A Defect of Kap104 Alleviates the Requirement of Mitotic Exit Network Gene Functions in *Saccharomyces cerevisiae*

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ABSTRACT

A subgroup of the karyopherin β (also called importin β) protein that includes budding yeast Kap104 and human transportin/karyopherin β2 is reported to function as a receptor for the transport of mRNA-binding proteins into the nucleus. We identified *KAP104* as a responsible gene for a suppressor mutation of *cdc15-2*. We found that the *kap104-E604K* mutation suppressed the temperature-sensitive growth of *cdc15-2* cells by promoting the exit from mitosis and suppressed the temperature sensitivity of various mitotic-exit mutations. The cytokinesis defect of these mitotic-exit mutants was not suppressed by *kap104-E604K*. Furthermore, the *kap104-E604K* mutation delays entry into DNA synthesis even at a permissive temperature. In *cdc15-2 kap104-E604K* cells, *SWI5* and *SIC1*, but not *CDH1*, became essential at a high temperature, suggesting that the *kap104-E604K* mutation promotes mitotic exit via the Swi5-Sic1 pathway. Interestingly, *SPO12*, which is involved in the release of Cdc14 from the nucleolus during early anaphase, also became essential in *cdc15-2 kap104-E604K* cells at a high temperature. The *kap104-E604K* mutation caused a partial delocalization of Cdc14 from the nucleolus during interphase. This delocalization of Cdc14 was suppressed by the deletion of *SPO12*. These results suggest that a mutation in Kap104 stimulates exit from mitosis through the activation of Cdc14 and implies a novel role for Kap104 in cell-cycle progression in budding yeast.

KARYOPHERINS (also known as importins/exportins/transportins), a family of soluble and structurally related proteins, serve as receptors in nucleocytoplasmic transport. Karyopherins bind their cargoes and transport them into and out of the nucleus. Ran GTPase regulates the interaction between karyopherins and their cargoes. Ran in its GTP-bound form, which is enriched in the nucleus, promotes the assembly of karyopherin/cargo complexes in the export processes or the disassembly of karyopherin/cargo complexes in the import processes (Sazer and Dasso 2000; Macara 2001).

In the budding yeast *Saccharomyces cerevisiae*, some mutations in karyopherin genes affect cell-cycle progression. Srp1, the sole importin α protein in this organism, is required for the G_2/M transition and for the degradation of the mitotic cyclin Clb2 in G_1 (Loeb *et al.* 1995; Hood and Silver 1998). Cse1/Kap109, a karyopherin β protein, is required for the progression through mitosis and for faithful chromosome segregation (Xiao *et al.* 1993; Schroeder *et al.* 1999). Yrb1, a budding yeast homolog of Ran-binding protein 1 (Ran BP1; Coutavas *et al.* 1993), is required for cell-cycle progression of G_1 phase and mitosis (Ouspenski 1998; Baumer *et al.* 2000). Involvement of karyopherins in exit from mitosis is suggested from the observation that the mutation either in *SRP1* or in *MTR10*, encoding a karyopherin β protein, bypasses the requirement of Cdc15 kinase for exit from mitosis (Shou and Deshaies 2002).

A signaling system called the mitotic exit network (MEN), which includes Cdc15 and Cdc14, eventually inactivates mitotic cyclin-dependent kinases (CDKs) at the end of mitosis by promoting the expression of the CDK inhibitor Sic1 and the activation of the anaphase-promoting complex (APC)/cyclosome, which brings about the degradation of mitotic cyclins (Bardin and Amon 2001). Cdc15 functions as an effector of Tem1 GTPase, whose activation triggers the signal for mitotic exit, and activates the Mob1-Dbf2 complex by phosphorylation (Asakawa *et al.* 2001; Lee *et al.* 2001; Mah *et al.* 2001). The protein phosphatase Cdc14 is localized to the nucleolus (Shou *et al.* 1999; Visintin *et al.* 1999) and the spindle-pole body (SPB; Yoshida *et al.* 2002). The localization of Cdc14, which is regulated by the cdc fourteen early anaphase release (FEAR) network, which includes Cdc5, Esp1, Slk19, and Spo12 during early anaphase, and by MEN during anaphase/telophase, is dynamically changed upon its release from the nucleolus at the onset of anaphase (Stegmeier *et al.* 2002). Once activated during anaphase/telophase, Cdc14 promotes accumulation of Swi5, a major transcriptional activator for *SIC1*, in the nucleus by dephosphorylation of Swi5 (Nasmyth *et al.* 1990; Moll *et al.* 1991; Knapp *et al.* 1996; Toyn *et al.* 1997; Visintin *et al.* 1998). Cdc14 also activates APC^{Cdh1} through dephosphorylation of Cdh1, which thereby promotes the ubiquitination of

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mitotic cyclins (Zachariae *et al.* 1998; Jaspersen *et al.* 1999).

A subgroup of the karyopherin β (also called importin β) protein, which includes transportin/karyopherin β2 (hereafter referred to as transportin), is reported to function as a receptor for the transport of mRNA-binding proteins into the nucleus in mammalian cells (Bonifaci *et al.* 1997; Siomi *et al.* 1997). Kap104/transportin from budding yeast also imports mRNA-binding proteins, Nab2 and Hrp1/Nab4, into the nucleus (Aitchison *et al.* 1996). From our search for the factors that genetically interact with *CDC15*, we identified *KAP104*. The *kap104-E604K* mutation suppressed the temperature-sensitive growth of *cdc15-2* cells by promoting the exit from mitosis. Furthermore, the present study suggests that Kap104/transportin-related protein is required for the maintenance of the mitotic state. We also found that the *kap104-E604K* mutation caused a partial delocalization of Cdc14 from the nucleolus during interphase, suggesting that a mutation in Kap104/transportin-related protein stimulates exit from mitosis through the activation of Cdc14.

MATERIALS AND METHODS

Microbial manipulation: The principal yeast strains used in this study are listed in Table 1. Strains derived from them were also used as described in the text. Yeast cells were grown either in rich medium (YPD) consisting of yeast extract (DIFCO, Detroit), polypeptone (Nihon Seiyaku, Tokyo), and glucose or in synthetic glucose medium (SC), which is SD containing appropriate supplements (Sherman *et al.* 1986). Yeast transformations were performed by the method of Ito *et al.* (1983), and other standard yeast genetic manipulations were performed as described by Sherman *et al.* (1986). The *Escherichia coli* strain used is DH5α[*supE44*Δ*lacU169* (ϕ*80lacZ*Δ*M15*) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1*].

Plasmid and strain construction: The *Sal*I^{-643}-*Sca*I^{+3319} genomic fragment containing the *KAP104* gene was cloned into the *Sal*I- and the *Sma*I-digested pRS316 (Sikorski and Hieter 1989), generating pKZ006 (for the sequence coordinate, see below). The gap-repair plasmid for the *kap104-E604K* gene was constructed as follows. The *Cla*I^{-208}-*Cla*I^{+2308} fragment of pKZ006 was deleted by *Cla*I digestion and religation, generating pKZ011. *kap104-E604K* cells (YKZ0239) were transformed with the *Cla*I-digested pKZ011 and the plasmid that contains the *kap104-E604K* gene was rescued from the resulting Ura$^+$ transformants and designated pKZ012. The site of the *kap104-E604K* mutation was determined by DNA sequencing (Sanger *et al.* 1977) using a Thermo Sequenase dye terminator cycle sequencing premix kit (Amersham Life Science, Cleveland) and the ABI 373A DNA sequencer (Applied Biosystems, Foster City, CA).

Number +1 indicates the position of the adenine residue of the start codon. To generate the Δ*swi5* strain, the DNA fragment of the *SWI5* locus was amplified by PCR using the yeast genome of the *swi5::URA3* strain (K1999, the *Nco*I^{+449}-*Hin*dIII^{+2041}, was replaced by the *URA3* gene) and a pair of primers, SWI5 1as/Bm (5′-CGGGATCCATGGATACATCAA ACTCT-3′) and SWI5 2127ss/Bm (5′-CGGGATCCCCTTTGA TTAGTTTTCATTG-3′). Wild-type cells (YKZ0517) were transformed with the amplified PCR products. The Ura$^+$ transformant was isolated and the strains with the *URA3* gene integrated at the *SWI5* locus were selected (YKZ0594).

