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# The Multiple Roles of Cyk1p in the Assembly and Function of the Actomyosin Ring in Budding Yeast

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The budding yeast IQGAP-like protein Cyk1p/Iqg1p localizes to the mother-bud junction during anaphase and has been shown to be required for the completion of cytokinesis. In this study, video microscopy analysis of cells expressing green fluorescent protein-tagged Cyk1p/Iqg1p demonstrates that Cyk1p/Iqg1p is a dynamic component of the contractile ring during cytokinesis. Furthermore, in the absence of Cyk1p/Iqg1p, myosin II fails to undergo the contraction-like size change at the end of mitosis. To understand the mechanistic role of Cyk1p/Iqg1p in actomyosin ring assembly and dynamics, we have investigated the role of the structural domains that Cyk1p/Iqg1p shares with IQGAPs. An amino terminal portion containing the calponin homology domain binds to actin filaments and is required for the assembly of actin filaments to the ring. This result supports the hypothesis that Cyk1p/Iqg1p plays a direct role in F-actin recruitment. Deletion of the domain harboring the eight IQ motifs abolishes the localization of Cyk1p/Iqg1p to the bud neck, suggesting that Cyk1p/Iqg1p may be localized through interactions with a calmodulin-like protein. Interestingly, deletion of the COOH-terminal GTPase-activating protein-related domain does not affect Cyk1p/Iqg1p localization or actin recruitment to the ring but prevents actomyosin ring contraction. In vitro binding experiments show that Cyk1p/Iqg1p binds to calmodulin, Cmd1p, in a calcium-dependent manner, and to Tem1p, a small GTP-binding protein previously found to be required for the completion of anaphase. These results demonstrate the critical function of Cyk1p/Iqg1p in regulating various steps of actomyosin ring assembly and cytokinesis.

## INTRODUCTION

The cleavage of eukaryotic cells during mitosis is accomplished by a concerted process of membrane constriction and addition along the plane that bisects the telophase spindle. The force that drives the membrane constriction is thought to come from the mechanochemistry that occurs within an actomyosin-based contractile ring (reviewed in Schroeder, 1990; Satterwhite and Pollard, 1992; Fishkind and Wang, 1995). Although a myosin II independent mechanism may also exist to drive membrane constriction (Neujahr *et al.*, 1997), the importance of myosin II and actin filaments in cleavage furrow formation and progression is supported by several studies involving inhibitors of actin and myosin in dividing eggs and cultured cells (Zurek *et al.*, 1990; Patterson *et al.*, 1993) as well as genetic manipulations in *Dictyostelium* and yeast (De Lozanne and Spudich, 1987; Knecht and Loomis, 1987; Watts *et al.*, 1987; Kitayama *et al.*, 1997)

The contractile ring is a transient structure whose assembly and disassembly are under stringent temporal and spatial regulation. The molecular mechanisms for actin and myosin II recruitment to the cleavage furrow are not known. Microinjection of fluorescently labeled actin monomers or filaments has shown that contractile ring formation may involve the recruitment of actin filaments to (and their transport along) the cell cortex, but little de novo filament formation at the furrowing site (Cao and Wang, 1990a,b); however, cytochalasin treatment of cells either before or after the initiation of furrowing prevents cytokinesis, suggesting that actin polymerization is important for the formation and maintenance of the contractile ring

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(Schroeder, 1972; Martinez *et al.*, 1989; Schroeder, 1990; Andreassen *et al.*, 1991).

It is not known which furrow component(s) directly recruits actin filaments. One idea has been that myosin II itself can fulfill this role and might further organize actin filaments into antiparallel bundles by applying force (Hayashi *et al.*, 1977; Pollard *et al.*, 1990). This hypothesis, however, is not supported by genetic analysis in *Dictyostelium* and yeast, which showed that actin filaments can accumulate to the predicted cleavage furrow site in myosin II null mutant cells (Kitayama *et al.*, 1997; May *et al.*, 1997; Neujahr *et al.*, 1997). Along with myosin II, a number of well known F-actin binding proteins have been found in the contractile ring, such as α-actinin and tropomyosin (Fujiwara *et al.*, 1978; Balasubramanian *et al.*, 1992). Although some of these proteins may be important for the organization and stabilization of actin filaments in the ring, it is not clear whether these proteins localize to the cleavage site before and independent of actin. Identification of the actin recruiting protein is likely to be a key step toward understanding the mechanism and regulation of contractile ring formation.

Once assembled, it is not clear whether the actomyosin ring contracts spontaneously or whether specific signals are provided to trigger contraction. Calcium signaling may play a role in triggering cleavage furrow ingression. Increases in intracellular $Ca^{2+}$ have been shown to correlate with cleavage in embryos (Kubota *et al.*, 1993; Striker, 1995). $Ca^{2+}$ waves have been observed along the cleavage furrow, and blocking changes in $Ca^{2+}$ concentration using heparin or calcium buffers delays or inhibits cleavage (Snow and Nuccitelli, 1993; Striker, 1995; Muto *et al.*, 1996). Calmodulin, a mediator of $Ca^{2+}$ signaling, has been implicated in cytokinesis in *Dictyostelium*, because calmodulin antisense RNA blocks completion of cell division (Liu *et al.*, 1992). $Ca^{2+}$/calmodulin-dependent myosin light chain kinase stimulates myosin filament formation and force generation through the phosphorylation of Ser19 of myosin light chain (MLC)[1] (Sellers *et al.*, 1982). Phosphorylation of two adjacent sites by $p34^{cdc2}$ inhibits Ser19 phosphorylation, and this may be a mechanism that prevents cytokinesis before anaphase (Satterwhite *et al.*, 1992; Yamakita *et al.*, 1994). Direct in vivo evidence has yet to be gained to support the importance of MLC phosphorylation in regulating actomyosin contraction.

In the past few years, genetic studies in yeast have provided new information on the molecules that are important for actomyosin ring assembly and cytokinesis. We and others have shown previously that an IQGAP-related protein, Cyk1p/Iqg1p, plays a direct role during cytokinesis in *Saccharomyces cerevisiae* (Epp and Chant, 1997; Lippincott and Li, 1998; Osman and Cerione, 1998). We identified a ring structure that contains Cyk1p/Iqg1p, actin, and Myo1p and exhibits contraction-like size change during cytokinesis in this organism. The assembly of this ring occurs at two different stages in the cell cycle: 1) at the G1-S transition, Myo1p, a type II myosin, assembles into a ring at the presumptive bud site, the future site of cell division; and 2) during anaphase the recruitment of actin filaments to the ring occurs subsequent to chromosome segregation (Bi *et al.*, 1998; Lippincott and Li, 1998). Cyk1p/Iqg1p plays a critical role in the second step of actomyosin ring assembly. During anaphase, Cyk1p/Iqg1p becomes concentrated in the ring slightly before and independent of actin recruitment (Epp and Chant, 1997; Lippincott and Li, 1998). Gene disruption of *CYK1/IQG1* is either lethal or causes temperature sensitivity, depending on strain background, but in all cases deletion of *CYK1/IQG1* results in cytokinesis defects (Epp and Chant, 1997; Lippincott and Li, 1998; Osman and Cerione, 1998).

The mammalian IQGAP family proteins all contain a calponin homology domain (CHD), which in IQGAP1 has been shown to bind actin filaments (Bashour *et al.*, 1997; Fukata *et al.*, 1997), multiple IQ motifs that are presumed to interact with calmodulin (Brill *et al.*, 1996; Hart *et al.*, 1996), and a GTPase-activating protein (GAP)-related domain (GRD) that binds Cdc42p, a Rho family small GTPase (McCallum *et al.*, 1996). Because Cyk1p/Iqg1 also has the above homology domains, we speculated that Cyk1p/Iqg1p may directly recruit actin filaments and may mediate important signaling events that regulate cytokinesis. Here we report a structure/function study of Cyk1p/Iqg1p through a combination of genetic and real time video microscopy analyses. We show that Cyk1p/Iqg1p regulates different steps of actomyosin ring assembly and activation through different parts of the molecule. We have also identified candidate proteins that interact with the conserved domains of Cyk1p/Iqg1p, which we will henceforth refer to as Cyk1p for convenience.

## MATERIALS AND METHODS

### *Media and Genetic Manipulations*

Yeast cell culture and genetic techniques were carried out by methods described previously (Sherman *et al.*, 1974). YPD contained 2% glucose, 1% yeast extract, and 2% Bactopeptone (Difco Laboratories, Detroit, MI). YPG contained 2% galactose, 2% raffinose, 1% yeast extract, and 2% Bactopeptone. Synthetic complete (SC) media was prepared by the method described (Kaiser *et al.*, 1994).

### *Plasmid Construction*

pRL166, the centromere plasmid expressing Cyk1p tagged at the COOH terminus with green fluorescent protein (GFP) under the control of *CYK1* promoter, was constructed by cloning the *Xho*I-*Eag*I

[1] Abbreviations used: CHD, Calponin homology domain; GAP, GTPase activating protein; GFP, green fluorescent protein; GRD, GAP-related domain; GST, glutathione *S*-transferase.

(blunted) fragment bearing *CYK1* promoter and open reading frame into the GFP expression vector pRL73 (Lippincott and Li, 1998), between the *Xho*I-*Bam*HI (blunted sites) sites. A *Xho*I-*Not*I fragment bearing CYK1-GFP was subcloned into vector pRS313 (Sikorski and Hieter, 1989) between the *Xho*I-*Not*I sites to yield pRL166.

Deletion mutants of *CYK1* were first constructed in bluescript vectors (Stratagene, La Jolla, CA). A deletion of the COOH terminus was made by digesting pRL143 (Lippincott and Li, 1998) with *Xba*I and religating, removing the sequence coding for amino acids 698-1425 to yield pKT1. A deletion of a portion of Cyk1p containing the CHD was made by PCR against yeast genomic DNA with primers DELCHD (5′-CCG CTC GAG ATG ACA GAG GAA CAA-3′) and YIG4 (5′-CGC GCG GCC GTA CAA AGC GTT CCT TTT ATA GA-3′). The PCR fragment was digested with *Xho*I and *Eag*I and cloned into the *Xho*I and *Eag*I sites in bluescript KS to give pKT9. A PCR product of primers YIG1 (5′-GCG CGC CTC GAG CGC TTT ATA TTG AGC TAC GC-3′) and CHDR (5′-CCG CTC GAG GGG CGT TTT GCC TGG-3′) was cut with *Xho*I and *Rsa*I, blunted, and cloned into pKT9 cut with *Xho*I and blunted to give pKT11. A deletion of the Cyk1 IQ motifs was constructed by digesting PCR product generated from primers CHDF (5′-CCG CTC GAG GAG TTT TTA TGC AGA-3′) and YIG4 with *Xho*I and *Eag*I and cloning into the bluescript KS *Xho*I and *Eag*I sites. The resulting plasmid was cut with *Xho*I, and PCR product from primers YIG1 and CHΔDR digested with *Xho*I was inserted, resulting in pKT12.

