

# New yeast genes important for chromosome integrity and segregation identified by dosage effects on genome stability

Ilia I. Ouspenski\*, Stephen J. Elledge<sup>1</sup> and B. R. Brinkley

Department of Cell Biology and <sup>1</sup>Department of Biochemistry, Department of Molecular and Human Genetics and Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030, USA

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## ABSTRACT

**Phenotypes produced by gene overexpression may provide important clues to gene function. Here, we have performed a search for genes that affect chromosome stability when overexpressed in the budding yeast *Saccharomyces cerevisiae*. We have obtained clones encompassing 30 different genes. Twenty-four of these genes have been previously characterized. Most of them are involved in chromatin dynamics, cell cycle control, DNA replication or mitotic chromosome segregation. Six novel genes obtained in this screen were named *CST* (chromosome stability). Based on the pattern of genomic instability, interaction with checkpoint mutations and sensitivity to chromosome replication or segregation inhibitors, we conclude that overexpression of *CST4* specifically interferes with mitotic chromosome segregation, and *CST6* affects some aspect of DNA metabolism. The other *CST* genes had complex pleiotropic phenotypes. We have created deletions of five genes obtained in this screen, *CST9*, *CST13*, *NAT1*, *SBA1* and *FUN30*. None of these genes is essential for viability, and deletions of *NAT1* and *SBA1* cause chromosome instability, a phenotype not previously associated with these genes. This work shows that analysis of dosage effects is complementary to mutational analysis of chromosome transmission fidelity, as it allows the identification of chromosome stability genes that have not been detected in mutational screens.**

## INTRODUCTION

Accurate propagation of genetic information requires the coordinated function of many cellular mechanisms. These include replication of the genome, detection and repair of spontaneous DNA damage and segregation of the duplicated and condensed chromosomes by the mitotic spindle. All of these events depend on proper control of chromatin architecture. High fidelity of these events is assured by monitoring and control by cell cycle regulatory mechanisms. Thus the genes

important for maintenance of genome stability include the components and regulators of chromatin, DNA replication and repair machinery, mitotic apparatus components and cell cycle controls.

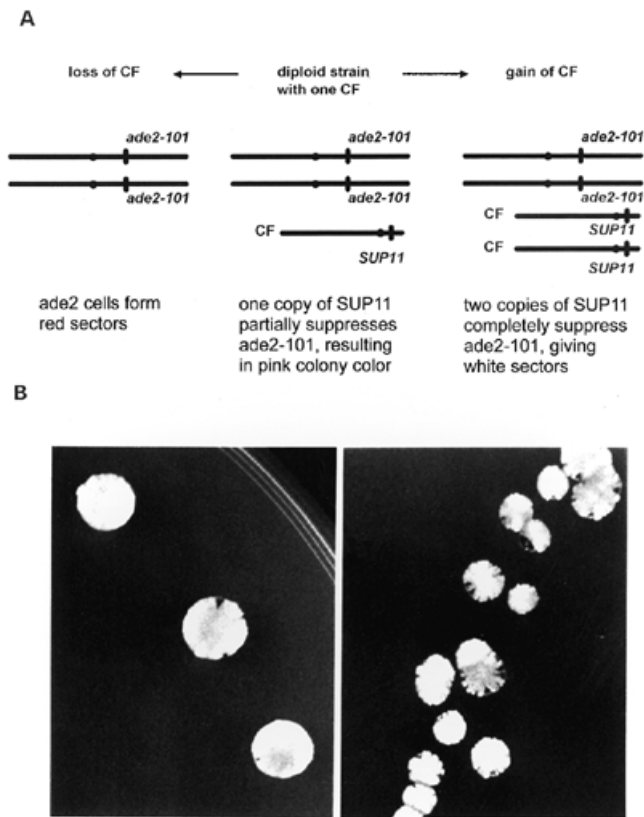
A large number of genes that affect chromosome stability in budding yeast have been identified by mutation screens (1–5). Additionally, many genes identified by other criteria were shown to be necessary for genome stability. The examples include DNA metabolism enzymes, such as polymerases and a ligase (6,7), and components of chromosome segregation machinery, including tubulins, mitotic spindle motors, components of the kinetochore, and spindle pole bodies (8–11).

In studies of mutations that affect chromosome stability, some genes important for this process may have been missed, either because their functions are redundant, or because only dominant mutations would have a phenotype. Genes affected by dominant mutations are difficult to clone because individual libraries are required. In this work, we broaden the mutational analysis of chromosome stability by the use of a dominant genetic approach.