Cell-cycle synchronization by mating pheromone or hydroxyurea treatment: α-Factor (Sigma, St. Louis) was used to arrest cell growth at late G_1 phase. *BAR1* cells or *bar1* cells growing asynchronously (OD_{600} = ~0.3) in 5–10 ml medium at 25° were treated with 10 μg/ml or 1 μg/ml of α-factor for 2.5–3 hr at 25°, respectively. After the treatment, α-factor was removed by washing cells three times (for *BAR1* cells) or four times (for *bar1* cells) with 5 ml of prewarmed medium. Then cells were released into fresh medium prewarmed at an indicated temperature. Hydroxyurea (Sigma) was used to arrest cell growth at S phase. Cells growing asynchronously (OD_{600} = ~0.3) in 5–10 ml medium at 25° were treated with 0.2 M hydroxyurea for 2.5–3 hr at 25°. After the treatment, hydroxyurea was removed by washing cells three times with 5 ml of prewarmed releasing medium. Then cells were released into fresh medium at the indicated temperature. Each washing step of these experiments took 8–9 min. The time point 0 min indicates the time when the cells were released from arrest.

Flow cytometry: Yeast cells were prepared for flow cytometry essentially as described by Hutter and Eipel (1979). Cells (430 μl of the culture at OD_{600} = ~0.5) were collected, fixed with 70% of ethanol, and washed with 0.2 M Tris-HCl (pH 7.5) solution. The fixed cells were sonicated thoroughly and treated with 1 mg/ml RNase A at 37° overnight. Before analysis, the cells were stained with 100 μg/ml propodium iodide for 30 min at room temperature and then analyzed on a FACScan/CellFIT DNA system (Becton Dickinson). Each histogram showing distribution of DNA contents was based on the accumulation of 20,000 nuclei.

Preparation of samples for Western blotting: Protein extraction for Western blotting analysis was performed as described by Kushnirov (2000). Yeast cells growing at log phase (OD_{600} = 0.3) were immediately placed on ice and harvested by centrifugation. These cells were resuspended in 100 μl of ice-cold distilled water, to which ice-cold 0.2 M NaOH (100 μl) was added, and incubated on ice for 10 min. After the incubation, the cells were pelleted by centrifugation and boiled in 50 μl of SDS-PAGE sample buffer (Laemmli 1970).

Microscopic analysis: For the indirect immunofluorescence method, cells (~300 μl of cell culture at OD_{600} = 0.3–0.6) were fixed by adding 37% formaldehyde to the culture (the final concentration was 3.7% formaldehyde) and incubated further for 20 min at the incubation temperature. The medium containing formalin was replaced with KPO_4 buffer containing formalin [0.1 M KPO_4 (pH 6.4), 3.7% formalin] and the cells were incubated at room temperature for 1 hr to overnight. For spheroplasting, cells were incubated with 200 μl of SP [1.2 M sorbitol, 0.1 M KPO_4 (pH 7.5)] containing zymolyase 100T (30 μg/ml; Seikagaku, Tokyo) and 0.2% of 2-mercaptoethanol (Wako, Osaka, Japan) for 1 hr at 30°. Mouse monoclonal anti-α-tubulin antibody (1/50 dilution; clone DM 1A, Sigma) or mouse monoclonal anti-myc antibody (1/50 dilution: 9E10, Calbiochem, Cambridge, MA) was used as primary antibody. Goat anti-mouse IgG antibody conjugated with fluorescein (1/800 dilution; ICN Pharmaceuticals, Aurora, OH) was used as secondary antibody. Microscopic analyses were done using an Olympus IX70 epifluorescence microscope (Olympus, Tokyo) with a UPlanApo100x lens (Olympus) and a CCD camera (Sensystem, Photometrics, Tucson, AZ).

RESULTS

A mutation in *KAP104* suppressed the temperature sensitivity of *cdc15-2* cells: To isolate factors functioning

TABLE 1

Yeast strains used in this study

Strain	Relevant genotype	Reference or source
YKZ0181	*MAT***a** *tem1-3*	This study
YKZ0200	*MAT***a** *cdc15-2*	Derivative of K1993 (a gift from K. Nasmyth)
YKZ0239	*MAT***a** *kap104-E604K*	This study
YKZ0240	*MAT*α *kap104-E604K*	This study
YKZ0285	*MAT***a** *cdc15-2 rcf114*	This study
YKZ0286	*MAT***a** *cdc15-2 rcf5*	This study
YKZ0287	*MAT***a** *cdc15-2 rcf70*	This study
YKZ0341	*MAT***a** *cdc15-2 rcf137* (*rcf137* = *kap104-E604K*)	This study
YKZ0391	*MAT***a** *cdc14-1*	Toh-e lab stock
YKZ0396	*MAT***a** *cdc14-1 kap104-E604K*	This study
YKZ0424	*MAT***a** *tem1-3 kap104-E604K*	This study
YKZ0436	*MAT***a** *cdc15-2 kap104-E604K cdh1::HIS3Cg*	This study
YKZ0448	*MAT***a** *cdc15-2 kap104-E604K sic1::URA3*	This study
YKZ0497	*MAT***a** *bar1::HIS3*	This study
YKZ0490	*MAT***a** *CDC14-myc18::HIS3 NOP1-GFP::TRP1*	Derivative of K8439 (a gift from K. Nasmyth)
YKZ0498	*MAT***a** *bar1::HIS3 kap104-E604K*	This study
YKZ0503	*MAT***a** *msd2-1::HIS3*	Derivative of K5997 (a gift from K. Nasmyth)
YKZ0517	*MAT***a** *ade2-1 ura3 leu2-3, 12 his3-11, 15 trp1-1 can1-100*	Toh-e lab stock
YKZ0532	*MAT***a** *dbf2-2*	Toh-e lab stock
YKZ0536	*MAT***a** *msd2-1::HIS3 kap104-E604K*	This study
YKZ0537	*MAT***a** *dbf2-2 kap104-E604K*	This study
YKZ0576	*MAT***a** *cdc15-2 HA-SIC1*	Derivative of K4317 (a gift from K. Nasmyth)
YKZ0578	*MAT***a** *cdc15-2 kap104-E604K HA-SIC1*	This study
YKZ0587	*MAT***a** *cdc14-1 SW15-myc18::TRP1*	Derivative of K8190 (a gift from K. Nasmyth)
YKZ0580	*MAT*α *cdc15-2 kap104-E604K SW15-myc18::TRP1*	This study
YKZ0581	*MAT*α *cdc15-2 SW15-myc18::TRP1*	This study
YKZ0595	*MAT***a** *cdc15-2 kap104-E604K swi5::URA3*	This study
YKZ0622	*MAT***a** *cdc14-1 kap104-E604K SW15-myc18::TRP1*	This study
YKZ0644	*MAT***a** *kap104-E604K spo12::HIS3*	This study
YKZ0645	*MAT***a** *cdc15-2 kap104-E604K spo12::HIS3*	This study
YKZ0646	*MAT***a** *cdc15-2 spo12::HIS3*	This study
YKZ0647	*MAT***a** *kap104-E604K CDC14-myc18::HIS3 NOP1-GFP::TRP1*	This study
YKZ0648	*MAT***a** *spo12::HIS3 CDC14-myc18::HIS3 NOP1-GFP::TRP1*	This study
YKZ0648	*MAT***a** *kap104-E604K spo12::HIS3 CDC14-myc18::HIS3 NOP1-GFP::TRP1*	This study

All strains listed above are in the W303 background or have been crossed to the strain in the W303 background at least four times. *msd2-1* is an allele of the *CDC5* gene.

downstream of Cdc15 in mitotic exit, we screened for extragenic suppressor mutations of the temperature sensitivity of *cdc15-2* cells (the *rcf* mutation, for revertant of *cdc15-2*). Haploid *cdc15-2* cells were streaked on plates and incubated at a restrictive temperature of 34°. Of 216 spontaneous revertant strains isolated (*cdc15-2 rcf1-cdc15-2 rcf216*), 8 reproducibly generated two temperature-sensitive $(ts)^+$ and two ts^- progenies when crossed with a *cdc15-2* strain with the opposite mating type and subjected to tetrad analyses. These 8 suppressors were found to be recessive. Complementation analysis showed that the 8 *rcf* mutations were located in four different genes (*RCF5*, *RCF70*, *RCF114*, and *RCF137*). We observed that haploid progenies that were temperature sensitive at 34° due to the *cdc15-2* mutation were frequently obtained from crosses between the wild-type and any of the *cdc15-2 rcf5*, *cdc15-2 rcf70*, *cdc15-2 rcf114*, or *cdc15-2 rcf137* strains; this indicated that none of the four *rcf* mutations occurred in *CDC15*. Each of the four *rcf* mutations suppressed the temperature sensitivity of *cdc15-2* at 34° but not at the higher temperature of 37° (Figure 1A).