To express the Cyk1p deletions under the *GAL1* promoter with an $NH_2$-terminal myc epitope, pKT12, pKT1, and pKT11 were cut with *Afl*II and *Eag*I, blunted, and subcloned into the *Stu*I site of pRL196, an integration vector for expression of $NH_2$-terminus myc-tagged proteins under the *GAL1* promoter in yeast, to produce pKT27 (expressing Cyk1p amino acids 95–226, L, E, 818-1495), pKT28 (expressing Cyk1p amino acids 95–697, 1426–1495), and pKT29 (expressing Cyk1p amino acids 95–104, F, E, 411-1495), respectively.

To express the deletions under the *CYK1* promoter and tag them at the COOH terminus with the myc epitope, pKT1 was cut with *Xho*I and *Eag*I, and pKT12 and pKT11 were cut with *BssHI* and *Eag*I; all sites were blunted and ligated to pRL222, a HIS3 CEN plasmid for myc-tagging proteins at the COOH terminus, which was cut with *Bam*HI and blunted. The resulting plasmids are pKT30 (expressing amino acids 1–697 followed by 1426–1495), pKT31 (expressing amino acids 1–226, L, E, followed by 818-1495), and pKT34 (expressing amino acids 1–104, F, E, followed by 411-1495).

Cyk1ΔN was constructed by cloning the fragment expressing amino acids 705-1495 of Cyk1p into pRL62, a yeast integration vector carrying the URA3 marker gene and the *GAL1* promoter, with an HA epitope immediately downstream of the *GAL1* promoter. A blunted *BsaAI-Eag*I fragment from PCR with primers YIG1 and YIG4 was cloned into pRL62 cut with *Cla*I and blunted.

The IQ motifs of Cyk1p (amino acids 221–705) were cloned under the *GAL1* promoter by cutting PCR product from the primers DELCHD2 (5′-GAA GGC CTG GCC AGG CAA AAC GCC CGC-3′) and YIG4 with *Stu*I and *BsaAI*, then ligating into pRL62 cut with *Cla*I and blunted.

*CMD1* and*TEM1* were analyzed by PCR from genomic DNA using Yeast ORF Specific GENEPAIRS (Research Genetics, Huntsville, AL), cut with *Sma*I and *Pvu*II, and ligated into the blunted *Eco*RI site of pGEX-2TK (AMRAD) to generate pKT6 and pKT5, respectively.

A fragment carrying Myo1-GFP was cut from pLP8 (Lippincott and Li, 1998) using *Not*I and *Pst*I and subcloned into the corresponding sites of pRS304 to make pKT36

## Strain Construction

All strains used in this study are listed in Table 1. RLY230 (Lippincott and Li, 1998) was transformed with pRL166 and selected on FOA to produce RLY237. RLY261 was transformed with pKT27, 28, 29, 33, and 39 digested with *XcmI* to make RLY 397, 398, 399, 458, and 578, respectively. RLY277 was transformed with pTL12, pKT30, 31, and 34 to make RLY 565, 555, 556, and 557, respectively. RLY 277, 555, 556, and 557 were transformed with pKT36 cut with *AgeI* to produce RLY 558, 559, 560, and 561.

## Observation of Cyk1-GFP and Myo1-GFP–expressing Cells

RLY237 cells were cultured in SC-Leu liquid media. RLY558 grown in SC-Leu + 2% galactose and RLY559 grown overnight in SC-His + 2% galactose were arrested with α-factor for 3 h with the addition of glucose to 2%, then washed three times with sterile water and resuspended in SC-Trp + 2% glucose. Cells were placed on an agarose pad as described previously (Waddle *et al.*, 1996). Living cells were imaged at room temperature using a Nikon Eclipse E600 microscope with a 100 ×/1.40 oil differential interference contrast objective (Nikon, Melville, NY). Images were collected every 0.5–1 min (depending on the experiment) with 0.1-s exposure to fluorescent light filtered through an EXHQ450/50 DM480 LP/BA465LP GFP filter set (Chroma, Brattleboro, VT) using a cooled RTE/CCD 782Y Interline camera (Princeton Instruments, Trenton, NJ). The shutter was controlled automatically using a D122 shutter driver (UniBlitz, Rochester, NY) and WinView 1.6.2 software (Princeton Instruments) with custom software (courtesy of Aneil Mallavarapu, Harvard Medical School, Cambridge, MA).

## Fluorescence Staining of Deletion Mutants of Cyk1p

RLY 555, 556, and 557 were grown overnight in SC-His + Gal and then arrested for 3 h with 0.05 μg/ml α-factor with the addition of glucose to 2%. Cells were washed three times with sterile water and then resuspended in YPD for approximately 1.5 h. Once most cells were budded, 5-ml samples were taken every 15 min until cells rebudded and fixed with 5% formaldehyde for 1 h at room temperature with gentle rocking. Immunofluorescence staining using mouse anti-myc (Evan *et al.*, 1985) and FITC-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA) and rhodamine phalloidin was performed as described (Lippincott and Li, 1998). Cells were visualized on a Zeiss (Thornwood, NY) Axiophot microscope with an HB 100 W/Z high-pressure mercury lamp and a Zeiss 100 ×/1.40 oil objective. Image acquisition was carried out using Northern Exposure (Phase 3 Imaging Systems, Milford, MA).

## Expression and Purification of Recombinant Proteins

Cmd1p or Tem1p Glutathione *S*-transferase (GST) fusion proteins were produced in *Escherichia coli* carrying pKT6 or pKT5, respectively. To prepare Cmd1p or Tem1p beads, the bacteria extracts containing each of the fusion proteins were incubated with glutathione agarose beads for 1 h at 4°C. The beads were washed three times in PBS supplemented with 1 mM DTT, 1 mM PMSF, and 0.1% Tween 20 and then washed three times in PBS supplemented with 1 mM DTT and 1 mM PMSF. For GST-Tem1, 2 mM $MgCl_2$ and 1 mM GTP were present at all times.

GST-Cdc42Sc was purified from baculovirus-infected insect cells by sonication in 50 mM Tris, pH 7.5, 2 mM $MgCl_2$, 1 mM EGTA, 1 mM GTP, 1% NP-40, 1 mM DTT, and protease inhibitors (Li *et al.*, 1995). Lysates were spun at 40,000 rpm for 30 min, and incubated with glutathione agarose overnight at 4°C. Beads were washed with UB (0.05 M HEPES, pH 7.5, 0.1 M KCl, 3 mM $MgCl_2$, 1 mM EGTA) + 0.1% Tween 20, and then UB + 0.5 M KCl, and resuspended in UB + 0.5 mM GTP.

Baculovirus expressing Cyk1-myc or HA-Cyk1ΔN was constructed and amplified using the Bac-to-Bac expression system (Life Technologies, Gaithersburg, MD) following manufacturer's instructions. Insect cell lysates were prepared by sonication of the infected insect cells resuspended in UB + 1 mM DTT and protease inhibitors

**Table 1.** Yeast strains

| Name | Genotype | Source |
|---|---|---|
| RLY 230 | *MAT**a** ura3-52 leu2-3, 112 his3-Δ200 lys2-801 Δcyk1::LEU2* | Lippincott and Li, 1998 |
| RLY 237 | *MAT**a** ura3-52 leu2-3, 112 his3-Δ200 lys2-801 Δcyk1::LEU2 pCYK1-GFP (pRL166)* | This work |
| RLY 261 | *MAT**a** ura3-52 leu2-3, 112 his3-Δ200 trp1-1 ade2 Δbar1* | Elion lab |
| RLY 277 | *MAT**a** ura3-52 leu2-3, 112 his3-Δ200 lys2-801 trp1-1 Δbar1 Δcyk1::LEU2 pGAL1-CYK1-myc (pRL170)* | Lippincott and Li, 1998 |
| RLY 397 | *MAT**a** ura3-52 leu2-3, 112 his3-Δ200 trp1-1 ade2 Δbar1 pGAL1-myc-CYK1ΔIQ (pKT27)* | This work |
| RLY 398 | *MAT**a** ura3-52 leu2-3, 112 his3-Δ200 trp1-1 ade2 Δbar1 pGAL1-myc-CYK1ΔGRD (pKT28)* | This work |
| RLY 399 | *MAT**a** ura3-52 leu2-3, 112 his3-Δ200 trp1-1 ade2 Δbar1 pGAL1-myc-CYK1ΔCHD (pKT29)* | This work |
| RLY 458 | *MAT**a** ura3-52 leu2-3, 112 his3-Δ200 trp1-1 ade2 Δbar1 pGAL1-HA-CYK1ΔN (pKT33)* | This work |
| RLY 555 | *MAT**a** ura3-52 leu2-3, 112 his3-Δ200 lys2-801 trp1-1 Δbar1 Δcyk1::LEU2 pGAL1-CYK1-myc (pRL170) pCYK1ΔGRD-myc (pKT30)* | This work |
| RLY 556 | *MAT**a** ura3-52 leu2-3, 112 his3-Δ200 lys2-801 trp1-1 Δbar1 Δcyk1::LEU2 pGAL1-CYK1-myc (pRL170) pCYK1ΔIQ-myc (pKT31)* | This work |
| RLY 557 | *MAT**a** ura3-52 leu2-3, 112 his3-Δ200 lys2-801 trp1-1 Δbar1 Δcyk1::LEU2 pGAL1-CYK1-myc (pRL170) pCYK1ΔCHD-myc (pKT34)* | This work |
| RLY558 | *MAT**a** ura3-52 leu2-3, 112 his3-Δ200 lys-2-801 trp1-1 Δbar1 Δcyk1::LEU2 pGAL1-CYK1-myc (pRL170) pMYO1-GFP (pKT36)* | This work |
| RLY 559 | *MAT**a** ura3-52 leu2-3, 112 his3-Δ200 lys2-801 trp1-1 Δbar1 Δcyk1::LEU2 pGAL1-CYK1-myc (pRL170) pCYK1ΔGRD-myc (pKT30) pMYO1-GFP (pKT36)* | This work |
| RLY 560 | *MAT**a** ura3-52 leu2-3, 112 his3-Δ200 lys2-801 trp1-1 Δbar1 Δcyk1::LEU2 pGAL1-CYK1-myc (pRL170) pCYK1ΔIQ-myc (pKT31) pMYO1-GFP (pKT36)* | This work |
| RLY 561 | *MAT**a** ura3-52 leu2-3, 112 his3-Δ200 lys2-801 trp1-1 Δbar1 Δcyk1::LEU2 pGAL1-CYK1-myc (pRL170) pCYK1ΔCHD-myc (pKT34) pMYO1-GFP (pKT36)* | This work |
| RLY 565 | *MAT**a** ura3-52 leu2-3, 112 his3-Δ200 lys2-801 trp1-1 Δbar1 Δcyk1::LEU2 pGAL1-CYK1-myc (pRL170) pCYK1-myc (pTL12)* | This work |
| RLY 578 | *MAT**a** ura3-52 leu2-3, 112 his3-Δ200 trp1-1 ade2 Δbar1 pGAL1-HA-IQmotifs (pKT39)* | This work |

All strains are in the W303 background, except RLY 230 and RLY 237, which are the S288C background.

