Drosophila Arf1 homologue Arf79F is essential for lamellipodia formation

Running title: Arf79F is essential for lamellipodia (40 characters)

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Summary

The WAVE regulatory complex (WRC) drives formation of newly polymerised actin filaments beneath the plasma membrane to generate lamellipodia that are pivotal to cell architecture and movement. By reconstituting WRC-dependent actin assembly at the membrane, we recently discovered that several classes of Arf family GTPases directly recruit and activate WRC in cell extract, and that Arf cooperates with Rac1 to trigger actin polymerisation. Here, we demonstrate that the Class 1 Arf1 homologue Arf79F colocalises with the WRC at dynamic lamellipodia. We report Arf79F is required for lamellipodia formation in Drosophila S2R+ cells which only express one Arf isoform for each class. Impeding Arf function either by dominant-negative Arf expression or by Arf dsRNAi-mediated knockdown uncovered that Arf-dependent lamellipodia formation was specific to Arf79F, establishing that Class 1 Arfs but not Class 2 or Class 3 Arfs, are crucial for lamellipodia. Lamellipodia formation in Arf79F-silenced cells was restored by expressing mammalian Arf1 but not by constitutively active Rac1, showing that Arf79F does not act via Rac1. Abolition of lamellipodia formation in Arf79F-silenced cells was not due to Golgi disruption. Blocking Arf79F activation with guanine nucleotide exchange factor inhibitors impaired WRC localisation to the plasma membrane and concomitant generation of lamellipodia. Our data indicate that the Class I Arf GTPase is a central component in WRC-driven lamellipodia formation.
Introduction

Lamellipodia are sheet-like protrusions generated at the leading edge of a cell which play crucial roles in cell morphology and motility (Ridley, 2011; Weijer, 2009). The protrusive force is supplied by polymerisation of actin filaments initiated via the ubiquitous Arp2/3 complex which is activated by nucleation promoting factors (NPFs) (Campellone and Welch, 2010). In mammalian and Drosophila cells, the critical NPF underlying lamellipodia formation is the WRC, which is thought to be activated at the plasma membrane by the Rho GTPase Rac1 (Kunda et al., 2003; Miki et al., 1998). Purified mammalian WRC is inactive, but can be activated in buffer by high concentrations of Rac1 (Ismail et al., 2009). The low affinity of Rac1 for WRC (Chen et al., 2010) suggested that additional unknown factors are required to facilitate WRC-dependent actin polymerisation (Davidson and Insall, 2011). We showed that active Rac1 was indeed insufficient for recruitment and activation of the WRC at the membrane in mammalian cell extract (Koronakis et al 2011). This led to our discovery that Arf family GTPases directly recruit and activate the WRC, which is enhanced when Arf and Rac1 work together to trigger actin polymerisation. Here, we sought to demonstrate that Arf family GTPases play a role in WRC-dependent generation of lamellipodia.

Results

WRC is composed of five subunits, namely SCAR/WAVE, Sra1/PIR121, Nap1, Abi and HSPC300 (Gautreau et al., 2004). In human cells, paralogous genes give rise to SCAR/WAVE1, WAVE2 and WAVE3, Sra1 and PIR121, and Abi1 and Abi2 to generate divergent WRC combinations. The mammalian Arf GTPase family is also divergent and can be divided into three subfamilies: Class 1 (Arf1, Arf3), Class 2 (Arf4, Arf5) and Class 3 (Arf6) (Donaldson and Jackson, 2011), which display significant functional redundancy (Volpicelli-Daley et al., 2005). In Drosophila cells however, each Arf class is represented by a single isoform, namely Arf79F (Class 1), Arf102F (Class 2) and Arf51F (Class 3). Therefore, to bypass this additional layer of complexity, we used Drosophila S2R+ cells where only single WRC subunits (SCAR, Sra1, Kette/Nap1, Abi and HSPC300) are encoded, all of which are required for lamellipodia formation, which is very prominent in this cell type (Kunda et al., 2003; Rogers et al., 2003; Zallen et al., 2002). We first examined whether the
Drosophila Arf1 homologue Arf79F localised at lamellipodia of S2R+ cells by immunofluorescence using antibodies against human Arf1 and Drosophila Sra1 (Fig 1A). While Arf79F was predominantly found at the perinuclear Golgi apparatus, Arf79F was also observed enriched at the actin-rich cell edge where it co-localised with the WRC component Sra1 (inset). Anti-Arf1 specifically recognised Arf79F as detection of Arf at the Golgi and plasma membrane was abolished when Arf79F gene expression was silenced by double-stranded RNA interference (dsRNAi) (Fig S1A, S2A). Like endogenous Arf79F, recombinant RFP-tagged Arf79F was also detected by immunoblotting with Arf1 antibody (Fig S2B) and was visualised at the cell edge where it colocalised with Sra1 and actin (insets) (Fig 1B). This was not the case in cells expressing RFP alone which was predominantly cytosolic relative to Arf79F (Fig S2C, S2D). The dynamics of fluorescent Arf79F was also monitored in real time where it was enriched at the plasma membrane and the Golgi (Supplementary Movie 1) which was further corroborated when Arf79F-RFP cells were stained with anti-Arf1 antibody (Fig S2E).

