F-BAR domain protein Syndapin regulates actomyosin dynamics during apical cap remodeling in syncytial Drosophila embryos
Aparna Sherlekar, Gayatri Mundhe, Prachi Richa, Bipasha Dey, Swati Sharma, Richa Rikhy

To cite this version:

HAL Id: hal-03141809
https://hal-amu.archives-ouvertes.fr/hal-03141809
Submitted on 15 Feb 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Copyright
SHORT REPORT

F-BAR domain protein Syndapin regulates actomyosin dynamics during apical cap remodeling in syncytial Drosophila embryos

Aparna Sherlekar*, Gayatri Mundhe*, Prachi Richa‡,¶, Bipasha Dey, Swati Sharma and Richa Rikhy**

ABSTRACT

Branched actin networks driven by Arp2/3 interact with actomyosin filaments in processes such as cell migration. Similar interactions occur in the syncytial Drosophila blastoderm embryo where expansion of apical caps by Arp2/3-driven actin polymerization occurs in interphase, and cap buckling at contact edges by Myosin II to form furrows takes place in metaphase. Here, we study the role of Syndapin (Synd), an F-BAR domain-containing protein, in apical cap remodeling prior to furrow extension. We found that depletion of synd resulted in larger apical caps. Super-resolution and TIRF microscopy showed that control embryos had long apical actin protrusions in caps during interphase and short protrusions during metaphase, whereas synd depletion led to formation of sustained long protrusions, even during metaphase. Loss of Arp2/3 function in synd mutants partly reverted defects in apical cap expansion and protrusion remodeling. Myosin II levels were decreased in synd mutants, an observation consistent with the expanded cap phenotype previously reported for Myosin II mutant embryos. We propose that Synd function limits branching activity during cap expansion and affects Myosin II distribution in order to bring about a transition in actin remodeling activity from apical cap expansion to lateral furrow extension.

KEY WORDS: Actomyosin, Syndapin, Cap, Metaphase furrow, Drosophila

INTRODUCTION

Branched and bundled actin networks, along with contractile proteins, mediate processes such as cell migration and cell shape remodeling in embryogenesis. Directional motility in crawling cells is characterized by Arp2/3-driven branched actin polymerization at the leading edge of the lamellipodial sheet and by actomyosin-driven contractile forces in the lamella (Pollard and Borisy, 2003; Ponti et al., 2004). Apical actin rings in 16-cell-stage mouse embryos expand through polymerization by formins and trigger zipperping of junctions at edges where they contact actomyosin (Zenker et al., 2018). Apical actin caps in Drosophila syncytial blastoderm embryos (Karr and Alberts, 1986) also expand, as a result of Arp2/3 complex activity, and meet at syncytial borders to form lateral furrows with distinct polarity (Mavrakis et al., 2009; Schmidt and Grosshans, 2018; Zenker et al., 2018; Zhang et al., 2018). BAR domain-containing proteins are known to play a role in actin remodeling (Carman and Dominguez, 2018; Sherlekar and Rikhy, 2016; Stanishneva-Konovalova et al., 2016). The F-BAR domain-containing protein Syndapin (Synd) interacts with N-WASP via its SRC homology 3 (SH3) domain, thereby activating Arp2/3-mediated actin polymerization (Modregger, 2000; Kessels and Qualmann, 2002; Dharmalingam et al., 2009). However, its function in regulating transitions between different actin networks has not been studied. Here, we analyze the role of Synd in Arp2/3 complex- and actomyosin-driven actin cap remodeling in Drosophila syncytial blastoderm embryos.

The Drosophila syncytial embryo consists of cortical nuclei surrounded by an apical actomyosin-rich cap with microvilli (protrusions) that resemble filopodia (Foe and Alberts, 1983; Karr and Alberts, 1986; Mavrakis et al., 2009; Turner and Mahowald, 1976; Wann et al., 1980, 1984; Young et al., 1991). Apical caps expand during each nuclear cycle (NC) and come into contact with each other at sites where lateral furrows then extend between adjacent nuclei. Arp2/3 complex mutants have smaller apical caps, whereas Rho-kinase inhibition leading to loss of Myosin II results in larger caps (Cao et al., 2010; Stevenson et al., 2002; Zallen et al., 2002; Zhang et al., 2018). Expanding caps formed by cortical actomyosin assembly are also enriched in Anillin, Syndapin, the septin Peanut and the formin Diaphanos. Mutant embryos in which these proteins are disrupted show decreased furrow extension (Afshtar et al., 2000; Fares et al., 1995; Field and Alberts, 1995; Foe et al., 2000; Sherlekar and Rikhy, 2016; Silverman-Gavrilva et al., 2008).

