Novel Role for a *Saccharomyces cerevisiae* Nucleoporin, Nup170p, in Chromosome Segregation

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ABSTRACT

We determined that a mutation in the nucleoporin gene *NUP170* leads to defects in chromosome transmission fidelity (*ctf*) and kinetochore integrity in *Saccharomyces cerevisiae*. A *ctf* mutant strain, termed *s141*, shows a transcription readthrough phenotype and stabilizes a dicentric chromosome fragment in two assays for kinetochore integrity. Previously, these assays led to the identification of two essential kinetochore components, Ctf13p and Ctf14p. Thus, *s141* represents another *ctf* mutant involved in the maintenance of kinetochore integrity. We cloned and mapped the gene complementing the *ctf* mutation of *s141* and showed that it is identical to the *S. cerevisiae NUP170* gene. A deletion strain of *NUP170* (*nup170*Δ*::HIS3*) has a *Ctf*$^-$ phenotype similar to the *s141* mutant (*nup170-141*) and also exhibits a kinetochore integrity defect. We identified a second nucleoporin, *NUP157*, a homologue of *NUP170*, as a suppressor of the *Ctf*$^-$ phenotype of *nup170-141* and *nup170*Δ*::HIS3* strains. However, a deletion of *NUP157* or several other nucleoporins did not affect chromosome segregation. Our data suggest that *NUP170* encodes a specialized nucleoporin with a unique role in chromosome segregation and possibly kinetochore function.

CHROMOSOME segregation in the budding yeast *Saccharomyces cerevisiae* requires the coordinated interplay of proteins involved in chromosome structure, DNA replication, checkpoint control, spindle assembly, nuclear structure, and cell cycle progression. The nuclear envelope and the nuclear pore complexes (NPC) remain intact during mitosis in *S. cerevisiae*. Chromosome segregation takes place within the confines of the nuclear envelope and requires the nuclear transport of proteins, mRNA, and other substrates to ensure the fidelity of chromosome segregation. In *S. cerevisiae* and *Schizosaccharomyces pombe*, centromeres and telomeres are reported to be clustered near the nuclear envelope (Galy *et al.* 2000; Jin *et al.* 2000).

Proteins and mRNA enter and leave the nucleus through the NPC, a megadalton translocase embedded in the nuclear envelope (Fabre and Hurt 1997; Bucci and Wente 1997; Rout *et al.* 2000; Ryan and Wente 2000; Wente 2000). Often, directional transport of proteins through the NPC is facilitated by karyopherins and the RanGTP system (Gorlich and Kutay 1999; Hood and Silver 1999; Nakielny and Dreyfuss 1999). In *S. cerevisiae* several karyopherins (Srp1p and Cse1p) and components of the RanGTP system (Gsp1p and Rna1p) are determinants of chromosome segregation fidelity (Xiao *et al.* 1993; Loeb *et al.* 1995; Ouspenski *et al.* 1999). It has been reported recently that regulated nuclear transport of cyclins and other proteins is required for cell cycle progression (Jones *et al.* 2000; Yuste-Rojas and Cross 2000). At least two proteins required for proper chromosome segregation are associated with the nuclear envelope. These include Mps2p and Ndc1p, which are required for spindle pole body (SPB) formation and function in the nuclear envelope (Thomas and Botstein 1986; Winey *et al.* 1993; Chial *et al.* 1998; Munoz-Centeno *et al.* 1999). Ndc1p represents a shared component between the SPB and the NPC. The SPB and the kinetochore (centromere DNA and associated proteins) provide sites of attachment for microtubules, thereby allowing directed chromosome movement during mitosis, which is tightly regulated by checkpoint proteins (for review, see Skibbens and Hieter 1998).

