

Syndapin bridges the membrane-cytoskeleton divide during furrow extension

Aparna Sherlekar  and Richa Rikhy 

Biology, Indian Institute of Science Education and Research, Pune, India

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.

ARTICLE HISTORY

Received 3 August 2016
Revised 24 October 2016
Accepted 24 October 2016



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.¹ 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.² 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.³ 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.⁴ 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.⁵ This could allow Syndapins to recruit to and regulate

vesicle formation during endocytosis,⁶ cilia length in hair cells,⁷ dendritic spines,⁸ filopodia,⁹ lamellipodia¹⁰ and the cytokinetic furrow.¹¹

PFs form between adjacent syncytial spindles unlike conventional cytokinetic furrows which form at the central spindle region.¹ The interphase apical membrane is lined by a mesh of cortical actin in a cap over each nucleus.¹² 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.^{13,14} RacGAP50C and RhoGEF2 overlap with astral microtubules at the cap periphery and initiate PF formation.¹⁵ 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.¹⁵ Activated Rho1 is present in an apical ring during interphase that extends basally (at future sites of PF ingression) 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

CONTACT Richa Rikhy  richa@iiserpune.ac.in  Biology, Indian Institute of Science Education and Research, Dr. Homi Bhabha Road, Pashan, Pune, 411008, India.

Addendum to: Sherlekar A, Rikhy R. Syndapin promotes pseudocleavage furrow formation by actin organization in the syncytial *Drosophila* embryo. *Molecular Biology of the Cell*, 27(13), 2064–2079; <http://dx.doi.org/10.1091/mbc.E15-09-0656>

© 2017 IISER. Published with license by Taylor & Francis.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited, and is not altered, transformed, or built upon in any way.

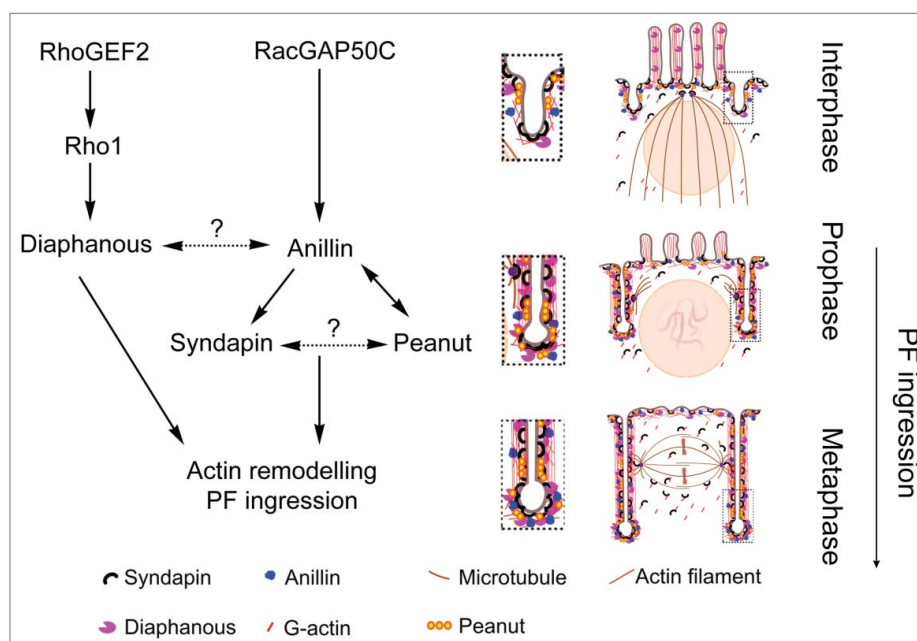


Figure 1. The syncytial *Drosophila* nuclear cycle proceeds from interphase to prophase during which the apical caps expand and meet to form PFs.^{13,33} PF initiation involves recruitment of RhoGEF2 to the membrane²² and subsequent activation of Rho1 by RhoGEF2.³⁴ From prophase to metaphase, PF extension 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.³⁴ RacGAP50C recruits Anillin³⁵ which interacts with Peanut and Syndapin which, in turn, interact with actin and regulate actin dynamics and PF extension.^{16,36}

that it has a role in PF extension.¹⁶ Our data along with previous studies show that in the absence of formin Diaphanous (Dia), Anillin and septins, PF extension fails¹⁶⁻¹⁸ (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.¹⁶ Syndapin may also interact with N-WASP¹⁹ in PFs²⁰ and regulate Arp2/3-based actin polymerization, thus, also affecting PF extension, but this function remains to be ascertained.