Syndapin mutants show defects in recruitment of Peanut to the furrow (Sherlekar and Rikhy, 2016). The Anillin–Peanut complex compensates for Myosin II loss, favoring the transition of F-actin from the cap to the furrow and promoting furrow ingestion (Zhang et al., 2018). We therefore performed an analysis of Syndapin function in regulating actin remodeling during cap expansion and furrow extension. Using a combination of live imaging by TIRF microscopy (TIRFM) and stimulated emission depletion (STED) microscopy, we show that interphase caps contain longer F-actin protrusions compared to metaphase caps. We find that synd mutants contain larger caps and sustained protrusions with increased F-actin remodeling and Arp2/3 activity. Syndapin function limits Arp2/3 activity during cap expansion to facilitate buckling by Myosin II for furrow extension.

RESULTS AND DISCUSSION

Syndapin mutant embryos contain enlarged caps, short furrows and defects in F-actin dynamics

We expressed a GFP-tagged version of the PH domain of GRP1 (tGPH, also known as Step), which binds the phospholipid phosphatidylinositol (3,4,5)-trisphosphate (Britton et al., 2002), in control and synd mutants to visualize apical cap area and furrow length dynamics during NC12 (Fig. 1A; Movie 1). Apical caps...
expanded from interphase to prophase, contacted each other and lateral furrow extension occurred at the edges (Fig. 1B–D). Cap area increased in tGPH/+ embryos (Movie 1) until ~6 min, after which the membranes came into contact and the area plateaued (~6.5 min) while the lateral furrows continued to extend (Fig. 1D). We visualized F-actin dynamics during cap expansion in apical sections of embryos labeled with LifeAct–GFP (Fig. 1E). LifeAct–GFP levels increased at the cap periphery as they expanded but started to decrease once the caps were in contact (Fig. 1E,F). Depletion of the Arp3 subunit of the Arp2/3 complex resulted in significantly smaller caps that formed but did not expand and did not initiate lateral furrow formation (Fig. S1A–C, Movie 2). LifeAct–GFP failed to accumulate at the cap periphery in arp3 mutants (Fig. S1D,E).

To test the role of Syndapin in cap and furrow dynamics, we used embryos from a previously reported hypomorphic allele of Syndapin in combination with a deficiency (synd^1/Df, hereafter termed synd) (Kumar et al., 2009b; Sherlek and Rikhy, 2016). Interestingly, on Syndapin depletion, caps were significantly larger and adjacent caps were already in contact in interphase (at 0 min). Cap expansion in synd reached a steady state earlier (~4.5 min) than in controls (~6.5 min) (Fig. 1B,C; Movie 3). Furrows were shorter in synd compared to those in controls (Fig. 1D). LifeAct–GFP localization increased at the cap periphery similar to controls (0-4 min) but remained at a significantly higher level in synd, even after the caps came into contact (~4 min) (Fig. 1E,F). The nuclear signal was visible in synd and not in controls, suggesting that the caps were flatter in synd (Fig. 1E). Syndapin function is likely to be important for limiting cap expansion and regulating F-actin dynamics at the cap periphery for the transition to furrow formation.

