F-BAR Cdc15 Promotes Cdc42 Activation During Cytokinesis and Cell Polarization in *Schizosaccharomyces pombe*

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ABSTRACT Cdc42, a Rho-family GTPase, is a master regulator of cell polarity. Recently, it has been shown that Cdc42 also facilitates proper cytokinesis in the fission yeast *Schizosaccharomyces pombe*. Cdc42 is activated by two partially redundant GEFs, Gef1 and Scd1. Although both GEFs activate Cdc42, their deletion mutants display distinct phenotypes, indicating that they are differentially regulated by an unknown mechanism. During cytokinesis, Gef1 localizes to the division site and activates Cdc42 to initiate ring constriction and septum ingression. Here, we report that the F-BAR protein Cdc15 promotes Gef1 localization to its functional sites. We show that *cdc15* promotes Gef1 association with cortical puncta at the incipient division site to activate Cdc42 during ring assembly. Moreover, *cdc15* phospho-mutants phenocopy the polarity phenotypes of *gef1* mutants. In a hypermorphic *cdc15* mutant, Gef1 localizes precociously to the division site and is readily detected at the cortical patches and the cell cortex. Correspondingly, the hypermorphic *cdc15* mutant shows increased bipolarity during interphase and precocious Cdc42 activation at the division site during cytokinesis. Finally, loss of *gef1* in hypermorphic *cdc15* mutants abrogates the increased bipolarity and precocious Cdc42 activation phenotype. We did not see any change in the localization of the other GEF Scd1 in a Cdc15-dependent manner. Our data indicate that Cdc15 facilitates Cdc42 activation at the division site during cytokinesis at the cell cortex to promote bipolarity and this is mediated by promoting Gef1 localization to these sites.

KEYWORDS Cdc15; Cdc42; Gef1; cytokinesis; polarity

THE conserved Cdc42 is a master regulator of polarized cell growth in fission yeast (Miller and Johnson 1994; Johnson 1999; Estravís *et al.* 2012; Das and Verde 2013). Recently, it has also been shown that Cdc42 has a role in cytokinesis—the final step in cell division (Wei *et al.* 2016). Through the regulation of actin and membrane trafficking, Cdc42 controls cellular processes such as growth, cell polarity, and cytokinesis (Martin *et al.* 2007; Harris and Tepass 2010; Estravís *et al.* 2011, 2012). Given the complexities of these cellular processes, Cdc42 activation needs to be precisely regulated in a spatiotemporal manner. A prime example of this precise regulation is the oscillation of Cdc42 activation between the two cell ends during bipolar growth (Das *et al.* 2012; Das and Verde 2013). Disrupting Cdc42 activation patterns lead to defects in cell shape and cytokinesis (Das *et al.* 2012; Wei *et al.* 2016; Onwubiko *et al.* 2019). While much is known about how Cdc42 promotes actin organization and polarization, the spatiotemporal manner in which regulation of Cdc42 is fine-tuned is not well understood.

Cdc42 is activated by GEFs (guanine nucleotide exchange factors), which exchange GDP for GTP, and inactivated by GAPs (GTPase activating proteins), which enhance the intrinsic rate of GTP hydrolysis (Bos *et al.* 2007). Fission yeasts have two GEFs, Scd1 and Gef1, that control polarization and cytokinesis (Chang *et al.* 1994; Coll *et al.* 2003). While the double deletion of the two GEFs is not viable (Coll *et al.* 2003; Hirota *et al.* 2003), *scd1Δ* and *gef1Δ* mutants exhibit distinct phenotypes, indicating that they differentially activate Cdc42. *scd1Δ* cells are depolarized and exhibit defects in septum morphology (Chang *et al.* 1994; Wei *et al.* 2016).

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In contrast, *gef1Δ* mutants exhibit monopolar growth and a delayed onset of ring constriction (Coll *et al.* 2003; Das *et al.* 2015; Wei *et al.* 2016; Onwubiko *et al.* 2019). This suggests that the two GEFs allow for distinct Cdc42 activation patterns that regulate different aspects of cell polarity establishment and cytokinesis. It is unclear how the two Cdc42 GEFs result in distinct phenotypes given that they both activate the same GTPase. One potential explanation could be differential regulation of these GEFs. Indeed, during cytokinesis, first Gef1 localizes to the membrane proximal to the actomyosin ring, where it activates Cdc42 to promote timely onset of ring constriction and septum initiation (Wei *et al.* 2016). Next, Scd1 localizes to the ingressing membrane to promote proper septum maturation (Wei *et al.* 2016).

It is unknown what gives rise to the temporal localization pattern of the GEFs. Gef1 contains a BAR domain that is required for its function but not for its localization (Das *et al.* 2015). The N-terminal region of Gef1 is necessary and sufficient for its localization (Das *et al.* 2015). Phosphorylation of the N-terminal region by Orb6 kinase generates a 14-3-3 binding site that results in the sequestration of Gef1 in the cytoplasm (Das *et al.* 2009, 2015). While it is known how Gef1 is removed from its site of action, it is unclear what localizes Gef1 to these sites.

Here, we show that Gef1 localization to its site of action is aided by the F-BAR protein Cdc15. Cdc15 localizes to endocytic patches during interphase and to the division site, where it scaffolds the actomyosin ring (Wu *et al.* 2003; Arasada and Pollard 2011; McDonald *et al.* 2017). We report that Gef1 localizes to cortical patches at the division site during ring assembly in a *cdc15*-dependent manner. Similarly, we find that *cdc15* promotes Gef1 localization to the cortical patches and cell tips. We show that *cdc15* phospho-mutants phenocopy *gef1* polarity phenotypes. A hypermorphic *cdc15* allele shows precocious Cdc42 activation at the division site during cytokinesis, and increased bipolarity during interphase. Finally, we show that enhanced bipolarity and premature Cdc42 activation is abrogated upon deletion of *gef1* in the hypermorphic *cdc15* mutant. Here, we show that Cdc15 regulates cell polarization by promoting Cdc42 activation through the regulation of Gef1. We did not see any change in the localization of the other GEF, Scd1, in a *cdc15*-dependent manner. Taken together, our data indicate that Cdc15 specifically promotes Gef1 localization to the division site and the cell cortex to promote Cdc42 activation.

Materials and Methods

Strains and cell culture

The *Schizosaccharomyces pombe* strains used in this study are listed in Supplemental Material, Table S1. All strains are isogenic to the original strain PN567. Cells were cultured in yeast extract (YE) medium and grown exponentially at 25°, unless specified otherwise. Standard techniques were used for genetic manipulation and analysis (Moreno *et al.* 1991). Cells were grown exponentially for at least three rounds of eight generations before imaging.

Microscopy

Cells were imaged at room temperature (23–25°) with an Olympus IX83 microscope equipped with a VTHawk two-dimensional array laser scanning confocal microscopy system (Visitech International, Sunderland, UK), electron-multiplying charge-coupled device digital camera (Hamamatsu, Hamamatsu City, Japan), and 100×/numerical aperture 1.49 UAPO lens (Olympus, Tokyo, Japan). Images were acquired with MetaMorph (Molecular Devices, Sunnyvale, CA) and analyzed by ImageJ [National Institutes of Health, Bethesda, MD (Schneider *et al.* 2012)]. For still and z-series imaging, the cells were mounted directly on glass slides with a #1.5 coverslip (Fisher Scientific, Waltham, MA) and imaged immediately; fresh slides were prepared every 10 min. Z-series images were acquired with a depth interval of 0.4 μm. For time-lapse images, the cells were placed in 3.5-mm glass-bottom culture dishes (MatTek, Ashland, MA) and overlaid with YE medium plus 0.6% agarose with 100 μM ascorbic acid as an antioxidant to minimize toxicity to the cell, as reported previously (Frigault *et al.* 2009; Wei *et al.* 2017).

Analysis of fluorescence intensity

Mutants expressing fluorescent proteins were harvested from midlog phase cultures at $OD_{(595)}$ 0.5, and imaged on slides. Depending on the mutant and the fluorophore, 16–18 z-planes were collected at a z-interval of 0.4 μm for either (or both of) the 488 and 561 nm channels. The respective controls were grown and imaged in an identical manner. ImageJ was used to generate sum projections from the z-series, and to measure the fluorescence intensity of a selected region. The cytoplasmic fluorescence of the same cell was subtracted to generate the normalized intensity. Mean normalized intensity was calculated for each image from all measurable cells ($n > 5$) within each field.

Statistical tests

Statistical tests were performed using GraphPad Prism software. When comparing two samples, a Student's *t*-test (two-tailed, unequal variance) was used to determine significance. When comparing three or more samples, one-way ANOVA was used, followed by a Tukey's multiple comparisons *post hoc* test to determine individual *P*-values.

Cell staining

To stain the septum and cell wall, cells were stained in YE liquid with 50 μg/ml Calcofluor White M2R (Sigma-Aldrich, St. Louis, MO) at room temperature.

Latrunculin A treatment

Cells were treated with 10 μM latrunculin A (LatA) in dimethyl sulfoxide (DMSO) in YE medium for 30 min before

imaging. Control cells were treated with only 0.1% DMSO in YE medium.

Analysis of sin and cdc12 mutants

plo1-25, *sid2-250*, and control cells were grown in YE at 25° to OD 0.2, then shifted to the restrictive temperature at 35.5°. Slides were then prepared and imaged from the cultures at 0, 1, 2, and 4 hr time points. Cells expressing cdc12ΔC-GFP were initially grown in EMM (Edinburgh minimal medium) with 150 μM thiamine. Induction of cdc12ΔC-GFP expression was performed as described previously (Yonetani and Chang 2010). Briefly, cultures were harvested by low speed centrifugation, rinsed, and then grown in EMM without thiamine for 18 hr prior to imaging.