The *rcf137* mutation alone caused temperature-sensitive growth (see below), which was suppressed by the introduction of a low-copy-number plasmid carrying the *KAP104* gene (we isolated such a plasmid from our gene library), and the mutant locus showed a strong genetic linkage with the *KAP104* locus on chromosome II (data not shown). Kap104 is a member of transportin-related

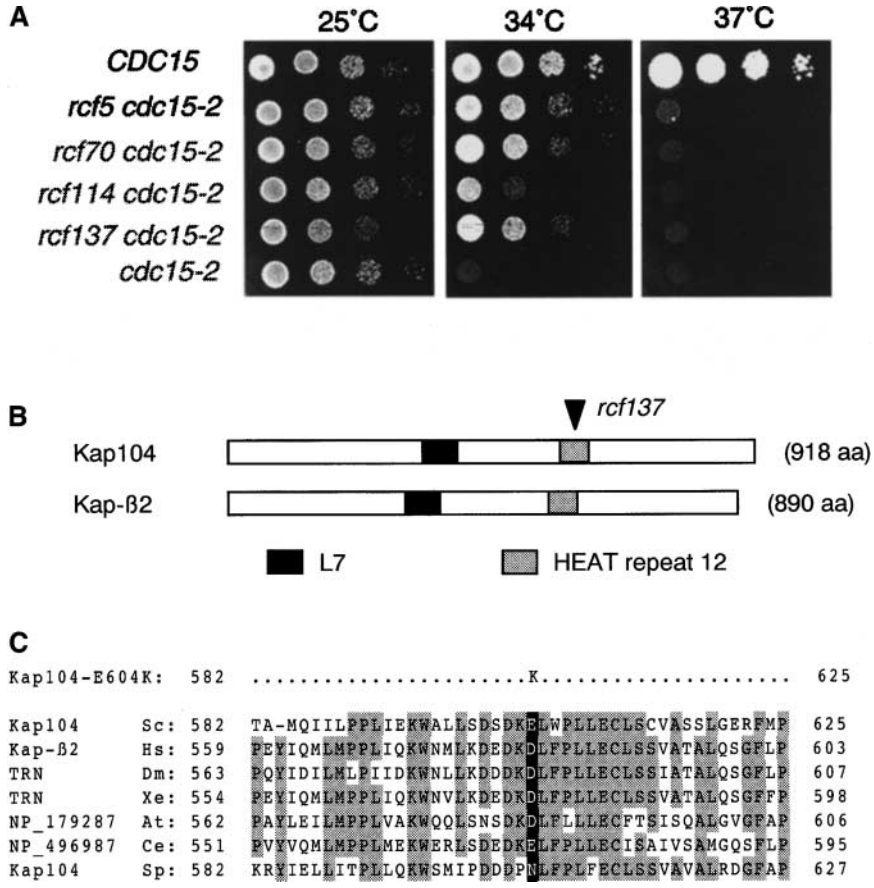


FIGURE 1.—*rcf137* is a mutation in *KAP104*. (A) Isolation of extragenic suppressors of *cdc15-2*. *cdc15-2* cells (YKZ0200) were streaked on YPAD (yeast extract, peptone, adenine, dextrose) plates and incubated at 34° for 3–4 days. Spontaneous revertant strains (216 colonies) were picked up. Each *rcf* mutation that occurred in a single gene was analyzed. Late log-phase cells (OD_{600} = 1) with the indicated genotypes (from the top, YKZ0517, YKZ0286, YK0287, YKZ0285, YKZ0341, and YKZ0200) were serially diluted by 10-fold and spotted onto YPAD plates. Plates were incubated at 25° or 37° for 3 days or at 34° for 2 days. (B) A schematic diagram of transportins from *S. cerevisiae* (Kap104) and *Homo sapiens* (Kap-β2). The mutation site of the *kap104-E604K* gene is indicated by an arrowhead. Stippled boxes and solid boxes indicate the twelfth HEAT repeat and L7, respectively. The L7 of Kap-β2 is required for the interaction with Ran (CHOOK and BLOBEL 1999). (C) Glutamate 604 of Kap104 was changed to lysine by the *kap104-E604K* mutation. Alignment of the twelfth HEAT repeat of transportin-related proteins from various species was shown. Shaded letters are conserved amino acid residues. The amino acid residues that correspond to the *kap104-E604K* mutation site are highlighted by a solid background. The numbers show amino acid positions where the methionine residue encoded by the start codon AUG is 1. NP_179287 and NP_496987 are accession numbers for putative transportin-related proteins registered at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). Sc, *S. cerevisiae*; Hs, *H. sapiens*; Dm, *Drosophila melanogaster*; Xe, *Xenopus laevis*; At, *Arabidopsis thaliana*; Ce, *Caenorhabditis elegans*; Sp, *Schizosaccharomyces pombe*.

proteins and Kap104 and human transportin/karyopherin β2 sequences are schematically shown in Figure 1B. DNA sequencing analysis of the open reading frame of *KAP104* retrieved from the *rcf137* strain revealed that E604 in the HEAT (*H*untingtin, *e*longation factor 3, *A* subunit of protein phosphatase 2A, and *T*OR1 lipid kinase) repeat of Kap104 was changed into K in the *rcf137* strain (Figure 1C). Introduction of this mutation in the *KAP104* gene abolished its ability to suppress the temperature sensitivity of *rcf137* cells, indicating that *rcf137* is a mutation in *KAP104*, which we designated *kap104-E604K*. The fact that Δ*cdc15 kap104-E604K* cells were inviable at 25°, 30°, or 34° (data not shown) indicated that the *kap104-E604K* mutation was not able to bypass the requirement of Cdc15.

The *kap104-E604K* mutation promoted mitotic exit, but not cytokinesis, in *cdc15-2* cells: To understand precisely the feature of the *kap104-E604K*-dependent suppression of the temperature sensitivity of *cdc15-2* cells, we investigated the cell-cycle progression of *cdc15-2 kap104-E604K* cells at 34°. *cdc15-2* cells and *cdc15-2 kap104-E604K* cells were released in fresh medium (34°) from the α-factor (G_1 phase) arrest. After budding, α-factor was added back to prevent cells from entering the next cell cycle. *cdc15-2* cells arrested at telophase with the elongated spindle and a high level of mitotic cyclin Clb2 (Figure 2A). On the contrary, *cdc15-2 kap104-E604K* cells depolymerized the spindle and Clb2 was degraded as the cells proceeded through mitosis (Figure 2A), indicating that the *kap104-E604K* mutation promoted mitotic exit in *cdc15-2* cells. In the case in which pheromone was not added back, *cdc15-2 kap104-E604K* cells continued the mitotic cycle and became multinucleated (Figure 2B, e and f) while *cdc15-2* cells remained arrested at telophase with an extraordinarily elongated spindle (Figure 2B, b and c). Even though the mitotic cycle proceeded, ~90% of *cdc15-2 kap104-E604K* cells displayed a defect in cytokinesis and became multi-budded (Figure 2B, d). Considering that the *kap104-E604K* mutation alone does not cause a significant cytokinetic defect (see below), these results show that the *cdc15* defect in cytokinesis is not suppressed by the *kap104-E604K* mutation. We concluded that the *kap104-E604K* mutation suppresses the temperature sensitivity of *cdc15-2* cells by promoting the exit from mitosis, but does not suppress the *cdc15* defect in cytokinesis.

