Genome-Wide Synthetic Lethal Screens Identify an Interaction Between the Nuclear Envelope Protein, Apq12p, and the Kinetochore in *Saccharomyces cerevisiae*

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ABSTRACT

The maintenance of genome stability is a fundamental requirement for normal cell cycle progression. The budding yeast *Saccharomyces cerevisiae* is an excellent model to study chromosome maintenance due to its well-defined centromere and kinetochore, the region of the chromosome and associated protein complex, respectively, that link chromosomes to microtubules. To identify genes that are linked to chromosome stability, we performed genome-wide synthetic lethal screens using a series of novel temperature-sensitive mutations in genes encoding a central and outer kinetochore protein. By performing the screens using different mutant alleles of each gene, we aimed to identify genetic interactions that revealed diverse pathways affecting chromosome stability. Our study, which is the first example of genome-wide synthetic lethal screening with multiple alleles of a single gene, demonstrates that functionally distinct mutants uncover different cellular processes required for chromosome maintenance. Two of our screens identified *APQ12*, which encodes a nuclear envelope protein that is required for proper nucleocytoplasmic transport of mRNA. We find that *apq12* mutants are delayed in anaphase, rereplicate their DNA, and rebud prior to completion of cytokinesis, suggesting a defect in controlling mitotic progression. Our analysis reveals a novel relationship between nucleocytoplasmic transport and chromosome stability.

The stable inheritance of chromosomes during the mitotic cell cycle requires faithful duplication of the genetic material and equal separation of sister chromatids. Chromosome instability occurs due to defects in multiple cellular functions including DNA replication, chromosome cohesion, chromosome-microtubule (MT) attachment, nucleocytoplasmic transport, and cell cycle control. The nuclear envelope (NE) houses the MT organizing centers, which in the budding yeast *Saccharomyces cerevisiae* are known as spindle pole bodies (SPBs). SPBs duplicate concurrently with DNA replication, allowing for the creation of a bipolar spindle onto which chromosomes attach. A large protein complex, known as the kinetochore, resides on centromere (*CEN*) DNA and mediates attachment of chromosomes to spindle MTs. Once all kinetochores have formed bipolar attachments with spindle MTs, the metaphase-to-anaphase transition proceeds and chromosome segregation occurs. Failure of even a single kinetochore to attach to the spindle alerts the spindle checkpoint machinery, which responds by halting cell cycle progression. Errors in chromosome segregation lead to abnormal numbers of chromosomes, or aneuploidy, which is a hallmark of cancerous cells. Mitotic spindle checkpoint failure is an important determinant in the development of aneuploid cells (Lew and Burke 2003; Rajagopalan and Lengauer 2004) and mutations in kinetochore proteins have recently been associated with a subset of aneuploid colon tumors (Wang et al. 2004).

The kinetochore is a hub of activity where centromere, chromatin, cohesin, spindle checkpoint, and MT-associated proteins gather to coordinate chromosome segregation. Inner and central kinetochore complexes assemble in a hierarchical manner onto *CEN* DNA and serve as a link to the Dam1 outer kinetochore complex that encircles MTs (Biggins and Walczak 2003; McAinsh et al. 2003; Miranda et al. 2005; Westermann et al. 2005). The Ndc80 complex is a highly conserved central kinetochore complex that is required for attachment of chromosomes to MTs (He et al. 2001; Janke et al. 2001; Wigge and Kilmartin 2001; Le Masson et al. 2002). Studies in multiple eukaryotic systems have shown that the Ndc80 complex is required to localize spindle checkpoint proteins to the kinetochore and that cells carrying mutations in

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Ndc80 complex components are defective in checkpoint signaling (Gillett et al. 2004; Maiato et al. 2004). Two of the spindle checkpoint proteins, Mad1p and Mad2p, localize to the NE, specifically to the nuclear pore complex (NPC) (Iouk et al. 2002). Upon activation of the spindle checkpoint, Mad2p and a portion of Mad1p relocalize to the kinetochore (Iouk et al. 2002; Gillett et al. 2004). In human cells, checkpoint proteins also localize to the NPC and upon NE break-down relocalize to the kinetochore until MT attachment has occurred (Campbell et al. 2001).

During mitosis in budding yeast, the NE does not break down and growing evidence suggests that the NPC has an active role in controlling mitotic progression and chromosome segregation (Ouspenski et al. 1999; Kerscher et al. 2001; Maknevych et al. 2003). NE-associated proteins that are required for chromosome stability, but do not appear to be structural components of the kinetochore, have been identified. For example, the nucleoporin Nup170p was identified in a genetic screen for mutants that exhibit an increased rate of chromosome loss and was subsequently shown to be required for kinetochore integrity, despite the fact that Nup170p does not interact with CEN DNA (Spencer et al. 1999; Kerscher et al. 2001). Mps2p localizes to both the SPB and the NE and is required for SPB insertion into the NE (Winey et al. 1991; Munoz-Centeno et al. 1999). Interestingly, Mps2p interacts in vivo with Spe24p, a component of the Ndc80 complex, suggesting that the kinetochore may physically interact with components of the NE (Le Masson et al. 2002). In support of a kinetochore-NE connection, CENs cluster near the SPB/NE in interphase and colocalize with SPBs during late anaphase (Goshima and Yangida 2000; He et al. 2000; Jin et al. 2000; Pearson et al. 2001).

