

Altered dosage of the *Saccharomyces cerevisiae* spindle pole body duplication gene, *NDC1*, leads to aneuploidy and polyploidy

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ABSTRACT *Saccharomyces cerevisiae* cells are exquisitely sensitive to altered dosage of the spindle pole body duplication gene, *NDC1*. We show that the *NDC1* locus is haploinsufficient because diploid yeast cells cannot survive with a single chromosomal copy of the *NDC1* gene. Diploid cells with a single copy of *NDC1* can survive by gaining an extra copy of the *NDC1*-containing chromosome. *NDC1* haploinsufficiency is a dominant loss-of-function phenotype that leads to aneuploidy. Furthermore, we report that overexpression of *NDC1* leads to spindle pole body duplication defects indistinguishable from those observed in *ndc1-1* mutant cells. Cells overexpressing *NDC1* arrest with monopolar spindles and exhibit increase-in-ploidy phenotypes. Thus, both increased and decreased *NDC1* dosage can lead to aneuploidy. The striking sensitivity of yeast cells to changes in *NDC1* gene dosage suggests a model for the behavior of some tumor suppressor genes and oncogenes in which loss-of-function mutations and overexpression, respectively, lead to increased genetic instability.

Many different events can lead to aneuploidy, including multipolar mitosis, monopolar mitosis, and nondisjunction of chromosomes during mitosis. Until recently, aneuploidy has been considered a consequence, rather than a cause, of cellular transformation. However, recent studies of chemically transformed cells (1), colorectal cancer cells (2, 3), and papillary renal carcinomas (4) suggest that aneuploidy can cause cancer.

Centrosomes play a critical role in the maintenance of genomic integrity. A study of centrosome morphology in human breast tumor cells reveals a compelling correlation between aberrant centrosome structure and aneuploidy in advanced tumors (5). A direct link between abnormal centrosome number, aneuploidy, and cellular transformation was revealed by studies of *aurora2*/*STK15*/*BTAK*, a centrosome-associated protein kinase (6, 7). It is amplified in many human tumor cells, and overexpression of *aurora2* in nontumor cells leads to increased centrosome number, aneuploidy, and tumorigenesis (6, 7). This suggests that an oncogene may induce cellular transformation by perturbing centrosome function.

In the budding yeast *S. cerevisiae*, the spindle pole body (SPB) functions as the centrosome-equivalent organelle. In early G₁, yeast cells contain a single SPB that must be duplicated precisely for chromosome segregation to occur properly. Mutations that disrupt SPB duplication lead to aneuploidy and polyploidy because of monopolar mitosis (reviewed in ref. 8).

The *NDC1* (nuclear division cycle) gene (9) is required for a late step in SPB duplication. Although SPB duplication is initiated in *ndc1-1* strains at the nonpermissive temperature, the newly synthesized SPB is not inserted into the nuclear envelope (10). All of the chromosomes remain associated with

the preexisting, functional SPB in these cells. In response to their monopolar spindles, *ndc1-1* cells arrest in mitosis because they activate the mitotic spindle assembly checkpoint (11). Eventually, the cells break through this mitotic arrest and all of their chromosomes segregate with the single, functional SPB (9). As a result, one cell doubles in ploidy and the other cell lacks chromosomal DNA. *NDC1* encodes an essential 74-kDa protein with six to seven predicted transmembrane domains (10). Ndc1p is a shared component of SPBs and nuclear pore complexes (NPCs) (12).

We report that the *NDC1* gene establishes a connection between gene dosage, SPB duplication, and genetic stability. Yeast cells are sensitive to both increased and decreased *NDC1* dosage, often resulting in aneuploidy and polyploidy. This study has led to insights concerning the mechanisms by which yeast cells can exhibit aneuploidy and polyploidy and has also demonstrated how specific types of aneuploidy can allow cells to survive under conditions that are normally lethal.

MATERIALS AND METHODS

Yeast Strains and Media. Yeast strains are listed in Table 1 and were constructed by using standard techniques (13). The *ndc1Δ::HIS3* allele was constructed by using a one-step gene-replacement technique (14). The *ndc1Δ::KANMX* allele was constructed by using a two-step gene-replacement technique (15) to replace the entire *NDC1* ORF with the *KANMX* gene (16). Yeast strains in which the *NDC1* locus contained two linked genetic markers, *TRP1* and *KANMX*, were constructed by transformation of a wild-type yeast strain with a *TRP1* integrative plasmid containing the *ndc1Δ::KANMX* allele (pRS304-*ndc1Δ::KANMX*, see paragraph on plasmids). The yeast strain containing *GAL-NDC1-3xpk* at the *LEU2* locus [HC14-10c(1235); see Table 1] was constructed by transformation of a wild-type yeast strain with a *LEU2* integrative vector containing *GAL-NDC1-3xpk* (pRS305-*GAL-NDC1-3xpk*; see paragraph on plasmids). The yeast strain that contained both *GAL-NDC1-3xpk* and *SPC42-GFP* was constructed by crossing yeast strain HC14-10c(1235) to yeast strain IAY18 (a kind gift from I. Adams and J. Kilmartin, MRC Laboratory of Molecular Biology, Cambridge, UK) that contains *spc42Δ::LEU2* and *TRP1::SPC42-GFP(3x)* (17).

All yeast strains were grown as described, in YPD (1% yeast extract/2% bacto-peptone/2% glucose), YPR (1% yeast extract/2% bacto-peptone/3% raffinose), or synthetic medium supplemented with the appropriate amino acids and 2% glucose. For galactose induction, we used YPR containing 2% galactose. Yeast plates containing 5-fluoroorotic acid (5-

This paper was submitted directly (Track II) to the *Proceedings* office. Abbreviations: SPB, spindle pole body; NDC, nuclear division cycle; NPC, nuclear pore complex; 5-FOA, 5-fluoroorotic acid; GFP, green fluorescent protein.

