[Cell Cycle 6:14, 1687-1691, 15 July 2007]; ©2007 Landes Bioscience

Extra View

Spatial Regulation of Cdc42 During Cytokinesis

Sergio Rincon
Pedro M. Coll
Pilar Perez*

Instituto de Microbiología Bioquímica; Consejo Superior de Investigaciones Científicas (CSIC)/Departamento de Microbiología y Genética; Universidad de Salamanca; Edificio Departamental; Salamanca, Spain

*Correspondence to: Pilar Perez; Instituto de Microbiología Bioquímica; Consejo Superior de Investigaciones Científicas (CSIC)/Departamento de Microbiología y Genética; Universidad de Salamanca; Edificio Departamental; Salamanca 37007 Spain; Tel.: 34.923.121644; Fax: 34.923.224876; Email: piper@gugu.usal.es

Original manuscript submitted: 05/21/07
Manuscript accepted: 05/21/07

Previously published online as a *Cell Cycle* E-publication:
http://www.landesbioscience.com/journals/cc/abstract.php?id=4481

KEY WORDS

Cdc42, Rho GTPases, amphiphysin, BAR, GEF, cytokinesis, fission yeast

ACKNOWLEDGEMENTS

We thank J.C. Arevalo, J.C. Ribas, and B. Santos for useful comments. Thanks to D. Posner for English correction of the manuscript. This work was supported by Grant BIO2004-0834 from the Comision Interministerial de Ciencia y Tecnología, Spain.

ABSTRACT

Cdc42 GTPase plays a critical role in the establishment of cell polarity in most eukaryotic organisms. Cdc42 active state, as that of other GTPases, depends on the bound nucleotide. The protein with GTP is active, and only in this state can it interact with different target effector proteins. The spatio-temporal control of Cdc42 activity is therefore necessary to generate growth polarity. In fission yeast cells, Cdc42 mainly localizes to the division area, and also to the growing tips and to some internal membranes. While the role of Cdc42 in apical growth is well defined, no role has been described for Cdc42 in the process of cell division. Fission yeast Cdc42 activity is regulated by two specific guanidine nucleotide exchange factors (GEFs), Scd1, and Gef1. We discuss here how Hob3, a BAR domain containing protein similar to human BIN3 and *S. cerevisiae* Rsv161, may be required to recruit Cdc42 to the cell division site as well as for the activation of this GTPase mediated by Gef1. We also discuss the possible role of Cdc42 in the contraction of the actomyosin ring necessary for cytokinesis.

Cell polarity is an essential feature of all eukaryotic cells, both during development and in the adult cells. The Rho family of GTPases are key molecules in the regulation of the polarized cellular organization required for differentiation, cell motility, cell adhesion, cell growth, cell division, and morphogenesis.[1-4] Cdc42 GTPase regulates cell morphology and polarization of the actin cytoskeleton in all eukaryotic cells. A precise and local activation of this GTPase at the presumptive growth site is required in order to selectively regulate the multiple signal transduction pathways that will generate an asymmetric growth in that site.[5-7] Cdc42 is structurally similar to other GTPases from the Ras superfamily. The presence of a carboxy-terminal CAAX sequence is critical for initiating a series of post-translational modifications that promote the association of the GTPases with the plasma or other cellular membranes.[8,9] Two distinct motifs immediately upstream of the CAAX box have also been implicated in promoting membrane association. Additionally, some results indicate that the hypervariable carboxy-terminal region plays a role in the spatial regulation of Rho GTPases. Thus, mammalian RhoA and RhoB share strong sequence identity, and interact with common regulators and effectors but have functionally distinct roles. RhoA is associated to the plasma membrane and regulates the actomyosin contractility, whereas RhoB associates with endosomes and regulates cytokine trafficking and cell survival.[10]

Cdc42 shares with other GTPases the molecular mechanism by which they are turned on when bound to GTP and off when this nucleotide is hydrolysed to GDP.[11] The balance between both forms of the GTPase is regulated by the opposing activities of guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs). GEFs promote the exchange of GDP for GTP, rendering the protein active,[12,13] whereas GAPs increase the intrinsic hydrolysis rate of bound GTP, turning the GTPase off.[14] In addition, there are other regulatory proteins called guanine nucleotide dissociation inhibitors (GDIs) that prevent nucleotide exchange and membrane association.[15]

The fission yeast *Schizosaccharomyces pombe* is a highly polarized eukaryotic cell extensively used as a model for morphogenetic studies. Cells grow by apical extension until mitosis and they undergo three main morphological transitions during the cell cycle (Fig. 1). Cells initiate growth in a monopolar fashion elongating from the "old end" that existed prior to septation. This monopolar growth continues until early G_2 phase. At this time, a transition to bipolar growth occurs by using the new end originated during cell division (this change is known as "new end take off" or NETO).[16] Finally, when the cell reaches its maximal size, tip elongation ceases and mitosis occurs followed by cytokinesis (Fig. 1). *S. pombe*, as all animal cells, organizes an actomyosin contractile ring necessary for