We next investigated the requirement for Arf79F in lamellipodia formation. Actin-rich lamellipodia were clearly apparent in ~95% of control cells (Fig 1C, 1D) but Sra1 dsRNAi (Fig S1B) impaired lamellipodia in ~93% of cells giving rise to a characteristic “starfish” phenotype (Kunda et al., 2003), typified by multiple thin extensions and disruption of cortical actin filaments (Fig 1C, 1D). This was also seen in SCAR or Kette dsRNAi cells (data not shown). Rac1-silencing also impaired lamellipodia formation in ~59% of cells (Fig 1C, 1D), a much milder phenotype than in WRC-silenced cells as previously reported (D’Ambrosio and Vale, 2010; Kunda et al., 2003; Rohn et al., 2011). In Arf79F-silenced cells, lamellipodia were abolished in ~87% of cells showing that Arf79F is critical for lamellipodia formation (Fig 1C, 1D).

We next tested the requirement for specific classes of Arf GTPases in lamellipodia formation using dsRNAi (Fig S1C). In contrast to Arf79F dsRNAi, lamellipodia were unaffected by Arf102F and Arf51F dsRNAi (Fig 1E, 1F). To confirm a role for Class 1 Arf GTPases in lamellipodia formation, we examined the morphology of cells equivalently expressing a readily available bank of dominant-negative human Arf GTPases (hArf(TN)) (Fig S3A). These are known to bind and sequester endogenous Arf
guanine nucleotide exchange factors (GEF), thereby blocking Arf activation (Dascher and Balch, 1994; Hart et al., 1994). As human and Drosophila Arfs retain high amino acid identity, we speculated that hArfT31N would also sequester class specific Drosophila Arf GEFs. While lamellipodia were not compromised in cells expressing hArf3T31N, hArf4T31N, hArf5T31N or hArf6T27N, their formation was impaired in ~84% of hArf1T31N cells (Fig 2A, 2B, Fig S3B) further supporting a specific role for class 1 Arf79F.

We next sought to restore lamellipodia formation in Arf79F-silenced cells. Human Arf1 shares 95% amino acid identity with Arf79F, but only possesses 77% mRNA identity making it ideal for Arf79F dsRNAi rescue experiments. Like Arf79F (Fig 1B), RFP-tagged hArf1 was observed at the Golgi and at actin-rich lamellipodia (Fig 2C: arrows) which were formed equivalently in non-transfected (~91%) and hArf1-transfected cells (hArf1; ~93%) (Fig 2D: control dsRNAi). When hArf1 was expressed in Arf79F-silenced cells, lamellipodia formation was restored from ~15% to ~62% (Fig 2C: arrows, Fig 2D: Arf79F dsRNAi). Importantly, while ~93% of control cells expressing constitutively-active Rac1G12V formed lamellipodia, Rac1G12V failed to restore lamellipodia formation in Arf79F-silenced cells (Fig 2E: arrows, Fig 2F) showing that Arf79F-dependent lamellipodia formation is not mediated via upstream activation of Rac1.

