Identification of Yeast IQGAP (Iqg1p) as an Anaphase-Promoting-Complex Substrate and Its Role in Actomyosin-Ring-Independent Cytokinesis[□]

Nolan Ko,^{*†} Ryuichi Nishihama,^{*†} Gregory H. Tully,[‡] Denis Ostapenko,[§] Mark J. Solomon,[§] David O. Morgan,[‡] and John R. Pringle^{*†}

^{*}Department of Biology, University of North Carolina, Chapel Hill, NC 27599; [†]Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305; [‡]Department of Physiology, University of California, San Francisco, San Francisco, CA 94143; and [§]Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520

Submitted May 30, 2007; Revised October 1, 2007; Accepted October 9, 2007

Monitoring Editor: Orna Cohen-Fix

In the yeast *Saccharomyces cerevisiae*, a ring of myosin II forms in a septin-dependent manner at the budding site in late G1. This ring remains at the bud neck until the onset of cytokinesis, when actin is recruited to it. The actomyosin ring then contracts, septum formation occurs concurrently, and cytokinesis is soon completed. Deletion of *MYO1* (the only myosin II gene) is lethal on rich medium in the W303 strain background and causes slow-growth and delayed-cell-separation phenotypes in the S288C strain background. These phenotypes can be suppressed by deletions of genes encoding nonessential components of the anaphase-promoting complex (APC/C). This suppression does not seem to result simply from a delay in mitotic exit, because overexpression of a nondegradable mitotic cyclin does not suppress the same phenotypes. Overexpression of either *IQG1* or *CYK3* also suppresses the *myo1Δ* phenotypes, and Iqg1p (an IQGAP protein) is increased in abundance and abnormally persistent after cytokinesis in APC/C mutants. In vitro assays showed that Iqg1p is ubiquitinated directly by APC/C^{Cdh1} via a novel recognition sequence. A nondegradable Iqg1p (lacking this recognition sequence) can suppress the *myo1Δ* phenotypes even when expressed at relatively low levels. Together, the data suggest that compromise of APC/C function allows the accumulation of Iqg1p, which then promotes actomyosin-ring-independent cytokinesis at least in part by activation of Cyk3p.

INTRODUCTION

Cytokinesis is the process that divides the cell surface and cytoplasm of one cell into two cells. Although the list of proteins known to be involved in cytokinesis has expanded significantly in recent years, a complete molecular understanding of this process remains elusive. Among the mysteries are the roles of the septins and of the actomyosin contractile ring. The septins are a family of GTP-binding proteins that have been found at the division site in all fungal and animal cells examined (Longtine *et al.*, 1996; Hall and Russell, 2004; Gladfelter, 2006). Although their roles are still imperfectly understood, the septins seem to function as both a scaffold and a diffusion barrier for the localization and organization of other proteins (Gladfelter *et al.*, 2001; Longtine and Bi, 2003; Dobbelaere and Barral, 2004; Versele and Thorner, 2005; Spiliotis and Nelson, 2006). Surprisingly, although the septins are indispensable for cytokinesis in

some cell types, they are dispensable in others (Longtine *et al.*, 1996; Adam *et al.*, 2000; Nguyen *et al.*, 2000; Kinoshita and Noda, 2001; An *et al.*, 2004). Similarly, it has recently become clear that the actomyosin ring at the division site is also dispensable for cytokinesis in a variety of cell types (Bi *et al.*, 1998; Nagasaki *et al.*, 2002; Kanada *et al.*, 2005). In the budding yeast *Saccharomyces cerevisiae*, the septins are essential for formation of the actomyosin ring. However, this cannot be their only role, because they are essential for cytokinesis in this organism, whereas the actomyosin ring is not essential in most strain backgrounds (Bi *et al.*, 1998; Schmidt *et al.*, 2002; Lord *et al.*, 2005; our unpublished results). A major challenge at present is to elucidate the actomyosin-ring-independent role(s) of the septins in cytokinesis in yeast and (presumably) in other cell types.

In *S. cerevisiae*, Myo1p (the only type II myosin in this organism) forms a ring at the presumptive budding site in late G1 (Bi *et al.*, 1998; Lippincott and Li, 1998). This ring remains at the mother-bud neck until the onset of cytokinesis, when actin and other proteins are recruited to it to form the mature actomyosin ring, which soon contracts. Concurrent with this contraction, the plasma membrane invaginates and the primary cell-wall septum is synthesized, principally by the chitin synthase Chs2p (Cabib *et al.*, 2001; Schmidt *et al.*, 2002). Secondary septa are then deposited on both sides of the primary septum to form the mature trilaminar septum, and the mother and daughter cells are separated by the action of a chitinase that partially hydrolyzes the primary septum (Kuranda and Robbins, 1991;

This article was published online ahead of print in *MBC in Press* (<http://www.molbiolcell.org/cgi/doi/10.1091/mbc.E07-05-0509>) on October 17, 2007.

[□] The online version of this article contains supplemental material at *MBC Online* (<http://www.molbiolcell.org>).

Address correspondence to: John R. Pringle (jpringle@stanford.edu).

Abbreviations used: APC/C, anaphase-promoting complex or cyclosome; DB, destruction box; FACS, fluorescence-activated cell sorter; 5-FOA, 5-fluoroorotic acid.

Colman-Lerner *et al.*, 2001). In viable *myo1Δ* cells, no actomyosin ring forms, and the septa that form are typically disorganized and often lack well defined primary septum-like structures (Schmidt *et al.*, 2002; Tolliday *et al.*, 2003; our unpublished results). These disorganized septa are presumably the reason that *myo1Δ* cells grow more slowly than wild type and fail to separate efficiently, resulting in the formation of multicell clusters.

Among the proteins recruited to the division site in a septin-dependent manner just before cytokinesis are Iqg1p and Cyk3p. Iqg1p is the only member of the IQGAP family (Brown and Sacks, 2006) in *S. cerevisiae*, and it has been reported to be essential both for formation of the actomyosin ring and for cytokinesis (Epp and Chant, 1997; Shannon and Li, 1999; Luo *et al.*, 2004). Because the actomyosin ring itself is not essential for cytokinesis, Iqg1p must have at least one cytotkinetic function that is actomyosin-ring independent. The function of Cyk3p is not known, but its overexpression suppresses the *iqg1Δ* lethality without restoring the actomyosin ring, suggesting that Cyk3p also promotes cytokinesis through an actomyosin-ring-independent pathway (Korinek *et al.*, 2000; our unpublished results).

For successful cellular reproduction, cytokinesis must be coordinated with other late cell-cycle events such as the completion of chromosome segregation and the exit from mitosis. Not surprisingly, cells have sophisticated regulatory pathways to ensure this coordination. Regulation of late mitotic events depends largely on the anaphase-promoting complex (or cyclosome) (APC/C), an essential multisubunit ubiquitin ligase that targets specific cell cycle-related proteins for degradation (Peters, 2006). The APC/C, with its activating subunit Cdc20p, initiates anaphase by triggering sister-chromatid separation via degradation of the securin Pds1p (Nasmyth, 2005), which unleashes the protease separase. In *S. cerevisiae*, separase activation also initiates release of the protein phosphatase Cdc14p from its nucleolar inhibitor Net1p. Activation of the mitotic-exit network further activates Cdc14p, which then activates Cdh1p, a second APC/C-activating subunit that targets Clb2p and other mitotic cyclins for degradation in late mitosis and early G1. In addition to these major roles, the APC/C also regulates other cell-cycle proteins, such as the spindle-associated kinases Kip1p and Cin8p (Harper *et al.*, 2002).

Thirteen subunits of the APC/C have been identified in *S. cerevisiae*. Many of the core subunits are essential for APC/C function and thus for viability. However, some subunits, including Cdc26p, Apc9p, Doc1p, Swm1p, and Mnd2p, are not essential. Cdc26p and Apc9p seem to be involved in the assembly of the APC/C (Passmore, 2004). Doc1p is involved in promoting the association between the APC/C and its substrates (Carroll and Morgan, 2002; Passmore *et al.*, 2003; Carroll *et al.*, 2005). Swm1p is required for full catalytic activity of the APC/C (Schwickart *et al.*, 2004; Page *et al.*, 2005), and Mnd2p seems to have little role in mitotic control but is important in regulating the APC/C during meiosis (Oelschlaegel *et al.*, 2005; Page *et al.*, 2005).

To investigate the mechanisms of septin-dependent, actomyosin-ring-independent cytokinesis in *S. cerevisiae*, we have been conducting synthetic-lethal and dosage-suppression screens starting with septin and *myo1Δ* mutants. During these studies, we unexpectedly observed that mutations in nonessential subunits of the APC/C could suppress the phenotypes of *myo1Δ* mutants. We present evidence that Iqg1p is a direct substrate of the APC/C and that the elevation of Iqg1p levels in APC/C mutants accounts for the suppression of *myo1Δ* phenotypes.

MATERIALS AND METHODS

Strains, Plasmids, Growth Conditions, and Genetic Methods

The strains and plasmids used in this study are listed in Tables 1 and 2; their construction is described below or in the tables. Yeast were grown on liquid or solid synthetic complete (SC) medium lacking specific nutrients as needed to select plasmids or transformants, YP rich liquid or solid medium, or YM-P rich, buffered liquid medium (Lillie and Pringle, 1980; Guthrie and Fink, 1991). Two percent glucose was used as carbon source except for experiments involving induction of gene expression under GAL promoter control, for which 1 or 2% raffinose plus 0.5 or 2% galactose was used, as indicated. Yeast strains were grown at 23°C except as noted. The antibiotic Geneticin (G-418; Lonza Walkersville, Walkersville, MD) was used to select for cells containing the *kan^R* marker, and 5-fluoroorotic acid (5-FOA; Research Products International, Mt. Prospect, IL) was used to select for *ura3*-mutant cells. To arrest cells in G1, α -factor (Sigma-Aldrich, St. Louis, MO) was used at the concentration indicated. The microtubule-depolymerizing drug benomyl (DuPont, Wilmington, DE) was used at 50 μ g/ml to arrest cells in M phase. To block translation, cycloheximide (MP Biomedicals, Solon, OH [Figure 6] or Sigma-Aldrich [Figure 9]) was added to the growth medium at the concentration indicated. Standard procedures were used for growth of *Escherichia coli*, genetic manipulations, polymerase chain reaction (PCR), and other molecular biological procedures (Sambrook *et al.*, 1989; Guthrie and Fink, 1991; Ausubel *et al.*, 1995).

Strain and Plasmid Constructions

Genes were deleted using the PCR method (Baudin *et al.*, 1993; Longtine *et al.*, 1998), and the primers are indicated in Supplemental Table 1; in each case, the entire coding region was deleted. The success of each deletion was confirmed by two PCR tests that used check primers that were upstream and downstream, respectively, of the deleted region together with primers internal to the selectable markers (Longtine *et al.*, 1998; Supplemental Table 1). C-terminal tagging with sequences encoding the 3HA epitope or GFP(F64L,S65T,V163A) was also done using the PCR method with plasmid pFA6a-3HA-His3MX6 (Longtine *et al.*, 1998) or pFA6a-GFP(F64L,S65T,V163A)-His3MX6 (see below) as template. The success of the tagging was confirmed by two PCR tests, essentially as described above. To construct strains DOY138-141, an IQG1-TAP::His3MX6 C-terminal fragment was PCR amplified using genomic DNA from strain IQG1-TAP as template and the primers indicated in Supplemental Table 1. This cassette was then transformed into appropriate parental strains as indicated in Table 1.