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Table 1. Yeast strains

Yeast strain	Genotype
HC14-10c/5d	<i>MATa/MATα ura3-52/ura3-52 his3Δ200/his3Δ200 trp1Δ63/TRP1 leu2Δ1/leu2Δ1 LYS2/lys2-801 adeΔ426/ade2Δ426 ade3Δ/ade3Δ</i>
HC14-10c/HC5-8c(799)	<i>MATa/MATα NDC1/ndc1-1 ura3-52/ura3-52 his3Δ200/his3Δ200 trp1Δ63/TRP1 leu2Δ1/leu2Δ1 LYS2/lys2-801 ade2Δ426/ade2Δ426 ade3Δ/ade3Δ (pALR10-NDC1)</i>
HC7-31c(1134)/HC14-5d	<i>MATa/MATα ndc1Δ::HIS3/NDC1 ura3-52/ura3-52 his3Δ200/his3Δ200 trp1Δ63/TRP1 leu2Δ1/leu2Δ1 LYS2/lys2-801 ade2Δ426/ade2Δ426 ade3Δ/ade3Δ (pALR10-NDC1)</i>
HC7-31c(1134)/HC5-8c	<i>MATa/MATα ndc1Δ::HIS3/ndc1-1 ura3-52/ura3-52 his3Δ200/his3Δ200 trp1Δ63/TRP1 leu2Δ1/leu2Δ1 LYS2/lys2-801 ade2Δ426/ade2Δ426 ade3Δ/ade3Δ (pALR10-NDC1)</i>
HC14-10c/5d(1197)	<i>MATa/MATα NDC1/ndc1::ΔKANMX ura3-52/ura3-52 his3Δ200/his3Δ200 trp1Δ63/TRP1 leu2Δ1/leu2Δ1 LYS2/lys2-801 ade2Δ426/ade2Δ426 ade3Δ/ade3Δ (pALR10-NDC1)</i>
HC14-10c(1405)/HC29-6b	<i>MATa/MATα NDC1::TRP1::ndc1Δ::KANMX/ndc1Δ::HIS3 ura3-52/ura3-52 his3Δ200/his3Δ200 trp1Δ63/TRP1 leu2Δ1/leu2Δ1 LYS2/lys2-801 ade2Δ426/ade2Δ426 ade3Δ/ade3Δ (pALR10-NDC1)</i>
HC14-10c(1404)/HC29-6b	<i>MATa/MATα NDC1::TRP1::ndc1Δ::KANMX/ndc1Δ::HIS3 ura3-52/ura3-52 his3Δ200/his3Δ200 trp1Δ63/TRP1 leu2Δ1/leu2Δ1 LYS2/lys2-801 ade2Δ426/ade2Δ426 ade3Δ/ade3Δ (pALR10-NDC1)</i>
HC14-10c(1405)/HC29-8a	<i>MATa/MATα NDC1::TRP1::ndc1Δ::KANMX/ndc1Δ::HIS3 ura3-52/ura3-52 his3Δ200/his3Δ200 trp1Δ63/TRP1 leu2Δ1/leu2Δ1 LYS2/lys2-801 ade2Δ426/ade2Δ426 ade3Δ/ade3Δ (pALR10-NDC1)</i>
HC-14c(1404)/HC29-8a	<i>MATa/MATα NDC1::TRP1::ndc1Δ::KANMX/ndc1Δ::HIS3 ura3-52/ura3-52 his3Δ200/his3Δ200 trp1Δ63/TRP1 leu2Δ1/leu2Δ1 LYS2/lys2-801 ade2Δ426/ade2Δ426 ade3Δ/ade3Δ (pALR10-NDC1)</i>
WX257-2d	<i>MATa ura3-52 his3Δ200 leu2-3,112</i>
WX257-4c	<i>MATα ura3-52 his3Δ200 leu2-3,112</i>
HC14-10c	<i>MATa ura3-52 his3Δ200 trp1Δ63 leu2Δ1 ade2Δ426 ade3Δ</i>
HC14-5d	<i>MATα ura3-52 his3Δ200 leu2Δ1 lys2-801 ade2Δ426 ade3Δ</i>
HC14-10c(1235)	<i>MATa leu2Δ1::GAL-NDC1-3xpk::LEU2 ura3-52 his3Δ200 trp1Δ63 ade2Δ426 ade3Δ</i>
HC44-2a	<i>MATa leu2Δ1::GAL-NDC1-3xpk::LEU2 TRP1::SPC42-GFP(3x) ura3-52 his3Δ200 lys2-801 ade2Δ426 ade3Δ</i>

FOA) or geneticin (G418) were prepared as in refs. 16 and 18. Cells were arrested in G₁ by using α -factor (Macromolecular Resources, Fort Collins, CO) at a concentration of 11 μ g/ml.

Diploid strains that are heterozygous for the *ndc1* null allele and contain a plasmid-borne copy of *NDC1* with the *URA3* marker [HC14-10c(1405)/HC29-6b, HC14-10c(1404)/HC29-6b, HC14-10c(1405)/HC29-8a, and HC14-10c(1404)/HC29-8a; see Table 1] were used to determine the frequency of survivors of *NDC1* haploinsufficiency. Various dilutions of overnight cultures were plated to 5-FOA-containing plates to isolate haploinsufficiency survivors and to YPD control plates. The number of cells plated on the 5-FOA-containing plates was normalized by using the YPD control plates, and a frequency of survival was determined. Similar results were obtained in two independent experiments by using all four strains.

