Nuclear sequestration of the exchange factor Cdc24 by Far1 regulates cell polarity during yeast mating

Yukiko Shimada*, Marie-Pierre Gulli* and Matthias Peter*†

*Swiss Institute for Experimental Cancer Research (ISREC), Chemin des Boveresses 155, 1066 Epalinges/VD, Switzerland
†e-mail: matthias.peter@isrec.unil.ch

Cytoskeletal rearrangements during the cell cycle and in response to signals are regulated by small Rho-type GTPases, but it is not known how these GTPases are activated in a spatial and temporal manner. Here we show that Cdc24, the guanine-nucleotide exchange factor for the yeast GTPase Cdc42, is sequestered in the cell nucleus by Far1. Export of Cdc24 to a site of cell polarization is mediated by two mechanisms. At bud emergence, activation of the G1 cyclin-dependent kinase Cdc28–Cln triggers degradation of Far1 and, as a result, relocation of Cdc24 to the cytoplasm. Cells overexpressing a non-degradable Far1 were unable to polarize their actin cytoskeleton because they failed to relocate Cdc24 to the incipient bud site. In contrast, in response to mating pheromones, the Far1–Cdc24 complex is exported from the nucleus by Msn5. This mechanism ensures that Cdc24 is targeted to the site of receptor-associated heterotrimeric G-protein activation at the plasma membrane, thereby allowing polarization of the actin cytoskeleton along the morphogenetic gradient of pheromone. Either degradation of Far1 or its nuclear export by Msn5 was sufficient for cell growth, suggesting that the two mechanisms are redundant for cell viability. Taken together, our results indicate that Far1 functions as a nuclear anchor for Cdc24. This sequestration regulates cell polarity in response to pheromones by restricting activation of Cdc42 to the site of pheromone receptor activation.

The actin cytoskeleton of eukaryotic cells is a highly dynamic structure which is required for maintenance of cell shape and polarity, cell motility, chemotaxis, intracellular trafficking, cytokinesis and phagocytosis[1]. Its rapid assembly and disassembly is controlled by intracellular and extracellular signals and involves a complex network of signalling pathways and actin-binding proteins. Members of the Rho family of GTPases, including Rho, Rac and Cdc42, have recently emerged as key regulators of the establishment of cell polarity and of the organization and dynamics of the actin cytoskeleton. It is thought that local activation of these Rho GTPases regulates cell polarity, but little is known about their spatial and temporal regulation.

In the budding yeast *Saccharomyces cerevisiae*, activation of Cdc42 by its guanine-nucleotide exchange factor Cdc24 is required to orientate the actin cytoskeleton towards the incipient bud site or towards the pheromone-secreting partner during mating. In the absence of Cdc42 function, cells fail to grow in a polarized manner and instead increase in size isotropically[2]. Activated Cdc42 interacts with several effector proteins, which in turn organize the actin cytoskeleton[3,4]. Cytoskeletal polarization also requires Bem1, a protein with two Src homology 3 (SH3) domains, which is thought to function as an adaptor for Cdc42 and Cdc24 (refs 5, 6), and co-immunoprecipitates with actin[7]. At bud emergence, Cdc24 is targeted to the incipient bud site on the cell cortex[8,9], possibly by interacting with the small GTPase Rsr1/Bud1 (ref. 10).

In contrast, during mating cells ignore the positional information at the bud site and instead polarize the actin cytoskeleton towards the site of the highest pheromone concentration[11]. Mating pheromones activate a mitogen-activated protein kinase (MAP kinase) signal transduction pathway, culminating in arrest of the cell cycle, changes in gene expression and altered cell polarity and morphology[12]. These responses are initiated by a cell-surface receptor coupled to a heterotrimeric GTP-binding protein (G protein). Activation of the pheromone receptor triggers dissociation of the heterotrimeric G protein into Gα and Gβγ which, in turn, signal to downstream effectors to induce cellular responses[13]. Far1 and Cdc24 are necessary for cell polarization towards the mating partner. Alleles of *FAR1* (*far1-s*) and *CDC24* (*cdc24-m*) have been identified whose sole defect is a failure to locate the mating partner[14,15]. Far1 binds Gβγ and the polarity-establishment proteins Cdc24, Bem1 and Cdc42, suggesting that Far1 links Gβγ to the polarity-establishment proteins during mating. Supporting this idea, Far1-s mutant proteins fail to interact with either Gβγ or the polarity-establishment proteins and, likewise, Cdc24-m proteins fail to bind Far1 (refs 8, 16). On the basis of these results, a model has been proposed to explain how yeast cells orientate cell polarity in a morphogenetic gradient. It is proposed that the presence of pheromones activates the receptors, leading to local formation of Gβγ at the plasma membrane. Far1 is recruited to the site by binding to Gβγ, thereby targeting the polarity-establishment proteins Cdc24, Bem1 and Cdc42 to the site of receptor activation[8,16]. As a result, Cdc42 becomes locally activated, which triggers polymerization of the actin cytoskeleton towards the mating partner. Consistent with this model, Cdc24 assembles at the incipient bud site instead of the mating site in cells that fail to form the Far1–Cdc24 complex[14,15]. Taken together, these results suggest that Far1 is necessary for correct targeting of Cdc24 to Gβγ, but it may also prevent use of the incipient bud site in response to pheromones.

Bud growth and morphogenesis during different phases of the cell cycle are regulated by cyclin-dependent kinases (CDKs). Bud emergence and polarized growth in G1 is triggered by the activated CDK–cyclin complexes Cdc28–Cln1 and Cdc28–Cln2, whereas activation of Cdc28–Clb kinases reverses the polar growth pattern to uniform bud growth in G2 (ref. 17). The substrates that regulate these cytoskeletal transitions are not known, however. At bud emergence, Cdc28–Cln antagonizes Far1 by at least two mechanisms. First, phosphorylation of Far1 by Cdc28–Cln is required to initiate its ubiquitin-dependent degradation[18,19]. Phosphorylated Far1 binds to the SCF^{Cdc4} complex, which ubiquitinates Far1 and targets it to the 26S proteasome. Second, activation of the Cdc28–Cln kinase represses *FAR1* transcription, which is induced at the end of mitosis and maintained throughout the G1 phase[20]. However, neither down-regulation of *FAR1* mRNA nor its ubiquitin-dependent degradation explains the dependence of bud emergence on Cdc28–Cln. Far1 is also regulated in a cell-type-specific manner. In diploid cells, expression of *FAR1* mRNA is abolished, presumably by the a/α mating fac-

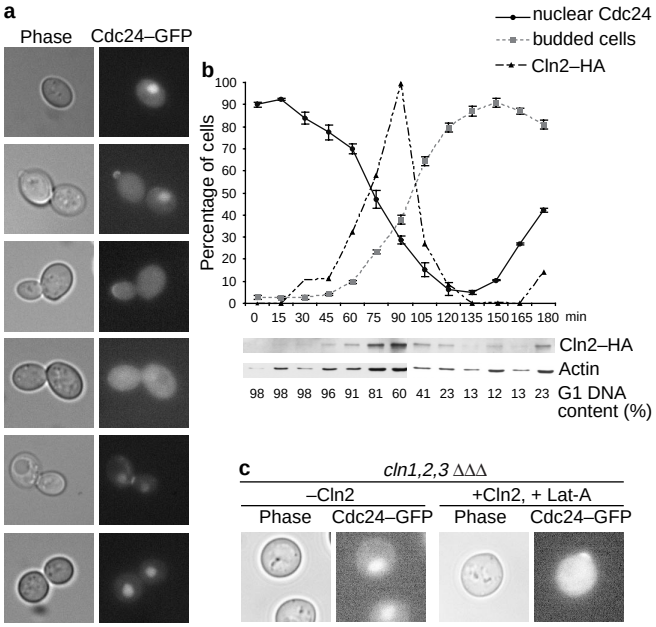

Figure 1 **Cell-cycle-dependent localization of Cdc24.** **a**, The localization of Cdc24–GFP (right column) was analysed by fluorescence microscopy at different stages of the cell cycle. The cell-cycle stage was determined by bud morphology viewed in phase contrast (left column). Note that Cdc24 is nuclear in the G1 phase of the cell cycle. **b**, Redistribution of Cdc24 to the cytoplasm occurs at bud emergence and coincides with expression of Cln2. Small G1 cells were collected by elutriation and monitored as they synchronously progressed through bud emergence. At every time point the percentage of cells with nuclear Cdc24 was determined by GFP staining. The budding index was determined microscopically; at least 300 cells were counted at each time point. An aliquot of cells was lysed and the proteins analysed by immunoblotting for the presence of Cln2–HA (upper gel) and actin (lower gel). DNA replication was followed by FACS analysis and is represented as percentage cells with a G1 DNA content. **c**, Expression of Cln2 is sufficient to trigger relocation of Cdc24. Cells deleted for the G1 cyclin genes *CLN1*, *CLN2* and *CLN3* but expressing Cln2 from the regulatable *MET* promoter (YMG258) (right two panels) were transformed with a plasmid expressing Cdc24–GFP. Cells were arrested by repression of the *MET* promoter (left two panels); activation of Cln2 triggered relocation of Cdc24 from the nucleus into the cytoplasm even in the presence of Lat-A to prevent bud emergence.

