Integrating high-throughput genetic interaction mapping and high-content screening to explore yeast spindle morphogenesis

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We describe the application of a novel screening approach that combines automated yeast genetics, synthetic genetic array (SGA) analysis, and a high-content screening (HCS) system to examine mitotic spindle morphogenesis. We measured numerous spindle and cellular morphological parameters in thousands of single mutants and corresponding sensitized double mutants lacking genes known to be involved in spindle function. We focused on a subset of genes that appear to define a highly conserved mitotic spindle disassembly pathway, which is known to involve Ipl1p, the yeast aurora B kinase, as well as the cell cycle regulatory networks mitotic exit network (MEN) and fourteen early anaphase release (FEAR). We also dissected the function of the kinetochore protein Mcm21p, showing that sumoylation of Mcm21p regulates the enrichment of Ipl1p and other chromosomal passenger proteins to the spindle midzone to mediate spindle disassembly. Although we focused on spindle disassembly in a proof-of-principle study, our integrated HCS-SGA method can be applied to virtually any pathway, making it a powerful means for identifying specific cellular functions.

Introduction

A major challenge in post-genome biology is to exploit genome sequence information to produce reagents and technologies that decipher the molecular basis of gene function through an unbiased and systematic analysis. Although functional genomic approaches have been applied productively with yeast, the integration of multiple datasets is typically required to accurately define gene function. Combining data from many large-scale studies remains problematic because individual screens may not be saturating or conducted under comparable experimental conditions. To facilitate integration of large-scale phenotypic and genetic datasets, we combined an automated form of yeast genetics, synthetic genetic array (SGA) analysis (Tong et al., 2001), with a high-content screening (HCS) system, which automates image acquisition and the quantification of specific morphological phenotypes.

We examined the morphological phenotypes of the growing mitotic spindle in both single gene deletion mutants and in selected double mutant arrays, sensitized for spindle defects. In addition, we also examined a subset of strains carrying conditional alleles of essential genes at both restrictive and permissive temperatures. For the implementation of the platform, each step, from sample processing to image acquisition and scoring of phenotypes, was automated and adapted for both live-cell and fixed-cell analysis. The cell biological phenotype of each yeast mutant
was represented by a quantitative readout of cellular parameters, called a morphological profile. Using this information, we identified 182 mutants that influence spindle dynamics, 90 of which had defects apparent only in the double mutant backgrounds. Our results identify new genes involved in spindle disassembly and outline an intricate pathway involving the SUMO machinery required for efficient relocalization of the Ipl1p kinase to the spindle midzone. Our SGA-HCS approach offers a general and powerful method for quantifying the activity of specific pathways in the context of complex genetic backgrounds.

Results

Systematic identification of mutants with aberrant spindle morphology

SGA methodology enables marked genetic elements to be combined in a single haploid cell through standard yeast mating and meiotic recombination via an automated procedure (Boone et al., 2007). Here, our goal was to systematically survey the yeast deletion collection for defects in spindle morphogenesis. To do so, we applied SGA to introduce a GFP-tubulin (GFP-Tub1p) reporter into the arrayed collection of deletion mutants. To sharpen our focus on spindle function, we also constructed double mutant arrays harboring GFP-Tub1p as well as a deletion allele of BNI1, which encodes a formin protein that participates in spindle orientation by nucleating actin cables, or a deletion of BIM1, which encodes a protein that links microtubules to a myosin motor that walks along the actin cables (Pruyne et al., 2004). We chose to assay double mutants with bni1Δ and bim1Δ because genetic interactions involving BNI1 and BIM1 have been well characterized (Tong et al., 2001) and the mutants have subtle defects in spindle function that appear mechanistically distinct.

We used automated image acquisition and analysis to quantify cell shape with respect to spindle morphology and score aberrant spindle defects (Fig. 1 A and Fig. S1; MetaXpress version 1.63, see Materials and methods). In brief, we used background fluorescence and a low threshold for GFP intensity to identify the individual cells or objects in each image (whole cell segmentation). Next, we identified spindles in the same image by varying the GFP threshold (spindle segmentation). After this, a minimal set of features such as area and shape factor were used to train the imaging software so that it could efficiently classify an unseen segmented image into two categories such as budded and unbudded cells. Each budded cell or unbudded cell was earmarked as a region of interest, and corresponding morphometric features were logged separately using the built-in functions of MetaXpress. An image-processing tool was then used to identify the bud neck region. After object identification, quantitative measurements allowed us to extract numerous morphometric features (Fig. S1) to generate a unique profile for each mutant strain (Tables S3–S5; see Materials and methods for more details on image analysis and morphometric feature extraction; for review see Vizeacoumar et al., 2009).

