Note

Uncovering Novel Cell Cycle Players Through the Inactivation of Securin in Budding Yeast

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ABSTRACT

Budding yeast securin/Pds1p, an inhibitor of the anaphase activator separase/Esp1p, is involved in several checkpoint pathways and in promoting Esp1p's nuclear localization. Using a modified synthetic genetic array (SGA) screen for genes that become essential in the absence of Pds1p, we uncovered roles for uncharacterized genes in cell cycle processes, including Esp1p activation.

THE fidelity of the cell division cycle is ensured, in part, by conserved regulatory mechanisms known as checkpoints that govern the timely execution of numerous cell cycle processes. Distinct checkpoints are activated in response to specific cellular defects, resulting in a cell cycle delay that persists until the defect is repaired. Thus, cells can compensate for certain intracellular deficiencies by activating a checkpoint response; conversely, in the absence of checkpoint pathways, innocuous mutations may become deleterious. This notion is the basis of the present study, in which we wished to identify novel genes involved in key cell cycle processes by sensitizing cells to internal damage through checkpoint inactivation.

The separation of sister chromatids at the metaphase-to-anaphase transition marks the commitment of cells to the completion of mitosis. As a result, cellular defects that could adversely affect chromosome segregation must be dealt with before anaphase takes place.

Anaphase initiation occurs when securin (Pds1p in budding yeast) is targeted for degradation by a ubiquitin ligase, the anaphase promoting complex/cyclosome (APC/C) (Figure 1; for review see PETERS 2002). Securin degradation leads to the activation of the protease separase (Esp1p in budding yeast), which is responsible for inducing sister chromatid separation (reviewed in UHLMANN 2003). Three checkpoint pathways are known

to use the budding yeast Pds1p as a mediator of a mitotic cell cycle delay (Figure 1): the spindle checkpoint pathway, which is activated in the absence of proper kinetochore-spindle microtubule interactions (reviewed in LEW and BURKE 2003); the DNA damage checkpoint pathway, which is activated in the presence of DNA damage (reviewed in MELO and TOCZYSKI 2002); and the DNA replication checkpoint pathway, which is activated in response to stalled replication forks (reviewed in LONGHESE et al. 2003). The checkpoint-induced cell cycle delay is accomplished, at least in part, by the stabilization of Pds1p, either by affecting APC/C activity [in the case of the spindle checkpoint pathway (YU 2002)] or by modifying Pds1p itself [in the case of the DNA damage checkpoint pathway (WANG et al. 2001; AGARWAL et al. 2003)]. Deletion of the *PDS1* gene results in the complete ablation of the spindle checkpoint pathway and the partial inactivation of the DNA damage and DNA replication checkpoint pathways, for which there are Pds1p-independent mechanisms for inducing a cell cycle delay (MELO and TOCZYSKI 2002).

Pds1p is also required for promoting the nuclear localization of Esp1p (Figure 1; JENSEN et al. 2001; AGARWAL and COHEN-FIX 2002) and, possibly, by analogy to higher eukaryotes, for Esp1p activation (JAGER et al. 2001; JALLEPALLI et al. 2001; MEI et al. 2001). In the absence of Pds1p, cells fail to grow at elevated temperatures (>28°) due to a deficiency in Esp1p activity (YAMAMOTO et al. 1996; JENSEN et al. 2001). However, unlike Esp1p, Pds1p is not essential for viability. Thus, under permis-

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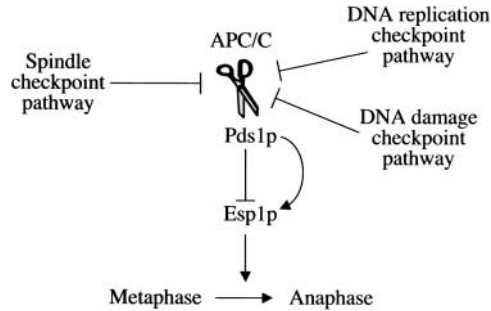
FIGURE 1.—The role of Pds1p in mitotic regulation. Pds1p is an inhibitor of the protease Esp1p that activates anaphase by inducing sister chromatid separation. At the onset of anaphase, Pds1p is degraded in a process that involves the APC/C. At least three checkpoint pathways inhibit mitotic progression by affecting the degradation of Pds1p. Pds1p not only inhibits Esp1p but also serves as its activator by promoting the nuclear localization of Esp1p (indicated by the curved arrow). See text for more details.

sive conditions, the nuclear localization, and perhaps activation, of Esp1p in *pds1Δ* strains is carried out by Pds1p-independent mechanisms.

Given the involvement of Pds1p in several checkpoint pathways, we reasoned that by inactivating Pds1p we could uncover novel proteins involved in spindle function, DNA metabolism, or DNA replication. Mutations in genes coding for these proteins may be tolerated in an otherwise wild-type cell through the presence of one or more checkpoint pathways. However, in a *pds1Δ* background, where checkpoint responses are either abolished or compromised, these mutations could cause a severe growth inhibition or death. This type of genetic interaction, known as synthetic lethality, has been traditionally used to identify proteins acting in redundant pathways (HARTWELL 2004). Here, we used the synthetic lethality scheme to uncover mutations whose harmful affects are normally masked by regulatory pathways. We also anticipated that this screen would lead to the identification of genes involved in Pds1p-independent mechanisms for Esp1p nuclear localization and/or activation. In this case, a synthetic interaction is likely to reflect the inactivation of pathways that are redundant or partially overlapping with Pds1p function.