Our current understanding of chromosome segregation has benefited from many studies focused on protein purification and mass spectrometry analysis that have been instrumental in identifying structural components of the kinetochore (Biggs and Walczak 2003; McAnsh et al. 2003). However, the identification of proteins or pathways that affect chromosome segregation via transient or indirect interactions has remained elusive. Genetic studies, however, have the ability to identify interactions that do not rely on direct protein-protein interaction yet still affect chromosome segregation. For this reason we used the systematic genetic analysis (SGA) approach for identifying non-essential mutants from the haploid yeast gene deletion set that have a role in chromosome stability (Tong et al. 2001). SGA is a method to automate the isolation of synthetically lethal (SL) or synthetically sick (SS) interactions that occur when the combination of two viable nonallelic mutations results in cell lethality or slower growth than either individual mutant. Two mutants that have a SL/SS interaction often function in the same or parallel biological pathways (Hartman et al. 2001). As a starting point for our SGA analysis, we created three independent temperature-sensitive (Ts) alleles in members of both the Ndc80 and the Dam1 kinetochore complex. We performed genome-wide SL screens using SGA methodology with all six Ts mutants as query strains. Our study represents the first series of comparative genome-wide SL screens performed on different mutant alleles of the same gene. Using this approach, we have uncovered a novel role for the Apq12p protein, which resides in the NE, in maintaining chromosome stability and proper progression through anaphase.

MATERIALS AND METHODS

Creation of Ts mutants and integration into yeast strains: The SPC24 and SPC34 ORFs (642 and 888 bp, respectively) including ~250 bp of upstream sequence were amplified by PCR and cloned into pRS316 (Sikorski and Hieter 1989) to create pRS316-SPC24 (BVM95a) and pRS316-SPC34 (BVM95c). Both BVM93a and BVM95c were sequenced to ensure that they carried wild-type SPC24 and SPC34 sequence, respectively. SPC24 and SPC34 were PCR amplified from BVM93a and BVM95c using mutagenic conditions [100 ng template, Taq polymerase (GIBCO BRL, Gaithersburg, MD), 200 μM of dNTPs with either limiting dATP (40 μM) or dGTP (40 μM), 2 mM MgCl2, and 25 pmol primers]. Next, mutagenized SPC24 and SPC34 were cloned into pRS315 using homologous recombination in strains VYM503 and VYM509 that contained deletions of SPC24 and SPC34 covered by the URA3-marked plasmids carrying wild-type versions of SPC24 (BVM93a) and SPC34 (BVM95c) (Muhlrad et al. 1992). Wild-type plasmids were removed from VYM503 and VYM509 by successive incubation on media containing 5-fluoroorotic acid. Colonies now carrying mutagenized pRS315-SPC24 or pRS315-SPC34 as the sole source of either gene were incubated at 37°C to identify Ts mutants, and plasmids rescued from these strains were retransformed to confirm the Ts phenotype. FACS analysis was performed on each Ts mutant after incubation at 37°C for 2–6 hr. Mutants representing different FACS profiles at 37°C—three spe24 (spe24-4, spe24-9, spe24-10) and three spe34 (spe34-5, spe34-6, spe34-7) mutants—were chosen for further analysis. Mutants were next integrated in the genome, replacing the wild-type SPC24 or SPC34 loci in both the SGA starting strain (Y2454) and our lab S288C background strain (YPI499) as described (Tong et al. 2001). Mutants were sequenced and the corresponding amino acid changes are illustrated in Figures 1 and 2. All yeast strains used in this study are listed in Table 1.

SL screen using SGA methodology: The deletion mutant array was manipulated via robotics and the SL screens were performed as described (Tong et al. 2001). Each SL screen was performed twice and double mutants were detected visually for SS/SL interactions. For each query gene, all deletion mutants isolated in the first and second screen were condensed onto a mini-array and a third SL screen was performed. SS/SL interactions that were scored at least twice were first confirmed by random spore analysis (Tong et al. 2004) and then subsequently by tetrads analysis on YPD medium at 25°C.

Two-dimensional hierarchical cluster analysis: Two-dimensional (2D) hierarchical clustering was performed as described (Tong et al. 2001, 2004).

Chromosome fragment loss assay: Quantitative half-sector analysis was performed as described (Koshland and Hieter 1987; Hyland et al. 1999). Homozygous diploid strains
containing a single chromosome fragment were plated to isolate single colonies on solid media containing limiting adenine (Spencer et al. 1990). Colonies were grown at either 30° or 35° (see Table 3) for 3 days before incubation at 4° for red pigment development. Chromosome loss or 1:0 events were scored as colonies that were half red and half pink, nondisjunction or 2:0 events were scored as colonies that were half red and half white, and overreplication or

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2:1 events were scored as colonies that were half white and half pink.

Preparation of yeast cell lysate and immunoblot analysis: Cells were grown to log phase and then lysed by bead beating (Tyers et al. 1992). Fifty micrograms of protein was loaded per lane, and Western blots were performed with α-GFP monoclonal antibodies (Roche) and with α-Cdc28 antibodies (gift from Raymond Deshaies).

Cell cycle synchronization: To assess the arrest phenotype of spe24 and spe34 Ts mutants (Figures 1 and 2), strains were grown to an OD600 of 0.2 at 25°C in YPD media. α-Factor (αF) was added (5 μg/ml) and cultures were incubated for 2 hr and then released into YPD at 37°C. Samples were taken every hour for FACS and immunofluorescence microscopy. For αF and nocodazole (Nz) arrest-release experiments (Figure 6), strains were grown to an OD600 of 0.3 at 30°C. αF or Nz was added to a final concentration of 5 μg/ml and 20 μg/ml, respectively. Cultures were incubated for 2 hr and then released into YPD. Samples were taken every 20 min for FACS and fluorescence microscopy.

FACS analysis: FACS analysis was performed as described (Haase and Lew 1997).