Cell synchronization

Cells expressing Gef1-tdTomato and Cdc15-GFP or Cdc15-27A-GFP were grown in YE at 25° to OD 0.2, then treated with 10 mM hydroxyurea (HU) for 4 hr. Cells were harvested by low speed centrifugation and washed three times in fresh YE to release them from S-phase arrest. Fresh slides were prepared and imaged in 30 min intervals until they entered M-phase.

Data availability

Strains are available upon request. The authors affirm that all data necessary for confirming the conclusions of the article are present within the article, figures, and tables. Supplemental material available at figshare: https://doi.org/10.25386/genetics.7722092.

Results

Gef1 localizes to cortical puncta

While we have previously characterized the distinct localization pattern and phenotypes of the Cdc42 GEFs Gef1 and Scd1 during cytokinesis (Wei *et al.* 2016), what facilitates their localization to the division site at the appropriate time is unknown. Since Gef1 is detectable at the membrane proximal to the assembled actomyosin ring, we posited that the ring is required for Gef1 localization. To test this, we treated cells with 10 μM LatA for 30 min to depolymerize actin structures, then observed the localization of Gef1-mNeonGreen (Gef1-mNG). Gef1-mNG localizes to the membrane proximal to the actomyosin ring, marked by Rlc1-tdTomato, in mock DMSO-treated cells (Figure 1A). Rlc1-tdTomato rings fragment upon treatment with LatA, as does Gef1-mNG, indicating that an intact ring is necessary for proper Gef1 localization. We observe that, upon LatA treatment, Gef1-mNG does not diffuse away into the cytosol, but instead localizes to cortical nodes about the cortex with Rlc1-tdTomato. Upon closer examination of these nodes, one population of Gef1 can be seen to partially colocalize with Rlc1, while the other population of Gef1 puncta do not overlap with Rlc1 (Figure 1B). These findings indicate that Gef1 localizes to cortical puncta near, or overlapping with, Rlc1-containing puncta.

Since Gef1 promotes timely onset of ring constriction (Wei *et al.* 2016), we asked if Gef1 localization itself was under a temporal control. Given that Gef1 arrives at the division site during anaphase as the actomyosin ring assembles (Wei *et al.* 2016), we asked whether Gef1 localization is cell-cycle-dependent. To test this, we induced ectopic ring formation in interphase cells using the constitutively active formin mutant, cdc12ΔC-GFP (Yonetani and Chang 2010). In the presence of thiamine, cdc12ΔC-GFP expression is repressed. In these conditions, Gef1-tdTomato localizes to the division site of mitotic cells, which are ∼14 μm in length (Figure 1C). However, Gef1-tdTomato also localizes to ectopic rings that form in cdc12ΔC-GFP expressing mononucleate interphase cells <10 μm long (Figure 1C). This indicates that Gef1 localization to the ring is not cell-cycle-dependent, but rather that formation of the actomyosin ring is sufficient for Gef1 localization.

Next, we asked what pathway localizes Gef1 to the division site. The Septation Initiation Network (SIN) is a protein signaling network that coordinates the timing of cytokinesis with chromosome segregation (Roberts-Galbraith and Gould 2008; Johnson *et al.* 2012; Simanis 2015). The SIN pathway promotes the localization and activity of proteins involved in ring constriction and the coordinated process of septum formation (Jin *et al.* 2006; Roberts-Galbraith *et al.* 2010; Bohnert *et al.* 2013). To determine whether the SIN is required for Gef1 localization to the division site, we examined the localization of Gef1-3YFP in two SIN protein kinase *ts* mutants, *plo1-25 and sid2-250* (Bähler *et al.* 1998; Jin *et al.* 2006; Hachet and Simanis 2008). In *plo1-25 and sid2-250*, Gef1-3xYFP localizes normally to the division site at the permissive temperature of 25°. Surprisingly, Gef1-3YFP still localizes to ring like structures in *plo1-25* and *sid2-250* cells shifted to the restrictive temperature of 35.5° for 1, 2, or 4 hr (Figure 1D). We imaged *plo1-25 and sid2-250* cells expressing Gef1-mNG and the ring marker Rlc1-tdTomato to better visualize the ring-like Gef1 structures, and to determine whether these structures represented components of the actomyosin ring. Indeed, Gef1-mNG colocalizes with Rlc1-tdTomato in cells shifted to 35.5° for 1, 2, or 4 hr, demonstrating that Gef1 localization to the actomyosin ring is not dependent upon the SIN pathway (Figure 1E). Since we had observed Gef1 localization to cortical nodes, we examined whether Gef1 localization was Mid1-dependent. Mid1 is an anillin-like protein that is exported from the nucleus to form cortical nodes that define the division plane (Bähler *et al.* 1998; Paoletti and Chang 2000). It is to these nodes that various contractile ring components are recruited, before coalescing to form the actomyosin ring (Coffman *et al.* 2009; Laporte *et al.* 2011). In *mid1Δ* cells, Gef1-3xYFP localizes to misplaced, extended ring-like structures (Figure 1D). Gef1-mNG and Rlc1-tdTomato colocalize at these extended ring-like structures, similarly to the *sin* mutants (Figure

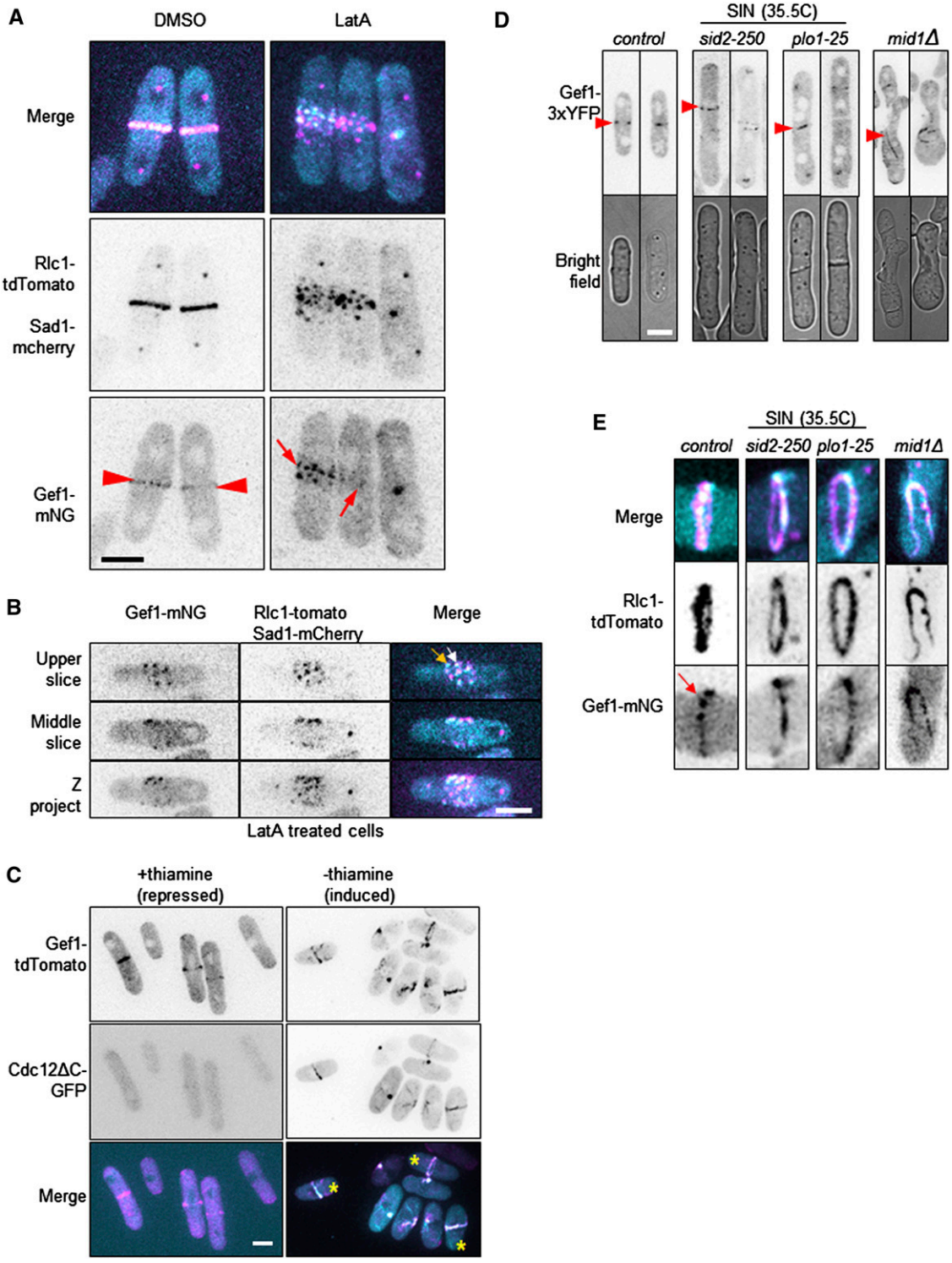

Figure 1 Gef1 localizes to cortical puncta. (A) Gef1-mNG and Rlc1-tdTomato localization was examined in cells treated with 10 μM LatA for 30 min. In DMSO-treated control cells, Gef1 (red arrowheads) localizes normally to the actomyosin ring. Upon treatment with the actin depolymerizing drug LatA, the ring fragments and Gef1 appears to localize to cortical nodes at division site (red arrows). (B) Top and middle z-series show node-like organization of Gef1-mNG and Rlc1-tdTomato about the cortex at the division site in cells treated with LatA. White arrow shows colocalized Gef1 and Rlc1, while orange arrow shows Gef1 alone. (C) Expression of cdc12ΔC-GFP induces ectopic actomyosin ring formation and constriction in interphase cells. Gef1-tdTomato ectopically localizes to these rings that form in interphase (yellow asterisks). (D) Gef1 localizes to aberrant ring-like structures formed in *sin* and *mid1Δ* mutants. Indicated genotypes were shifted to the restrictive temperature of 35.5° for 4 hr. Top row: Inverted max projections of Gef1-3xYFP (red arrowheads). Bottom row: Brightfield images of the representative images above. (E) Gef1 colocalizes with Rlc1-tdTomato in the aberrant rings formed in *sin* and *mid1Δ* mutants. Red arrow shows node like organization of Gef1 at the actomyosin ring in *sid2*$^+$ *plo1*$^+$ *mid1*$^+$ control cells. Merge of the division site of *control* and *sin* and *mid1Δ* mutant cells expressing Gef1-mNG and Rlc1-tdTomato. Bar, 5 μm.