The screen for synthetic interactions with *pds1Δ* was carried out by a modified synthetic genetic array (SGA) analysis (TONG *et al.* 2001; see legend to Figure 2). In the original method, a strain carrying a mutation in a query gene was mated with a collection of ~4700 strains, each carrying a single deletion in a nonessential gene (designated gene$_n$Δ, where gene$_n$ is one of the ~4700 nonessential genes that is deleted). This array of heterozygous diploids was induced to undergo meiosis, and the inability to obtain a double mutant, namely a cell with the queried mutation and the deleted gene from the collection (gene$_n$Δ), was evidence for a synthetic lethal interaction. In this study we modified the SGA

method and incorporated a plasmid-based step. Briefly, the queried mutation, in our case *pds1Δ*, was combined with mutations from the deletion collection in the presence of a centromeric plasmid, pPDS1, coding for *URA3* and a wild-type copy of the *PDS1* gene. Following meiosis and germination, *pds1Δ* gene$_n$Δ double-mutant strains carrying pPDS1 were tested for synthetic lethality by examining their ability to survive without the plasmid (Figure 2). The advantages of using a plasmid-based assay were threefold:

1. It eliminated many false positives that arose from the inability to obtain double mutants due procedural reasons unrelated to synthetic interactions.
2. It allowed us to circumvent synthetic interactions that cause lethality not during growth *per se* but at the time of spore germination, the requirements and regulation of which are poorly understood but could be significantly different from those for vegetative growth.
3. Cells lacking Pds1p are prone, in and of themselves, to high levels of chromosome instability and exhibit poor growth even at the permissive temperature (23°). Including a wild-type copy of the *PDS1* gene ensured that cells were genetically stable throughout the experiment, thus reducing the likelihood of suppression or exacerbation of a synthetic interaction due to aneuploidy.

The modified SGA screen was carried out twice, resulting in 260 putative synthetic interactions (*e.g.*, synthetic lethality or synthetic sickness), with only a 10% overlap between the two screens. A total of 220 diploid parents containing pPDS1 were subjected to tetrad analysis as described in the legend to Figure 2. Each gene$_n$Δ mutant strain was tested for growth by itself and in combination with the *pds1Δ* mutation with pPDS1 (on YPD) or without pPDS1 [on plates containing 5-fluoro-orotic acid (5-FOA), which allows growth of cells that have lost the *URA3*-containing plasmid; Figure 2]. To ensure that the 5-FOA sensitivity reflected dependence on *PDS1*, all double-mutant combinations carrying pPDS1 were further tested for their ability to grow on 5-FOA when another centromeric plasmid carrying *PDS1* and a selectable marker other than *URA3* was introduced (see legend for Figure 2). Under this condition, all strains with double-mutant combinations that resulted in synthetic lethality or sickness should be able to lose pPDS1 and grow on 5-FOA. On the basis of the growth phenotype, interactions were classified as "synthetically lethal" (for examples, see Figure 2, *cin8Δ* and *slk19Δ*), "synthetically sick" (Figure 2, *chl1Δ* and *sti1Δ*), or "noninteractors" (*i.e.*, if growth in the presence or absence of pPDS1 were indistinguishable; not shown). The side-by-side analysis of the gene$_n$Δ mutant strain and the gene$_n$Δ *pds1Δ* double-mutant strain allowed us to eliminate false positives that resulted from a severe growth defect of the gene$_n$Δ mutation alone.

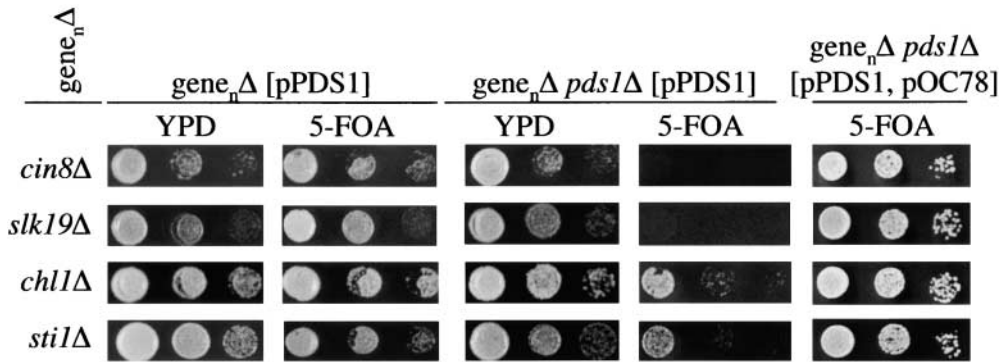FIGURE 2.—A screen for synthetic lethal interactions with *pds1Δ* using a modified SGA analysis. A *pds1Δ* strain (*MATα* MFα::MFα$_{promoter}$*LEU2* *can1Δ::* MFA$_{promoter}$*HIS3* *his3Δ* *ura3Δ* *met15Δ* *lys2Δ* *pds1::NAT*, S288C background) carrying a centromeric plasmid encoding *PDS1* and *URA3* (pPDS1) was crossed individually to ~4700 *MATa* strains of the yeast deletion collection, each containing a single deletion of a nonessential gene (designated gene$_n$Δ). Following diploid selection, the strains were sporulated and selected for double mutants (*pds1Δ* gene$_n$Δ) containing pPDS1 (for double-mutant selection procedure see TONG *et al.* 2001). Of the 4700 crosses, ~90% gave rise to double-mutant strains containing the plasmid. Of those, 260 strains showed a putative synthetic interaction as scored by the inability to lose pPDS1 (see below). A total of 220 of these strains were further examined by tetrad analysis. For each of these strains, four double-mutant isolates (*pds1Δ* gene$_n$Δ) and two single-mutant isolates (gene$_n$Δ) were assayed by three 10-fold serial dilutions for their ability to survive in the absence of Pds1p function by counterselecting for pPDS1 on 5-FOA media. Because 5-FOA is toxic to cells expressing *URA3*, only cells that have spontaneously lost pPDS1 would be able to grow on the 5-FOA plates. The inability to grow on 5-FOA, indicative of a requirement for Pds1p for growth, was manifested by either synthetic lethality (for example, see *cin8Δ* or *slk19Δ*) or severe synthetic sickness (see *chl1Δ* or *sti1Δ*). Double-mutant combinations that were unable to grow on 5-FOA were transformed with pOC78, a centromeric plasmid coding for *PDS1 LEU2 HIS3*. If the inability of the gene$_n$Δ *pds1Δ* pPDS1 strain to grow on 5-FOA was due to a requirement for *PDS1* (and not due to the integration of the *URA3* gene into the chromosome, for example), then the presence of pOC78 should allow these cells to grow on 5-FOA. Of the 220 strains examined by tetrad analysis, 21 showed a true synthetic interaction. The identity of the gene disruptions in these strains was verified by sequencing. The high false-positive rate, which is in line with recent reports (TONG *et al.* 2004), may be due to technical challenges associated with the automated procedure (TONG *et al.* 2001) that can be affected by various factors such as growth rate differences and morphology effects on transfer efficiency.