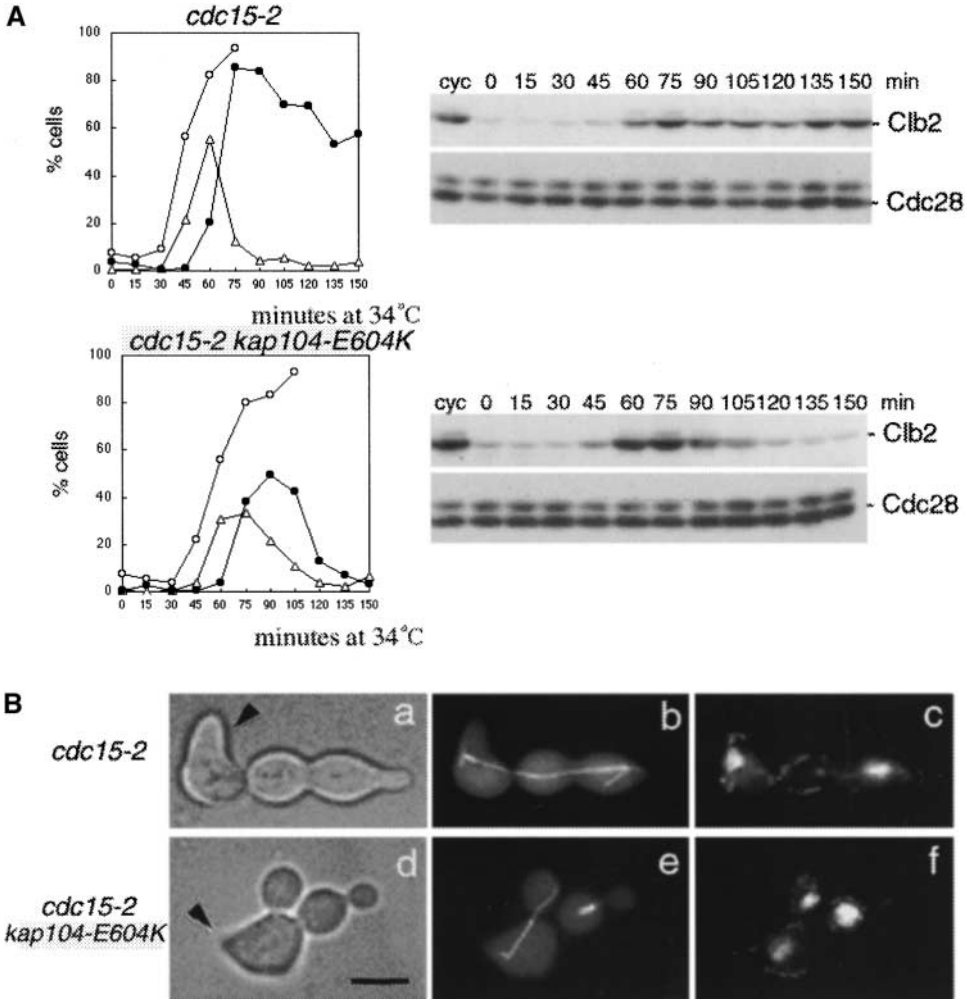


FIGURE 2.—The *kap104-E604K* mutation promoted mitotic exit in *cdc15-2* cells at 34°. (A) *cdc15-2 kap104-E604K* cells exited mitosis at 34°. *MAT***a** *cdc15-2* Δ*bar1* cells (YKZ-0635) and *MAT***a** *cdc15-2 kap104-E604K* Δ*bar1* cells (YKZ0640) were arrested in G_1 phase with mating pheromone α-factor (1 μg/ml, treated for 3 hr at 25°) and released in fresh medium at 34°. Pheromone was added after budding to arrest cells in the next G_1 phase (at 60 min to *cdc15-2* cells and at 75 min to *cdc15-2 kap104-E604K* cells). Samples were taken at indicated time points for microscopic analysis and for Western blotting analysis (see MATERIALS AND METHODS). Budding index (open circle) and the population of the cells with short spindle (<3 μm, triangle) and elongated spindle (>3 μm, solid circle) are shown. Spindles were detected by the indirect immunofluorescence method using antitubulin antibody. Amounts of the mitotic cyclins Clb2 and Cdc28 (control) were determined by Western blotting using anti-Clb2 antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and anti-PSTAIRE antibody (Santa Cruz Biotechnology), respectively. The sample taken from the asynchronous culture at 25° is shown as "cyc." (B) *cdc15-2 kap104-E604K* cells continued the mitotic cycle but displayed a cytokinetic defect at 34°. The α-factor arrest/release experiment was performed as described in A except that pheromone was not added after budding. Cells taken at 140 min after the release from the α-factor arrest are shown. Spindles and DNA were detected by the indirect immunofluorescence method using antitubulin antibody (b and e) and DAPI staining (c and f), respectively. A multi-budded *cdc15-2 kap104-E604K* cell containing both a short and an elongated spindle within a single cell (*i.e.*, undergoing the second mitosis in the absence of cytokinesis) are shown (d, e, and f). *cdc15-2* cells maintained the elongated spindle after nuclear division but continued the budding cycle by producing the bud only from the previous daughter cell (a, b, and c). Arrowheads (a and d) indicate the original mother cells that had been treated with mating pheromone. We confirmed that the multi-budded morphology of the *cdc15-2 kap104-E604K* cells remained after sonication. Bar, 5 μm.

Suppression spectrum of the *kap104-E604K* mutation: To test whether *KAP104* interacts genetically with other MEN genes, double-mutant strains were constructed by crossing the *kap104-E604K* strain with the temperature-sensitive MEN mutant strains other than the *cdc15-2* strains. We found that temperature-sensitive phenotypes of these MEN mutant strains were suppressed by the *kap104-E604K* mutation at a low restrictive temperature although the suppression of *msd2-1* (allelic to *CDC5*) was less efficient than that of the other MEN mutations (Figure 3). The *cdc14-1* mutation was suppressed by the *kap104-E604K* mutation at a lower restrictive temperature. Additionally, all of these double-mutant strains showed a multi-budded phenotype at a low restrictive temperature, as *cdc15-2 kap104-E604K* cells did (data not shown). These results indicate that the *kap104-E604K* mutation suppresses the defect in mitotic exit, but not in cytokinesis, of MEN in general.

The *kap104-E604K* mutation affects cell-cycle progression: The results described above established that Kap104 is involved in cell-cycle progression at least in cells defective in mitotic exit. To address the possibility that Kap104 itself is involved in cell-cycle progression, as an initial attempt we examined the phenotypes of *kap104-E604K* cells at the restrictive temperature. During a 9-hr incubation at 37°, *kap104-E604K* cells showed slow but continued growth until ~6 hr and then gradually stopped growing without a decline of viability (Figure 4A and data not shown). We found, however, that the population of the cells with the elongated spindle

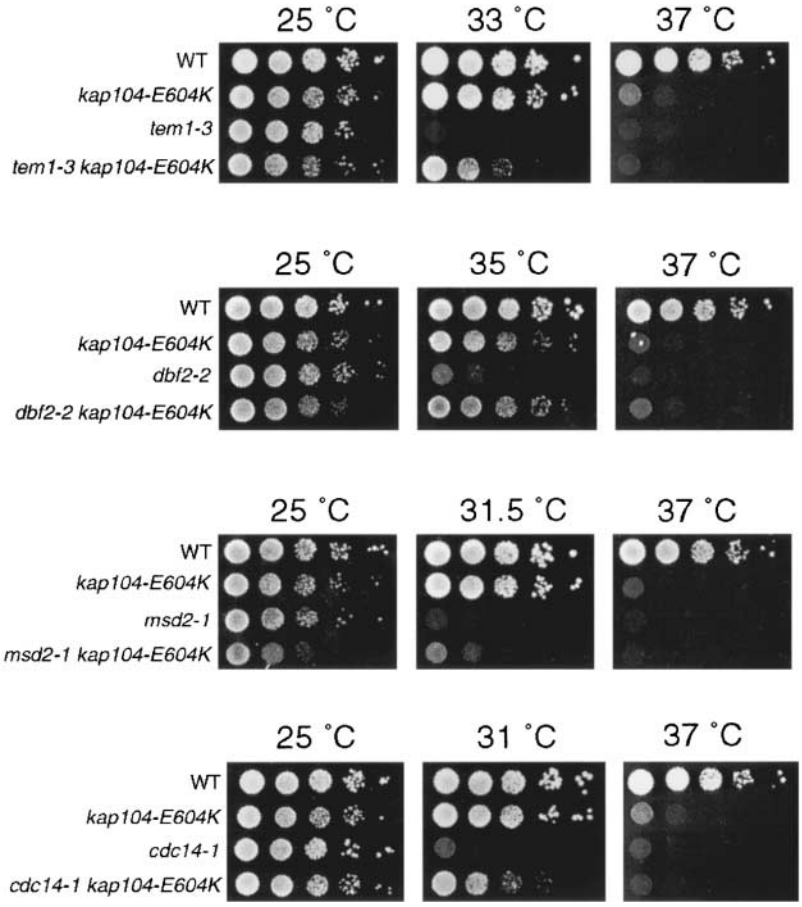
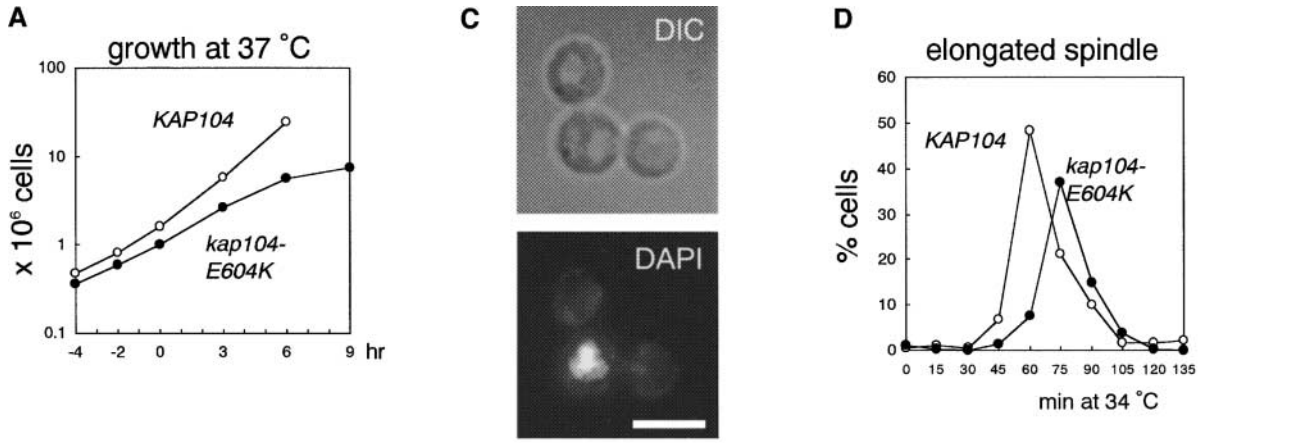