Plasmid pFA6a-GFP(F64L,S65T,V163A)-His3MX6 was constructed by subcloning a MscI-BstBI fragment containing the three mutations from YEeGFP*-BUD8F (Schenkman *et al.*, 2002) into MscI/BstBI-cut pFA6a-GFP(S65T)-His3MX6 (Wach *et al.*, 1997). Plasmid pTSV31A-MYO1 was constructed by subcloning the 7.0-kb Sall-BamHI MYO1 fragment from pBS-MYO1 (a gift from E. Bi, University of Pennsylvania, Philadelphia, PA) into Sall/BamHI-cut pTSV31A (a 2 μ URA3 ADE3 plasmid; Tibbetts and Pringle, unpublished data). Plasmid pGT04 was constructed using two steps of PCR. In the first step, a fragment of IQG1 (nucleotides -262 to +3 relative to the A of the start codon) was amplified from genomic DNA with a BamHI site incorporated into the 5' primer and a 3' primer that included nucleotides corresponding to positions +127 to +141 of IQG1. A second fragment (nucleotides +127 to +277) was also amplified from genomic DNA using a 5' primer that included nucleotides corresponding to positions -15 to +3 of IQG1 and a 3' primer that included an XbaI site. In the second step, the PCR products from the first step were purified and used as template with the BamHI site-containing 5' primer and the XbaI site-containing 3' primer. The resulting product, which contained 262 nucleotides of the IQG1 promoter, a start codon, and 151 nucleotides (from +127 to +277) of open reading frame sequence, was cut with BamHI and XbaI, gel purified, and inserted into BamHI/XbaI-cut pRS305 (Sikorski and Hieter, 1989). Plasmids pGAL-IQG1-TAP and pGAL-*iqg1Δ42*-TAP were constructed by transforming yeast cells with BamHI/HindIII-cut pRSAB1234 (see Supplemental Materials and Methods of Gelperin *et al.*, 2005) with PCR-amplified full-length or truncated (lacking codons 2–42) IQG1; the amplified fragment contained 22 (5') and 21 (3') base pairs of flanking vector sequences to allow the in vivo recombination.

Growth Rates, Cell-Cluster Indices, Colony-Sectoring Assays, and Fluorescence-Activated Cell Sorter (FACS) Analysis

To determine growth rates, exponential phase cultures (OD₆₀₀ $\approx$ 0.4) in YM-P medium were diluted twofold with fresh YM-P, and incubation was continued. The times needed to return to the original OD₆₀₀ were recorded as the doubling times.

To determine cell-cluster indices, strains were streaked onto the indicated media, grown overnight, scraped from the plates, washed once with water by centrifugation, resuspended, sonicated briefly, and observed by differential-interference-contrast (DIC) microscopy. Each unbudded (one cell body) or

Table 1. *S. cerevisiae* strains used in this study^{a,b}

Strain	Genotype	Source
YEF473 ^a	<i>alα his3-Δ200/his3-Δ200 leu2-Δ1/leu2-Δ1 lys2-801/lys2-801 trp1-Δ63/trp1-Δ63 ura3-52/ura3-52</i>	Bi and Pringle (1996)
YEF473A ^a	<i>a his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ63 ura3-52</i>	Segregant from YEF473
YEF473B ^a	<i>α his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ63 ura3-52</i>	Segregant from YEF473
BY4741 ^{a,c}	<i>a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	Brachmann <i>et al.</i> (1998)
IQG1-TAP ^a	as BY4741 except IQG1-TAP:His3MX6	Ghaemmaghami <i>et al.</i> (2003)
W1588-4C ^b	<i>a ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1</i>	Zhao <i>et al.</i> , 1998
JMY314.1-4b ^b	<i>α ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1</i>	T. Petes (Duke University, Durham, NC)
AFS92 ^b	as W1588-4C except <i>bar1Δ</i>	Jaspersen <i>et al.</i> (1998)
DOY138 ^b	as W1588-4C except <i>bar1Δ IQG1-TAP:His3MX6</i>	This study ^d
DOY139 ^b	as W1588-4C except <i>bar1Δ cdc23-1:URA3 IQG1-TAP:His3MX6</i>	This study ^d
DOY140 ^b	as W1588-4C except <i>bar1Δ IQG1-TAP:His3MX6 leu2-3,112:P_{GAL}-pds1-mdb:LEU2</i>	This study ^d
DOY141 ^b	as W1588-4C except <i>cdc28-13:TRP1 cdh1Δ::LEU2 IQG1-TAP:His3MX6</i>	This study ^d
GT24 ^b	as W1588-4C except <i>bar1Δ [pGAL-IQG1-TAP]</i>	This study ^d
GT70 ^b	as W1588-4C except <i>bar1Δ [pGAL-iqg1Δ42-TAP]</i>	This study ^e
GT112 ^b	as W1588-4C except <i>bar1Δ MYO1-GFP:His3MX6 iqg1Δ42:LEU2</i>	This study ^f
GT124 ^a	as BY4741 except <i>iqg1Δ42-TAP:His3MX6:LEU2</i>	This study ^g
RNY112 ^a	as YEF473 except <i>myo1Δ::kanMX6/+</i>	Our unpublished results
RNY501 ^b	<i>alα ade2-1/ade2-1 can1-100/can1-100 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 trp1-1/trp1-1 ura3-1/ura3-1</i>	W1588-4C X JMY314.1-4b
RNY509 ^b	as RNY501 except <i>myo1Δ::kanMX6/+</i>	Our unpublished results
RNY798 ^b	as W1588-4C except <i>myo1Δ::kanMX6 ade3Δ::His3MX6 [pTSV31A-MYO1]</i>	This study ^h
RNY1001 ^a	as YEF473B except <i>myo1Δ::kanMX6 [pRS315-IQG1]</i>	This study ⁱ
RNY1002 ^a	as YEF473B except <i>myo1Δ::kanMX6 [YEpl81-IQG1]</i>	This study ⁱ
RNY1003 ^a	as YEF473B except <i>myo1Δ::kanMX6 [pRS315-CYK3]</i>	This study ⁱ
RNY1004 ^a	as YEF473B except <i>myo1Δ::kanMX6 [pRS425-CYK3]</i>	This study ⁱ
RNY1006 ^a	as YEF473B except <i>myo1Δ::kanMX6 [pRS315]</i>	This study ⁱ
RNY1007 ^a	as YEF473B except <i>myo1Δ::kanMX6 [pRS425]</i>	This study ⁱ
KO139 ^a	as YEF473A except <i>myo1Δ::kanMX6 pds1Δ::TRP1</i>	This study ^j
KO199 ^a	as YEF473 except <i>myo1Δ::kanMX6/+ net1Δ::TRP1/+</i>	This study ^j
KO213 ^b	as RNY501 except <i>myo1Δ::kanMX6/+ cdc26Δ::TRP1/+</i>	This study ^j
KO216 ^b	as RNY501 except <i>myo1Δ::kanMX6/+ apc9Δ::TRP1/+</i>	This study ^j
KO246 ^b	as RNY501 except <i>myo1Δ::kanMX6/+ pds1Δ::TRP1/+</i>	This study ^j
KO257 ^a	as YEF473A except <i>myo1Δ::kanMX6 cdh1Δ::TRP1</i>	This study ^j
KO275 ^a	as YEF473 except <i>myo1Δ::kanMX6/+ net1Δ::TRP1/+ apc9Δ::His3MX6/+</i>	This study ^k
KO296 ^b	as W1588-4C	Segregant from RNY509
KO313 ^b	as W1588-4C except <i>myo1Δ::kanMX6 ade3Δ::His3MX6 apc9Δ::TRP1 [pTSV31A-MYO1]</i>	This study ^j
KO336 ^b	as RNY501 except <i>myo1Δ::kanMX6/+ P_{GAL}-clb2ΔDB:LEU2</i>	This study ^m
KO342 ^b	as RNY501 except <i>P_{GAL}-clb2ΔDB:LEU2</i>	This study ^m
KO346 ^b	as RNY501 except <i>myo1Δ::kanMX6/+ swm1Δ::TRP1/+</i>	This study ^j
KO351 ^b	as RNY501 except <i>myo1Δ::kanMX6/+ mnd2Δ::TRP1/+</i>	This study ^j
KO354 ^b	as RNY501 except <i>myo1Δ::kanMX6/+ cdh1Δ::TRP1/+</i>	This study ^j
KO360 ^b	as W1588-4C except <i>myo1Δ::kanMX6 ade3Δ::His3MX6 P_{GAL}-clb2ΔDB:LEU2 [pTSV31A-MYO1]</i>	This study ^m
KO369 ^b	as W1588-4C except <i>P_{GAL}-clb2ΔDB:LEU2</i>	Segregant from KO342
KO372 ^b	as W1588-4C	Segregant from KO342
KO388 ^a	as YEF473 except <i>P_{GAL}-clb2ΔDB:LEU2</i>	This study ^m
KO397 ^a	as YEF473A except <i>myo1Δ::kanMX6 cdc26Δ::TRP1</i>	This study ^j
KO399 ^a	as YEF473A except <i>doc1Δ::TRP1</i>	This study ^j
KO401 ^a	as YEF473A except <i>myo1Δ::kanMX6 doc1Δ::TRP1</i>	This study ^j
KO405 ^a	as YEF473A except <i>myo1Δ::kanMX6 swm1Δ::TRP1</i>	This study ^j
KO415 ^a	as YEF473A except <i>myo1Δ::kanMX6 mnd2Δ::TRP1</i>	This study ^j
KO421 ^a	as YEF473A except <i>myo1Δ::kanMX6 apc9Δ::TRP1</i>	This study ^j
KO460 ^a	as YEF473B except <i>IQG1-3HA:His3MX6 doc1Δ::TRP1</i>	This study ⁿ
KO463 ^a	as YEF473A except <i>IQG1-3HA:His3MX6 swm1Δ::TRP1</i>	This study ⁿ
KO465 ^a	as YEF473B except <i>IQG1-3HA:His3MX6 swm1Δ::TRP1</i>	This study ⁿ
KO470 ^a	as YEF473B except <i>IQG1-3HA:His3MX6 cdc26Δ::TRP1</i>	This study ⁿ
KO471 ^a	as YEF473A except <i>IQG1-3HA:His3MX6</i>	This study ⁿ
KO474 ^a	as YEF473B except <i>IQG1-3HA:His3MX6</i>	This study ⁿ
KO477 ^b	as JMY314.1-4b except <i>IQG1-3HA:His3MX6 apc9Δ::TRP1</i>	This study ⁿ
KO481 ^b	as W1588-4C except <i>IQG1-3HA:His3MX6 swm1Δ::TRP1</i>	This study ⁿ
KO482 ^b	as JMY314.1-4b except <i>IQG1-3HA:His3MX6 swm1Δ::TRP1</i>	This study ⁿ
KO486 ^b	as JMY314.1-4b except <i>IQG1-3HA:His3MX6 cdc26Δ::TRP1</i>	This study ⁿ
KO487 ^b	as W1588-4C except <i>IQG1-3HA:His3MX6</i>	This study ⁿ
KO490 ^b	as JMY314.1-4b except <i>IQG1-3HA:His3MX6</i>	This study ⁿ
KO492 ^a	as YEF473A	Segregant from KO388
KO494 ^a	as YEF473A except <i>P_{GAL}-clb2ΔDB:LEU2</i>	Segregant from KO388

Continued

Table 1. *Continued*

Strain	Genotype	Source
KO529 ^b	as W1588-4C except <i>cdh1Δ::TRP1</i>	Segregant from KO354
KO561 ^a	as YEF473 except <i>myo1Δ::kanMX6/+ P_{GAL}-clb2ΔDB:LEU2</i>	This study ^m
KO563 ^a	as YEF473A except <i>IQG1-GFP:His3MX6 doc1Δ::TRP1</i>	This study ^o
KO566 ^a	as YEF473A except <i>IQG1-GFP:His3MX6</i>	This study ^o
KO570 ^a	as YEF473A except <i>myo1Δ::kanMX6 P_{GAL}-clb2ΔDB:LEU2</i>	Segregant from KO561
KO588 ^b	as W1588-4C except <i>IQG1-3HA:His3MX6 swm1Δ::TRP1 bar1Δ::kanMX6</i>	This study ^p
KO589 ^b	as W1588-4C except <i>IQG1-3HA:His3MX6 bar1Δ::kanMX6</i>	This study ^p
KO608 ^a	as YEF473A except <i>myo1Δ::kanMX6</i>	Segregant from RNY112
KO625 ^a	as YEF473A except <i>IQG1-GFP:His3MX6 swm1Δ::TRP1</i>	This study ^o
KO671 ^a	as YEF473A except <i>IQG1-3HA:His3MX6 swm1Δ::TRP1 bar1Δ::kanMX6</i>	This study ^p
KO672 ^a	as YEF473A except <i>IQG1-3HA:His3MX6 bar1Δ::kanMX6</i>	This study ^p
KO1226 ^a	<i>alα his3-Δ200/his3Δ1 leu2-Δ1/leu2Δ0 lys2-801/+ met15Δ0/+ trp1-Δ63/+ ura3-52/ura3Δ0 myo1Δ::kanMX6/+ iqg1Δ42-TAP:His3MX6:LEU2/+</i>	This study ^q
KO1228 ^b	as RNY501 except <i>bar1Δ/+ iqg1Δ42:LEU2/+ myo1Δ::kanMX6/MYO1-GFP:His3MX6</i>	This study ^q
KO1229 ^b	as RNY501 except <i>bar1Δ/+ iqg1Δ42:LEU2/+ myo1Δ::kanMX6/MYO1-GFP:His3MX6</i>	This study ^q
KO1248 ^a	as YEF473A except <i>myo1Δ::kanMX6</i>	Segregant from KO1226
KO1249 ^a	as YEF473A except <i>myo1Δ::kanMX6 iqg1Δ42-TAP:His3MX6:LEU2</i>	Segregant from KO1226

^a Denotes strains of the S288C genetic background (Mortimer and Johnston, 1986).