Microscopy: Genes carrying C-terminal epitope tags were designed according to Longtine et al. (1998). For immunofluorescence microscopy, cells were fixed by adding an equal volume of 4% paraformaldehyde and incubating for 10 min at 25°C. Cells were imaged using a Zeiss Axioplan 2 microscope equipped with a CoolSNAP HQ camera (Photometrics, Tucson, AZ) and Metamorph (Universal Imaging, West Chester, PA) software. Cells used for fluorescence microscopy were fixed by adding an equal volume of 4% paraformaldehyde and incubating for 10 min at 25°C. Typically, a stack of 10 images was taken at 0.3-μm spacing and then the images were displayed as maximum projections for analysis. SPB-to-SPB distances were determined using Metamorph software by measuring the straight-line distance between the brightest Spc29p-CFP pixels.

RESULTS

Isolation of Ts alleles in central and outer kinetochore proteins: To identify pathways that are important for proper chromosome segregation, we performed a series of SL screens using mutants in essential kinetochore components as query strains. We decided to focus on one member of the Ndc80 complex, Spc24p, which has previously been shown to have a role in MT attachment and spindle checkpoint control, and one member of the outer kinetochore Dam1 complex, Spc34p, which is required for MT attachment, spindle stability, and prevention of monopolar attachment (Janke et al. 2001, 2002; Wigge and Kilmartin 2001; Le Masson et al. 2002). We were concerned that genome-wide screening using only a single mutant of spe24 and spe34 might limit our results, depending on the nature of the mutation and the resulting defect in protein function. Thus we created a series of Ts mutations in both genes and selected for mutants that displayed different arrest phenotypes upon shift to nonpermissive temperature. We isolated three alleles in both SPC24 (spe24-8, spe24-9, spe24-10) and SPC34 (spe34-5, spe34-6, spe34-7) and assessed their effects on DNA content and cell morphology at 37°C. Strains were arrested in G1 phase using the mating pheromone αF and then released to 37°C and monitored over a period of 4 hr. Multiple spe24 Ts mutants have been created yet only one arrest phenotype has been published (Janke et al. 2001; Wigge and Kilmartin 2001; Le Masson et al. 2002). Unexpectedly, we found that all three of our spe24 alleles displayed different arrest profiles (Figure 1, A and B). The spe24-10 mutant, which carries four point mutations (Figure 1C), duplicates its chromosomes and elongates its spindles but DNA remains in the mother cells, suggesting a lack of MT attachment (Figure 1, A and B). In addition, DNA rereplicates to 4N due to continuation of the cell cycle despite the defect in chromosome segregation (Figure 1A). Previous groups have described the spe24-10 mutant arrest phenotype and have shown that the failure to arrest at the metaphase-to-anaphase transition is due to a defect in activation of the spindle checkpoint (Janke et al. 2001; Wigge and Kilmartin 2001; Le Masson et al. 2002). The other two Ts mutants of spe24 exhibited previously undescribed spe24 arrest phenotypes. spe24-8 carries one mutation in the second predicted coiled-coil domain of Spc24p (Figure 1C) and arrests with a short spindle and DNA at the bud neck indicative of an active checkpoint arrest. The third allele, spe24-9, carries one mutation in the C-terminus of Spc24p (Figure 1C) and displays a pleiotropic arrest phenotype. Three hours after shift to nonpermissive temperature, ~30% of spe24-9 cells have discontinuous spindles that extend into the mother and break (Figure 1B). DAPI staining revealed that 3 hr after the temperature shift, unequal amounts of DNA have segregated, suggesting that partial MT attachment has occurred. The spindle defect phenotypes of spe24-9 mutants are reminiscent of the effects of some mutations in the Dam1 complex (see below). The three spe34 mutants that we generated fall into two phenotypic classes. spe34-6 and spe34-7 mutants have a metaphase arrest phenotype at restrictive temperature with a short spindle and DNA at the bud neck as described for spe24-8 (Figure 2, A and B). In addition to having a similar arrest phenotype, spe34-6 and spe34-7 have a common amino acid mutation (S18P). However, they do not display similar genetic interactions (see below), possibly due to the K198E mutation in spe34-6, which is directly adjacent to an Ipl1p phosphorylation site (T199) (Cheeseman et al. 2002). After 3 hr at nonpermissive temperature, spe34-5 mutants have a population of mixed cells that have either a discontinuous spindle in the mother that progresses into the daughter cell and breaks (Figure 2B, first three panels from the left) or a short spindle and a MT projection (Figure 2B, fourth panel from the left). The spe34-5 mutant phenotypes are similar to those caused by the spe34-3 allele described by Janke et al. (2002). Other
members of the Dam1 complex have phenotypes similar to all three of our spc34 mutants (Hofmann et al. 1998; Jones et al. 1999; Cheeseman et al. 2001a,b; Enquist-Newman et al. 2001; Janke et al. 2002).

Genome-wide SL screen with spc24 and spc34 alleles: We introduced the spc24 and spc34 mutations into the SGA query strain and reconfirmed the mutations by sequencing and checking the arrest phenotypes at restrictive temperature (data not shown). Genome-wide SL screens were performed twice using SGA methodology by mating each query strain to the yeast deletion set and selecting for double mutants (Tong et al. 2001).