Genetic analysis of *S. cerevisiae* mutants with a chromosome transmission fidelity (*Ctf*$^-$) phenotype has identified several kinetochore proteins, including Ctf13p, Ctf14p/Ndc10p, Ctf19p, Skp1p, Sgt1p, a cohesion factor Ctf7p/Eco1p (Doheny *et al.* 1993; Connelly and Hieter 1996; Hyland *et al.* 1999; Kitagawa *et al.* 1999; Skibbens *et al.* 1999; Pidoux and Allshire 2000), and Spt4p (Basrai *et al.* 1996). Two *in vivo* assays for kinetochore integrity were developed and used to further characterize the *ctf* mutants (Doheny *et al.* 1993). The first assay detects relaxation of a transcriptional block formed at the centromere (the transcription readthrough assay) and the second assay examines the mitotic stability of

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a test dicentric chromosome (the dicentric stabilization assay). To identify additional determinants of kinetochore function we examined 26 *ctf* mutants in these assays. One *ctf* mutant, *spt4-138,* led to the identification of Spt4p, a putative regulator of chromatin structure, as a determinant of chromosome segregation (Basrai *et al.* 1996).

In this article we describe our analysis of a *ctf* mutant, *s141,* which tested positive in the two *in vivo* assays for kinetochore integrity. We determined that the *s141* mutation is allelic to the nucleoporin *NUP170*. *NUP170* was previously identified in genetic and biochemical screens for components of the NPC (Aitchison *et al.* 1995; Kenna *et al.* 1996). Our data show that Nup170p is a determinant of high-fidelity chromosome segregation and possibly kinetochore integrity, thereby defining a novel function for a nucleoporin. We envision several possibilities by which Nup170p could influence the fidelity of chromosome segregation. In this work we present genetic evidence to define Nup170p as a specialized nucleoporin and discuss its potential role in chromosome segregation and possibly kinetochore function.

MATERIALS AND METHODS

Yeast media, strains, and plasmids: Media for yeast growth and sporulation were as described (Adams 1997) except where indicated. A large reference set of chromosome transmission fidelity mutants, the *ctf* mutants of *S. cerevisiae,* has been described previously (Spencer *et al.* 1990). For experiments monitoring the loss of the chromosome fragment (CF), adenine was added to 6 μg/ml to enhance the color of the red pigment in *ade2-101* strains (Gerring *et al.* 1990). *S. cerevisiae* strains used in this study are listed in Table 1. Plasmid B1820 containing a *Spe*I-*Not*I fragment of *NUP170* in pRS316, generously provided by L. Davis, was used as a *NUP170* control plasmid (Kenna *et al.* 1996). Plasmids pMB259 and pMB261 containing the *NUP157* and *NUP170* genes, respectively, were identified by functional complementation of the *Ctf*⁻ phenotype of the *s141* (*nup170-141*) strain as described below. Plasmids containing *NUP53* (pRS244-*NUP53*), *NUP59* (pRS244-*NUP59*), and *KAP121* (pRS314-*KAP121*) were provided generously by M. Marelli and R. Wozniak.

Cloning and characterization of the gene(s) complementing the *Ctf*⁻ phenotype of *s141*: The gene complementing the *Ctf*⁻ phenotype in mutant strain *s141* was cloned from a yeast genomic library inserted into a pBR322-based *LEU2 CEN4 ARS1* shuttle vector, pSB32 (F. Spencer and P. Hieter, unpublished data). Two transformants with a wild-type phenotype were identified from a total of ~10,000 transformants that were screened at 30°. Plasmid DNA isolated from the two transformants was able to complement the *Ctf*⁻ phenotype of the *s141* mutant. One of the plasmids contained the *NUP170* (pMB261) gene and the second contained the *NUP157* (pMB259) gene along with other sequences. Subcloning experiments confirmed that the genes *NUP170* and *NUP157* complemented the *Ctf*⁻ phenotype of the *s141* mutant. Genetic linkage analysis was done by subcloning a fragment from plasmids pMB259 and pMB261 into pRS305 (*LEU2*; Sikorski and Hieter 1989). The resulting plasmids, pMB269 and pMB271, respectively, were linearized and integrated into the genome of YPH102 wild-type cells. After confirmation of the integration event, the YPH102 transformants were mated to the *s141* strain. The diploids were sporulated and tetrad analysis showed that the *LEU2* marker from pMB271 (*NUP170*) segregated away from the *Ctf*⁻ phenotype (due to the *s141* mutation) in 36 tetrads that were analyzed. These results showed that the *s141* mutation is linked to the *NUP170* gene.