^b Denotes strains of the W303 genetic background (Thomas and Rothstein, 1989). Note that although the original W303 contained a *rad5* mutation, all of the W303-family strains used here are *RAD5+* like W1588-4C (Zhao *et al.*, 1998).

^c American Type Culture Collection (Manassas, VA) strain ATCC201388 or ATCC4040002.

^d Strains YJB14 and YJB115 (Burton and Solomon, 2000), OCF1517.2 (Cohen-Fix *et al.*, 1996), and YJB368 (Burton and Solomon, 2001) were transformed with an *IQG1-TAP:His3MX6* C-terminal-tagging cassette (see *Materials and Methods*).

^e Strain AFS92 was transformed with the indicated plasmids (Table 2).

^f Strain AFS92 was transformed with a *MYO1-GFP:His3MX6* C-terminal-tagging cassette and then with pGT04 (Table 2) after cutting with *NheI* to target integration to the *IQG1* coding sequence. The site of integration was confirmed by PCR.

^g Strain *IQG1-TAP* was transformed with pGT04 after cutting with *NheI*, and the site of integration was confirmed by PCR.

^h Strain RNY509 was transformed with pTSV31A-MYO1 (Table 2). A *myo1Δ::kanMX6* segregant carrying the plasmid was isolated by tetrad dissection and transformed with an *ade3Δ::His3MX6* deletion cassette.

ⁱ Strain RNY112 was transformed with pRS316-MYO1. A *myo1Δ::kanMX6* segregant carrying the plasmid was then isolated by tetrad dissection and transformed with the indicated plasmids (Table 2); pRS316-MYO1 was then eliminated by growth on 5-FOA.

^j Strains RNY112 and RNY509 were transformed with various *TRP1*-marked deletion cassettes (see *Materials and Methods*). Single-mutant and double-mutant segregants from the RNY112 derivatives were isolated by tetrad dissection.

^k Strain KO199 was transformed with an *apc9Δ::His3MX6* deletion cassette.

^l Strain RNY798 was transformed with an *apc9Δ::TRP1* deletion cassette.

^m Strains YEF473, RNY112, RNY501, RNY509, and RNY798 were transformed with plasmid Yip-GAL-*clb2ΔDB* (Table 2) after cutting with *KpnI* to target integration to the *LEU2* locus. Although the actual sites of integration were not checked, each diploid transformant was heterozygous for a single copy of the *P_{GAL}-clb2ΔDB:LEU2* allele, as shown by subsequent tetrad analysis.

ⁿ Strains YEF473A and KO296 were transformed with an *IQG1-3HA:His3MX6* C-terminal-tagging cassette (see *Materials and Methods*). The resulting strains were crossed to various APC/C mutant strains, and segregants were isolated by tetrad dissection.

^o Strain YEF473A was transformed with an *IQG1-GFP:His3MX6* C-terminal-tagging cassette (see *Materials and Methods*). The resulting strain was crossed to *doc1Δ* and *swm1Δ* strains (from the same tetrads that yielded KO401 and KO405), and single-mutant and double-mutant segregants were isolated by tetrad dissection.

^p Strains KO463, KO471, KO481, and KO487 were transformed with a *bar1Δ::kanMX6* deletion cassette (see *Materials and Methods*).

^q Strains RNY112 and RNY509 were transformed with plasmids pRS316-MYO1 and pTSV31A-MYO1, respectively. A *myo1Δ::kanMX6* segregant carrying the plasmid was then isolated from each strain by tetrad dissection and mated to strain GT124 or GT112, as appropriate to maintain the S288C or W303 genetic background. pRS316-MYO1 or pTSV31A-MYO1 was then eliminated by growth on 5-FOA.

budded (two cell bodies) cell was scored as one nonclustered unit, and entities with three, four, five, or six or more cell bodies were scored as one, two, three, or four clusters, respectively. Each count was continued until the number of clusters plus nonclustered units was 100, and the number of clusters was recorded as the cluster index. To minimize the possibility that the cell-cluster index determined for the *myo1Δ* single mutant would be influenced by spontaneously arising suppressors, we examined nine different *myo1Δ* strains that were obtained as segregants from RNY112 and its double-mutant derivatives.

The colony-sectoring assay was based on that described by Bender and Pringle (1991). *ade2-1 ade3Δ ura3-3 myo1Δ* strains carrying a *URA3 ADE3 MYO1* plasmid were grown on SC-Ura plates and then streaked onto YP or YPGalRaf plates to observe sectoring or nonsectoring single colonies. In some experiments, a *LEU2*-marked plasmid (empty or carrying *IQG1* or *CYK3*) was

also present. In these cases, the strains were first grown on SC-Ura-Leu plates and then streaked onto SC-Leu plates to observe sectoring or nonsectoring single colonies.

To assess the extent of G2 delay by using FACS, cells were grown to exponential phase ($OD_{600} \approx 0.4$) in YM-P medium, and then they were collected, fixed, stained with SYTOX Green (Invitrogen, Carlsbad, CA), and examined using standard procedures (Haase and Reed, 2002).

Screen for Dosage Suppressors of *myo1Δ*

A W303-background *myo1Δ* haploid strain, RNY798, which contained an *ADE3 MYO1* plasmid and could only form nonsectored viable colonies (see above), was transformed with a genomic-DNA library in the low-copy plasmid YCp50-LEU2 (Bi and Pringle, 1996; the library was constructed using

Table 2. Plasmids used in this study

Plasmid	Description	Source
pRS315	<i>CEN6 ARS4 LEU2</i> (low copy)	Sikorski and Hieter (1989)
pRS425	2 μ , <i>LEU2</i> (high copy)	Christianson <i>et al.</i> (1992)
YEpl181-IQG1	2 μ , <i>LEU2 IQG1</i> (high copy)	Our unpublished results
pRS315-IQG1	<i>IQG1</i> in pRS315	Our unpublished results
pRS315-CYK3	<i>CYK3</i> in pRS315	Our unpublished results
pRS425-CYK3	<i>CYK3</i> in pRS425	Our unpublished results
pRS316-MYO1	<i>CEN6 ARS4 URA3 MYO1</i>	Our unpublished results
pTSV31A-MYO1	2 μ , <i>URA3 ADE3 MYO1</i>	See text
YIp-GAL- <i>clb2ΔDB</i>	<i>LEU2 P_{GAL}-clb2ΔDB</i> (integrating)	S. Reed (Scripps Research Institute, La Jolla, CA)
pGT04	<i>LEU2 iqq1Δ42</i> (integrating)	See text
pGAL-IQG1-TAP	2 μ , <i>URA3 P_{GAL}-IQG1-TAP</i>	See text
pGAL- <i>iqg1Δ42</i> -TAP	2 μ , <i>URA3 P_{GAL}-iqg1Δ42-TAP</i>	See text

DNA from an S288C-background strain and was kindly provided by F. Spencer and P. Hieter, Johns Hopkins University, Baltimore, MD). From ~20,000 transformants screened on SC-Leu plates, 85 reproducibly sectoring transformants were identified. Isolation of plasmids and retransformation of strain RNY798 yielded 14 plasmids that rescued the lethality of the *myo1Δ* strain. Sequencing and subcloning of the inserts revealed that nine plasmids contained full-length *MYO1* or C-terminal *MYO1* fragments, and that in three other plasmids, the gene responsible for suppression was either *IQG1* (two cases) or *CYK3* (one case). The suppressing genes in the final two plasmids have not yet been identified.

Protein Analysis and Ubiquitination Assays

Iqg1p-3HA levels were determined by immunoblotting in both asynchronous and synchronous cultures. For synchronous cultures, the supersensitive *bar1Δ* (Sprague, 1991) strains were grown to exponential phase in SC-His medium, treated for 3 h with 70 ng/ml α -factor to arrest cells in G1, released from arrest by centrifugation and resuspension in SC-His medium, and sampled at 20-min intervals. At each time point, one sample was taken for protein extraction, and a second sample was fixed for 10 min in 70% ethanol at 0°C, resuspended in phosphate-buffered saline (PBS), stained with 4,6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich), and used to evaluate cell-cycle progression by scoring the percentages of large-budded cells with two well separated chromosome sets. To extract proteins, cells were collected by centrifugation, resuspended in 1.85 M NaOH containing 2% β -mercaptoethanol, and incubated for 10 min at 0°C. Trichloroacetic acid was then added to 50%, and incubation was continued for 15–25 min at 0°C. Insoluble material was collected by centrifugation, mixed with SDS-sample buffer, boiled for 5 min, and analyzed on 7% SDS-polyacrylamide gels. After transferring proteins electrophoretically to nitrocellulose transfer membrane (GE Healthcare, Piscataway, NJ), Iqg1p-3HA was detected using the rat monoclonal anti-HA antibody 3F10 (Roche Molecular Biochemicals, Indianapolis, IN) as primary antibody. Protein bands were then visualized using either mouse anti-rat immunoglobulin (IgG) secondary antibody (Jackson ImmunoResearch, West Grove, PA), alkaline phosphatase-conjugated goat anti-mouse-IgG tertiary antibody (Sigma-Aldrich), and the AttoPhos AP fluorescent substrate system (Promega, Madison, WI) (Figure 6D), or horseradish peroxidase (HRP)-conjugated goat anti-rat-IgG secondary antibody (GE Healthcare) and the enhanced chemiluminescence (ECL) system (GE Healthcare) (Figure 7). Actin was detected using either a goat polyclonal anti-actin antibody (Karpova *et al.*, 1993) (Figures 6D and 7B) or the mouse monoclonal anti-actin antibody MAB1501 (Chemicon International, Temecula, CA) (Figure 7A) as primary antibody. Protein bands were then visualized using alkaline phosphatase-conjugated rabbit anti-goat-IgG secondary antibody (Sigma-Aldrich) and the AttoPhos AP system (Figure 6D), HRP-conjugated donkey anti-goat-IgG secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and the ECL system (Figure 7B), or alkaline phosphatase-conjugated goat anti-mouse-IgG secondary antibody and the AttoPhos AP system (Figure 7A). In Figure 6D, the intensities of the protein bands were measured using the Storm Scanner model 840 (GE Healthcare), and the values for Iqg1p-3HA were normalized using the actin bands from the same samples.