Since some of the spc24 and spc34 mutants are inviable at temperatures >32°C, we analyzed the double-mutant phenotypes at 25°C. Any nonessential mutants that were either inviable or slow growing in combination with spc24 or spc34 mutants were placed on a miniarray and rescreened against the query strains. From our three screens, we chose genetic interactors that were identified in at least two screens and performed random spore analysis and then tetrad dissection to confirm double-mutant phenotypes. Next, the data were organized by 2D hierarchical clustering, which orders both query genes and array genes on the basis of the number of

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**Figure 1.** — Characterization of spc24 Ts mutants. (A) Wild-type (SPC24, YVM1370), spc24-8 (YVM1448), spc24-9 (YVM1580), and spc24-10 (YVM1363) strains were arrested with the mating pheromone αF at 25°C and then released to 37°C and samples were taken at 2, 3, and 4 hr. DNA content was analyzed by flow cytometry for 1N, 2N, or 4N DNA content. (B) Immunofluorescence microscopy performed on cells incubated at 37°C for 2 or 3 hr post-αF release, as described in A. Cells were stained for tubulin and DNA (DAPI). (C) Schematic of amino acid (aa) substitutions in spc24 mutants. Shading identifies aa regions of Spc24p predicted to be coiled-coil domains (aa 51–aa 61 and aa 87–aa 122) on the basis of MultiCoil analysis (http://multicoll.lcs.mit.edu/cgi-bin/multi coil).

**Figure 2.** — Characterization of spc34 Ts mutants. (A) Wild-type (SPC34, YM61), spc34-5 (YM40), spc34-6 (YVM1864), and spc34-7 (YM41) strains were arrested with the mating pheromone αF at 25°C and then released to 37°C and samples were taken at 2, 3, and 4 hr. DNA content was analyzed by flow cytometry for 1N, 2N, or 4N DNA content. (B) Immunofluorescence microscopy performed on cells incubated at 37°C for 3 hr post-αF release, as described in A. Cells were stained for tubulin and DNA (DAPI). (C) Schematic of aa substitutions in spc34 mutants.
common interactions (Figure 3A). The profile of genetic interactions varied considerably depending on the allele screened.

Three central kinetochore mutants (ctf3, ctf19, and mcm21) were identified in both spc24-9 and spc34-6 SL screens while two additional kinetochore mutants (iml3 and chl4) were identified in the spc24-9 screen. We were curious about whether central kinetochore mutants interact specifically with spc24-9 and spc34-6 mutants or if they were false negatives in the other spc24 and spc34 screens. To simplify our analysis we focused on the spc24 mutants by directly testing for genetic interactions via tetrad analysis. Neither spc24-8 nor spc24-10 displayed an SL or SS interaction with the chl4, ctf3, ctf19, or mcm21 central kinetochore mutant or the mad1 spindle checkpoint mutant at 25°C, suggesting that the interactions with spc24-9 are indeed allele specific at this temperature (Table 2). Thus the defect in the spc24-9 mutant is more sensitive to loss of central kinetochore proteins than that in the other spc24 mutants.

Our 2D clustering analysis revealed that the spc24-9 and spc34-6 screens have the most genetic interactions in common. We organized the array genes that were identified in both the spc24-9 and spc34-6 screens on the basis of functional groups (Figure 3B). Many of the genes have known roles in chromosome segregation, anaphase, and spindle checkpoint functions.

### TABLE 2
Genetic interactions between spc24 and gene deletion mutants

<table>
<thead>
<tr>
<th>ORF</th>
<th>SGD name</th>
<th>Function</th>
<th>Query strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>YIL040W</td>
<td>APQ12</td>
<td>mRNA export</td>
<td>SL*</td>
</tr>
<tr>
<td>YDR254W</td>
<td>CHL4</td>
<td>Central kinetochore</td>
<td>SL</td>
</tr>
<tr>
<td>YLR381W</td>
<td>CTF3</td>
<td>Central kinetochore</td>
<td>SL</td>
</tr>
<tr>
<td>YPL018W</td>
<td>CTF19</td>
<td>Central kinetochore</td>
<td>SS</td>
</tr>
<tr>
<td>YGL086W</td>
<td>MAD1</td>
<td>Spindle checkpoint</td>
<td>SS</td>
</tr>
<tr>
<td>YDR318W</td>
<td>MCM21</td>
<td>Central kinetochore</td>
<td>SL</td>
</tr>
</tbody>
</table>

Note: All interactions were determined at 25°C on YPD media. SL, synthetic lethal; SS, synthetic sick; —, no phenotype.
chromatin structure, MT dynamics, and spindle checkpoint function. We identified three members of the GimC/Prefoldin complex (gmt3, gmt4, and yke2), which is a molecular chaperone that promotes MT and actin filament folding (Geissler et al. 1998; Vainberg et al. 1998; Siegers et al. 1999; Hartl and Hayer-Hartl 2002). Genome-wide SL screens have been performed using members of the GimC/Prefoldin complex as query strains and have identified genetic interactors representing a wide variety of cellular processes (Tong et al. 2004). We also identified two members of the SWR1 chromatin-remodeling complex (arp6, swc6) that incorporates the histone H2A variant Htz1p (which was also identified in our SL screen) into nucleosomes (Korber and Horz 2004). We further identified two components of the Paf1 elongation complex, rtf1 and leol, plus three additional mutants that interact with RNA polymerase II (etch2, rtt103, and sub1) (Sterner et al. 1995; Henry et al. 1996; Knaus et al. 1996; Müller and Jaehning 2002; Km et al. 2004). Interestingly, we also identified the apq12 mutant in both the spe24-9 and spe34-6 screens. Apq12 mutants are known to have defects in nuclear transport (Baker et al. 2004), and recent evidence suggests that nuclear transport is specifically regulated during mitosis (Makhnevych et al. 2003). Thus, we were interested in analyzing this potential link between nuclear transport and kinetochore function.