**Syndapin mutants show sustained F-actin protrusions in apical caps**

Scanning electron microscopy and TIRFM have shown protrusions in the apical domain of caps in interphase of the NC (Turner and Mahowald, 1976; Webb et al., 2009). To investigate the dynamics of actin-rich protrusions we observed the apical region of LifeAct–GFP-labeled caps using TIRFM and phalloidin-labeled F-actin structures using STED microscopy. In TIRFM, an evanescent excitation wave is propagated at the interface between two materials with different refractive indices and this results in the excitation of fluorophores within ~100 nm of the interface (Axelrod, 2001). In LifeAct–GFP-expressing embryos, this wave illuminated the apical region of the cap. In control embryos, we saw apical villi-like protrusions (Fig. 2A), as previously observed in syncytial embryos using TIRFM (Webb et al., 2009). The protrusions appeared as puncta in the middle of the cap and were longer at the edge of the interphase cap, reflecting different orientations, similar to those observed apically by TIRFM during cellularization (Figard et al., 2016). In contrast, shorter protrusions in the form of puncta were observed throughout the metaphase cap. Only optically separable protrusions were used to quantitify their observable length (Fig. S1F). The average protrusion length was 0.86 μm in interphase, and a few protrusions at the periphery were as long as ~2.6 μm. The protrusions were significantly longer in interphase as compared to their length in metaphase where the average was 0.67 μm with a maximum of ~1.5 μm (Fig. 2B). This suggested that long villi-like protrusions at the apical cap are remodeled at the time of furrow ingressation.

STED super-resolution microscopy showed phalloidin-labeled, F-actin-containing protrusions at the cap periphery in control embryos. Apical structures in the form of puncta seen in TIRFM were not visible with phalloidin labeling in STED (Fig. S1F,G). Furthermore, in control metaphase caps protrusions were significantly shorter or absent, and F-actin was organized at cap edges along the depth of the furrow (Fig. 2C,D). In summary, the periphery of the interphase caps showed longer actin-rich protrusions, and this distribution was coincident with neighboring cap membranes approaching each other. Shorter protrusions were seen in metaphase when actin was enriched at the furrow (Fig. 2E).

In Arp3-depleted embryos, the majority of protrusions in interphase and metaphase caps were significantly shorter than controls (Fig. 2B) and were of similar length in interphase (average of 0.57 μm) and metaphase (average of 0.55 μm) (Fig. S1H,I). Thus, formation of long protrusions is Arp2/3 dependent, and their absence may result in loss of cap expansion (Stevenson et al., 2002; Zhang et al., 2018) (Fig. 2E, Fig. S1J).

In synd, the mean protrusion lengths in interphase (0.81 μm) and metaphase (0.75 μm) caps were not significantly different from each other and from the mean length in control interphase caps (Fig. 2A,B). The lack of a significant difference was also seen in phalloidin-labeled caps imaged with STED (Fig. 2C,D). Because protrusions remained long during metaphase in synd, we tested whether Arp2/3 localization was affected in synd mutants. In controls, Arp3 was enriched around the rim of the apical actin cap. In synd, Arp3 was spread across the entire cap surface (Fig. S2A), however, Arp3 levels were unchanged in interphase as compared to levels in controls (Fig. S2B).

We also performed fluorescence loss in photobleaching (FLIP) experiments using LifeAct–GFP to assess actin dynamics across the cap in control and synd embryos. LifeAct–GFP exchange during cap expansion was significantly faster in synd than in controls (Fig. S3A–D) suggesting an increase in F-actin remodeling across the apical domain. The spread of Arp3 localization throughout the cap in synd correlated with higher F-actin remodeling and may contribute to cap expansion and protrusion remodeling.

**ArpC1 depletion in synd rescues apical cap expansion dynamics and furrow length**

Vertebrate syndapins interact with N-WASP and activate Arp2/3-mediated actin polymerization (Modregger, 2000; Kessels and Qualmann, 2002). However, our data shows that depletion of Syndapin and Arp3 do not phenocopy each other. Syndapin mutants had larger caps, whereas Arp3 mutant caps failed to expand. This indicates that Syndapin and the Arp2/3 complex do not function in a linear pathway. To further confirm this, we depleted ArpC1, an essential component of the Arp2/3 complex, in the synd mutant background (arpC1/+; synd) and observed cap expansion and furrow extension using tGPH. Interestingly, we found that arpC1/+; synd had smaller caps (Fig. 3A,B) as compared to synd (Fig. 1C; Movie 4). In addition to this partial rescue of the cap size, furrow extension was also rescued (Fig. 3C). LifeAct–GFP accumulated at the cap periphery in arpC1/+; synd even though it remained at a lower level than in control embryos (Fig. S1D,E).