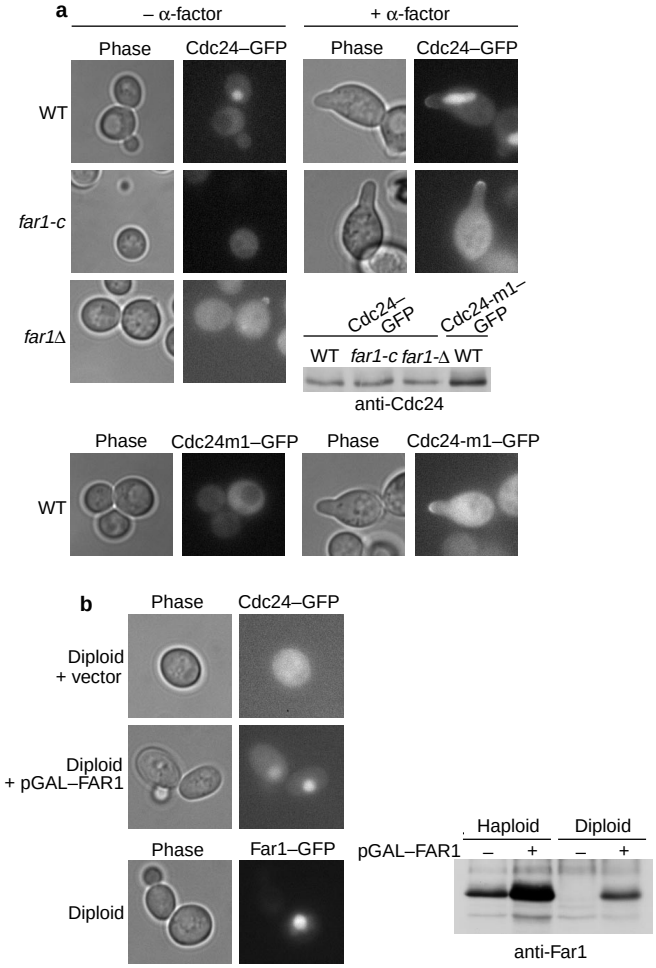

Figure 2 **Nuclear localization of Cdc24 depends on its ability to bind Far1.** **a**, The localization of Cdc24–GFP (top three rows) or Cdc24-m1–GFP (bottom row) was analysed by GFP fluorescence microscopy in wild-type (WT; K699), *far1-c* (YS68) or *far1*Δ (YMP1054) cells as indicated. Where indicated, cells were exposed to α-factor for 2 h (right panels). Left column of each pair, phase contrast; right column, GFP fluorescence. The levels of Cdc24–GFP or Cdc24-m1–GFP in the different strains were determined by immunoblotting with anti-Cdc24 antibodies (see gel). Only the relevant part of the gel is shown. **b**, The localization of Cdc24–GFP was analysed in **a**/α diploid cells transformed with an empty vector (top row) or a plasmid expressing Far1 from the *GAL* promoter (second row). For control, a diploid strain was also transformed with a plasmid expressing Far1–GFP from the inducible *GAL* promoter (third row). Left column, phase contrast; right column, GFP fluorescence. The expression of Far1 was also monitored by immunoblotting with anti-Far1 antibodies (right); + indicates addition of galactose to induce expression of Far1 from the *GAL* promoter.

tor heterodimer[21]. In haploid cells, the expression of *FAR1* mRNA is strongly induced in response to pheromones by the transcription factor Ste12, leading to accumulation of Far1 during mating.

Finally, Far1 is regulated at the level of subcellular localization. In the absence of α-factor, Far1 is predominantly nuclear, but partially relocates to the cytoplasm in response to pheromones[16,22]. Nuclear localization is accomplished by a bipartite nuclear localization signal (NLS) in the amino terminus of Far1 (ref. 22). In response to pheromones, Far1 is exported to the cytoplasm by the exportin Msn5, which interacts with Far1 in a Gsp1-dependent manner (where Gsp1 is the yeast homologue of the Ran GTPase of other systems). Cells expressing a Far1 mutant (Far1-ΔNES) that is unable to interact with Msn5 exhibit a bilateral mating defect, presumably because they are unable to orientate growth towards the mating partner[22]. Thus, nuclear export of Far1 by Msn5 is important for efficient mating.

We have investigated the roles of Far1 and Cdc24 in establishing cell polarity during budding and mating. We found that Cdc24 was localized in the nucleus of haploid G1 cells, but was rapidly redistributed to the cytoplasm at bud emergence. Interestingly, the interaction of Cdc24 with Far1 was required for its nuclear localization[9], suggesting that Far1 regulates Cdc24 *in vivo*. Relocation of Cdc24 at bud emergence was triggered by activation of the Cdc28–Cln kinase, which initiates ubiquitin-dependent degradation of Far1. In contrast, Msn5 exported the Cdc24–Far1 complex in response to pheromones. This mechanism ensures that Far1 targets Cdc24 to the site of activated Gβγ, enabling polarization of the actin cytoskeleton towards the mating partner. Thus, by regulating the subcellular localization of Cdc24, Far1 has an important role in the local activation of Cdc42 during vegetative growth and in response to extracellular signals.

Results

Cdc24 is nuclear in the G1 phase of the cell cycle. To examine the localization of Cdc24 through the cell cycle we analysed *cdc24*Δ cells expressing a fully functional Cdc24 fused to green fluorescent pro-

tein (Cdc24–GFP; Fig. 1a). Consistent with earlier results[8,9], Cdc24 was found at the presumptive bud site and in a crescent at the tip of small-budded cells (Fig. 1a). Bud-tip staining was weak or undetectable in large-budded cells, suggesting that Cdc24 may be released during the switch from polar to isotropic bud growth[23]. In addition, Cdc24 was detected at the mother–bud neck at the end of mitosis, supporting a possible role for Cdc24 during cytokinesis. Of greatest importance, however, Cdc24 was nuclear in G1 cells; nuclear Cdc24 abruptly disappeared at the time of bud emergence, after which Cdc24 was predominantly cytoplasmic[9]. Cdc24 reaccumulated in the nucleus at later stages of cytokinesis (Fig. 1a), at a time that coincides with inactivation of Cdc28–Clb kinase. Taken together, these results suggest that Cdc24 is nuclear in the G1 phase of the cell cycle, but rapidly relocates to the site of polarization at the time of bud emergence.

Activation of Cdc28–Cln kinase or the mating pheromone pathway induces nuclear export of Cdc24. To determine the timing of Cdc24 relocation with respect to activation of the Cdc28–Cln2 kinase, we elutriated wild-type cells (YMT263) expressing Cdc24–GFP and monitored nuclear localization of Cdc24, bud emergence and expression of haemagglutinin-tagged Cln2 (Cln2-HA) (Fig. 1b) as cells synchronously progressed through the cell cycle. Clearly, Cdc24 was nuclear in small G1 cells, but was redistributed to the cytoplasm immediately before bud emergence, concomitant with expression of Cln2. Cdc24 remained predominantly cytoplasmic after bud emergence, even after the disappearance of Cln2, demonstrating that the continued presence of Cln2 was not necessary to maintain Cdc24 in the cytoplasm. Cdc24 was nuclear in cells arrested in G1 by depletion of the G1 cyclins (Fig. 1c), suggesting that activation of Cdc28–Cln was required for relocation of Cdc24. Indeed, expression of Cln2 in these cells triggered rapid relocation of Cdc24 to the incipient bud site, even when actin polarization and resulting bud emergence were prevented by the addition of latrunculin A (Lat-A). Taken together, these results indicate that activation of Cdc28–Cln2 kinase is necessary and sufficient to trigger export of Cdc24 at bud emergence.