In all, 21 of the 220 mutations tested were verified as having a *bona fide* synthetic interaction with *pds1Δ*. The full list of synthetic interactions is shown in Table 1. These could be divided into five functional groups: microtubule dynamics, chromosome segregation, cell cycle regulators, heat-shock proteins, and proteins of unknown function. One gene identified in this screen that was previously unnamed was designated as *SWS2*, for *s*ick *w*ithout *s*ecurin. Importantly, our screen was based on the premise that the absence of Pds1p function would uncover proteins involved in spindle- or DNA-related processes. This approach was validated by the isolation of mutant strains defective in microtubule-associated proteins (*e.g.*, *cin8Δ*) that were known to require the spindle checkpoint pathway for viability (GEISER *et al.* 1997).

Next, we wished to determine which of the synthetic interactions listed in Table 1 was due to checkpoint failure. To this end, each of the single gene$_n$Δ strains was crossed to a strain defective in the spindle checkpoint pathway (*mad2Δ*), the DNA damage checkpoint pathway (*rad9Δ*), or the DNA replication checkpoint pathway (*mrc1Δ*). In each case, the checkpoint mutant strain harbored a wild-type copy of the mutant checkpoint gene (*MAD2, RAD9,* or *MRC1*, respectively) on a *CEN URA3* plasmid. A synthetic interaction was evident by the ability of the double mutant (*i.e.*, gene$_n$Δ with a checkpoint mutant) to grow in the presence, but not in the absence, of the plasmid, as described above (leg-

end of Figure 2). As shown in Table 1, 11 mutants exhibited a synthetic interaction with at least 1 checkpoint mutant. As expected, mutations in most of the genes coding for microtubule-associated proteins showed a synthetic interaction (lethality or sickness) with *mad2Δ*. Moreover, among those that showed a synthetic interaction with any checkpoint mutant, most were with *mad2Δ* (8 of 11). This is consistent with the more prominent role played by Pds1p in the spindle assembly checkpoint than in the DNA replication or DNA damage checkpoint pathways. Interestingly, mutations in two genes coding for proteins of unknown function, *APQ12* and *VPS64* (also known as *FAR9*), also exhibited synthetic interactions with *mad2Δ*, suggesting that the proteins encoded by these genes may play a role in kinetochore or spindle function. Apq12p is an endoplasmic-reticulum-associated protein of unknown function (T. SLABY and O. COHEN-FIX, unpublished results) and Vps64p/Far9p was previously shown to have a role in the response to mating-pheromone-induced arrest (KEMP and SPRAGUE 2003). Thus, in both cases, a connection to the spindle checkpoint pathway is intriguing. Likewise, a mutation in a gene coding for another protein of unknown function, Dia2p, a predicted F-box protein, was synthetically lethal with *rad9Δ*. Mutation of *DIA2* causes a mild invasive growth phenotype (PALECEK *et al.* 2000) and a modest stabilization of ectopically expressed cyclin E (KOEPP *et al.* 2001). Intriguingly, both the *pds1Δ dia2Δ* and the

TABLE 1

Synthetic interaction results

ORF	Gene name	Function[a]	SI with *pds1*Δ[b]	SI with *mad2*[c,d]	SI with *rad9*Δ[c,e]	SI with *mrc1*Δ[c,f]	Rescue of SI with *pds1*Δ by 2μ*ESP1*[g]
Microtubule dynamics							
YMR198W	*CIK1*	Kar3 kinesin-binding protein	Lethal	Lethal	None	Sick	No
YOR349W	*CIN1*	Tubulin-folding chaperone	Lethal	Lethal	None	None	No
YPL241C	*CIN2*	Tubulin-folding chaperone	Lethal	None	None	None	No
YEL061C	*CIN8*	Spindle-associated kinesin	Lethal	Lethal	None	None	No
YML094W	*GIM5*	Tubulin folding	Lethal	Sick	None	None	No
Chromosome segregation							
YPL008W	*CHL1*	Helicase, sister chromatid cohesion	Sick	None	None	None	No
YPR135W	*CTF4*	Associated with DNA polα	Sick	None	None	Lethal	No
YRM078C	*CTF18*	Alternative RF-C subunit	Lethal	None	None	None	No
YPL018W	*CTF19*	Kinetochore protein	Lethal	Lethal	None	None	No
YCL016C	*DCC1*	Alternative RF-C subunit	Lethal	None	None	None	No
YOR195W	*SLK19*	Kinetochore and spindle associated	Lethal	None	None	None	No
Cell cycle							
YPR119W	*CLB2*	Mitotic cyclin	Sick	None	None	None	No
YCR065W	*HCM1*	Transcription factor	Sick	Sick	None	None	No
Heat Shock							
YMR186W	*HSC82*	Hsp90-type chaperone/heat shock	Sick	None	None	None	Yes
YOR027W	*STI1*	Hsp90 cochaperone	Sick	None	None	None	Yes
YPL106C	*SSE1*	Hsp70-type chaperone/heat shock	Lethal	None	None	None	No
Unknown function							
YOR080W	*DIA2*	F-box protein	Lethal	None	Lethal	None	No
YLR204W	*QRI5*	Unknown	Sick	None	None	None	No
YNL081C	*SWS2*	Unknown	Sick	None	None	None	No
YIL040W	*APQ12*	Unknown	Lethal	Lethal	None	None	No
YDR200C	*VPS64*	Mating-pheromone response	Sick	Lethal	None	None	No

SI, synthetic interaction.