Many of the events critical for genomic stability are known to be dependent on proper stoichiometric ratios of the components involved. It was shown, for example, that altered levels of histones or tubulins affect the fidelity of chromosome segregation, presumably by interfering with chromatin assembly and mitotic spindle functions, respectively (8–12).

Overproduction or inappropriate production of a normal gene product can affect cellular functions by different mechanisms. For components of multisubunit assemblies, an excess of one subunit can interfere with the formation of an active stoichiometric complex (dominant-negative effect). In this case, the phenotype produced by overexpression of the gene is similar to that of loss of function of the same gene. Numerous examples of this mechanism have been documented, including genes involved in chromosome segregation (3,8,13–15). Alternatively, the elevated amount of a protein can increase the activity of a normal cellular process, which might in itself lead to a detectable phenotype. This allows the identification of genes involved in maintenance of genomic stability by the chromosome loss phenotype resulting from their overexpression (15–17). Examples of successful overexpression-based screens include identification of proteins required for meiosis in budding yeast, and for mitotic cell division and cell morphogenesis in fission yeast

\*To whom correspondence should be addressed at: Department of Cell Biology, 126A, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA. Tel: +1 713 798 5295; Fax: +1 713 790 0545; Email: iliao@bcm.tmc.edu



**Figure 1.** Colony color sectoring assay for chromosome loss (2;21). (A). The mechanism of colony color changes resulting from loss or gain of the CF. (B) An example of sectoring phenotype induced by overexpression of *CST* genes. Control cells (left) and cell overexpressing *CST1/UBI2* (right), which shows an intermediate level of chromosome loss (10-fold increase over parental cells, compare to data in Table 3).

(16,18,19). In particular, *MIF2*, a gene important for centromere function, and a possible homolog of human centromeric protein CENP-C, has been isolated and characterized through an overexpression-based screen (15,17). Overexpression has also been used successfully to pinpoint specific functional interactions between components of the kinetochore, and between proteins involved in DNA replication (20). In this work, we have exploited this approach to systematically search for genes important for chromosome stability (*CST* genes) in the budding yeast *Saccharomyces cerevisiae*.

## MATERIALS AND METHODS

### Yeast strains, media and procedures

The genotypes of the yeast strains used in this work are listed in Table 1. Strain YIIL39 was obtained by one-step gene replacement of *MAD1* with *HIS3*, using pKH149 (provided by K. Hardwick and A. Murray). YIL40 was produced by replacing a 450 bp fragment of *MAD3* with *LEU2*, using pKH515 (provided by K. Hardwick and A. Murray). Strain YF451 was obtained

by backcrossing the *mad2-1* allele into the S288C background (F. Spencer, personal communication).

### Colony color sectoring assay

Colony color sectoring assay was performed as described by (21), using the diploid yeast strain YPH275 (2) (Fig. 1). The frequency of loss and non-disjunction of the chromosome fragment (CF) was quantified by determining the frequency of half-red colonies within a population of pink sectoring colonies.

### Identification of *CST* clones

We used a yeast cDNA library expressed from the *GALI* promoter in a *CEN* (single copy) vector (22). This library is derived from cells logarithmically growing in rich medium. The cDNA representation is estimated to be ~50-fold larger than the mRNA population (22). The library was transformed into the chromosome loss tester strain YPH275, and the cells were allowed to recover in liquid SC-Ura for 4 h. Transformants were plated onto SC-Ura containing 2% galactose to induce the expression of cDNA and a reduced concentration of adenine (5 mg/l) to facilitate red color development by *ade2* cells. Cells were plated at a low density (500–1000 transformants per 150 mm plate). After 10 days at 30°C the plates were scored for the presence of sectoring phenotypes (Fig. 1B). Colonies showing consistent sectoring phenotype on galactose, but not on glucose, were selected. Plasmids were rescued in *Escherichia coli* and re-transformed into YPH275. The inserts of the plasmids, which consistently induced galactose-dependent chromosome loss, were compared to each other by restriction mapping and Southern hybridization. The identity of unique inserts was established by partial sequencing.