Pheromones induced relocation of Cdc24 from the nucleus to the shmoo site (the site of cell elongation during mating) shortly before polarization of the actin cytoskeleton and hence to the tips of mating projections (Fig. 2a). Cdc24 was, however, present in the nucleus, as judged by specific staining, even after extended exposure of cells to pheromones. This suggests that, in contrast to the situation at bud emergence, Cdc24 is not quantitatively exported in response to α-factor. These results indicate that nuclear export of Cdc24 may be regulated by distinct mechanisms in the two situations (see below).

Nuclear accumulation of Cdc24 requires its ability to bind Far1. We found that the mutant Cdc24-m1 and m3 proteins[15] did not accumulate in the nucleus of G1 cells (Fig. 2a, and data not shown)[9] although they were correctly localized to sites of polarized growth during budding and shmoo formation. Because Cdc24-m mutants fail to interact with Far1 (refs 8, 16), these results suggested that Far1 may be required for nuclear localization of Cdc24. In support of this idea, no nuclear staining of Cdc24 was detected in *far1-c* cells, which express a truncated Far1 unable to interact with Cdc24 (ref. 16). In all these situations, the protein levels of Cdc24–GFP remained constant (Fig. 2, gel), demonstrating that this relocation is not caused by degradation of Cdc24. Interestingly, Cdc24 was cytoplasmic in **a**/α diploid cells[9], which do not express *FAR1* (Fig. 2b). Nuclear Cdc24 staining was, however, restored upon expression of Far1 from the *GAL* promoter, indicating that Far1 is sufficient to retain Cdc24 in the nucleus. Nevertheless, Cdc24 was nuclear only in the G1 phase of the cell cycle, demonstrating that the cell-cycle dependence of Cdc24 localization was maintained. *cln*ΔΔΔ cells harbouring the *far1-c* allele were unable to polarize their actin cytoskeleton, although Cdc24 was distributed throughout the cytoplasm, indicating that export of Cdc24 does not seem to be the essential function of Cdc28–Cln kinase with respect to cytoskeletal polarization and bud emergence (M.-P.G., Y.S. and M.P., unpublished observations). Thus, whereas activation of Cdc28–Cln is required for export of Cdc24, cytoplasmic Cdc24 alone is not sufficient for production of Cdc42–GTP in the absence of Cdc28–Cln activity. Taken

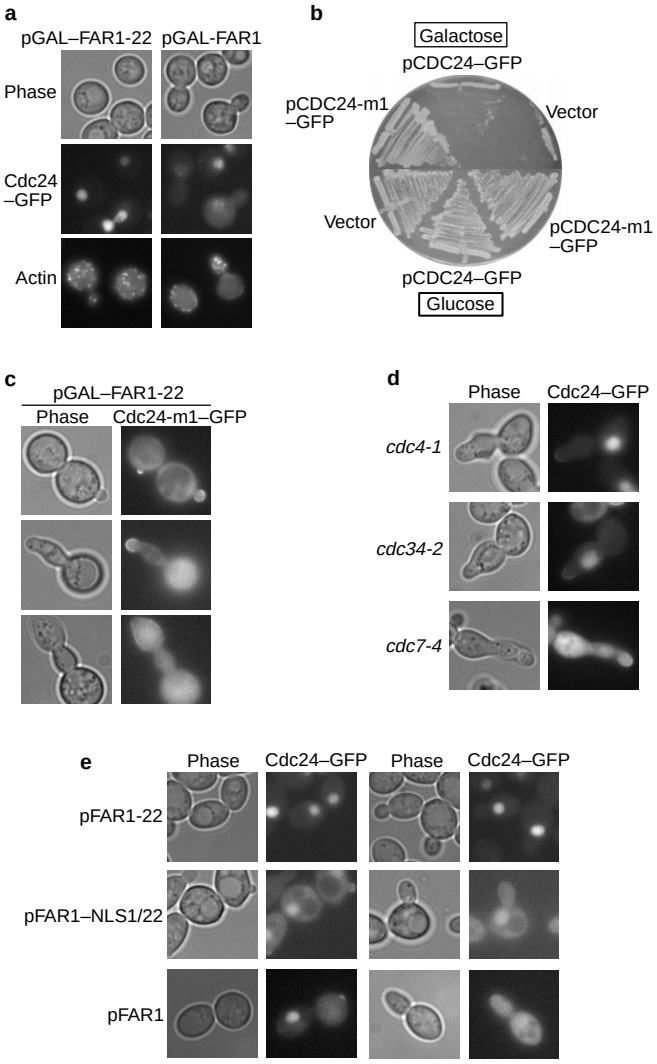

Figure 3 **Relocation of Cdc24–GFP at bud emergence requires degradation of Far1. a–c**, Cells expressing stable Far1-22 (YMP126) or wild-type Far1 (YMP128) from the inducible *GAL* promoter were transformed with an empty control vector or plasmids encoding wild-type Cdc24–GFP or cytoplasmic Cdc24-m1–GFP. The cell morphology and localization of Cdc24–GFP (**a**) or Cdc24-m1–GFP (**c**) was analysed by phase-contrast (phase) and GFP microscopy (GFP) 6 h after addition of galactose. The actin cytoskeleton of the cells was visualized with rhodamine–phalloidin staining (**a**, bottom row). The three rows in **c** represent three different cells at the same time point (6 h). Cells were also plated on medium containing glucose (*GAL* promoter off, lower half of plate) or galactose (*GAL* promoter on, upper half of plate), and photographed after 3 days at 30 °C (**b**). Note that expression of cytoplasmic Cdc24-m1 partially restores viability of cells overexpressing non-degradable Far1-22. **d**, Temperature-sensitive *cdc4-1* (YMT668) or *cdc34-2* (YMT670) cells unable to degrade Far1 or, for control, *cdc7-2* cells (YMP586) were arrested at 37°C and the localization of Cdc24–GFP was examined by GFP microscopy. Note that nuclear staining of Cdc24 persists after bud emergence in the absence of Far1 degradation. **e**, The localization of Cdc24–GFP was examined in wild-type cells expressing non-degradable Far1-22 (top row), Far1-22/nls1 with a mutated nuclear localization signal (middle row) or wild-type Far1 (bottom row) from their native promoter. Note that nuclear localization of Cdc24 is detectable throughout the cell cycle in the presence of stable Far1.