Arf GTPases are predominantly observed at the perinuclear Golgi apparatus (Fig 1A, 1B) where they are best characterised as having roles in membrane trafficking and Golgi organisation (Donaldson and Jackson, 2011). We therefore addressed whether it is Arf79F-mediated membrane trafficking from the Golgi that is imperative for lamellipodia by examining the Golgi apparatus in Arf79F-silenced cells (Fig 3A). Immunofluorescence of the cis-Golgi marker GM130 following Arf79F dsRNAi revealed a clearly defined Golgi network (~84% of cells) comparable to that seen in control cells (~93%) with lamellipodia. This was not the case in cells expressing dominant-negative hArf4T31N where the Golgi was impaired in ~75% of cells (Fig 3B). Despite this Golgi disruption, cells expressing hArf4T31N still displayed lamellipodia and Sra1 localisation at the cell cortex in ~90% of cells (Fig 3C). These results suggest that Arf79F may facilitate lamellipodia formation independently of its role in Golgi organisation and membrane trafficking.
We recently demonstrated that mammalian Arf1 directly binds the structural homologues Sra1 and Nap1 (Koronakis et al 2011) and therefore wondered whether Arf79F drives lamellipodia formation by recruiting WRC to the plasma membrane. First, purified *in vitro* myristoylated Arf79F was anchored to silica beads coated in a phospholipid bilayer consisting of equal amounts of phosphatidylcholine and phosphatidylinositol (PC:PI). Anchored Arf79F was locked in an active state by loading with non-hydrolysable GTPγS before addition of the beads to S2R+ cell extract. The beads were isolated from the extract, washed and the recruited proteins extracted from the membrane before analysis by SDS-PAGE and immunoblotting to detect WRC components Sra1 and Kette (Nap1 homologue) (Fig 3D). In contrast to control beads with no Arf79F (-), active Arf79F (+) recruited Sra1 and Kette demonstrating active Arf79F acts like mammalian Arf GTPases by recruiting the WRC to the membrane. We next tested whether Arf79F could also recruit the WRC to the cell plasma membrane by examining Sra1 localisation in Arf79F-silenced cells by immunofluorescence. In control cells, Sra1 was observed enriched at the perinuclear region and at the plasma membrane (arrows) in ~92% of cells (Fig 3E, 3F). Sra1 was virtually non-detectable in control Sra1-silenced cells confirming the specificity of the Sra1 antibodies (Fig 3E). In Arf79F-silenced cells, Sra1 was still found at the perinuclear area but cell cortex Sra1 was abolished in ~79% of cells.

WRC recruitment from cell extract by GTP bound Arf79F (Fig 3D) indicated that Arf79F activation would be key to Sra1 plasma membrane localisation and therefore lamellipodia formation. To test this hypothesis, we employed Arf GEF inhibitors SecinH3 and BrefeldinA (BFA) (Fig 4A, 4B). Lamellipodia were generated by ~95% of control cells which was reflected in Sra1 plasma membrane localisation in ~92% of cells. SecinH3 treatment abrogated lamellipodia and cell cortex Sra1 by ~35% (insets), while ~50% of remaining SecinH3-treated cells displayed an abnormal cell edge resulting in disjointed lamellipodia (white arrows), likely representing incomplete pathway inhibition as indicated by Sra1 plasma localisation in these cells (white arrows). BFA eliminated lamellipodia and Sra1 at the cell cortex in ~84% of cells. Differences in effectiveness between inhibitors is probably due to their mechanism where SecinH3 targets the cytohesin-family of Arf GTPase activators, Steppke in *Drosophila* (Donaldson and Jackson, 2011; Fuss et al., 2006), while BFA forms a ternary complex with Arf1-GDP and Arf GEFs that would sequester class 1
Arf GTPase(s) (Mossessova et al., 2003). To dissect whether the reduction in Sra1 localisation ensues directly from inhibiting Arf or indirectly as a consequence of inhibiting lamellipodia, we uncoupled the two with actin polymerisation inhibitors latrunculinB (LatB) and cytochalasinD (CytoD) (Fig 4A, 4B). While both inhibitors abrogated lamellipodia formation, cell cortex Sra1 still remained in ~44% of LatB-treated cells and ~51% of CytoD-treated cells (insets) demonstrating that Sra1 still localised to the cell periphery upon inhibition of lamellipodial actin polymerisation. Consistent with our finding, WRC components have been previously observed at the plasma membrane of mammalian cell treated with LatB (Weiner et al., 2007).