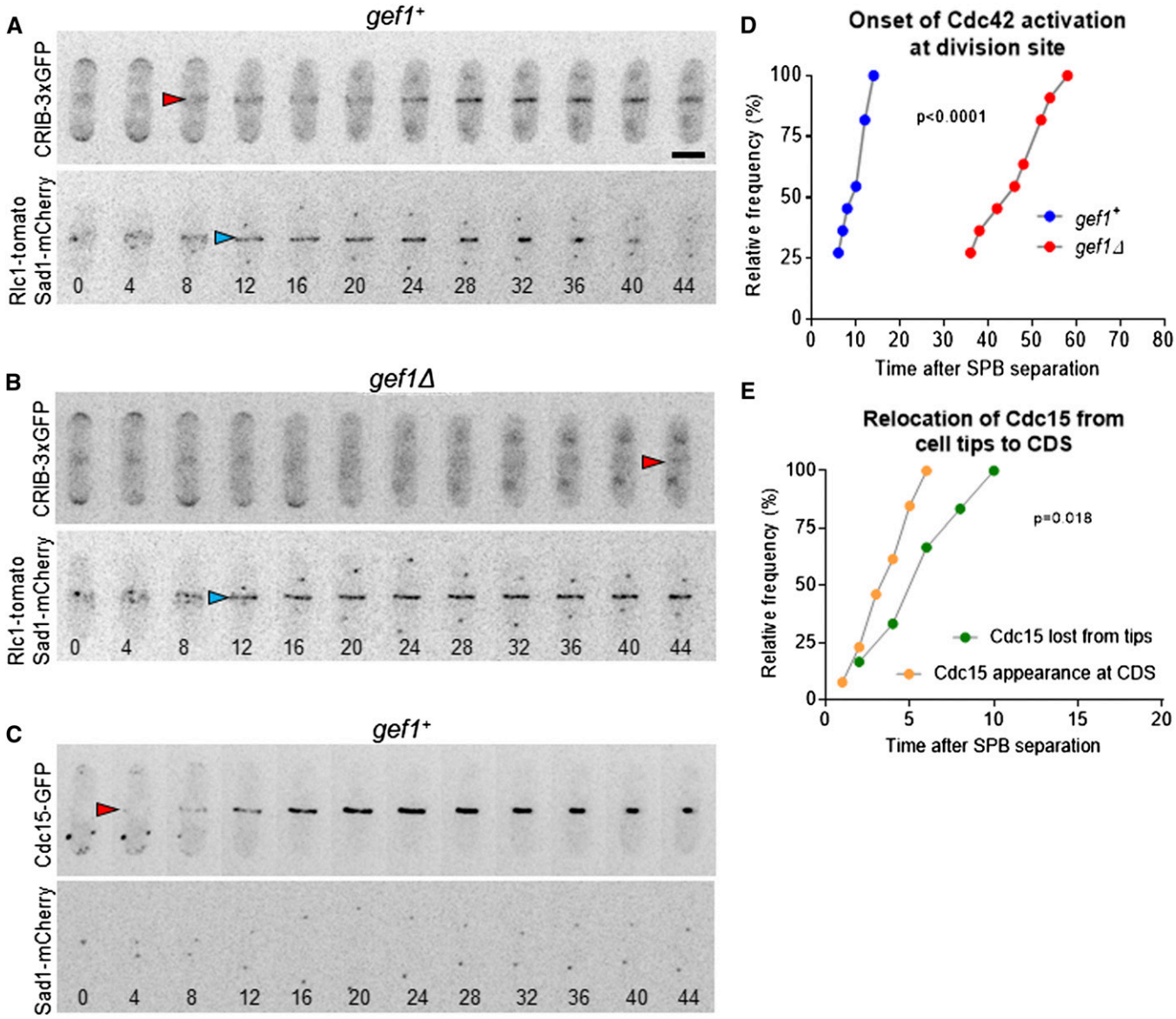

Figure 2 Cdc42 activation at the division site initiates during actomyosin ring formation. (A) In *gef1*$^{+}$ cells, CRIB-3xGFP appears at the division site prior to ring assembly. (B) In *gef1Δ* cells, CRIB-3xGFP does not appear at the division site until the onset of ring constriction. (C) Cdc15-GFP appears at the division site and begins to condense into the ring just prior to Cdc42 activation. Montages are inverted z-projections of the same cells imaged over time. Numbers beneath montages represent time in minutes with respect to SPB separation. Red arrowheads mark the time at which CRIB-3xGFP is first detected at the division site (A) and (B) or Cdc15-GFP (C). Blue arrowheads mark ring formation. (D) Frequency distribution plot of the percentage of cells in the indicated strains with CRIB-3xGFP at the division site as a function of time since SPB separation. (E) Frequency distribution plot of the relocation of Cdc15 from the tips to the division site as a function of time since SPB separation. Reported *P*-values from Student's *t*-test. Bar, 5 μm.

1E). This demonstrates that the early node protein Mid1 is not required for localization of Gef1 to the actomyosin ring.

Gef1-dependent Cdc42 activation appears at the division site prior to ring assembly

Gef1 is localized mainly in the cytoplasm and is not easily detected when present in small quantities at the membrane. Gef1 is the first GEF to localize to the division site and activate Cdc42 (Wei *et al.* 2016). To determine precisely when Gef1 localizes to the division site, we carefully examined Gef1-mediated Cdc42 activity during ring assembly. We monitored Cdc42 activity with the CRIB-3xGFP bio-probe that specifically binds to active GTP-bound Cdc42 (Tatebe *et al.* 2008). In *gef1*$^{+}$ cells, CRIB-3xGFP first appears as a broad band at the division site, as it is lost from the cell tips, 8 min after the Sad1-mCherry labeled spindle pole bodies (SBP) separate (Figure 2A, red arrowhead). However, the actomyosin ring, visualized by Rlc1-tdTomato, does not fully assemble for another 4 min (Figure 2A, blue arrowhead). This suggests that Cdc42 is activated at the membrane at the division site before the cortical nodes completely condense to form the cytokinetic ring. In contrast, CRIB-3xGFP

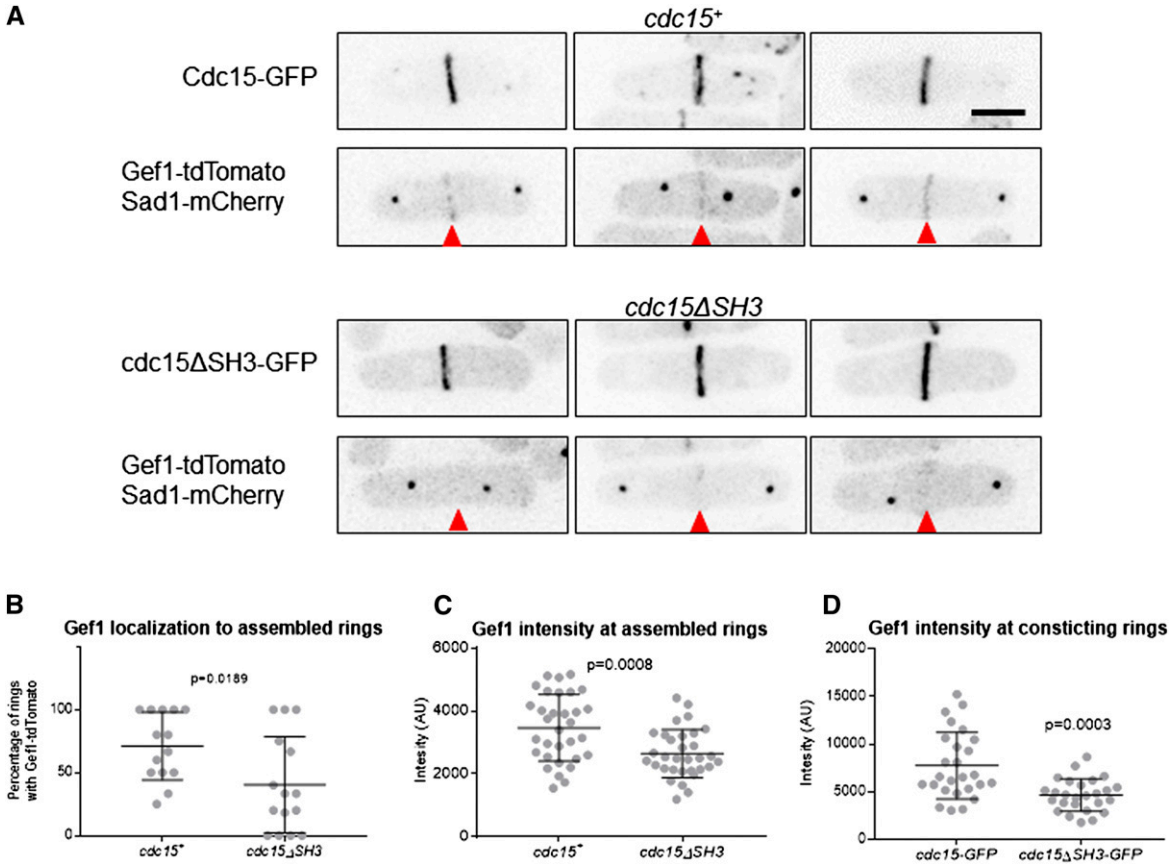

Figure 3 Cdc15 promotes Gef1 localization to the division site. (A) Inverted max projections of *cdc15*$^{+}$ and *cdc15ΔSH3* expressing Cdc15-GFP, Gef1-tdTomato, and Sad1-mCherry. Red arrowheads mark the division site. (B) Quantification of fields of cells of the indicated genotypes that have Gef1-tdTomato present at the assembled actomyosin ring. (C) Quantification of Gef1-tdTomato intensity at assembled, but not constricting, rings in the indicated genotypes. (D) Quantification of Gef1-tdTomato intensity at constricting rings in the indicated genotypes. Reported *P*-values from Student's *t*-test. Bar, 5 μm.

does not become active at the division site until ∼44 min after SPB separation in *gef1Δ* mutants (Figure 2B, red arrowhead). Thus, although Gef1 cannot be directly detected at the division site during this period, our findings suggest that Gef1 specifically activates Cdc42 as the ring assembles (Figure 2B).