(Li *et al.*, 1995) followed by centrifugation at 200,000 × *g* for 1 h at 4°C.

### *In Vitro Binding Experiments*

Yeast extracts were prepared by the liquid nitrogen grinding method (Sorger and Pelham, 1987) in UB with 1 mM DTT and 1 mM PMSF followed by centrifugation at 14,000 rpm for 30 min. For Cmd1 binding assays, 5 μl GST-Cmd1 beads were added to extracts plus or minus 5 mM $CaCl_2$. After incubation at 4°C for 1 h, the beads were washed three times in UB + 0.1% Triton X-100 before boiling in SDS sample buffer. The proteins were separated by SDS gel electrophoresis and analyzed by immunoblot analysis using the enhanced chemiluminescence detection kit (Amersham, Arlington Heights, IL).

For Tem1p or Cdc42p binding assays, 10 μl beads were loaded with nucleotide by incubation in 100 μl exchange buffer (50 mM Tris, pH 7.5, 5 mM EDTA) with 1 mM GDPβS or GTPγS for 10 min at 30°C. $MgCl_2$ was then added to 10 mM, and beads were placed on ice and pelleted to remove buffer. Yeast extracts in UB with 1 mM DTT and 1 mM PMSF were added and incubated for 1 h at 4°C. The beads were washed and analyzed as described above.

### *Actin Pelleting*

Insect cells expressing Cyk1-myc or HA-Cyk1ΔN were lysed in UB with 1 mM DTT and protease inhibitors by sonication, then spun at 200,000 × *g* for 1 h. Yeast or rabbit muscle actin was prepared as described (Holtzman *et al.*, 1994; Pardee and Spudich, 1982), and polymerized in UB with 1 mM ATP and 0.4 μM phallodin for 30 min at room temperature. Actin was added to the lysates (yeast actin at 3 μM and rabbit actin at 4.5 μM final concentrations), and incubated at 4°C for 1 h. Pelleting was performed by centrifugation at 200,000 × *g* for 1 h. The pellet was resuspended in sample buffer and analyzed by immunoblot analysis using mouse anti-myc (Evan *et al.*, 1985), mouse anti-HA (Babco, Richmond, CA), and mouse anti-actin (Boehringer Mannheim, Indianapolis, IN) antibodies.

## RESULTS

### *Cyk1p Is a Component of the Actomyosin Ring and Is Required for Ring Contraction*

We have recently shown, using GFP-tagged Myo1p, that the budding yeast actomyosin ring exhibits a contraction-like size change during cytokinesis (Lippincott and Li, 1998). For convenience, this event will be referred to as contraction throughout this paper, albeit the lack of a rigorous demonstration of the underlying mechanism. Immunofluorescence staining revealed that Cyk1p colocalizes with this ring during anaphase and disappears from the bud neck sometime later in the cell cycle (Lippincott and Li, 1998). To determine whether Cyk1p is a component of the contractile ring and its precise time of delocalization, a strain was constructed in which Cyk1p was tagged at its COOH terminus with GFP, under the control of the *CYK1* promoter. Cyk1-GFP is capable of rescuing the lethality of Δ*cyk1* cells (our unpublished results). The Cyk1-GFP–expressing cells were observed by time-lapse video microscopy. Figure 1 shows a representa-

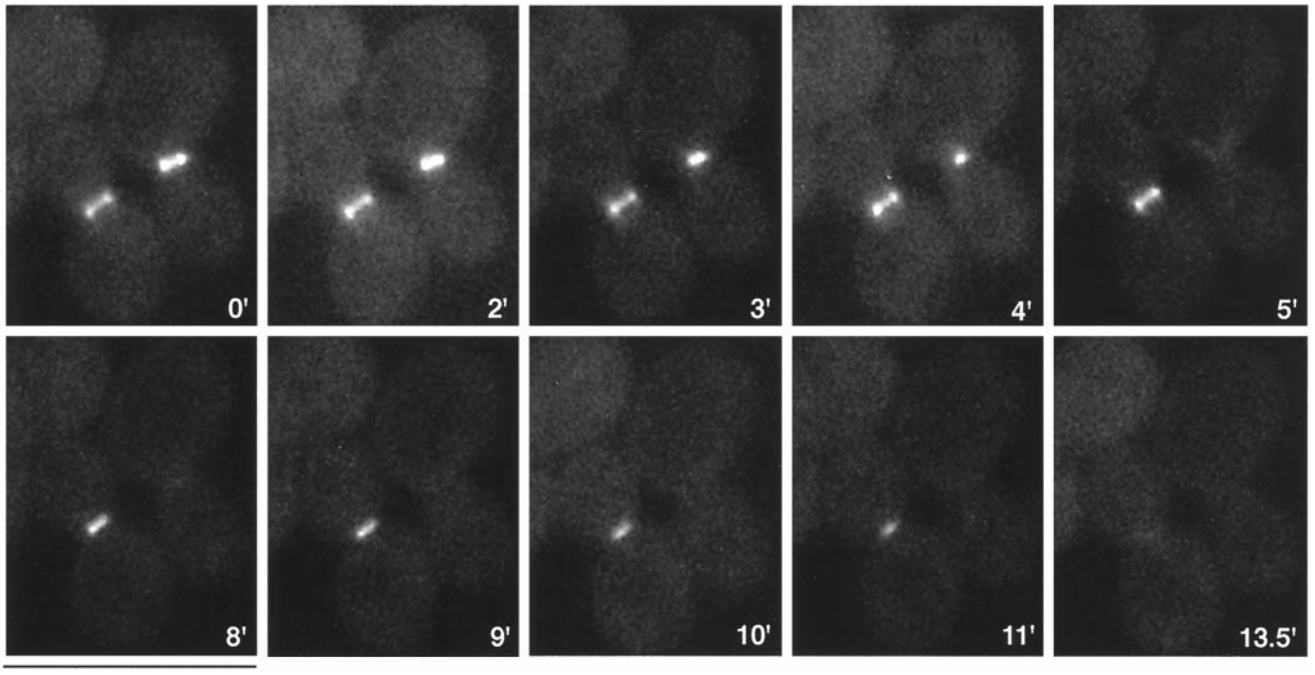


**Figure 1.** In vivo dynamics of the Cyk1-GFP ring. RLY 237 (Cyk1-GFP–expressing) cells were observed by video microscopy as described in MATERIALS AND METHODS. A representative sequence is shown. Bar, 10 $\mu$m.

tive series of two large budded cells expressing Cyk1-GFP undergoing cytokinesis. Over a period of approximately 4 min, the pattern of Cyk1-GFP changed from a band (the side view of a ring) across the neck to a small dot in the center (panels 3′ and 4′ for the right cell, and 8′–11′ for the left cell) and then disappeared within the next minute. Cytokinesis occurred during this time, because a clear septation could be observed between the mother and the daughter by Nomarski optics (our unpublished results). This ring to dot change and the rapid disassembly thereafter are similar to the dynamics observed with Myo1-GFP (Lippincott and Li, 1998), suggesting that Cyk1p is part of the contractile ring during cytokinesis.

To determine whether Cyk1p was required for the contraction of Myo1p, Myo1-GFP was introduced into a strain in which the sole source of Cyk1p was under the control of the repressible *GAL1* promoter. Cells were grown overnight in media containing galactose and raffinose and then arrested in G1 with $\alpha$-mating factor in the presence of 2% glucose to repress the promoter for 3 h. We had demonstrated previously that these conditions lead to a complete turnover of Cyk1p from the cells (Lippincott and Li, 1998). After release of the cells from the G1 arrest into glucose-containing media, Myo1-GFP was observed by video microscopy in cells going through the late stages in the cell cycle in the absence of Cyk1p. In contrast to the behavior of the Myo1p in wild-type cells, the Myo1-GFP ring in all Cyk1$^-$ cells observed did not constrict to a dot, but remained as a band of constant diameter. In 74.5% of cells the Myo1p band diminished in intensity for 4–5 min before disappearing (Figure 2A, panels 5′–7′ for the bottom cell, and 14′–18′ for the top cell). Approximately 45–55 min later, a new bud emerged from one of the cells (our unpublished results), suggesting that the time of ring disappearance coincided roughly with the normal time of cytokinesis. The failure of Myo1p to contract in the absence of Cyk1p is consistent with the observations that Cyk1$^-$ cells lack actin in the ring and that Myo1p cannot contract in the absence of actin (Bi *et al.*, 1998; Lippincott and Li, 1998). In 23% of the cells observed, the uncontracted Myo1p band remained at the old neck, whereas Myo1p also formed a ring at the new bud neck (Figure 2B, the left two panels show two focal planes). A smaller population of cells (2.5%) had a ring of Myo1p at the old neck, but either no Myo1p (Figure 2C, bottom panels) or only a small dot of Myo1p appearing at the neck of the emerging bud (Figure 2C, top panels). This observation suggests that Myo1p disassembly from the ring may be partially impaired in the absence of Cyk1p or contraction.