TIRFM revealed longer protrusions during interphase and shorter protrusions during metaphase in arpC1/+; synd (Fig. 3D,E) similar to the situation in control caps and unlike the protrusions observed for synd (Fig. 2A,B). Measurements of protrusion lengths in arpC1/+; synd revealed an average length of 0.72 μm in interphase (with a few protrusions reaching 2.4 μm) and 0.6 μm in metaphase (Fig. 3E). This significant difference in protrusion lengths between interphase and metaphase caps that reducing Arp2/3 complex activity in synd mutants allowed F-actin remodeling from long protrusions to short protrusions to occur during cap expansion and lateral furrow extension as the embryos progressed from interphase to metaphase. STED microscopy also revealed that,
unlike in synd embryos (Fig. 2C,D), long F-actin protrusions were not seen during metaphase of arpC1/+; synd embryos, an observation consistent with the rescue of cap expansion and furrow extension (Fig. 3F,G). The rescue of cap expansion and furrow extension in arpC1/+; synd also suggested that these embryos were able to remodel their protrusions to transition from cap expansion to furrow ingression (Fig. 3H).

**Myosin II is reduced at the cortex and remains associated with the metaphase furrow membrane in synd mutants**

Non-muscle Myosin II is implicated in restricting cap expansion, driving buckling at cap edges during the transition to furrow formation and creating tension across the cap (Sommi et al., 2011; Zhang et al., 2018). We used an anti-Zipper (Myosin II heavy chain) antibody and Sqh–GFP (Myosin II light chain reporter) to observe the Myosin II distribution in fixed and live samples of control and synd embryos. Zipper and Sqh–GFP (Fig. 4A,B) displayed distinct dynamic localization during the NC compared to the localization of the membrane marker Dlg; they localized to the cap periphery during expansion, and were cytosolic at metaphase with enrichment in the spindle region when the lateral furrows were at their longest (Royou et al., 2002). Zipper staining was reduced overall and was not present in the spindle region in synd. Zipper continued to be localized at vertices in metaphase – the points of highest tension in

Fig. 1. synd shows defects in cap and furrow remodeling and F-actin dynamics. (A) Imaging Drosophila syncytial NCs using an inverted microscope (left). NC schematic (right) is rotated by 180° with apical at the top to represent the orientation in sagittal views. Interphase embryos have apical centrioles and short furrows, and metaphase embryos have lateral centrosomes and long furrows. Dashed line indicates the plane of membrane shown in the top panel. (B–D) synd embryos expressing tGPH show significantly increased NC12 cap area (B,C) and significantly shorter furrow length (B,D). (E,F) Peripheral LifeAct–GFP intensity in synd embryos is significantly higher than in controls. Graphs show mean±s.e.m. for n=3 embryos, with three caps quantified per embryo. ***P=0.001 (one-way ANOVA and Tukey’s multiple comparison test). Scale bars: 10 μm.
the cell membrane (Higashi and Miller, 2017) (Fig. 4A). Sqh–GFP intensities in the caps were also significantly lower in synd compared to controls (Fig. 4B,D; Movies 5–7), and Sqh–GFP remained on the membrane well after metaphase onset in synd mutant embryos (Fig. 4B,C). Sqh–GFP intensity remained low in arpC1/+; synd embryos but was lost from the furrow membrane in metaphase, similar to the Sqh–GFP dynamics observed in controls (Fig. 4B–D).

Cap and furrow dynamics were observed in sqhRNAi embryos using tGPH. We observed that sqhRNAi embryos phenocopied synd mutants; the cap area was larger, and furrow lengths significantly shorter, than in controls (Fig. S4A–C, Movie 8). In sqhRNAi embryos, Syndapin levels were reduced on the membrane, with some Syndapin remaining at the vertices (Fig. S2A), and Arp3 was present on the membrane. Thus, the expanded caps in synd showed decreased Myosin II intensity (Fig. 4D) despite a small amount of Myosin II being retained on the metaphase membrane.

Our work can be summarized in a model where we predict that actin-rich apical protrusions in interphase retract into the cortex to supplement membrane and/or actin for furrow extension during metaphase. The growth of these long protrusions is dependent on activity of the Arp2/3 complex. Syndapin restricts Arp2/3 complex activity in cap expansion thereby supporting furrow extension in a timely manner. Once the caps come into contact, these protrusions are remodeled to drive furrow formation by buckling forces regulated by Myosin II contractile activity (Zhang et al., 2018), a transition step that requires Syndapin.
Fig. 3. See next page for legend.
controls, and unlike measured per embryo, *** shows decreased protrusion length and increased furrow lengths in metaphase.