Since Gef1-mediated Cdc42 activation initiates during ring formation, we asked if a protein involved in ring assembly regulates Gef1 localization to the division site. The F-BAR protein Cdc15 is recruited to the cortical nodes involved in ring formation, and acts as a scaffold for multiple proteins involved in cytokinesis (Fankhauser *et al.* 1995; Carnahan and Gould 2003; Wachtler *et al.* 2006; Roberts-Galbraith *et al.* 2009; Ren *et al.* 2015). During cytokinesis, Cdc15 is redistributed from the cell tips to the division site. We find that, while Cdc15 localizes to the division site ∼4 min after SPB separation, patches of Cdc15 remain at the polarized growth regions until ∼10 min after SPB separation (Figure 2, C and E). This suggests that Cdc42 is activated at the division site as Cdc15 is redistributed within the cell. Since Cdc42 activation is solely Gef1-mediated during this period, we asked whether Cdc15 may promote Gef1 localization to the division site to activate Cdc42. A recent report indicates that Gef1 regulates Cdc15 distribution along the actomyosin ring (Onwubiko *et al.* 2019). It is possible that Gef1 in turn depends on Cdc15 for its localization.

Cdc15 promotes Gef1 localization to the division site

Cdc15 associates with the membrane via its F-BAR domain and acts as a scaffold that associates with proteins at the actomyosin ring (McDonald *et al.* 2015, 2017; Ren *et al.* 2015). The scaffolding ability of Cdc15 is conferred primarily through its C-terminal SH3 domain, through which it interacts with other proteins (Roberts-Galbraith *et al.* 2009; Ren *et al.* 2015). While *cdc15* is essential for fission yeast, a *cdc15ΔSH3* mutant is viable but displays defects in septum ingression and ring constriction (Roberts-Galbraith *et al.* 2009). Similar to *gef1Δ* mutants, onset of ring constriction and Bgs1 localization to the division site is delayed in *cdc15ΔSH3* mutants (Roberts-Galbraith *et al.* 2009; Arasada and Pollard 2014; Cortés *et al.* 2015; Wei *et al.* 2016). Since *cdc15ΔSH3* and *gef1Δ* displayed similar cytokinetic defects, we asked if Cdc15 promotes Gef1 localization to the division site. To test this, we examined Gef1-tdTomato localization to

Table 1 Characterization of Gef1 recruitment to the cell division site (CDS) in a *cdc15*-dependent manner

	SPB distance at which Gef1 first appears at CDS	% of Anaphase B cells with Gef1 at CDS[a]	% of cells with assembled rings with Gef1 at CDS[a]	Relative Gef1-tdTomato intensity in assembled rings	% of cells with constricting rings with Gef1 at CDS	Relative Gef1-tdTomato intensity in constricting rings
cdc15⁺	3 μm *N* = 26	61% *N* = 26	78% *N* = 56	1.0 *N* = 32	100% *N* = 24	1.0 *N* = 26
cdc15ΔSH3	7 μm *N* = 26	12% *N* = 26	48% *N* = 37	0.76 *N* = 32	100% *N* = 43	0.6 *N* = 26

[a] The actomyosin ring assembles during anaphase B; hence, while all cells with assembled rings are in anaphase B, not all cells in anaphase B have an assembled ring.

assembled but not constricting rings, in cells expressing either Cdc15-GFP or cdc15ΔSH3-GFP. Gef1-tdTomato is present in ~70% of Cdc15-GFP rings, while Gef1-tdTomato is present in only ~40% of cdc15ΔSH3-GFP rings (Figure 3, A and B). Furthermore, Gef1-tdTomato fluorescent intensity is also reduced at the assembled rings of the *cdc15ΔSH3* mutant, with a relative intensity of only 76% that of *cdc15⁺* cells (Figure 3, A and C and Table 1). We find that Gef1-tdTomato localizes to the division site in cells with a minimum SPB distance of 3 μm in *cdc15⁺* cells. In contrast, in *cdc15ΔSH3* mutants Gef1-tdTomato appears at the division site with a minimum SPB distance of 7 μm (Table 1). Moreover, in *cdc15⁺* cells, 61% of cells in anaphase B displayed Gef1-tdTomato at the division site, while in *cdc15ΔSH3* mutants only 12% of anaphase B cells showed Gef1 localization (Table 1). In fission yeast, ring assembly completes during anaphase B. While Gef1 localization to assembled rings is initially impaired in cells expressing cdc15ΔSH3-GFP, all constricting rings have Gef1-tdTomato (Table 1). If Gef1 localization to the ring was impaired solely due to the delayed onset of ring constriction defect exhibited by *cdc15ΔSH3* mutants, Gef1 intensity should increase as soon as the actomyosin ring initiates constriction. However, even in the constricting rings, Gef1-tdTomato levels in *cdc15ΔSH3* mutants are only 60% that of *cdc15⁺* cells (Figure 3D, $P = 0.003$, Table 1). This suggests that Cdc15 likely promotes Gef1 localization to the division site.

cdc15 phenocopies gef1 polarity phenotypes

Our data indicate a functional relationship between Gef1 and Cdc15 during cytokinesis. This is further supported by the fact that *cdc15ΔSH3* and *gef1* share a common phenotype: a delay in the onset of ring constriction and Bgs1 localization at the division site (Arasada and Pollard 2014; Cortés *et al.* 2015; Wei *et al.* 2016). It is possible that, during cytokinesis, Cdc15 recruits Bgs1 to the division site through Gef1. *gef1Δ* cells are primarily monopolar, growing only from the old end (Coll *et al.* 2003; Das *et al.* 2015). In contrast, the hypermorphic allele *gef1S112A* exhibits precocious new end growth, producing primarily bipolar cells (Das *et al.* 2015). We asked if this functional relationship between Gef1 and Cdc15 is specific to cytokinesis, or whether it is also observed during polarized growth. Indeed, as compared to control cells, *cdc15ΔSH3* mutants show decreased bipolarity in interphase cells, similar to *gef1Δ* cells (Figure 4, A and B). Next, we asked if an increase in bipolarity was also observed in *cdc15* mutants with increased cortical localization. When oligomerized, the F-BAR domain enables Cdc15 to properly interact with the membrane (Roberts-Galbraith *et al.* 2010; McDonald *et al.* 2015). Cdc15 is a phospho-protein where hyper-phosphorylation disrupts proper oligomerization and, at least in part, impairs function (Roberts-Galbraith *et al.* 2010). In contrast, the dephosphorylated form of Cdc15 shows increased oligomerization and increased localization at cortical patches (Roberts-Galbraith *et al.* 2010). We find that, similar to *gef1Δ* and *cdc15ΔSH3* mutants, the phosphomimetic *cdc15-27D* allele exhibits decreased bipolarity (Figure 4, A and B). Further, the nonphosphorylatable *cdc15-27A* allele is primarily bipolar, similar to *gef1S112A* mutants (Figure 4, A and B).

To determine whether *gef1* is epistatic to *cdc15*, we generated double mutants of *gef1Δ* with different hypomorphic *cdc15* alleles (Figure 4C and Figure S1). We found that *gef1Δ cdc15-27D* and *gef1Δ cdc15ΔSH3* double mutants show a decrease in bipolarity similar to that observed in *gef1Δ*, *cdc15-27D*, and *cdc15ΔSH3* single mutants. Moreover, the increased bipolarity in the *cdc15-27A* mutant was reversed when combined with a *gef1Δ* mutant (Figure 4C and Figure S1). Together, this suggests that these proteins functionally interact, and that *gef1* is epistatic to *cdc15*. In contrast, when we analyzed the relationship between *gef1S112A* and *cdc15* mutant alleles, the morphological defects were further enhanced (Figure S2). The *gef1S112A cdc15-27A* double mutant displayed an increase in aberrant morphology and depolarized cells (Figure S2, A and B), while single *gef1S112A* and *cdc15-27A* mutants displayed normal cell morphology with enhanced bipolarity (Figure 4, A–C). Similarly, *gef1S112A cdc15-27D* and *gef1S112A cdc15ΔSH3* double mutants showed aberrant cell morphology defects with multiple cell poles, and large cell size (Figure S2, A and B). The *gef1S112A* mutant has a mutation in the Orb6 kinase phosphorylation site, and, in the dephosphorylated form, this allele does not interact with the 14-3-3 binding protein Rad24, and remains localized to the plasma membrane (Das *et al.* 2009, 2015). Our observations indicate that Cdc15 functionally interacts with Gef1 via a mechanism distinct from that of the Orb6 kinase-*gef1S112A* allele.