Iqg1p-TAP levels were determined by immunoblotting using the peroxidase anti-peroxidase soluble complex produced in rabbit (catalog no. P1291; Sigma-Aldrich) and the SuperSignal West Pico chemiluminescence system (Pierce Chemical, Rockford, IL). For the experiments depicted in Figure 6, B and C, cell lysates were prepared as described previously (Ostapenko and Solomon, 2005), and Iqg1p-TAP was precipitated from equal amounts of lysate by incubation for 90 min at 4°C with IgG-Sepharose (GE Healthcare) in 10 mM Tris-Cl, pH 7.5, 150 mM NaCl, containing 1% NP-40 and protease inhibitors (10 mg/ml each of leupeptin, chymostatin, and pepstatin; all from Chemicon International). Precipitated proteins were separated on 10% SDS-

PAGE, transferred to Immobilon-P membranes (Millipore, Billerica, MA), and detected as described above using an overnight incubation at 4°C in 10 mM Tris-Cl, pH 7.5, 150 mM NaCl, containing 0.1% Tween 20 and 5% dry milk. For the loading control in Figure 6B, Cdc28p was detected using a rabbit polyclonal anti-PSTAIR primary antibody (Solomon, unpublished data), an HRP-conjugated goat anti-rabbit-IgG secondary antibody (Santa Cruz Biotechnology), and the SuperSignal West Pico system. For the experiments depicted in Figure 9, C and D, protein extracts were prepared and analyzed by 10% SDS-PAGE and immunoblotting as described previously (Ubersax *et al.*, 2003). Iqg1p-TAP and Iqg1Δ42p-TAP were detected as described above, and Clb2p was detected using a rabbit polyclonal anti-Clb2p primary antibody (Kellogg and Murray, 1995), an HRP-conjugated donkey anti-rabbit-IgG secondary antibody (GE Healthcare), and the SuperSignal West Pico system.

To perform ubiquitination assays, reaction components were expressed and purified as described previously (Charles *et al.*, 1998; Carroll and Morgan, 2002, 2005). Substrates were produced in rabbit reticulocyte lysates by coupled transcription and translation in the presence of [³⁵S]methionine, following the manufacturer's instructions (Promega). Truncated *IQG1* constructs with a T7 promoter sequence and Kozak site added upstream were made using two steps of PCR. In the first step, the desired *IQG1* fragment was PCR amplified using a 5' primer containing a sequence of 23 nucleotides that overlapped the 5' primer used in the second step plus 30–37 nucleotides of *IQG1* coding sequence beginning with a start codon. The 3' primer contained ~20 nucleotides of coding sequence ending with a stop codon. In the second step, the product from the first step was used as template with the same 3' primer and a 5' primer of 119 nucleotides that included a T7 promoter and a Kozak site. Point mutations were introduced by incorporation into the 5' primer used in the first step. Similarly, full-length *PDS1* (from the start codon to 99 nucleotides downstream of the stop codon) and *CYK3* (from the start codon to 64 nucleotides downstream of the stop codon) constructs were generated with the T7 promoter sequence and Kozak site upstream of the start codons. Ubiquitination reactions were performed and monitored using a Molecular Dynamics PhosphorImager (GE Healthcare) as described previously (Carroll and Morgan, 2002, 2005; Carroll *et al.*, 2005).

Microscopy and Quantitation of Green Fluorescent Protein (GFP) Fluorescence

DIC and fluorescence microscopy were performed using a Nikon (Tokyo, Japan) Eclipse E600-FN microscope and an ORCA-2 cooled charge-coupled-device camera (Hamamatsu Photonic Systems, Bridgewater, NJ). To quantitate Iqg1p-GFP signal intensities, cells were grown to exponential phase in SC-His medium, collected by centrifugation, and resuspended in water before observation by DIC and fluorescence microscopy. The average intensities of the GFP signals at the mother-bud neck were determined for each strain using MetaMorph version 5.0 (Molecular Devices, Sunnyvale, CA); a box of fixed size was drawn to contain the neck of each large-budded cell with a detectable signal, and the total fluorescence of the boxed area was measured and recorded using the regional-measurement function of MetaMorph.

RESULTS

Suppression of *myo1Δ* Phenotypes by APC/C Mutations

A *myo1Δ* mutation is not lethal in the S288C strain background at 23°C (see *Introduction*). We used the colony-sectoring method of Bender and Pringle (1991) to screen for mutations synthetically lethal with *myo1Δ* under these conditions (our unpublished results). The first several mutants

analyzed proved to be temperature sensitive for growth (i.e., unable to grow at 37°C even when the *MYO1* plasmid was present), which seemed to offer an easy method to clone the genes harboring the synthetic-lethal mutations. Using a genomic-DNA library to rescue the temperature sensitivity of one mutant, we recovered *CDC26*, which encodes a subunit of the APC/C that is nonessential at 23°C but essential at 37°C. Further investigation revealed that the parental *myo1Δ* strain used in the screen was itself temperature sensitive and harbored a *cdc26* mutation [a *YJRWdelta11* or *YJR-Wdelta13* sequence (338 nucleotides; *Saccharomyces* Genome Database) inserted at nucleotide -7 relative to the start site

of the *CDC26* ORF] that accounted for the temperature-sensitive phenotype. Because *myo1Δ* strains grow less well than wild type (Rodriguez and Paterson, 1990; Bi *et al.*, 1998; Lippincott and Li, 1998) and can accumulate spontaneous suppressor mutations (Tolliday *et al.*, 2003), this suggested that the *cdc26* mutation might have been selected because it alleviated the phenotype of the original *myo1* mutant. Indeed, when we intentionally introduced deletions of *CDC26* or other nonessential APC/C subunits into freshly prepared *myo1Δ* strains, we found that these mutations could suppress both the clustering and slow-growth phenotypes in the S288C background (Figure 1) and the lethal phenotype (Tolliday *et al.*, 2003; our unpublished results) in the W303 background (Figure 2). Similar results were obtained with deletions of *CDH1*, the APC/C activator for exit from mitosis (Figures 1, B and C, and 2A). In contrast, deletion of *PDS1*, which encodes securin, the APC/C target whose degradation triggers anaphase onset, did not alter the pheno-

Figure 1. Suppression of *myo1Δ* clustering and slow-growth phenotypes by APC/C mutations in the S288C strain background. (A) Presence of multicell clusters in a *myo1Δ* strain but not in a congenic wild type (WT). Strains YEF473A and KO608 were grown overnight on YP plates and observed by DIC microscopy as described in *Materials and Methods*. (B) Suppression of clustering phenotype. Strains were grown as described in A, and clustering was scored as described in *Materials and Methods*. Strains were YEF473A, KO608 and related strains (for *myo1Δ*; see *Materials and Methods*), KO397, KO421, KO401, KO405, KO415, KO257, and KO139 and contained deletions of the indicated genes. Values shown are the means of three to nine counts except for *myo1Δ* (mean of 25 counts); standard deviations are also indicated. (C) Suppression of slow-growth phenotype. Strains were the same as in B. The doubling times shown are the averages of two measurements made as described in *Materials and Methods*.

Figure 2. Suppression of *myo1Δ* lethality by APC/C mutations in the W303 strain background. (A) Suppression of spore lethality. Tetrads were dissected from strains heterozygous for *myo1Δ::kanMX6* or for both *myo1Δ::kanMX6* and an APC/C or *pds1* deletion marked by *TRP1*. All viable *myo1Δ* segregants (scored by growth on YP+G418) also carried an APC/C mutation (scored by growth on SC-Trp). The strains analyzed were RNY509, KO213, KO216, KO346, KO351, KO354, and KO246. (B) Demonstration of suppression by a colony-sectoring assay. Strain RNY798 (*myo1Δ ade2 ade3*; MYO1 ADE3 plasmid) and its *apc9Δ* derivative KO313 were streaked onto YP plates. Only strain KO313 could form viable subclones (sectors) that had lost the plasmid and thus were white due to the loss of Ade3p activity.

types in either background (Figures 1, B and C, and 2A). Together, these results suggested that APC/C mutations do not suppress *myo1Δ* phenotypes by affecting the timing of anaphase.

Lack of *myo1Δ* Suppression by Delayed Mitotic Exit

It also seemed possible that APC/C mutations might suppress *myo1Δ* phenotypes by delaying mitotic exit and thus allowing more time for an inefficient process of cytokinesis to be completed successfully. To explore this possibility, we first introduced a deletion of *NET1* into an S288C-background strain that was heterozygous for a *myo1Δ* mutation. Because *NET1* encodes an inhibitor of Cdc14p, which activates the APC/C for mitotic exit, it seemed possible that a *net1* mutation might exacerbate the *myo1Δ* phenotypes by accelerating mitotic exit. Indeed, the doubly heterozygous diploid yielded only synthetic-lethal or synthetic-sick *myo1Δ net1Δ* double-mutant segregants (Figure 3A), and the poor viability was suppressed by deletion of a nonessential APC/C subunit (Figure 3B). These data were consistent with the hypothesis that APC/C mutations might suppress the *myo1Δ* phenotypes by delaying mitotic exit. However, this hypothesis was difficult to reconcile with the observation that deletion of *MND2*, which seems to have little effect on mitotic exit (Oelschlaegel *et al.*, 2005; Page *et al.*, 2005), suppressed the *myo1Δ* phenotypes as effectively as did mutations of other APC/C subunits (Figures 1, B and C, and 2A).

Figure 3. Synthetic lethality between *myo1Δ* and *net1Δ* in the S288C background and its suppression by an APC/C mutation. (A) Tetrads were dissected from a strain (KO199) heterozygous for the unlinked mutations *myo1Δ::kanMX6* and *net1Δ::TRP1*. Most tetrads, like the six shown here, yielded no viable double-mutant spores. In 18 additional tetrads, only two viable spores were recovered that carried both mutations and thus grew, albeit very slowly, on both YP+G418 and SC-Trp plates. (B) Tetrads were dissected from a strain (KO275) heterozygous for *myo1Δ::kanMX6*, *net1Δ::TRP1*, and *apc9Δ::His3MX6*. In 54 tetrads dissected, 24 viable segregants were recovered that carried both *myo1Δ* and *net1Δ* (see examples in circles); 20 of these also carried *apc9Δ* (as shown by growth on SC-His plates), and the other four grew very slowly.

Figure 4. Lack of *myo1Δ* suppression by expression of a nondegradable mitotic cyclin. All galactose media also contained 1% raffinose. (A and B) Delayed mitotic exit produced by APC/C mutations or by expression of a nondegradable cyclin. (A) S288C-background strains KO492, KO399, and KO494 were grown to exponential phase in YM-P medium containing glucose or galactose, as indicated, and FACS analysis was performed as described in *Materials and Methods*. (B) W303-background strains KO372, KO529, and KO369 were grown and analyzed as described for A. (C–E) Lack of suppression of *myo1Δ* phenotypes. (C) Clustering phenotype in the S288C background. Strains YEF473A, KO608, and KO570 were grown overnight on YP plates containing the indicated sugars, and clustering was scored as described in *Materials and Methods*. Values shown are the means of three to five counts; standard deviations are also indicated. (D and E) Lethal phenotype in the W303 background. (D) Three sets of tetrads from strain KO336 (*myo1Δ::kanMX6/+* and heterozygous for *P_{GAL}-clb2ΔDB::LEU2*; see Table 1) were dissected (top panels) on YP + 2% glucose (tetrads 1–5), YP + 0.5% galactose (tetrads 6–10), or YP + 2.0% galactose (tetrads 11–15). Tetrads were then replica-plated to YP plates containing the indicated sugars plus G-418 (to identify any segregants carrying *myo1Δ*) and to SC-Leu plates containing the indicated sugars (to identify segregants carrying *P_{GAL}-clb2ΔDB*). No viable *myo1Δ* segregants were observed in 24 tetrads dissected on 0.5% galactose and 18 tetrads dissected on 2% galactose. (E) Strain KO360 was streaked onto YP plates containing the indicated sugars. The absence of white sectors (none were observed in >300 colonies observed) indicates an inability to form viable subclones that have lost the *MYO1 ADE3* plasmid even when *Clb2pΔDB* is expressed.