To identify genes that play a role in spindle dynamics, we concentrated on three basic spindle-specific features derived from the GFP-Tub1p reporter: (1) the ratio of spindle to cell length, (2) the orientation of the spindle with respect to the cell axis, and (3) the distance between the center of the spindle and the bud neck. We assessed these features relative to cell cycle stage and searched for mutants that showed an altered distribution of cells for a given feature compared with wild type (see Materials and methods). For example, Fig. 1 B shows a 2D histogram of spindle orientation and budding index (cell cycle stage) for wild-type, bim1Δ, and bni1Δ cells. As expected, the bim1Δ culture showed a larger population of cells in the mid-upper region of the histogram (Fig. 1 B), representing cells that are in the medium-to-large budded stage with misoriented spindles. The distribution of bni1Δ cells was also deviant but more similar to that of wild-type cells due to a subtle spindle defect (Lee et al., 1999). Machine learning was used to classify the signal from the wild-type cells and used to rank the deviant mutants based on a significant p-value (see Materials and methods). Fig. 1 C shows the distribution of a collection of cells from two different mutant cultures: a control strain (rcr2Δ), which has normal spindle function, and dyn2Δ, a known spindle orientation mutant (Vallee et al., 2004). The budded cells examined from the mutant cultures are represented by a blue X and overlaid on the wild-type histogram. As expected, the rcr2Δ mutant fell into the wild-type category, with a p-value >0.1, whereas the dyn2Δ mutant was classified as having a spindle defect, with a p-value < 0.1.

Using a p-value cutoff of 0.1, we identified 1,962 mutants in our three screens as being defective in at least one of the three spindle-specific features (length, orientation, and distance to the bud neck). We applied a filter using 78 additional spindle-specific features, and by demanding a defect in at least 10 features, we honed our list to 745 mutants. Manual inspection confirmed obvious defects for 419/745 mutants (with a false negative rate of 44%). The known mutants that were missed using these criteria had p-values in the range of 0.1–0.5 and had significant p-values for some individual features.

To hone our list for more detailed analysis, we selected mutants with a highly penetrant nuclear positioning or delayed anaphase phenotype, the two most common defects seen in our screens. We also selected any mutants that showed a relatively rare bi-nucleation, spindle misorientation, or fish hook spindle phenotypes (Fig. 2). In this way, we selected 182 mutants from manual inspection for more detailed analysis; 92 genes were identified from screening the single mutant deletion array. We scored double mutants that enhanced spindle phenotypes relative to either the bni1Δ or bim1Δ single mutants and identified an additional 90 mutants. As expected, most mutants identified in the single mutant screen were also identified in the double mutant screens, and the bni1Δ and bim1Δ double mutant screens overlapped extensively, by 122 genes (Fig. 1 D). Analysis of gene ontology annotations revealed a clear enrichment for genes encoding proteins implicated in spindle-related processes, including genes with roles in microtubule-based processes (P = 1.021 × 10−18), tubulin folding (P = 4.701 × 10−10), cytoskeleton organization and biogenesis (P = 5.702 × 10−11), mitotic anaphase (P = 9.394 × 10−7), and chromosome segregation (P = 7.699 × 10−7), among others (Robinson et al., 2002). We also observed enrichment for genes with annotated functions in metabolism, ribosomal biogenesis, and RNA processing.
which is consistent with reported roles for nutrient signaling (Ras–cAMP pathway) and RNA-dependent protein complexes in regulating mitotic exit and mitotic spindle assembly, respectively (Morishita et al., 1995; Yoshida et al., 2003; Kittler et al., 2004; Blower et al., 2005).

To summarize the mutant phenotypes, we categorized each of the top-ranked mutants based on five major phenotypes by manual inspection (Fig. 2): (1) delayed nuclear positioning, in which the spindle fails to position close to the bud neck in medium/large budded cells; (2) delayed anaphase, in which the metaphase spindle persists in large budded cells; (3) bi-nucleated cells, where we observed two GFP dots, representing the spindle poles, within the mother of large budded cells; (4) misoriented spindle, in which the mitotic spindle is misaligned with the axis of the cell; and (5) fish hook spindle, in which the anaphase spindle is hyperelongated. Several genes with known roles in spindle dynamics were detected by our analysis, validating our approach (Fig. 2). Although our method allows rapid assessment of deletion collections and the detection of many new spindle mutants, false negatives may occur because of a cell-sampling issue, especially if the single or the double mutants show a severe fitness defect. For example, some known spindle mutants were just below the cutoff range as assessed by the p-values generated (Table S6).

**Novel candidate genes required for spindle disassembly**

We identified a relatively rare subset of mutants that exhibited fish hook spindles, including five members of the CTF19 complex (MCM21, MCM16, MCM22, CTF3, and CHL4), which is associated with the yeast kinetochore. This hyper-extended spindle phenotype resembles that of spindle disassembly mutants defective for IPL1, which encodes the Aurora B kinase (Buvelot et al., 2003), or KIP3, which encodes a kinesin that...
that activates the early meiotic-specific transcription factor, IME1 (Enyenih and Saunders, 2003). We performed further analysis on these three genes, as well as MCM21, a nonessential component of the CTF19 complex, which had not been specifically linked to spindle disassembly.