### Estimation of the number of *CST* genes

To estimate the minimal number of yeast genes that may have dosage effects on chromosome stability (*CST*-type genes), we used a simplifying assumption that all genes are equally represented in our cDNA library. In reality, unequal representation of cDNA species means that the number of *CST* genes is greater than estimated by this method. Given the equal representation assumption, the probability  $P_x$  of recovering any gene exactly  $x$  times will be described by the Poisson distribution:

$$P_x = m^x/x!e^{-m} \quad 1$$

where  $m$  is the Poisson parameter. The observed frequency of obtaining a particular gene  $x$  times should be proportional to the probability  $P_x$  of this event. If  $n_x$  is the number of genes recovered  $x$  times (e.g.  $n_0$  genes were not encountered in this screen,  $n_1$  genes were recovered once each,  $n_2$  genes twice each, etc.), then

$$\begin{aligned} n_0/n_1 &= P_0/P_1, \\ n_1/n_2 &= P_1/P_2, \text{ etc.} \end{aligned} \quad 2$$

Substituting the values of  $P_0$ ,  $P_1$  and  $P_2$  from 1 into 2 gives

$$\begin{aligned} n_0/n_1 &= 1/m \\ \text{and } n_1/n_2 &= 2/m \end{aligned} \quad 3$$

Thus  $n_0$ , the number of *CST* genes missed by this screen, can be

**Table 1.** Yeast stains used in this study

Strain	Genotype	Source
YPH275	<i>MATa/MATα ura3-52 lys2-80 ade2-101 trp1-Δ1 his3-Δ200 leu2-Δ1 CF [TRP1 SUP11 CEN4]</i>	P. Hieter
LH33OSD	<i>MATα ade2 leu2 trp1 hom3 ura3 can1 fcy1 sap3 (lys5 + cyh2 CEN7 ade6 +)(+ aro2 + CEN7 + ade3)</i>	L. Hartwell
TWY308	<i>MATa mec1-1 ura3 trp1</i>	T. Weinert
TWY398	<i>MATa Δrad9::LEU2 his7 ura3 leu2 trp1</i>	T. Weinert
YIL39	<i>MATa Δmad1::HIS3 ura3-52 lys2-801 ade2-101 trp1-Δ1 his3-Δ200 leu2-Δ1 CF [TRP1 SUP11 CEN4]</i>	This study
YF451	<i>MATa mad2-1 ura3-52 lys2-801 ade2-101 his3-Δ200 trp1-Δ63 leu2-Δ1 CF [URA3 SUP11 CEN3]</i>	F. Spencer
YIL40	<i>MATa Δmad3::LEU2 ura3-52 lys2-801 ade2-101 trp1-Δ1 his3-Δ200 leu2-Δ1 CF [TRP1 SUP11 CEN4]</i>	This study

estimated from  $n_1$  and  $n_2$ , the numbers of genes recovered once and twice each, respectively:

$$n_0 = n_1^2/2n_2 \quad 4$$

Since the abundance of different cDNAs in the library is not equal, this estimate provides a lower limit for the number of possible *CST* genes.

#### Quantification of mitotic recombination frequency

The frequency of mitotic recombination was determined as described (12), after introducing *CST*-overexpressing plasmids into the strain LH33OSD (Table 1). This is a haploid strain with an extra copy of chromosome VII. One of the copies of chromosome VII has an *ade3* mutation on the right chromosome arm, and the other has *ade6* on the right arm and a recessive cycloheximide resistance marker *cyh2* on the left arm. This strain is also mutant for *ade2* and therefore forms red colonies. If it becomes hemizygous or homozygous for *ade3* and/or *ade6*, due to recombination, gene conversion or chromosome loss, the colony color changes to white. Cells that lose the wild-type *CYH2* cycloheximide-sensitivity allele by loss can be selected on cycloheximide-containing plates. The mechanism of the *ade3* and/or *ade6* marker loss is then determined by the color of the colony, with chromosome loss producing white colonies, and recombination or gene conversion, red colonies (12). The frequency of mitotic recombination between *cyh2* and the pluscentromere, plus the frequency of gene conversion at the *CYH2* locus are thus proportional to the frequency at which red cycloheximide-resistant colonies arise (12).

#### Dosage interaction of *CST* genes with checkpoint mutations

*CST*-expressing plasmids were introduced into checkpoint mutant strains and the corresponding isogenic checkpoint-proficient strains. The cells were streaked on SC-Ura or SC, Gal-Ura plates, incubated at 30°C for 3–5 days (SC-Ura) or 7–14 days (SC, Gal-Ura), and scored for independent colony formation and colony size.