***In vivo* assay for kinetochore integrity—centromere transcriptional readthrough assay:** The kinetochore integrity reporter plasmids pMB203 and pMB204, containing a *GAL10* promoter that initiates transcription of an actin-LacZ fusion gene, have been described previously (Basrai *et al.* 1996). The two plasmids are identical except that pMB203 has a wild-type centromere (*CEN*) *VI* and pMB204 has a mutant *CENVI* (CDEIII G to C mutation). The plasmids were digested with *Not*I and integrated into chromosome III at the *leu2* locus. The structurally dicentric plasmids were maintained in a functionally monocentric state by keeping the transformed strains on galactose-containing media, thereby causing transcriptional inactivation of the test centromere. After confirming the site of integration by PCR, at least two independent transformants were tested on media containing the chromogenic substrate X-Gal as described previously (Doheny *et al.* 1993; Basrai *et al.* 1996). The color of colonies on X-Gal-containing plates was recorded after incubation at 25° for 5–6 days. Quantitation of the β-galactosidase activities was performed in triplicate using liquid *o*-nitrophenyl-β-D-galactopyranoside (ONPG) assays as outlined in the Clontech two-hybrid analysis manual (Clontech, Palo Alto, CA). Briefly, cells were grown in synthetic galactose-containing media at 30° to early log-phase, harvested, washed, and resuspended in Z buffer. Cells were then frozen in liquid nitrogen and thawed, and ONPG and β-mercaptoethanol were added to start the color reactions. Color reactions were stopped by addition of Na_2CO_3, and the OD_{420} was determined spectrophotometrically. β-Galactosidase units were calculated with respect to the number of cells used in each sample. We determined the activity for a wild-type strain containing a wild-type *CENVI* (β-galactosidase units = 0.23), which was arbitrarily set to 1.0, and used this to normalize values for the other strains.

***In vivo* assay for kinetochore integrity—dicentric stabilization assay:** The reporter constructs (pMB205-wild-type *CENVI* and pMB206-mutant *CENVI*) were integrated at the *MES1* locus on the CF, thereby creating a dicentric chromosome. The presence of a dicentric chromosome was confirmed by Southern blot analysis of orthogonal field alternating gel electrophoresis (OFAGE) gels. The GAL-*CEN* of the reporter allowed control of *CEN* activity; the reporter is not on in galactose-containing media (*CEN* OFF) and on in glucose-containing media (*CEN* ON). Strains containing the dicentric CF were plated on synthetic complete medium with glucose and results were recorded after incubation at 30° for 5–6 days.

Quantitation of chromosome loss phenotype: Homozygous diploid *nup170*Δ*::HIS3/nup170*Δ*::HIS3* (YMB589), *nup170-141/nup170*-141 (YMB514), and *NUP170/NUP170* (YPH279) strains were used to quantitate the chromosome missegregation rate as previously described (Gerring *et al.* 1990; Basrai *et al.* 1996). Strains were plated on synthetic media containing 6 μg/ml adenine and the numbers of 1:0 chromosome loss events $[(hR/hP)/CF_{tot}]$ and 2:0 chromosome nondisjunction events $[(hR/hW))/CF_{tot}]$ were determined. In this calculation hR represents the number of half-red colonies, hP represents the number of half-pink colonies, hW represents the number of half-white colonies, and CF_{tot} represents the number of colonies "born" with at least one chromosome fragment (pink). Mating phenotypes of homozygous diploid strains to assess possible loss of endogenous chromosome III were determined as descibed previously (Gerring *et al.* 1990). In this assay we mated a known number of diploid cells (*ctf* mutants and wild-type cells) with a fixed number of *MAT***a** haploid