[a] Function is based on the gene ontology annotation of the *Saccharomyces cerevisiae* database (http://www.yeastgenome.org/) and references therein.

[b] *pds1*Δ strain used was described in legend to Figure 2.

[c] Crosses to *mad2*Δ, *rad9*Δ, and *mrc1*Δ strains were done with single mutants (gene_nΔ) that came out of the tetrad analysis done in the cross with *pds1*Δ.

[d] Deletion of the *MAD2* gene results in the inactivation of the spindle checkpoint pathway. The *mad2*Δ strain used was *MATα can1*Δ *leu2*Δ *his3*Δ *lys2*Δ *ura3*Δ *mfa1*Δ::*MFApr-HIS3 mad2*::NAT[pRL18] (pRL18: *CEN URA3 MAD2* plasmid). Strain and plasmid were a gift from Dan Burke (University of Virginia).

[e] Deletion of the *RAD9* gene results in the inactivation of the DNA damage checkpoint pathway. The *rad9*Δ strain used was *MAT*a *ura3*Δ *leu2*Δ *his3*Δ *met15*Δ *rad9*::NAT [pRAD9] (pRAD9: *CEN URA3 RAD9* plasmid). pRAD9 was a gift from Steve Elledge (Harvard Medical School).

[f] Deletion of the *MRC1* gene results in the inactivation of the DNA replication checkpoint pathway. The *mrc1*Δ strain used was *MAT*a *ura3*Δ *leu2*Δ *his3*Δ *met15*Δ *mrc1*::NAT [pMRC1] (pMRC1: *CEN URA3 MRC1* plasmid). pMRC1 was a gift from Steve Elledge (Harvard Medical School).

[g] Plasmid 2μESP1 is a pRS426 (2μURA3)-based plasmid encoding for *ESP1* in which the *URA3* gene was substituted for *LYS2* by PCR-mediated recombination. This plasmid was transformed into *pds1*Δ gene_nΔ *lys2* strains carrying pPDS1 that were isolated in the original tetrad analysis.

*rad9*Δ *dia2*Δ synthetic lethal interactions were observed only in crosses with *dia2*Δ cells that had been propagated for numerous cell divisions, a finding that reflects the progressive accumulation of DNA damage that occurs in the absence of Dia2p (D. BLAKE and M. TYERS, unpublished results).

The synthetic lethality with *pds1*Δ and mutations in checkpoint genes suggested that in a checkpoint-proficient background, several of the mutations isolated in this screen might exhibit cell cycle delays, especially in G_2/M, where the aforementioned checkpoint pathways are known to act. This was of particular interest in the case of *apq12*Δ, *vps64*Δ, and *dia2*Δ, mutations that were previously uncharacterized with regards to cell cycle
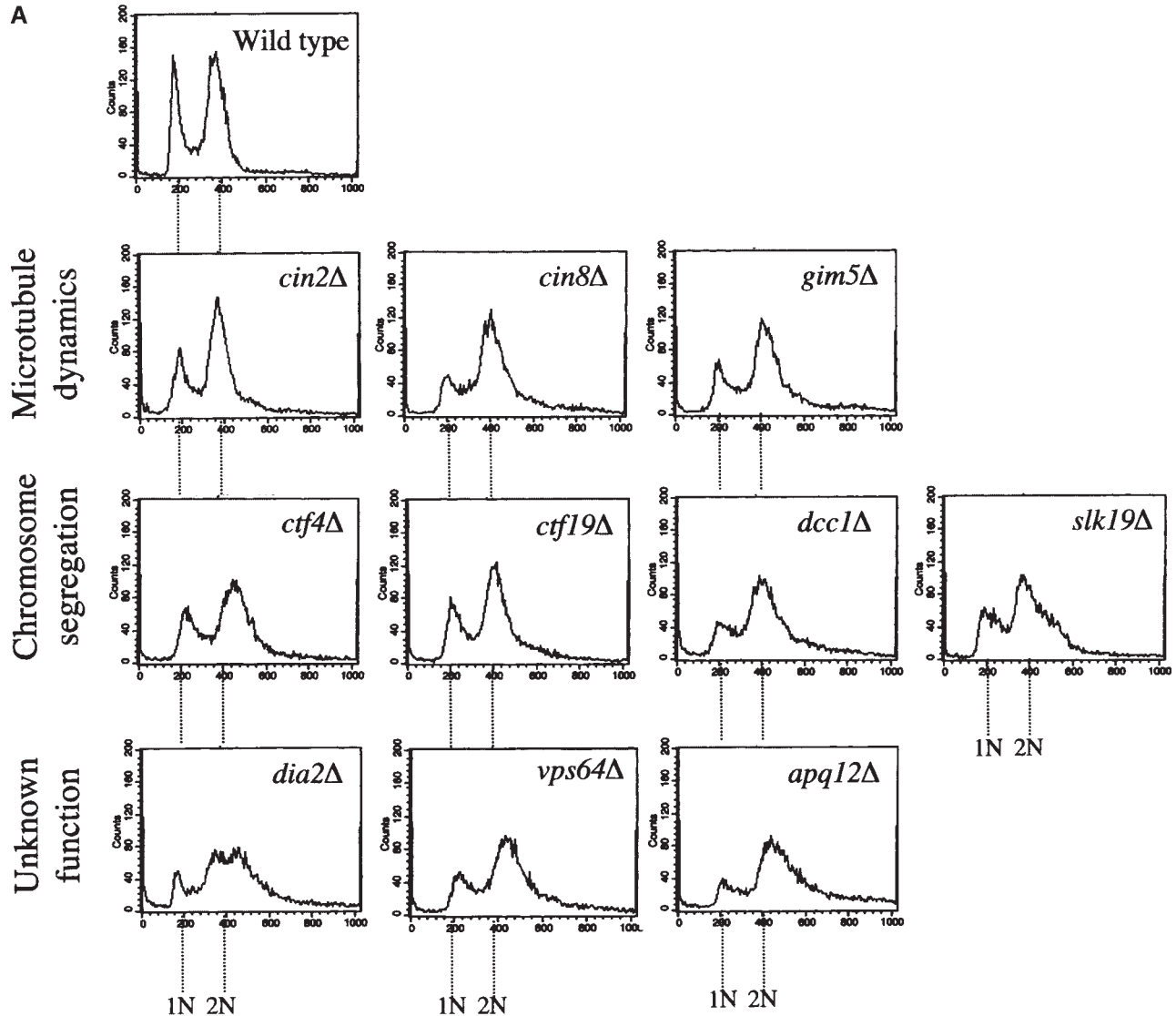
FIGURE 3.—Cell cycle distribution of mutants that show a genetic interaction with *pds1Δ*. Wild-type and mutant strains in A and B were grown to early log phase, fixed in 70% ethanol, and analyzed by flow cytometry (A) and fluorescent microscopy (B) for cell cycle distribution. (A) Only strains whose flow cytometry profiles are different from wild type are shown. Note the relatively high fraction of cells with 2N DNA content in the mutants shown relative to wild type. (B) Histograms represent the percentage of cells with a large bud and an undivided nucleus, indicative of a G_2/M phase. (C) Images of wild-type, *dia2Δ*, *vps64Δ*, and *apq12Δ* cells. Bright white areas indicate DNA (DAPI staining).