FIGURE 3.—The *kap104-E604K* mutation generally suppressed the MEN defect. Late log-phase cells (OD_{600} = 1) with the indicated genotypes were serially diluted by 10-fold and spotted onto YPAD plates. Plates were incubated for 2 or 3 days at indicated temperatures. WT (YKZ0517); *kap104-E604K* (YKZ-0239); *tem1-3* (YKZ0181); *tem1-3 kap104-E604K* (YKZ-0424); *dbf2-2* (YKZ0532); *dbf2-2 kap104-E604K* (YKZ0537); *msd2-1* (YKZ0503); *msd2-1 kap104-E604K* (YKZ0536); *cdc14-1* (YKZ0391); *cdc14-1 kap104-E604K* (YKZ0396). *msd2-1* is an allele of the *CDC5* gene.

(*i.e.*, anaphase/telophase cells) was substantially decreased in the *kap104-E604K* culture as compared with that in the wild-type culture, regardless of the incubation temperatures (Figure 4B, type IV; 47–64% reduction). The population of the cells with the short spindle, although less prominent, was also decreased by the *kap104-E604K* mutation (Figure 4B, type III; 35–44% reduction). Interestingly, cells with two buds and a large nucleus, which were totally absent in the wild-type culture, had accumulated in a certain population (6%) of *kap104-E604K* cells after a 9-hr incubation at 37° (Figure 4B, type VI, and Figure 4C), suggesting that the *kap104-E604K* mutation leads to a weak defect in nuclear division.

Because *kap104-E604K* cells show a similar or slightly slower growth rate than the wild-type cells do at 25° or 34° (Figure 3), the reduced population of the cells with the mitotic spindle in the asynchronous *kap104-E604K* culture raises the possibility that the *kap104-E604K* cells undergo shortened mitosis. To confirm that the *kap104-E604K* mutation shortens mitosis, an α-factor arrest/release experiment was performed. Although the start of spindle elongation delayed by ~15 min, *kap104-E604K* cells completed the depolymerization of the spindle at almost the same time as wild-type cells did (Figure 4D), showing that *kap104-E604K* cells undergo a shorter mitosis than wild-type cells do. Fluorescence-activated cell sorter (FACS) analysis revealed that the 15-min delay occurred before initiation of DNA replication (Figure 4E), suggesting that *kap104-E604K* cells have a defect in G_1/S transition.

Swi5, Sic1, and Spo12, but not APC^{Cdh1}, are essential for the viability of *cdc15-2 kap104-E604K* cells at 34°: To address how Kap104 is involved in mitosis, we searched the factor(s) required for the *kap104-E604K*-dependent promotion of mitotic exit. We found that the deletion of *SIC1* (encoding an inhibitor for mitotic CDKs) abolished the growth of *cdc15-2 kap104-E604K* cells at 34° while that of *CDH1* (a specificity factor for APC^{Cdh1}) did not (Figure 5A). In addition, the deletion of *SWI5* (encoding a major transcriptional activator for *SIC1*) also impaired the growth of *cdc15-2 kap104-E604K* cells at 34° (Figure 5B), suggesting that the Swi5-Sic1 pathway plays a major role in the *kap104-E604K*-dependent mitotic exit in *cdc15-2* cells. Deletion of neither *ACE2* (encoding another transcriptional activator for *SIC1*) nor *SWE1* (encoding a CDK-inhibitory kinase) affected the growth of *cdc15-2 kap104-E604K* cells at 34°, showing that *ACE2* and *SWE1* are dispensable for the suppression of *cdc15-2* by the *kap104-E604K* mutation (data not shown). Interestingly, *SPO12*, which is required for meiosis and for the release of Cdc14 from the nucleolus



B

		I	II	III	IV	V	VI
	37 °C						
KAP104	0 h	36	25	**17**	**16**	6	--
	3 h	40	23	**23**	**22**	3	--
	9 h	40	25	**16**	**15**	4	--
kap104-E604K	0 h	64	17	**10**	**6**	4	--
	3 h	55	15	**15**	**8**	2	--
	9 h	40	28	**9**	**8**	8	6

(% cells, n > 205)

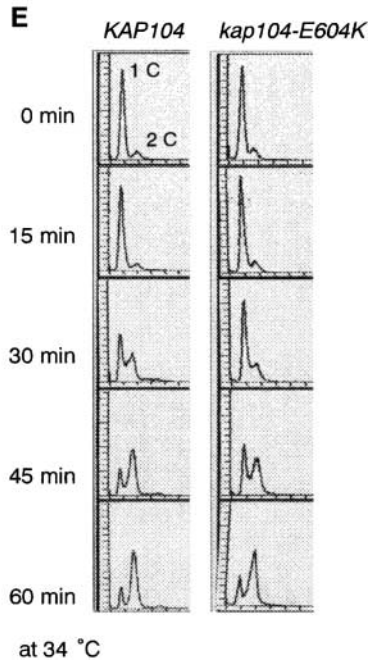


FIGURE 4.—Cell-cycle analysis of the *kap104-E604K* mutant. (A) Temperature-sensitive growth of *kap104-E604K* cells. Log-phase culture of *KAP104* cells (YKZ0517) and *kap104-E604K* cells (YKZ0240) at 25° was shifted up to 37° (at the time point 0 hr) and the cell number (per milliliter) at each of the indicated time points was plotted. (B) Population of *kap104-E604K* cells at various cell-cycle stages at 37°. Samples taken at indicated times in A were subjected to double staining of tubulin (by the indirect immunofluorescence method using antitubulin antibody) and DNA (DAPI staining). Cells at various cell-cycle stages were categorized into six groups (I–VI). I, unbudded cells; II, budded cells with the single SPB; III, cells with the short mitotic spindle; IV, cells with the elongated mitotic spindle; V, binucleate cells without the elongated mitotic spindle; VI, cells with a single nucleus and two buds. Nucleus and microtubule structure are indicated by shaded circles and solid bars, respectively. The result shown is representative of two independent experiments. (C) Typical image of *kap104-E604K* cells of type VI in B. The cells of type VI had two buds (top, DIC) and the nucleus was rather large. DNA was stained with DAPI (bottom, DAPI). Bar, 5 μm. (D and E) Cell-cycle progression of *kap104-E604K* cells at 34°. *MAT***a** *KAP104* Δ*bar1* cells (YKZ0497) and *MAT***a** *kap104-E604K* Δ*bar1* cells (YKZ0498) were arrested in G_1 phase with mating pheromone α-factor (1 μg/ml, treated for 3 hr at 25°) and were synchronously released in fresh medium at 34°. At 50 min after release, α-factor was added back to prevent cells from entering the next cell cycle. Spindles were detected by the indirect immunofluorescence method using antitubulin antibody. Spindle index indicating the proportion of cells with the elongated mitotic spindle (>3 μm) is shown. The result is representative of three independent experiments. DNA contents of the cells were investigated by FACS analysis (E). *kap104-E604K* cells delayed entering S phase by ~15 min.

during early anaphase, was essential for the growth of *cdc15-2 kap104-E604K* cells at 34° (Figure 5C). Any deletion of *SWI5*, *SIC1*, *CDH1*, *ACE2*, *SWE1*, or *SPO12* did not cause synthetic lethality with the *kap104-E604K* mutation at 34° (data not shown and Figure 5C).