cdc15-27A enhances Gef1 localization at cortical patches and division site

Gef1 is predominantly a cytosolic GEF during interphase, and localizes only transiently to sites of polarized growth (Das *et al.* 2015). Given that Gef1 localization at the division site is dependent on Cdc15, we asked whether such a relationship

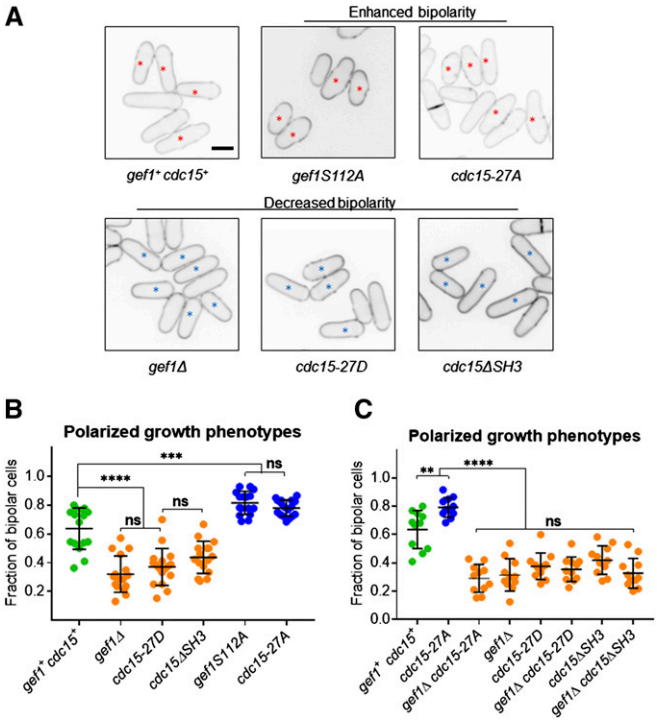

Figure 4 *cdc15* phenocopies *gef1* polarity phenotypes. (A) Representative images of the indicated genotypes stained with calcofluor to visualize polarized growth. Red asterisks denote bipolar cells, blue asterisks mark monopolar cells. (B) Quantification of the polarized growth phenotypes in the indicated genotypes. (C) Quantification of the monopolar cells in the indicated double mutants. (**** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, ns, not significant, *P*-values reported from ANOVA with Tukey's multiple comparisons *post hoc* test). Bar, 5 μm.

also occurs at the sites of polarized growth, as suggested by the polarity phenotypes exhibited by *cdc15* mutants. While Cdc15-GFP is clearly visible at endocytic patches at the cell tips, Gef1-tdTomato is seldom observed (Figure 5A, i). The nonphoshorylatable *cdc15-27A* mutants tagged to GFP show increased localization at cortical patches during interphase. Correspondingly, in cells expressing cdc15-27A-GFP, Gef1-tdTomato is readily observed at the cell cortex (Figure 5A, ii, iii). Moreover, we also observed that Gef1-tdTomato and cdc15-27A-GFP localize to the same cortical patches (Figure 5A, iv, v, red arrow). In addition to these patches, some regions of the cortex contain only Gef1-tdTomato or Cdc15-GFP (Figure 5A, iv, v, green and orange arrowheads, respectively). Next, we asked if Cdc15 also promoted Gef1 localization to the division site. We observe Gef1-mediated Cdc42 activation at the division site well before Gef1 itself is detectable (Figure 1A). Similar to a previous report, Gef1-tdTomato can be detected at the division site only in rings that have completed assembly (Wei *et al.* 2016). We find that in *cdc15-27A* mutants, Gef1-tdTomato localizes to the division site before the ring completes assembly. Gef1-tdTomato colocalizes with cdc15-27A-GFP as the latter condenses into a ring, while it is not yet detectable at this stage in *cdc15*$^+$ cells (Figure 5B). Since it is hard to distinguish Gef1 signal at the division site from the cytoplasmic signal, it is not possible to precisely determine when Gef1 localizes to the division site by time-lapse microscopy. We therefore synchronized the cells in the S phase using HU treatment, and then washed out the drug to allow for cell cycle progression. We used Cdc15-GFP or cdc15-27A-GFP-labeled actomyosin rings to determine cell cycle stage. During early cytokinesis Cdc15 appears at the ring, or at the precursor cytokinetic nodes around the nucleus. We find that, as the percentage of cells in cytokinesis increases, the fraction of cells with cdc15-27A-GFP patches around the nucleus containing Gef1-tdTomato also increased (Figure 5, C and D, green arrows). Thus, it is possible that Gef1 localizes earlier to the division site in *cdc15-27A* mutants, most likely to the cytokinetic nodes.

Cdc15 promotes Gef1-mediated Cdc42 activation

Given that Gef1 appears to precociously localize to nodes at the division site of cells expressing *cdc15-27A*, we asked whether this was concomitant with precocious Cdc42 activation. Normally, Cdc42 activity, visualized by CRIB-3xGFP, first appears at the division site only after the cell initiates anaphase A (Wei *et al.* 2016). However, we find that, in *cdc15-27A* mutants, CRIB-3xGFP signal was visible at the medial region in 33% of late G2-phase cells, prior to the separation of the Sad1-mCherry labeled SPB (Figure 6, A and B). In these cells, CRIB-3xGFP signal appeared as a broad band that overlapped with the nucleus (Figure 6A, red arrows). Next, we performed time-lapse microscopy to determine when Cdc42 was activated at the division site in *cdc15-27A* mutants. Cdc42 is first activated ~10 min after SBP separation in *cdc15*$^+$ cells. We find that, in *cdc15-27A* mutants, Cdc42 is activated earlier at ~4 min after SPB separation, as determined by CRIB-3xGFP localization (Figure 6C, red arrowhead, Figure 7D). Further, similar to previous reports, in *cdc15*$^+$ cells CRIB-3xGFP signal at the division site appears concurrent with the loss of signal from the cell tips. We find that, in *cdc15-27A* mutants, CRIB-3xGFP signal appears at the division site well before the signal is lost from the cell tips (Figure 6C, yellow asterisk). While CRIB-3xGFP signal at the cell medial region is clearly detected in cells with a single SPB by still imaging, we did not detect CRIB-3xGFP signal at the division site prior to SPB separation by time-lapse imaging. This could be due to low abundance or photobleaching of the signal, or it is possible that Cdc42 is activated only transiently at the medial region during interphase in *cdc15-27A* cells.

Finally, we asked if the premature CRIB-3xGFP signal at the division site and the increased bipolarity observed in *cdc15-27A* mutants was due to Gef1-mediated Cdc42 activation. To test this, we deleted *gef1* in *cdc15-27A* mutants. We find that *cdc15-27A* cells display an increase in bipolar CRIB-3xGFP localization at the cell tips relative to *cdc15*$^+$ cells (Figure 7, A and B, $P = 0.039$). This is consistent with our data indicating that bipolar growth is enhanced by *cdc15-27A* (Figure 4). Deletion of *gef1* in *cdc15-27A* mutants reduces bipolar CRIB-3xGFP localization, similar to that