#### Sensitivity to growth inhibitors

Haploid cells containing *CST*-expressing plasmids or the control parental plasmid were streaked to single colonies on SC-Ura or SC, Gal-Ura plates, supplemented with the growth inhibitors in the following concentrations: 2.00–2.25 μg/ml methylmethane sulfonate (MMS); 125–175 mM hydroxyurea (HU); 12–17 μg/ml

benomyl. The plates were incubated for 6–8 days at 30°C (HU and MMS) or for 10–12 days at 23°C (benomyl), and scored for independent colony growth and colony size.

#### Deletions of *CST* genes

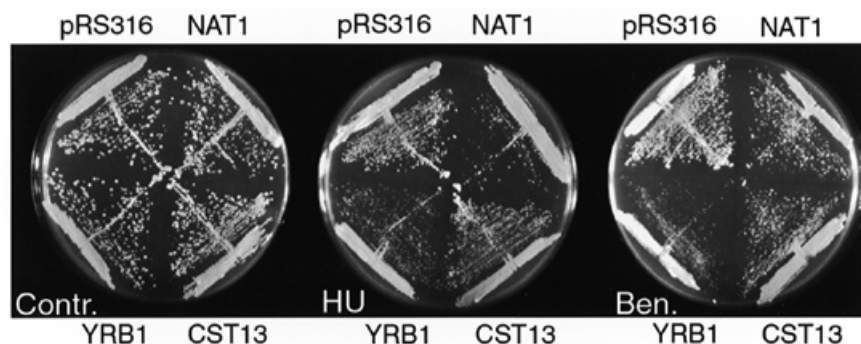
Disruption fragments were generated by PCR amplification of the KanMX4 G418-resistance module, using the appropriate gene-specific primers with 40–50 bp identity to the corresponding gene at their 5' ends (23). The resulting PCR fragments were transformed into YPH275, and G418-resistant colonies were selected. Correct replacement was verified by PCR amplification of the two replacement junction regions. Heterozygous diploid cells were sporulated and gene disruption was verified in haploid progeny by G418 resistance and PCR.

## RESULTS

#### Genes with dosage effect on chromosome stability

We have performed a search for genes that affect chromosome stability when overexpressed in budding yeast *S.cerevisiae*. We used a library of *S.cerevisiae* cDNA in a centromere-containing vector pRS316, under the control of a strong inducible promoter *GALI* (22). This promoter is induced when cells are grown on galactose as the carbon source, and repressed on glucose. The library was introduced into the YPH275 strain, which contains a supernumerary CF that is dispensable for viability (2). The CF is telocentric, containing a long arm derived from a native yeast chromosome and functional centromere and telomere sequences (Fig. 1A). Mitotic stability of the CF is comparable to that of native yeast chromosomes (24). The CF is marked with an ochre suppressor tRNA gene *SUP11*, and the strain is homozygous for an ochre allele *ade2-101*, which results in the accumulation of a red pigment. In diploid cells, one copy of *SUP11* partially suppresses the red color phenotype, making pink colonies, while two copies of the suppressor produce white colonies. The loss of the CF during colony growth is manifested by the appearance of red sectors on a pink colony background (Fig. 1B), while gain of extra copies of the CF appears as white sectors (21).

cDNA library transformants were plated on SC-Ura with galactose as the carbon source. Clones showing plasmid-dependent and galactose-dependent increases in the rate of chromosome loss or non-disjunction were selected, and the corresponding plasmids were recovered. The inserts were compared to each other using restriction mapping, Southern hybridization and partial sequencing.



**Figure 2.** Examples of selective interaction of *CST* overexpression with growth inhibitors. Cells containing the control plasmid pRS316 or the indicated *CST* genes were streaked on SC, galactose-Ura plates containing HU or Benomyl (Ben.) and grown at 30°C.

Screening of approximately  $10^5$  transformants resulted in the identification of 30 different genes, which we named *CST* (chromosome stability). One gene (*CST1/UBI2*) was recovered nine times, five genes were recovered twice each and 24 genes were obtained once each.

To estimate the minimal number of *CST* genes that may have dosage effects on chromosome stability, we disregarded the apparently overabundant *CST1* gene and assumed that the remaining genes have identical abundance in the cDNA library. An estimate based on Poisson distribution shows that at least 58 additional *CST* genes have not been encountered in this screen (Materials and Methods). Since different cDNAs vary in abundance, and efficiency of cDNA synthesis varies, the actual number of *CST* genes in yeast genome is higher than provided by this estimate.