### *The IQGAP Homology Domains Are All Essential for Cyk1p Function*

The data presented previously (Lippincott and Li, 1998) and the finding that Cyk1p is required for Myo1p contraction suggest a critical role of Cyk1p in

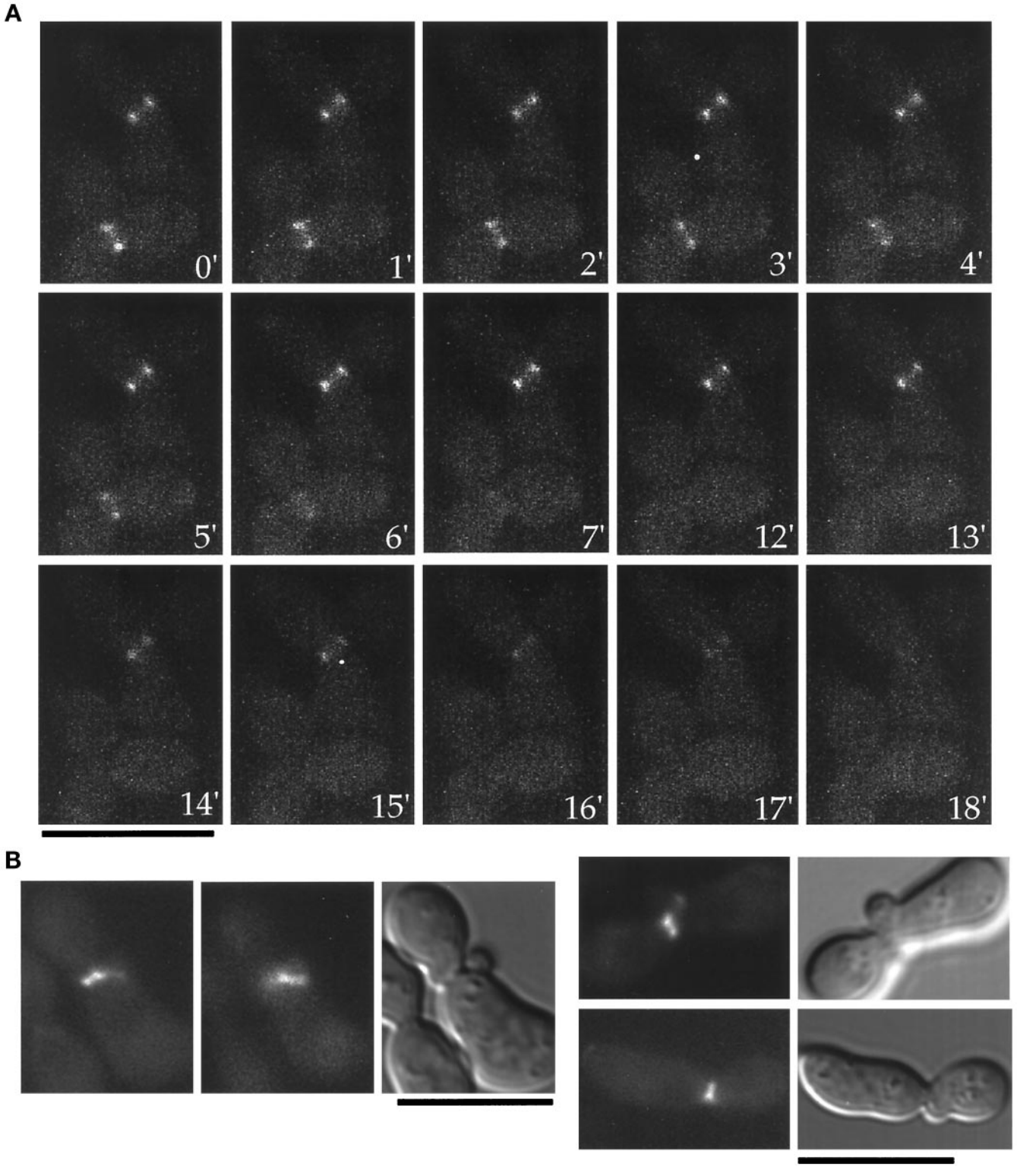


**Figure 2.** Myo1-GFP in Cyk1$^-$ cells. (A) A representative time lapse sequence of the Myo1-GFP ring after *GAL1* promoter shut off of Cyk1p expression. (B) An example of a Cyk1p$^-$ cell with Myo1-GFP located at both old and new bud necks in a cell. Two focal planes of Myo1-GFP are shown, showing old (left) and new (right) rings. (C) Examples of Cyk1p$^-$ cells with Myo1-GFP remaining at old bud neck and failing to form a ring at the new neck. Bars, 10 $\mu$m.

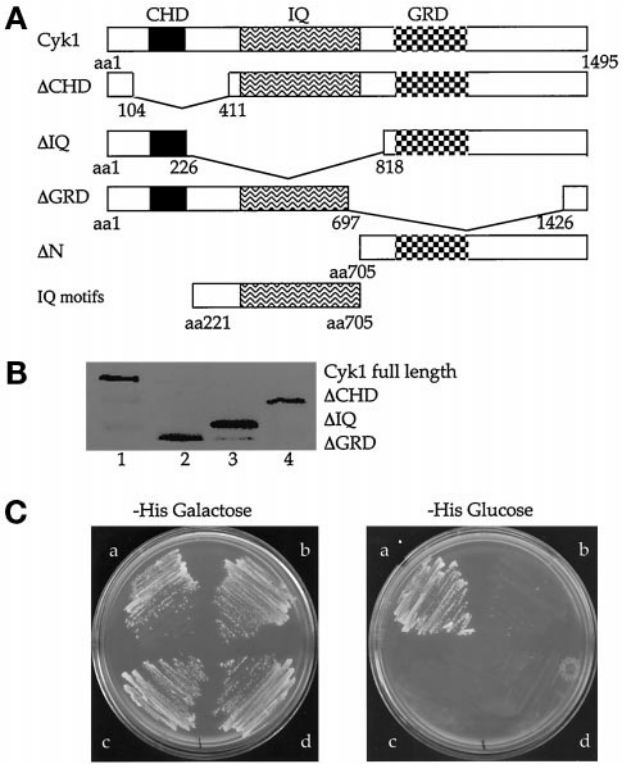


**Figure 3.** Deletion of Cyk1 domains. (A) Schematic diagrams of CYK1 deletion constructs: ΔCHD (pKT 34), ΔIQ (pKT31), ΔGRD (pKT 30), ΔN (pKT33), and IQ motifs (pKT39). (B) Expression of Cyk1 deletions. Extracts were prepared after release from α-factor arrest in glucose (to shut off expression of the full-length Cyk1p expressed under the *GAL1* promoter) and blotted with mouse anti-myc antibody. Lane 1, RLY 565 (full-length Cyk1-myc); lane 2, RLY 555 (ΔGRD); lane 3, RLY 556 (ΔIQ); lane 4, RLY 557 (ΔCHD). (C) Cyk1 deletions do not rescue the Cyk1$^-$ cells. (a) RLY 565, (b) RLY 555, (c) RLY 556, and (d) RLY 557 cells grown on an SC-His galactose plate (left), or on an SC-His glucose plate (right) at 30 °C for 3 d.

regulating the assembly and dynamics of the actomyosin ring. To better understand this role, we have carried out an analysis of the in vivo function of each of the Cyk1 domains homologous to other IQGAPs and implicated in protein interactions. Members of the IQGAP family, including Cyk1p, all contain a CHD, numerous IQ motifs, and a GRD (reviewed in Machesky, 1998). To determine the requirement of these domains in Cyk1p function, a series of deletion constructs were made (Figure 3A). The Cyk1 deletion mutants are all controlled under the *CYK1* promoter and tagged at the COOH terminus with six myc epitopes. RLY277, the strain whose only copy of *CYK1* is under the *GAL1* promoter, was used as the parental strain for the phenotypic analysis. This strain grows as well as wild type on plates containing galactose but is unable to grow in media containing glucose, because *CYK1* is an essential gene in our strain background (Lippincott and Li, 1998). The deletion constructs under the *CYK1* promoter were transformed into RLY277, and all the truncated proteins were expressed at normal levels (Figure 3B). Growth of the strains on galactose plates was normal, but on glucose plates, cells expressing any one of the deletion constructs failed to form colonies (Figure 3C, b–d) and died as chains of lysed cells (our unpublished results), similar to those observed for RLY277 cells on glucose media (Lippincott and Li, 1998). This result indicates that the CHD, IQ motifs, and GRD-containing regions are all essential for Cyk1p function.

### *The Domain Containing the IQ motifs Is Required for the Localization of Cyk1p*

The localization of Cyk1p to the contractile ring at the mother-bud junction is temporally regulated: Cyk1p localizes during anaphase slightly before the appearance of F-actin in the ring. To better understand how Cyk1p is targeted and what role it plays in the assembly of other ring components, we assayed the ability of each of the Cyk1 deletion mutants to localize.

Because IQGAP1 has been shown to oligomerize (Fukata *et al.*, 1997), making it possible for the wild-type protein to bring the deletion mutants to the ring, it was necessary to examine the localization of the mutants in the absence of the wild-type protein. Therefore, the strains constructed by transforming the deletion into RLY277, as described above, were used in this study. Cells were cultured overnight in galactose-containing media also selecting for the plasmid bearing the deletions, and then arrested in G1 with α-factor for 3 h to synchronize the cells. During the arrest, glucose was added to 2% to repress the *GAL1* promoter. The full-length Cyk1p was completely depleted after this arrest (Lippincott and Li, 1998), as confirmed by immunoblot analysis (our unpublished results). Cells were released from the G1 arrest in the glucose-containing media, and multiple time points were taken as cells progressed through M phase. The localization of the deletion mutants was determined by immunofluorescence staining of cells from all time points.

In the parent strain, i.e., RLY277, no Cyk1p localization to the ring was detected after *GAL1* promoter repression (Table 2). The Cyk1ΔGRDp and Cyk1ΔCHDp were able to localize to a ring at the bud neck in anaphase cells (Figure 4 and Table 2), suggesting that the GRD and CHD are not required for Cyk1p localization, although a smaller number of Cyk1ΔCHD rings were observed (Table 2). The deletion of the IQ motifs, by contrast, completely abolished Cyk1p localization: more than 1000 cells were observed by immunofluorescence, and none contained a Cyk1ΔIQ-myc ring (Table 2). This result suggests that Cyk1p is localized to the contractile ring through the domain that contains eight IQ motifs. Actin was also not localized to rings in these cells, consistent

**Table 2.** The formation of Cyk1 (myc) or actin rings in strains bearing Cyk1 deletions

| Strain | No. of cells counted | No. of myc rings | No. of actin rings |
|---|---|---|---|
| RLY 277 (full-length) (arrested and released in galactose) | 655 | 23 | 23 |
| RLY 277 (full-length) | 580 | 0 | 0 |
| RLY 555 (ΔGRD) | 1390 | 35 | 35 |
| RLY 556 (ΔIQ) | 1375 | 0 | 0 |
| RLY 557 (ΔCHD) | 1665 | 25 | 0 |

Cells were arrested with α-factor in the presence of 2% glucose (except as noted), released from arrest, and fixed at 15-min intervals beginning when most cells were budded until cells rebudded. Because of the transient nature of the structure and because we did not want to bias toward a particular timepoint, the numbers are totals from five time points. The numbers are consistent with the observation that approximately 5% of cells in an exponentially growing population have Cyk1p rings. Cyk1p was visualized by immunofluorescence using anti-myc antibody, and actin was stained with rhodamine phallodin.

with the previous conclusion that the localization of Cyk1p is a prerequisite for actin recruitment.