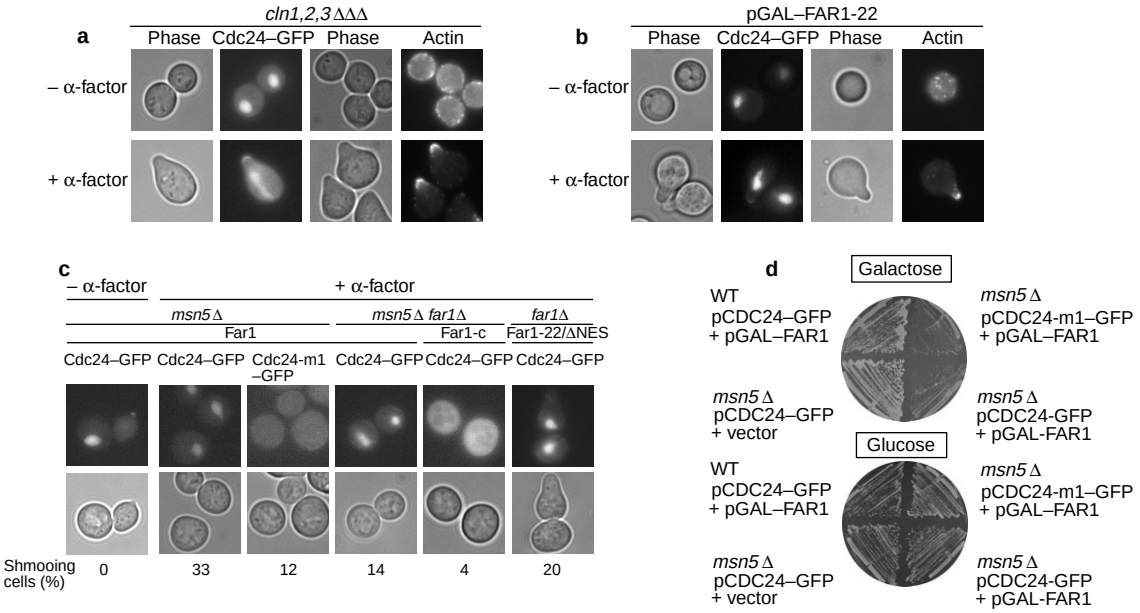

Figure 4 **Export of Far1 by Msn5/Ste21 is required to relocate Cdc24 in response to pheromones.** **a**, **b**, The localization of Cdc24–GFP was determined by GFP microscopy in cells arrested by depletion of the G1 cyclins (YMG258; **a**) or overexpression of non-degradable Far1-22 (YMP126; **b**). Where indicated, cells were exposed to α-factor for 2 h. The morphology of the cells was examined by phase-contrast microscopy (phase); actin polarization was analysed after staining with rhodamine–phalloidin (actin). Note that the G1 cyclins and degradation of Far1 are not required to polarize the actin cytoskeleton in response to pheromones. **c**, The localization of Cdc24–GFP was examined by GFP microscopy in *msn5*Δ (PAY20), *msn5*Δ *far1*Δ (YMP1067), or *far1*Δ (YMP1054) cells expressing Far1, Far1-c, or Far1-22/ΔNES from the inducible *GAL* promoter. Where indicated, cells were exposed to α-factor for 3 h. The percentage of cells with mating projections (shmooing cells) was determined microscopically and is shown below. Note that Cdc24 is nuclear in *msn5*Δ cells exposed to pheromones, but cytoplasmic Cdc24 is unable to localize to the plasma membrane and is thus not sufficient to rescue the shmoo defect of *msn5*Δ cells. **d**, Wild-type (K699) or *msn5*Δ (PAY20) cells harbouring Cdc24–GFP (pYS47) or cytoplasmic Cdc24-m1–GFP (pYS51) were transformed with an empty control plasmid (vector) or a plasmid expressing Far1 from the inducible *GAL* promoter (ACB435). The cells were grown on selective medium containing galactose (upper plate) or glucose (lower plate) and grown for 3 days at 30 °C.

together, these results suggest that activation of the Cdc28–Cln kinase overcomes nuclear sequestration of Cdc24 by Far1.

Export of Cdc24 at bud emergence requires degradation of Far1. Far1 is rapidly degraded at bud emergence by a ubiquitin-dependent mechanism, and ubiquitination requires phosphorylation of Far1 by the Cdc28–Cln kinase[18,19]. Thus, Cdc28–Cln2 kinase may release Cdc24 by inducing degradation of its binding partner Far1. In support of this model, overexpression of a non-phosphorylated, stable form of Far1 (Far1-22) is highly toxic and leads to accumulation of large unbudded cells with an unpolarized actin cytoskeleton[19], a phenotype reminiscent of loss of Cdc24 function. Indeed, Cdc24 was efficiently retained in the nucleus of cells overexpressing Far1-22 (Fig. 3a), but not in that of cells overexpressing wild-type Far1. Expression of cytoplasmic Cdc24-m1 was able to partially restore growth of these cells (Fig. 3b, c), suggesting that non-degradable Far1 prevents export of Cdc24 to the cytoplasm *in vivo*. Most cells exhibited an aberrant morphology, however, probably because Far1 still interferes with the function of the G1 cyclins. Furthermore, Cdc24 was cytoplasmic in *cdc7-4* cells, but nuclear in *cdc34-2* or *cdc4-1* cells (Fig. 3d), which are defective in ubiquitination and degradation of Far1 (ref. 19) and arrest with long buds before S phase. Finally, nuclear staining of Cdc24 was sustained throughout the cell cycle in cells expressing low levels of stable Far1 (Fig. 3e), confirming that the loss of nuclear Cdc24 in cells after bud emergence results from degradation of Far1. Nuclear localization of Cdc24 is dependent on the ability of Far1 to localize to the nucleus, as mutating one of the nuclear localization signals (nls1)[22] of Far1 led to partial accumulation of Cdc24 in the cytoplasm. Taken together, these results demonstrate that export of Cdc24 at bud emergence is triggered by degradation of Far1, which is induced by activation of the Cdc28–Cln kinase.

Nuclear export of Far1 by Msn5 triggers relocation of Cdc24 in response to pheromones. To determine whether degradation of Far1 is involved in the export of Cdc24 in response to pheromones, we next examined the localization of Cdc24 in *cln*ΔΔΔ cells treated with α-factor (Fig. 4a). Clearly, the Cln-depleted cells were able to polarize the actin cytoskeleton and localize Cdc24 to the shmoo tip, suggesting that, in contrast to bud emergence, export of Cdc24 in response to pheromones is independent of the G1 cyclins. To corroborate these results, we arrested cells by overexpression of non-degradable Far1-22 in the presence or absence of α-factor. Interestingly, these cells were able to form mating projections when treated with α-factor (Fig. 4b) and, as in wild-type cells, Cdc24 was found at the shmoo tip and in the nucleus. Likewise, *far1*Δ cells expressing Far1-22 from the endogenous promoter were able to relocate Cdc24 efficiently when treated with α-factor. Finally, *cdc34-2* cells shifted to the restrictive temperature were able to form mating projections (data not shown). We conclude that export of Cdc24 in response to pheromones does not require degradation of Far1. We found previously that Far1 is exported by Msn5 in response to α-factor[22]. Interestingly, Cdc24 remained nuclear in *msn5*Δ cells (Fig. 4c), suggesting that Msn5 exports the Far1–Cdc24 complex in response to pheromones. In contrast, export of Cdc24 is not dependent on Msn5 in vegetatively growing cells, suggesting that Msn5 does not function as an export factor for Cdc24 and is not required to degrade Far1 at bud emergence. In addition, Cdc24 remained predominantly nuclear in *far1*Δ cells expressing Far1-22/ΔNES (Fig. 4c), which is unable to interact with Msn5 (ref. 22), suggesting that nuclear export of Cdc24 in response to pheromones requires Far1. Taken together, these results indicate that during mating a fraction of Cdc24 is transported into the cytoplasm bound to Far1, which is exported by Msn5 in response to pheromones.

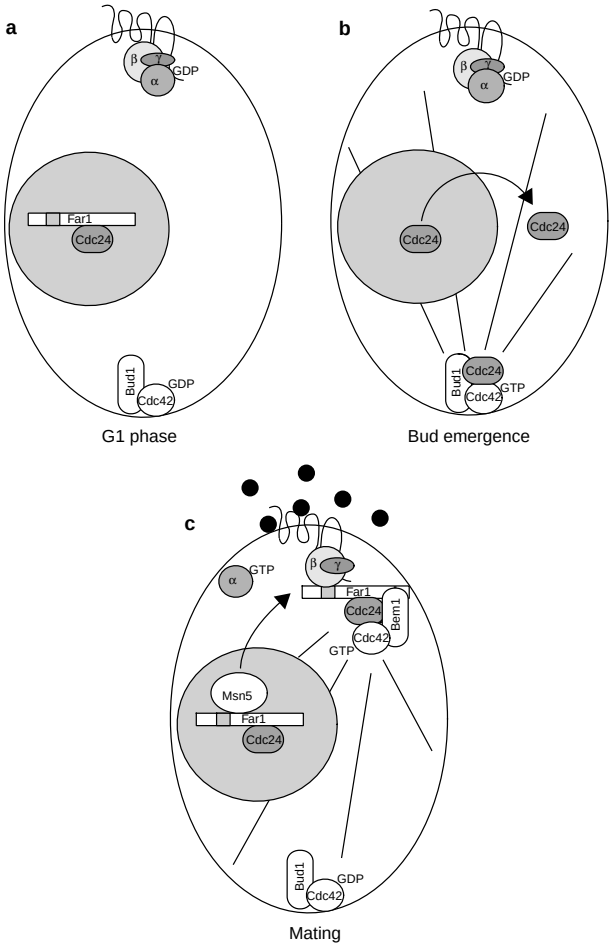

Figure 5 **Model for the localization of Cdc24 during budding and mating.** **a**, In the G1 phase of the cell cycle, Far1 localizes Cdc24 in the nucleus. **b**, Activation of the Cdc28–Cln kinase triggers degradation of Far1 in the nucleus, allowing rapid export of Cdc24 to the cytoplasm. Cytoplasmic Cdc24 is then recruited to the incipient bud site, possibly marked by Rsr1/Bud1-GTP. **c**, In the presence of pheromones, the Far1–Cdc24 complex is exported by Msn5 into the cytoplasm, where Far1 targets Cdc24 to Gβγ at the site of activated receptor. As a result, the actin cytoskeleton polarizes towards the position of the mating partner, signalled by the morphogenetic pheromone gradient.