Twenty-four *CST* genes correspond to previously described genes, and six are new (Table 2). Most are represented by full-length open reading frames, while some are expressed as C-terminal fragments. As expected, the known *CST* genes are involved in chromatin organization, cell cycle control, mitotic chromosome segregation or DNA replication. Four genes are involved in ubiquitin-directed proteolysis, which controls key steps in cell cycle progression and other processes. An unexpected finding is the identification of three components of the Ran GTPase molecular switch system. These proteins are involved in nucleocytoplasmic transport; however, no other proteins essential for this process (e.g. nucleoporins) have been obtained in this screen. This may be an indication that the Ran GTPase system also controls processes other than nucleocytoplasmic transport, as suggested previously (25).

### Cellular functions affected by *CST* overexpression

Chromosomal instability may result from defects in cell cycle progression, DNA replication or repair or mitotic chromosome segregation. To establish which chromosomal functions are primarily affected by overexpression of the *CST* genes, we have determined the patterns of genomic instability, interactions with checkpoint mutations and sensitivity to hydroxyurea and benomyl, which selectively affect chromosomal functions (Table 3 and Fig. 2).

Genomic instability may result in chromosome loss (1:0 segregation) or non-disjunction (2:0), and may increase the rate of

mitotic recombination. Chromosome loss is characteristic of problems in DNA metabolism, while non-disjunction reflects defects in mitotic segregation. The rate of mitotic recombination is directly related to the presence of DNA lesions (6).

Genes that cause DNA damage, replication problems or chromosome segregation defects are expected to differ from each other in the way they interact with cell cycle checkpoint mutations. The product of *RAD9* gene is necessary to delay cell cycle progression in response to DNA damage, and *MEC1* controls cell cycle progression in response to both DNA damage and incomplete DNA replication (26,27). Certain mutations, including *mad1*, *mad2* and *mad3*, relieve the dependence of cell cycle progression on proper mitotic spindle function, allowing the cell to attempt division in the absence of chromosome segregation, which leads to cell death (28,29).

HU is an inhibitor of ribonucleotide reductase, an enzyme necessary for DNA synthesis (reviewed in 30). Benomyl is a microtubule depolymerizing drug which prevents formation of the mitotic spindle.

A specific defect in DNA metabolism should be manifested as increased 1:0 but not 2:0 segregation, elevated mitotic recombination, interaction with *rad9* and/or *mec1* but not *mad* checkpoint mutations, and sensitivity to HU but not benomyl. A defect in mitotic segregation should have the opposite effect by the above criteria.

We have applied these criteria to the *CIN8* kinesin-like mitotic motor, a DNA replication factor *RFC2*, and to *YRB1*, which affects multiple nuclear functions. As expected, *CIN8* fits the 'mitotic' set of characteristics, in that it has increased mitotic non-disjunction (2:0), no increase in mitotic recombination, interacts with a mitotic checkpoint but not with DNA damage nor replication checkpoints, and is sensitive to benomyl but not HU (Table 3). The effects of overproduction of the novel gene *CST4* are very similar, indicating that this gene is likely specifically involved in mitosis. Replication factor *RFC2* fits the profile of a 'DNA metabolism' gene, with increased recombination but not mitotic non-disjunction, interaction with a DNA replication checkpoint, and sensitivity to HU but not benomyl. *CST6* fits the same profile, and thus may also be involved in some aspect of DNA metabolism. *YRB1* has a complex pleiotropic phenotype, being positive in all assays (Table 3). This is similar to overexpression phenotypes