To explore this matter further, we wanted to delay mitotic exit by means independent of effects on the APC/C. To this end, we constructed strains in both backgrounds in which the nondegradable mitotic cyclin Clb2p Δ DB (Amon *et al.*, 1994) is expressed from the inducible *GAL1* promoter. FACS analysis on asynchronous cultures showed that in glucose medium, APC/C-mutant strains showed a higher ratio of G2/M cells to G1 cells than did wild-type strains (Figure 4A and B, panels 1 and 4), indicating a delay in mitotic exit in the APC/C mutants. A shift to galactose medium produced a modest decrease in the G2/M-to-G1 ratio in these strains (Figure 4, A and B, panels 2, 3, 5, and 6), presumably reflecting a delay in cell-cycle initiation on the poorer carbon source (Pringle and Hartwell, 1981). In contrast, the *P_{GAL}-clb2 Δ DB* strains showed no accumulation of G2/M cells on glucose medium (Figure 4, A and B, panels 7), but, as expected, they showed a significant accumulation of such cells when production of Clb2p Δ DB was induced by growth in galactose medium (Figure 4, A and B, panels 8 and 9). The apparent delay in mitotic exit was similar to (W303 background) or greater than (S288C background) the delay produced by APC/C mutations. However, in contrast to APC/C mutations, induction of Clb2p Δ DB produced little or no suppression of either the *myo1 Δ* clustering phenotype in the S288C background (Figure 4C) or the *myo1 Δ* lethal phenotype in the W303 background (Figure 4, D and E). Thus, neither reduced degradation of the APC/C^{Cdh1} target Clb2p nor the resulting delay in mitotic exit seems to suppress *myo1 Δ* phenotypes. These results suggested that some other APC/C target(s) and/or pathway(s) might be involved in the suppression.

Suppression of *myo1 Δ* Phenotypes by Overexpression of *Iqg1p* or *Cyk3p*

In parallel with the synthetic-lethal screen, we also screened for dosage suppressors of *myo1 Δ* lethality in the W303 background (see *Materials and Methods*). From this screen, low-copy plasmids carrying *IQG1* and *CYK3* were isolated. Further investigation showed that both low-copy and high-copy *IQG1* and *CYK3* plasmids could indeed suppress *myo1 Δ* inviability in the W303 background, as judged by tests either of spore viability (Figure 5A) or of the ability of vegetative *myo1 Δ* cells to lose a *MYO1* plasmid (Figure 5B). Sequencing of both genes in the W303 background revealed no mutations (our unpublished results), suggesting that the suppression of *myo1 Δ* lethality was due simply to increased amounts of one or the other wild-type protein. In further support of this hypothesis, either low-copy or high-copy plasmids containing either *IQG1* or *CYK3* could also suppress the clustering phenotype of the *myo1 Δ* mutant in the S288C background (Figure 5C). These results suggested that *Iqg1p*, *Cyk3p*, or both might be a previously unrecognized target(s) of the APC/C, so that APC/C mutations would suppress *myo1 Δ* phenotypes by increasing the amount of that protein(s).

Role of APC/C in Postmitotic Degradation of *Iqg1p*

To ask whether *Iqg1p* or *Cyk3p* might be a target of the APC/C, we first searched their sequences for the known APC/C recognition motifs called the destruction box (DB) (Glotzer *et al.*, 1991; King *et al.*, 1996; Burton and Solomon, 2001; Burton *et al.*, 2005), KEN box (Pfleger and Kirschner, 2000), A box (Littlepage and Ruderman, 2002), O box (Araki *et al.*, 2005), and GxEN motif (Castro *et al.*, 2003). No such sequences were found in *Cyk3p*; one 5-amino-acid sequence resembles the core of the O box, but the adjacent amino acids are very different. In contrast, two possible DBs, a possible

Figure 5. Suppression of *myo1 Δ* phenotypes by overexpression of *IQG1* or *CYK3*. The genes were carried on *LEU2*-marked low-copy (pRS315-based) or high-copy (pRS425- or YEp181-based) plasmids. (A and B) Suppression of *myo1 Δ* lethality in the W303 background. (A) Suppression of spore inviability. Tetrads were dissected from strain RNY509 (*myo1 Δ ::kanMX6/+*) carrying either an empty vector (top two rows) or an *IQG1* or *CYK3* plasmid (bottom four rows). With the empty vectors, no viable *myo1 Δ* segregants were observed in 43 tetrads dissected (or in 67 tetrads of strain RNY509 dissected in the absence of a plasmid). With the *IQG1* and *CYK3* plasmids, 34 viable *myo1 Δ* segregants (scored by growth on YP+G418) were observed in 168 tetrads dissected; all such segregants also carried an *IQG1* or *CYK3* plasmid (scored by growth on SC-Leu). (B) Suppression demonstrated by a colony-sectoring assay. Strain RNY798 (*myo1 Δ ade2 ade3; MYO1 ADE3* plasmid) was transformed with the indicated plasmids and streaked onto SC-Leu plates. Only transformants carrying an *IQG1* or *CYK3* plasmid could form viable subclones (sectors) that had lost the *MYO1 ADE3* plasmid and thus were white due to the loss of Ade3p activity. (C) Suppression of clustering phenotype in the S288C background. Strains RNY1006, RNY1007, RNY1001, RNY1002, RNY1003, and RNY1004 were grown overnight on SC-Leu plates, and clustering was scored as described in *Materials and Methods*. Values shown are the means of three counts; standard deviations are also indicated.

KEN box, and a GKEN sequence (a possible KEN box or GxEN motif) were found in *Iqg1p* (Figure 6A). Thus, we focused on this protein. Wild-type cells arrested in G1 phase had significantly lower levels of *Iqg1p* and a significantly higher rate of *Iqg1p* turnover than cells arrested in M phase

Figure 6. Increased Iqg1p levels in M-phase cells and in APC/C mutants. All strains expressed either Iqg1p-TAP or Iqg1p-3HA from the chromosomal *IQG1* promoter. (A) Domain structure of Iqg1p. CH, calponin-homology domain; DB1 (191RFELQDLYN199) and DB2 (276RSLIKDFN284) (black lines), putative destruction-box sequences; KEN (600-602) (black line), putative KEN-box; GKEN (627-630) (black line), putative KEN-box or GxEN motif; IQ, IQ repeats; GRD, GAP-related domain. (B) Cell cycle dependence of Iqg1p levels. Strains BY4741 (lane 1; untagged control) and IQG1-TAP (lanes 2 and 3) were grown to exponential phase in YP medium at 30°C, and strain IQG1-TAP was then treated for 2 h either with 100 ng/ml α -factor or with 50 μ g/ml benomyl to arrest cells in G1 or M phase. Proteins were then extracted, immunoprecipitated, and analyzed by SDS-PAGE and immunoblotting as described in *Materials and Methods*. Cdc28p was used as a loading control (see *Materials and Methods*). (C) Regulation of Iqg1p degradation by APC/C^{Cdh1}. Strains DOY138, DOY139, DOY140, and DOY141 were grown to exponential phase in YP medium. Strains DOY138 (top panel) and DOY139 (third panel) were then treated for 2 h with 100 ng/ml α -factor to arrest cells in G1 phase; the last 30 min of this incubation was at 37°C to inactivate the temperature-sensitive APC/C subunit Cdc23-1p in DOY139 while providing an appropriate control. Strain DOY140 (second panel) was arrested in M phase by shifting to YP medium containing 2% galactose + 2% raffinose for 2 h at 30°C to induce expression of the nondegradable Pds1p^{mdb}. Strain DOY141 (bottom panel) was arrested in G1 phase by shifting to 37°C for 2 h to inactivate Cdc28-13p. After these incubations, translation in all four strains was blocked by addition of 500 μ g/ml cycloheximide. Samples were taken at the time of cycloheximide addition and at 10-min intervals thereafter, and proteins were extracted and analyzed as described in B. The blot in the top panel was exposed ~10 times longer than the others in order to allow visualization of the much lower amount (cf. B) of Iqg1p-TAP in the wild-type G1 cells at time 0. (D) Increased Iqg1p levels in APC/C-mutant strains. Strains KO474, KO470, KO460, and KO465 (left four lanes) are in the S288C background; strains KO490, KO486, KO477, and KO482 (right four lanes) are in the W303 background. Each

(Figure 6B; Figure 6C, top two panels), suggesting that the APC/C might target Iqg1p for degradation at the end of mitosis, as it does other proteins. Consistent with this hypothesis, we found that Iqg1p levels were significantly elevated in asynchronous populations of APC/C-mutant cells (Figure 6D) and that the rapid rate of Iqg1p degradation in G1 cells was eliminated in APC/C mutants (Figure 6C, bottom two panels). Moreover, in synchronous cultures, Iqg1p levels were found to increase before mitosis and drop precipitously after mitosis in wild-type cells (Figure 7, A and B, left), and the postmitotic decrease was largely eliminated in an APC/C mutant (Figure 7, A and B, right).

Finally, we also examined cells expressing Iqg1p-GFP from the chromosomal *IQG1* promoter. As reported previously (Epp and Chant, 1997; Shannon and Li, 1999), Iqg1p localized to a ring at the mother-bud neck in medium-budded and large-budded wild-type cells. However, under the conditions used, the neck signal was rather weak (Figure 8A), and it was observed in only ~50% of the medium-budded and large-budded cells. Moreover, the Iqg1p-GFP did not seem to persist through cell division, because signal was never observed in unbudded cells (Figure 8B, left). In contrast, in APC/C mutants examined under identical conditions, Iqg1p-GFP signal was observed at the neck in a higher percentage (~80%) of medium-budded and large-budded cells, and it was significantly brighter there (Figure 8A). Moreover, Iqg1p-GFP seemed to persist through cell division in at least some cells, because patches of signal were sometimes observed in unbudded cells (Figure 8B, right).

Together, the results strongly suggest that Iqg1p is degraded in a cell-cycle-dependent manner that depends, directly or indirectly, on the APC/C.

Ubiquitination of Iqg1p by APC/C and Identification of a Novel APC/C-Recognition Motif

To ask whether Iqg1p is a direct target of the APC/C, in vitro ubiquitination assays were performed using various fragments of Iqg1p and wild-type APC/C with its activator Cdh1p. Under conditions in which the well characterized APC/C substrate Pds1p was ubiquitinated effectively (Figure 9A, lanes 1 and 2), Iqg1p(1-750) and Iqg1p(33-250) were ubiquitinated in an APC/C-dependent manner (Figure 9A, lanes 3 and 4, and B, lanes 1 and 2), whereas Iqg1p(43-750), Iqg1p(400-1100), and Iqg1p(741-1495) were not (Figure 9A, lanes 5-10). These data indicated that Iqg1p is indeed a direct target of the APC/C and that a recognition sequence between amino acids 1 and 42 is required for this ubiquitination. Although this region contains no clear DB or other known APC/C-recognition sequence (see above), amino acids 34-42 have some resemblance (RxxxxxxN) to previously characterized DBs (RxxLxxxxN/D/E), and mutating residues within this sequence reduced (single substitutions: Figure 9B, lanes 3-10) or eliminated (multiple substitutions: Figure 9B, lanes 11-14) APC/C^{Cdh1}-dependent ubiquitination. We also performed similar experiments using full-length Cyk3p. However, consistent with the lack of recognizable APC/C-recognition sequences in this protein, no ubiquitination was detected (data not shown).

To ask whether the apparent APC/C^{Cdh1}-recognition sequence in Iqg1p is also important for its turnover in vivo, we

strain was grown to exponential phase in SC-His medium, and proteins were extracted and analyzed by SDS-PAGE, immunoblotting, and quantitation as described in *Materials and Methods*. Iqg1p-3HA levels are expressed relative to those in the appropriate wild type, using the actin band for normalization.