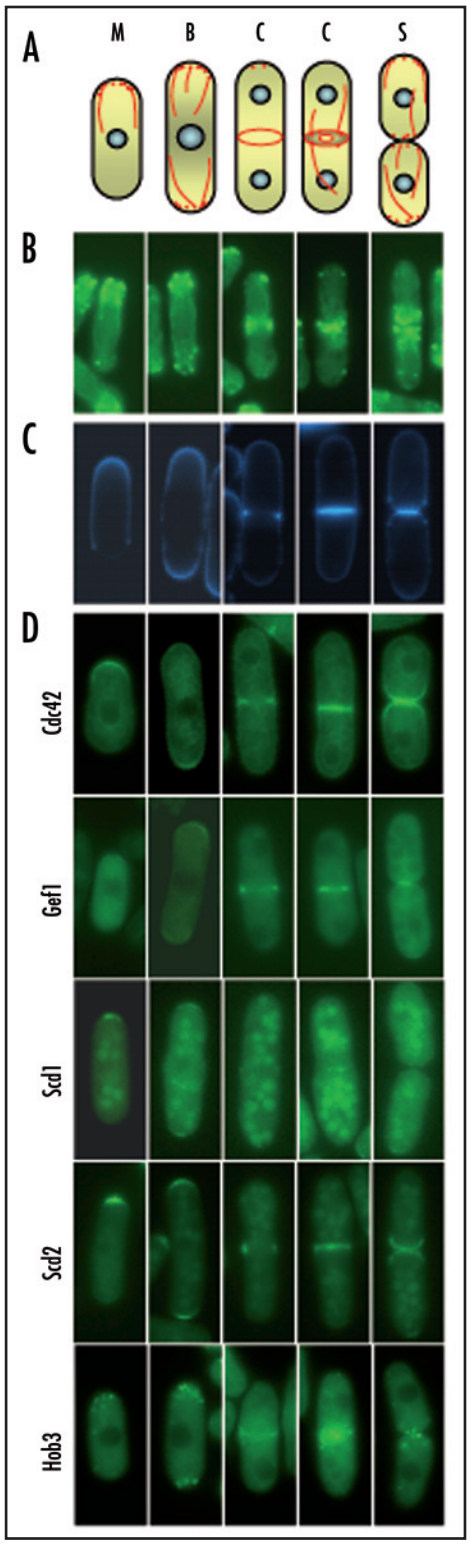

Figure 1. (A) Scheme of growth stages during the fission yeast *Schizosaccharomyces pombe* cell cycle. M, monopolar; B, Bipolar; C cytokinesis; S, separation. Actin patches are shown as red dots and actin cables as red filaments. (B) Actin staining with Alexafluor 488-Phalloidin of fixed cells during different cell cycle stages. (C) Calcofluor staining of fission yeast indicating sites of newly synthesized wall. (D) Localization of GFP-Cdc42, the two GEFs: GFP-Gef1 and Scd1-GFP, the adaptor Scd2-GFP, and Hob3-GFP during the cell cycle.

cell cleavage.[17] Most of the components of this contractile ring are conserved among eukaryotes.[18] Concomitant with ring constriction and addition of new membranes, synthesis of the primary and secondary septa occurs. Several regulatory proteins form a signaling pathway, named septation initiation network (SIN), which triggers the contraction of the F-actin ring and coordinates cytokinesis with the nuclear cycle.[19-22] The biosynthesis of the primary septum, mainly composed of linear (1,3)-β-D-glucan is performed by Bgs1, a β-1,3-glucan synthase subunit[23,24] that is activated by Rho1.[25] The primary septum is flanked on both sides by the new cell wall that forms the secondary septum. Upon septum assembly completion, local erosion of the adjacent regions of the cell wall accompanied by rapid autolytic degradation of the primary septum occurs and causes separation of the two new cells. This last step of cytokinesis is accomplished by polarized secretion of hydrolytic enzymes such as the glucanases Eng1 and Agn1.[26,27] Polarized secretion during cytokinesis requires the organization of the septin cytoskeleton, the exocyst, and Rho4 GTPase.[28,29]