**Table 2.** Identities of *CST* genes

Gene	Identity	ORF (first/total amino acids)	Relevant functions or properties
Chromatin components or regulators			
<i>CST3</i>	<i>HOS1</i>	Complete	Histone deacetylase
<i>CST5</i>	<i>RME1</i>	Complete	Transcription factor affecting chromatin structure
<i>CST8</i>	<i>NAT1</i>	590/854	N-terminal acetyltransferase, required for silencing
<i>CST10</i>	<i>ANCI</i>	Complete	Component of SNF/SWI remodeling complex
<i>CST19</i>	<i>HHT2</i>	Complete	Histone H3
<i>CST21</i>	<i>RPA34</i>	78/233	Interacts with RNA polymerase I and topo I
<i>CST22</i>	<i>HHF2</i>	Complete	Histone H4
<i>CST24</i>	<i>FUN30</i>	550/1131	Homolog of chromatin remodeling factor <i>SNF2</i>
<i>CST25</i>	<i>EGD1</i>	Complete	Regulator of Gal4p DNA-binding activity
Ubiquitin-directed proteolysis			
<i>CST1</i>	<i>UBI2</i>	Complete	Polyubiquitin precursor
<i>CST2</i>	<i>UBI1</i>	Complete	Polyubiquitin precursor
<i>CST27</i>	<i>PUP3</i>	Complete	Proteasome subunit
<i>CST28</i>	<i>PRE1</i>	Complete	Proteasome subunit
Cell cycle-related factors			
<i>CST7</i>	<i>CLN3</i>	475/580	G1 cyclin
<i>CST23</i>	<i>CDC19</i>	Complete	Pyruvate kinase, necessary for G1/S transition
DNA replication			
<i>CST15</i>	<i>RFC2</i>	Complete	Replication factor C subunit
<i>CST30</i>	<i>RNR2</i>	Complete	Ribonucleotide reductase subunit
Microtubule-associated proteins			
<i>CST14</i>	<i>YTM1</i>	Complete	Microtubule-interacting protein
<i>CST16</i>	<i>CIN8</i>	829/1038	Kinesin-like mitotic motor
Nucleocytoplasmic transport (Ran GTPase system)			
<i>CST12</i>	<i>RNA1</i>	Complete	Ran GTPase activating protein
<i>CST17</i>	<i>GSP1</i>	Complete	Ran GTPase
<i>CST20</i>	<i>YRB1</i>	Complete	Ran-binding protein 1
Other			
<i>CST11</i>	<i>PAB1</i>	Complete	Poly(A) <sup>+</sup> RNA-binding protein
<i>CST18</i>	<i>SBA1</i>	Complete	Hsp90-associated co-chaperone
Novel <i>CST</i> genes			
<i>CST4</i>	<i>YLR226W</i>	321/395	Transcript peaks at mitosis <sup>a</sup>
<i>CST6</i>	<i>YIL036W</i>	207/587	
<i>CST9</i>	<i>YLR394W</i>	268/482	
<i>CST13</i>	<i>YBR158W</i>	Complete	
<i>CST26</i>	<i>YBR042C</i>	Complete	Transcript level is increased at G1/S <sup>a</sup>
<i>CST29</i>	<i>YLR222C</i>	459/817	

<sup>a</sup>According to Cho *et al.* (38).

of *GSP1* or *RNA1*, two other components of the Ran regulatory system obtained in this screen (31; data not shown). *CST29* also probably affects multiple nuclear functions, since it is positive in all assays (Table 3). Phenotypes of *CST13* and

*CST26* are not sufficiently informative to assign a specific function to these genes.

We have also determined the sensitivity of *CST*-over-expressing cells to MMS-induced DNA damage, and the

**Table 3.** Phenotypes and checkpoint interactions produced by overexpression of selected *CST* genes

Gene	Genomic instability (fold increase)			Checkpoint interactions					Drug sensitivity	
	1:0	2:0	Rec.	<i>Mec1</i>	<i>rad9</i>	<i>mad1</i>	<i>mad2</i>	<i>mad3</i>	HU	Ben.
Mitotic chromosome segregation phenotypes										
<i>CIN8</i>	w.t.	14	w.t.	+	+	+	-	+	+	+/-
<i>CST4</i>	10	5	w.t.	+	+	+	+	+	+	+/-
DNA metabolism phenotypes										
<i>RFC2</i>	10	w.t.	6	-	+	+	+	+	+/-	+
<i>CST6</i>	12	4	6	-	+	+	+	+	+/-	+
Combination phenotypes										
<i>YRB1</i>	25	50	40	-	-	-	-	+/-	-	-
<i>NAT1</i>	6	w.t.	8	-	+/-	+	+	+	-	+/-
<i>CST9</i>	10	8	9	+/-	+	+	+	+	+/-	+/-
<i>CST29</i>	12	5	12	+/-	+	+/-	+/-	+/-	+/-	+/-
Uninformative phenotypes										
<i>CST13</i>	6	5	6	+	+	+	+	+	+	+
<i>CST26</i>	6	4	w.t.	+	+	+	+	+	+	+

1:0, chromosome loss frequency; 2:0, chromosome non-disjunction frequency; Rec., mitotic recombination frequency; Ben., benomyl; HU, hydroxyurea; w.t., wild type level of chromosome stability. +, no effect on growth; +/-, reduced independent colony formation or colony size (Fig. 2); -, substantially impaired growth.