We scored the fish hook spindle phenotype in a synchronized population of cells (Fig. 3 A) and by time-lapse imaging (Fig. S2 A, yellow arrows). Analysis of the kinetics of spindle elongation in mcm21Δ, hnt3Δ, emi1Δ, and emi2Δ mutant strains (Fig. S2, B–E and G) revealed a clear delay in anaphase spindle disassembly showing a fish hook spindle with varying degrees of defect. A small percentage of hnt3Δ bni1Δ double mutant cells also showed swapping of spindle poles between the mother and

![Figure 2. Categorization of top-ranked spindle mutants identified in high-content screens.](image)

The list of mutant strains identified computationally as spindle defective is shown. Mutants were manually assessed in a secondary assay and classified into five major categories: (1) delayed nuclear positioning; (2) delayed anaphase; (3) binucleated; (4) misoriented spindle; and (5) fish hook spindle. Blue, a phenotype seen only in the indicated single deletion mutant; green, a phenotype seen in the relevant bni1Δ double deletion strain; red, a phenotype for the corresponding bim1Δ double deletion mutant. Mutants are organized according to gene ontology "process" annotations.

depolymerizes microtubules (Gupta et al., 2006). In addition, this hyperextended spindle phenotype is observed in several mitotic exit mutants as well (Toyn and Johnston, 1994; Jensen et al., 2004; Stoepel et al., 2005). We also identified three other genes that were not linked previously to spindle disassembly but whose deletion, either singly or in the context of a bni1Δ double mutant, resulted in a significant fish hook spindle phenotype (see Table S6). They were HNT3, which encodes a member of the HIT (histidine triad) superfamily of nucleotide-binding proteins, whose distant mammalian homologue, FHIT, binds microtubules (Chaudhuri et al., 1999); and EMI1 and EMI2, which were identified in our double deletion screen with BNI1 (Fig. 2) and have been implicated in control of the developmental program that activates the early meiotic-specific transcription factor, IME1 (Enyenih and Saunders, 2003). We performed further analysis on these three genes, as well as MCM21, a nonessential component of the CTF19 complex, which had not been specifically linked to spindle disassembly.

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Ipl1p-GFP association with the spindle midzone is delayed in mcm21Δ cells

As noted earlier, yeast strains carrying mutations in the gene encoding the essential Aurora B kinase, *IPL1*, exhibit fish hook spindles, which is consistent with a role for Ipl1p in anaphase spindle disassembly (Buvelot et al., 2003). The products of *IPL1* (Aurora B), *SLI15* (IN-CENP), and *BIR1* (SURVIVIN) interact to form the chromosomal passenger complex (CPC), which performs key mitotic roles through dynamic relocalization in a dividing cell (Zeng et al., 1999; Sullivan et al., 2001; Buvelot et al., 2003; Pereira and Schiebel, 2003; Ruchaud et al., 2007).