S. pombe cdc42$^+$ is an essential gene. Cells lacking *cdc42*$^+$ are rounded, small, uninucleated and more dense, suggesting that incorporation of new material to the membranes was inhibited.[30] Cdc42 mainly localizes to the division area, and also to the growing tips and to some internal membranes[31] (Fig. 1). In spite of this localization, no role has been described yet for Cdc42 in the cytokinesis process. There are two specific Cdc42 GEFs in *S. pombe*, Scd1[32] and Gef1.[33] The double deletion of these genes is not viable, suggesting that Scd1 and Gef1 share the essential role of Cdc42 activation. Scd1 is necessary for apical growth,[32] and activates the polarity signalling pathway: Ras1-Scd1-Cdc42-Shk1.[34] The scaffold protein Scd2 facilitates the interaction between Cdc42, the exchange factor Scd1, and the p21 activated kinase Shk1 that regulates apical growth.[35] Gef1, the other Cdc42 GEF, does not regulate apical growth but plays a role in septation and NETO activation.[33,36]

Most Rho GEFs contain a Dbl homology domain (DH) responsible for their exchange activity.[12] The DH module is located upstream a pleckstrin homology (PH) domain, whose lipid-binding properties are thought to play a critical role in the protein localization and in the regulation of the DH domain activity.[37] Scd1 contains the canonical tandem DH-PH domains; by contrast, Gef1 does not have a PH domain downstream the DH domain. In a recent work we identified Hob3 as a protein interacting with the C-terminal region of Gef1, downstream of the DH domain. Hob3 localizes to the division area forming a ring, and also forms some patches at the growing poles[38] (Fig. 1). Deletion of *hob3*$^+$ causes cell elongation and multiseptation.[39] Hob3 is structurally similar to *S. cerevisiae* Rvs161 and human Bin3.[39] All of them are the simplest members of the BAR (BIN/Amphiphysin/Rvs) domain containing protein family, since the whole protein is formed by a single BAR domain.[40] This evolutionarily conserved domain was initially found in yeast Rvs (reduced viability upon starvation) proteins and in metazoan amphiphysins.[40] The first members of this extensive protein family were found to be regulators of the early steps in endocytosis.[41] These domains can form dimers with a crescent-shaped structure and can bind to negatively charged phospholipid heads in membranes through two pairs of basic residues. They act both as a curvature-generating and a curvature-sensing module[42,43] generating a protrusion, and facilitating the split of the vesicle by the recruitment of GTPases like dynamin.[44] Dynamin is often associated to sites of actin nucleation and its disruption affects actin dynamics.[42,43] BAR domain can also interact with other GTPases such as Rac, Rab, and Arfs.[45] It