**Table 4.** *CST* gene deletion phenotypes

Gene	Chromosome loss	Other phenotypes
<i>CST9</i>	No	
<i>CST13</i>	No	Slow growth
<i>NAT1</i>	Yes	ts <sup>-</sup> , HU sensitive
<i>SBA1</i>	Yes	
<i>FUN30</i>	No	ts <sup>-</sup>

frequency of point mutations using canavanine resistance assay. No significant effects of *CST* overexpression on these parameters were detected (data not shown).

### *CST* gene deletions

We have created deletions of five genes obtained in this screen, *CST9*, *CST13*, *NAT1*, *SBA1* and *FUN30*. These genes are not essential for viability. Deletions of *NAT1* and *SBA1* result in an increase in chromosome loss, while the other three genes have no apparent effect on chromosome stability (Table 4). Cell deleted for *CST13* grow more slowly than wild type at all temperatures. Other phenotypes include temperature sensitivity of *nat1* and *fun30* deletion mutants, and HU-sensitivity of *nat1* deletion.

### Human genes that affect chromosome stability in yeast

Since most *CST* genes are conserved between yeast and humans, we sought to determine whether the system based on

overexpression in yeast can be used to identify human genes important for genomic stability, as suggested by (17). We used two human cDNA libraries, one in a low copy (*CEN*) vector under the control of the *ADH* promoter (32), and the other in a high copy (2  $\mu$ m) vector with the *GAP* promoter (33).

In the screen of approximately  $3 \times 10^5$  clones from the first library (*CEN ADH*) we identified three clones, which contained histone H2B, the C-terminal part of the small subunit of ribonucleotide reductase (the human homolog of *RNR2* obtained in the yeast screen), and a mitotic cyclin B2.

The screen of approximately  $4 \times 10^5$  transformants with the second library (2  $\mu$ m *GAP*) yielded about 20 consistently sectoring clones. All of them grew slowly, with frequent appearance of revertants for both chromosome loss and slow growth. We were unable to rescue plasmids from these clones which would confer chromosome loss upon re-introduction back into the tester yeast strain. It is possible that the constitutively high level of expression from the high-copy library precludes the identification of proteins affecting chromosome. Alternatively, the difference between the results of the two screens may be related to different cDNA representation in the two libraries.

## DISCUSSION

### Parameters and specificity of the screen

We employed the colony color sectoring assay using a *SUP11*-marked CF. It was previously demonstrated that segregation of the CF is representative of behavior of native yeast chromosomes (2). Mitotic stability of the CF is very high, although slightly reduced compared to native yeast chromosomes, making the system more sensitive to conditions affecting chromosome stability. An additional advantage of this selection system is the

possibility to visually distinguish between two types of chromosome transmission defects: non-disjunction (2:0 segregation) and chromosome loss (1:0), by the colony color sectoring. Since the *SUP11* marker on the CF is flanked by pBR322 plasmid sequences, the marker loss generally occurs by loss of the whole CF, and not by recombination or gene conversion. This system has been successfully used to isolate a collection of mutants with reduced chromosome transmission fidelity (*ctf* mutants) (2). The *CTF* genes proved very informative for understanding the molecular bases of chromosome stability. For example, some *CTF* genes encode components of kinetochore, or subunits of the DNA replication complex (10,34).

Protein overexpression is a valuable tool in studying gene function. Using a high-copy yeast genomic library, two new genes that affect chromosome stability were identified (17). One of these, *MIF2*, is required for mitotic spindle function in anaphase, and shares sequence similarity to human kinetochore protein CENP-C (15,35). Genetic interaction between gene overexpression and temperature-sensitive mutations has proved useful in identifying specific interactions between proteins involved in kinetochore function, and between components of DNA replication machinery (20).

In the present work, we applied this approach to a yeast cDNA library under the control of strong inducible promoter, which facilitates the identification of genes with deleterious effects on cell growth and circumvents some of the limitations of high-copy libraries (discussed in 36).

Since there are numerous examples of protein overexpression producing a phenotype similar to a mutation in the corresponding gene, we expected to recover a set of genes partially overlapping that of mutations that affect chromosome segregation. This turned out to be the case. Defects in chromosome transmission have been reported for several previously described genes obtained in this screen. These include a mitotic motor *CIN8*, core histones *HHT2* and *HHF2*, chromatin regulator *ANCI*, and the components of the Ran regulatory system, *GSP1*, *RNAI* and *YRBI*. This shows that the approach based on overexpression can identify genes important for genomic stability.