### *The CHD-containing Region Is Required for the Recruitment of Actin to the Ring*

The observations that Cyk1p localizes slightly before and independently of actin and the lack of an actin ring in Cyk1$^-$ cells have indicated that Cyk1p may play a direct role in F-actin recruitment (Epp and Chant, 1997; Lippincott and Li, 1998). The domain of Cyk1p that is likely to carry out this role is the CHD, which in IQGAP1 has been shown to bind actin filaments with high affinity in vitro (Bashour *et al.*, 1997; Fukata *et al.*, 1997). We tested this possibility by examining the presence of actin in the ring in Cyk1ΔCHDp-expressing cells by staining with rhodamine phalloidin. Although Cyk1ΔCHDp can localize to a ring at the bud neck, as described above, none of the rings with Cyk1ΔCHDp contained actin (Figure 4A and Table 2). The Cyk1ΔGRDp rings, by contrast, all contained F-actin as indicated by phalloidin staining (Figure 4B and Table 2). This result suggests that Cyk1p recruits actin filaments through the CHD-containing region to the site of cytokinesis.

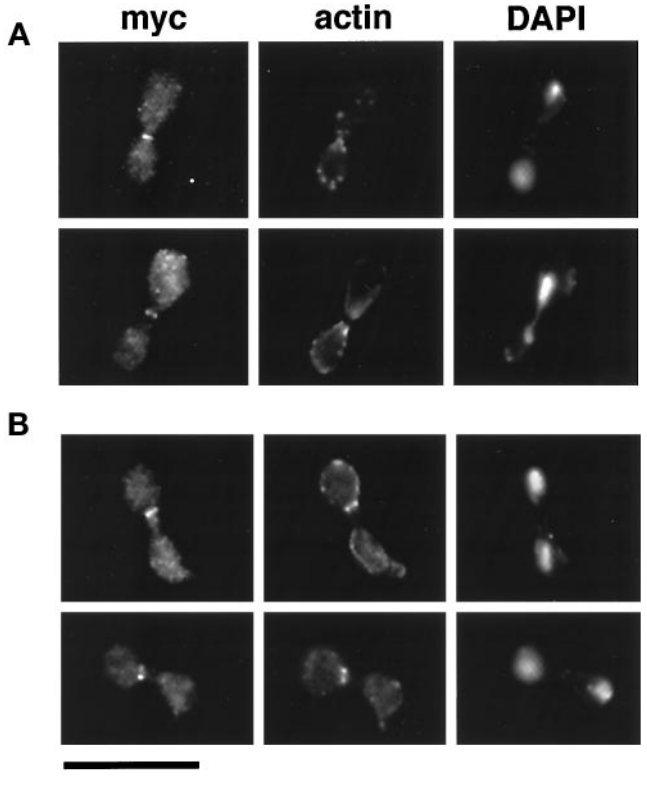


**Figure 4.** Effects of ΔCHD (A) and ΔGRD (B) on the formation of the Cyk1p (myc) and actin rings. Cells were fixed and stained with anti-myc primary, FITC-conjugated anti-mouse secondary antibody (left), rhodamine phallodin (middle), and DAPI (right). Representative cells that have a myc ring are shown. Bar, 10 μm.

### *The GAP-related Domain of Cyk1p Plays a Distinct Role in Actomyosin Ring Contraction*

The finding that Cyk1ΔGRDp was localized to a ring that also contained actin was curious, because like Cyk1$^-$ cells, these cells still had a complete cytokinesis defect: 3 h after the release from the G1 arrest in the glucose media, all of the cells had at least two buds connected with one mother, and the connection was not severed after cells were fixed and the cell wall was removed (our unpublished results). This raised the possibility that the recruitment of actin to the ring is not sufficient for contraction and Cyk1p may play a separate role in ring contraction mediated through the GRD. To test this possibility, we examined the dynamics of Myo1-GFP in cells expressing only Cyk1ΔGRDp but not the full-length Cyk1p. After RLY 555 cells were arrested with α-factor in the presence of glucose for 3 h, cells were released from the arrest into glucose media and observed by time-lapse video microscopy. In these cells, Myo1p localized to a ring encircling the bud neck, indistinguishable from wild-type cells (Figure 5); however, the myosin rings in the Cyk1ΔGRD-

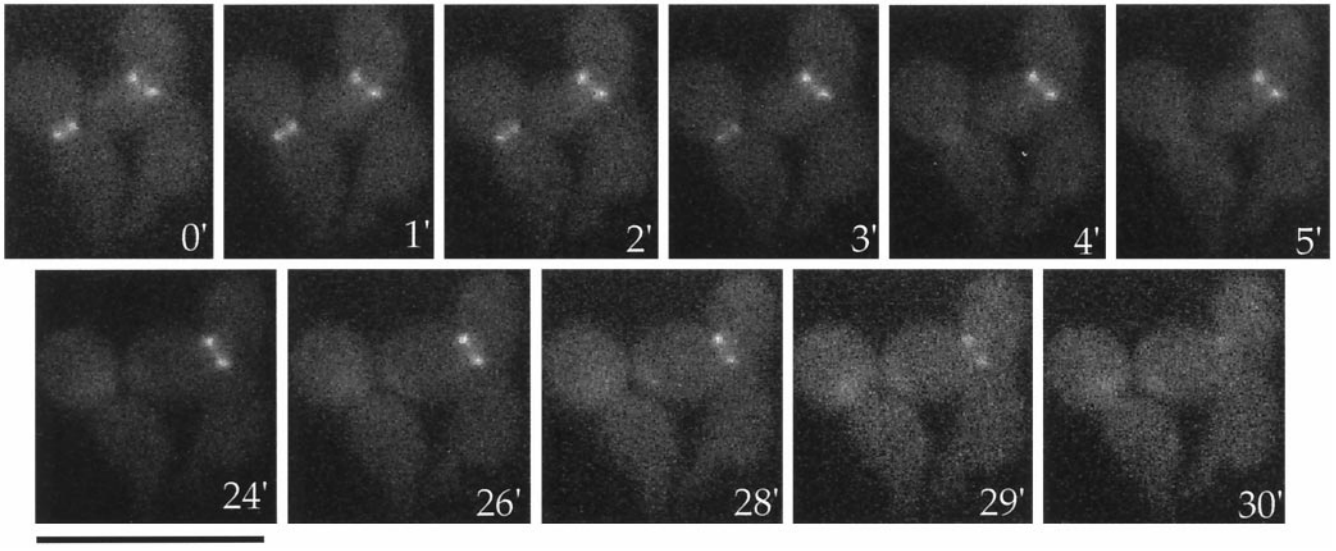


**Figure 5.** Myo1p fails to contract in Cyk1$^-$ cells expressing Cyk1ΔGRD. Myo1-GFP was viewed in RLY558 by time-lapse microscopy after depletion of the full-length protein as described. A representative sequence is shown. Bar, 10 $\mu$m.

expressing cells never initiated contraction before diminishing over a period of a few minutes (panels 3′–5′ for the left cell, 28′–30′ for the bottom cell). These cells later budded again, with Myo1-GFP at the new bud neck, indicating that ring assembly in the next cell cycle was not affected (our unpublished results). This result suggests that the GRD of Cyk1p may be involved in signaling ring contraction or in arranging actin filaments in a configuration critical for contraction.

### *Cyk1p-interacting Proteins Revealed by In Vitro Binding Assays*

Further understanding of the mechanism of Cyk1p function relies on the identification of binding partners of each of the functional domains. Because a number of proteins, including calmodulin, F-actin, and Cdc42p, have been demonstrated to interact directly with the mammalian IQGAP proteins (Brill *et al.*, 1996; Hart *et al.*, 1996; Bashour *et al.*, 1997), we set out to determine whether Cyk1p can bind to a similar set of proteins. First, we tested whether Cyk1p is able to interact with the yeast calmodulin Cmd1p. A GST-Cmd1 fusion protein was expressed in bacteria and purified by affinity with glutathione agarose. The GST-Cmd1 beads were incubated with a yeast extract prepared from the strain that expresses the myc-tagged Cyk1p under the *CYK1* promoter. The beads were then washed, and the bound protein was analyzed by immunoblot analysis using anti-myc as the primary antibody. No interaction was detected between GST-Cmd1 and Cyk1p-myc in the presence of 1 mM EGTA without calcium, or between GST and Cyk1p-myc with or without the added calcium. When 5 mM calcium was added to the extract containing 1 mM EGTA, an interaction between GST-Cmd1 and Cyk1p-myc became apparent (Figure 6A). Surprisingly, the interaction of Cmd1p and Cyk1p is not dependent on the IQ motifs of the latter, because the GST-Cmd1 beads were able to pull down Cyk1ΔIQp (Figure 6B). Furthermore, GST-Cmd1 could not interact with the IQ motifs expressed using the *GAL1* promoter in yeast extracts (Figure 6C). Figure 6D summarizes the interaction of Cmd1p with Cyk1p deletion mutants. These results suggest that the IQ motifs of Cyk1p are neither necessary nor sufficient for interacting with Cmd1.

A copelleting assay was used to test the interaction of Cyk1p with actin filaments. Because Cyk1p from yeast lysates pellets on its own after centrifugation at 200,000 $\times$ *g*, which is necessary to pellet actin filaments, we expressed Cyk1p and Cyk1ΔNp by the baculovirus expression system. A small fraction of the recombinant Cyk1p or Cyk1ΔNp stayed in the high speed supernatant of the baculovirus-infected insect cell lysate. The soluble Cyk1p copelleted with preassembled yeast actin filaments but not with rabbit muscle actin filaments (Figure 7A). Cyk1ΔNp, on the other hand, did not copellet with yeast actin filaments (Figure 7B), demonstrating that the $NH_2$ terminus of Cyk1p is required for actin binding. Although Cyk1ΔNp is a much larger deletion than Cyk1ΔCHDp, the combined results of the actin pelleting and localization experiments are consistent with the idea that the CHD mediates a direct role of Cyk1p in recruiting actin filaments.