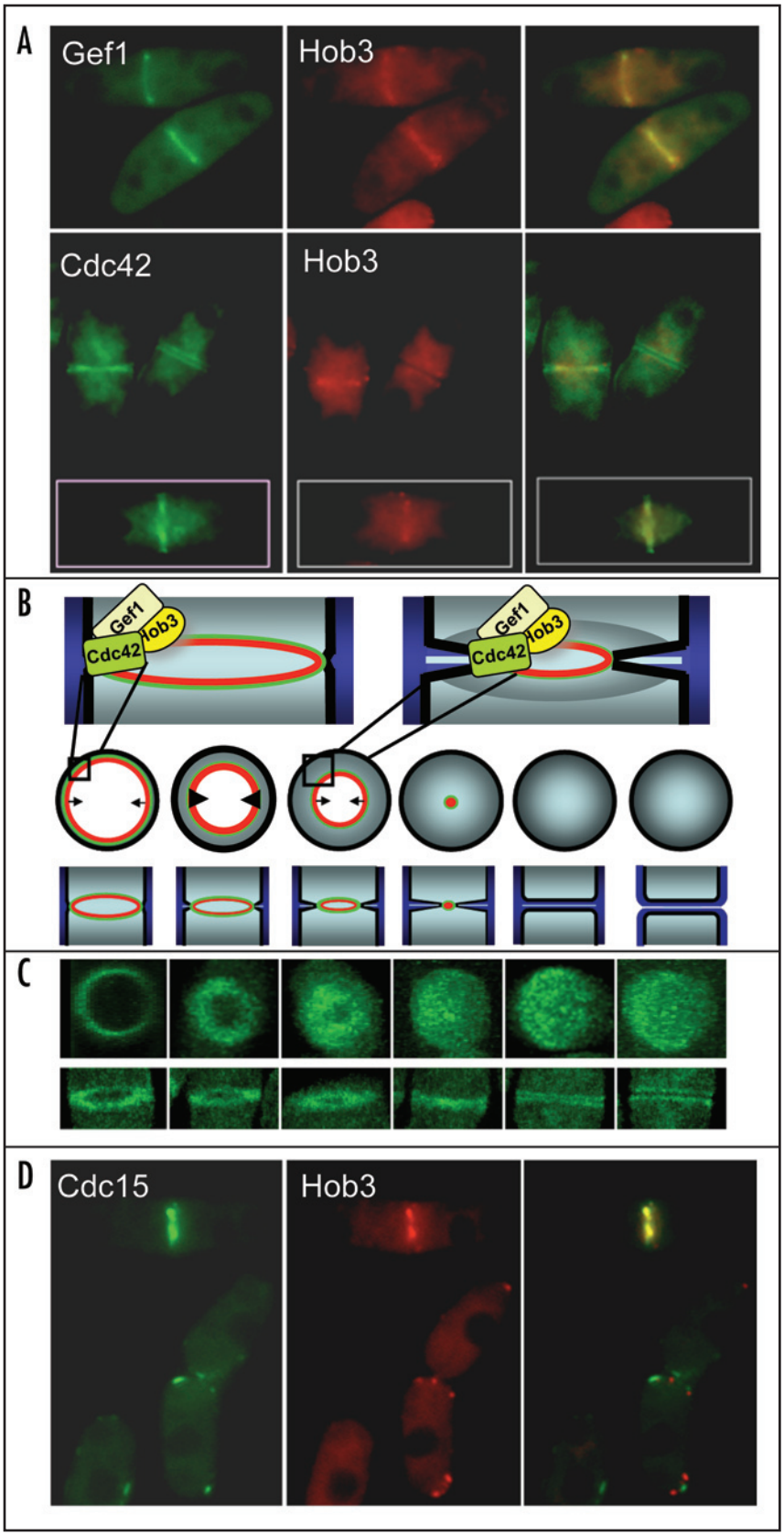

Figure 2. (A) Colocalization of Hob3-RFP with GFP-Gef1 and GFP-Cdc42 during cytokinesis. (B) Scheme of ring constriction and membrane closure during cytokinesis. In red is represented the actomyosin ring, and in green the membrane edge contacting the ring during cytokinesis. The position of the Gef1-Cdc42-Hob3 complex is indicated. (C) Confocal microscopy images of GFP-Cdc42 during cytokinesis. (D) Colocalization of Hob3-RFP with Cdc15-GFP at the medial ring.

is not known if binding to membranes and binding to GTPases are mutually exclusive or they are related functions. In the latter case, they might be sequential processes in which the BAR protein recognizes a membrane region with a certain curvature; the interaction with the small GTPase could then take place there. This hypothesis supposes that the initial location of the BAR protein is independent of its interaction with the GTPase. Another possibility would be that the interaction with the GTPase would promote the binding of the BAR domain to a membrane region, where the BAR domain would regulate the curvature of this membrane region.[45] In this second case the BAR protein would be an effector of the GTPase.

An interesting BAR domain containing protein in mammalian cells is Tuba, a unique Cdc42 GEF that promotes accumulation of F-actin.[46] Tuba DH domain is not followed by a PH domain but it is followed by a BAR domain that is required for its interaction with the membrane and with Cdc42.[46,47] The NH2-terminal region of Tuba has four SH3 (Src Homology 3) domains that bind to dynamin. Tuba also interacts with several proteins that regulate the actin cytoskeleton, such as N-WASP, through other SH3 domains at the C-terminal region. The presence in Tuba of a BAR domain, rather than a PH domain, may reflect its action at certain plasma membrane regions where Tuba might regulate actin cytoskeleton and endocytosis.[46] Our recent results suggest that Hob3 facilitates the recruitment of Cdc42 to the membrane area that contacts the contractile ring where Cdc42 can be activated by Gef1 and would in turn activate other target molecules required for cytokinesis.[38] Hob3 is necessary for the Gef1 mediated activation of Cdc42, and colocalizes with Gef1 in a ring that undergoes shrinkage during cytokinesis[38] (Fig. 2). Moreover, Hob3 also colocalizes with the inner part of the broader ring formed by Cdc42 (Fig. 2). The tandem Gef1-Hob3 could be similar to the protein Tuba in mammalian cells activating Cdc42 at the site of contact with the contractile ring.

How Hob3 is localized to the division area remains an open question. To date it is only known that its localization depends on actin and Cdc15 and that is independent of Gef1 and the SIN proteins.[38] Hob3 does not have other protein interaction domains besides BAR, and BAR domains do not have a strong phospholipid binding specificity. Heterodimerization has been described among BAR proteins and this could be a means for Hob3 to localize. In *S. cerevisiae*, Rvs161 and Rvs167 form heterodimers in vivo,[48] and some *rvs161*Δ or *rvs167*Δ mutant phenotypes are very similar to each other. Hob1, the other *S. pombe* BAR domain containing protein, localizes to actin patches at the cell ends during growth and to the medial site during cell division.[49] However, elimination of the coding gene failed to implicate Hob1 in cytokinesis,[50] and both Hob3 and Cdc42 are properly localized in cells lacking Hob1.[38]