Plasmids. DNA was manipulated by using standard techniques as described in ref. 19. A subclone of the *NDC1* ORF was made by cloning a 2.8-kb *AgeI-SacI* *NDC1* fragment into the *XbaI/SmaI* restriction sites of the pRS315 vector (20). The pALR10-NDC1 plasmid is described in ref. 12. The 2- μ m *LEU2* vector containing *NDC1* (pRS425-NDC1) was constructed by cloning a *XhoI-SpeI* *NDC1* fragment into *XhoI/SpeI*-digested pRS425 (21). The pRS304-ndc1 Δ ::KANMX plasmid is a derivative of pRS315-NDC1 wherein the *NDC1* ORF has been replaced with the *KANMX* gene (16). The 2- μ m *URA3* vectors containing either *NDC1* or *NDC1-3xmyc* (pRS426-NDC1 and pRS426-NDC1-3xmyc, respectively) were made by cloning a *XhoI-SpeI* fragment containing either *NDC1* or *NDC1-3xmyc*, respectively, into *XhoI/SpeI*-digested pRS426 (21). The pRS305-GAL-NDC1-3xpk plasmid was made by using a modified pRS305 vector (20) that contains the *GAL1-10* promoter [derived from the pBM272 vector (22)] cloned into the *EcoRI* and *BamHI* sites. A *BamHI* site was introduced at the start of the *NDC1* ORF, and a *BamHI-SpeI* fragment was cloned into the *BamHI* and *SpeI* sites in this modified vector. The 3xpk epitope tag (23) was introduced as a *XbaI* fragment into an *AvrII* linker engineered at the 3' end of *NDC1*.

Cytological Techniques. Indirect immunofluorescence microscopy was carried out as described in ref. 12, except the cells

were fixed for 45 min and were not extracted with methanol/acetone. The primary antibody was YOL1/34 (Accurate Chemicals; diluted 1:150 in blocker), and the secondary antibody was Texas Red-conjugated donkey anti-rat IgGs (The Jackson Laboratory; diluted 1:75 in blocker). Deconvolution microscopy was carried out as in ref. 12. Immunoelectron microscopy analysis was carried out as in ref. 12. Thin sections were labeled with an anti-pk mouse monoclonal antiserum (a kind gift from I. Hagan, University of Manchester, Manchester, UK) diluted 1:1 and goat anti-mouse 15-nm colloidal gold conjugate (Ted Pella, Redding, CA) diluted 1:20. Flow cytometry was carried out as in ref. 24 by using propidium iodide to stain DNA (Sigma). Stained cells were analyzed by using a Becton-Dickinson FACScan flow cytometer and the CELL QUEST software package for data analysis.

RESULTS

The *NDC1* Locus Is Haploinsufficient. Early studies of the *S. cerevisiae* *NDC1* gene suggested that this locus might be haploinsufficient. It was difficult to construct a diploid strain that was heterozygous for a disruption allele of *NDC1*. We analyzed this phenotype and found that the *NDC1* locus is indeed haploinsufficient. We tested whether diploid strains heterozygous for a *ndc1* null allele require a plasmid-borne copy of *NDC1* for viability. The *NDC1* plasmid also contained the *URA3* and *ADE3* markers, allowing plasmid dependency to be monitored by a failure to grow in the presence of 5-FOA because of the *URA3* marker (18) and by a failure to sector because of the *ADE3* marker (25). Using both criteria, diploid yeast strains could not survive with a single chromosomal copy of *NDC1* (Fig. 1). This plasmid dependency was due to a specific requirement for *NDC1*, because the 5-FOA sensitive phenotype (data not shown) and the nonsectoring phenotype (Fig. 1B) were both suppressed by introducing a second plasmid-borne copy of *NDC1*. This also suggests that *NDC1* haploinsufficiency is due to lowered *NDC1* expression, rather than a perturbation of chromatin structure at the *NDC1* locus.

Characterization of *NDC1* Haploinsufficiency Survival Mechanisms. After extended growth in the presence of 5-FOA to induce loss of the *NDC1*-containing plasmid, strains het-

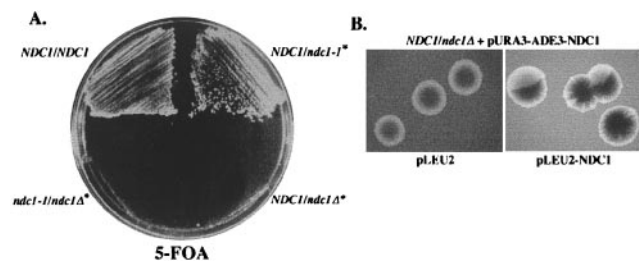
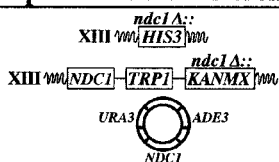