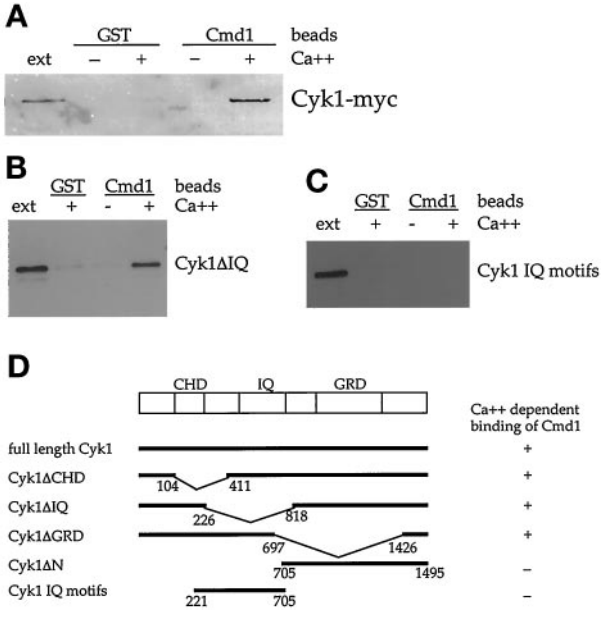


**Figure 6.** Calmodulin binds Cyk1p in the presence of calcium. Cmd1-GST or GST control beads were incubated with extract plus or minus 5 mM $CaCl_2$ as described in MATERIALS AND METHODS. Samples were analyzed by immunoblot analysis. (A) Cmd1-GST binds full-length Cyk1p in the presence of calcium. ext, RLY230 extract. All lanes were immunoblotted with mouse anti-myc antibody. (B) The IQ motifs of Cyk1p are not required for Cmd1p binding. ext, RLY556 extract. Samples were immunoblotted with mouse anti-myc antibody. (C) IQ motifs of Cyk1p are not sufficient for Cmd1p binding. ext, RLY578. Samples were immunoblotted with mouse anti-HA antibody. (D) A schematic diagram summarizing the results from the GST-Cmd1 pull-down experiments using extracts expressing various Cyk1 deletions. Cmd1-GST was able to bind Cyk1ΔIQp, Cyk1ΔGRDp, and Cyk1ΔCHDp in the presence (but not absence) of calcium. Cmd1p was unable to bind either Cyk1ΔN or IQ motifs overexpressed in yeast extracts.

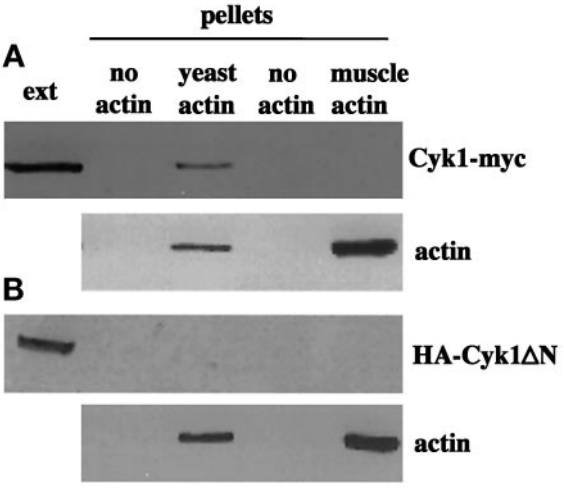


**Figure 7.** Cyk1p copellets with yeast actin filaments. Fifty microliters of insect cell lysate were incubated with or without prepolymerized yeast actin (3 $\mu$M) or rabbit muscle actin (4.5 $\mu$M), and then centrifuged for 1 h at 200,000 $\times$ *g*. Pellets were resuspended in 40 $\mu$l sample buffer and analyzed by immunoblot. (A) Top panel, insect cell extract expressing full-length Cyk1-myc incubated with or without actin, pelleted, and blotted with anti-myc antibody. Bottom panel, the same membrane blotted with mouse anti-actin antibody. (B) Top panel, insect cell extract expressing Cyk1ΔN incubated with or without actin, pelleted, and blotted with anti-HA antibody. Bottom panel, the same membrane blotted with anti-actin. ext, extracts after clearing spin, before addition of actin.

The mammalian IQGAPs have been reported to bind Cdc42p (Brill *et al.*, 1996; Hart *et al.*, 1996; McCallum *et al.*, 1996), a Rho-family small GTP-binding protein implicated in regulating actin cytoskeleton organization (reviewed in Nobes and Hall, 1995). To test the interaction of budding yeast Cdc42p with Cyk1p, we expressed GST-Cdc42 protein by the baculovirus expression system and coupled the protein to glutathione beads. Two other small GTP-binding proteins, Rho1p (required for bud growth) (Yamochi *et al.*, 1994) and Tem1p (required for the completion of anaphase) (Shirayama *et al.*, 1994) were expressed in *E. coli* as GST fusion proteins and coupled to glutathione beads. The beads, carrying equivalent amounts of each of the small GTP-binding proteins, were preloaded with either GTP-$\gamma$S or GDP-$\beta$S and incubated with a yeast extract prepared from the strain that expresses the myc-tagged Cyk1p under the *CYK1* promoter. The beads were then washed, and the bound protein was analyzed by immunoblot analysis. Surprisingly, no interaction was detected between Cdc42p and Cyk1p or Rho1p and Cyk1p, but Tem1p loaded with either nucleotide showed strong interaction with Cyk1p (Figure 8A). The interaction between Tem1p and Cyk1p was abolished by GRD deletion but not by CHD or IQ motif deletions, suggesting that the interaction is dependent specifically on the GRD (Figure 8B). GST-Tem1p was also able to interact with Cyk1ΔNp expressed either in yeast or in baculovirus-infected insect cells (Figure 8, C and D). GST-Cdc42, on the other hand, did not show any affinity with Cyk1ΔN overexpressed in yeast using the *GAL1* promoter (Figure 8C) and only a weak interaction with Cyk1ΔN expressed at a much higher level in baculovirus-infected cells (Figure 8D). Because the same concentration of Tem1p and Cdc42p (2 $\mu$M) was used in this experiment, it is evident that the affinity between Tem1p and the GRD of Cyk1p is higher than that between Cdc42 and the GRD of Cyk1p. These results suggest that Tem1p is a more plausible in vivo partner for Cyk1p than Cdc42p.

## DISCUSSION

### *Cyk1p Has Multiple Essential Roles in the Assembly and Contraction of the Actomyosin Ring*

Three previous observations had led us to suspect that Cyk1p may be the actin filament-recruiting protein in

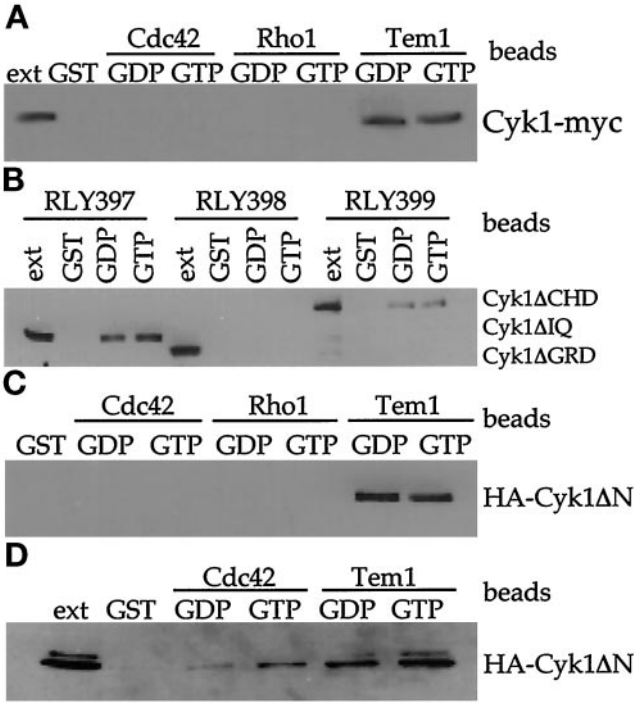


**Figure 8.** Tem1p interacts with Cyk1p through the GRD. (A) Cyk1p interacts with Tem1p. RLY230 (full-length Cyk1-myc–expressing) extract was incubated with GST fusion proteins coupled to glutathione agarose beads and loaded with nucleotide (GDP$\beta$S or GTP$\gamma$S) as indicated above each lane. Beads were washed, boiled, and analyzed by immunoblotting with mouse anti-myc. ext, RLY230 extract. (B) The GRD of Cyk1p is required for Tem1p interaction. Extracts from RLY397($\Delta$IQ), 398($\Delta$GRD), or 399($\Delta$CHD) were incubated with GST or GST-TEM1 proteins coupled to glutathione agarose beads and loaded with GDP$\beta$S or GTP$\gamma$S as indicated. Beads were washed, boiled, and analyzed by immunoblotting with mouse anti-myc antibody. ext, extract from RLY 397, 398, or 399. (C) Tem1p interacts with Cyk1$\Delta$Np expressed in yeast. RLY458 extract was incubated with GST fusion proteins coupled to glutathione agarose beads and loaded with GDP$\beta$S or GTP$\gamma$S as indicated. Beads were washed, boiled, and analyzed by immunoblotting with mouse anti-HA antibody. (D) Tem1p interacts with Cyk1$\Delta$N expressed in insect cells. Extract was prepared from insect cells infected with Cyk1$\Delta$N virus and incubated with GST-fusion proteins coupled to glutathione agarose beads and loaded with GDP$\beta$S or GTP$\gamma$S as indicated. Beads were washed, boiled, and analyzed by immunoblotting with mouse anti-HA antibody. ext, Cyk1$\Delta$N-expressing insect cell extract.

the "cleavage furrow," the bud neck of *S. cerevisiae* cells. First, the localization of Cyk1p to a ring at the mother-bud junction occurs before the appearance of a superimposable actin ring (Lippincott and Li, 1998a). Second, Cyk1p localization is independent of actin filaments (Epp and Chant, 1997). Third, actin was not localized to the ring in cells that went through the cell cycle in the absence of Cyk1p (Lippincott and Li, 1998a). Because Cyk1p shares the IQGAP domains that interact with Cdc42p (Brill *et al.*, 1996; Hart *et al.*, 1996; McCallum *et al.*, 1996), a Rho family small GTP-binding protein (Johnson and Pringle, 1990), and calmodulin (Brill *et al.*, 1996; Hart *et al.*, 1996), a key mediator of calcium signaling (Head, 1992), we hypothesized that Cyk1p may link multiple signaling pathways to actomyosin ring activity during anaphase. In this study, we have investigated the role of Cyk1p in the assembly and function of the actomyosin ring. Video microscopy analysis of Cyk1-GFP–expressing cells revealed that Cyk1p is a component of the actomyosin ring during contraction, because the Cyk1-GFP ring, like the Myo1-GFP ring (Bi *et al.*, 1998; Lippincott and Li, 1998a), undergoes a contraction-like size change during cytokinesis. This is in contrast to the septins that are also required for cytokinesis, but the structure that they form around the bud neck does not undergo contraction-like changes in size during cytokinesis (Lippincott and Li, 1998b), suggesting that different components of the bud neck are involved in structures with different dynamic properties.