Syndapin mutant phenotypes are rescued upon loss of ArpC1. Cell spreading is inhibited by low concentrations of cytochalasin D, a drug that slows the rate of actin polymerization (Wakatsuki, 2003). We have previously shown that furrow lengths are rescued when synd mutants are treated with low concentrations of cytochalasin D (Sherlekar and Rikhy, 2016). Here we have shown, using photobleaching experiments, that turnover of F-actin is higher in synd caps. Syndapin function is therefore required to limit Arp2/3 complex-mediated F-actin dynamics in the cap and to promote furrow ingestion.

Myosin II is decreased in synd mutant caps. A theoretical model of cell spreading predicts that Myosin II in apical regions of the cell periphery facilitates spreading, whereas it opposes spreading when present in deeper regions of the lamella (Nisenholz et al., 2016). We have demonstrated that Syndapin is required to limit apical cap expansion, resulting in optimal Myosin II distribution to allow inward growth of the lateral furrow, in a manner analogous to regulation of cell spreading. Future studies on the role of Syndapin in fine tuning actin-remodeling protein activity and Myosin II-dependent contractility in different contexts will shed light on the diversity of processes that this protein can regulate in development.

**MATERIALS AND METHODS**

*Drosophila* stocks

Flies were raised on standard cornmeal agar at 25°C. Embryos from Canton S flies were used in control experiments. Homozygous escaper adults from the transposon-tagged mutant synd/Df(3R)BSC43, Tb1 (Vinmlesh Kumar, IISER, Bhopal, India; Kumar et al., 2009a) or adults in trans-allelic combination of synd/Df(3R)BSC43 (Bloomington stock number BL-7413) were used to generate synd mutant embryos. To visualize actin in apical caps we used LifeAct–GFP driven by nanos-Gal4 (UASp LifeAct-GFP/+; nanos-Gal4/+). The following lines were obtained from Bloomington Stock Center: tGPH (BL-8163), Arp3 RNAi (BL-32921), hsFLP; The following lines were obtained from Bloomington Stock Center: tGPH (BL-8163), Arp3 RNAi (BL-32921), hsFLP; (Bl-mannose; (Chougule et al., 2016), anti-Zipper [rabbit, 1:200; Jeffrey H. Thomas (Texas Tech University Health Sciences Center, TX); (Chougule et al., 2016)], anti-Dlg [mouse, 1:10; DSHB-4F3; (Pamna et al., 2001)]. Fluorescently coupled secondary antibodies (Alexa Fluor 488, 568 and 633; Life technologies; 1:1000) were used. DNA was labeled with Hoechst 33424 (10 mg/ml; 1:1000; Life technologies) and F-actin was labeled with Alexa Fluor 488/568-conjugated phalloidin (1:100, Life technologies). The embryos were mounted in SlowFade Gold (Life technologies). Samples were imaged using a Plan-Apochromat 40×/1.3 NA oil-immersion objective on a confocal Laser Scanning Microscope (LSM 710/780, Zeiss). z-stacks were acquired on sequentially scanned images with a spacing of 1.08 mm between slices.

**Live-embryo time-lapse imaging**

Embryos were collected for 1 h, dechorionated and mounted in a LabTek chamber with PBS (Mavrikas et al., 2008) imaged in 4D (every 30 s with 0.48 or 1.08 mm between z-sections) by a PlanApochromat 40×/1.3 NA oil immersion objective on a Zeiss LSM 710/780 or a Leica SP8 inverted confocal microscope. Specific stages of the cell cycle were recognized by marking entry of fluorescent molecules in tGPH and LifeAct–GFP transgenes into the nuclear region during nuclear envelope breakdown and by observing extension of metaphase furrow length. The cap area and corresponding furrow length were measured with respect to time from interphase to metaphase transition in NC12.