**APQ12 genetically interacts with the kinetochore:**

APQ12 encodes a small 16.5-kDa protein with a predicted transmembrane domain that localizes to the NE (Hu et al. 2003; Baker et al. 2004). Recently, it was shown that apq12 deletion mutants produce hyperadenylated mRNAs and accumulate poly(A)+ RNA in the nucleus, suggesting a defect in mRNA export (Baker et al. 2004). Unlike most mRNA transport mutants however, apq12 mutants also have an aberrant cell morphology, suggesting that Apq12p has additional cellular functions (Baker et al. 2004; Saito et al. 2004). Apq12 deletion mutants are specifically SS/SL in combination with either spe24-9 or spe34-6 mutants but do not display a phenotype when combined with the other spe24 or spe34 mutants at 25°C (Table 2 and data not shown). However, when we tested for genetic interactions between apq12Δ and the other spe24 alleles at temperatures higher than that used in our genome-wide screens (25°C), we found that spe24-10 was SS in combination with apq12Δ at 30°C and that the spe24-8 apq12Δ double mutant was SS at 33°C (Figure 4A). Thus all three of the spe24 mutants exhibit an unexpected genetic interaction with a mutant that has mRNA export defects.

Conceivably, Apq12p could contribute to the nuclear export of SPC24 mRNA and thereby affect Stp24p cellular protein levels. Combining an SPC24 mRNA export defect with a spe24 Ts mutation could result in low levels of the Stp24p protein, which is essential, and may explain the lethality of apq12 spe24 Ts double mutants. To determine if Stp24p protein levels are perturbed in apq12Δ strains, we analyzed the stability and localization of GFP-tagged Stp24p in a log-phase apq12Δ cell population. We found that Stp24p-GFP protein levels are not notably different between a wild-type and an apq12Δ strain (Figure 5A). We also tagged the spe24-8 mutant allele with GFP and found that spe24p-8 mutant protein levels are not perturbed in apq12Δ strains (Figure 5A). Stp24p-GFP still remains Ts and the strain arrests with the same FACS profile as that in Figure 1A, suggesting that the GFP tag does not alter the behavior of the spe24-8 mutant (data not shown). In addition, we found that both C-terminally GFP epitope-tagged spe24p-8 and spe24p-10 remain at wild-type protein levels when incubated at restrictive temperature (data not shown; spe24p-9 does not tolerate a C-terminal tag and therefore could not be assessed). Stp24p-GFP localizes to punctate foci that are close to each other in small budded cells and separated in the mother and daughter in large budded cells (Figure 5B, wild type). Spe24p-GFP still localized to distinct foci in an apq12 mutant, suggesting that kinetochore clustering and the structure of the Ndc80 complex is not significantly altered in apq12Δ strains (Figure 5B, apq12Δ). Thus, the Stp24p protein stability and localization data suggest that the Stp24p protein is not severely affected in apq12Δ strains. Therefore, apq12 mutants are unlikely to have a specific defect in SPC24 mRNA export.
To determine if \( apq12 \) mutants have a defect in overall kinetochore structure, we analyzed the localization of a central kinetochore protein fused to the Venus fluorescent protein (VFP) Okp1p-VFP in relation to an SPB protein fused to the cyan fluorescent protein (CFP) Spc29p-CFP in \( apq12 \Delta \) cells. Kinetochore proteins localize to the nuclear side of each SPB in cells with short spindles and colocalize with SPBs in cells with long spindles (Goshima and Yanagida 2000; He et al. 2000; Pearson et al. 2001). Okp1p-VFP Spc29p-CFP localization was unperturbed in \( apq12 \Delta \) cells, suggesting that kinetochore structure and dynamics are not greatly altered in \( apq12 \) mutants (Figure 5C).

\( apq12 \) mutants have aberrant chromosome segregation: Since Spc24p protein levels and localization appear to be normal in \( apq12 \) mutants, we asked if \( apq12 \) mutants might have phenotypes that suggest a defect in chromosome stability. We used a colony-color-based half-sector assay to determine if \( apq12 \Delta \) strains were able to maintain a nonessential chromosome fragment (CF) at wild-type levels (Koshland and Hieter 1987). We also assayed CF missegregation in the three \( spc24 \) Ts mutants to compare CF loss phenotypes among the different mutants. As expected, the \( spc24 \) mutants showed an increased rate of chromosome loss (1:0 events) compared to wild type (Table 3). Interestingly, \( spc24-9 \) had a much higher rate of chromosome loss (380-fold increase) compared to \( spc24-8 \) and \( spc24-10 \) (33- and 14-fold increase, respectively) at 30°. \( apq12 \) mutants did not display a significant increase in chromosome loss compared to the wild-type strain but did show a dramatic increase (58.8-fold over wild type) in 2:1 segregation events when plated at 35°. For 2:1 segregation to occur in the first mitotic division (which gives rise to half-sectored colonies), the parental cell must overreplicate the CF to three or more copies and segregate two or more copies to one cell and one copy to the other cell. We were concerned that there might be a selective advantage for \( apq12 \) deletion strains to acquire an extra copy of the CF (CFIII CEN3.L) that contains part of chromosome III, even though the wild-type copy of APQ12 is located on chromosome IX and thus not on the CF. We therefore performed the sector assay using a different CF (CFVII RAD2.d) and still saw a significant increase (43.5-fold over wild type) in 2:1 segregation events for \( apq12 \Delta \) strains (Table 3).

Mutants that have defects in chromosome segregation, including kinetochoore mutants, are often sensitive to the microtubule-depolymerizing drug benomyl. Although the precise mechanism of benomyl action is not known, evidence suggests that it may bind to the \( \alpha \)- and \( \beta \)-tubulin heterodimers, thereby inhibiting MT formation (Richards et al. 2000). We plated the \( apq12 \Delta \) mutant and the three \( spc24 \) Ts mutants, as well as tubulin mutant control strains, on plates to test their sensitivity to benomyl. To our surprise, we found that \( apq12 \Delta \) strains are resistant to benomyl and grow nearly as well as a \( \text{tub}2-104 \)-resistant allele on 20 \( \mu \)g/ml benomyl plates at 30° (Figure 4B). In contrast, we found that the \( spc24-9 \) and \( spc24-10 \) mutants are sensitive to 15 and 20 \( \mu \)g/ml benomyl (Figure 4B). Resistance to benomyl suggests that \( apq12 \) mutants may have stabilized MTs or high levels of tubulin. We performed antitubulin immuno-fluorescence on fixed \( apq12 \) log-phase cells but did not note any striking differences in MT formation (data not shown), although the resolution may not have been sufficient to detect minor changes in MT levels or structure. Thus, \( apq12 \Delta \) strains may have stabilized MTs or be resistant to benomyl due to an indirect mechanism.