A potential partner to interact and localize Hob3 could be Cdc15. This *S. pombe* protein is the founder member of the PCH family of proteins expressed in many eukaryotes.[51] They contain an N-terminal FCH (Fes/CIP4 Homology) domain followed by a coiled coil (CC) region and by one or two C-terminal SH3 domains. The FCH and CC regions form what is called an F-BAR domain that is conserved in all PCH proteins and mediates their oligomerization and interaction with membrane phospholipids.[52] The SH3 domain mediates the interaction with proline-rich motifs found in many proteins, including members of the Wiskott-Aldrich syndrome protein (WASP) family and dynamin.[53] Recent work has shown that F-BAR has a similar predicted tertiary structure to that of BAR domain. F-BAR also binds to phospholipids and induces membrane tubulation, as BAR domains.[52] *S. pombe* Cdc15 promotes ring formation during cytokinesis, recruits both the formin Cdc12 and the Arp2/3 complex activator Myo1 to the medial region of the cells, and coordinates the formation of the cytokinetic actin ring.[54] Cdc15 is also required for Hob3 localization, and using Cdc15-GFP and Hob3-RFP we have shown that they colocalize during cytokinesis (Fig. 2). Additionally, Hob3 immunoprecipitates with Cdc15 (our unpublished results). It is therefore tempting to speculate that Cdc15 recruits Hob3 to the division area but additional experimental data are needed in order to prove it. Interestingly, Cdc15 and Hob3 form cytoplasmic patches during interphase but those patches do not colocalize (Fig. 2).

Hob3 regulates cytokinesis and recruits Cdc42 to the division site via the BAR domain. This is a novel mechanism for spatially regulating Cdc42 signalling. In *S. cerevisiae*, Cdc42 clusters at the septum region prior to actomyosin ring contraction[55] but it is not known how it concentrates there. It might be possible that Rvs161 participates in that clustering. It is interesting that Bin3, the human ortholog of Hob3, partially complements the defects of *hob3*Δ mutants, suggesting a conserved function for both proteins.[50] Bin3 transcript is expressed ubiquitously in embryos and all adult tissues with the exception of the brain. Unlike Bin1, whose expression is lost in a significant percentage of cultured cancer cell lines and primary tumors, all tumor cell lines tested retain Bin3 expression.[39] There has not yet been a thorough characterization of Bin3 and its cellular functions remain to be identified. An obvious question to ask is if Bin3 is also able of recruiting Cdc42 for cytokinesis in human cells. Hob3 is an example of proteins other than GEFs that spatially regulate Rho GTPases. Another recently described example is mammalian Scrib, an evolutionnarily conserved adaptor protein that controls localization and activation of the small GTPase Cdc42 at the front edge of polarizing astrocytes, and regulates Cdc42-dependent polarization pathways by interacting with bPIX, a Cdc42 and Rac GEF.[56,57]

A main question derived from our results is: how does Cdc42 regulate cytokinesis? A possible role in septin organization, as in *S. cerevisiae*,[58] is ruled out since septins are well organized in *hob3*Δ cells and the cytokinesis defects caused by septin malfunction is additive to that caused by *hob3* deletion.[38] Cells lacking Hob3, and therefore Cdc42 in the cytokinesis area, have a delay in ring contraction that can cause the increase in septating cells and the appearance of multiseptated cells after several cell cycles.[38] It is possible that Cdc42 participates in the actin polymerization needed for the maintenance and closure of the contractile ring.[59] Other mutant strains with defects in actin polimerization, such as *arp2-1* showed increased septating cells at the restrictive temperature.[60] In *S. cerevisiae*, Cdc42 regulates the proper spatiotemporal activation of the Arp2/3 complex, which is the main regulator of actin polymerization described to date.[61] Cdc42 also activates Arp2/3 in animal cells via the Wiskott-Aldrich syndrome proteins (WASPs).[62] In *S. pombe*, Wsp1 (a WASP homolog) and Myo1 stimulate actin assembly by Arp2/3, and *wsp1*Δ or *myo1*Δ logarithmic cultures also have an increased percentage of septating cells.[63] We have found that double deletion of *wsp1* and *hob3* is lethal; cells die upon germination without losing polarity (our unpublished results), suggesting that Hob3 signaling pathway participates with Wsp1 in an essential process. Cdc42 might also participate in cytokinesis by regulating either targeted membrane addition or endocytosis during ring constriction since these are fundamental and widely conserved processes required in cytokinesis.[64] In yeast cells, ring constriction and septum formation are coordinated, and both septum biosynthetic and septum degrading enzymes are delivered by polarized secretion. It is not known how membrane-trafficking pathways, either membrane delivery or endocytosis, are regulated during cytokinesis but Cdc42 has also been implicated in intracellular trafficking regulation.[6] Although it has been described that *hob3*Δ cells do not have endocytosis defects,[39] this strain could still have specific defects in endocytosis during cytokinesis. *hob3*Δ cells have not been observed by electron microscopy to see if there is accumulation of vesicles in the septum area but a much greater proportion of *S. cerevisiae rvs161*Δ and *rvs167*Δ mutant than wild type cells are seen by electron microscopy in the process of depositing septa, suggesting that they are involved in the late targeting of vesicles carrying proteins required for cell wall construction.[65] Future studies will reveal how the spatially restricted Cdc42 activity and the Gef1-Cdc42-Hob3 complex works at the actomyosin ring-membrane interphase contributing to the ring constriction and to the correct conclusion of cell division.