The *kap104-E604K* mutation promoted the nuclear accumulation of Swi5 in a Cdc14-dependent manner: To verify that the Swi5-Sic1 pathway is activated by the *kap104-E604K* mutation in the first place, we tested whether the *kap104-E604K* mutation results in the nuclear accumulation of

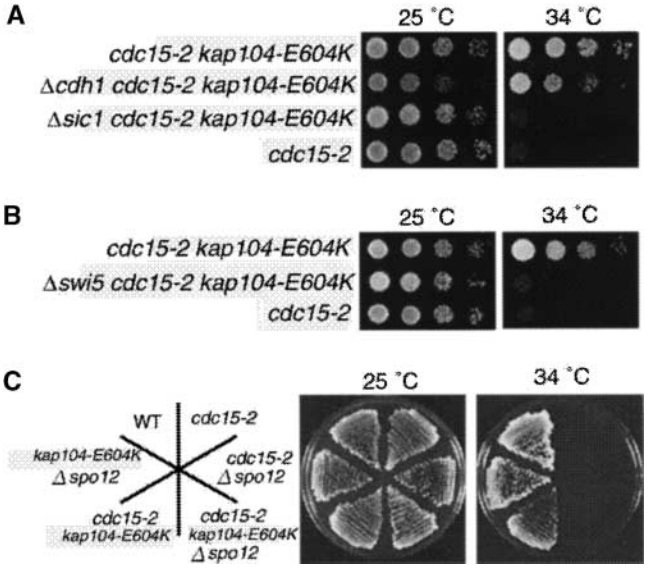


FIGURE 5.—Swi5, Sic1, and Spo12, but not APC^{Cdh1}, are essential for the viability of *cdc15-2 kap104-E604K* cells at 34°. (A) *CDH1* was not required for *cdc15-2 kap104-E604K* cells to grow at 34°. Late log-phase cells (OD_{600} = 1) with the indicated genotype were serially diluted by 10-fold and spotted onto YPAD plates. Plates were incubated for 2 days at 34° or for 3 days at 25°. *cdc15-2 kap104-E604K* (YKZ0341), Δ*cdh1 cdc15-2 kap104-E604K* (YKZ0436), Δ*sic1 cdc15-2 kap104-E604K* (YKZ0448), and *cdc15-2* (YKZ0200). (B) *SWI5* was indispensable for *cdc15-2 kap104-E604K* cells to grow at 34°. Late log-phase cells (OD_{600} = 1) with the indicated genotype were serially diluted by 10-fold and spotted onto YPAD plates. Plates were incubated for 2 days at 34° or for 3 days at 25°. *cdc15-2 kap104-E604K* (YKZ 0341), Δ*swi5 cdc15-2 kap104-E604K* (YKZ0595), and *cdc15-2* (YKZ0200). (C) *SPO12* was indispensable for *cdc15-2 kap104-E604K* cells to grow at 34°. Cells with the indicated genotypes were streaked onto YPAD plates and incubated for 2 days at 34° or for 3 days at 25°. WT (YKZ0517), *cdc15-2* (YKZ0200), *cdc15-2* Δ*spo12* (YKZ0646), *cdc15-2 kap104-E604K* Δ*spo12* (YKZ0645), *cdc15-2 kap104-E604K* (YKZ0341), and *kap104-E604K* Δ*spo12* (YKZ0644).

Swi5, which promotes the expression of *SIC1*. As previously reported (NASMYTH *et al.* 1990; MOLL *et al.* 1991), the nuclear accumulation of Swi5 was hardly observed in *cdc15-2* cells at 37° (Figure 6A). On the contrary, the nuclear accumulation of Swi5 was observed in *cdc15-2 kap104-E604K* cells at telophase (Figure 6, A and B; 20% of binucleate cells, at the time point 120 min). Because the nuclear accumulation of Swi5 was detectable in 20–23% of binucleate cells in the wild-type culture (data not shown), we conclude that the *kap104-E604K* mutation promotes the nuclear accumulation of Swi5.

Next we investigated the amount of Sic1 in *cdc15-2 kap104-E604K* cells at 37°. Even at the elevated temperature at which the spindle depolymerization and the degradation of Clb2 were inefficient, the expression of Sic1 did occur in *cdc15-2 kap104-E604K* cells (Figure 6, C and D), showing that the *kap104-E604K* mutation primarily leads to the expression of Sic1 rather than to the degradation of Clb2. The stabilization of Clb2 occurred in *cdc15-2 kap104-E604K* cells as soon as the temperature shifted to 37°, which was less remarkable in the shift to 34° (Figure 2A). Additionally, in *cdc15-2 kap104-E604K* cells, a degradation of Sic1 after release from the α-factor arrest delayed 20–40 min longer than in *cdc15-2* cells (Figure 6C). This is consistent with the delay in initiation of DNA replication observed in *kap104-E604K* cells (Figure 4E). In these assays, we chose a higher restrictive temperature for *cdc15-2* cells (37°), because our tagging construct (*SWI5-myc* or *HA-SIC1*) alone partially suppressed the temperature sensitivity of *cdc15-2* cells at 34° (data not shown).

Cdc14-dependent dephosphorylation of Swi5 is crucial for the nuclear accumulation of Swi5 (VISINTIN *et al.* 1998). In either *cdc14-1* cells or *cdc14-1 kap104-E604K* cells, the nuclear accumulation of Swi5 was not at all observed at 37° irrespective of the cell cycle (Figure 6E and data not shown). These results show that the *kap104-E604K* mutation promotes the nuclear accumulation of Swi5 in a manner dependent on Cdc14 and suggest that the *kap104-E604K* mutation promotes mitotic exit through Cdc14 function. This observation seems controversial because the *kap104-E604K* mutation suppresses the temperature sensitivity of the *cdc14-1* mutation at a low restrictive temperature (Figure 3; for interpretation, see DISCUSSION). Unexpectedly, most (~70%) *cdc14-1 kap104-E604K* cells remained arrested with the short spindle (<3 μm) at 37°, suggesting that Kap104 is required for the onset of anaphase when the Cdc14 function is compromised.

Kap104 is required for the tight sequestration of Cdc14 to the nucleolus during interphase: The result mentioned above raises the possibility that the *kap104-E604K* mutation leads to the activation of Cdc14. We therefore investigated the localization of Cdc14 in *kap104-E604K* cells at the permissive temperature of 34° using chromosomally integrated 18 myc-tagged *CDC14*. In *kap104-E604K* cells, Cdc14 was released from the nucleolus when the nuclear division occurred, as observed in wild-type cells (Figure 7A). However, a faint but distinct Cdc14 stain was detectable around a discrete nucleolar stain in nonmitotic *kap104-E604K* cells (Figure 7, A and B; 11–13% of unbudded cells and 31–41% of budded cells from three independent assays), while such a pattern of the Cdc14 stain was not prominent in the wild-type cells (Figure 7A; 0–4% of unbudded cells and 3–6% of budded cells from three independent assays). Triple staining of Cdc14, a nucleolar protein Nop1, and DNA clearly revealed that the faint Cdc14 stain was in the 4′,6-diamidino-2-phenylindole (DAPI)-staining region of the nucleus (Figure 7B), showing that the *kap104-E604K* mutation results in a partial delocalization of Cdc14 from the nucleolus during interphase. We performed the same assay, except that the cells were incubated at 37° for 3 hr, and observed a similar pattern of the Cdc14 stain in *kap104-E604K* cells (data not shown).