\( apq12 \) mutants have defects in exiting mitosis: The 2:1 CF segregation phenotype and benomyl resistance of \( apq12 \Delta \) mutants are indicative of problems during mitosis. We compared the progression of a wild-type vs.
TABLE 3
Chromosome loss events in spc24 and apq12 mutants

<table>
<thead>
<tr>
<th>Strain no.</th>
<th>Genotype (a/a)</th>
<th>Temperature</th>
<th>Total colonies</th>
<th>Chromosome loss (1:0 events)</th>
<th>Nondisjunction (2:0 events)</th>
<th>Overreplication or nondisjunction (2:1 events)</th>
</tr>
</thead>
<tbody>
<tr>
<td>YM196</td>
<td>spc24-8::kanMX6/spc24-8::kanMX6</td>
<td>30(^\circ)</td>
<td>24,976</td>
<td>(2 \times 10^{-4}) (1.0)</td>
<td>(2.8 \times 10^{-4}) (1.0)</td>
<td>0</td>
</tr>
<tr>
<td>YM192</td>
<td>spc24-8::kanMX6/spc24-8::kanMX6</td>
<td>30(^\circ)</td>
<td>25,488</td>
<td>(6.6 \times 10^{-3}) (33)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VVM1892</td>
<td>spc24-9::kanMX6/spc24-9::kanMX6</td>
<td>30(^\circ)</td>
<td>7,606</td>
<td>(7.6 \times 10^{-2}) (380)</td>
<td>(1.3 \times 10^{-4}) (0.5)</td>
<td>0</td>
</tr>
<tr>
<td>YM194</td>
<td>spc24-10::kanMX6/spc24-10::kanMX6</td>
<td>30(^\circ)</td>
<td>23,824</td>
<td>(2.8 \times 10^{-3}) (14)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>YPH982</td>
<td>Wild type CFIII CEN3.L URA3</td>
<td>35(^\circ)</td>
<td>17,440</td>
<td>(4.6 \times 10^{-4}) (1.0)</td>
<td>(4.1 \times 10^{-3}) (1.0)</td>
<td>(1.7 \times 10^{-4}) (1.0)</td>
</tr>
<tr>
<td>VVM1893</td>
<td>apq12Δ/apq12Δ CFIII CEN3.L URA3</td>
<td>35(^\circ)</td>
<td>47,612</td>
<td>(6.5 \times 10^{-4}) (1.4)</td>
<td>(2.0 \times 10^{-3}) (0.5)</td>
<td>(1.0 \times 10^{-2}) (588)</td>
</tr>
<tr>
<td>YPH272</td>
<td>Wild type CFII RAD2.d URA</td>
<td>35(^\circ)</td>
<td>29,224</td>
<td>(1.7 \times 10^{-4}) (1.0)</td>
<td>(1.4 \times 10^{-5}) (1.0)</td>
<td>(1.7 \times 10^{-4}) (1.0)</td>
</tr>
<tr>
<td>YM160</td>
<td>apq12Δ/apq12Δ CFII RAD2.d URA</td>
<td>35(^\circ)</td>
<td>35,759</td>
<td>(8.1 \times 10^{-1}) (4.8)</td>
<td>0</td>
<td>(7.4 \times 10^{-5}) (43.5)</td>
</tr>
</tbody>
</table>

an apq12Δ strain through the cell cycle by synchronizing cells in G1 with the mating pheromone αF and releasing them into the cell cycle. Each strain carried a kinetochore and a SPB marker (Okp1p-VFP and Spc29p-CFP, respectively). Samples were taken every 20 min for DNA profiling by FACS analysis and for kinetochore-SPB localization analysis by fluorescence microscopy. After release from the αF arrest we found that wild-type and apq12Δ strains showed similar timing of bud emergence and SPB duplication by analyzing fixed cells (data not shown). FACS analysis indicated that the timing of DNA replication was also similar (Figure 6A, compare 40-min time points). However, we found that apq12Δ cells showed a reproducible delay in the reappearance of 1N cells by ~20 min (Figure 6A; compare 100-min time points) and that the population of 1N cells remained small compared to wild type throughout the time course (Figure 6A). Wild-type cells showed a transient appearance of 4N DNA, which disappeared by 160 min, whereas the apq12Δ cells had a 4N population of cells from 40 min onward, suggesting that a percentage of apq12Δ mutants rereplicate DNA prior to exiting mitosis (Figure 6A). Finally, we repeatedly found that apq12Δ cells had an increased proportion of 2N cells when arrested with αF, suggesting that a percentage of apq12Δ G1 cells are carrying an extra copy of all chromosomes or that a percentage of apq12Δ cells do not respond to mating pheromone (Figure 6A, 0 min, apq12Δ). Our observations are consistent with a delay for apq12Δ mutants in progression through mitosis and a failure to complete mitosis prior to initiating replication.