A (S288C background) **B** (W303 background)

Figure 7. Cell-cycle regulation of Iqg1p levels and the effect of APC/C mutations on this regulation. All strains expressed Iqg1p-3HA from the chromosomal *IQG1* promoter. Strains were grown, synchronized in G1 by treating with α -factor, and sampled as described in *Materials and Methods*. Iqg1p-3HA levels were assessed as described in Figure 6D (but without quantitation in this case), and the percentages of anaphase cells were assessed by DAPI staining as described in *Materials and Methods*. (A) S288C-background strains KO672 and KO671. (B) W303-background strains KO589 and KO588.

compared the stabilities of full-length Iqg1p and Iqg1p(43-1495) in both the W303 and S288C strain backgrounds. As expected, the truncated protein was substantially more stable both during prolonged arrest in G1 (Figure 9C) and at the end of mitosis in a synchronized population (Figure 9D).

Figure 8. Stronger (A) and more persistent (B) Iqg1p-GFP signal in APC/C mutants. Strains KO566 (wild type), KO563 (*doc1Δ*), and KO625 (*swm1Δ*) are in the S288C background and express Iqg1p-GFP from the chromosomal *IQG1* promoter. Cells were grown and observed as described in *Materials and Methods*. (A) For each strain, all medium- and large-budded cells with detectable Iqg1p-GFP signal at the mother-bud neck were imaged using identical exposure times (4 s) and scaling factors. Representative images are shown together with a histogram of average signal intensities (determined as described in *Materials and Methods*) for 80–100 of the imaged cells of each strain; standard errors of the mean are also indicated. (B) Unbudded cells of strains KO566 and KO563 were imaged using identical exposure times (4 s) and scaling factors. Representative fluorescence images are shown.

Suppression of *myo1Δ* Phenotypes by Nondegradable Iqg1p

The data presented above suggest that the elevated levels of Iqg1p resulting from APC/C mutations might explain the suppression of *myo1Δ* phenotypes by such mutations. In this case, synthesis at normal levels of a nondegradable Iqg1p should also be able to suppress *myo1Δ* phenotypes. In agreement with this prediction, we found that expression of Iqg1p(43-1495) from the chromosomal *IQG1* promoter could suppress both the *myo1Δ* lethality in the W303 background (Figure 10A) and the *myo1Δ* clustering phenotype in the S288C background (Figure 10B). Somewhat surprisingly, segregants expressing the nondegradable Iqg1p in an otherwise wild-type background showed little or no defect in growth rate (Figure 10A) or in cell division, although subtle defects in septum formation were observed (our unpublished data). However, overexpression of the nondegradable Iqg1p from a *GAL* promoter did cause severe growth defects (our unpublished data).

DISCUSSION

Suppression of Actomyosin-Ring Defects by APC/C Mutations

Although an actomyosin contractile ring is involved in cytokinesis in most if not all animal and fungal cell types, it has recently become clear that actomyosin-ring-independent mechanisms are also critical, and in some cases sufficient, for cytokinesis (see *Introduction*). In an attempt to elucidate these mechanisms, we are performing genetic studies in *S. cerevisiae*. In this organism, the phenotypes associated with loss of the actomyosin ring (due to deletion of the single myosin II gene, *MYO1*) vary greatly in severity depending on the strain background, presumably due to differences among the strains in the efficiency of the actomyosin-ring-independent mechanisms, as we will discuss in more detail elsewhere.

During our studies, we made the surprising observation that mutations in genes encoding nonessential subunits of the APC/C could suppress both the lethal phenotype of *myo1Δ* in the W303 strain background and the slow-growth and delayed-cell-separation phenotypes of *myo1Δ* in the S288C strain background. Initially, we thought that this suppression probably resulted simply from changes in the timing of late-cell-cycle events; for example, a delay in mitotic exit due to APC/C malfunction might allow more time for an inefficient process of cytokinesis to be completed

Figure 9. Ubiquitination of Iqg1p by APC/C^{cdh1} and identification of a novel APC/C^{cdh1}-recognition motif. (A and B) Ubiquitination of Iqg1p in vitro and its dependence on Iqg1p residues 33–42. Full-length Pds1p and various Iqg1p fragments (as indicated) were synthesized in vitro and incubated with E2-methyl-ubiquitin and purified APC/C^{cdh1} as described in *Materials and Methods*. Proteins were then separated by SDS-PAGE and visualized using a PhosphorImager. The slower migrating proteins seen in the presence of APC/C^{cdh1} (lanes A2, A4, B2, B4, B6, B8, and B10) are Pds1p or Iqg1p ubiquitination products. In B, the point mutations introduced into Iqg1p(33–250) are indicated (see *Materials and Methods*); 3A denotes the triple mutation R34A,S37A,N42A, and 4A denotes the quadruple mutation R34A,S37A,K40A,N42A. (C and D) Dependence of Iqg1p turnover in vivo on the N-terminal APC/C^{cdh1}-recognition sequence. (C) Strains GT24 (pGAL-IQG1-TAP) and GT70 (pGAL-iqg1Δ42-TAP) were grown to exponential phase in YP medium containing 2% raffinose as sole carbon source and arrested in G1 phase by treatment with 1 μg/ml α-factor for 4 h. Galactose (2%) was then added to induce Iqg1p-TAP or Iqg1Δ42p-TAP for 1 h, after which glucose (2%) and cycloheximide (100 μg/ml) were added to block both transcription and translation of the IQG1 constructs. Samples were taken at the time of glucose and cycloheximide addition and at intervals thereafter, and Iqg1p-TAP was extracted and analyzed by immunoblotting as described in *Materials and Methods*. (D) Strains IQG1-TAP (IQG1-TAP) and GT124 (iqg1Δ42-TAP) were grown to exponential phase in YP medium at 30°C, arrested in G1 phase by treatment with 10 μg/ml α-factor for 4 h, and released from the arrest by washing once and resuspending in fresh YP medium. When 90% of the cells had budded, 10 μg/ml α-factor was added again to prevent the initiation of new cell cycles. Samples were taken at the time of release from α-factor arrest and at intervals thereafter, and proteins (including Clb2p as a control) were analyzed by immunoblotting as described in *Materials and Methods*.

successfully. However, several lines of evidence (the suppression by *cdh1* and *mmd2* mutations; the lack of effect of a

pds1 mutation or of expression of a nondegradable Clb2p; see *Results* for details) suggested strongly that the suppression was not due to changes in the timing of either anaphase or mitotic exit, and hence that it was likely to involve some novel APC/C target(s), pathway(s), or both. Indeed, we have now obtained strong evidence that suppression occurs because the APC/C defects result in an increased abundance of Iqg1p, a novel APC/C^{cdh1} target that is important in actomyosin-ring-independent cytokinesis, as discussed further below.

This model also provides a plausible explanation for the observation that a *net1* mutation exacerbates the *myo1Δ* phenotype in the S288C strain background. We initially thought that an acceleration of mitotic exit due to loss of Net1p might allow insufficient time for an inefficient cytokinesis process to be completed. However, it now seems more likely that premature activation of Cdc14p resulting from the absence of Net1p leads to premature activation of Cdh1p (Visintin *et al.*, 1998) and thus to targeting of Iqg1p for degradation by APC/C^{cdh1} before its role in cytokinesis can be completed. Of course, the effects of *net1* mutations could also be more complex.

Identification of Iqg1p as the Relevant APC/C Target

In a dosage-suppressor screen, we observed that even low-copy plasmids containing *IQG1* or *CYK3* could suppress the *myo1Δ* phenotypes in both the S288C and W303 genetic backgrounds. These observations suggested that Iqg1p, Cyk3p, or both might be previously unidentified targets of APC/C. Thus, mutations that compromised APC/C func-

Figure 10. Suppression of *myo1Δ* phenotypes by expression of a nondegradable Iqg1p at endogenous levels. (A) Suppression of *myo1Δ* lethality in the W303 background. Tetrads were dissected from two strains (KO1228 and KO1229) that are heterozygous for both *myo1Δ::kanMX6* and *iqg1Δ42::LEU2*. From 44 tetrads dissected, 34 viable *myo1Δ* segregants were recovered, as shown by growth on YP+G418; each of these also carried *iqg1Δ42*, as shown by growth on SC-Leu. (B) Suppression of clustering phenotype in the S288C background. Strains YEF473A, KO1248, and KO1249 were grown overnight on YP plates and clustering was scored as described in *Materials and Methods*.

tion might allow the intracellular concentrations of Iqg1p and/or Cyk3p to rise to levels that could suppress the *myo1Δ* phenotypes, by mechanisms that we will consider in more detail elsewhere. Although it remains possible that Cyk3p is an APC/C target, there is as yet no evidence to support this possibility: Cyk3p contains no clearly recognizable APC/C-recognition sequences, and we failed to detect ubiquitination *in vitro* by APC/C^{Cdh1}. In contrast, a variety of *in vivo* and *in vitro* experiments indicate that Iqg1p is a target of APC/C^{Cdh1}. First, in wild-type cells, both the levels of Iqg1p and the rates of Iqg1p degradation fluctuate during the cell cycle in a manner consistent with APC/C-triggered degradation at the end of mitosis. Second, in APC/C (including *cdh1*) mutants, the rates of Iqg1p degradation are drastically reduced; correspondingly, Iqg1p is present at higher levels and persists abnormally through cell division. Finally, *in vitro* assays showed that Iqg1p can be ubiquitinated directly by APC/C^{Cdh1}. The observation that the *myo1Δ* phenotypes can be suppressed by expression from the chromosomal *IQG1* promoter of a stabilized Iqg1p (lacking the APC/C^{Cdh1} recognition site) provides strong support for the hypothesis that the suppression of *myo1Δ* by APC/C mutations indeed results from the elevated and more persistent levels of Iqg1p in the mutant strains.

Insight into APC/C Function

Interestingly, recognition of Iqg1p by APC/C^{Cdh1} does not seem to depend on any of the sequences that correspond to previously characterized APC/C-recognition motifs. Instead, the ubiquitination of Iqg1p depends on a novel sequence (33-LRPQSSSKIN-42) near the N terminus that is similar but not identical to the consensus DB motif (RxxLxxxxN/D/E; Burton and Solomon, 2001). We observed only modest effects on ubiquitination when single residues in the Iqg1p sequence were replaced by alanine, but mutants in which positions 34, 37, and 42 were all altered showed an essentially complete loss of ubiquitination *in vitro*. Further dissection of the amino acids important for recognition of this site by APC/C^{Cdh1} should shed light on the mechanisms of APC/C-target interaction. In the meantime, the results highlight the danger of attempting to identify APC/C targets solely on the basis of the known recognition motifs.

Another interesting conclusion from this study concerns the role of Mnd2p. Although this APC/C subunit seems to have little or no role in mitotic control (Oelschlaegel *et al.*, 2005; Page *et al.*, 2005), we found that an *mnd2* mutation suppressed the *myo1Δ* phenotypes as strongly as did other APC/C mutations. This apparent discrepancy might be explained if particular APC/C subunits are important for the interactions with different specific targets. However, it also seems possible that the explanation might simply involve differential strengths of the interactions of the APC/C with different targets. In particular, because the APC/C roles in anaphase promotion and mitotic exit are essential for cell survival, the interactions with the targets relevant to these processes might be stronger than the interaction with a target like Iqg1p whose APC/C-mediated degradation is not essential for cell survival. The loss of an accessory subunit like Mnd2p might have little effect on the strong interactions but significantly compromise the weak interactions. An attraction of this model is that it might also help to explain the otherwise puzzling observation that loss of each of the non-essential APC/C subunits produces a very similar suppression of the *myo1Δ* phenotypes, even though each subunit seems to have a distinct role in APC/C function (Carroll and

Morgan, 2002; Passmore *et al.*, 2003; Schwickart *et al.*, 2004; Carroll *et al.*, 2005; Page *et al.*, 2005).