Table 1 Strain list

Strain name	Relevant genotype	Background	Source
YMG315	*Mat* **a** *cdc24Δ-1:: HIS5* pGN6	S288C	This study
YMT263	*Mat* **a** *cln2::CLN2-HA3::LEU2*	W303	M. Tyers
YMG258	*Mat* **a** *cln1::HisG, cln2::Δ, cln3::leu2::HisG, trp1::MET-CLN2::TRP1*	W303	This study
K699	*Mat* **a**	W303	K. Nasmyth
YS68	*Mat* **a** *far1-c::URA3*	W303	This study
YMP1054	*Mat* **a** *far1::LEU2*	W303	Ref. 19
YMP562	*Mat* **a**/α	W303	Ref. 19
YMP126	*Mat* **a** *trp1::GAL-FAR1-22::TRP1 bar1-1*	W303	Ref. 19
YMP128	*Mat* **a** *trp1::GAL-FAR1::TRP1 bar1-1*	W303	Ref. 19
YMT668	*Mat* **a** *cdc4-1*	W303	M. Tyers
YMT670	*Mat* **a** *cdc34-2*	W303	M. Tyers
YMP586	*Mat* **a** *cdc7-4*	W303	R. Deshaies
PAY20	*Mat* **a** *msn5::HIS3*	W303	Ref. 22
YMP1067	*Mat* **a** *msn5::HIS3, far1::LEU2*	W303	Ref. 22

*msn5*Δ cells exhibit a strongly reduced ability to form mating projections[22], raising the possibility that this defect may be caused by a failure to export Cdc24. However, *msn5*Δ *far1-c* cells still failed to form mating projections, although, as expected, Cdc24 was cytoplasmic in these cells (Fig. 4c). Similarly, *msn5*Δ cells expressing cytoplasmic Cdc24-m1 were unable to form mating projections. In both situations, Cdc24 was distributed uniformly throughout the cytoplasm, suggesting that these cells fail to recruit Cdc24 to the plasma membrane. Thus, cytoplasmic Cdc24 is not sufficient to suppress the shmoo defect of *msn5*Δ cells, suggesting that besides Far1, additional proteins involved in shmoo formation must be exported by Msn5.

Degradation of Far1 and Msn5-dependent nuclear export are redundant for viability. Why are cells expressing stable Far1 at physiological levels viable? Whereas nuclear export of Far1 by Msn5 is stimulated by activation of the MAP kinase Fus3, low levels of Far1 are exported constitutively[22]. Thus, export of Far1 by Msn5 may partially compensate for a lack of degradation of Far1 in the absence of pheromones. Indeed, *msn5*Δ cells were unable to grow with a plasmid expressing Far1-22 (data not shown). Similarly, overexpression of wild-type Far1 interfered with growth of *msn5*Δ cells (Fig. 4d), and the cells accumulated as unpolarized cells with nuclear Cdc24 (Fig. 4c). These results suggest that cellular polarization of haploid cells requires export of Cdc24, triggered either by Cln-dependent degradation of Far1 or by nuclear export of Far1 by Msn5. By analogy with the response of *msn5*Δ cells to pheromones, however, expression of cytoplasmic Cdc24-m was unable to restore growth of the haploid cells, suggesting that the polarization defect is not solely due to a failure to export Cdc24. Cdc24-m was uniformly distributed throughout the cytoplasm in these cells (data not shown), indicating that Msn5 may export an additional factor(s) involved in recruitment of Cdc24 to the membrane. Because Far1 antagonizes the activity of Cdc28–Cln in the nucleus[22,24] and Cdc28–Cln is needed for membrane recruitment of Cdc24 at bud emergence (M.-P.G., Y.S. and M.P., unpublished observations), we speculate that this factor may be Cdc28–Cln.

Discussion

Local activation of Cdc42 is thought to be responsible for directed cell polarization, but it is not known how Cdc42 is activated at spatially restricted sites. In yeast, cell polarity is manifested at bud emergence during vegetative growth and projection formation during mating[3]. During vegetative growth, the site of bud formation at the cell cortex (landmark) is defined in a genetically programmed manner[25], whereas during mating, cells polarize towards their mating partner by responding to a morphogenetic pheromone gradient[26,27]. Here we show that the Cdc42 guanine-nucleotide exchange factor, Cdc24, is sequestered in the nucleus by the adaptor protein Far1 (ref. 9), but is rapidly exported to the cytoplasm after degradation of Far1 at bud emergence or by Msn5-dependent export of Far1 in response to pheromones. Our results suggest that nuclear sequestration of Cdc24 by Far1 ensures efficient targeting of Cdc24 to the site marked by Gβγ where the pheromone signal is received.

It is clear that nuclear accumulation of Cdc24 is not essential for viability. Cells with cytoplasmic Cdc24 are able to grow efficiently and exhibit no obvious defects in bud growth, morphogenesis or bud-site selection[15]. What, therefore, is the function of nuclear Cdc24? Several lines of evidence suggest that nuclear sequestration of Cdc24 by Far1 may be crucial for establishing cell polarization along a pheromone gradient during yeast mating (Fig. 5). First, *FAR1* is a haploid-specific gene whose transcription is repressed in **a**/α diploid cells, which are the product of the mating reaction[21]. Second, formation of a Far1–Cdc24 complex is required to polarize growth along a pheromone gradient. Cells expressing either mutant

Table 2 Plasmids and oligonucleotides

Plasmid	Relevant characteristics	Source
pGN6	CDC24 URA3 CEN	This study
pYS24	CYC1 CDC24–GFP LEU2 CEN	This study
pYS37	CDC24–GFP LEU2 CEN	This study
pYS65	CYC1 CDC24–GFP URA3 integrative	This study
pYS49	CYC1 CDC24–GFP TRP1 CEN	This study
pYS47	CYC1 CDC24–GFP URA3 CEN	This study
pYS51	CYC1 CDC24m1–GFP URA3 CEN	This study
pYS52	CYC1 CDC24m3–GFP URA3 CEN	This study
ACB435	GAL FAR1 LEU2 CEN	Ref. 16
pTP62	GAL FAR1 URA3 CEN	Ref. 19
pTP68	GAL FAR1–GFP URA3 CEN	Ref. 19
pNP162	ADH FAR1 URA3 CEN	This study
pNP160	ADH FAR1-22 URA3 CEN	This study
pBM18	FAR1 URA3 CEN	Ref. 22
pBM19	FAR1-22 URA3 CEN	Ref. 22
pBM29	FAR1-NLS1/22 URA3 CEN	Ref. 22
pBM125	GAL FAR1 22/ΔNES URA3 CEN	This study
pTP77	GAL FAR1-C URA3 CEN	This study
Oligonucleotide	**Sequence 5′ to 3′**	
oTP463	CCAAGGATCCAGATCATGGCGATCCAAACC	
oTP516	CATGATCTGGATCCTTGGATTAGCAGACAGGTCTTGATAGGG	
oTP517	CGCACCGCGGCCGCCGGGCTGATCACTTTATCGTGC	
oTP600	TAGCTCGAGGATACAGACGAATGTTCAAGAATTTCTC	