Syndapin functions in endocytosis;⁶ Dynamin-mediated endocytosis and vesicle recycling by Rab11 are implicated in PF formation during the syncytial cycles.^{21,22} Rab11 recruits Nuf and this, in turn, affects RhoGEF2 recruitment and Rho1 activation.²² 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.¹⁶ 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 actin²³ 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.²⁴ There exists a competition, albeit weak, between negatively charged liposomes and F-actin for interaction with Syndapin's F-BAR domain,²³ hence, Syndapin could shuttle between membrane and actin-bound states to coordinate PF extension.

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.²⁵ Cip4 functions synergistically with a related F-BAR protein Nostrin and their depletion forms tubular vesicles at adherens junctions.²⁶ 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.²⁷ RalA is a small-GTPase that functions through the exocyst complex to control directed

membrane addition²⁸ and embryos mutant for RalA contain shorter furrows and have defective actin polymerization.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 ingression.³⁰ Assembly/disassembly of BAR proteins depends on plasma membrane tension and can, therefore, buffer membrane tension during mechanical stress.³¹ Since Syndapin-2 associates with proteins that regulate microvillar length,³² it is possible that other dynamic actin protrusions like microvilli present on the apical surface of *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.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.

Acknowledgments

AS thanks the Council of Scientific and Industrial Research, India for funding. RR thanks IISER, Pune, DBT and DST for funding.

ORCID

Aparna Sherlekar  <http://orcid.org/0000-0003-3413-9599>
Richa Rikhy  <http://orcid.org/0000-0002-4262-0238>

References

- [1] Sisson JC, Rothwell WF, Sullivan W. Cytokinesis: lessons from rappaport and the *Drosophila* blastoderm embryo. *Cell Biol Int* 1999; 23:871-6; PMID:10772762; <http://dx.doi.org/10.1006/cbir.1999.0484>
- [2] Bezanilla M, Gladfelter AS, Kovar DR, Lee WL. Cytoskeletal dynamics: A view from the membrane. *J Cell Biol* 2015; 209:329-37; PMID:25963816; <http://dx.doi.org/10.1083/jcb.201502062>
- [3] Wang Q, Navarro MVAS, Peng G, Molinelli E, Goh SL, Judson BL, Rajashankar KR, Sondermann H. Molecular mechanism of membrane constriction and tubulation mediated by the F-BAR protein Pacsin/Syndapin. *Proc Natl Acad Sci U S A* 2009; 106:12700-5; PMID:19549836; <http://dx.doi.org/10.1073/pnas.0902974106>
- [4] Qualmann B, Kelly RB. Syndapin isoforms participate in receptor-mediated endocytosis and actin organization. *J Cell Biol* 2000; 148:1047-62; PMID:10704453; <http://dx.doi.org/10.1083/jcb.148.5.1047>
- [5] Bai X, Zheng X. Tiptotip interaction in the crystal packing of PACSIN 2 is important in regulating tubulation activity. *Protein Cell* 2013; 4:695-701; PMID:23888307; <http://dx.doi.org/10.1007/s13238-013-3041-x>
- [6] Quan A, Robinson PJ. Syndapin - a membrane remodeling and endocytic F-BAR protein. *FEBS J* 2013; 280:5198-212; PMID:23668323; <http://dx.doi.org/10.1111/febs.12343>
- [7] Schüler S, Hauptmann J, Perner B, Kessels MM, Englert C, Qualmann B. Ciliated sensory hair cell formation and function require the F-BAR protein syndapin I and the WH2 domain-based actin nucleator Cobl. *J Cell Sci* 2013; 126:196-208; PMID:23203810; <http://dx.doi.org/10.1242/jcs.111674>
- [8] Schneider K, Seemann E, Liebmann L, Ahuja R, Koch D, Westermann M, Hübner CA, Kessels MM, Qualmann B. ProSAP1 and membrane nanodomain-associated syndapin1 promote postsynapse formation and function. *J Cell Biol* 2014; 205:197-215; PMID:24751538; <http://dx.doi.org/10.1083/jcb.201307088>
- [9] Shimada A, Takano K, Shirouzu M, Hanawa-Suetsugu K, Terada T, Toyooka K, Umehara T, Yamamoto M, Yokoyama S, Suetsugu S. Mapping of the basic amino-acid residues responsible for tubulation and cellular protrusion by the EFC/F-BAR domain of pacsin2/Syndapin II. *FEBS Lett* 2010; 584:1111-8; PMID:20188097; <http://dx.doi.org/10.1016/j.febslet.2010.02.058>
- [10] de Kreuk B-J, Anthony EC, Geerts D, Hordijk PL. The F-BAR Protein PACSIN2 Regulates Epidermal Growth Factor Receptor Internalization. *J Biol Chem* 2012; 287:43438-53; PMID:23129763; <http://dx.doi.org/10.1074/jbc.M112.391078>
- [11] Takeda T, Robinson IM, Savoian MM, Griffiths JR, Whetton AD, McMahon HT, Glover DM. *Drosophila* F-BAR protein Syndapin contributes to coupling the plasma membrane and contractile ring in cytokinesis.