Another interesting question concerns the mechanisms by which the APC/C, which resides predominantly or exclusively in the nucleus (Sikorski *et al.*, 1993; Zachariae *et al.*, 1996; Jaquenoud *et al.*, 2002; Melloy and Holloway, 2004), ubiquitinates proteins, such as Hsl1p (Burton and Solomon, 2000, 2001) and Iqg1p (this study), that seem to reside predominantly or exclusively in the cytoplasm (Epp and Chant, 1997; Barral *et al.*, 1999; Shannon and Li, 1999, 2000; Shulewitz *et al.*, 1999; Longtine *et al.*, 2000; Theesfeld *et al.*, 2003). Although it remains possible that some APC/C acts in the cytoplasm, a more attractive model is that cytoplasmic APC/C targets must enter the nucleus in order to be ubiquitinated. At least for APC/C^{Cdh1} targets, such nuclear entry may occur after the target is already bound to Cdh1p, a model supported both by studies on the order of assembly of APC/C-Cdh1p-substrate complexes (Burton *et al.*, 2005) and by the observation that Cdh1p, unlike other APC/C components, shuttles in and out of the nucleus during the cell cycle (Jaquenoud *et al.*, 2002). In this regard, it is also striking that a substantial fraction of the cytoplasmic Cdh1p is concentrated at the mother-bud neck (Jaquenoud *et al.*, 2002), the very site to which both Hsl1p and Iqg1p are localized before their ubiquitination-dependent degradation.

Role of Timely APC/C-Mediated Degradation of Iqg1p

An interesting and unresolved question is the role of the APC/C-dependent proteolysis of Iqg1p, a question that is underscored by the observation that expression of the nondegradable Iqg1p from the chromosomal *IQG1* promoter had little effect on the growth or division of wild-type cells. One possible answer involves the multiple roles of Mlc1p, which is a light chain for Iqg1p, Myo1p, and the type V myosin Myo2p, and whose interaction with Myo2p is critical for septum formation, cell separation, and new bud formation (Stevens and Davis, 1998; Boyne *et al.*, 2000; Shannon and Li, 2000; Wagner *et al.*, 2002; Luo *et al.*, 2004). *S. cerevisiae* cells are highly sensitive to the cellular levels of this protein: heterozygous *mlc1Δ* diploid cells display haploinsufficiency that can be suppressed by reducing the copy number of *MYO2* (Stevens and Davis, 1998), and overexpression of a Myo1p C-terminal tail (containing the Mlc1p-binding IQ motifs) leads to cytokinetic defects that can be alleviated by overexpressing Mlc1p (Tolliday *et al.*, 2003). In addition, our own recent studies have shown that overexpression of wild-type Iqg1p from its own promoter on a high-copy plasmid in wild-type cells can cause abnormalities in cytokinesis (our unpublished results). Finally, overexpression of the nondegradable Iqg1p from the *GAL* promoter on a high-copy plasmid seems to cause severe growth defects (see above). Together, these results suggest that the association of Mlc1p with each binding partner may need to be fine-tuned temporally and that the timely APC/C-mediated degradation of Iqg1p may be important to release Mlc1p, particularly for interaction with Myo2p during secondary-septum formation and/or the formation of the new bud.

Possible Conserved Regulation of IQGAPs by the APC/C

IQGAPs and the APC/C are both widely conserved, so it is possible that IQGAPs in other organisms are also regulated by the APC/C. This hypothesis is particularly attractive for Rng2p in *Schizosaccharomyces pombe*, IQGAP1 in mammalian

cells, and PES-7 (F09C3.1) in *Caenorhabditis elegans*, because these proteins are also involved in cytokinesis (Eng *et al.*, 1998; Wu *et al.*, 2003; Skop *et al.*, 2004). However, although each protein has one or more DB consensus sequences, none of them has a sequence corresponding to the novel APC/C-recognition motif identified in Iqg1p. The possible regulation of these proteins by the APC/C will require further investigation.

ACKNOWLEDGMENTS

We thank I-Ching Yu for initiating the synthetic-lethal screen; Erfei Bi, Forrest Spencer, Phil Hieter, Erin O'Shea, Jonathan Weissman, and John Cooper for strains, plasmids, and antibodies; Steve Haase and members of his laboratory for help with the FACS analysis; and Akanksha Gangar for a protein extraction protocol. We also thank Danny Lew, Pat Brennwald, Steve Haase, Matt Sullivan, and members of their and our own laboratories for helpful suggestions and support. This work was supported by National Institutes of Health grants GM-31006 (to J.R.P.), GM-53270 (to D.O.M.), and GM-76200 (to M.J.S.) and by March of Dimes grant 0455851T (to M.J.S.). R.N. was supported in part by a long-term fellowship from the Human Frontier Science Program.

REFERENCES

- Adam, J. C., Pringle, J. R., and Peifer, M. (2000). Evidence for functional differentiation among *Drosophila* septins in cytokinesis and cellularization. *Mol. Biol. Cell* 11, 3123–3135.
- Amon, A., Irniger, S., and Nasmyth, K. (1994). Closing the cell cycle circle in yeast: G2 cyclin proteolysis initiated at mitosis persists until the activation of G1 cyclins in the next cycle. *Cell* 77, 1037–1050.
- An, H., Morrell, J. L., Jennings, J. L., Link, A. J., and Gould, K. L. (2004). Requirements of fission yeast septins for complex formation, localization, and function. *Mol. Biol. Cell* 15, 5551–5564.
- Araki, M., Yu, H., and Asano, M. (2005). A novel motif governs APC-dependent degradation of *Drosophila* ORC1 in vivo. *Genes Dev.* 19, 2458–2465.
- Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1995). *Current Protocols in Molecular Biology*, New York: John Wiley & Sons.
- Barral, Y., Parra, M., Bidlingmaier, S., and Snyder, M. (1999). Nim1-related kinases coordinate cell cycle progression with the organization of the peripheral cytoskeleton in yeast. *Genes Dev.* 13, 176–187.
- Baudin, A., Ozier-Kalogeropoulos, O., Denouel, A., Lacroute, F., and Cullin, C. (1993). A simple and efficient method for direct gene deletion in *Saccharomyces cerevisiae*. *Nucleic Acids Res.* 21, 3329–3330.
- Bender, A., and Pringle, J. R. (1991). Use of a screen for synthetic lethal and multicopy suppressor mutants to identify two new genes involved in morphogenesis in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 11, 1295–1305.
- Bi, E., and Pringle, J. R. (1996). *ZDS1* and *ZDS2*, genes whose products may regulate Cdc42p in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 16, 5264–5275.
- 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.
- Boyne, J. R., Yusuf, H. M., Bieganowski, P., Brenner, C., and Price, C. (2000). Yeast myosin light chain, Mlc1p, interacts with both IQGAP and Class II myosin to effect cytokinesis. *J. Cell Sci.* 113, 4533–4543.
- Brachmann, C. B., Davies, A., Cost, G. J., Caputo, E., Li, J., Hieter, P., and Boeke, J. D. (1998). Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* 14, 115–132.
- Brown, M. D., and Sacks, D. B. (2006). IQGAP1 in cellular signaling: bridging the GAP. *Trends Cell Biol.* 16, 242–249.
- Burton, J. L., and Solomon, M. J. (2000). Hsl1p, a Swe1p inhibitor, is degraded via the anaphase-promoting complex. *Mol. Cell Biol.* 20, 4614–4625.
- Burton, J. L., and Solomon, M. J. (2001). D box and KEN box motifs in budding yeast Hsl1p are required for APC-mediated degradation and direct binding to Cdc20p and Cdh1p. *Genes Dev.* 15, 2381–2395.
- Burton, J. L., Tsakraklides, V., and Solomon, M. J. (2005). Assembly of an APC-Cdh1-substrate complex is stimulated by engagement of a destruction box. *Mol. Cell* 18, 533–542.
- Cabib, E., Roh, D.-H., Schmidt, M., Crotti, L. B., and Varma, A. (2001). The yeast cell wall and septum as paradigms of cell growth and morphogenesis. *J. Biol. Chem.* 276, 19679–19682.
- Carroll, C. W., and Morgan, D. O. (2002). The Doc1 subunit is a processivity factor for the anaphase-promoting complex. *Nat. Cell Biol.* 4, 880–887.
- Carroll, C. W., and Morgan, D. O. (2005). Enzymology of the anaphase-promoting complex. *Methods Enzymol.* 398, 219–230.
- Carroll, C. W., Enquist-Newman, M., and Morgan, D. O. (2005). The APC subunit Doc1 promotes recognition of the substrate destruction box. *Curr. Biol.* 15, 11–18.
- Castro, A., Vigneron, S., Bernis, C., Labbé, J.-C., and Lorca, T. (2003). Xkid is degraded in a D-box, KEN-box, and A-box-independent pathway. *Mol. Cell Biol.* 23, 4126–4138.
- Charles, J. F., Jaspersen, S. L., Tinker-Kulberg, R. L., Hwang, L., Szidon, A., and Morgan, D. O. (1998). The Polo-related kinase Cdc5 activates and is destroyed by the mitotic cyclin destruction machinery in *S. cerevisiae*. *Curr. Biol.* 8, 497–507.
- Christianson, T. W., Sikorski, R. S., Dante, M., Shero, J. H., and Hieter, P. (1992). Multifunctional yeast high-copy-number shuttle vectors. *Gene* 110, 119–122.
- Cohen-Fix, O., Peters, J.-M., Kirschner, M. W., and Koshland, D. (1996). Anaphase initiation in *Saccharomyces cerevisiae* is controlled by the APC-dependent degradation of the anaphase inhibitor Pds1p. *Genes Dev.* 10, 3081–3093.
- Colman-Lerner, A., Chin, T. E., and Brent, R. (2001). Yeast Cbk1 and Mob2 activate daughter-specific genetic programs to induce asymmetric cell fates. *Cell* 107, 739–750.
- Dobbelare, J., and Barral, Y. (2004). Spatial coordination of cytokinetic events by compartmentalization of the cell cortex. *Science* 305, 393–396.
- Eng, K., Naqvi, N. I., Wong, K.C.Y., and Balasubramanian, M. K. (1998). Rng2p, a protein required for cytokinesis in fission yeast, is a component of the actomyosin ring and the 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.
- Glötzer, M., Murray, A. W., and Kirschner, M. W. (1991). Cyclin is degraded by the ubiquitin pathway. *Nature* 349, 132–138.
- Gelperin, D. M. *et al.* (2005). Biochemical and genetic analysis of the yeast proteome with a movable ORF collection. *Genes Dev.* 19, 2816–2826.
- Ghaemmaghami, S., Huh, W.-K., Bower, K., Howson, R. W., Belle, A., Dehoure, N., O'Shea, E. K., and Weissman, J. S. (2003). Global analysis of protein expression in yeast. *Nature* 425, 737–741.
- Gladfelter, A. S. (2006). Control of filamentous fungal cell shape by septins and formins. *Nat. Rev. Microbiol.* 4, 223–229.
- Gladfelter, A. S., Pringle, J. R., and Lew, D. J. (2001). The septin cortex at the yeast mother-bud neck. *Curr. Opin. Microbiol.* 4, 681–689.
- Guthrie, C., and Fink, G. R., eds. (1991). *Guide to Yeast Genetics and Molecular Biology*, Volume 194, *Methods in Enzymology*, New York: Academic.
- Haase, S. B., and Reed, S. I. (2002). Improved flow cytometric analysis of the budding yeast cell cycle. *Cell Cycle* 1, 132–136.
- Hall, P. A., and Russell, S.E.H. (2004). The pathobiology of the septin gene family. *J. Pathol.* 204, 489–505.
- Harper, J. W., Burton, J. L., and Solomon, M. J. (2002). The anaphase-promoting complex: it's not just for mitosis any more. *Genes Dev.* 16, 2179–2206.
- Jaquenoud, M., van Drogen, F., and Peter, M. (2002). Cell cycle-dependent nuclear export of Cdh1p may contribute to the inactivation of APC/C^{dh1}. *EMBO J.* 21, 6515–6526.
- Jaspersen, S. L., Charles, J. F., Tinker-Kulberg, R. L., and Morgan, D. O. (1998). A late mitotic regulatory network controlling cyclin destruction in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* 9, 2803–2817.
- Kanada, M., Nagasaki, A., and Uyeda, T.Q.P. (2005). Adhesion-dependent and contractile ring-independent equatorial furrowing during cytokinesis in mammalian cells. *Mol. Biol. Cell* 16, 3865–3872.
- Karpova, T. S., Lepetit, M. M., and Cooper, J. A. (1993). Mutations that enhance the *cap2* null mutant phenotype in *Saccharomyces cerevisiae* affect the actin cytoskeleton, morphogenesis and pattern of growth. *Genetics* 135, 693–709.
- Kellogg, D. R., and Murray, A. W. (1995). NAP1 acts with Clb2 to perform mitotic functions and to suppress polar bud growth in budding yeast. *J. Cell Biol.* 130, 675–685.