profiles. To test this possibility, the cell cycle distribution of unperturbed logarithmic-phase cultures of the single mutants (*i.e.*, a wild-type *PDS1* and checkpoint-proficient background) was determined by flow cytometry analysis (Figure 3A) and cell morphology analysis (Figure 3B). As expected, many of the mutations in processes related to microtubule dynamics and chromosome segregation (Table 1) exhibited a G_2/M accumulation either by flow cytometry or by cell morphology (Figure 3, A and B). This was also true for *dia2Δ*, *apq12Δ*, and *vps64Δ*, further supporting an involvement in DNA metabolism (for Dia2p) or spindle/kinetochore function (for Apq12p and Vps64p), as suggested by their genetic interactions with the respective checkpoint pathway mu-

tations. Interestingly, the growth defect in *dia2Δ*, *apq12Δ*, or *vps64Δ* was also manifested in their relatively large cell size (Figure 3C), typical of cells that are experiencing a cell cycle delay. Taken together, these results strongly suggest that Dia2p, Apq12p, and Vps64p are involved in processes that affect cell cycle progression.

While this manuscript was in preparation, TONG *et al.* (2004) reported the results of an extensive SGA analysis involving numerous checkpoint mutations. The TONG *et al.* (2004) screen, done without the plasmid loss step, uncovered synthetic interactions between *mad2Δ* and many of the gene disruptions reported here, but not with *apq12Δ*, *vps64Δ*, or *dia2Δ*. Furthermore, the TONG *et al.* (2004) screen reported synthetic interactions be-
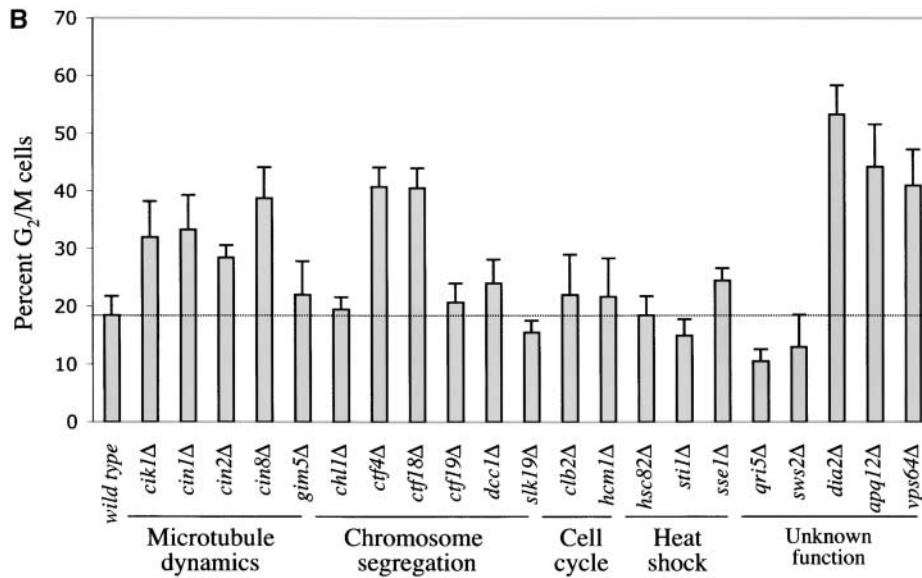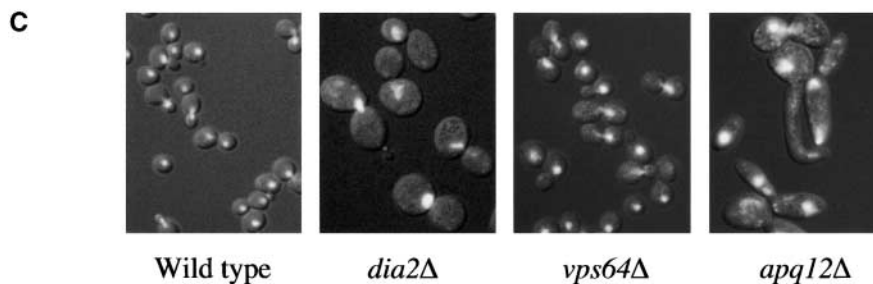
FIGURE 3.—*Continued.*