To address whether impaired lamellipodia following BFA- and SecinH3-treatment was due to inhibiting Arf79F, we examined whether expressing constitutively active Arf1Q71L would restore lamellipodia (Fig 4C, 4D). Relative to non-expressors (yellow arrows), cells expressing Arf1Q71L generated ~2.5-fold more lamellipodia in BFA-treated cells (white arrows) and ~2-fold more in SecinH3-treated cells demonstrating that the Arf GEF inhibitors target Drosophila Arf1 (ie. Arf79F).

Having established that Arf79F and its activation is required for maintaining lamellipodia, we investigated whether active Arf79F triggers re-generation of lamellipodia following their destruction by CytoD (Fig 4E). When CytoD-treated cells were washed then subsequently incubated with either growth media (control), SecinH3 or BFA, lamellipodia were produced in ~83% of control cells but only in ~52% SecinH3-treated cells and ~4% of BFA-treated cells demonstrating a role for active Arf79F in establishing new lamellipodia. The impairment in lamellipodia formation caused by Arf GEF inhibition was mirrored in the cell cortex localisation of Sra1 indicating that recruitment of Sra1 to the plasma membrane by Arf79F is key for triggering lamellipodia.

Arf GTPases are best known for their roles in membrane trafficking and organelle organisation (Donaldson and Jackson, 2011) but they have also been implicated in actin-dependent processes at the plasma membrane (Boulay et al., 2008; Cohen et al., 2007; Myers and Casanova, 2008; Stalder et al., 2011). Here, we establish that Arf79F not only localises at cell edge with the WRC component Sra1 but is also essential for lamellipodia formation. This builds on our recent demonstration that Arf1 directly recruits and activates mammalian WRC in vitro (Koronakis et al., 2011).
High-throughput RNAi screening of *Drosophila* S2R+ cells has enabled the discovery of cellular factors driving lamellipodia (D'Ambrosio and Vale, 2010; Kiger et al., 2003; Rogers et al., 2003; Rohn et al., 2011); though as yet, Arf79f has not been implicated. This could be due to a 8% false negative rate in RNAi screens (Booker et al., 2011) and the relatively long time required for complete Arf79F dsRNAi (Fig 1C, Fig S1A). Our discovery that Arf79F is key to lamellipodia establishes a new platform to dissect the Arf-WRC pathway. This role appears to be specific for Arf79F as Class 1 but not Class 2 or 3 Arfs were required for lamellipodia. This was surprising as WRC can be activated in cell extract by divergent Arf GTPases such as Arf5 (Arf102F homologue) and Arl1 (Koronakis et al., 2011). However, Gautier and colleagues showed that Arf79F, but not related *Drosophila* Arf isoforms, was found bound to immunoprecipitated WRC (Gautier et al., 2011) underlining Arf specificity for the WRC. Since Arf1 alone is insufficient for WRC activation *in vitro* (Koronakis et al., 2011) and Arf must cooperate with Rac1 for WRC-driven actin assembly (Humphreys et al., 2012; Koronakis et al., 2011), it seems that it is only active Arf79F which is temporally and spatially coincident with Rac1 activation in *Drosophila* cells.

Recent structural studies of WRC indicates its plasma membrane localisation is mediated by Sra1-Rac1 interactions that would release the SCAR/WAVE VCA domain for Arp2/3 activation (Chen et al., 2010). However, WRC-Rac1 interactions are of low affinity suggesting recruitment and activation of WRC requires additional factors (Davidson and Insall, 2011). This is supported by a recent report revealing a role for clathrin in WRC recruitment which is independent of its connection to Arf-AP1/AP2 trafficking (Gautier et al., 2011). However, we demonstrated that Rac1 is insufficient for WRC activation (Koronakis et al., 2011) so while clathrin could promote the recruitment of WRC to the plasma membrane, its activation would only be triggered when both active Arf and Rac1 cooperate. We showed the role for Arf79F in lamellipodia formation is independent of Rac1 activation and trafficking. Consistent with this, we confirmed effects on lamellipodia were not due to altered Golgi function implying direct Arf-WRC interactions and not indirect Arf-mediated membrane trafficking triggers WRC-signalling. A direct mechanism involving Arf79F is further supported by its ability to recruit WRC components *in vitro* which was reflected in cells where active Arf79F was critical for Sra1 localisation and
concomitant generation of lamellipodia. The data indicate Arf GTPase is a key component of the WRC signalling pathway in cells.