Figure 3. *hmt3Δ, mcm21Δ, emi1Δ,* and *emi2Δ* mutants have spindle elongation phenotypes and components of the CPC become partially mislocalized in mcm21Δ mutant cells. (A) Quantitation of number of cells with fish hook spindles in single and double mutant cultures. A histogram illustrating the percentage of cells in the indicated mutant cultures with fish hook spindles is shown. Cells were synchronized with α factor for 90 min, released into fresh medium, and assessed for fish hook spindles after 60 min; images of at least 100 anaphase cells for each culture were captured and quantified. Error bars show the standard deviation from three independent experiments. (B, top) Ipl1p-GFP localization to the elongating spindle and spindle midzone is disrupted in mcm21Δ mutant cells. Mid-log phase cells were treated with nocodazole for 2.5 h at room temperature and released into fresh medium before imaging. DIC images, fluorescent micrographs, and merged images are shown of representative single cells of wild-type (left) or mcm21Δ (right) strains expressing Ipl1p-GFP and RFP-Tub1p. Yellow arrowheads indicate the diffused localization of Ipl1p-GFP (for the mcm21Δ cells) and the spindle midzone. Bar, 5 µm. (B, bottom) SLI15p-GFP is mislocalized, and Bir1p-GFP localization is unaffected in mcm21Δ mutant cells. Mid-log phase cells were treated with nocodazole for 2.5 h and released into fresh medium before imaging. DIC images, fluorescent micrographs, and merged images of a representative wild-type or mcm21Δ anaphase cell expressing SL15p-GFP/Bir1p-GFP and RFP-Tub1p are shown. Yellow arrowheads indicate the localization of SL15p-GFP. (C and D) Quantitation of the defect in Ipl1p-GFP localization at the kinetochore during metaphase and the spindle midzone during anaphase in wild-type and mcm21Δ mutant cells. (C) For kinetochore localization, cells with spindles between <2 and 2–4 µm were quantified, as there was an increased mislocalization of Ipl1p-GFP/SL15p-GFP during early anaphase. (D) For midzone localization, cells with spindles 4 µm and longer were assessed. Cells with Ipl1p-GFP signal throughout the spindle were also considered as enriched for midzone in the quantitation. *n* > 60–100 cells in each spindle length category.
Given that Mcm21p functions at the yeast kinetochore, we wondered whether the fish hook spindle defect in \textit{mcm21\Delta} mutants might be associated with mislocalization of Ipl1p. As expected, in wild-type cells, Ipl1p-GFP was localized to discrete dots at the kinetochore during metaphase, along the spindle as it elongated, at the midzone of the spindle during late anaphase, and finally to the trailing end of the disassembling microtubules (Fig. 3 B). In \textit{mcm21\Delta} cells, during metaphase, Ipl1p-GFP association with the kinetochore was reduced and remained diffusely localized throughout the nucleus (Fig. 3, B and C). During late anaphase, although Ipl1p appeared properly relocated to the spindle, we observed a delay in its spindle midzone accumulation (Fig. 3, B and D). Similar results were obtained for Sli15p-GFP (Fig. 3 B, bottom left). In contrast, Bir1p-GFP localized to the anaphase spindle normally in \textit{mcm21\Delta} cells with a mild delay (Fig. 3 B, bottom right), which is consistent with previous work suggesting that CPC components may have distinct functions (Gassmann et al., 2004; Thomas and Kaplan, 2007). These findings suggest that the fish hook phenotype in \textit{mcm21\Delta} cells may be caused by the mislocalization of the CPC, particularly the failure to localize Ipl1p to the spindle midzone. Consistent with our results, Knockleby and Vogel (2009) recently reported that Sli15p association with the kinetochore was reduced in mutants lacking the COMA complex, which is composed of Mcm21p and three other members of the CTF19 complex.

**Genetic interactions involving \textit{MCM21} and late mitotic regulators**

Sli15p dephosphorylation at the kinetochore is necessary for the relocation of the CPC to the spindle midzone (Pereira and Schiebel, 2003). This reaction is mediated by the Cdc14p phosphatase, which is activated during early anaphase by the fourteen early anaphase release (FEAR) network proteins (Fig. 4 A). Although the release of Cdc14p from the nucleolus is initiated during early anaphase in a FEAR-dependent manner, maintenance of Cdc14p in the released state is controlled by the mitotic exit network (MEN) during the late stages of anaphase (Fig. 4 A; Buonomo et al., 2003; Pereira and Schiebel, 2003; Sullivan and Uhmann, 2003; D’Amours and Amon, 2004). Ultimately, Cdc14p is resquestered back into the nucleolus concomitant with spindle disassembly after degradation of the Polo kinase Cdc5p, a key factor in releasing Cdc14p from its inhibitor in the nucleolus (Visintin et al., 2008).

To examine the functional relationship between Mcm21p and the FEAR network, we first tested for genetic interactions between \textit{MCM21} and \textit{SLK19}, which encodes a signaling component of the FEAR network (Fig. 4 A; Stegmeier et al., 2002; Pereira and Schiebel, 2003). The \textit{mcm21\Delta} and \textit{slk19\Delta} mutations combined to cause a more extreme double mutant phenotype in comparison to the corresponding single mutants. The \textit{mcm21\Delta slk19\Delta} double mutant had a significant fitness defect, and \(\sim 90\%\) of the anaphase cells exhibited severely coiled fish hook spindles (Fig. 4, B and C). This double mutant analysis highlights the critical roles of both the kinetochore and Cdc14p activation in spindle disassembly.

To generalize our conclusions, we examined spindle morphology in a panel of mutants defective for genes encoding other components of the CTF19 complex, the FEAR network, and the MEN network. All the mutants we examined exhibited fish hook spindles to varying degrees (Fig. S3 A and Fig. 4 D). As previously described (Buvelot et al., 2003), we also found that mutant alleles of \textit{IPL1} (\textit{ipl1-1} and \textit{ipl1-2}) were associated with a fish hook spindle phenotype (Fig. S3 A). Our observations combined with previous findings (Pereira and Schiebel, 2003; Stegmeier and Amon, 2004; Stoepel et al., 2005) suggest a model in which MEN and FEAR pathway components activate Cdc14p, which regulates the CPC after its docking at the kinetochore in a CTF19 complex–dependent manner.