Far1 unable to bind Cdc24 or mutant Cdc24 unable to interact with Far1 fail to polarize the cytoskeleton towards the mating partner. Finally, *msn5*Δ cells, which are unable to export the Far1–Cdc24 complex, fail to polarize their actin cytoskeleton in response to pheromones[22]. In the G1 phase of the haploid cell cycle, Far1 localizes Cdc24 to the nucleus (Fig. 5a). In the absence of Far1, Cdc24 is predominantly cytoplasmic and is able to recognize the landmark at the incipient bud site (Fig. 5b). The biochemical nature of the budding landmark is poorly understood, but available evidence suggests a crucial role for the small GTPase Rsr1/Bud1 (refs 3, 25). Rsr1/Bud1 was originally identified as a multicopy suppressor of *cdc24-5* cells[28] and was subsequently shown to bind Cdc24 in a GTP-dependent manner[29]. In addition, Rsr1/Bud1 is necessary for shmoo formation in pheromone-treated *cdc24-m* cells, which have lost their ability to polarize towards the mating partner and instead use the Rsr1/Bud1 GTPase module[8]. Thus, after degradation of Far1, Cdc24 is rapidly exported to the cytoplasm, where it may interact with Rsr1/Bud1-GTP at the plasma membrane, thereby promoting activation of Cdc42 at the incipient bud site (Fig. 5b). In contrast, in response to pheromones, Cdc24 is exported in a complex with Far1 (Fig. 5c); in the cytoplasm, the adaptor Far1 binds directly to Gβγ (refs 7, 14) (Fig. 5c), which is formed at the site of activated pheromone receptor. Thus, Far1 targets Cdc24 to the site of polarization established by the activated pheromone receptor and Gβγ, leading to local activation of Cdc42 at the mating landmark and thus polarization of the actin cytoskeleton towards the mating partner. By analogy with Ste5 (ref. 30), it is possible that nuclear localization of Far1 and/or Cdc24 is required to allow their recruitment to the plasma membrane. Why is Cdc24 not targeted to the budding landmark in response to pheromones? In the absence of Far1 or in cells expressing Cdc24-m proteins that are unable to interact with Far1 (refs 8, 16), Cdc24 is found at the incipient bud site, suggesting that Far1 is not only required to target Cdc24 to Gβγ but may also prevent mistargeting of Cdc24 to the budding landmark. Consistent with this hypothesis, overexpression of cytoplasmic Far1 was found to interfere with the predetermined budding pattern[22]. We speculate that Cdc24 may be unable to assemble at the budding landmark when it is bound to Far1. This model would explain why cells need two distinct mechanisms to relocate Cdc24 during the cell cycle and in response to pheromones (Fig. 5): degradation of Far1 at bud emergence enables Cdc24 to assemble at the incipient bud site (Fig. 5b), whereas export of Far1 by Msn5 targets Cdc24 to Gβγ in response to pheromones (Fig. 5c).

Our results suggest that degradation of Far1 or its export from the nucleus by Msn5 are redundant for cell viability. Thus, although export of Far1 is stimulated by pheromones[22], basal rates of Far1 export must accumulate sufficient cytoplasmic Cdc24 to trigger bud emergence. The G1 cyclins target Far1 for ubiquitin-dependent degradation by phosphorylation of Ser 87 (ref. 19). Interestingly, *cln1*Δ *cln2*Δ *msn5*Δ or *swi4*Δ *msn5*Δ cells are non-viable[31], possibly because they fail to relocate Cdc24 into the cytoplasm. Degradation of Far1 is not, however, the function of G1 cyclins that is essential for bud emergence, and available evidence suggests that G1 cyclins also have a key role in membrane recruitment of Cdc24 (M.-P.G., Y.S. and M.P., unpublished observations). Interestingly, cytoplasmic Cdc24-m failed to localize to the plasma membrane in *msn5*Δ cells overexpressing Far1, perhaps because Msn5 may be involved in exporting the G1 cyclins. Thus, although degradation of Far1 and Msn5-dependent export of Far1 are redundant mechanisms in the export of Cdc24 at bud emergence, Msn5 may also be involved in efficient membrane recruitment of Cdc24. In contrast, ubiquitin-dependent degradation of Far1 cannot compensate for the loss of Msn5 during mating. Cells deleted for *MSN5* are unable to polarize efficiently in response to pheromones[22], possibly because, in the absence of an active export system, increased levels of Far1 produced in response to pheromones trap Cdc24 in the nucleus. In addition to inducing *FAR1* transcription[21], activation of the pheromone pathway may also decrease ubiquitin-dependent degradation of Far1 (ref. 18, and M. Blondel and M.P., unpublished observations). As with bud emergence, however, the shmoo defect of *msn5*Δ cells could not be restored by expression of cytoplasmic Cdc24, suggesting that Msn5 may export an additional target required for shmoo formation. Alternatively, Cdc24 may be unable to localize to Gβγ at the plasma membrane in the absence of cytoplasmic Far1, although cells deleted for Far1 are able to polarize towards the bud site[14,32]. In addition to Far1, Msn5 is required to export the MAP kinase scaffold Ste5 (ref. 30) and, interestingly, expression of cytoplasmic Ste5 restores shmoo formation of *msn5*Δ cells (G. Ammerer, personal communication). Thus, formation of a mating projection in response to pheromones may require export of Ste5, whereas correct orientation of polarized growth in a morphogenetic gradient requires cytoplasmic Far1 (ref. 22).

Several possible mechanisms could explain how nuclear localization of Cdc24 depends on its ability to interact with Far1. First, Far1 may be required for nuclear import of Cdc24. Consistent with this possibility, Far1 contains a functional bipartite NLS in its N-terminal domain[22]. Nuclear localization of Cdc24 is not, however, abolished in *far1*Δ cells, demonstrating that Cdc24 still travels to the nucleus in the absence of Far1. In addition, Cdc24 is nuclear in cells unable to export Far1, suggesting that shuttling of Far1 between the nucleus and cytoplasm is not required for nuclear import of Cdc24. The sequence of Cdc24 contains several potential bipartite NLSs, but without a functional characterization of these putative signals and/or the identification of the importin(s) for Cdc24 it is difficult to distinguish between direct or indirect mechanisms for nuclear import of Cdc24. Alternatively, Far1 may function as a nuclear anchor for Cdc24. Cdc24 may simply have a greater binding affinity for Far1 than for the export system, or binding of Far1 to Cdc24 in the nucleus may mask a nuclear export signal (NES) on Cdc24. In both cases, degradation of Far1 or its export by Msn5 in response to pheromones would enable Cdc24 to leave the nucleus. We favour the latter possibility because it would explain the observation that in response to pheromones Cdc24 is exported only when complexed with Far1. Distinguishing between these models will, however, require identification of the proteins responsible for nuclear import and export of Cdc24.

Relocation of Cdc24 at bud emergence occurs very rapidly and is likely to require an active nuclear export system. Ubiquitin-dependent degradation of Far1 takes place in the nucleus (M. Blondel and M.P., unpublished observations), suggesting that export of Cdc24 is independent of Far1. The exportin mediating this relocation is unknown, but may not be Msn5, because *msn5*Δ *far1*Δ cells are able to bud efficiently and do not accumulate Cdc24 in the nucleus (data not shown, and ref. 20). In addition, the binding sites for Msn5 and Cdc24 on Far1 are distinct[16,22], indicating that Cdc24 is not necessary for export of Far1. Thus, unless Far1 is required for nuclear import of Cdc24 (see above), two distinct exportins may be involved in nuclear export of Cdc24. At bud emergence, an unknown exportin relocates Cdc24 after degradation of Far1, whereas in response to pheromones, Cdc24 is exported together with Far1 by Msn5. Because Cdc24 is predominantly cytoplasmic in the absence of Far1, its nuclear export rate must exceed the import rate. It is conceivable that cytoplasmic retention of Cdc24 contributes to this distribution. Good candidates for cytoplasmic anchors may be Rsr1/Bud1 or Bem1, which both interact with Cdc24 at the cell cortex. Further work will be required to determine whether these proteins influence the localization of Cdc24 *in vivo*.