To identify the function associated with each of the IQGAP homology domains of Cyk1p, we generated Cyk1p deletion mutants lacking each domain and analyzed their effects on the assembly and activity of the actomyosin ring. All of the deletions abolished Cyk1p function and resulted in a cytokinesis failure. Deletion of an $NH_2$-terminal portion that contains the CHD does not affect the localization of Cyk1p but prevents the accumulation of actin filaments to the ring. This result, together with the findings that Cyk1p binds specifically to yeast actin filaments and that the $NH_2$-terminal half is required for this interaction, strongly supports the hypothesis that Cyk1p directly recruits actin filaments to the actomyosin ring. The Cyk1p domain rich in IQ motifs, on the other hand, is required for the localization of Cyk1p to the bud neck. Actin is also absent from the ring in cells lacking this domain, most likely as a consequence of the Cyk1p localization defect. The IQ-rich domain may bind directly to a preexisting bud neck protein or to a protein that mediates the binding of other parts of Cyk1p with the bud neck. The presence of the eight IQ motifs is significant because calcium signaling has been implicated in cytokinesis (Snow and Nuccitelli, 1993; Muto *et al.*, 1996), but the target of calcium and the event that it regulates are not clear. Our result raises the possibility that a calmodulin-like protein may mediate calcium regulation of actin filament recruitment to the cleavage furrow. In vitro, Cyk1p does bind calmodulin in a calcium-dependent manner, but this interaction is not dependent on the IQ-rich domain. The identification of the IQ domain-interacting protein(s) may reveal how Cyk1p is recruited to the bud neck and how Cyk1p localization is regulated.

The role of Cyk1p in cytokinesis is not simply to recruit actin filaments to the contractile ring, because cells lacking the COOH terminal portion of Cyk1p harboring the GRD can still assemble actin filaments at

the neck, and yet the actomyosin ring fails to contract and cytokinesis does not occur. Two potential functions of Cyk1p may account for this defect. First, Cyk1p may mediate a specific signal required to trigger ring contraction after the proper assembly of actin and myosin II filaments to the cleavage furrow. Such a signal may be important for ensuring that cytokinesis does not occur until the completion of chromosome segregation to the poles. The GRD of Cyk1p may also function in a structural capacity, because it may be involved in organizing the recruited actin filaments into structures capable of generating contractile force. The mammalian IQGAP1 has been shown to form oligomers that can cross-link actin filaments, and Cdc42 binding to the GRD enhances the oligomerization (Fukata *et al.*, 1997). It remains to be tested whether Cyk1p has a similar activity mediated by the GRD.

### *Cyk1-interacting Proteins*

As mentioned above, Cyk1p shares two of the IQGAP-interacting proteins, F-actin and calmodulin. The F-actin binding activity of IQGAP1 has been attributed to the CHD, although a recent mutagenesis study showed that the minimum calponin homology region of calponin is not sufficient for actin binding (Gimina and Mital, 1998). The F-actin binding ability of Cyk1p depends on the $NH_2$-terminal portion containing the CHD. Cyk1p binds only yeast actin and not rabbit muscle actin, which are 86% identical. The structural basis for this binding specificity may be of interest. Calmodulin was a predicted binding partner of IQGAPs because of the presence of tandem IQ motifs. Consistently, IQGAP1 and IQGAP2 were shown to bind calmodulin in a calcium-independent manner, and the domain responsible for the interaction was mapped to the $NH_2$-terminal half of the proteins that harbor the IQ motifs (Brill *et al.*, 1996; Hart *et al.*, 1996). In vitro, calmodulin binding appears to modulate the interaction of IQGAP1 with Cdc42 and actin (Bashour *et al.*, 1997; Joyal *et al.*, 1997). We have detected a calcium-dependent interaction of the yeast calmodulin (Cmd1p) with Cyk1p, but surprisingly, the IQ-rich domain of Cyk1p is neither necessary nor sufficient for calmodulin interaction. The interaction between Cyk1p and calmodulin is likely to bear in vivo significance, because Cmd1p also localizes to the mother-bud junction during cytokinesis. Cmd1 localization is dependent on actin and the IQ motifs of Myo2p (Brockerhoff and Davis, 1992; Stevens and Davis, 1998). Calmodulin has also been reported to be delocalized in a strain lacking Cyk1p (Osman and Cerione, 1998). An interaction between calmodulin and Rng2p, the fission yeast IQGAP homologue, was recently demonstrated by coimmunoprecipitation experiments, and this interaction appears to be important for the localization of calmodulin to the site of septation (Eng *et al.*, 1998). It is not yet known whether calmodulin has a direct role in actomyosin ring assembly or contraction.

IQGAPs all share a COOH terminal portion highly homologous to Sar1p, a RasGAP from *Schizosaccharomyces pombe* (Wang *et al.*, 1991); however, neither IQGAP1 nor IQGAP2 exhibits GAP activity toward Ras, but both bind Cdc42 and Rac, two Rho family small GTP-binding proteins (Brill *et al.*, 1996; Hart *et al.*, 1996; McCallum *et al.*, 1996). One property that may distinguish IQGAP1 and IQGAP2 is that the former binds the GTP-bound Cdc42 or Rac with higher affinity than the GDP-bound form, whereas the latter does not seem to have a nucleotide preference. This property has led to the hypothesis that IQGAP1 may be an important target of the activated Cdc42 in the regulation of actin cytoskeleton organization. Consistent with this idea, IQGAP1 appears to concentrate in actin-rich structures such as membrane ruffles (Hart *et al.*, 1996). Direct demonstration of the in vivo function of mammalian IQGAPs is still lacking. Genetic analysis in both budding yeast and fission yeast have demonstrated an essential and specific function of IQGAP-like proteins in cytokinesis (Epp and Chant, 1997; Eng *et al.*, 1998; Lippincott and Li, 1998a), suggesting that an involvement in actin-dependent processes is conserved for all IQGAP family members; however, we could not detect any interaction of the budding yeast Cdc42p with Cyk1p expressed at endogenous levels in yeast extracts. Cyk1p did show a strong interaction with Tem1p, another Ras superfamily small GTPase (Shirayama *et al.*, 1994). Although this interaction does not seem to be affected by the nucleotide-bound state of Tem1p, Tem1-GTP and Tem1-GDP could have different structural effects on Cyk1p. Furthermore, the GRD of Cyk1p was necessary and sufficient for this interaction. An interaction with Cdc42p was only detected when the GRD was expressed at a much higher level using the baculovirus expression system, but the affinity appears to be at least threefold lower than that between the GRD and Tem1p. These results suggest that Tem1p is a more likely GRD-binding protein in vivo than Cdc42p. Consistent with this possibility, it was reported that overexpression of the GRD-containing COOH-terminal portion of IQGAP1 in yeast results in a cell polarization defect that can be rescued by Cdc42 overexpression (Hart *et al.*, 1996), but overexpression of the analogous region of Cyk1p, Cyk1ΔNp, does not have such a detrimental effect on cells (our unpublished results), consistent with its lack of interaction with the endogenous Cdc42p.

Tem1p and its fission yeast homolog, Spg1p, represent a subfamily of Ras-like small GTP-binding proteins that do not seem to contain prenylation sites at the COOH terminus (Shirayama *et al.*, 1994; Schmidt *et al.*, 1997). Spg1p is concentrated in the spindle pole

body and has been shown to be specifically required for cytokinesis but not for cell cycle progression (Schmidt *et al.*, 1997). Tem1p-deficient budding yeast cells also cannot carry out cytokinesis; however, this defect has been thought to result from an anaphase arrest (Shirayama *et al.*, 1994). Determining whether Tem1p plays a direct role in cytokinesis requires the ability to bypass the cell cycle arrest when the function of the protein is impaired. Cdc42p regulates the establishment of cell polarity in budding yeast. Its role in the reorganization of the actin cytoskeleton has been demonstrated in many cell types (reviewed in Nobes and Hall, 1995). We have not detected, however, any effect of the dominant negative *CDC42*$^{A118}$ mutation on cytokinesis once the bud has formed in its absence (our unpublished result). Recently, it was suggested that Iqg1p (Cyk1p) mediates Cdc42 effects on the actin cytoskeleton on the basis of a two-hybrid interaction (Osman and Cerione, 1998). The in vivo relevance of this interaction remains to be demonstrated.

## ACKNOWLEDGMENTS

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## REFERENCES

Andreassen, P.R., Palmer, D.K., Wener, M.H., and Margolis, R.L. (1991). Telophase disk: a new mammalian mitotic organelle that bisects telophase cells with a possible function in cytokinesis. J. Cell Sci. *99*, 523–534.

Balasubramanian, M.K., Helfman, D.M., and Hemmingsen, S.M. (1992). A new tropomyosin essential for cytokinesis in the fission yeast *S. pombe*. *360*, 84–87.

Bashour, A.M., Fullerton, A.T., Hart, M.J., and Bloom, G.S. (1997). IQGAP1, a Rac and Cdc42-binding protein, directly binds and cross-links microfilaments. J. Cell Biol. *137*, 1555–1566.

Bi, E., Maddox, P., Lew, D.J., Salmon, E.D., McMillan, J.N., Yeh, E., and Pringle, J.R. (1998). Involvement of an actomyosin contractile ring in *Saccharomyces cerevisiae* cytokinesis. J. Cell Biol. *142*, 1301–1312.

Brill, S., Li, S., Lyman, C.W., Church, D.M., Wasmuth, J.J., Weissbach, L., Bernards, A., and Snijders, A.J. (1996). The Ras GTPase-activating-protein-related human protein IQGAP2 harbors a potential actin binding domain and interacts with calmodulin and Rho family GTPases. Mol. Cell. Biol. *16*, 4869–4878.

Brockerhoff, S.E., and Davis, T.N. (1992). Calmodulin concentrates at regions of cell growth in *Saccharomyces cerevisiae*. J. Cell Biol. *118*, 619–629.

Cao, L.G., and Wang, Y.L. (1990a). Mechanism of the formation of contractile ring in dividing cultured animal cells. II. Cortical movement of microinjected actin filaments. J. Cell Biol. *111*, 1905–1911.

Cao, L.G., and Wang, Y.L. (1990b). Mechanism of the formation of contractile ring in dividing cultured animal cells. I. Recruitment of preexisting actin filaments into the cleavage furrow. J. Cell Biol. *110*, 1089–1095.

De Lozanne, A., and Spudich, J.A. (1987). Disruption of the Dictyostelium myosin heavy chain gene by homologous recombination. Science *236*, 1086–1091.