Materials and Methods

Plasmids and recombinant proteins

The following plasmids were generated by Invitrogen Gateway methodology: pET15b-DEST-Arf79F, pAHW-Rac-V12 (Rac1G12V) and pMT-RFP (Liu et al., 2010) was used to create RFP-tagged Arf79F, human Arf1, Arf1Q71L Arf1T31N, Arf3T31N, Arf4T31N, Arf5T31N and Arf6T27N. pET15b-DEST-Arf79F and pBB131 encoding the Arf family N-myristoyltransferase (Prof Martin Spiess) were co-expressed in E.coli Rosetta (Novagen) at 16°C before affinity purification of His-tagged Arf79F (Koronakis et al., 2011).

Drosophila cell culture, transfection and dsRNAi

S2R+ cells were cultured at 25°C in M3 insect medium (Sigma) with 10% heat-inactivated fetal bovine serum (JRH Biosciences) and 1% penicillin-streptomycin (Sigma-Aldrich) as described (Liu et al., 2010). When appropriate cells were incubated with 0.5 µM LatrunculinB or cytochalasinD, 5 µM BrefeldinA (Sigma-Aldrich) or 25 µM SecinH3 (Merck) for 1h. Drosophila cell transfection and RNAi experiments were carried out on concanavalinA-coated coverslips as previously described (Liu et al., 2010). RNAi was performed with double-stranded RNA generated from the following primer pairs: Arf79F

(Arf79F_01_f:TAATACGACTCACTATAGGGTTGGATGGTTGGA, Arf79F_01_r:TAATACGACTCACTATAGGGACATCAGAAGGAATTTTCACAAA and Arf79F_02_f:TAATACGACTCACTATAGGAAGGGGTGGAGGAGGAACCC, Arf79F_02_r:TAATACGACTCACTATAGGAAGGGGTGGAGGAGGAACCC, Arf79F_02_r:TAATACGACTCACTATAGGAAGGGGTGGAGGAGGAACCC, Arf79F_02_r:TAATACGACTCACTATAGGAAGGGGTGGAGGAGGAACCC)

Arf102

(Arf102F_f:TAATACGACTCACTATAGGATGCTGCTGGAAAAACGACT, Arf102F_r:TAATACGACTCACTATAGGCCATGTTCTGTAGTTCTCTCTCGAC); Arf51

(Arf51F_f:TAATACGACTCACTATAGGGTGAACCCCATGAAATCCAG, Arf51F_r:TAATACGACTCACTATAGGGTGAACCCCATGAAATCCAG)

SCAR

(Scar_f:TAATACGACTCACTATAGGGTGATGAGGACGCACTAATT, Scar_r:TAATACGACTCACTATAGGGTGATGAGGACGCACTAATT, Scar_r:TAATACGACTCACTATAGGGTGATGAGGACGCACTAATT)

Sra1

(Sra1_f:TAATACGACTCACTATAGGGTGAACCCCATGAAATCCAG, Sra1_r:TAATACGACTCACTATAGGGTGAACCCCATGAAATCCAG)

Kette

(Kette_f:TAATACGACTCACTATAGGGTGAACCCCATGAAATCCAG, Kette_r:TAATACGACTCACTATAGGGTGAACCCCATGAAATCCAG)
Kette_r:TAATACGACTCCTATAGG ATTCGATGCCACCGATCTTA); Rac1  
(Rac1_f:TAATACGACTCCTATAGGTCGAACACGGTGGGTATGTA,  
Rac1_r:TAATACGACTCCTATAGGAGCAACAGTCCTCCGCCG). RNAi was quantified  
in each case by EXPRESS One-Step SYBR® GreenER qRT-PCR according to  
manufacturers instructions (Invitrogen) with RP49 as an internal control.

