A role for cell polarity proteins in mitotic exit

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Supplement

Results

Overexpression of *STE20* induces expression of the *FAR1* gene, which encodes an inhibitor of Cdk/Cln kinases (Peter and Herskowitz, 1994). We wanted to exclude the possibility that induction of Far1 facilitates ME in Δlte1 cells by inactivating Cdk-Clb. If this were the case *STE20* should not be able to suppress the cold sensitive growth defect of Δfar1 Δlte1 cells. However, Δfar1 Δlte1 2µm-*STE20* cells were able to grow at low temperatures and so we conclude that Far1 is not involved in this process.

To confirm a role of Cdc42 in the binding of Lte1 to the cell cortex, we assayed GFP-Lte1 localization in the presence of the constitutively active Cdc42<sup>G12V</sup>. Cdc42<sup>G12V</sup> is not susceptible to the action of a GTPase-activating protein, and thus the protein is locked in the GTP-bound, active form (Benton *et al.*, 1997). Overexpression of Cdc42<sup>G12V</sup> targeted GFP-Lte1 in about 32% of unbudded G1 cells to the cell cortex (Figure 9A top row, middle cell and 9B). Lte1 is never seen at the cell cortex of wild type cells without a bud.
Moreover, in \(CDC42^{G12V}\) cells with a large bud, GFP-Lte1 was frequently associated with the mother-bud neck as a double ring (Figure 9A top row, middle cell and 9B). In contrast, overexpression of \(CDC42\) or the inactive \(CDC42^{D118A}\) was unable to target GFP-Lte1 to the cell cortex of unbudded cells or the bud neck of large budded cells (Figure 9A, B).

Expression of \(CDC42^{G12V}\) but not of CDC42 or \(CDC42^{D118A}\) increases activity of the PAK Cla4 2.5-fold (Benton et al., 1997). This raised the possibility that activation of Cla4 through Cdc42\(^{G12V}\) recruited GFP-Lte1 to the cell cortex. We therefore asked whether Cdc42\(^{G12V}\) required Cla4 to target GFP-Lte1 to the cell cortex. When Cdc42\(^{G12V}\) was overexpressed in \(\Delta cla4\) Gal1-GFP-LTE1 cells, GFP-Lte1 failed to bind to the cell cortex of unbudded cells or the mother-bud neck of cells with a large bud (Figure 9A, B). Thus, Cdc42\(^{G12V}\) requires Cla4 activity to target GFP-Lte1 to the cell cortex.

**Additional materials and methods**

**Growth conditions and plasmids**

Basic yeast methods and growth media were as described (Sherman, 1991). \(CLA4^{K594R-9Myc}\) was constructed by recombinant PCR using plasmid pBB131 (pRS416-CLA4-9Myc) as template (Benton et al., 1997). \(CLA4^{K594R-9Myc}\) was subcloned into pRS316 (Sikorski and Hieter, 1989) and the \(CLA4\) sequence confirmed by sequence analysis.
Suppression analysis and test for synthetic lethality

For the high copy suppression screen Δlte1 cells were transformed with a 2 µm LEU2-based YEpl3 library (from K. Nasmyth) and grown on selective plates at 30°C for 3 days. Transformants were replica plated onto selective plates and incubated for 10 d at 10°C. Genes were subcloned after PCR amplification into 2 µm pRS426 or pRS425 plasmids (Christianson et al., 1992). Shuffle strains were constructed by transforming strain YPH499 with the gene of interest on the URA3-based plasmid pRS316 (Sikorski and Hieter, 1989). The chromosomal gene was then disrupted. Cell density was determined with a Beckman Coulter Counter Z1. A final concentration of $10^4$ cells was serially diluted (1:10) and spotted on 5'-fluoroorotic acid (5-FOA) or selective plates.

Analysis of cells for a ME defect

Yeast cells with CDC14-GFP were incubated at 30°C for 3 h with α-factor (10 µg/ml) to arrest cells in G1 phase of the cell cycle. α-Factor was removed by washing the cells twice with medium (t=0). Cells were then incubated at the indicated temperature. At the indicated times the budding index and nucleolar Cdc14-GFP of fixed cells were determined by phase contrast and fluorescence microscopy.

Dephosphorylation of Lte1-ProA

A yeast lysate of CLA4 and Δcla4 cells with LTE1-ProA was prepared using glass beads (Knop et al., 1999). In brief, yeast cells were resuspended in UB buffer (0.05 M Hepes, pH 7.5, 0.1 M KCl, 3 mM MgCl₂, 1 mM EGTA, 1 mM DTT, 1 mM PMSF, 0.2% Triton X-100, EDTA-free protease inhibitors cocktail tablets (Roche)) and acid washed glass
beads (Sigma) were added. Cells were lysed using a vortex mixer until >90% of the cells were sheared. The lysate was cleared by centrifugation (5,100 g, 10 min, 4°C). The supernatant was incubated for 60 min at 4°C with the IgG Sepharose beads (Amersham Pharmacia). After washing, beads were resuspended in 50 μl buffer P (150 mM NaCl, 50 mM Tris-Cl pH 8.0, 1 mM MgCl₂, 0.1 mM ZnCl₂, 0.4 % SDS) and incubated for 3 min at 65°C. Cooled samples were incubated for 45 min at 30°C with no further addition, 10 U alkaline phosphatase, or 10 U alkaline phosphatase with phosphatase inhibitor mix (100 mM β-glycerophosphate, 50 mM NaF, 200 μM sodium orthovanadate). Samples were mixed with HU buffer and heated for 15 min at 65°C. Lte1-ProA was detected by immunoblotting with rabbit IgG's coupled to horse radish peroxidase (Jackson Research Laboratories) and the use of the ECL+Plus western blotting detection system (Amersham Pharmacia).

References


**Fig. 9.** Constitutively active Cdc42<sup>G12V</sup> targets GFP-Lte1 to the cell cortex in a Cla4 dependent manner. (A) *CLA4* and *Δcla4* cells with Gal1-GFP-LTE1 cells were transformed with Gal1-CDC42, Gal1-CDC42<sup>G12V</sup> and Gal1-CDC42<sup>D118A</sup> on pRS315 (Benton et al., 1997). The transformants were grown in selective medium with 3% raffinose at 30°C. The Gal1 promoter was induced for 1 h by the addition of 2% galactose. Fixed cells were analysed by fluorescence microscopy to determine the distribution of GFP-Lte1. Bar: 5 µm. (B) Quantification of (A). n>100.