References

1 Hall A. Rho GTPases and the control of cell behaviour. Biochem Soc Trans 2005; 33:891-5.
2 Ridley AJ. Rho GTPases and actin dynamics in membrane protrusions and vesicle trafficking. Trends Cell Biol 2006; 16:522-9.
3 Park HO, Bi E. Central roles of small GTPases in the development of cell polarity in yeast and beyond. Microbiol Mol Biol Rev 2007; 71:48-96.
4 Charest PG, Firtel RA. Big roles for small GTPases in the control of directed cell movement. Biochem J 2007; 401:377-90.
5 Irazoqui JE, Gladfelter AS, Lew DJ. Cdc42p, GTP hydrolysis, and the cell's sense of direction. Cell Cycle 2004; 3:861-4.
6 Cerione RA. Cdc42: New roads to travel. Trends Cell Biol 2004; 14:127-32.
7 Etienne-Manneville S. Cdc42—the centre of polarity. J Cell Sci 2004; 117:1291-300.
8 Der CJ, Cox AD. Isoprenoid modification and plasma membrane association: Critical factors for ras oncogenicity. Cancer Cells 1991; 3:331-4.
9 Fu HW, Casey PJ. Enzymology and biology of CaaX protein prenylation. Recent Prog Horm Res 1999; 54:315-42.
10 Wheeler AP, Ridley AJ. Why three Rho proteins? RhoA, RhoB, RhoC, and cell motility. Exp Cell Res 2004; 301:43-9.
11 Bourne HR, Sanders DA, McCormick F. The GTPase superfamily: Conserved structure and molecular mechanism. Nature 1991; 349:117-27.
12 Hoffman GR, Cerione RA. Signaling to the Rho GTPases: Networking with the DH domain. FEBS Lett 2002; 513:85-91.
13 Rossman KL, Der CJ, Sondek J. GEF means go: Turning on RHO GTPases with guanine nucleotide-exchange factors. Nat Rev Mol Cell Biol 2005; 6:167-80.
14 Moon SY, Zheng Y. Rho GTPase-activating proteins in cell regulation. Trends Cell Biol 2003; 13:13-22.
15 Dovas A, Couchman JR. RhoGDI: Multiple functions in the regulation of Rho family GTPase activities. Biochem J 2005; 390:1-9.
16 Mitchison JM, Nurse P. Growth in cell length in the fission yeast *Schizosaccharomyces pombe*. J Cell Sci 1985; 75:357-76.
17 Feierbach B, Chang F. Cytokinesis and the contractile ring in fission yeast. Curr Opin Microbiol 2001; 4:713-9.
18 Glotzer M. The molecular requirements for cytokinesis. Science 2005; 307:1735-9.
19 Balasubramanian MK, Bi E, Glotzer M. Comparative analysis of cytokinesis in budding yeast, fission yeast and animal cells. Curr Biol 2004; 14:R806-18.
20 Krapp A, Gulli MP, Simanis V. SIN and the art of splitting the fission yeast cell. Curr Biol 2004; 14:R722-30.

21 Krapp A, Simanis V. Cell division: SIN, cytokinesis and ethanol dependency. Curr Biol 2005; 15:R605-7.

22 Wolfe BA, Gould KL. Split decisions: Coordinating cytokinesis in yeast. Trends Cell Biol 2005; 15:10-8.

23 Ishiguro J, Saitou A, Duran A, Ribas JC. *cps1*$^{+}$, a *Schizosaccharomyces pombe* gene homolog of *Saccharomyces cerevisiae FKS* genes whose mutation confers hypersensitivity to cyclosporin A and papulacandin B. J Bacteriol 1997; 179:7653-62.

24 Liu J, Wang H, McCollum D, Balasubramanian MK. Drc1p/Cps1p, a 1,3-beta-glucan synthase subunit, is essential for division septum assembly in *Schizosaccharomyces pombe*. Genetics 1999; 153:1193-203.

25 Arellano M, Durán A, Perez P. Rho1 GTPase activates the (1-3)β-D-glucan synthase and is involved in *Schizosaccharomyces pombe* morphogenesis. EMBO J 1996; 15:4584-91.