Wild type   *dia2Δ*   *vps64Δ*   *apq12Δ*

tween double-mutant combinations involving check-point genes that were viable in our hands, including *mad2Δ* with *chl1Δ*, *ctf18Δ*, or *dcc1Δ* and *rad9Δ* with *ctf18Δ* or *dcc1Δ*. There could be at least two reasons for these differences:

1. Due to the temperature sensitivity of the *pds1Δ* strain, all of our analyses were done at 23°, whereas the TONG *et al.* (2004) assays were done at 30°. We know that at least one synthetic interaction, between *apq12Δ* and *mad2Δ*, is temperature dependent (T. SLABY and O. COHEN-FIX, unpublished results).
2. The TONG *et al.* (2004) analysis was done without a plasmid carrying a wild-type copy of the queried gene, and therefore it is possible that in some cases the ob-served synthetic interaction stemmed from a require-ment for germination rather than for viability *per se*. In other words, certain double mutants may not have displayed a synthetic lethal interaction during vegeta-tive growth had they been able to pass the germina-tion step.

In our screen, the inclusion of the plasmid circumvents germination issues, as we were testing only double mu-tants that have passed this stage. In this regard, during tetrad analysis we observed that several double-mutant combinations displayed a higher-than-expected rate of spore death that was not observed when the double-mutant strain carried the plasmid.

The role of Pds1p in Esp1p nuclear localization prompted us to examine whether any of the genes iden-tified in this study were associated with Esp1p function. In the absence of Pds1p, cells are unable to grow at elevated temperatures due, at least in part, to Esp1p's inability to enter the nucleus (JENSEN *et al.* 2001). This phenotype could be partially suppressed by overexpress-ing *ESP1* (JENSEN *et al.* 2001), suggesting that high levels of Esp1p can compensate for defects in pathways in-volved in Esp1p activation. Therefore, we examined whether elevated levels of Esp1p expressed from a high-copy plasmid (2μESP1) could rescue any of the syn-thetic interactions between *pds1Δ* and the mutants isolated in our screen. Indeed, the synthetic sickness between *pds1Δ* and mutations in two heat-shock genes, *HSC82*, encoding an Hsp90-type chaperone, and *STI1*, encoding a cochaperone of Hsc82p (CHANG and LIND-QUIST 1994; CHANG *et al.* 1997), was suppressed by 2μESP1, as is evident by the ability of the *pds1Δ hsc82Δ* 2μESP1 or *pds1Δ sti1Δ* 2μESP1 strains to survive in the absence of pPDS1 (Figure 4A). Interestingly, the syn-thetic interaction between *pds1Δ* and a disruption in
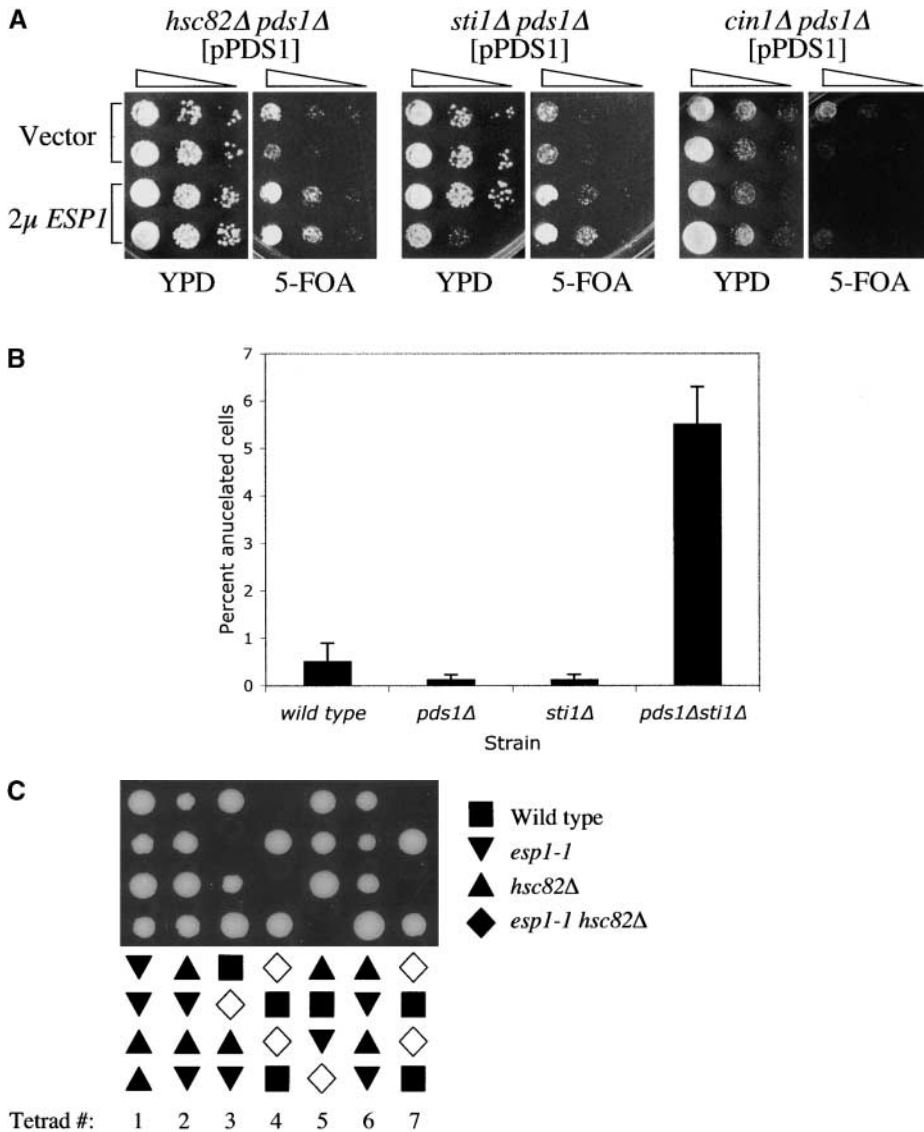
FIGURE 4.—The genetic interactions between *pds1Δ* and mutants in genes coding for Hsp90-type chaperones reflect a role for the latter in Esp1p activity. (A) Double-mutant strains (*pds1Δ* gene_nΔ) carrying pPDS1 were transformed with 2μESP1 (described in Table 1) or with vector alone and analyzed for their ability to survive in the absence of Pds1p by growth on media containing 5-FOA. The synthetic lethality of two double mutants, *hsc82Δ pds1Δ* and *sti1Δ pds1Δ*, could be rescued by this approach. An example of a double mutant that was not rescued (*cin1Δ pds1Δ*) is also shown. (B) Percentage of anucleate cells (*i.e.*, cells without a distinct DNA mass as determined by DAPI staining) was determined as described in Figure 3A. (C) An *hsc82Δ/HSC82 esp1-1/ESP1* diploid strain was induced to undergo meiosis and form tetrads. The haploid meiotic products (spores) were dissected onto a rich media plate (YPD). The growth of spores from seven such tetrads is shown. The symbols denote the genotypes of these spores as determined subsequently. Open symbols indicate the predicted genotypes of spores that failed to germinate. Similar results were obtained when analyzing a *sti1Δ/STI1 esp1-1/ESP1* diploid strain (data not shown).