- King, R. W., Glotzer, M., and Kirschner, M. W. (1996). Mutagenic analysis of the destruction signal of mitotic cyclins and structural characterization of ubiquitinated intermediates. *Mol. Biol. Cell* 7, 1343–1357.
- Kinoshita, M., and Noda, M. (2001). Roles of septins in the mammalian cytokinesis machinery. *Cell Struct. Funct.* 26, 667–670.
- Korinek, W. S., Bi, E., Epp, J. A., Wang, L., Ho, J., and Chant, J. (2000). Cyk3, a novel SH3-domain protein, affects cytokinesis in yeast. *Curr. Biol.* 10, 947–950.
- Kuranda, M. J., and Robbins, P. W. (1991). Chitinase is required for cell separation during growth of *Saccharomyces cerevisiae*. *J. Biol. Chem.* 266, 19758–19767.
- Lillie, S. H., and Pringle, J. R. (1980). Reserve carbohydrate metabolism in *Saccharomyces cerevisiae*: responses to nutrient limitation. *J. Bacteriol.* 143, 1384–1394.
- Lippincott, J., and Li, R. (1998). 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.
- Littlepage, L. E., and Ruderman, J. V. (2002). Identification of a new APC/C recognition domain, the A box, which is required for the Cdh1-dependent destruction of the kinase Aurora-A during mitotic exit. *Genes Dev.* 16, 2274–2285.
- Longtine, M. S., and Bi, E. (2003). Regulation of septin organization and function in yeast. *Trends Cell Biol.* 13, 403–409.
- Longtine, M. S., DeMarini, D. J., Valencik, M. L., Al-Awar, O. S., Fares, H., De Virgilio, C., and Pringle, J. R. (1996). The septins: roles in cytokinesis and other processes. *Curr. Opin. Cell Biol.* 8, 106–119.
- Longtine, M. S., McKenzie, III, A., DeMarini, D. J., Shah, N. G., Wach, A., Brachat, A., Philippsen, P., and Pringle, J. R. (1998). Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. *Yeast* 14, 953–961.
- Longtine, M. S., Theesfeld, C. L., McMillan, J., Weaver, E., Pringle, J. R., and Lew, D. J. (2000). Septin-dependent assembly of a cell cycle-regulatory module in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 20, 4049–4061.
- Lord, M., Laves, E., and Pollard, T. D. (2005). Cytokinesis depends on the motor domains of myosin-II in fission yeast but not in budding yeast. *Mol. Biol. Cell* 16, 5346–5355.
- Luo, J., Vallen, E. A., Dravis, C., Tcheperegine, S. E., Drees, B., and Bi, E. (2004). Identification and functional analysis of the essential and regulatory light chains of the only type II myosin Myo1p in *Saccharomyces cerevisiae*. *J. Cell Biol.* 165, 843–855.
- Melloy, P. G., and Holloway, S. L. (2004). Changes in the localization of the *Saccharomyces cerevisiae* anaphase-promoting complex upon microtubule depolymerization and spindle checkpoint activation. *Genetics* 167, 1079–1094.
- Mortimer, R. K., and Johnston, J. R. (1986). Genealogy of principal strains of the yeast genetic stock center. *Genetics* 113, 35–43.
- Nagasaki, A., de Hostos, E. L., and Uyeda, T.Q.P. (2002). Genetic and morphological evidence for two parallel pathways of cell-cycle-coupled cytokinesis in *Dictyostelium*. *J. Cell Sci.* 115, 2241–2251.
- Nasmyth, K. (2005). How do so few control so many? *Cell* 120, 739–746.
- Nguyen, T. Q., Sawa, H., Okano, H., and White, J. G. (2000). The *C. elegans* septin genes, *unc-59* and *unc-61*, are required for normal postembryonic cytokinesis and morphogenesis but have no essential function in embryogenesis. *J. Cell Sci.* 113, 3825–3837.
- Oelschlaegel, T., Schwickart, M., Matos, J., Bogdanova, A., Camasses, A., Havlis, J., Shevchenko, A., and Zachariae, W. (2005). The yeast APC/C subunit Mnd2 prevents premature sister chromatid separation triggered by the meiosis-specific APC/C-Ama1. *Cell* 120, 773–788.
- Ostapenko, D., and Solomon, M. J. (2005). Phosphorylation by Cak1 regulates the C-terminal domain kinase Ctk1 in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 25, 3906–3913.
- Page, A. M., Aneliunas, V., Lamb, J. R., and Hieter, P. (2005). In vivo characterization of the nonessential budding yeast anaphase-promoting complex/cyclosome components Swm1p, Mnd2p, and Apc9p. *Genetics* 170, 1045–1062.
- Passmore, L. A. (2004). The anaphase-promoting complex (APC): the sum of its parts? *Biochem. Soc. Trans.* 32, 724–727.
- Passmore, L. A., McCormack, E. A., Au, S.W.N., Paul, A., Willison, K. R., Harper, J. W., and Barford, D. (2003). Doc1 mediates the activity of the anaphase-promoting complex by contributing to substrate recognition. *EMBO J.* 22, 786–796.
- Peters, J.-M. (2006). The anaphase promoting complex/cyclosome: a machine designed to destroy. *Nat. Rev. Mol. Cell Biol.* 7, 644–656.
- Pfleger, C. M., and Kirschner, M. W. (2000). The KEN box: an APC recognition signal distinct from the D box targeted by Cdh1. *Genes Dev.* 14, 655–665.
- Pringle, J. R., and Hartwell, L. H. (1981). The *Saccharomyces cerevisiae* cell cycle. In: *The Molecular Biology of the Yeast Saccharomyces. Life Cycle and Inheritance*, ed. J. N. Strathern, E. W. Jones, and J. R. Broach, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 97–142.
- Rodriguez, J. R., and Paterson, B. M. (1990). Yeast myosin heavy chain mutant: maintenance of the cell type specific budding pattern and the normal deposition of chitin and cell wall components requires an intact myosin heavy chain gene. *Cell Motil. Cytoskeleton* 17, 301–308.
- Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- Schenkman, L. R., Caruso, C., Pagé, N., and Pringle, J. R. (2002). The role of cell cycle-regulated expression in the localization of spatial landmark proteins in yeast. *J. Cell Biol.* 156, 829–841.
- Schmidt, M., Bowers, B., Varma, A., Roh, D.-H., and Cabib, E. (2002). In budding yeast, contraction of the actomyosin ring and formation of the primary septum at cytokinesis depend on each other. *J. Cell Sci.* 115, 293–302.
- Schwickart, M., Havlis, J., Habermann, B., Bogdanova, A., Camasses, A., Oelschlaegel, T., Shevchenko, A., and Zachariae, W. (2004). Swm1/Apc13 is an evolutionarily conserved subunit of the anaphase-promoting complex stabilizing the association of Cdc16 and Cdc27. *Mol. Cell Biol.* 24, 3562–3576.
- Shannon, K. B., and Li, R. (1999). The multiple roles of Cyk1p in the assembly and function of the actomyosin ring in budding yeast. *Mol. Biol. Cell* 10, 283–296.
- Shannon, K. B., and Li, R. (2000). A myosin light chain mediates the localization of the budding yeast IQGAP-like protein during contractile ring formation. *Curr. Biol.* 10, 727–730.
- Shulewitz, M. J., Inouye, C. J., and Thorner, J. (1999). Hsl7 localizes to a septin ring and serves as an adapter in a regulatory pathway that relieves tyrosine phosphorylation of Cdc28 protein kinase in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 19, 7123–7137.
- 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* 112, 19–27.
- Sikorski, R. S., Michaud, W. A., Hieter, P. (1993). p62^{cdc23} of *Saccharomyces cerevisiae*: a nuclear tetratricopeptide repeat protein with two mutable domains. *Mol. Cell Biol.* 13, 1212–1221.
- Skop, A. R., Liu, H., Yates, III, J., Meyer, B. J., and Heald, R. (2004). Dissection of the mammalian midbody proteome reveals conserved cytokinesis mechanisms. *Science* 305, 61–66.
- Spiliotis, E. T., and Nelson, W. J. (2006). Here come the septins: novel polymers that coordinate intracellular functions and organization. *J. Cell Sci.* 119, 4–10.
- Sprague, G. F., Jr. (1991). Assay of yeast mating reaction. *Methods Enzymol.* 194, 77–93.
- 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.
- Theesfeld, C. L., Zyla, T. R., Bardes, E.G.S., and Lew, D. J. (2003). A monitor for bud emergence in the yeast morphogenesis checkpoint. *Mol. Biol. Cell* 14, 3280–3291.
- Thomas, B. J., and Rothstein, R. (1989). Elevated recombination rates in transcriptionally active DNA. *Cell* 56, 619–630.
- Tolliday, N., Pitcher, M., and Li, R. (2003). Direct evidence for a critical role of myosin II in budding yeast cytokinesis and the evolvability of new cytokinetic mechanisms in the absence of myosin II. *Mol. Biol. Cell* 14, 798–809.

- Ubersax, J. A., Woodbury, E. L., Quang, P. N., Paraz, M., Blethrow, J. D., Shah, K., Shokat, K. M., and Morgan, D. O. (2003). Targets of the cyclin-dependent kinase Cdk1. *Nature* 425, 859–864.
- Versele, M., and Thorner, J. (2005). Some assembly required: yeast septins provide the instruction manual. *Trends Cell Biol.* 15, 414–424.
- Visintin, R., Craig, K., Hwang, E. S., Prinz, S., Tyers, M., and Amon, A. (1998). The phosphatase Cdc14 triggers mitotic exit by reversal of Cdk-dependent phosphorylation. *Mol. Cell* 2, 709–718.
- Wach, A., Brachat, A., Alberti-Segui, C., Rebischung, C., and Philippsen, P. (1997). Heterologous *HIS3* marker and GFP reporter modules for PCR-targeting in *Saccharomyces cerevisiae*. *Yeast* 13, 1065–1075.
- Wagner, W., Bielli, P., Wacha, S., and Ragnini-Wilson, A. (2002). Mlc1p promotes septin closure during cytokinesis via the IQ motifs of the vesicle motor Myo2p. *EMBO J.* 23, 6397–6408.
- Wu, J.-Q., Kuhn, J. R., Kovar, D. R., and Pollard, T. D. (2003). Spatial and temporal pathway for assembly and constriction of the contractile ring in fission yeast cytokinesis. *Dev. Cell* 5, 723–734.
- Zachariae, W., Shin, T. H., Galova, M., Obermaier, B., and Nasmyth, K. (1996). Identification of subunits of the anaphase-promoting complex of *Saccharomyces cerevisiae*. *Science* 274, 1201–1204.
- Zhao, X., Muller, E.G.D., and Rothstein, R. (1998). A suppressor of two essential checkpoint genes identifies a novel protein that negatively affects dNTP pools. *Mol. Cell* 2, 329–340.