26 Martin-Cuadrado AB, Duenas E, Sipiczki M, Vazquez de Aldana CR, del Rey F. The endo-beta-1,3-glucanase eng1p is required for dissolution of the primary septum during cell separation in *Schizosaccharomyces pombe*. J Cell Sci 2003; 116:1689-98.

27 Dekker N, Speijer D, Grun CH, van den Berg M, de Haan A, Hochstenbach F. Role of the alpha-glucanase Agn1p in fission-yeast cell separation. Mol Biol Cell 2004; 15:3903-14.

28 Martin-Cuadrado AB, Morrell JL, Konomi M, An H, Petit C, Osumi M, Balasubramanian M, Gould KL, Del Rey F, Vazquez de Aldana CR. Role of septins and the exocyst complex in the function of hydrolytic enzymes responsible for fission yeast cell separation. Mol Biol Cell 2005; 16:4867-81.

29 Santos B, Martin-Cuadrado AB, Vazquez de Aldana CR, del Rey F, Perez P. Rho4 GTPase is involved in secretion of glucanases during fission yeast cytokinesis. Eukaryot Cell 2005; 4:1639-45.

30 Miller PJ, Johnson DI. Cdc42p GTPase is involved in controlling polarized growth in *Schizosaccharomyces pombe*. Mol Cell Biol 1994; 14:1075-83.

31 Merla A, Johnson DI. The Cdc42p GTPase is targeted to the site of cell división in the fission yeast *Schizosaccharomyces pombe*. Int J Cell Biol 2000; 79:469-77.

32 Chang EC, Barr M, Wang Y, Jung V, Xu HP, Wigler MH. Cooperative interaction of *S. pombe* proteins required for mating and morphogenesis. Cell 1994; 79:131-41.

33 Coll PM, Trillo Y, Ametzazurra A, Perez P. Gef1p, a new guanine nucleotide exchange factor for Cdc42p, regulates polarity in *Schizosaccharomyces pombe*. Mol Biol Cell 2003; 14:313-23.

34 Marcus S, Polverino A, Chang E, Robbins D, Cobb MH, Wigler M. Shk1, a homolog of the *Saccharomyces cerevisiae* Ste20 and mammalian p65PAK protein kinases, is a component of a Ras/Cdc42 signaling module in the fission yeast *Schizosaccharomyces pombe*. Proc Natl Acad Sci USA 1995; 92:6180-4.

35 Chang E, Bartholomeusz G, Pimental R, Che J, Lai H, Wang L, Yang P, Marcus S. Direct binding and In vivo regulation of the fission yeast p21-activated kinase shk1 by the SH3 domain protein scd2. Mol Cell Biol 1999; 19:8066-74.

36 Hirota K, Tanaka K, Ohta K, Yamamoto M. Gef1p and Scd1p, the Two GDP-GTP exchange factors for Cdc42p, form a ring structure that shrinks during cytokinesis in *Schizosaccharomyces pombe*. Mol Biol Cell 2003; 14:3617-27.

37 Schmidt A, Hall A. Guanine nucleotide exchange factors for Rho GTPases: Turning on the switch. Genes Dev 2002; 16:1587-609.

38 Coll PM, Rincon SA, Izquierdo RA, Perez P. Hob3p, the fission yeast ortholog of human BIN3, localizes Cdc42p to the division site and regulates cytokinesis. EMBO J 2007; 26:1865-77.

39 Routhier EL, Burn TC, Abbaszade I, Summers M, Albright CF, Prendergast GC. Human BIN3 complements the F-actin localization defects caused by loss of Hob3p, the fission yeast homolog of Rvs161p. J Biol Chem 2001; 276:21670-7.

40 Ren G, Vajjhala P, Lee JS, Winsor B, Munn AL. The BAR domain proteins: Molding membranes in fission, fusion, and phagy. Microbiol Mol Biol Rev 2006; 70:37-120.

41 David C, McPherson PS, Mundigl O, De Camilli P. A role of amphiphysin in synaptic vesicle endocytosis suggested by its binding to dynamin in nerve terminals. Proc Natl Acad Sci USA 1996; 93:331-5.

42 Kruchten AE, McNiven MA. Dynamin as a mover and pincher during cell migration and invasion. J Cell Sci 2006; 119:1683-90.

43 Konopka CA, Schleede JB, Skop AR, Bednarek SY. Dynamin and cytokinesis. Traffic 2006; 7:239-47.

44 Yoshida Y, Kinuta M, Abe T, Liang S, Araki K, Cremona O, Di Paolo G, Moriyama Y, Yasuda T, De Camilli P, Takei K. The stimulatory action of amphiphysin on dynamin function is dependent on lipid bilayer curvature. Embo J 2004; 23:3483-91.