Several proteins have now been shown to interact with proteins that regulate their subcellular localization[33,34]. For example, the transcription factor NF-κB is kept in an inactive form in the cytoplasm by association with IκB which blocks its nuclear import[35,36] and promotes nuclear export[37]. In *S. pombe*, the stress-activated kinase Arf1 was proposed to function as a nuclear anchor for the transcription factor Spc1 in the presence of osmotic stress[38]. Nuclear compartmentalization of the exchange factor Cdc24 by Far1 provides an elegant mechanism for targeting Cdc24 to distinct sites, thereby spatially restricting assembly of the actin cytoskeleton at bud emergence or in response to extracellular signals (Fig. 5). Interestingly, the putative Cdc42-exchange factor Scd1/Ral1 in fission yeast is also found in the nucleus[39], suggesting that nuclear sequestration of exchange factors may be conserved. In mammalian cells, G-protein-coupled receptors are required for directed cell migration, invasion and locomotion. In all these cases, local activation of Cdc42 is involved in polarizing the actin cytoskeleton towards the morphogenetic signal, but it is not known how Cdc42 is activated in a spatially restricted manner. Although no mammalian Far1 homologues have been discovered to date, the binding site for Far1 on Cdc24 has been conserved and is present in the human exchange factor Dbl[15]. Very little is currently known about the subcellular localization of mammalian exchange factors but, intriguingly, a few studies reported a nuclear fraction of Vav[40]. It will thus be interesting to investigate whether compartmentalization of exchange factors may also be involved in directed cell polarization in higher eukaryotes. □

Methods

Yeast strains and genetic experiments.

Yeast strains are described in Table 1. The genotypes of the yeast strains are: W303: *ade2-1*, *trp1-1*, *can1-100*, *leu2-3,112*, *his3-11,15*, *ura3*, *GAL+*, *psi+*, *ssd1-d2*; and S288C: *ade2-101*, *ura3-52*, *lys2-801*, *trp1*-Δ1, *his3*Δ*200*, *leu2*-Δ, unless noted otherwise. Standard yeast growth conditions and genetic manipulations were used as described[41]. Pheromone response and mating assays were performed as described[42]. Replacements of the *FAR1* locus with *FAR1-C* marked with *URA3* were performed with the plasmid pCY340 digested with *Not*I[21].

DNA manipulations.

Plasmids and oligonucleotides are described in Table 2. Standard procedures were used for recombinant DNA manipulations[43]. PCR reactions were performed with the Expand polymerase kit as recommended by the manufacturer (Boehringer Mannheim). Oligonucleotides were synthesized by Genset (France). *CDC24* fused to green fluorescent protein (GFP) at the carboxy terminus was constructed as follows. The *CDC24* coding sequence was amplified by PCR using the primers oTP463 and oTP600 and genomic DNA isolated from either wild-type or *cdc24-m* strains[15], and cloned as a *Bam*HI/*Xho*I fragment into pRS315 containing the GFP coding sequence with the S35T mutation[44]. Expression of Cdc24 was driven by the constitutive *CYC1* or *ADH* promoters[45] or from its endogenous promoter, which was amplified by PCR using the primers oTP517 and oTP516. The Cdc24–GFP fusion protein was able to fully rescue the growth defect of cells deleted for *CDC24* (YMG315). The *CYC1–CDC24–GFP* DNA fragment was also cloned into the integrating vector pRS306; integration was targeted to the *URA3* locus by digesting the plasmid with *Stu*I. Plasmids expressing wild-type Far 1 or various mutant forms[19,22] from the constitutive *ADH* promoter were constructed by replacing the *Sac*I–*Xho*I fragment encoding the *GAL1* promoter[22] with the *ADH* fragment isolated from pRS416(ADH)[45].

Antibodies and western blots.

Standard procedures were used for yeast cell extract preparation and immunoblotting[46,47]. Polyclonal anti-Far1 and anti-Cdc24 antibodies have been described previously[16,19]. Antibodies specific for actin or the haemagglutinin (HA) epitope (HA11) were purchased from Boehringer Mannheim or Babco (Berkeley Antibody Company), respectively, and used as recommended by the manufacturer.

Analysis of Cdc24–GFP in cells depleted of G1 cyclins.

The strain YMG258 harbouring a plasmid encoding *CDC24–GFP* (pYS47) was grown to early log phase at 30°C in selective medium (SD-URA/MET), at which time methionine (final concentration 2 mM) was added to shut off expression of Cln2 (ref. 48). After 3 h (time 0), cells were washed twice and released into medium lacking methionine in the presence or absence of latrunculin A (Lat-A, 200 μM final concentration; stock solution 10 mM in DMSO). The localization of Cdc24–GFP was visualized by GFP microscopy at time 0 or 1 h after removal of methionine.

Cell synchronization.

Wild-type cells (YMT263) carrying *CDC24–GFP* (pYS65) integrated at the *URA3* locus were grown in selective medium (SD-URA) to early log phase and elutriated using a Beckman JS 5.0 system essentially as described[49]. Small unbudded cells were inoculated into prewarmed selective medium (25 °C); samples were collected as indicated and cell-cycle progression was monitored by FACS analysis. Cells were examined microscopically and the actin cytoskeleton was visualized after staining with rhodamine–phalloidin. At least 300 cells were counted for each time point. Protein extracts were prepared as described above; western blots were quantified using the Aida200 software (Raytest Isotopenmessgeräte GmbH).

Microscopy and flow cytometry.

Yeast actin was visualized with rhodamine–phalloidin (Molecular Probes) as described previously[22]. Proteins tagged with GFP were visualized using a Zeiss axiophot fluorescence microscope using a Chroma GFPII filter (excitation 440–470 nm), photographed with a Photometrics CCD camera and analysed with Photoshop 4.0 software (Adobe).

Flow cytometry was carried out as described[50]. Briefly, cells were fixed in 70% ethanol, washed and digested with RNase A for 5 h. The DNA was then stained with propidium iodide and cells analysed on a FACScan (Becton-Dickinson).

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1. Hall, A. Rho GTPases and the actin cytoskeleton. *Science* **279,** 509–514 (1998).
2. Adams, A. E., Johnson, D. I., Longnecker, R. M., Sloat, B. F. & Pringle, J. R. CDC42 and CDC43, two additional genes involved in budding and the establishment of cell polarity in the yeast *Saccharomyces cerevisiae*. *J. Cell Biol.* **111,** 131–142 (1990).
3. Bähler, J. & Peter, M. in *Frontiers in Molecular Biology: Cell Polarity* (ed. Drubin, D. G.) (Oxford Univ. Press, Oxford, in the press).
4. Johnson, D. I. Cdc42: An essential Rho-type GTPase controlling eukaryotic cell polarity. *Microbiol. Mol. Biol. Rev.* **63,** 54–105 (1999).
5. Chenevert, J., Corrado, K., Bender, A., Pringle, J. & Herskowitz, I. A yeast gene (BEM1) necessary for cell polarization whose product contains two SH3 domains. *Nature* **356,** 77–79 (1992).
6. Peterson, J., Zheng, Y., Bender, L., Myers, A., Cerione, R. & Bender, A. Interactions between the bud emergence proteins Bem1p and Bem2p and Rho-type GTPases in yeast. *J. Cell Biol.* **127,** 1395–1406 (1994).
7. Leeuw, T. *et al.* Pheromone response in yeast: association of Bem1p with proteins of the MAP kinase cascade and actin. *Science* **270,** 1210–1213 (1995).
8. Nern, A. & Arkowitz, R. A. A Cdc24-Far1-G beta gamma protein complex required for yeast orientation during mating. *J. Cell Biol.* **144,** 1187–1202 (1999).
9. Toenjes, K. A., Sawyer, M. M. & Johnson, D. I. The guanine-nucleotide-exchange factor Cdc24 is targeted to the nucleus and polarized growth sites. *Curr. Biol.* **9,** 1183–1186 (1999).
10. Chant, J. & Stowers, L. GTPase cascades choreographing cellular behavior: movement, morphogenesis, and more. *Cell* **81,** 1–4 (1995).
11. Segall, J. E. Polarization of yeast cells in spatial gradients of alpha mating factor. *Proc. Natl Acad. Sci. USA* **90,** 8332–8336 (1993).
12. Sprague, G. F. & Thorner, J. W. in *The Molecular and Cellular Biology of the Yeast Saccharomyces* (eds Jones, E.W., Pringle, J.R. & Broach, J.R.) 657–744 (Cold Spring Harb. Lab. Press, Cold Spring Harbor, 1992).
13. Herskowitz, I. MAP kinase pathways in yeast: for mating and more. *Cell* **80,** 187–197 (1995).
14. Valtz, N., Peter, M. & Herskowitz, I. FAR1 is required for oriented polarization of yeast cells in response to mating pheromones. *J. Cell Biol.* **131,** 863–873 (1995).
15. Nern, A. & Arkowitz, R. A. A GTP-exchange factor required for cell orientation. *Nature* **391,** 195–198 (1998).
16. Butty, A. C., Pryciak, P. M., Huang, L. S., Herskowitz, I. & Peter, M. The role of Far1 in linking the heterotrimeric G protein to polarity establishment proteins during yeast mating. *Science* **282,** 1511–1516 (1998).
17. Lew, D. J. & Reed, S. I. Cell cycle control of morphogenesis in budding yeast. *Curr. Opin. Genet. Dev.* **5,** 17–23 (1995).
18. McKinney, J. D. & Cross, F. R. FAR1 and the G1 phase specificity of cell cycle arrest by mating factor in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **15,** 2509–2516 (1995).
19. Henchoz, S., Chi, Y., Catarin, B., Herskowitz, I., Deshaies, R. J. & Peter, M. Phosphorylation- and ubiquitin-dependent degradation of the cyclin-dependent kinase inhibitor Far1 in budding yeast. *Genes Dev.* **11,** 3046–3060 (1997).
20. McKinney, J. D., Chang, F., Heintz, N. & Cross, F. R. Negative regulation of FAR1 at the Start of the yeast cell cycle. *Genes Dev.* **7,** 833–843 (1993).
21. Chang, F. & Herskowitz, I. Identification of a gene necessary for cell cycle arrest by a negative growth factor of yeast: FAR1 is an inhibitor of a G1 cyclin, CLN2. *Cell* **63,** 999–1011 (1990).