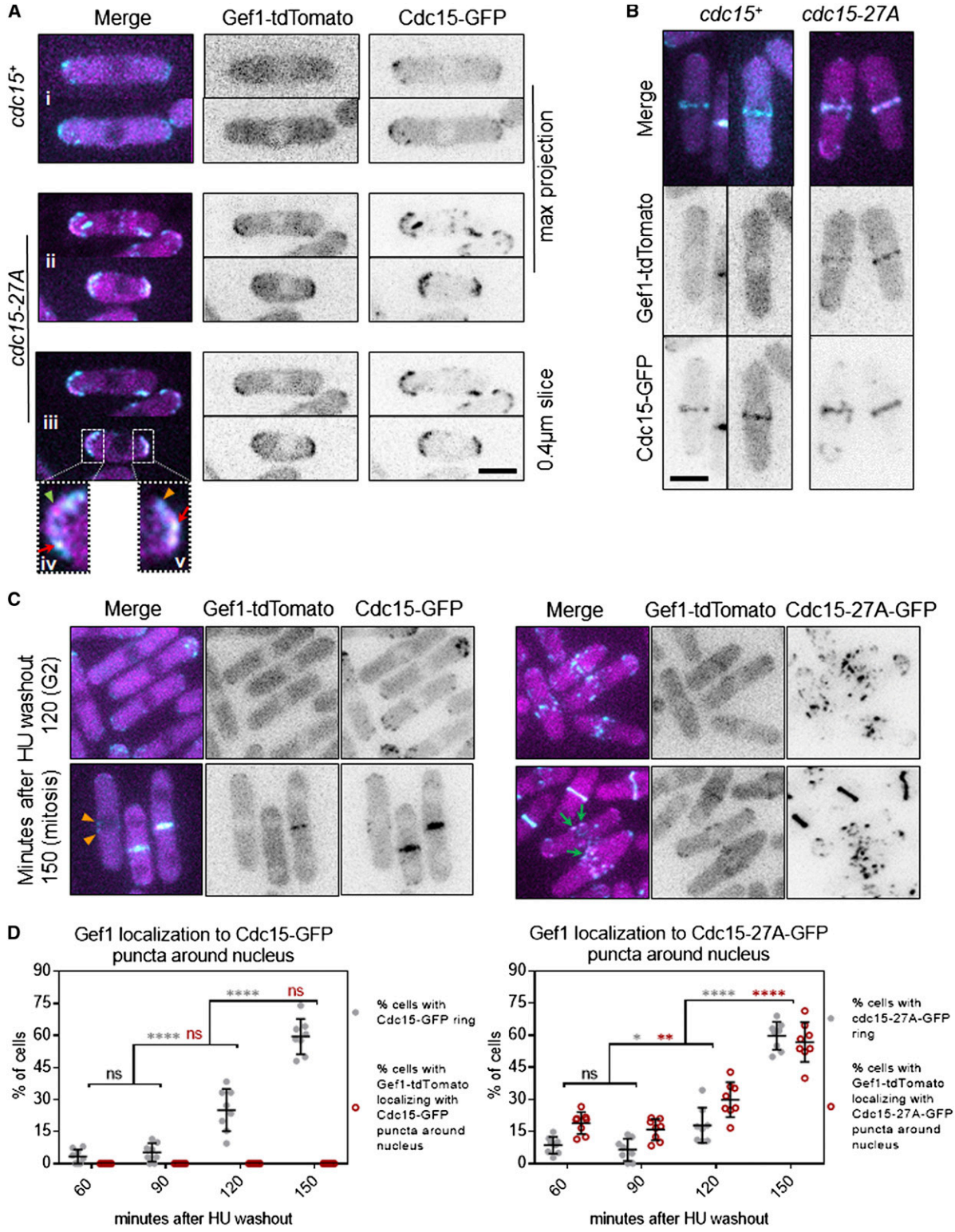

Figure 5 *cdc15-27A* enhances Gef1 localization at cortical patches. (A) Gef1-tdTomato and Cdc15-GFP localization to cortical patches in interphase *cdc15*$^{+}$ and *cdc15-27A* cells. i and ii are max projections, while iii is a single 0.4 μm z-plane of the same cell in ii. Insets iv and v are enlarged regions of the cell poles marked by white boxes. Red arrows indicate colocalization of Gef1 and Cdc15 patch. Green arrowhead indicates a Gef1 patch that does not colocalize with Cdc15. Orange arrowhead indicates a Cdc15 patch that does not colocalize with Gef1. (B) Gef1-tdTomato and Cdc15-GFP localization to the division site in *cdc15+* and *cdc15-27A* cells. (C) Gef1-tdTomato and Cdc15-GFP localization to cortical patches at the division site in *cdc15*$^{+}$ and *cdc15-27A* cells after synchronization with 10 mM HU and subsequent washout. Red arrows indicate cells with cortical puncta around nucleus of Gef1-tdTomato and Cdc15-27A-GFP at the division site of early mitotic cells. Orange arrowheads indicate puncta around the nucleus that contain Cdc15-GFP but lack Gef1-tdTomato. Green arrows indicate puncta around the nucleus that contain both Cdc15-27A-GFP and

observed in *gef1Δ* cells (Figure 7, A and B, $P < 0.0001$). Likewise, premature Cdc42 activation at the division site in *cdc15-27A* mutants is also abrogated in *gef1Δ cdc15-27A* cells. In *gef1Δ cdc15-27A* mutants, CRIB-3xGFP did not appear at the division site until ~45 min after SPB separation, as was also observed in *gef1Δ* (Figure 7, C and D). Together, these results indicate that Cdc15 promotes Gef1-mediated Cdc42 activation during cytokinesis and cell polarization.

Cdc15 regulates Cdc42 activation likely independently of the other GEF Scd1

Next, we asked if Cdc15 also promoted Scd1-dependent Cdc42 activation. To address this, we first examined the localization of the Cdc42 GEF Scd1 in *cdc15-27A* mutants. Under normal conditions, Scd1-tdTomato appears as a cap at the cell tips during interphase (Kelly and Nurse 2011; Das *et al.* 2012). We find that Scd1-tdTomato localization in *cdc15-27A* cells does not differ from *cdc15*$^+$ cells and does not localize to interphase cortical patches and nodes (Figure 8A). We did not observe any change in the number of Cdc15 labeled actomyosin rings with Scd1-tdTomato (Figure 8B). Moreover, Scd1-tdTomato levels at the cell poles are similar for *cdc15*$^+$ and *cdc15-27A* mutants (Figure 8C). This indicates that Cdc15 specifically regulates Gef1 localization during cytokinesis and cell polarization but not that of Scd1.

Next, we combined *scd1Δ* with *cdc15* mutant alleles. Scd1 is essential for mating and hence *scd1Δ* cells are sterile (Chang *et al.* 1994; Bendezú and Martin 2013). Thus, in order to generate double mutants, we transformed *scd1Δ* strains with a plasmid bearing *scd1* and mated this to *cdc15* mutants. Once *scd1Δ cdc15* double mutants were selected, we tried to remove the *scd1*-bearing plasmid. In *scd1Δ cdc15-27A* double mutants, cells that lost the plasmid were not viable (Figure 8E) and appeared more depolarized compared to *scd1Δ* mutants (Figure 8D). A recent report indicates that, in *scd1Δ* mutants, Gef1 cortical localization is enhanced and is ectopic (Hercyk *et al.* 2019). In the absence of *scd1*, *gef1* is the only remaining GEF, and *scd1Δ gef1Δ* double mutants are inviable. It is possible that, in the *scd1Δ cdc15-27A* mutant, Gef1 localization is severely impaired due to the combined effect of the two mutations, thus leading to loss of viability. In agreement with this, we find that *cdc15-27D* and *cdc15ΔSH3* mutants that phenocopy *gef1Δ* are also synthetically lethal with *scd1Δ*. Terminal colonies of *scd1Δ cdc15-27D* and *scd1Δ cdc15ΔSH3* without the *scd1*-bearing plasmid were observed under the microscope. These mutants display severe morphological defects (Figure 8E). Thus, these observations indicate that Scd1 and Cdc15 function independently to activate Cdc42.

Discussion

The two Cdc42 GEFs, while partially redundant, show distinct phenotypes during cell polarity and cytokinesis (Chang *et al.* 1994; Coll *et al.* 2003; Wei *et al.* 2016). This suggests that the GEFs may be regulated in different ways to precisely activate Cdc42 at its site of function. Although the role of the Cdc42 GEF, Gef1, in cytokinesis and cell polarity is well established (Das and Verde 2013; Chiou *et al.* 2017), it is not clear how Gef1 localizes to its site of action. Here, we show that Gef1, but not the other GEF, Scd1, localizes to its site of action in a manner dependent on the F-BAR Cdc15.

While disintegration of the actomyosin ring by LatA treatment results in Gef1 localizing to the cortical puncta, Gef1 does not become visible at the division site until the cytokinetic nodes coalesce into the actomyosin ring (Wei *et al.* 2016). However, Gef1-dependent Cdc42 activity can be observed at the membrane overlapping the nodes a few minutes before the nodes fully coalesce to form the ring. Given that Gef1 is a low abundance protein, it is possible that Gef1 may be present at quantities beneath our detection limit at the cortical nodes during the initial stages of ring assembly. Alternately, it is possible that cytoplasmic Gef1 near the division site may activate Cdc42 at the membrane overlapping the nucleus by an as yet unknown mechanism.

Given the timing of Cdc42 activation, Gef1 appears to be recruited late during the ring assembly process. Thus, we looked at other proteins that are likewise recruited to the division site during a similar time frame. It has previously been reported that the F-BAR protein Cdc15 is one of the last proteins to be recruited to the cytokinetic nodes before the ring assembles (Wu *et al.* 2003). We find that Cdc15 localizes to the division site shortly before Gef1-dependent Cdc42 activity initiates. Since Cdc15 serves as a scaffold and promotes localization for many other proteins during cytokinesis, we asked if Cdc15 also promoted Gef1 localization at the division site. We find that Gef1 localization is delayed in the hypomorphic *cdc15ΔSH3* mutant, and Gef1 levels at the division site remain low throughout constriction. Thus, our data suggest that Gef1 localization to the division site is *cdc15* dependent. While our data indicate a relationship between Gef1 and Cdc15, we did not observe any physical interaction between these proteins. This suggests that Cdc15 does not promote Gef1 localization by physically recruiting it to its site of action. It is possible that Cdc15 promotes Gef1 localization via indirect means. Cdc15 is required for endocytosis and also regulates the organization of lipid-rich domains in the plasma

Gef1-tdTomato. (D) Quantifications of Gef1-tdTomato localization to Cdc15-27A-GFP cortical puncta around nucleus in a cell cycle-dependent manner in the synchronized populations depicted in (C). (**** $P < 0.0001$, ** $P < 0.01$, * $P < 0.05$, ns, not significant, one-way ANOVA with Tukey's multiple comparisons *post hoc* test). Bar, 5 μm.