**Emi2p participates in Cdc14p activation**

Because the MEN and FEAR networks control spindle disassembly, we asked if the fish hook spindle mutants that we identified in our screens might participate in these pathways. Emi2p is a possible MEN network component because affinity purification/mass spectrometry suggested an interaction with two MEN network proteins, Dbf2p and Lte1p (Graumann et al., 2004). Emi2p encodes a candidate hexose kinase (Johnston, 1999), which suggests a potential connection between nutrient sensing and cell cycle control. To assay for a functional linkage between Emi2p and the MEN or FEAR pathway, we made emi2\Delta double mutants with deletion alleles of genes encoding either MEN or FEAR components. Double mutants combining emi2\Delta with deletion alleles of either MEN (\textit{lte1\Delta}, \textit{dbf2\Delta}) or FEAR (\textit{bns1\Delta}, \textit{spo12\Delta}, \textit{slk19\Delta}) components showed an exaggerated fish hook spindle (Fig. 4 D and Fig. S3 B). An emi2\Delta dbf2\Delta double mutant strain was inviable, providing genetic evidence that Emi2p may function in parallel with \textit{DBF2}, perhaps in the FEAR pathway (unpublished data). These genetic data, and the possible physical interaction between Emi2p and MEN components, suggest involvement of Emi2p in both the MEN and FEAR networks.

To contrast these observations, we also examined genetic conditions that activate the MEN network. Bub2p and Bfa1p are inhibitors of MEN and the subsequent release of Cdc14p, as they form a GTPase-activating complex (GAP), or an inhibitor for the Tem1p GTPase (Fesquet et al., 1999; Pereira et al., 2000; Geymonat et al., 2002; Ro et al., 2002). In the emi2\Delta \textit{bub2\Delta} double mutant, the anaphase spindles extended normally, and cells underwent mitosis like wild-type cells (Fig. 4 D and Fig. S3 B). Consistent with this finding, overexpression of \textit{MOB1}, an activating MEN component, also rescued the fish hook spindle in emi2\Delta cells (Fig. 4 D). Thus, defects in MEN and FEAR signaling accentuate the emi2\Delta mutant phenotype, whereas activation of the MEN pathway appears to suppress it. Finally, we examined Cdc14p localization in emi2\Delta cells to evaluate its role in Cdc14p release. Wild-type cells largely release Cdc14p from the nucleolus as the spindles start to elongate, and, concomitant with spindle disassembly, they resquester Cdc14p back into the nucleolus even before spindle disassembly (Fig. 4, E and F, blue arrows). Thus, like mutants in the MEN and FEAR network genes, emi2\Delta cells appear to have a defect in...
mcm21Δ
slk19Δ
slk13Δ mcm21Δ

Figure 4. Genetic interactions involving the FEAR network, MEN, and kinetochore components. (A) Diagram of key components of the FEAR network and MEN. Cdc14p is released from the nucleolus during early anaphase by the FEAR network and maintained in a released state by the MEN proteins. (B) Exacerbation of the fish hook spindle phenotype of mcm21Δ mutant cells by deletion of a FEAR network activator. Representative fluorescence micrographs of GFP-Tub1p in mcm21Δ, slk19Δ, and slk19Δ mcm21Δ cells are shown. Bar, 5 µm. (C) Quantitation of the fish hook spindle phenotype for the mutants shown in B. The plot shows the percentage of anaphase cells for the indicated mutant cultures with a clear fish hook spindle phenotype. Quantitation of the defect in an mcm21Δ mutant overexpressing MOB1 is also shown. Error bars show the standard deviation from three independent experiments counting ~100 cells for each strain. (D) Quantitation of the fish hook spindle phenotype for single and double mutants of EMI2 with FEAR and MEN components. The plot shows the percentage of anaphase cells for the indicated mutant cultures with a clear fish hook spindle phenotype. Error bars show the standard deviation from three independent experiments counting ~100 cells for each strain. (E) Quantitation of the percentage of cells with intact spindles that show premature resequestration of Cdc14p-GFP into the nucleolus. Cells were synchronized using α factor, released into fresh medium, and monitored using live-cell imaging every 15 min for 180 min. RFP-Tub1p was used to monitor the spindle and identify the cell cycle stage. Error bars show the standard deviation from three independent experiments counting ~80 cells for each strain in all the time points. (F) Release of Cdc14p-GFP in mcm21Δ, emi2Δ, and dbf2Δ mutant cells. Cells were synchronized using α factor, released into fresh medium, and monitored using live-cell imaging every 15 min for 180 min. RFP-Tub1p was used to monitor the spindle and identify the cell cycle stage. Representative wild-type, emi2Δ, mcm21Δ, and dbf2Δ mutant cells in late anaphase are shown. Blue arrows indicate the premature sequestration of Cdc14p to the nucleolus before spindle disassembly. Note that unlike emi2Δ and dbf2Δ cells, mcm21Δ cells maintained the released state of Cdc14p even when the spindle was hyperelongated. Bar, 5 µm.

maintaining Cdc14p in an active released state, which suggests that Emi2p may modulate one of these pathways.