Fig. 1. The *NDC1* locus is haploinsufficient. (A) Diploid yeast strains that were either *NDC1/NDC1* [HC14–10c/5d; see Table 1], *NDC1/ndc1-1* [HC14–10c/HC5–8c(799)], *NDC1/ndc1Δ* [HC7–31c(1134)/HC14–5d], or *ndc1-1/ndc1Δ* [HC7–31c(1134)/HC5–8c] were streaked to 5-FOA-containing plates to test whether they are able to lose a plasmid-borne copy of *NDC1* (pURA3-ADE3-*NDC1*; pALR10-*NDC1*) at 30°C. Asterisks indicate strains that initially contained the plasmid-borne copy of *NDC1*. (B) A *NDC1/ndc1Δ* yeast strain [HC14–10c/5d(1197); see Table 1] containing pALR10-*NDC1* (pURA3-ADE3-*NDC1*) was transformed with either a *LEU2* vector (pLEU2; pRS425) or with the *LEU2* vector containing *NDC1* (pLEU2-*NDC1*; pRS425-*NDC1*), and the resulting strains were used in a plasmid-sectoring assay. Solid, red colonies indicate a requirement for the plasmid-borne copy of *NDC1*, whereas sectoring colonies indicate that the cells can lose the *NDC1*-containing plasmid.

erozygous for the *ndc1* null allele produced “survivor” colonies. The frequency of survival was approximately 1 in 500 cells plated (see *Materials and Methods*). These yeast strains contain a complete deletion of the *NDC1* ORF that is replaced with *HIS3*, so simple reversion is not the mechanism of generating survivors. We reasoned that the cells could use a number of mechanisms to survive, including aneuploidy, mitotic recombination, an extragenic suppressor mutation, or mutation of the *URA3* marker on the plasmid (Fig. 2 and see below).

We constructed diploid strains that were heterozygous for the *ndc1* null allele in which both copies of chromosome XIII contained specific genetic markers linked to the *NDC1* loci.

NDC1 Haploinsufficient Parent Strain:



Survivors:

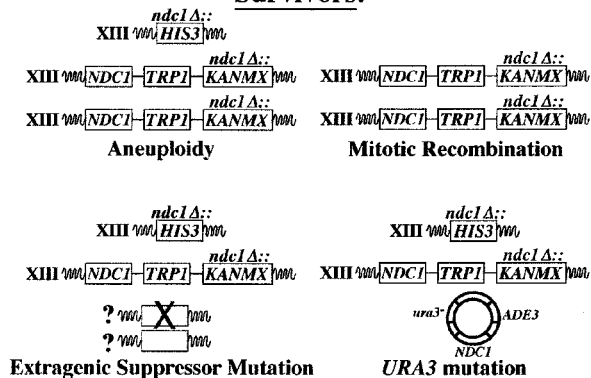


Fig. 2. *NDC1* haploinsufficiency survival mechanisms. Schematic of the mechanisms by which diploid strains that are heterozygous for the *ndc1* null allele survive in the absence of a plasmid-borne copy of *NDC1*. The starting yeast strain (Haploinsufficient Parent Yeast Strain) has both copies of chromosome XIII, where *NDC1* resides, marked with specific genes corresponding to the *ndc1* null-containing chromosome (*HIS3*) or the *NDC1*-containing chromosome (*TRP1* and *KANMX*). This yeast strain also contains a plasmid-borne copy of *NDC1* with the *URA3* and *ADE3* markers.

The *NDC1* allele was marked by using both *TRP1* and *KANMX* (G418 resistance), and the *ndc1* null allele was marked by using *HIS3* (Fig. 2). These strains containing a plasmid-borne copy of *NDC1* were streaked to 5-FOA-containing plates to select cells that lost the plasmid-borne *URA3* gene. We selected five individual survivors and examined their meiotic products ($n = 20$ tetrads). The segregation of the *NDC1*-linked genetic markers, ploidy of survivors and spore clones, and PCR confirmation of markers all were used to determine the possible mechanism(s) used for surviving the *NDC1* haploinsufficient phenotype.

The survivors used all of the suggested mechanisms, individually or in combinations, to overcome the *NDC1* haploinsufficient phenotype. Three survivors were aneuploid, because a number of their spore clones contained both the *NDC1* chromosome and the *ndc1* null chromosome. One of the survivors had undergone mitotic recombination to replace the *ndc1* null allele with the *NDC1* allele. Another survivor appeared to have acquired a suppressor mutation that, in addition to its ability to suppress the *NDC1* haploinsufficient phenotype, also functioned as a weak bypass suppressor of the *ndc1* null allele; some slow-growing spore clones derived from this survivor did not contain the *NDC1* gene, and flow cytometry showed that they exhibited a variety of DNA contents (data not shown). This survivor also exhibited aneuploidy for the *NDC1*-containing chromosome. Finally, we identified survivors that mutated the *URA3* marker carried on the *NDC1*-containing plasmid. None of the survivors corresponded to diploid cells that were heterozygous for the *ndc1* null allele. Furthermore, heterogeneity in the ploidy of spore clones indicated that these cells continue to display genetic instability as they are cultured (data not shown).

Moderate Constitutive Overexpression of *NDC1* Causes Haploid Cells to Increase in Ploidy. *NDC1* was moderately overexpressed (5 to 10 times) in cells by transforming them with a 2- μ m plasmid containing either *NDC1* or *NDC1* tagged with three repeats of the myc epitope. When haploid cells were transformed with the 2- μ m vector alone, all of the transformants exhibited a stable haploid DNA content as determined by flow cytometry ($n = 33$) (Fig. 3). However, when haploid cells were transformed with the 2- μ m vector containing *NDC1*, only 2% of the transformants exhibited a typical haploid DNA content ($n = 112$); 91% of the transformants showed a mixed ploidy population of typical haploid and diploid DNA peaks, and 7% exhibited a diploid DNA content (Fig. 3). Although increased *NDC1* dosage caused cells to increase in ploidy, it did not appear to affect viability.