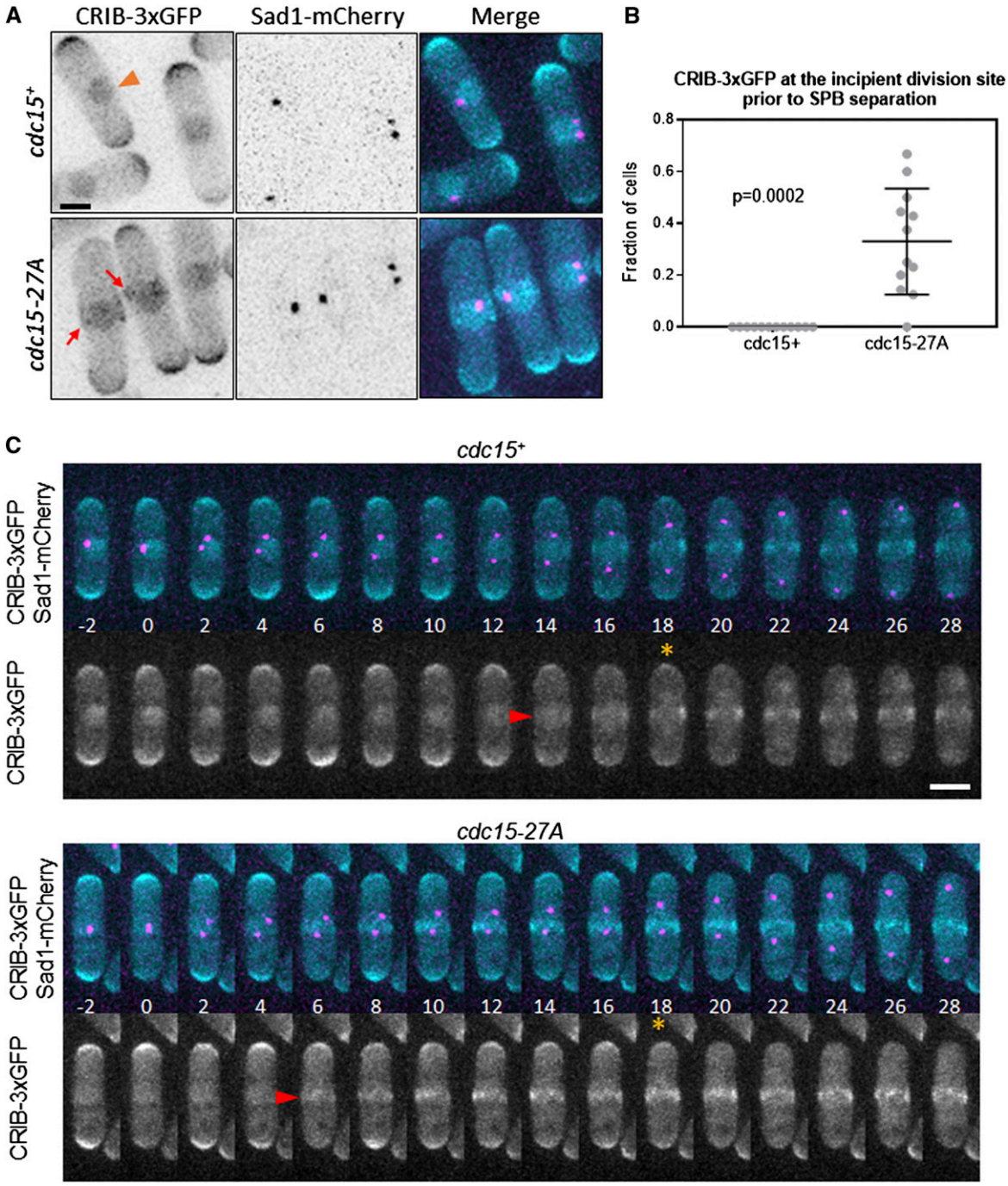

Figure 6 Cdc42 is prematurely activated in *cdc15-27A* cells during cytokinesis. (A) Inverted max projections of the indicated genotypes expressing CRIB-3xGFP and Sad1-mCherry. Orange arrowheads mark interphase cells without CRIB-3xGFP at the division site. Red arrows mark interphase cells with premature Cdc42 activation at the division site. (B) Quantification of Cdc42 activation at the division site prior to spindle pole body (SPB) separation in the indicated genotypes. Reported *P*-values from Student's *t*-test. (C) Time lapse montages of *cdc15+* and *cdc15-27A* cells expressing CRIB-3xGFP and Sad1-mCherry. Red arrowheads mark onset of Cdc42 activation at the division site. Orange asterisks mark last time points before Cdc42 is completely lost from the cell tips. Numbers beneath montages represent time in minutes with respect to SPB separation. Bar, 5 μm.

membrane (Wachtler *et al.* 2003; Takeda *et al.* 2004; Arasada and Pollard 2011). It is possible that Cdc15 may regulate Gef1 localization via either of these processes. Indeed, in a recent report, Gef1 has been shown to be involved in endocytosis (Onwubiko *et al.* 2019). Further analysis will be necessary to understand the molecular mechanism of Cdc15-dependent Gef1 localization.

We have previously reported that the β-1,3-glucan synthase Bgs1—the septum synthesizing enzyme that drives membrane ingression—is delayed in *gef1Δ* cells (Wei *et al.*

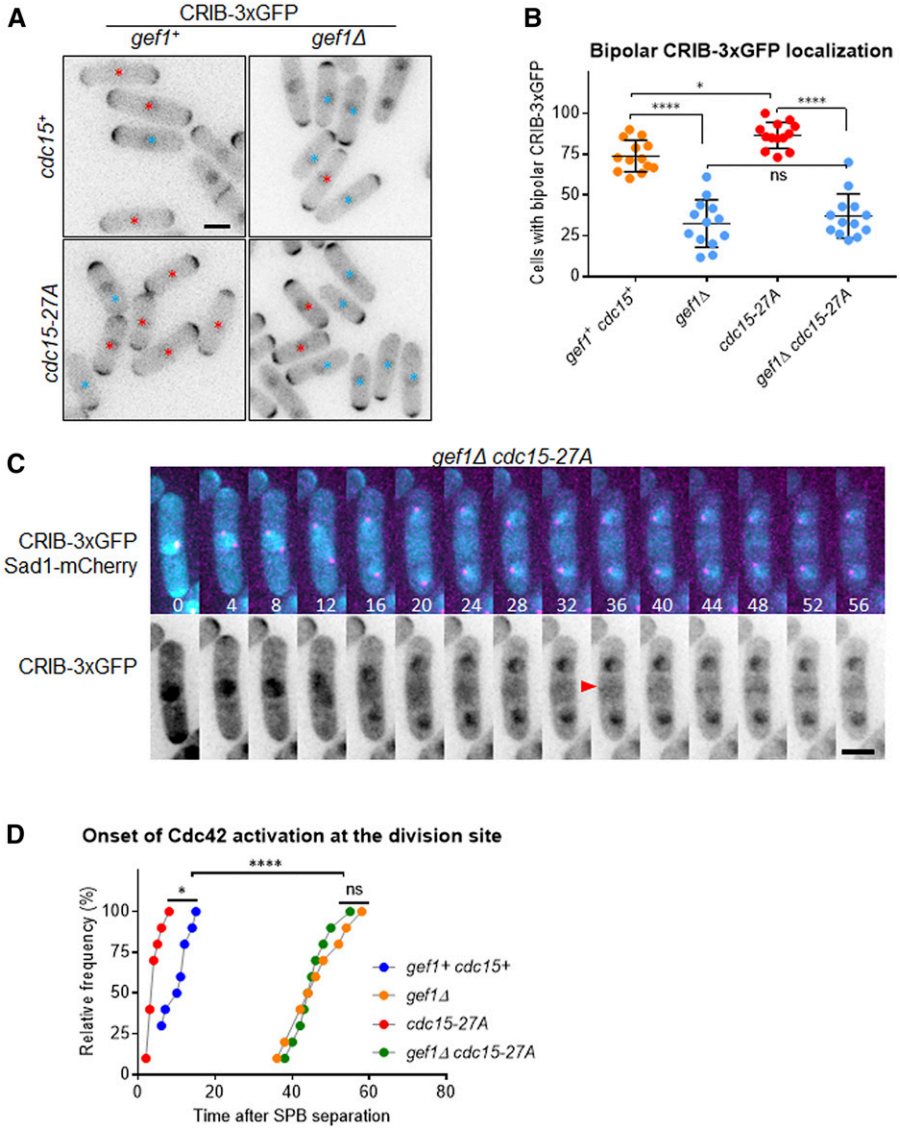

Figure 7 Cdc15 promotes Gef1-mediated Cdc42 activation. (A) Inverted max projections of the indicated strains expressing CRIB-3xGFP. Red asterisks mark cells with bipolar CRIB, blue asterisks show cells with monopolar CRIB localization. (B) Quantification of the bipolar CRIB-3xGFP in the indicated genotypes. (C) Time lapse montages of *gef1Δ cdc15-27A* cells expressing CRIB-3xGFP and Sad1-mCherry. Red arrowheads mark onset of Cdc42 activation at the division site. Numbers beneath montages represent time in minutes with respect to SPB separation. (D) Quantification of Cdc42 activation at the division site from time lapse images in the indicated genotypes expressing CRIB-3xGFP and Sad1-mCherry. **** $P < 0.0001$, *** $P < 0.001$, * $P < 0.05$, ns = not significant, one-way ANOVA with Tukey's multiple comparisons *post hoc* test. Bar, 5 μm.

2016). A similar defect is observed in *cdc15ΔSH3* mutants (Roberts-Galbraith *et al.* 2009; Cortés *et al.* 2015). Given that Cdc15 promotes Gef1 localization to the division site, and that *cdc15ΔSH3* also exhibits the delayed onset of ring constriction, characteristic of *gef1Δ* cells, we posited that Cdc15 acts upstream of Gef1 during cytokinesis. Apart from its role in cytokinesis, Gef1 is also required for proper cell polarity establishment (Coll *et al.* 2003). In fission yeast, immediately after division, the cells grow in a monopolar manner from the old end, and as the cells reach a certain size, bipolarity ensues (Das *et al.* 2012, 2015). Loss of *gef1* leads to a delay in initiation of bipolarity and as a result a large number of the cells in interphase are monopolar (Coll *et al.* 2003; Das *et al.* 2015). While Gef1 localizes mainly to the cytoplasm, its cortical localization is enhanced in *gef1S112A* mutants rendering the cells bipolar. We find that the gain of function *cdc15-27A* mutant resembles *gef1S112A* mutants, in which the cells are predominantly bipolar. In contrast, *cdc15-27D* and *cdc15ΔSH3* mutants mimic *gef1Δ* mutants, in which cells are predominantly monopolar. Moreover, both gain of function (*cdc15-27A*) and hypomorphic (*cdc15ΔSH3* and *cdc15-27D*) *cdc15* mutants displayed monopolarity similar to *gef1Δ* single mutants when combined with *gef1Δ*. This provides further evidence that *gef1* is epistatic over *cdc15* and that the two proteins functionally interact.