Sumoylation of Mcm21p targets the CPC specifically to the spindle midzone
In addition to the nonessential gene set, we also screened a collection of temperature-sensitive mutants covering ~100 essential genes for defects in spindle morphology (Table S7). By screening this collection, we identified a spindle disassembly defect (fish hook spindle) associated with a mutant allele of MMS21 (Fig. S3 A), which encodes one of the known E3 SUMO ligases (Zhao and Blobel, 2005). We were intrigued by this observation because recent work from our laboratory identified genetic interactions between MCM21 and genes encoding SUMO pathway components, which suggested a link between sumoylation and Mcm21p function (Makhnevych et al., 2009). Mcm21p is the only protein in the CTF19 complex that is known to be sumoylated (Panse et al., 2004; Wohlschlegel et al., 2004; Zhou et al., 2004; Denison et al., 2005; Hannich et al., 2005; Wykoff and O’Shea, 2005), raising the possibility that sumoylation may...
regulate CPC localization and that the sumoylation of Mcm21p may be regulated by the E3 ligase MMS21. Consistent with this idea, two observations suggested that factors in addition to Cdc14p influenced CPC localization in the mcm21Δ mutant. First, unlike emi2Δ cells, we found that the kinetics of Cdc14p localization were unaffected in mcm21Δ cells, yet Ipl1p-GFP failed to properly localize to the spindle (Fig. 4 F and Fig. 3 B). Second, hyperactivation of the Cdc14p signaling pathway through overexpression of a MEN component, MOB1 (Komarnitsky et al., 1998), resulted in only partial rescue of either the Ipl1p-GFP mislocalization phenotype or the fish hook spindle phenotype (Fig. 4 C and Fig. S3, C and D). These findings indicate that signaling from Cdc14p alone is not sufficient to target the CPC to the spindle midzone, which suggests that Mcm21p might play additional roles at the kinetochore.

We explored this idea by first assessing Mcm21p and its closely associated CTF19 complex proteins Okp1p, Amel1p, and Ctf19p (the COMA subcomplex), and confirmed that only Mcm21p was detectably sumoylated in our assay (Fig. 5 A). To dissect the role of Mcm21p sumoylation in spindle function, we first mutated the best matches to the consensus site for sumoylation in Mcm21p (Jeram et al., 2009), and observed no significant effect on Mcm21p sumoylation (unpublished data). Because >40% of the published yeast SUMO conjugation sites occur at nonconsensus lysine residues (Jeram et al., 2009), we next synthesized a sumoylation-deficient allele of MCM21, mcm21-32R. We made mutations that changed all 32 Mcm21p lysine residues to arginine (Mcm21p-32R), and we examined the functionality of the Mcm21p-32R mutant in three different ways. First, we found that the Mcm21p-32R mutant was expressed at normal levels but was not sumoylated (Fig. 5 A). Second, when expressed as a GFP chimera, Mcm21p-32R localized normally to the kinetochore (Fig. 5 B). Third, tetrad dissection revealed that expression of MCM21-32R rescued the synthetic lethality of a mcm21Δ mad1Δ double mutant strain (unpublished data).

Unlike in the mcm21Δ deletion mutant, the kinetochore association of Ipl1p-GFP and Sli15p-GFP was not affected during metaphase in the mcm21-32R mutant, which suggests
that Mcm21p-32R still retains the scaffolding function associated with wild-type Mcm21p at the kinetochore (Fig. 5, C–F). Nevertheless, like the mcm21Δ deletion mutant, the mcm21-32R mutant was defective for targeting Ipl1p-GFP and Sli15p-GFP to the spindle midzone, resulting in the formation of extended fish hook spindles (Fig. 6, A–D). Wild-type cells were able to target Ipl1p-GFP and Sli15p-GFP to the midzone, such that the spindle reached a maximum length of 6–8 µm before disassembly, whereas the mcm21Δ and the mcm21-32R mutant cells were defective in this localization and showed an extended fish hook spindle that often grew beyond 9 µm (Fig. 6, A–D). Invariably, every cell that formed a fish hook spindle did not localize Ipl1p-GFP to the midzone in both the mcm21Δ deletion mutant and the mcm21-32R mutant, which suggests that sumoylation of Mcm21p may signal the efficient relocation of the CPC to the spindle midzone.

To further assess the role of sumoylation in CPC localization, we examined a ubc9-2 temperature-sensitive mutant defective for the SUMO E2 ligase. Sumoylation is involved in many cellular processes (Melchior, 2000; Müller et al., 2001; Seeler and Dejean, 2003), and most ubc9-2 mutant cells arrest in metaphase and early anaphase (Fig. 6 E), precluding analysis of the fish hook spindle phenotype. Nonetheless, the large budded ubc9-2 cells clearly failed to efficiently localize both Ipl1p-GFP and Sli15p-GFP to the spindle at the restrictive temperature (Fig. 6, E and F). After release from the restrictive temperature,
Ipl1p-GFP rapidly enriched along the spindles and specifically concentrated at the spindle midzone as expected (Fig. 6E). Thus, sumoylation of Mcm21p appears to play a specific role in targeting the CPC to the spindle midzone.