Induced *NDC1* Overexpression in Synchronized Cells Leads to Monopolar Spindle Phenotypes. To study overexpression phenotypes further, we used a haploid strain containing *NDC1* with three repeats of the pk epitope at its COOH terminus expressed under the control of the *GAL1*-inducible promoter (*GAL-NDC1-3xpk*) integrated at the *LEU2* chromosomal locus. This tagged allele of *NDC1* is fully functional when expressed by using the endogenous *NDC1* promoter (data not shown). This yeast strain still contains the endogenous chromosomal copy of *NDC1*. *NDC1-3xpk* was overexpressed in synchronized cells to examine phenotypes in the first SPB duplication cycle. Cells were synchronized in G_1 by using α -factor (see *Materials and Methods*) before the *NDC1* execution point (10). Cells were held at the arrest for 1.5 hr to induce expression of *NDC1-3xpk* and then were released into the cell cycle to determine the effect of excess Ndc1p on SPB duplication.

Cells from the uninduced culture released synchronously from the α -factor arrest and eventually became asynchronous (Fig. 4A Left). Cells overexpressing *NDC1-3xpk* released synchronously from the α -factor arrest, but then arrested with a G_2 DNA content in the first cell cycle (Fig. 4A Right). The arrested cells were large-budded (Fig. 4B), contained a single

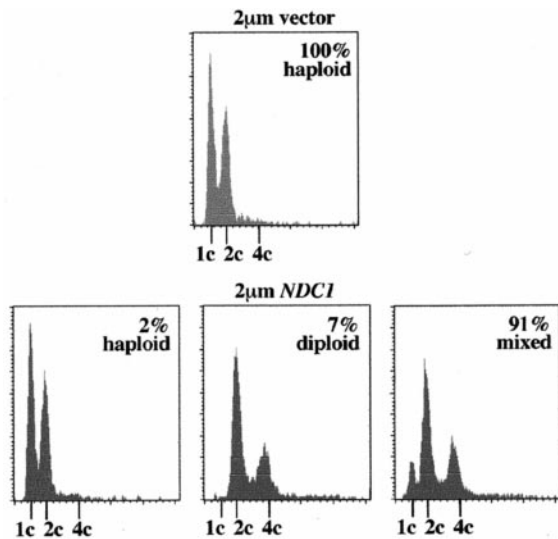


FIG. 3. Mild overexpression of *NDC1* causes haploid cells to increase in ploidy. Haploid yeast strains (WX257-2d, WX257-4c, HC14-10c, and HC14-5d; see Table 1) were transformed with either a 2- μ m *URA3* vector (2- μ m vector; pRS426) or with the 2- μ m *URA3* vector containing *NDC1* (2- μ m *NDC1*; pRS426-*NDC1*). After transformation, individual colonies ($n = 33$ for vector alone; $n = 112$ for 2- μ m *NDC1*) were selected and inoculated into Ura⁻ broth overnight, and samples were prepared for flow cytometry the following day. Shown here are examples of flow cytometric profiles for haploid strains transformed with either the 2- μ m vector or with the 2- μ m vector containing *NDC1*.

region of unsegregated nuclear DNA, and exhibited abnormal microtubule staining, all characteristic of cells containing monopolar spindles. Cells overexpressing *NDC1-3xpk* did not exhibit a dramatic decrease in viability, but a large percentage of the surviving cells exhibited increased ploidy (Fig. 4B). Immunoblot analysis showed that Ndc1p-3xpk was highly overexpressed (20 to 30 times) in these cells (data not shown).

We examined microtubule staining and the localization of an SPB component, Spc42p tagged with green fluorescent protein (Spc42p-GFP) (17), in cells overexpressing *NDC1-3xpk*. Four hours after release from the α -factor block, the uninduced control cells were asynchronous, and large-budded cells typically contained a long mitotic spindle with separated DNA (Fig. 5A). Cells overexpressing *NDC1-3xpk*, however, exhibited a G₂ arrest, and 83% of the cells were large-budded. Of these large-budded cells, 89% ($n = 90$) contained unsegregated nuclear DNA and 81% contained two spots of Spc42p-GFP signal. Only one spot of Spc42p-GFP signal was associated with the nuclear DNA, and it nucleated an abnormal array of microtubules (Fig. 5B); the second spot of Spc42p-GFP signal was not associated with the nuclear DNA, but a small amount of microtubule staining emanated from it.

The phenotypes associated with *NDC1* overexpression are strikingly similar to those observed previously for the *ndc1-1* allele (9, 10). We used electron microscopy to examine SPB morphology in cells overexpressing *NDC1-3xpk* 1 hr after release from the α -factor block and found that these cells contain monopolar spindles and defective SPBs similar to what has been reported for *ndc1-1* cells at the nonpermissive temperature (10) (data not shown).

Ndc1p Is Mislocalized When Overexpressed. Normally, Ndc1p exhibits punctate nuclear peripheral staining, corresponding to its localization to both NPCs and SPBs (12). We examined the localization of overexpressed Ndc1p-3xpk by using indirect immunofluorescence microscopy and found that Ndc1p-3xpk was mislocalized; it formed large spots in the cytoplasm (data not shown). We carried out immunoelectron microscopy analysis to examine this localization more closely.