To define the stage of mitosis that is delayed in apq12Δ mutants, we performed a similar experiment using the MT-depolymerizing drug Nz to arrest cells in metaphase. apq12 mutants arrest primarily with a 2N population of cells after 2 hr exposure to Nz and with a peak of 4N cells that persists throughout the apq12Δ time course (Figure 6B). Although a small peak of 4N cells is detectable in wild-type cells responding to Nz, a greater proportion of apq12Δ cells contain a 4N quantity of DNA in the Nz-imposed G2 arrest (Figure 6B). The DNA profile of apq12Δ cells released from Nz arrest showed an ~20 min delay in the reappearance of 1N cells compared to wild-type cells as was seen in the G1 synchronisation experiment (Figure 6B; compare 20- and 40-min time points). We also monitored the distance between two Spc29p-CFP foci, as an indicator of spindle length, and found that wild-type and apq12Δ cells entered anaphase with similar kinetics. However, anaphase spindles persisted longer in the apq12Δ cell population, suggesting that apq12Δ cells are delayed during mitosis (Figure 6C). More specifically, at 60 min post-Nz release, 70% of wild-type cells had a spindle length of 1–1.99 μm, suggesting that they had progressed from anaphase to G1 (Figure 6D). In contrast, only 19% of the apq12Δ cells had a 1- to 1.99-μm spindle and 28% of the cells had a spindle between 8 and 10 μm, suggesting that the cells were still in anaphase (Figure 6D). In addition, 12% of apq12Δ mutants had spindle lengths >10 μm whereas none of the wild-type spindles reached this length. Finally, we also noted that the apq12Δ mutant population contained multibudded cells throughout the time course, even immediately after release from Nz exposure, suggesting that some cells might be breaking through the mitotic checkpoint arrest (Figure 6E). The appearance of apq12Δ cells with multiple buds and 4N DNA content suggests that cells attempt to reenter the cell cycle prior to completing cytokinesis.

DISCUSSION

We employed genome-wide SL screens using novel mutations in kinetochore proteins to uncover genes important for chromosome stability when kinetochore function is compromised. We identified a component of
the NE called Apq12p that had not been previously linked to chromosome segregation. Our data demonstrate that Apq12p has a role in the timely execution of anaphase and the maintenance of chromosome stability and provide evidence that the NE is intimately linked with chromosome segregation.

The results of our SL screens highlight the importance of using multiple alleles of essential genes as queries in SGA analysis. Moreover, allele-specific interactions provide information about functional domains of the query protein. For instance, our SL data suggest that Spc24p can be divided into distinct functional domains. spc24-9, which carries a mutation in the C terminus of Spc24p, was SS or SL with the chl4, ctf3, ctf19, tim3, and mem21 central kinetochore mutants. spc24-8 and spc24-10, which carry mutations in the N-terminal region of Spc24p that contains two coiled-coil domains, did not display genetic interactions with central kinetochore mutants at 25°C. spc24-9 mutants also have a much higher rate of chromosome loss than spc24-8 and spc24-10 mutants (Table 3). Thus it is likely that the C-terminal mutation in spc24-9 affects a different Spc24p function

**Figure 6.**—apq12 mutants are delayed in anaphase and prematurely enter a new cell cycle. Wild-type (IPY1986) and apq12Δ (YM20) cells were released from αF or Nz arrest and sampled every 20 min. (A) αF- and (B) Nz-treated cells were assessed for DNA content by FACS analysis. (C) Average SPB-to-SPB distances in cells sampled from each Nz arrest time point. SPB distances were marked with Spc29p-CFP and the distance was quantified from immunofluorescent images. (D) Distribution of SPB-to-SPB distances at 60 min after Nz release. (E) Percentage of multi-budded cells at each time point after Nz release. (F) DIC image of multi-budded cells at 60 min post-Nz release in an apq12Δ mutant. For each time point (C–E), 100 cells were analyzed. Experiments were performed in duplicate with representative data from one experiment shown.
or protein-protein interaction than do the \textit{spc24-8} and \textit{spc24-10} mutants. Our data are consistent with a recently published structural analysis of the Ndc80 complex, which demonstrates that the C terminus of Spc24p is a globular domain that likely interacts with the kinetochore (\textit{Wei et al.} 2005).

In addition to identifying numerous central kinetochore mutants, we also identified two negative regulators of the cAMP pathway, \textit{ira2} and \textit{pde2}, in the \textit{spc24-9} genome-wide SL screen (Figure 3). Interestingly, \textit{PDE2} was recently identified as a high-copy suppressor of Dam1 complex mutants (\textit{Lx et al.} 2005). Five negative regulators of the cAMP pathway, including \textit{ira2} and \textit{pde2}, were also identified as benomyl-sensitive mutants in genome-wide screens (\textit{Pan et al.} 2004). Thus upregulation of the cAMP pathway by mutation of its negative regulators appears to have a deleterious effect on kinetochore function.

The \textit{apq12} mutant, which has defects in mRNA nucleocytoplasmic transport (\textit{Baker et al.} 2004), was identified in both the \textit{spc24-9} and \textit{spc34-6} screens. Given the role of Apq12p in mRNA transport, one possibility is that Apq12p could direct the nucleocytoplasmic export of specific mRNAs expressing kinetochore proteins. In this hypothesis, mutation of \textit{APQ12} could cause nuclear retention of these mRNAs and improper expression of their protein products. However, our data suggest that Spc24p protein levels and localization are not altered in \textit{apq12} mutants, further suggesting that Spc24p protein expression is not affected (Figure 5, A and B). In addition, the Okp1p central kinetochore protein displayed a typical kinetochore localization pattern in \textit{apq12Δ} cells, suggesting that the kinetochore is intact (Figure 5C). Finally, \textit{apq12} mutants are resistant to benomyl (Figure 4B), whereas cells carrying mutations in kinetochore components or spindle checkpoint proteins are often benomyl sensitive, suggesting that \textit{apq12} mutants do not contain reduced levels of kinetochore and spindle checkpoint proteins.