The previously known genes encountered in our screen belong to functional groups related to chromatin organization, cell cycle control, microtubule function, DNA replication and proteolysis. All of these functions are necessary for proper chromosome dynamics, a requisite of genomic stability. This further demonstrates the utility of overexpression-based approach for the study of the mechanisms of chromosome stability. Interestingly, a gene homologous to poly(A)<sup>+</sup> binding protein encoded by *PABI/CST11* and a component of ubiquitin-directed proteolysis machinery have been obtained in a similar overexpression screen in fission yeast (16).

Twenty-four out of 30 *CST* genes identified in this work were recovered only once each. This indicates that the screen is far from saturation, as can be expected for the relatively small number of clones screened ( $1.2 \times 10^5$ ). A minimal estimate puts the number of additional *CST*-type genes at 58. The spectrum of genes encountered in this screen also suggests that the screen is far from saturation. For example, given that two proteasome subunits (*PUP3* and *PRE1*) have dosage effects on chromosome stability, it is likely that so will many others. Thus the number of *CST*-type genes in yeast is likely high, and the overexpression-based screening provides a convenient way to identify them.

## Phenotypes produced by overexpression of *CST* genes

We have used several secondary criteria to identify the cellular processes affected by overexpression of *CST* genes: the patterns of chromosome instability, interaction with checkpoint mutations and sensitivity to agents affecting either DNA replication or mitotic spindle assembly.

Overexpression of the C-terminal portion of a kinesin-like mitotic spindle motor *Cin8p* produced a specifically mitotic set of traits, which may be due to its interference with the interaction between the endogenous *Cin8p* motor and its 'cargo'. The phenotype induced by overexpression of a replication factor C subunit *Rfc2p* clearly points to its function in DNA replication. Overexpression of *YRBI*, which affects multiple nuclear functions (31), caused a combination phenotype, as expected. A similar effect was produced by the overexpression of the C-terminal part of *NAT1*, a subunit of the N-terminal *N*-acetyl transferase. This enzyme modifies and presumably regulates numerous proteins (37), and likely affects many cellular functions. Thus our criteria can distinguish genes involved in different aspects of chromosome dynamics.

When applied to novel *CST* genes identified in this work, the same criteria pointed to *CST4* as a mitotic gene and to *CST6* as a DNA metabolism gene, and to *CST9* and *CST29* as possible global regulators of chromosome dynamics. The mitotic phenotype of *CST4* is in line with the regulation of its mRNA level, which is increased at mitosis (38). Interestingly, *CST9* has sequence similarity to the RING family of DNA-binding proteins. The specific functions of these genes remain to be determined, and the information obtained from their overexpression phenotype may provide a valuable guide in this process.

## Deletions of *CST* genes

We have created deletions of five *CST* genes, two of which are novel and three have been previously described, but not associated with chromosome stability functions. Deletions of *NAT1* and *SBA1* have a chromosome loss phenotype similar to that produced by their overexpression. In the case of *NAT1*, sensitivity to HU is a property of both the overexpression and deletion phenotypes. It appears that *NAT1* overexpression acts by a dominant negative mechanism. Since *Nat1p* is one of the two subunits of N-terminal *N*-acetyl transferase, it is likely that overexpression of the C-terminal portion of *Nat1p* interferes with the formation of the active heterodimer.

## Evolutionary conservation of *CST* genes

It was proposed that overexpression of heterospecific genes in yeast may be used to identify chromosome stability factors (17). We have tested this prediction for human cDNA libraries expressed in yeast, and obtained three genes similar to those recovered in the yeast cDNA screen: a core histone, a ribonucleotide reductase subunit and a cyclin. These proteins are very highly conserved in eukaryotes. The frequency of recovering human *CST*-type genes in yeast is ~10-fold lower than that of recovering yeast genes, which probably reflects both the greater number of genes in the human genome and the fact that the genes which are not sufficiently conserved may not have effect in a heterospecific environment. This means that screening for yeast *CST* genes, followed by identification of their human

homologs, may be the preferential route to identification of human genes involved in chromosome stability.

Our work shows that chromosome integrity and segregation are very sensitive to the balance of components of multi-molecular complexes involved in chromatin organization, ubiquitin-directed proteolysis, cell cycle control, DNA replication and mitotic spindle function. This may be one of the underlying mechanisms of genomic instability at the early stages of cancer, which are accompanied by deregulation of gene expression.

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