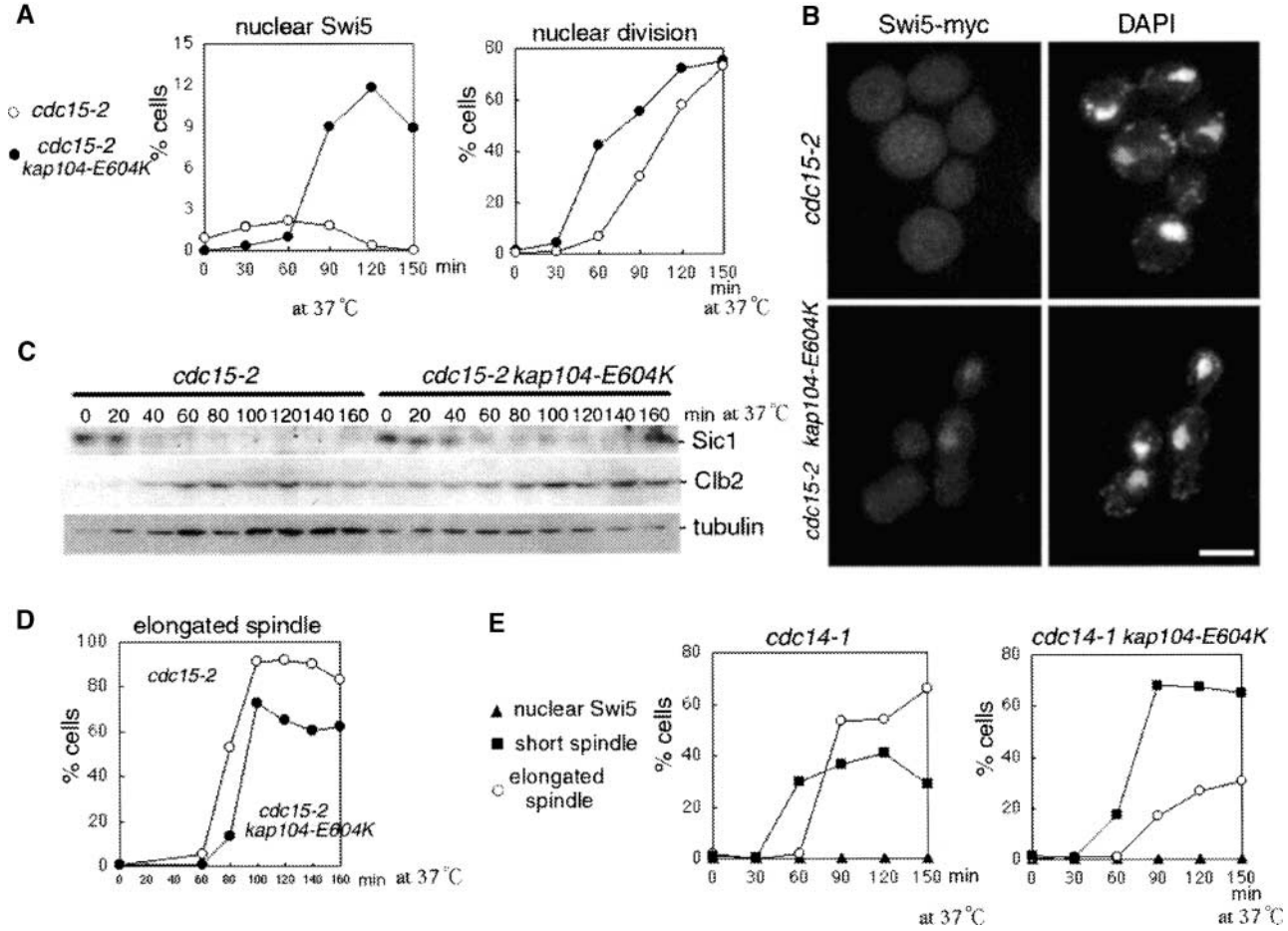


FIGURE 6.—The *kap104-E604K* mutation promoted the nuclear accumulation of Swi5 in a Cdc14-dependent manner. (A) The *kap104-E604K* mutation promoted the nuclear accumulation of Swi5-myc in *cdc15-2* cells at telophase at 37°. *MAT*α *cdc15-2 SWI5-myc18* cells (YKZ0581) and *MAT*α *cdc15-2 kap104-E604K SWI5-myc18* cells (YKZ0580) were arrested in S phase with hydroxyurea (0.2 M, treated for 2.5 hr at 25°) and were synchronously released in fresh medium at 37°. Samples were taken at indicated times and subjected to double staining. DNA was visualized by DAPI staining and Swi5-myc18 by the indirect immunofluorescence method using anti-myc antibody (9E10) as primary antibody and fluorescein-conjugated anti-mouse IgG antibody (FITC) as secondary antibody. Population of the cells with the nuclear Swi5-myc stain (left, nuclear Swi5) and divided nuclei (right, binucleate) at indicated time points was plotted ($n > 200$ at each time point). (B) Typical images of *cdc15-2 SWI5-myc* cells and *cdc15-2 kap104-E604K SWI5-myc* cells in A. Bar, 5 μm. (C) The *kap104-E604K* mutation promoted the expression of Sic1 in *cdc15-2* cells at telophase at 37°. *MAT***a** *cdc15-2 HA-SIC1* cells (YKZ0576) and *MAT***a** *cdc15-2 kap104-E604K HA-SIC1* cells (YKZ0578) were arrested in G_1 phase with mating pheromone α-factor (10 μg/ml, treated for 3 hr) and released in fresh medium at 37°. We carried out this experiment at 37°, because our tagging construct (*SWI5-myc* or *HA-SIC1*) alone partially suppressed the temperature sensitivity of *cdc15-2* cells at 34°. Samples were taken at indicated time points for Western blotting analysis using anti-HA antibody (16B12) for HA-Sic1, anti-Clb2 antibody, and antitubulin antibody (control). (D) Spindle index of *cdc15-2* (open circles) and *cdc15-2 kap104-E604K* (solid circles) in C. Spindles were detected by the indirect immunofluorescence method using antitubulin antibody. More than 90% of cells were budded at 60 min in the *cdc15-2* culture and at 80 min in the *cdc15-2 kap104-E604K* culture. (E) Swi5 was not accumulated in the nucleus in *cdc14-1 kap104-E604K* cells at 37°. *MAT***a** *cdc14-1 SWI5-myc18* cells (YKZ0587) and *MAT***a** *cdc14-1 kap104-E604K SWI5-myc18* cells (YKZ0622) were arrested in G_1 phase with mating pheromone α-factor (10 μg/ml, treated for 3 hr at 25°) and released in fresh medium at 37°. Samples were taken at indicated times and subjected to double staining as in A. Population of the cells with the nuclear Swi5-myc stain (triangle), the short spindle (<3 μm, square), and the elongated spindle (>3 μm, circle) at indicated time points was plotted ($n > 200$ at each time point) for each strain.

Partial delocalization of Cdc14 from the nucleolus caused by the *kap104-E604K* mutation is a Spo12-dependent phenomenon: Because *SPO12* is essential for the *kap104-E604K*-dependent suppression of *cdc15-2*, we tested whether *SPO12* was responsible for the partial delocalization of Cdc14 from the nucleolus. The deletion of *SPO12* from *kap104-E604K* cells almost completely diminished the Cdc14 stain in the DAPI-staining region of the interphase nucleus, showing that the partial delocalization of Cdc14 from the nucleolus in *kap104-E604K* cells occurred in a Spo12-dependent manner (Figure 7A). We noted that the population of the cells with a dividing nucleus or with divided nuclei (*i.e.*, mitotic cells) was almost at the same level in either

the Δ*spo12* or the Δ*spo12 kap104-E604K* culture, suggesting that the shortened mitosis caused by the *kap104-E604K* mutation is also a Spo12-dependent phenomenon.

DISCUSSION

We identified *KAP104* as a responsible gene for one of the suppressor mutations (the *rcf* mutation) of the temperature-sensitive *cdc15-2* mutation in a search for the downstream elements of Cdc15 (Figure 1A). Further genetic analyses revealed that the *kap104-E604K* mutation generally suppresses the MEN defect and that the Swi5-Sic1 pathway is essential for the *kap104-E604K*-dependent suppression of *cdc15-2* (Figures 3 and 6). Indeed, the *kap104-E604K* mutation promoted the nuclear accumulation of Swi5 and the expression of Sic1 in *cdc15-2* cells (Figure 6). The fact that Cdc14 is essential for the *kap104-E604K*-dependent nuclear accumulation of Swi5 at telophase suggests that the *kap104-E604K* mutation suppresses the MEN defect through the activation of Cdc14 (Figure 6). To our surprise, the *kap104-E604K* mutation suppressed the *cdc14-1* mutation at 31° (Figure 3). However, the *kap104-E604K* mutation was not able to bypass the requirement of Cdc14. This controversial phenomenon will be explained later. Since one of the critical outputs of the MEN signaling is believed to be the regulation of Cdc14, these observations may place the Kap104 function at or near the downstream of MEN, but not immediately after the Cdc15 function.