A recent report suggests that Gef1 is primarily a cytosolic GEF, where it activates Cdc42 (Tay *et al.* 2018). Rather, our data suggest that Cdc15 recruits Gef1 to the cortical patches to promote bipolar growth. During interphase, Cdc15 is localized to the endocytic patches, where it promotes vesicle internalization (Arasada and Pollard 2011). In the hypermorphic mutants, cdc15-27A-GFP levels are elevated at cortical patches (Roberts-Galbraith *et al.* 2010). Correspondingly, these mutants also show Gef1 localization to these patches. Moreover, Gef1 localization at the cortex is quite prominent in these mutants. In agreement with

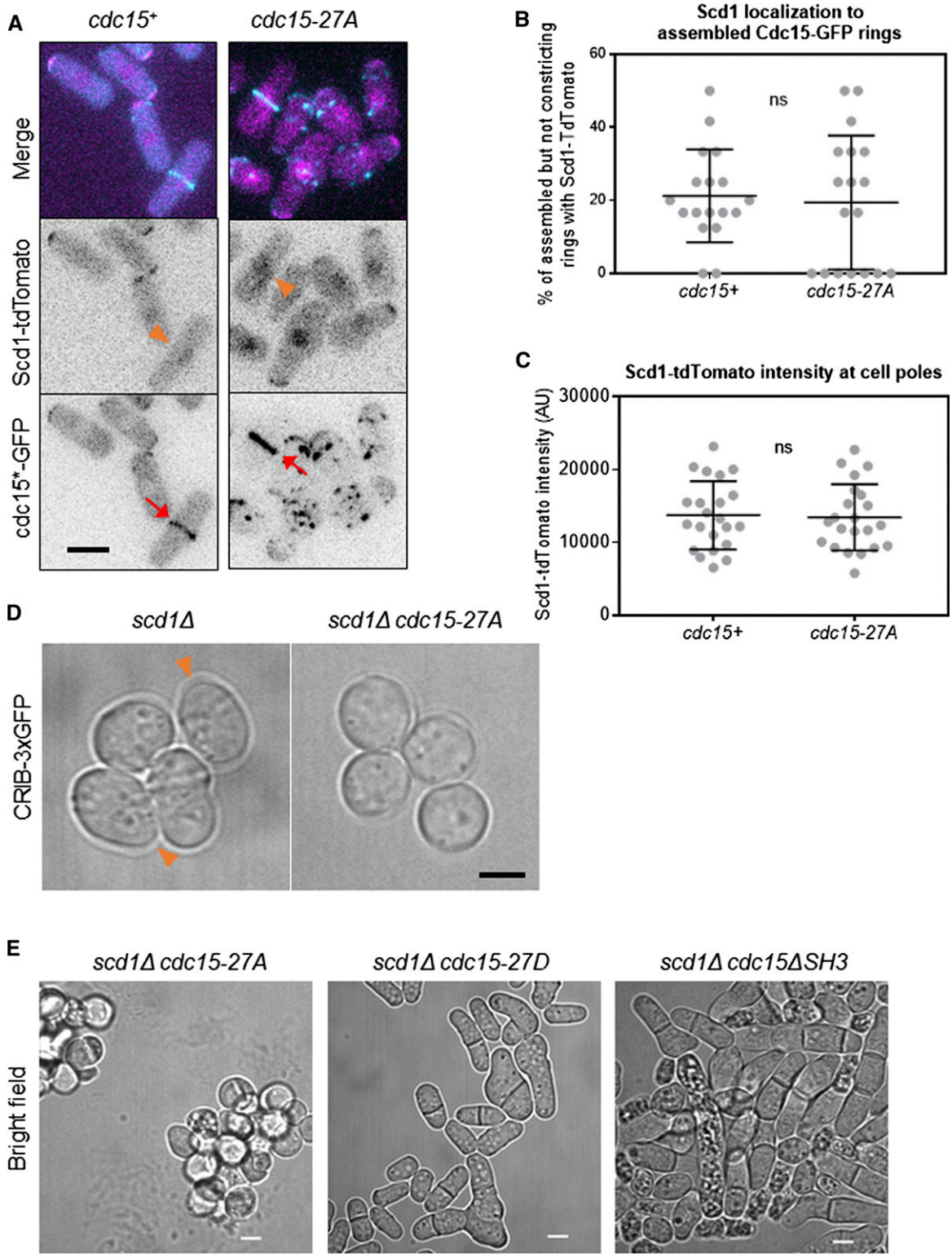

Figure 8 Cdc15 and Scd1 regulate cell polarity through parallel pathways. (A) Scd1-tdTomato localization in cdc15-GFP and cdc15-27A-GFP expressing cells. Red arrows mark cells with assembled Cdc15-GFP rings. Orange arrowheads indicate absence of Scd1-tdTomato localization at these rings. (B) Quantification of Scd1-tdTomato localization to assembled Cdc15-GFP rings in the indicated genotypes. (C) Quantification of Scd1-tdTomato intensity at the cell poles in *cdc15+* and *cdc15-27A* cells. (D) Bright field images of *scd1Δ* and *scd1Δ cdc15-27A* mutants. Orange arrowheads indicate sites of polarization. (E) Representative bright field images of the synthetic lethal polarity phenotypes of *scd1Δ cdc15-27A*, *scd1Δ cdc15-27D*, and *scd1Δ cdc15ΔSH3*. Reported *P*-values from Student's *t*-test. Bar, 5 μm.

increased Gef1 cortical localization, we also observe increased Cdc42 activation at both cell poles, resulting in increased bipolarity. Gef1 cortical localization has been shown to increase under stress conditions (Das *et al.* 2015; Tay *et al.* 2018). It is possible that, in *cdc15-27A*, the cells undergo stress, resulting in enhanced cortical localization of Gef1. However, given that hypomorphic *cdc15* mutants impair Gef1 localization and Gef1-dependent Cdc42 activation, and that Gef1 localizes to *cdc15-27A* containing patches, we propose that Cdc15 regulates Gef1-mediated Cdc42 activation. A recent paper demonstrates that Gef1 regulates Cdc15 by controlling the size and lifetime of Cdc15 cortical patches (Onwubiko *et al.* 2019). Above, we present data that demonstrate that Cdc15 is upstream of Gef1. These two observations are not contradictory, but rather reveal an elegant regulatory pattern: Cdc15 promotes Gef1 localization to endocytic patches, where Gef1, in turn, regulates the size of the Cdc15 patch via Cdc42 activation. Our observation that Gef1-tdTomato and Cdc15-27A-GFP do not perfectly colocalize at the cortex can be explained by the following model. Cdc15 initially recruits Gef1 to endocytic patches at the cortex, resulting in colocalization. Once Gef1 facilitates patch internalization, Cdc15 is lost from the cortex while Gef1 remains for a short time. Further investigations will determine how Gef1-mediated Cdc42 activity regulates Cdc15 cortical patch lifetime. Given the abundance of Gef1 in the cytoplasm, low levels of Gef1 are not easily detectable at cortical patches. Gef1 localization to the cortical patches and the cortex may be enhanced by the increased abundance of cdc15-27A at cortical patches. Gef1 localization at the cell cortex is also regulated by the NDR kinase Orb6 (Das *et al.* 2009). Orb6 kinase phosphorylates Gef1 at a serine at position 112, and this promotes sequestration of Gef1 to the cytoplasm by the 14-3-3 protein Rad24 (Das *et al.* 2015). We find that *gef1S112A* mutants that constitutively localize to the membrane show additive effects with *cdc15* mutants. This suggests that Gef1 localization to the site of action is regulated by multiple pathways. While Orb6 kinase is involved in preventing Gef1 localization to the membrane, Cdc15 likely promotes its localization. The cell shape defects with increased cell size observed in *gef1S112A cdc15-27D* and *gef1S112A cdc15ΔSH3* mutants suggests that, in the absence of proper Cdc15 function, constitutively localized *gef1S112A* can establish multiple growth poles.

Together, these results indicate that Cdc15 promotes Gef1-mediated Cdc42 activity at the cell poles and during cytokinesis. Cdc42 is activated by Gef1 and Scd1, and the *scd1Δ gef1Δ* double mutant is lethal (Coll *et al.* 2003; Hirota *et al.* 2003). Our observation that loss of function *cdc15* mutants are synthetically lethal with deletion of the other Cdc42 GEF *scd1* provides further evidence that Cdc15 promotes Gef1 function.

A mechanistic understanding of factors that control Gef1 localization is sorely lacking. Aside from the observation that the N-terminus of Gef1 is required for its localization to the membrane, no other factors have been identified (Das *et al.* 2015). It has also been reported that Gef1 activates Cdc42 with the help of BAR Hob3 protein interaction (Coll *et al.* 2007). Gef1 is a homolog of the mammalian GEF TUBA and contains a BAR domain (Das *et al.* 2015). However, previous reports show that the Gef1-BAR domain is not required for its localization to the division site, nor is Hob3 required for Gef1 localization (Figure S3) (Das *et al.* 2015). In contrast, the mechanism removing Gef1 from the membrane has been elucidated. Gef1 is phosphorylated by Orb6, generating a 14-3-3 binding site that results in Gef1 removal by Rad24 (Das *et al.* 2009, 2015). Here, we identify Cdc15 as a factor that promotes Gef1 localization to both the cell tips and the division site. The role of Cdc15 in the processes of cytokinesis and endocytosis is well established (Fankhauser *et al.* 1995; Carnahan and Gould 2003; Wachtler *et al.* 2006; Roberts-Galbraith *et al.* 2010; Arasada and Pollard 2011, 2014; Martin-Garcia *et al.* 2014; Ren *et al.* 2015; Willet *et al.* 2015). Here, we present data that reveals an additional role for Cdc15 in the regulation of Cdc42 activation during cell polarization and cytokinesis. Furthermore, this regulation is mediated by the specific regulation of Gef1 localization, but not that of the other GEF Scd1. These studies begin to explain how, through differential regulation and localization, two GEFs of the same GTPase can exhibit distinct phenotypes.

Acknowledgments

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