TABLE 1

Strains used in this study

S. cerevisiae strain	Genotype	Source or Reference
spt4-138	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ1 leu2Δ1 spt4-138 CFVIII(RAD2d.YPH277)URA3 SUP11*	Basrai *et al.* (1996)
s141	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ1 leu2Δ1 nup170-141 CFVIII(RAD2d.YPH277)URA3 SUP11*	This work
YPH102	*MATα ura3-52 lys2-801 ade2-101 his3-Δ200 leu2Δ1*	Spencer *et al.* (1990)
YPH254	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ1 his3-Δ200 leu2Δ1 CFVIII(RAD2d.YPH277)URA3 SUP11*	F. Spencer and P. Hieter
YPH272	*MATa/***a** *ura3-52 /ura3-52 lys2-801/lys2-801 ade2-101/ade2-101 TRP1/trp1Δ1 leu2Δ1/leu2Δ1 HIS3/his3-Δ200 CFVII(RAD2d.YPH277)URA3 SUP11*	F. Spencer and P. Hieter
YPH277	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ1 leu2Δ1 CFVII(RAD2d.YPH277)URA3 SUP11*	Spencer *et al.* (1990)
YPH278	*MATα ura3-52 lys2-801 ade2-101 his3-Δ200 leu2Δ1 CFIII (CEN3L.YPH278)URA3 SUP11*	Spencer *et al.* (1990)
YPH279	*MAT***a**/α *ura3-52 /ura3-52 lys2-801/lys2-801 ade2-101/ade2-101 TRP1/trp1Δ1 leu2Δ1/leu2Δ1 HIS3/his3-Δ200 CFVII(RAD2d.YPH277)URA3 SUP11*	Gerring *et al.* (1990)
YPH499	*MAT***a** *ura3-52 lys2-801 ade2-101 his3-Δ200 trp1Δ63 leu2Δ1*	Sikorski and Hieter (1989)
YPH500	*MATα ura3-52 lys2-801 ade2-101 his3-Δ200 trp1Δ63 leu2Δ1*	Sikorski and Hieter (1989)
YPH987	*MAT***a**/α *ura3-52 /ura3-52 lys2-801/lys2-801 ade2-101/ade2-101 trp1Δ63/trp1Δ63 leu2Δ1/leu2Δ1 his3-Δ200/his3-Δ200 CFIII (CEN3L.YPH983)TRP1 SUP11*	P. Hieter
YPH1022	*MATα ura3-52 lys2-801 ade2-101 his3-Δ200 trp1Δ63 leu2Δ1 CFIII (CEN3L.YPH987)TRP1 SUP11*	P. Hieter
YMB11	*MAT***a**/α *ura3-52 /ura3-52 lys2-801/lys2-801 ade2-101/ade2-101 trp1Δ1/TRP1 leu2Δ1/leu2Δ1 his3-Δ200/HIS3 ctf19-26/ctf19-58 CFIII (CEN3L.YPH983)TRP1 SUP11*	P. Hieter
YMB48	*MAT***a**/α *ura3-52 /ura3-52 lys2-801/lys2-801 ade2-101/ade2-101 trp1Δ1/TRP1 leu2Δ1/leu2Δ1 his3-Δ200/HIS3 spt4-138/spt4-138 CFVII(RAD2d.YPH277)URA3 SUP11*	Basrai *et al.* (1996)
YMB301	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ1 leu2Δ1 nup170Δ::HIS3 CFVIII(RAD2d.YPH277)URA3 SUP11*	This work
YMB328	*MAT***a**/α *ura3-52 /ura3-52 lys2-801/lys2-801 ade2-101/ade2-101 trp1Δ1/TRP1 leu2Δ1/leu2Δ1 his3-Δ200/his3-Δ200 spt4Δ2::HIS3/spt4Δ2::HIS3 CFVII(RAD2d.YPH277)URA3 SUP11*	Basrai *et al.* (1996)
YMB514	*MAT***a**/α *ura3-52 /ura3-52 lys2-801/lys2-801 ade2-101/ade2-101 trp1Δ1/trp1Δ1 leu2Δ1/leu2Δ1 his3Δ-200/his3-Δ200 nup170-141/nup170-141 CFVII(RAD2d.YPH277)URA3 SUP11*	This work
YMB571	*MATα ura3-52 lys2-801 ade2-10 trp1Δ1 leu2Δ1 his3Δ-200 nup170Δ::HIS3*	This work
YMB589	*MAT***a**/α *ura3-52 /ura3-52 lys2-801/lys2-801 ade2-101/ade2-101 TRP1/trp1Δ1 leu2Δ1/leu2Δ1 his3-Δ200/his3-Δ200 nup170Δ::HIS3/nup170Δ::HIS3 CFVII(RAD2d.YPH277)URA3 SUP11*	This work
YMB609	*MATα ura3-52 lys2-801 ade2-101 trp1Δ1 leu2Δ1 nup170-141 CFIII (CEN3L.YPH983)TRP1 SUP11*	This work
YMB640	*MATα his3Δ200 lys2-801 leu2Δ1 ura3-52 ade2-101 trp1Δ63 nup157Δ::HIS3 CFIII (CEN3L.YPH983)TRP1 SUP11*	This work
YMB689	*MATα ura3-52 lys2-801 ade2-101 trp1Δ1 leu2Δ1 nup2Δ::TRP1 CFVIII(RAD2d.YPH277)URA3 SUP11*	This work
YMB703	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ1 leu2Δ1 pom152Δ::kan CFVIII(RAD2d.YPH277)URA3 SUP11*	This work
YMB1223	*MAT***a** *his3Δ200 lys2-801 leu2Δ1 ura3-52 ade2-101 trp1Δ63 nup59Δ::HIS3 CFIII (CEN3L.YPH983)TRP1 SUP11*	This work
YMB1338	*MAT***a** *his3Δ200 lys2-801 leu2Δ1 ura3-52 ade2-101 trp1Δ63 nup53Δ::kan CFIII (CEN3L.YPH983)TRP1 SUP11*	This work
YMB1391	*MATα his3Δ200 lys2-801 leu2Δ1 ura3-52 ade2-101 trp1Δ63 nup188Δ::HIS3 CFIII (CEN3L.YPH983)TRP1 SUP11*	This work
17/14	*MAT***a** *his1*	G. Fink
17/17	*MATα his1*	G. Fink