**Quantification of apical caps**

All measurements were done on time-lapse movies of the apical caps in syncytial NC 12. Apical cap areas were measured at approximately 3 mm depth from the first visible z stack using the polygon tool of ImageJ (NIH, MD). The cap areas were plotted as a function of time using GraphPad Prism. The segmented line tool in ImageJ was used to measure intensity of LifeAct–GFP along the periphery of caps as they expand. The intensity values were normalized to the maximum and plotted against time using GraphPad Prism.

Myosin II intensities with Sqh–GFP were measured in caps similar to the method used to measure LifeAct–GFP intensities. Sqh–GFP movies were taken from different genotypes under similar settings. The image corresponding to the plot at 9 min was intentionally scaled to increase brightness so that Sqh–GFP association with the cap is clearly visible in synd compared to control and arpC1/+; synd (Fig. 4B). Average values of Sqh–GFP were obtained from an ROI drawn at the periphery of the cap from a section at 3 mm and normalized from 0–1 (Fig. 4C). Average values of Sqh–GFP were obtained from an ROI drawn at the periphery of the cap from interphase to metaphase from a section at 3 mm (Fig. 4D). The graphs were plotted using GraphPad Prism.

**TIRFM**

0–1 h embryos were collected and dechorionated with 50% sodium hypochlorite. Embryos were lined up on a LabTek chamber having their ventral side facing the coverslip, in order to maximise the contact area of the embryo to the coverslip, and immersed in 1 ml PBS. Embryos were then subjected to time-lapse TIRF imaging on an Olympus Biosystems cell TIRF using an Olympus Plan APO 100×/1.49. The angle of incidence was maintained at the critical angle for total internal reflection with small angular adjustments to sample illuminating angle in each experiment. In order to get optimized signals from the cortex/apical membrane, the TIRF illumination plane was set at approximately 100 mm optical section thickness from the coverslip. For time-lapse, images were acquired every 6 s. Post-acquisition, all time frames were deconvolved using the deconvolution algorithm provided by Olympus imaging and processing software package. TIRF images of LifeAct–GFP expressing embryos illuminated protrusions in apical caps in both interphase and metaphase. Longer protrusions were typically seen at the cap periphery and shorter protrusions were seen in the center of the cap in interphase of the syncytial cycle. The protrusions were shorter in length in metaphase. Protrusion lengths were measured manually for clearly visible and optically separable protrusions (crowded regions were omitted) (Fig. S1F) using ImageJ line tool, and their frequency distributions plotted using GraphPad Prism.

**STED**

Embryos were collected for 13.0–2 h at 25°C and were labeled with anti-Lamin DmO primary antibody (mouse, 1:75, AbCam) to allow observation...
of the nucleus in order to discern the stage of the syncytial NC. This was coupled with anti-mouse Alexa Fluor 488 secondary antibody (1:1000, Life technologies). F-actin was labeled with Alexa Fluor 568 phalloidin (1:100, Life technologies). The embryo preparations were embedded in Mowiol (Sigma-Aldrich) supplemented with 2.5% DABCO (Sigma-Aldrich) to reduce fading of the fluorescence signal. All the preparations were imaged within 1–2 days to prevent loss of signal on prolonged storage. The embryos were imaged with an inverted TCS SP8 3X microscope (Leica Microsystems, Mannheim, Germany) with a 100×1.4 NA oil immersion (refractive index 1.518) objective (Leica HC PL APO CS2-
STED WHITE) which is operated by the Leica LAS X software (version 3.5.3). Fluorophores were excited with 488 nm (for nuclear Lamin) and 561 nm (for phalloidin) laser light derived from an 80 MHz pulsed white light laser (Leica Microsystems) at 70% intensity, and the depletion was performed with a 775 nm pulsed laser (Leica Microsystems) with a maximum power of 820–860 mW. All images were acquired in 2D STED mode, optimizing it for maximum lateral resolution improvement. The fluorophore emission was collected with Hybrid Detectors (HyD, Leica Microsystems) with a time gate of 0.85 ≤ g ≤ 5.5 ns with respect to the excitation pulse. A typical spectral window of 495–550 nm was used for nuclear Lamin imaging, with 570–640 nm used for phalloidin imaging. The pinhole was set to 1 AU and the images were recorded in standard mode using 5× line averaging at a zoom of 3.5× with a scan speed of 200 Hz. The images were saved and exported as 1500×1500 TIFF format images with a pixel size of around 20–25 μm.