Since \textit{apq12} mutants appear to have normal levels of kinetochore proteins, Apq12p could have a direct role in chromosome segregation and cell cycle regulation by coordinating the localization of specific protein components to the NE. Recently, identified links between the NPC and the kinetochore have given precedent for communication between the NE and the spindle checkpoint machinery (\textit{Stukenberg and Macara} 2003; \textit{Loiodice et al.} 2004; \textit{Rabut et al.} 2004). For example, the Mad1p and Mad2p spindle checkpoint proteins localize to the NPC in both yeast and mammalian cells (\textit{Campbell et al.} 2001; \textit{Iouk et al.} 2002). The yeast Nup53 complex sequesters Mad1p and Mad2p in the NE (\textit{Iouk et al.} 2002). Interestingly, both mutants in the Nup53 complex and \textit{apq12} mutants are resistant to benomyl, whereas other Nup mutants are not benomyl resistant, suggesting that a specific class of NE proteins may have a role in chromosome segregation (Figure 4B; \textit{Iouk et al.} 2002). We analyzed the localization patterns of Mad1p and Mad2p in \textit{apq12} mutants both during normal cell growth and in response to the spindle checkpoint induced by Nz. However, we were unable to detect any changes in Mad1p or Mad2p localization in \textit{apq12} strains compared to a wild-type strain, suggesting that Apq12p is not required to sequester spindle checkpoint proteins in the NE (data not shown). Thus Apq12p has an alternative role at the NE, perhaps by sequestering or trafficking other chromosome segregation proteins via the NE and NPC.

The data presented in this article reveal a new role for Apq12p in cell cycle progression. \textit{apq12} mutants are delayed during mitosis and accumulate in anaphase, suggesting a defect in mitotic exit (Figure 6, C and D). Using two methods of cell synchrony, we found that a small percentage of \textit{apq12} mutants reeplicate their DNA and rebud prior to completing cytokinesis (Figure 6, A, B, and E). During mitosis, the transition from metaphase to anaphase is marked by degradation of the anaphase inhibitor protein Pds1p (\textit{Cohen-Fix et al.} 1996; \textit{Yamamoto et al.} 1996a,b). Stabilization of Pds1p is a hallmark of cells that are actively responding to the spindle checkpoint pathway; thus \textit{pds1} mutants are defective in the spindle checkpoint response. Recent genetic studies identified an SL interaction between \textit{pds1} and \textit{apq12} and between \textit{mad2} and \textit{apq12} (\textit{Sarin et al.} 2004). Therefore, the mitotic defects of \textit{apq12Δ} mutants render the spindle checkpoint pathway essential during normal cell growth.

Apq12p is one of a growing member of NE-associated proteins that have a role in chromosome stability and mitotic progression. For example, Sac3p is a nucleoporin-associated protein that connects transcription elongation with mRNA export (\textit{Fischer et al.} 2002). \textit{Sac3} deletion mutants accumulate in mitosis as large budded cells with extended MTs, are resistant to benomyl, and have an increased rate of chromosome loss compared to wild-type strains (\textit{Bauer and Kolling} 1996; \textit{Jones et al.} 2000). In a previous genome-wide SL screen, we identified a genetic interaction between \textit{Sac3} and \textit{cep3-2} (an inner kinetochore protein), further supporting a role for Sac3p in chromosome segregation (\textit{Measday et al.} 2005). A component of the SPB called Mps3p is another example of the connection between the NE and the chromosome. Mps3p interacts with the Cdc7p cohesin protein and is required to maintain wild-type levels of cohesion between chromosomes (\textit{Antoniacci et al.} 2004).

In mammalian cells, where the NE disassembles, multiple proteins located at the NPC relocate to the kinetochore upon NE breakdown (\textit{Stukenberg and Macara} 2003). Ran is a small GTPase that regulates the interaction of cargo proteins with nucleoporins. The Ran GTPase-activating protein RanGAP1 and its associated nucleoporin RanBP2 are targeted to kinetochores in a MT- and Ndc80-complex-dependent fashion.
(Joseph et al. 2004). In addition, RanGAP1 and RanBP2 are required for the kinetochore localization of both spindle checkpoint and kinetochore proteins and for maintaining kinetochore-MT interactions (Joseph et al. 2004). Although no nucleoporin has been shown to relocalize to the kinetochore in yeast, the Nnf1p kinetochore protein was originally identified from a purification of NE proteins, and yeast cells depleted of Nnf1p accumulate poly(A) + RNA (Shan et al. 1997). The molecular mechanism by which NE proteins, such as Apq12p, and kinetochore proteins interact may be a conserved cellular process that functions to promote proper chromosome segregation and mitotic progression.

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LITERATURE CITED


Fischer, T., K. Strasser, A. Racz, S. Rodriguez-Navarro, M. Oppizzi et al., 2002 The mRNA export machinery requires the novel Sac3p-Thp1p complex to dock at the nucleoplasmic entrance of the nuclear pores. EMBO J. 21: 5845–5852.


Jones, M. H., J. B. Bachant, A. R. Castillo, T. H. Giddings, Jr. and M. Wink, 1999 Yeast Dam1p is required to maintain spindle integrity during mitosis and interacts with the Mps1p kinase. Mol. Biol. Cell 10: 2577–2591.


Knaus, R., R. Pollock and L. Guarente, 1996 Yeast SUI1 is a suppressor of TFIIH mutations and has homology to the human co-activator PC4. EMBO J. 15: 1933–1940.

APQ12 Interacts With the Kinetochore


Lohman, A. I., A. Alves, G. Rabut, M. Van Overbeek, J. Ellenberg et al., 2004 The entire Nup107-160 complex, including three new members, is targeted as one entity to kinetochores in mitosis. Mol. Biol. Cell 15: 3335–3344.


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