Discussion

Integrating SGA analysis and HCS provides a general strategy for quantitative assessment of cell biological phenotypes on a genome-wide scale in sensitized genetic backgrounds. Automation and machine learning techniques are required to scale this analysis to multiple pathways, enabling objective, quantitative, and highly reproducible determination of detailed morphological phenotypes. Although the deletion collection has been assessed for morphological phenotypes through systematic analysis of fixed cells (Ohya et al., 2005), our study represents the first fully automated genome-scale mutant screen tracking a dynamic intracellular event. An unbiased quantitative readout allows for the identification of subtle but significant phenotypes, offering the potential to confirm and expand genetic networks based solely on fitness measurements. For example, with a BIM1 query mutation and combined SGA-HCS system, we scored 45 genes (~50%) previously identified as BIM1 synthetic lethal–sick interactions (Tong et al., 2004), but we also identified 122 novel genes involved in spindle function. A limitation of our system is that true synthetic lethal double mutants fail to divide and cannot be examined by HCS (Fig. S4A); however, this problem can be overcome if the double mutant is made conditional, as through the use of a temperature-sensitive allele of one member of the gene pair (Xu et al., 2007).

Morphological mutants, networks, and complexes

We compared our phenotypic profiling results to genetic and protein–protein interaction datasets, and found that mutant strains that had a high phenotypic deviation from wild type were more likely to be highly connected in the existing synthetic lethal genetic and protein–protein interaction networks (Fig. S4, B and C). Thus, an accurate assessment of phenotypic deviance from wild type may allow us to infer participation of proteins or genes in network hubs (Rutherford and Lindquist, 1998). The occurrence of a specific phenotypic change in response to a particular mutation was also correlated for proteins within the same complex (Fig. S4D). For example, we observed that members of the CTF19 complex exhibit a similar fish hook spindle phenotype, as do components of the FEAR and MEN pathways (Fig. S3A).

Spindle disassembly pathway

Our large-scale analysis of mutants displaying a fish hook spindle combined with previous observations elaborates a general model of spindle disassembly in yeast (Fig. 7; Pereira and Schiebel, 2003; Stegmeier and Amon, 2004; Stoepel et al., 2005). In particular, our study established a new role for sumoylation at the kinetochore associated with the CTF19 complex and modulation of spindle disassembly. We suggest that Mcm21p along with the members of the CTF19 complex may act as a scaffold for the CPC. Specifically, in cooperation with Cdc14p, sumoylation of Mmc21p may increase the efficiency of timed localization of the CPC to the spindle midzone for proper disassembly of the spindle.

Figure 7.  Model of mitotic exit and spindle disassembly. Major regulators of the CPC during mitosis are illustrated. For simplicity, MEN and FEAR pathway components are not shown. FEAR activates the partial release of Cdc14p during early anaphase, and MEN activates the complete release of Cdc14p during late anaphase. Cdc14p release from the nucleolus and sumoylation of Mm21p at the kinetochore seem to be two important signals that regulate the dynamic relocalization of the CPC components to the spindle midzone from the kinetochore. We suggest that Mmc21p along with the members of the CTF19 complex may act as a scaffold for the CPC. Specifically, in cooperation with Cdc14p, sumoylation of Mmm21p may increase the efficiency of timed localization of the CPC to the spindle midzone for proper disassembly of the spindle. 
Materials and methods

Automated image acquisition

A modification of the SGA method (Tong et al., 2001; Sopko et al., 2006) was used to introduce the GFP-αTub1p-expressing plasmid and the deletion allele of BNL1 into the deletion array. A strain harboring bnl1Δ (Table S2) was transformed with a GFP-αTub1p plasmid and mated to the yeast deletion array (Gioever et al., 2002) by replica pinning. Diploids were selected and sporulated using standard SGA selections (Tong et al., 2001). Mather bnl1Δ geneXΔ haploids expressing GFP-αTub1p were isolated through successive pinning onto selective media and then into liquid selection medium for overnight growth. To optimize cell density for image analysis, we used liquid-handling robots (BioMek FX Laboratory automation work station; Beckman Coulter) to dispense sample volumes based on the optical density of each strain (SpectraMax Plus 384 Microplate Spectrophotometer; MDS Analytical Technologies). Plates were imaged in 96-well format using glass-bottomed plates (MMI Greiner M plates), and each plate contained control strains at 19 positions amounting to 1,064 combinations of cell morphology (Tables S3–S5; cataloged measurements, 240 of which were attributed to cell morphology and 81 attributed to spindle morphology [Tables S3–S5]). Our measurements included description of cell shape, a feature that can serve as a proxy for cell cycle position (unbudded, small budded, medium budded, and large budded cells), characteristics of spindle pole bodies, and the mitotic spindle. The cataloged measurements in Tables S3–S5 are a mean of all the values measured in each mutant.