- Open Biol 2013; 3:130081; PMID:23926047; <http://dx.doi.org/10.1098/rsob.130081>
- [12] Foe VE, Field CM, Odell GM. Microtubules and mitotic cycle phase modulate spatiotemporal distributions of F-actin and myosin II in *Drosophila* syncytial blastoderm embryos. *Development* 2000; 127:1767-87; PMID:10751167
- [13] Stevenson V, Hudson A, Cooley L, Theurkauf WE. Arp2/3-dependent pseudocleavage [correction of pseudocleavage] furrow assembly in syncytial *Drosophila* embryos. *Curr Biol [Internet]* 2002; 12:705-11; PMID:12007413; [http://dx.doi.org/10.1016/S0960-9822\(02\)00807-2](http://dx.doi.org/10.1016/S0960-9822(02)00807-2)
- [14] Stevenson VA, Kramer J, Kuhn J, Theurkauf WE. Centrosomes and the Scrambled protein coordinate microtubule-independent actin reorganization. 2001; 3:68-75
- [15] Crest J, Concha-Moore K, Sullivan W. RhoGEF and Positioning of Rappaport-like Furrows in the Early *Drosophila* Embryo. *Curr Biol* 2012; 22:2037-41; PMID:23022066; <http://dx.doi.org/10.1016/j.cub.2012.08.046>
- [16] Sherlekar A, Rikhy R. Syndapin promotes pseudocleavage furrow formation by actin organization in the syncytial *Drosophila* embryo. *Mol Biol Cell* 2016; 27:2064-79; PMID:27146115; <http://dx.doi.org/10.1091/mbc.E15-09-0656>
- [17] Afshar K, Stuart B, Wasserman SA. Functional analysis of the *Drosophila* Diaphanous FH protein in early embryonic development. *Development* 2000; 127:1887-97; PMID:10751177
- [18] Silverman-Gavrila R V, Hales KG, Wilde A. Anillin-mediated targeting of peanut to pseudocleavage furrows is regulated by the GTPase ran. *Mol Biol Cell* 2008; 19:3735-44; PMID:18579688; <http://dx.doi.org/10.1091/mbc.E08-01-0049>
- [19] Kessels MM, Qualmann B. Syndapins integrate N-WASP in receptor-mediated endocytosis. *EMBO J* 2002; 21:6083-94; PMID:12426380; <http://dx.doi.org/10.1093/emboj/cdf604>
- [20] Rodriguez-Mesa E, Abreu-Blanco MT, Rosales-Nieves AE, Parkhurst SM. Developmental expression of *Drosophila* Wiskott-Aldrich Syndrome family proteins. *Dev Dyn* 2012; 241:608-26; <http://dx.doi.org/10.1002/dvdy.23899>
- [21] Rikhy R, Mavrikis M, Lippincott-Schwartz J. Dynamin regulates metaphase furrow formation and plasma membrane compartmentalization in the syncytial *Drosophila* embryo. *Biol Open* 2015; 4:301-11; PMID:25661871; <http://dx.doi.org/10.1242/bio.20149936>
- [22] Cao J, Albertson R, Riggs B, Field CM, Sullivan W. Nuf, a Rab11 effector, maintains cytokinetic furrow integrity by promoting local actin polymerization. *J Cell Biol* 2008; 182:301-13; PMID:18644888; <http://dx.doi.org/10.1083/jcb.200712036>
- [23] Kostan J, Salzer U, Orlova A, Törö I, Hodnik V, Senju Y, Zou J, Schreiner C, Steiner J, Meriläinen J, et al. Direct interaction of actin filaments with F-BAR protein pacsin2. *EMBO Rep* 2014; 15:1154-62; PMID:25216944; <http://dx.doi.org/10.15252/embr.201439267>
- [24] Peskin CS, Odell GM, Oster GF. Cellular motions and thermal fluctuations: the Brownian ratchet. *Biophys J* 1993; 65:316-24; PMID:8369439; [http://dx.doi.org/10.1016/S0006-3495\(93\)81035-X](http://dx.doi.org/10.1016/S0006-3495(93)81035-X)