CF denotes the nonessential chromosome fragment as described in Spencer *et al.* (1990).

mating testers and examined the formation of putative triploids. Mating was allowed for 6 hr at 25° in 75% YEPD medium. Cells were washed, resuspended in water, spread on SD plates, and incubated for 2 days at 30°, and the plate was photographed.

Nucleoporin gene deletions: Unless noted otherwise, complete chromosomal deletions of *NUP170*, *NUP157*, *NUP53*, *NUP188*, *NUP59*, *NUP2*, and *POM152* were generated in strain YPH987 using a modification of a PCR-based technique (Baudin *et al.* 1993). For deletions of *NUP170* and *NUP157*, we used a PCR product derived from 40 bp of sequences immediately upstream of the start and downstream of the stop codons of

the gene to be deleted plus 20 bp of sequence homologous to pRS305 (Sikorski and Hieter 1989) immediately adjacent to the vector-selectable marker. The oligonucleotides were used to amplify a *HIS3* marker from pRS303 and the PCR product was transformed into the diploid strain YPH272 for the deletion of *NUP170* and YPH987 for the deletion of *NUP157*. A deletion of *NUP53* was constructed using a PCR product that included ~200 bp upstream and downstream of *NUP53* plus the *KAN* deletion cassette derived from a *nup53Δ::kanMX4* deletion strain (strain no. 10734; Research Genetics, Huntsville, AL). A deletion of *NUP188* was constructed as the *nup53* deletion, except that strain *nup188Δ::HIS3(4-1-1)* (provided by R. Wozniak) was used as a template for primers annealing ~200 bp upstream and downstream of *nup188Δ::HIS3*. *NUP59* was deleted by transformation of a *Hin*dIII-linearized construct *pBS-Nup59Δ::HIS3* (provided by R. Wozniak) into strain YPH987. *NUP2* was deleted by transformation of a *Bam*HI-*Spe*I fragment containing *nup2Δ::TRP1* from LBD61 (provided by L. Davis) into strain YPH272. *POM152* was deleted by transformation of a PCR product derived from 40 bp of sequences immediately upstream of the start and stop codons of *POM152* plus 20 bp of sequence homologous to pRS400 (Brachmann *et al.* 1998) immediately adjacent to the *KAN* marker. The deletions were confirmed by Southern blot analysis and/or PCR analysis using the following primer pairs: (a) flanking the gene to be deleted, and (b) two sets of primer pairs, each of which contains one primer specific for the gene and the other for the marker used for the deletion. After confirmation of the deletions, the heterozygous diploid strains were sporulated and haploid meiotic progeny containing the deletion and a CF were assayed for a *Ctf*$^-$ phenotype at 25°, 30°, 32°, and 37°.