another heat-shock gene, *sse1Δ*, was not suppressed by 2μESP1 (data not shown).

The relatively mild sickness exhibited by the *pds1Δ sti1Δ* strain allowed us to test for further evidence in support of an Esp1p-related function for Sti1p. Cells compromised for Esp1p function are defective in sister chromatid separation, and Esp1p null cells exit mitosis in the complete absence of nuclear division, resulting in the accumulation of cells lacking a nucleus (anucleate cells; McGREW *et al.* 1992). Thus, if Pds1p and Sti1p contribute independently to Esp1p function, one would expect that the *pds1Δ sti1Δ* double-mutant strain would exhibit phenotypes reminiscent of loss of Esp1p activity. In keeping with this possibility, the *pds1Δ sti1Δ* strain showed levels of anucleate cells that were significantly higher than those of wild-type cells or either of the single-mutant strains alone (Figure 4B). These results suggest that Pds1p and Sti1p independently contribute to Esp1p

activation and that in the absence of both proteins Esp1p activity is reduced, resulting in the appearance of anucleate cells.

Finally, if Sti1p and Hsc82p are involved in Esp1p function, one might expect that in their absence, the phenotype of a compromised *esp1* allele may become more severe. To test this possibility, we crossed a *sti1Δ* or *hsc82Δ* strain to an *esp1-1* strain and the resulting diploid strains were induced to undergo meiosis. Analysis of the meiotic products revealed that the *sti1Δ esp1-1* or *hsc82Δ esp1-1* double-mutant combination was drastically underrepresented (Table 2), representing only 3 of 103 spores and 3 of 60 spores, respectively. In contrast, the other expected genotypes (wild type and each of the single mutants) appeared in roughly equal numbers and accounted for >95% of the spores. In most cases, the genotype of spores that failed to germinate could be inferred to be *sti1Δ esp1-1* or *hsc82Δ esp1-1* (Fig-

TABLE 2

Synthetic interactions between *esp1-1* and chaperone mutants

Spore genotype	No. observed
A. *hsc82Δ* × *esp1-1*	
hsc82Δ	20
esp1-1	20
hsc82Δ esp1-1	3
Wild type	17
Total no. of spores	60
B. *sti1Δ* × *esp1-1*	
sti1Δ	36
esp1-1	28
sti1Δ esp1-1	3
Wild type	36
Total no. of spores	103

ure 4C and data not shown). Taken together, these results suggest that the heat-shock/chaperone proteins Sti1p and Hsc82p play a role in promoting Esp1p function.

For four of the gene disruptions identified in our screen (*clb2Δ*, *sws2Δ*, *qri5Δ*, and *sse1Δ*) neither their putative function nor our secondary screens (synthetic interactions with checkpoint mutants or high-copy *ESP1*) shed light on the cause for their synthetic lethality or sickness with *pds1Δ*. Clb2p is one of the yeast mitotic cyclins and the synthetic lethality between *pds1Δ* and *clb2Δ* has been reported previously (Ross and Cohen-Fix 2003). *SWS2* and *QRI5* encode mitochondria-associated proteins of unknown function (Huh *et al.* 2003), and their genetic interaction with *PDS1* suggests that mitochondria may play a role in cell cycle regulation. Interestingly, flow cytomerty analysis suggested that *sws2Δ* and *qri5Δ* strains accumulate early in the cell cycle (data not shown). Finally, Sse1p is a heat-shock/chaperone protein of the Hsp70 family, and further studies are needed to determine how heat-shock proteins are involved in regulating cell cycle progression.