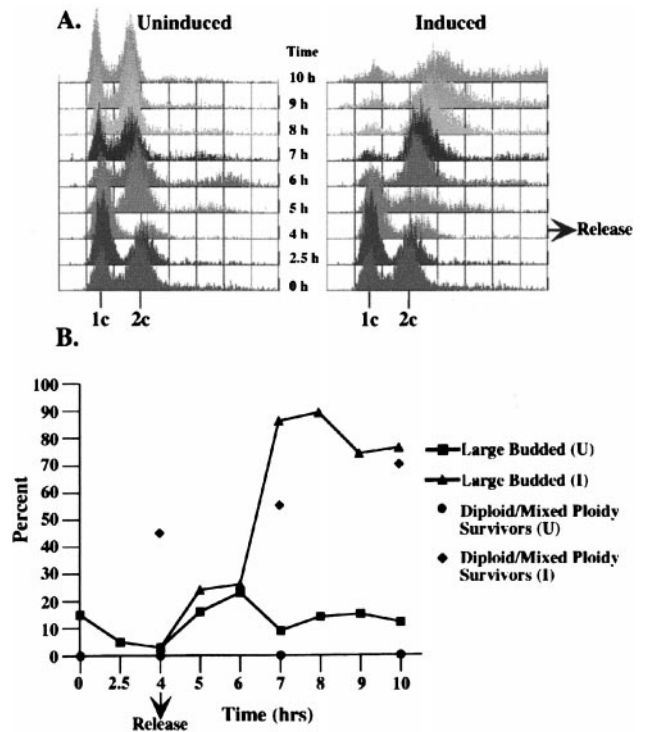


FIG. 4. Overexpression of *NDC1-3xpk* causes cells to arrest and increase in ploidy. Asynchronous cultures of yeast cells containing *GAL-NDC1-3xpk* integrated at the *LEU2* chromosomal locus [HC14-10c(1235); see Table 1] were arrested in G₁ by using α -factor for 2.5 hr. The culture then was split; one half was resuspended in medium containing α -factor with glucose (Uninduced), and the other half was resuspended in medium containing α -factor with galactose (Induced) for an additional 1.5 hr to induce the expression of *NDC1-3xpk* in the induced culture. The cells then were rinsed and resuspended in either glucose-containing (Uninduced) or galactose-containing (Induced) medium to release them from the α -factor block. Samples were taken for flow cytometry, budding index analysis, and viability every hour for 5 hr. (A) Flow cytometric profiles of uninduced and induced cultures. (B) A graph showing the percentages of large-budded cells and higher ploidy surviving cells, either diploid or mixed ploidy, at selected time points.

The uninduced control cells showed little or no immunogold labeling (data not shown). Cells overexpressing *NDC1-3xpk* showed large cytoplasmic membranous structures filled with immunogold labeling, corresponding to overexpressed Ndc1p-3xpk; these structures often were associated with the nuclear envelope (Fig. 6).

DISCUSSION

Yeast cells are strikingly sensitive to altered *NDC1* gene dosage. The *NDC1* locus is haploinsufficient, meaning that diploid cells cannot survive with a single chromosomal copy of *NDC1*. It is remarkable that yeast cells are so sensitive to a 2-fold change in *NDC1* gene dosage. This phenotype of haploinsufficiency resulting in lethality has been observed for only one other yeast gene, *MLC1*, which encodes a myosin light chain (26) and has no functional relationship to *NDC1*. Chromosome XIII, where *NDC1* resides, also contains the *TUB1* and *TUB3* genes, both of which encode α -tubulin. Interestingly, diploid strains with a single copy of *TUB1* exhibit aneuploidy for chromosome XIII (27). It is possible that changes in α -tubulin dosage also may contribute to *NDC1* haploinsufficiency survival.

We determined the mechanisms by which rare haploinsufficiency "survivors" arose. None of the surviving cells corresponded to typical diploid yeast strains that were heterozygous

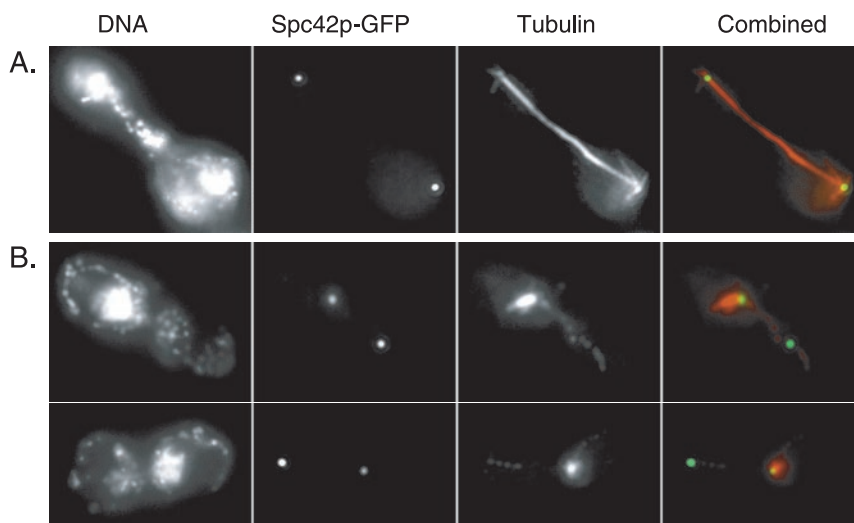


FIG. 5. Cells overexpressing *NDC1-3xp*k exhibit aberrant microtubule staining and Spc42p-GFP localization. Indirect immunofluorescence microscopy was used to examine microtubules, and SPBs were visualized by the autofluorescence of Spc42p-GFP in cells overexpressing *NDC1-3xp*k. This experiment was carried out as described in Fig. 4 by using a yeast strain that contains *GAL-NDC1-3xp*k integrated at the *LEU2* chromosomal locus, as well as a chromosomally integrated *SPC42-GFP* allele (HC44-2a; see Table 1). Shown here are representative examples of tubulin and Spc42p-GFP localization at 4 hr after release from the α -factor arrest. Tubulin was labeled indirectly by using Texas Red. Shown are both tubulin (red) and Spc42p-GFP (green) signals (overlap is yellow). DNA, tubulin, and Spc42p-GFP localization are shown in a large-budded, uninduced cell (A) and in large-budded cells from the induced culture (B).