Data analysis

To identify mutants whose morphological profiles differed from wild type, we used a mixture of Gaussian models to learn the probability density function of the control based on the four features (Bishop, 2006). The motherdaughter cell size ratio is used in the analysis as an indication of cell cycle stage (Byers and Goetsch, 1975; Kilmartin and Adams, 1984). Cross-validation revealed that 60 Gaussians was optimal for this data set. Mutant strains were evaluated by computing the likelihood under this learned model. A low likelihood indicates that the cells of the mutant strain exhibit different phenotypes noticeably different than that of the control. Because the number of identified and measured cells differs for each strain, the likelihoods must be normalized to compare the different strains as well as provide a measure of statistical significance. So for a strain with a sample size of n cells, a random sample of n cells from the mixture of Gaussians was drawn, and the likelihood of these randomly drawn cells was computed. This sampling procedure was conducted 2,000 times, and the likelihood of the mutant cells was compared with the likelihood of the random samples. A p-value for the mutant phenotype. The same procedure was applied to other measurements to find mutant strains for different phenotypes as well as for double knockout experiments.

To assess the correlation between gene connectivity and phenotype, we downloaded all available synthetic lethal interaction data from BioGrid (http://www.thebiogrid.org/) and protein–protein interaction data from the Database of Interacting Proteins (http://dip.doe-mbi.ucla.edu/). We identified the top 100 highly connected genes and the bottom 100 least-connected genes in each of these datasets. The mean phenotypic change and the 95% confidence interval of the mean are derived from 5,000 bootstrap resamplings. P-values were derived using a Mann-Whitney U test. Fig. 2B and C shows the mean phenotypic alterations in these gene datasets. Mean phenotypic variation is defined as the mean number of altered morphometric measurements for each gene, we counted the number of altered phenotypic features for both the single deletion and the double deletion. To determine whether or not the loss of proteins in the same complex tends to cause similar morphology profiles, we compared phenotypic correlation within complexes against random controls. A total of ~274 literature-curated protein complexes and a similar set of randomly generated protein complexes were examined for the correlation of their morphological profile. Phenotypic correlation is defined as the mean pairwise correlation among complex subunits. We evaluated phenotypic correlation upon deletion of genes involved in the same protein complexes. In brief, we calculated pairwise Pearson’s correlation for genes involved in protein complexes, and mean pairwise correlation was then compared against a set of random parameter estimates.
controls in which complex memberships were randomly shuffled. Fig. S4 D shows the correlation between true complexes and random complexes.

Confocal microscopy and image quantitation

Images were captured using a spinning disc confocal system (WaveFX, Quorum) with an ultra-cooled S12 back-tinned EM charge-coupled device camera, or a microscope (E-600FN; Nikon) with an Orca camera (Hamamatsu). Images were acquired with 63× HCX Plan-Apochromat oil objective with an NA of 1.4. Images were captured at room temperature after loading the cells on gelatin pads as described previously (Jones et al., 1993; Yeh et al., 1995). Stacks of 11 optical sections spaced 0.3 µm apart or 5 optical planes spaced 0.2 µm apart were captured every 1 min. GFP was excited using a 488-nm laser, and its emission was collected using a 505-nm long-pass filter. No further processing was needed, as raw images clearly showed the phenotype. The 2-axis images were converted to a single composite image by using the brightest pixel at every position in each of the image planes. This maximum pixel projection technique produced a 2D representation of the GFP fusion proteins within the cell from the 3D dataset. A macro written with the MetaMorph scripting language was used to input the entire stack of background-subtracted images and output a new stack of only the projected images, and to measure the spindle length in every z-stack plane.

Detection of SUMO conjugates and Western blot analysis

The mcm21-32R mutant was made by gene synthesis (DNA2.0, Inc.). The mcm21-32R and its associated promotor (500 bp), with a C-terminal tandem affinity purification (TAP) tag (543 bp) and downstream of 90 features for single mutants. Table S4 shows mean values of 90 features for double deletion with 50 features for single mutants. Fig. S1 shows steps involved in automated image analysis. Fig. S2 shows representative micrographs of fish hook spindle mutants. Fig. S4 shows correlation of the phenotypic changes in single mutant and bni1 or bim1 double mutant strains. Table S2 describes the features of the other datasets. Table S5 lists the features measured. Table S2 shows strains and plasmids used in this study. Table S3 shows mean values of 90 features for double deletion with bni1/1. Table S5 shows mean values of 90 features for double deletion with bim1/1. Table S6 shows pvalues generated for single mutants and bni1 and bim1 double mutants on three spindle specific features. Table S7 lists essential genes analyzed for spindle defect by HCS. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.200909013/DC1.

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