Uncovering genetic interactions with a mutation that confers genomic instability is challenging, as suppressors that could mask a severe growth deficiency can readily arise through aneuploidy. Nonetheless, our screen yielded 21 gene disruptions that showed genetic interactions with *pds1Δ* under very stringent conditions, namely tetrad analysis followed by a plasmid loss assay. A significant number (eight genes) were not previously known to have cell-cycle-related functions. We were able to identify new genes involved in spindle function or DNA metabolism, and we uncovered a previously unknown role for heat-shock/chaperone proteins in Esp1p function. Moreover, synthetic interactions with *pds1Δ* that were not mirrored by genetic interactions with other checkpoint mutants or suppressed by high levels of Esp1p may reflect novel roles of Pds1p; Pds1p may be involved in as-yet-uncharacterized checkpoint pathways or it may have additional targets that require the presence of Pds1p to sustain viability. Future work aimed at understanding these interactions at the molecular level is likely to yield insights into key cell cycle processes, such as spindle function and Esp1p activation, and possibly new roles for Pds1p in cell cycle regulation.

LITERATURE CITED

Agarwal, R., and O. Cohen-Fix, 2002 Phosphorylation of the mitotic regulator Pds1/securin by Cdc28 is required for efficient nuclear localization of Esp1/separase. Genes Dev. **16:** 1371–1382.

Agarwal, R., Z. Tang, H. Yu and O. Cohen-Fix, 2003 Two distinct pathways for inhibiting pds1 ubiquitination in response to DNA damage. J. Biol. Chem. **278:** 45027–45033.

Chang, H. C., and S. Lindquist, 1994 Conservation of Hsp90 macromolecular complexes in Saccharomyces cerevisiae. J. Biol. Chem. **269:** 24983–24988.

Chang, H. C., D. F. Nathan and S. Lindquist, 1997 In vivo analysis of the Hsp90 cochaperone Sti1 (p60). Mol. Cell. Biol. **17:** 318–325.

Geiser, J. R., E. J. Schott, T. J. Kingsbury, N. B. Cole, L. J. Totis *et al.*, 1997 Saccharomyces cerevisiae genes required in the absence of the CIN8-encoded spindle motor act in functionally diverse mitotic pathways. Mol. Biol. Cell **8:** 1035–1050.

Hartwell, L., 2004 Genetics. Robust interactions. Science **303:** 774–775.

Huh, W. K., J. V. Falvo, L. C. Gerke, A. S. Carroll, R. W. Howson *et al.*, 2003 Global analysis of protein localization in budding yeast. Nature **425:** 686–691.

Jager, H., A. Herzig, C. F. Lehner and S. Heidmann, 2001 Drosophila separase is required for sister chromatid separation and binds to PIM and THR. Genes Dev. **15:** 2572–2584.

Jallepalli, P. V., I. C. Waizenegger, F. Bunz, S. Langer, M. R. Speicher *et al.*, 2001 Securin is required for chromosomal stability in human cells. Cell **105:** 445–457.

Jensen, S., M. Segal, D. J. Clarke and S. I. Reed, 2001 A novel role of the budding yeast separin Esp1 in anaphase spindle elongation: evidence that proper spindle association of Esp1 is regulated by Pds1. J. Cell Biol. **152:** 27–40.

Kemp, H. A., and G. F. Sprague, Jr., 2003 Far3 and five interacting proteins prevent premature recovery from pheromone arrest in the budding yeast Saccharomyces cerevisiae. Mol. Cell. Biol. **23:** 1750–1763.

Koepp, D. M., L. K. Schaefer, X. Ye, K. Keyomarsi, C. Chu *et al.*, 2001 Phosphorylation-dependent ubiquitination of cyclin E by the SCFFbw7 ubiquitin ligase. Science **294:** 173–177.

Lew, D. J., and D. J. Burke, 2003 The spindle assembly and spindle position checkpoints. Annu. Rev. Genet. **37:** 251–282.

Longhese, M. P., M. Clerici and G. Lucchini, 2003 The S-phase checkpoint and its regulation in Saccharomyces cerevisiae. Mutat. Res. **532:** 41–58.

McGrew, J. T., L. Goetsch, B. Byers and P. Baum, 1992 Requirement for ESP1 in the nuclear division of Saccharomyces cerevisiae. Mol. Biol. Cell **3:** 1443–1454.

Mei, J., X. Huang and P. Zhang, 2001 Securin is not required for cellular viability but is for normal growth of mouse embryonic fibroblasts. Curr. Biol. **11:** 1197–1201.

Melo, J., and D. Toczyski, 2002 A unified view of the DNA-damage checkpoint. Curr. Opin. Cell Biol. **14:** 237–245.

Palecek, S. P., A. S. Parikh and S. J. Kron, 2000 Genetic analysis reveals that FLO11 upregulation and cell polarization independently regulate invasive growth in *Saccharomyces cerevisiae.* Genetics **156:** 1005–1023.

Peters, J. M., 2002 The anaphase-promoting complex: proteolysis in mitosis and beyond. Mol. Cell **9:** 931–943.

Ross, K. E., and O. Cohen-Fix, 2003 The role of Cdh1p in maintaining genomic stability in budding yeast. Genetics **165:** 489–503.

Tong, A. H., M. Evangelista, A. B. Parsons, H. Xu, G. D. Bader *et al.*, 2001 Systematic genetic analysis with ordered arrays of yeast deletion mutants. Science **294:** 2364–2368.

Tong, A. H., G. Lesage, G. D. Bader, H. Ding, H. Xu *et al.*, 2004 Global mapping of the yeast genetic interaction network. Science **303:** 808–813.

Uhlmann, F., 2003 Chromosome cohesion and separation: from men and molecules. Curr. Biol. **13:** R104–R114.

Wang, H., D. Liu, Y. Wang, J. Qin and S. J. Elledge, 2001 Pds1 phosphorylation in response to DNA damage is essential for its DNA damage checkpoint function. Genes Dev. **15:** 1361–1372.

Yamamoto, A., V. Guacci and D. Koshland, 1996 Pds1p is required for faithful execution of anaphase in the yeast, Saccharomyces cerevisiae. J. Cell Biol. **133:** 85–97.

Yu, H., 2002 Regulation of APC-Cdc20 by the spindle checkpoint. Curr. Opin. Cell Biol. **14:** 706–714.

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