**Immunofluorescence**

Immunofluorescence microscopy and images assembled as described (Humphreys et  
al., 2009). RFP intensity (arbitrary units) within regions-of-interest at the cell edge  
were compared to equivalent regions in the cytoplasm (4 regions per cell) using  
Volocity 3D Image Analysis Software (PerkinElmer). Antibodies used were Sra1 and  
Kette (gifts from Buzz Baum), anti-Arf1 (Abcam-ab58578) and anti-GM130 (Abcam-  
ab30637). AlexaFluor-labelled secondary antibodies, AlexaFluor- and TxRed-labelled  
phalloidin (Invitrogen).

**WRC recruitment to Arf79F anchored phospholipid beads**

*Drosophila* S2R+ cell extract was prepared by passage through a 22-guage needle in  
10mM HEPES (pH7.4), 100mM KCl, 1mM MgCl and Complete™ EDTA-free  
protease inhibitor cocktail (Roche) then ultracentrifugation (180,000g, 20min)  
separated membranes from the high-speed supernatant cytoplasm which was used for  
experiments. Preparation of phospholipid-coated beads and pulldowns have been  
described in detail (Koronakis et al., 2011).

**Statistics**

In all experiments (n ≥ 3), at least 300 cells were analysed for quantification per  
experiment. Comparisons between two data points were made using Student's t-test,  
between three or more data points using ANOVA combined with post-hoc  
Bonferroni’s multiple comparison test. Asterisks indicate p< 0.01 unless stated  
otherwise. Error bars represent ±SEM.

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template plasmid pUAS-RacV12.
**Figure Legends**

**Fig 1. Arf79F is required for lamellipodia formation in S2R+ cells**

(A) Arf79F and control Sra1 visualised at lamellipodia (insets) using Sra1 or Arf1 antibodies and fluorescent-phalloidin (actin). Scale bars 5µm. (B) Cells expressing RFP-tagged Arf79F labelled as in ‘A’. (C) Lamellipodia in control-, Sra1-, Rac1- and Arf79F-dsRNAi cells visualised by staining actin (arrows indicate starfish phenotype, scale bars 20µm) then quantified microscopically (D). Asterisks indicate a significant difference from control. Error bars represent ±SEM. (E) Lamellipodia in control-, Arf79F-, Arf102F- and Arf51F-dsRNAi cells visualised as in ‘B’ and quantified in (F) as in ‘D’.

**Fig 2. Rescue of lamellipodia formation in Arf79F-silenced cells by Arf1 and Rac1**

(A) Lamellipodia in cells expressing RFP-tagged human dominant-negative Arf GTPases (hArf), hArf1T31N or hArf5T31N, visualised by staining actin (scale bars 10µm) then quantified in (B) as Fig 1D. Lamellipodia in control- or Arf79F-dsRNAi cells expressing either (C) hArf1-RFP or (E) HA-tagged Rac1G12V visualised by staining actin and Rac1 with anti-HA antibody (arrows indicate transfected cells). Lamellipodia quantified in non-expressing (-) and either (D) hArf1-RFP or (F) Rac1G12V from experiments in ‘C’ and ‘E’ were quantified as in ’B’.

**Fig 3. Arf79F is required for Sra1 localisation to the plasma membrane**

(A) The cis-Golgi in control- or Arf79F-dsRNAi cells visualised with anti-GM130 antibodies and actin as control. Scale bars 10µm. Cells expressing RFP-tagged dominant-negative hArf44T31N labelled with antibodies to (B) GM130 for the cis-Golgi or (C) Sra1 (magnified in insets) with actin as control. Scale bars 10µm. (D) Arf79F recruitment of WRC components to the membrane. Silica beads coated with phospholipid bilayers alone (-) or with anchored myristoylated Arf79F loaded with GTPγS (+) were isolated from Drosophila cell extract then the recruited proteins analysed by SDS-PAGE and immunoblotting with antibodies to Sra1 and Kette. (E) Sra1 in control-, Sra1- or Arf79F-dsRNAi cells visualised at the cell cortex with Sra1 antibodies and actin as control (scale bar 20µm) then cell cortex Sra1 and lamellipodia quantified (F) as in Fig 1D (p< 0.001, t-test).
Fig 4. Active Arf79F recruits Sra1 to the cell membrane