22. Blondel, M., Alepuz, P. M., Huang, L. S., Shaham, S., Ammerer, G. & Peter, M. Nuclear export of Far1 in response to pheromones requires the export receptor Msn5/Ste21p. *Genes Dev.* **13,** 2284–2300 (1999).
23. Lew, D. J. & Reed, S. I. Morphogenesis in the yeast cell cycle: regulation by Cdc28 and cyclins. *J. Cell Biol.* **120,** 1305–1320 (1993).
24. Peter, M. & Herskowitz, I. Direct inhibition of the yeast cyclin-dependent kinase Cdc28-Cln by Far1. *Science* **265,** 1228–1231 (1994).
25. Chant, J. Generation of cell polarity in yeast. *Curr. Opin. Cell Biol.* **8,** 557–565 (1996).
26. Chenevert, J. Cell polarization directed by extracellular cues in yeast. *Mol. Biol. Cell* **5,** 1169–1175 (1994).
27. Arkowitz, R. A. Responding to attraction: chemotaxis and chemotropism in Dictyostelium and yeast. *Trends Cell Biol.* **9,** 20–27 (1999).
28. Bender, A. & Pringle, J. R. Multicopy suppression of the cdc24 budding defect in yeast by CDC42 and three newly identified genes including the ras-related gene RSR1. *Proc. Natl Acad. Sci. USA* **86,** 9976–9980 (1989).
29. Park, H. O., Bi, E., Pringle, J. R. & Herskowitz, I. Two active states of the Ras-related Bud1/Rsr1 protein bind to different effectors to determine yeast cell polarity. *Proc. Natl Acad. Sci. USA* **94,** 4463–4468 (1997).
30. Mahanty, S. K., Wang, Y. M., Farley, F. W. & Elion, E. A. Nuclear shuttling of yeast scaffold Ste5 is required for its recruitment to the plasma membrane and activation of the mating MAPK cascade. *Cell* **98,** 501–512 (1999).
31. Alepuz, P. M., Matheos, D., Cunningham, K. W. & Estruch, F. The *Saccharomyces cerevisiae* RanGTP binding protein Msn5 is involved in different signal transduction pathways. *Genetics* **153,** 1219–1231 (1999).
32. Dorer, R., Pryciak, P. M. & Hartwell, L. H. *Saccharomyces cerevisiae* cells execute a default pathway to select a mate in the absence of pheromone gradients. *J. Cell Biol.* **131,** 845–861 (1995).
33. Kaffmann, A. & O'Shea, E. K. Regulation of nuclear localization: A key to a door. *Annu. Rev. Cell Dev. Biol* (in the press).
34. Hood, J. K. & Silver, P. A. In or out? Regulating nuclear transport. *Curr. Opin. Cell Biol.* **11,** 241–247 (1999).
35. Beg, A. A., Ruben, S. M., Scheinman, R. I., Haskill, S., Rosen, C. A. & Baldwin, A. S., Jr I kappa B interacts with the nuclear localization sequences of the subunits of NF-kappa B: a mechanism for cytoplasmic retention. *Genes Dev.* **6,** 1899–1913 (1992).
36. Henkel, T., Zabel, U., van Zee, K., Muller, J. M., Fanning, E. & Baeuerle, P. A. Intramolecular masking of the nuclear location signal and dimerization domain in the precursor for the p50 NF-kappa B subunit. *Cell* **68,** 1121–1133 (1992).
37. Arenzana-Seisdedos, F. *et al.* Nuclear localization of I kappa B alpha promotes active transport of NF-kappa B from the nucleus to the cytoplasm. *J. Cell Sci.* **110,** 369–378 (1997).
38. Gaits, F., Degols, G., Shiozaki, K. & Russell, P. Phosphorylation and association with the transcription factor Atf1 regulate localization of Spc1/Sty1 stress-activated kinase in fission yeast. *Genes Dev.* **12,** 1464–1473 (1998).
39. Chen, C. R., Li, Y. C., Chen, J., Hou, M. C., Papadaki, P. & Chang, E. C. Moe1, a conserved protein in Schizosaccharomyces pombe, interacts with a Ras effector, Scd1, to affect proper spindle formation. *Proc. Natl Acad. Sci. USA* **96,** 517–522 (1999).
40. Bertagnolo, V., Marchisio, M., Volinia, S., Caramelli, E. & Capitani, S. Nuclear association of tyrosine-phosphorylated Vav to phospholipase C-gamma1 and phosphoinositide 3-kinase during granulocytic differentiation of HL-60 cells. *FEBS Lett.* **441,** 480–484 (1998).
41. Guthrie, C. & Fink, G. R. *Guide to Yeast Genetics and Molecular Biology* (Academic, San Diego, 1991).
42. Valtz, N. & Peter, M. Functional analysis of FAR1 in yeast. *Methods Enzymol.* **283,** 350–365 (1997).
43. Ausubel, F. M. *et al. Current Protocols in Molecular Biology* (Greene Publishing Associates and Wiley-Interscience, New York, 1991).
44. Posas, F., Witten, E. A. & Saito, H. Requirement of STE50 for osmostress-induced activation of the STE11 mitogen-activated protein kinase kinase kinase in the high-osmolarity glycerol response pathway. *Mol. Cell. Biol.* **18,** 5788–5796 (1998).
45. Mumberg, D., Muller, R. & Funk, M. Yeast vectors for the controlled expression of heterologous proteins in different genetic backgrounds. *Gene* **156,** 119–122 (1995).
46. Brown, J. L., Jaquenoud, M., Gulli, M. P., Chant, J. & Peter, M. Novel Cdc42-binding proteins Gic1 and Gic2 control cell polarity in yeast. *Genes Dev.* **11,** 2972–2982 (1997).
47. Harlow, E. & Lane, D. *Antibodies: A Laboratory Manual* (Cold Spring Harb. Lab. Press, Cold Spring Harbor, 1988).
48. Ronicke, V., Graulich, W., Mumberg, D., Muller, R. & Funk, M. Use of conditional promoters for expression of heterologous proteins in Saccharomyces cerevisiae. *Methods Enzymol.* **283,** 313–322 (1997).
49. Johnston, L. H. & Johnson, A. L. Elutriation of budding yeast. *Methods Enzymol.* **283,** 342–350 (1997).
50. Epstein, C. B. & Cross, F. R. CLB5: a novel B cyclin from budding yeast with a role in S phase. *Genes Dev.* **6,** 1695–1706 (1992).

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Correspondence and requests for materials should be addressed to M.P.