for the *ndc1* null allele; most exhibited dramatic changes in their chromosomal composition. We observed four mechanisms for surviving *NDC1* haploinsufficiency, including (i) aneuploidy for chromosome XIII, where *NDC1* resides, (ii) mitotic recombination to replace the *ndc1* null allele with a wild-type copy of *NDC1*, (iii) acquisition of an extragenic suppressor mutation, and (iv) mutation of the *NDC1*-containing plasmid, allowing it to be maintained even under negative selection. Additionally, we found that the survivors that exhibited aneuploidy were not genetically stable. Most survivors had effectively restored normal *NDC1* gene dosage. It is likely that the *NDC1* haploinsufficient phenotype is due, at least in part, to a defect in SPB duplication. It is interesting to note that a SPB duplication defect actually may allow more cells to overcome the *NDC1* haploinsufficient phenotype; it would contribute to the ability of these cells to exhibit aneuploidy, one of the main mechanisms by which they survive.

We also found that yeast cells are sensitive to increased *NDC1* gene dosage. When *NDC1* was moderately overex-

pressed, haploid cells exhibited increased ploidy. We tested whether the increase-in-ploidy phenotype was due to a SPB duplication defect. The overexpression of *NDC1-3xp*k in synchronized cells resulted in a SPB duplication defect that was strikingly similar to that observed in *ndc1-1* mutant yeast strains (9, 10). This phenomena of overexpression mimicking loss-of-function phenotypes is not unprecedented for a gene involved in SPB duplication; the overexpression of *KARI* leads to SPB duplication defects that are similar to *kar1* loss-of-function alleles (28).

It is possible that a precise distribution of Ndc1p among SPBs and NPCs is critical for its function and, ultimately, for cell viability. Changes in *NDC1* gene dosage might alter this distribution, leading to the phenotypes we observed. The depletion of Ndc1p in diploid strains that are heterozygous for the *ndc1* null allele may cause them to fail in SPB duplication because of an insufficient pool of Ndc1p at the SPB. Similarly, the overexpression of Ndc1p may lead to failed SPB duplication by depleting Ndc1p or a key cofactor from the SPB. A function for Ndc1p at NPCs is not known at this time, and we did not observe any obvious NPC-related phenotypes in cells with altered *NDC1* gene dosage (H.J.C. and M.W., unpublished observation). Our results suggest that the SPB-related function of *NDC1* is more sensitive to changes in *NDC1* gene dosage.

The striking sensitivity of yeast cells to changes in *NDC1* gene dosage suggests models for the behavior of genes involved in cellular transformation. The phenotypes associated with *NDC1* overexpression and *NDC1* haploinsufficiency mimic the behavior of oncogenes and tumor suppressor genes, respectively. Oncogenes typically encode positive regulators of cell cycle progression in which either gain-of-function mutations or overexpression of the wild-type gene leads to cellular transformation (29). The increase-in-ploidy phenotypes associated with *NDC1* overexpression could serve as a model for the behavior of an oncogene that normally functions to maintain centrosome fidelity, such as the *aurora2/STK15/BTAK* protein kinase (6, 7). The overexpression of this type of oncogene could lead to defects in chromosome segregation, resulting in aneuploidy or polyploidy, which are early genetic events in some cancers.

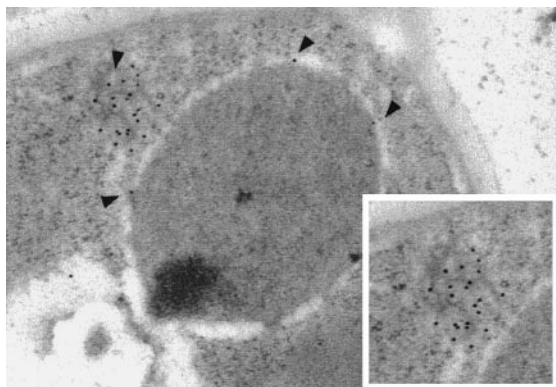


FIG. 6. *NDC1p-3xp*k is mislocalized when overexpressed. *NDC1-3xp*k was overexpressed by galactose induction, as described in Fig. 4. Immunoelectron microscopy analysis (see *Materials and Methods*) was used to examine the localization of overexpressed Ndc1p-3xp. Shown is a cell at 40 min after release from the α -factor arrest in the presence of galactose. Arrows indicate membranous cytoplasmic structures and NPCs that display immunogold labeling, corresponding to Ndc1p-3xp. Inset shows a closer view of the immunogold-labeled structure.

Contrasting with oncogenes, tumor suppressor genes generally encode negative regulators of cell cycle progression whose function is eliminated during cellular transformation (30). The behavior of these genes is the basis for Knudson's "two-hit" model for cellular transformation, in which the first hit is a mutation in the tumor suppressor gene and the second hit is a mutation in the remaining wild-type allele of this gene, leading to a loss of heterozygosity (31). Partial loss-of-function alleles of *NDC1*, such as the *ndc1-1* mutation, would adhere to this model. The *NDC1* haploinsufficient phenotype could serve as an example of a tumor suppressor gene in which complete loss-of-function mutations do not adhere to the traditional two-hit model. The *NDC1* model of cellular transformation would be that a complete loss-of-function mutation in a haploinsufficient gene immediately would give rise to aneuploid surviving cells that had restored the normal gene dosage of the remaining wild-type allele. These cells may also behave similarly to the survivors of *NDC1* haploinsufficiency and exhibit genetic instability in later cell cycles, possibly even eliminating the mutation that induced the aneuploidy. This model may be used to reinterpret the behavior of some genes involved in cancer, such as p53.