The *kap104-E604K* mutation causes cell-cycle phenotypes not only in the MEN-defective cells but also in otherwise wild-type cells, which suggests a novel role for Kap104 in cell-cycle progression; the duration of mitosis in *kap104-E604K* cells is shorter than that of wild-type cells (Figure 4, B and D), and the *kap104-E604K* mutation delays initiation of DNA replication (Figure 4C). The *kap104-null* mutation leads to the elevated rate of chromosome loss (Entian *et al.* 1999) and the *kap104-E604K* mutation results in the emergence of the cells with multiple buds and a large nucleus at the restrictive temperature, although at a low frequency (Figure 4, B and C). These phenotypes may be the result of the commitment of the next cell cycle after the short and unfaithful mitosis. On the basis of these observations, we speculate that Kap104 is required for the temporal control of mitosis. Interestingly, we found that the

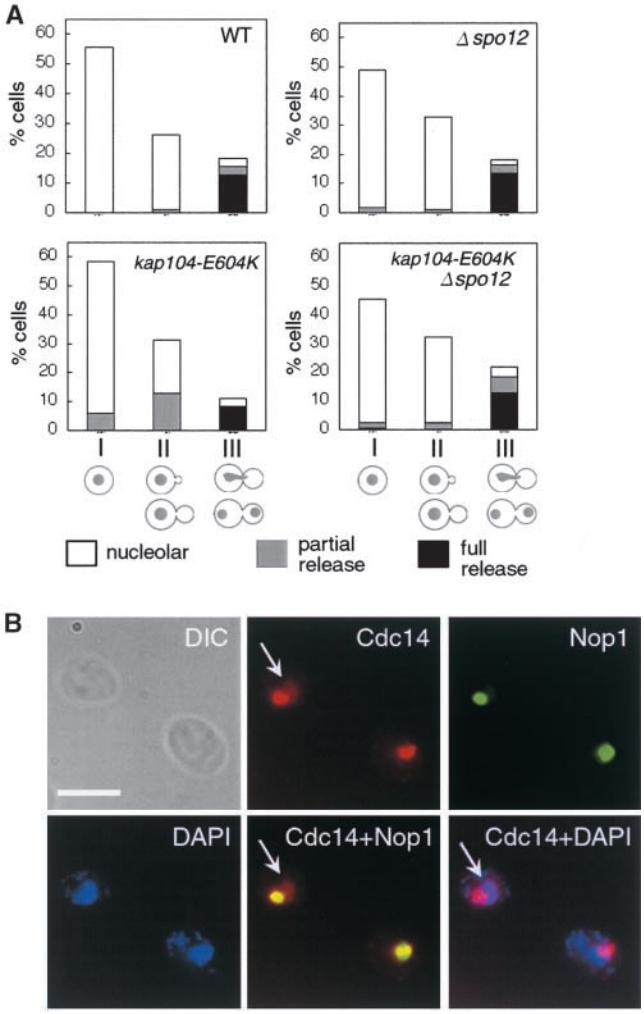


Figure 7.—The *kap104-E604K* mutation results in a Spo12-dependent delocalization of Cdc14 from the nucleolus during interphase. (A) The *kap104-E604K* mutation caused the partial delocalization of Cdc14 from the nucleolus during interphase, which was cancelled by the deletion of *SPO12*. Wild-type (WT, YKZ0490), *kap104-E604K* (YKZ0647), Δ*spo12* (YKZ0648), and Δ*spo12 kap104-E604K* (YKZ0649) cells, each of which contained the chromosomally integrated *CDC14-myc18* and *NOP1-GFP*, were shifted up to 34°, a permissive temperature for each strain. After the 3-hr incubation at 34°, cells were fixed with 3.7% formaldehyde and processed for the immunofluorescence assay for the Cdc14 localization using anti-myc antibody (9E10). From each of the three independent preparations, cells ($n > 100$) were randomly chosen and the localization of Cdc14 was determined by comparing with the nucleolar localization of Nop1-GFP. Cell cycle stages of the cells were classified into three groups: type I, unbudded cells; type II, budded cells with a nucleus before division; and type III, budded cells with a dividing nucleus or divided nuclei. Open bars, shaded bars, and solid bars indicate the population of cells that displayed only the nucleolar stain (nucleolar), both the nucleolar and the faint nuclear stain (partial release), and the uniform bright nuclear stain (full release) of Cdc14-myc18, respectively. Results shown are representative of three independent experiments for the wild-type strain and the *kap104-E604K* strain and of two independent experiments for the Δ*spo12* strain and the Δ*spo12 kap104-E604K* strain. (B) Typical images of the delocalization of Cdc14 from the nucleolus in *kap104-E604K* cells during interphase in A. Two unbudded *kap104-E604K* cells were selected (DIC), only one of which displayed the Cdc14-myc18 stain (red) in the DAPI-staining region as indicated by the arrow. Merged images between Nop1-GFP (green) and Cdc14-myc18 (Cdc14 + Nop1) and between DNA (DAPI, blue) and Cdc14-myc18 (Cdc14 + DAPI) are also shown. Bar, 5 μm.

kap104-E604K mutation severely enhanced the defect in the initiation of spindle elongation in *cdc14-1* cells at 37° (Figure 6E). This is the third cell-cycle phenotype caused by the *kap104-E604K* mutation, which implies that Kap104 has a role for the anaphase onset at least when the function of Cdc14 is compromised.

The fact that the *kap104-E604K* mutation caused a partial delocalization of Cdc14 from the nucleolus during interphase further suggests that the *kap104-E604K* mutation promotes the exit from mitosis through the activation of Cdc14 (Figure 7). Because the release of a small amount of Cdc14 from the nucleolus is believed to be sufficient for execution of Cdc14 function (Shou *et al.* 1999), the shortened mitosis in *kap104-E604K* cells can be explained by their partial delocalization of Cdc14 from the nucleolus before anaphase. Moreover, the delay in the G_1/S transition in *kap104-E604K* cells is also explained by the phosphatase activity derived from delocalized Cdc14 during G_1 phase, which is supposed to stabilize Sic1 to delay DNA replication initiation (Figures 4C and 6C).

One possible mechanism for the *kap104-E604K*-dependent delocalization of Cdc14 from the nucleolus is the activation by the *kap104-E604K* mutation of the Spo12 pathway, leading to the precocious release of Cdc14 from the nucleolus during interphase. It will be interesting to determine the localization of FEAR factors such as Spo12, Esp1, Cdc5, and Slk19 (Stegmeier *et al.* 2002) in *kap104-E604K* cells to learn whether Kap104 is involved in the transport of these factors. So far, Spo12 seems not to be the target of Kap104 in the nucleocytoplasmic transport as it was reported that the localization of Spo12 was not affected by the deletion of *KAP104* (Chaves and Blobel 2001). It was previously reported that some karyopherins (importin β and importin α) served as inhibitors for mitotic spindle assembly in Xenopus egg extract or in mammalian cells, in which the nuclear envelope does not exist during mitosis (Gruss *et al.* 2001; Nachury *et al.* 2001; Wiese *et al.* 2001). Given that an analogous feature of importin β is shared with transportin-related proteins, it is also a fascinating model that Kap104 serves as an inhibitor for a FEAR factor and that the *kap104-E604K* mutation attenuates this inhibitory effect to cause the precocious delocalization of Cdc14 from the nucleolus. Alternatively, it is possible that Kap104 is required for the return of Cdc14 (resequestration) to the nucleolus after mitotic exit and cell division because a defect in this process should extend the released state of Cdc14 and may cause the delocalization of Cdc14 during interphase.

As described above, a feature of the *kap104-E604K* mutation is that it causes partial constitutive delocalization of Cdc14. This phenomenon may explain our observation that *kap104-E604K* suppressed *cdc14-1* at 31°. According to the report by Jaspersen and Morgan (2000), the *cdc14-1* mutation delays but does not prevent the release of green fluorescent protein (GFP)-tagged Cdc14-1 protein from the nucleolus at 34°. Assuming that the release of Cdc14-1 from the nucleolus at 31° is similar to that at 34° and that Cdc14-1 shows a weak phosphatase activity at 31°, a slightly higher level of delocalized Cdc14-1 in *cdc14-1 kap104-E604K* cells at 31° due to the *kap104-E604K* mutation could fulfill a critical level of Cdc14 phosphatase activity needed for exit from mitosis.

Another model is that the defective transport of known cargoes of Kap104 (Nab2 and Hrp1/Nab4), or the defective nuclear architecture as a subsidiary consequence of the defective nucleocytoplasmic transport, causes the delocalization of Cdc14 during interphase because the deletion of *KAP104* results in an abnormal nuclear morphology (Aitchison *et al.* 1996). However, we failed to detect an aberration of nuclear morphology in *kap104-E604K* cells except that a larger nucleus was observed in a small population (6%) of *kap104-E604K* cells 9 hr after shift up to 37° (Figure 4, B and C). We also failed to notice any alteration of the nucleolar morphology through the microscopic observation of the Nop1-GFP signal in *kap104-E604K* cells (Figure 7B). Nonetheless, we cannot exclude the possibility that abnormal nucleolar architecture that is undetectable by microscopic analysis could cause the precocious delocalization of Cdc14.

This study provides the first evidence that transportin-related protein is involved in cell-cycle progression. It will be interesting to examine whether the defect in transportin-related protein causes similar cell-cycle phenotypes in eukaryotes other than budding yeast. Our results suggest that the *kap104-E604K* mutation activates Cdc14 in a Spo12-dependent fashion and that this leads primarily to the activation of the Swi5-Sic1 pathway rather than to that of APC^{Cdh1}. Identification of the binding partner(s) of Kap104 involved in the cell-cycle progression will deepen our understanding of the role of transportin-related proteins in the cell-cycle progression and the molecular mechanism of exit from mitosis.

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