- [25] Yan S, Lv Z, Winterhoff M, Wenzl C, Zobel T, Faix J, Bogdan S, Grosshans J. The F-BAR protein Cip4/Toca-1 antagonizes the formin Diaphanous in membrane stabilization and compartmentalization. *J Cell Sci* 2013; 126:1796-805; PMID:23424199; <http://dx.doi.org/10.1242/jcs.118422>
- [26] Zobel T, Brinkmann K, Koch N, Schneider K, Seemann E, Fleige A, Qualmann B, Kessels MM, Bogdan S. Cooperative functions of the two F-BAR proteins Cip4 and Nostrin in the regulation of E-cadherin in epithelial morphogenesis. *J Cell Sci* 2015; 128:499-515; PMID:25413347; <http://dx.doi.org/10.1242/jcs.155929>
- [27] Tran DT, Masedunskas A, Weigert R, Hagen KG Ten. Arp2/3-mediated F-actin formation controls regulated exocytosis in vivo. *Nat Commun* 2015; 6:1-10
- [28] Moskalenko S, Henry DO, Rosse C, Mirey G, Camonis JH, White MA. The exocyst is a Ral effector complex. *Nat Cell Biol* 2002; 4:66-72; PMID:11740492
- [29] Holly RM, Mavor LM, Zuo Z, Blankenship JT. A rapid, membrane-dependent pathway directs furrow formation through RalA in the early *Drosophila* embryo. *Development* 2015; 142:2316-28; PMID:26092850; <http://dx.doi.org/10.1242/dev.120998>
- [30] Gerald N, Dai J, Ting-Beall HP, De Lozanne A. A role for Dictyostelium race in cortical tension and cleavage furrow progression. *J Cell Biol* 1998; 141:483-92; PMID:9548725; <http://dx.doi.org/10.1083/jcb.141.2.483>
- [31] Simunovic M, Voth GA, Callan-Jones A, Bassereau P. When Physics Takes Over: BAR Proteins and Membrane Curvature. *Trends Cell Biol* 2015; 25:780-92; PMID:26519988; <http://dx.doi.org/10.1016/j.tcb.2015.09.005>
- [32] Schwintzer L, Koch N, Ahuja R, Grimm J, Kessels MM, Qualmann B. The functions of the actin nucleator Cobl in cellular morphogenesis critically depend on syndapin I. *EMBO J* 2011; 30:3147-59; PMID:21725280; <http://dx.doi.org/10.1038/emboj.2011.207>
- [33] Cao J, Crest J, Fasulo B, Sullivan W. Cortical Actin Dynamics Facilitate Early-Stage Centrosome Separation. *Curr Biol* 2010; 20:770-6; PMID:20409712; <http://dx.doi.org/10.1016/j.cub.2010.02.060>
- [34] Grosshans J, Wenzl C, Herz H-M, Bartoszewski S, Schnorrer F, Vogt N, Schwarz H, Müller HA. RhoGEF2 and the formin Dia control the formation of the furrow canal by directed actin assembly during *Drosophila* cellularisation. *Development* 2005; 132:1009-20; PMID:15689371; <http://dx.doi.org/10.1242/dev.01669>
- [35] D'Avino PP, Takeda T, Capalbo L, Zhang W, Lilley KS, Laue ED, Glover DM. Interaction between Anillin and RacGAP50C connects the actomyosin contractile ring with spindle microtubules at the cell division site. *J Cell Sci* 2008; 121:1151-8; <http://dx.doi.org/10.1242/jcs.026716>
- [36] Mavrikis M, Azou-Gros Y, Tsai F-C, Alvarado J, Bertin A, Iv F, Kress A, Brasselet S, Koenderink GH, Lecuit T. Septins promote F-actin ring formation by crosslinking actin filaments into curved bundles. *Nat Cell Biol* 2014; 16:322-34; PMID:24633326; <http://dx.doi.org/10.1038/ncb2921>