Recently, it was shown that mice that are heterozygous for a null allele of the p53 gene exhibit higher rates of tumor formation, even though they still maintain a single wild-type copy of the p53 gene, suggesting that decreased p53 gene dosage can lead to tumorigenesis (32). Perhaps p53 is haploinsufficient for some activity such that heterozygous null mice have higher levels of genetic instability. If mutations in p53 or other genes behave similarly to the complete loss-of-function alleles of *NDC1*, they would display a potent and dominant aneuploidy-generating phenotype that could lead to cellular transformation.

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1. Li, R., Yerganian, G., Duesberg, P., Kraemer, A., Willer, A., Rausch, C. & Hehlmann, R. (1997) *Proc. Natl. Acad. Sci. USA* **94**, 14506-14511.
2. Lengauer, C., Kinzler, K. W. & Vogelstein, B. (1997) *Nature (London)* **386**, 623-627.
3. Cahill, D. P., Lengauer, C., Yu, J., Riggins, G. J., Willson, J. K., Markowitz, S. D., Kinzler, K. W. & Vogelstein, B. (1998) *Nature (London)* **392**, 300-303.
4. Zhuang, Z., Park, W. S., Pack, S., Schmidt, L., Vortmeyer, A. O., Pak, E., Pham, T., Weil, R. J., Candidus, S., Lubensky, I. A., *et al.* (1998) *Nat. Genet.* **20**, 66-69.
5. Lingle, W. L., Lutz, W. H., Ingle, J. N., Maihle, N. J. & Salisbury, J. L. (1998) *Proc. Natl. Acad. Sci. USA* **95**, 2950-2955.
6. Zhou, H., Kuang, J., Zhong, L., Kuo, W. L., Gray, J. W., Sahin, A., Brinkley, B. R. & Sen, S. (1998) *Nat. Genet.* **20**, 189-193.
7. Bischoff, J. R., Anderson, L., Zhu, Y., Mossie, K., Ng, L., Souza, B., Schryver, B., Flanagan, P., Clairvoyant, F., Ginther, C., *et al.* (1998) *EMBO J.* **17**, 3052-3065.
8. Chial, H. J. & Winey, M. (1999) *Biol. Cell*, in press.
9. Thomas, J. H. & Botstein, D. (1986) *Cell* **44**, 65-76.
10. Winey, M., Hoyt, M. A., Chan, C., Goetsch, L., Botstein, D. & Byers, B. (1993) *J. Cell Biol.* **122**, 743-751.
11. Hardwick, K. G., Weiss, E., Luca, F. C., Winey, M. & Murray, A. W. (1996) *Science* **273**, 953-956.
12. Chial, H. J., Rout, M. P., Giddings, T. H. & Winey, M. (1998) *J. Cell Biol.* **143**, 1789-1800.
13. Sherman, F. G., Fink, G. R. & Hicks, J. B. (1986) *Methods in Yeast Genetics* (Cold Spring Harbor Lab. Press, Plainview, NY), p. 186.
14. Baudin, A., Ozier-Kalogeropoulos, O., Denouel, A., Lacroute, F. & Cullin, C. (1993) *Nucleic Acids Res.* **21**, 3329-3330.
15. Rothstein, R. (1991) *Methods Enzymol.* **194**, 281-301.
16. Wach, A., Brachat, A., Pohlmann, R. & Philippsen, P. (1994) *Yeast* **10**, 1793-1808.
17. Schutz, A. R. & Winey, M. (1998) *Mol. Biol. Cell* **9**, 759-774.
18. Boeke, J. D., Trueheart, J., Natsoulis, G. & Fink, G. R. (1987) *Methods Enzymol.* **154**, 164-175.
19. Ausubel, J. D., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (1994) *Current Protocols in Molecular Biology* (Wiley, New York).
20. Sikorski, R. S. & Hieter, P. (1989) *Genetics* **122**, 19-27.
21. Christianson, T. W., Sikorski, R. S., Dante, M., Shero, J. H. & Hieter, P. (1992) *Gene* **110**, 119-122.
22. Johnston, M. & Davis, R. W. (1984) *Mol. Cell. Biol.* **4**, 1440-1448.
23. Randall, R. E., Young, D. F., Goswami, K. K. & Russell, W. C. (1987) *J. Gen. Virol.* **68**, 2769-2780.
24. Hutter, K. J. & Eipel, H. E. (1979) *J. Gen. Microbiol.* **113**, 369-375.
25. Bender, A. & Pringle, J. R. (1991) *Mol. Cell. Biol.* **11**, 1295-1305.
26. Stevens, R. C. & Davis, T. N. (1998) *J. Cell Biol.* **142**, 711-722.
27. Schatz, P. J., Solomon, F. & Botstein, D. (1986) *Mol. Cell. Biol.* **6**, 3722-3733.
28. Rose, M. D. & Fink, G. R. (1987) *Cell* **48**, 1047-1060.
29. Hunter, T. (1997) *Cell* **88**, 333-346.
30. Weinberg, R. A. (1991) *Science* **254**, 1138-1146.
31. Knudson, A. G., Jr. (1971) *Proc. Natl. Acad. Sci. USA* **68**, 820-823.
32. Venkatchalam, S., Shi, Y. P., Jones, S. N., Vogel, H., Bradley, A., Pinkel, D. & Donehower, L. A. (1998) *EMBO J.* **17**, 4657-4667.