Eng, K., Naqvi, N.I., Wong, K.C.Y., and Balasubramanian, M.K. (1998). Rng2, a protein required for cytokinesis in fission yeast, is a component of the actomyosin ring and spindle pole body. Curr. Biol. *8*, 611–621.

Epp, J.A., and Chant, J. (1997). An IQGAP-related protein controls actin-ring formation and cytokinesis in yeast. Curr. Biol. *7*, 921–929.

Evan, G.I., Lewis, G.K., Ramsay, G., and Bishop, J.M. (1985). Isolation of monoclonal antibodies specific for human c-*myc* proto-oncogene product. Mol. Cell. Biol. *5*, 3610–3616.

Fishkind, D.J., and Wang, Y.L. (1995). New horizons for cytokinesis. Curr. Opin. Cell Biol. *7*, 23–31.

Fujiwara, K., Porter, M.E., and Pollard, T.D. (1978). $\alpha$-actinin localization in the cleavage furrow during cytokinesis. J. Cell Biol. *79*, 268–275.

Fukata, M., Kuroda, S., Fujii, K., Nakamura, T., Shoji, I., Matsuura, Y., Okawa, K., Iwamatsu, A., Kikuchi, A., and Kaibuchi, K. (1997). Regulation of cross-linking of actin filament by IQGAP1, a target for Cdc42. J. Biol. Chem. *272*, 29579–29583.

Gimina, M., and Mital, R. (1998). The single CH domain of calponin is neither sufficient nor necessary for F-actin binding. J. Cell Sci. *111*, 1813–1821.

Hart, M.J., Callow, M.G., Souza, B., and Polakis, P. (1996). IQGAP1, a calmodulin-binding protein with a rasGAP-related domain, is a potential effector for cdc42Hs. EMBO J. *15*, 2997–3005.

Hayashi, T., Silver, R.B., Ip, W., Cayer, M.L., and Smith, D.S. (1977). Actin-myosin interaction. Self-assembly into a bipolar "contractile unit." J. Mol. Biol. *111*, 159–171.

Head, J.F. (1992). A better grip on calmodulin. Curr. Biol. *2*, 609–611.

Holtzman, D.A., Wertman, K.F., and Drubin, D.G. (1994). Mapping actin surfaces required for functional interactions in vivo. J. Cell Biol. *126*, 423–432.

Johnson, D.I., and Pringle, J.R. (1990). Molecular characterization of *CDC42*, a *Saccharomyces cerevisiae* gene involved in the development of cell polarity. J. Cell Biol. *111*, 143–152.

Joyal, J.L., Annan, R.S., Ho, Y.-D., Huddleston, M.E., Carr, S.A., Hart, M.J., and Sacks, D.B. (1997). Calmodulin modulates the interaction between IQGAP1 and Cdc42. J. Biol. Chem. *272*, 15419–15425.

Kaiser, C., Michaelis, S., and Mitchell, A. (1994). Methods in Yeast Genetics, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Kitayama, C., Sugimoto, A., and Yamamoto, M. (1997). Type II myosin heavy chain encoded by the *myo2* gene composes the contractile ring during cytokinesis in *Schizosacchoramyces pombe*. J. Cell Biol. *137*, 1309–1319.

Knecht, D.A., and Loomis, W.F. (1987). Antisense RNA inactivation of myosin heavy chain gene expression in *Dictyostelium discoideum*. Science *236*, 1081–1086.

Kubota, H.Y., Yoshimoto, Y., and Hiramoto, Y. (1993). Oscillation of intracellular free calcium in cleaving and cleavage-arrested embryos of *Xenopus laevis*. Dev. Biol. *160*, 512–518.

Li, R., Zheng, Y., and Drubin, D. (1995). Regulation of cortical actin cytoskeleton assembly during polarized cell growth in budding yeast. J. Cell Biol. *128*, 599–615.

Lippincott, J., and Li, R. (1998a). Sequential assembly of myosin II, an IQGAP-like protein, and filamentous actin, to a ring structure involved in budding yeast cytokinesis. J. Cell Biol. *140*, 355–366.

Lippincott, J., and Li, R. (1998b). Dual function of Cyk2, a cdc15/PSTPIP family protein in regulating actomyosin ring dynamics and septin distribution. J. Cell Biol. *143*, 1947–1960.

Liu, T., Williams, J.G., and Clarke, M. (1992). Inducible expression of calmodulin antisense RNA in *Dictyostelium* cells inhibits the completion of cytokinesis. Mol. Biol. Cell *3*, 1403–1413.

Machesky, L.M. (1998). Cytokinesis: IQGAPs find a function. Curr. Biol. *8*, R202–205.

Martinez, P.M., Rodriguez, G., Reyna, G., Calvo, M.C., and Ruiz, H.J. (1989). Inhibition of the yeast-mycelial transition and the photogenesis of Mucorales by diamino butanone. Arch. Microbiol. *151*, 10–14.

May, K.M., Watts, F.Z., Jones, N., and Hyams, J.S. (1997). Type II myosin involved in cytokinesis in the fission yeast *Schizosaccharomyces pombe*. Cell Motil. Cytoskeleton *38*, 385–396.

McCallum, S.J., Wu, W.J., and Cerione, R.A. (1996). Identification of a putative effector for Cdc42Hs with high sequence similarity to the RasGAP-related protein IQGAP1 and a Cdc42Hs binding partner with similarity to IQGAP2. J. Biol. Chem. *271*, 21732–21737.

Muto, A., Kume, S., Inoue, T., Okano, H., and Mikoshiba, K. (1996). Calcium waves along the cleavage furrows in cleavage-stage *Xenopus* embryos and its inhibition by heparin. J. Cell Biol. *135*, 181–190.

Neujahr, R., Heizer, C., and Gerisch, G. (1997). Myosin II-independent processes in mitotic cells of *Dictyostelium discoideum*: redistribution of the nuclei, rearrangement of the actin system and formation of the cleavage furrow. J. Cell Sci. *110*, 123–137.

Nobes, C.D., and Hall, A. (1995). Rho, Rac, and Cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. Cell *81*, 53–62.

Osman, M.A., and Cerione, R.A. (1998). Igq1p, a yeast homologue of the mammalian IQGAPs, mediates Cdc42p effects on the actin cytoskeleton. J. Cell Biol. *142*, 443–455.

Pardee, J.D., and Spudich, J.A. (1982). Purification of muscle actin. Methods Enzymol. *85*, 164–181.

Patterson, G.M., Smith, C.D., Kimura, L.H., Britton, B.A., and Carmeli, S. (1993). Action of tolytoxin on cell morphology, cytoskeletal organization, and actin polymerization. Cell Motil. Cytoskeleton *24*, 39–48.

Pollard, T.D., Satterwhite, L., Cisek, L., Corden, J., Sato, M., and Maupin, P. (1990). Actin and myosin biochemistry in relation to cytokinesis. Ann. NY Acad. Sci. *582*, 120–130.

Satterwhite, L.L., Lohka, M.J., Wilson, K.L., Scherson, T.Y., Cisek, L.J., Corden, J.L., and Pollard, T.D. (1992). Phosphorylation of myosin-II regulatory light chain by cyclin-p34cdc2: a mechanism for the timing of cytokinesis. J. Cell Biol. *118*, 595–605.

Satterwhite, L.L., and Pollard, T.D. (1992). Cytokinesis. Curr. Opin. Cell Biol. *4*, 43–52.

Schmidt, S., Sohrmann, M., Hofmann, K., Woolard, A., and Simanis, V. (1997). The Spg1p GTPase is an essential, dosage-dependent inducer of septum formation in *Schizosaccharomyces pombe*. Genes Dev. *11*, 1519–1534.

Schroeder, T.E. (1972). The contractile ring. II. Determining its brief existence, volumetric changes, and vital role in cleaving Arbacia eggs. J. Cell Biol. *53*, 419–434.

Schroeder, T.E. (1990). The contractile ring and furrowing in dividing cells. Ann. NY Acad. Sci. *582*, 78–87.

Sellers, J.R., Eisenberg, E., and Adelstein, R.S. (1982). The binding of smooth muscle heavy meromyosin to actin in the presence of ATP. J. Biol. Chem. *257*, 13880–13883.

Sherman, F., Fink, G., and Lawrence, C. (1974). Methods in Yeast Genetics, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Shirayama, M., Matsui, Y., and Toh-E, A. (1994). The yeast TEM1 gene, which encodes a GTP-binding protein, is involved in termination of M phase. Mol. Cell. Biol. *14*, 7476–7482.

Sikorski, R.S., and Hieter, P. (1989). A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics *122*, 19–27.

Snow, P., and Nuccitelli, R. (1993). Calcium buffer injections delay cleavage in *Xenopus laevis* blastomeres. J. Cell Biol. *122*, 387–394.

Sorger, P.K., and Pelham, H.R. (1987). Purification and characterization of a heat-shock element binding protein from yeast. EMBO J. *6*, 3035–3041.

Stevens, R.C., and Davis, T.N. (1998). Mlc1p is a light chain for the unconventional myosin myo2p in *Saccharomyces cerevisiae*. J. Cell Biol. *142*, 711–722.

Striker, S.A. (1995). Time-lapse confocal imaging of calcium dynamics in starfish embryos. Dev. Biol. *170*, 496–518.

Waddle, J.A., Karpova, T.S., Waterson, R.H., and Cooper, J.A. (1996). Movement of cortical actin patches in yeast. J. Cell Biol. *132*, 861–870.

Wang, Y., Boguski, M., Riggs, M., Rodgers, L., and Wigler, M. (1991). sar1, a gene from *Schizosaccharomyces pombe* encoding a protein that regulates ras1. Cell Regul. *2*, 453–465.

Watts, F.Z., Shiels, G., and Orr, E. (1987). The yeast *MYO1* gene encoding a myosin-like protein required for cell division. EMBO J. *6*, 3499–3505.

Yamakita, Y., Yamashiro, S., and Matsumura, F. (1994). In vivo phosphorylation of regulatory light chain of myosin II during mitosis of cultured cells. J. Cell Biol. *124*, 129–137.

Yamochi, W., Tanaka, K., Nonaka, H., Maeda, A., Musha, T., and Takai, Y. (1994). Growth site localization of Rho1 small GTP-binding protein and its involvement in bud formation in *Sacharomyces cerevisiae*. J. Cell Biol. *125*, 1077–1093.

Zurek, B., Sanger, J.M., Sanger, J.W., and Jockusch, B.M. (1990). Differential effects of myosin-antibody complexes on contractile rings and circumferential belts in epitheloid cells. J. Cell Sci. *97*, 297–306.