roles in the context of furrow formation. Firstly, Syndapin can associate/dissociate from the membrane and modulate plasma membrane tension at the PF. It could release membrane tension from apical microvilli and organize actin in furrows allowing furrow elongation. Secondly, Syndapin can stabilize Anillin, Peanut and Dia to the PF for extension. Finally, Syndapin can directly regulate rates of actin depolymerization that drives furrow extension. Therefore, our model places Syndapin as an important player in the control of membrane and cytoskeletal remodeling that orchestrate PF extension.

Abbreviations

- Arp: Actin-related protein
- BAR: Bin-Amphiphysin-Rvs
- Dia: Diaphanous
- GEF: Guanine nucleotide exchange factor
- PF: Pseudocleavage furrow

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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