45 Habermann B. The BAR-domain family of proteins: A case of bending and binding? EMBO Rep 2004; 5:250-5.

46 Salazar MA, Kwiatkowski AV, Pellegrini L, Cestra G, Butler MH, Rossman KL, Serna DM, Sondek J, Gertler FB, De Camilli P. Tuba, a novel protein containing bin/amphiphysin/Rvs and Dbl homology domains, links dynamin to regulation of the actin cytoskeleton. J Biol Chem 2003; 278:49031-43.

47 Cestra G, Kwiatkowski A, Salazar M, Gertler F, De Camilli P. Tuba, a GEF for CDC42, links dynamin to actin regulatory proteins. Methods Enzymol 2005; 404:537-45.

48 Navarro P, Durrens P, Aigle M. Protein-protein interaction between the *RVS161* and *RVS167* gene products of *Saccharomyces cerevisiae*. Biochim Biophys Acta 1997; 1343:187-92.

49 Huang TY, Renaud-Young M, Young D. Nak1 interacts with Hob1 and Wsp1 to regulate cell growth and polarity in *Schizosaccharomyces pombe*. J Cell Sci 2005; 118:199-210.

50 Routhier EL, Donover PS, Prendergast GC. hob1+, the fission yeast homolog of Bin1, is dispensable for endocytosis or actin organization, but required for the response to starvation or genotoxic stress. Oncogene 2003; 22:637-48.

51 Chitu V, Stanley ER. Pombe Cdc15 homology (PCH) proteins: Coordinators of membrane-cytoskeletal interactions. Trends Cell Biol 2007; 17:145-56.

52 Tsujita K, Suetsugu S, Sasaki N, Furutani M, Oikawa T, Takenawa T. Coordination between the actin cytoskeleton and membrane deformation by a novel membrane tubulation domain of PCH proteins is involved in endocytosis. J Cell Biol 2006; 172:269-79.

53 Aspenstrom P, Fransson A, Richnau N. Pombe Cdc15 homology proteins: Regulators of membrane dynamics and the actin cytoskeleton. Trends Biochem Sci 2006; 31:670-9.

54 Carnahan RH, Gould KL. The PCH family protein, Cdc15p, recruits two F-actin nucleation pathways to coordinate cytokinetic actin ring formation in *Schizosaccharomyces pombe*. J Cell Biol 2003; 162:851-62.

55 Richman TJ, Sawyer MM, Johnson DI. *Saccharomyces cerevisiae* Cdc42p localizes to cellular membranes and clusters at sites of polarized growth. Eukaryot Cell 2002; 1:458-68.

56 Manser E, Loo TH, Koh CG, Zhao ZS, Chen XQ, Tan L, Tan I, Leung T, Lim L. PAK kinases are directly coupled to the PIX family of nucleotide exchange factors. Mol Cell 1998; 1:183-92.

57 Osmani N, Vitale N, Borg JP, Etienne-Manneville S. Scrib controls cdc42 localization and activity to promote cell polarization during astrocyte migration. Curr Biol 2006; 16:2395-405.

58 Gladfelter AS, Bose I, Zyla TR, Bardes ES, Lew DJ. Septin ring assembly involves cycles of GTP loading and hydrolysis by Cdc42p. J Cell Biol 2002; 156:315-26.

59 Pelham RJ, Chang F. Actin dynamics in the contractile ring during cytokinesis in fission yeast. Nature 2002; 419:82-6.

60 Morrell JL, Morphew M, Gould KL. A mutant of *Arp2p* causes partial disassembly of the *Arp2/3* complex and loss of cortical actin function in fission yeast. Mol Biol Cell 1999; 10:4201-15.

61 Lechler T, Jonsdottir GA, Klee SK, Pellman D, Li R. A two-tiered mechanism by which Cdc42 controls the localization and activation of an Arp2/3-activating motor complex in yeast. J Cell Biol 2001; 155:261-70.

62 Pollard TD, Borisy GG. Cellular motility driven by assembly and disassembly of actin filaments. Cell 2003; 112:453-65.

63 Lee WL, Bezanilla M, Pollard TD. Fission yeast myosin-I, Myo1p, stimulates actin assembly by Arp2/3 complex and shares functions with WASp. J Cell Biol 2000; 151:789-800.

64 Albertson R, Riggs B, Sullivan W. Membrane traffic: A driving force in cytokinesis. Trends Cell Biol 2005; 15:92-101.

65 Breton AM, Schaeffer J, Aigle M. The yeast Rvs161 and Rvs167 proteins are involved in secretory vesicles targeting the plasma membrane and in cell integrity. Yeast 2001; 18:1053-68.