The images were deconvolved using the STED module on Huygens Professional version 17.04 (Scientific Volume Imaging, Netherlands). To achieve maximum resolution, this software calculates the theoretical point spread function based on the given measured image and the imaging parameters from the metadata of the acquired image. For deconvolved images presented here we first estimated the average background using the In/near object module by controlling the radius parameter. Next, we used the Classical Maximum Likelihood Estimation (CMLE) algorithm by setting the maximum iterations at 40, the Signal to Noise Ratio at 7, Quality Threshold at 0.1 in the ‘fast’ iteration mode with possible bleaching correction. After the completion of iterations, the deconvolved images were saved as high quality TIFF images.

Phalloidin-labeled protrusions were predominantly seen at the periphery of the cap in interphase of the syncytial cycle. The most apical section was acquired in interphase and metaphase to compare the morphology. The protrusion density is reduced in metaphase of the syncytial cycle in phalloidin-labeled caps. Protrusion lengths were measured manually for clearly visible and optically separable protrusions (crowded regions were omitted) using the ImageJ segmented line tool (Fig. S1G). A scatter plot of the distribution of protrusion lengths was plotted using GraphPad Prism, with the bar representing the average and standard deviation shown.

**Comparison of actin-rich protrusions from TIRFM and STED**

Live embryos expressing LifeAct–GFP were used for imaging protrusions by TIRFM, and phalloidin-stained embryos were used to image protrusions by STED microscopy. Whereas LifeAct–GFP in living embryos highlighted protrusions on apical regions of caps in interphase and metaphase in TIRFM, phalloidin-labeled protrusions were enriched at the periphery in fixed embryos visualized in STED microscopy (Fig. S1F,G). The protrusions visible by TIRFM are likely to be in varied orientations. The actin filaments visible in STED images are at greater depths from the surface as compared to those visible in TIRF imaging. The longer protrusions at the periphery visible with TIRFM are likely to be the phalloidin-labeled protrusions seen in STED. Apical protrusions were not visible in STED imaging when samples were labeled with phalloidin. Faint phalloidin-labeled structures on the apical caps are bleached by the depletion laser in STED. The range of protrusion lengths seen in LifeAct–GFP-labeled embryos by TIRF followed by deconvolution is approximately 0.36 to 2.62 μm for interphase caps and approximately 0.25 to 1.46 μm for metaphase caps. The range of protrusion lengths seen in phalloidin-labeled embryos by STED followed by deconvolution is approximately 0.93 to 3.46 μm for interphase caps and approximately 0.24 to 2.52 μm for metaphase caps.

**FLIP**

Continuous photobleaching of the region of interest (ROI) of size 5 μm² was carried out on syncytial embryos expressing LifeAct–GFP using the 488 nm laser at 100% power for 30 iterations. The ROI was bleached after every second. The ROI and neighboring region were monitored for fluorescence depletion. The data was background corrected and normalized to the pre-bleach intensity, transformed on a 0–1 scale and mean intensities (a.u.) were plotted with standard error using GraphPad Prism. To represent the difference in depletion of LifeAct–GFP intensity between control and synd embryos, the ratio of intensities between the ROI and the neighbouring region at 50% ROI intensity depletion was plotted for control and synd embryos.

**Acknowledgements**

We thank Gaurav Diwan for help with experiments. We thank the Microscopy and Drosophila facilities of the Indian Institute of Science Education and Research, Pune. Stocks obtained from Bloomington Drosophila Stock Center (which is funded by National Institutes of Health grant P40OD18537) were used in this study.

**Competing interests**

The authors declare no competing or financial interests.

**Author contributions**


**Funding**

A.S., B.D. and S.S. thank the Council of Scientific and Industrial Research, India for fellowships. R.R. thanks funding from the Department of Biotechnology, Ministry of Science and Technology, India (BT/PR17317/BDB/10/1521/2016), and the Indian Institute of Science Education and Research Pune.

**Supplementary information**

Supplementary information available online at http://jcs.biologists.org/lookup/doi/10.1242/jcs.235846.supplemental

**References**


Kumar, V., Allla, S. R., Krishnan, K. S. and Ramaswami, M. (2009a). Syndapin is dispensable for synaptic vesicle endocytosis at the Drosophila larval...