(A) Sra1 in control-, SecinH3-, BFA-, LatB- and CytoD-treated cells visualised at the cell cortex (magnified in insets) with Sra1 antibodies and actin as control (arrows indicate disjointed lamellipodia, scale bars 20μm then cell cortex Sra1 and lamellipodia quantified (B) as in Fig 1A. (C) Lamellipodia in expressing cells constitutively active Arf1Q71L (white arrows) or adjacent non-expressing cells (yellow) visualised by staining actin (scale bar 10μm) then (D) as in Fig 1D (p< 0.05, t-test). (E) Lamellipodia and cell cortex Sra1 in cells treated with CytoD (30min) that were subsequently incubated with either growth media (control), SecinH3 or BFA (1h) were quantified as in ‘B’.

Fig S1. dsRNAi-mediated knockdown of small GTPases and Sra1

(A) qRT-PCR quantification of mRNA levels in control- or Arf79F-dsRNAi cells after either 3 or 6 days dsRNA incubation. qRT-PCR quantification of mRNA levels in (B) control-, Sra1-, Rac1- or Arf79F-dsRNAi cells or (C) control-, Arf79F-, Arf102F- or Arf51F-dsRNAi cells after 6 days dsRNA incubation.

Fig S2. Controls for Arf79F localisation in S2R+ cells

(A) Arf79F in Arf79F-dsRNA cells visualised with Arf1 antibodies and actin as control (scale bars 10μm). (B) Immunoblot of Arf79F-RFP with antibodies to Arf1 and tubulin as control. (C) Percentage increase in RFP or Arf79F-RFP fluorescence at cell edge relative to cytoplasm. Statistics as Fig 1D. Lamellipodia (white arrows) visualised in cells expressing (D) RFP or (E) Arf79F-RFP by staining actin (scale bars 10μm). Arf79F-RFP stained with Arf1 antibodies confirming Arf79F localisation at the Golgi (yellow) and plasma membrane (white).

Fig S3. Influence of dominant negative Arf GTPases on lamellipodia formation

(A) Relative expression of indicated RFP-tagged human Arf dominant negatives determined by immunoblotting whole cell lysates with antibodies to RFP and tubulin as control. (B) Lamellipodia in cells expressing human dominant-negative Arf GTPases (hArfGTN) visualised by staining actin (scale bars 10μm).

Movie 1. Arf79F localises to dynamic lamellipodia and macropinosomes
Live microscopy was used to image S2R+ cells expressing Arf79F-RFP at a rate of 8 frames/second.

References


Fig 1. Arf79F is required for lamellipodia formation in S2R+ cells

A. actin, Sra1, Arf79F

B. actin, Sra1, Arf79F-RFP

C. control, Sra1, Rac1, Arf79F

D. Bar chart showing lamellipodia (%)

E. control, Arf79F, Arf102F, Arf51F

F. Bar chart showing lamellipodia (%)

* indicates significant differences.
Fig 2. Rescue of lamellipodia formation in Arf79F-silenced cells by Arf1 and Rac1
Fig 3. Arf79F is required for Sra1 localisation to the plasma membrane

A

merge  actin  cis-Golgi

control dsRNAi

Arf79F dsRNAi

B

actin  Arf4T31N  cis-Golgi

C

actin  Arf4T31N  Sra1

D

merge  actin  Sra1

control dsRNAi

Arf79F dsRNAi

E

% of cells

lamellipodia

cell cortex Sra1

Arf79F - +

Sra1

Kette

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Fig 4. Active Arf79F recruits Sra1 to the cell membrane

A

merge  actin  Sra1

cell type

B

control

SecinH3

BFA

LatB

CytoD

C

merge  actin  Arf1^{Q71L}

BFA

SecinH3

D

non-expressors

Arf1-Q71L cells

E

control

SecinH3

BFA