Construction of green fluorescent protein-tagged *NUP170* and immunoelectron microscopy: *NUP170* was tagged with the gene encoding green fluorescent protein (GFP) as follows. *NUP170* and the adjoining promoter sequences were amplified from pMB261 with the following primers: 5′ *Xho*I primer for *NUP170* (5′-CCGCTCGAGTCTAGTTCCTACTCTGG-3′) and 3′ *Not*I primer for *NUP170* (5′-ATAGTTTAGCGGC CGCCTTCTTTGTAGAAACAAATC-3′). PCR amplification was achieved with Life Technologies (Rockville, MD) Elongase enzyme mix. The resulting PCR product was digested with *Xho*I and *Not*I and cloned into pBSSKII+ (Stratagene, La Jolla, CA) to form pBSN170. The *Xho*I/*Not*I *NUP170* fragment from pBSN170 was cloned into pAA3 (Sesaki and Jensen 1999). In pAA3, *NUP170* (containing its own promoter) is placed in frame with *GFP* to form pOKN170G. A second plasmid, pOKN170-HA, was constructed by cloning the *Xho*I/*Not*I *NUP170* fragment from plasmid pOKN170G in frame with the sequence encoding the hemagglutinin (HA) epitope tag in *LEU2/CEN* plasmid pAA1 (Sesaki and Jensen 1999). The plasmids pOKN170G and pOKN170-HA complemented the *nup170* growth and *Ctf*$^-$ phenotypes. For immunoelectron microscopy a strain containing a chromosomally tagged *NUP170-GFP* as the only source of Nup170p was analyzed as described previously (Chial *et al.* 1998).

Gel mobility shift assays: Gel mobility shift assays with whole cell yeast extracts and radiolabeled *CENIII* DNA probes containing centromere determinant elements (CDE) I + II or III were performed by previously described procedures (Doheny *et al.* 1993; Basrai *et al.* 1996). Binding reactions were done at 30° for 30 min and electrophoresed on 4% polyacrylamide gels.

Chromatin immunoprecipitation: Chromatin immunoprecipitations (ChIPs) were performed as described previously (Meluh and Koshland 1997; Megee *et al.* 1999). Briefly, cells expressing *NUP170-HA* or *MCD1-6HA* were grown in synthetic dextrose-containing medium to an OD_{600} of ~1.0 and cross-linked for 2 hr at 25° with 1% formaldehyde in the medium. Cells were spheroplasted and sonicated until DNA was in the 100–2000 bp range. The resulting mixture was saved as total chromatin. Crosslinked chromatin was immunoprecipitated with anti-HA antibodies (Roche Molecular Biochemicals, Indianapolis) coupled to agarose beads (Immunopure; Pierce, Rockford, IL) or mock precipitated without the addition of coupled antibodies. Immune complexes were harvested, washed, and eluted from the beads. Formaldehyde crosslinks were reversed for 4 hr at 65°. Precipitated chromatin was extracted with phenol/chloroform and finally analyzed for the presence of *CENIII* and nonspecific sequences with the following primer pairs: (*CENIII*) PM22, 5′-GATCAGCGC CAAACAATATGG-3′; and PM48, 5′-AACTTCCACCAGTAA ACGTTTC-3′; (*TUP1*) OMB209, 5′-CTAGCCGCTGCATCTG CATCTGTTC-3′; and OMB210, 5′-GGTGCCTTGACAGAGG GTAAAGTAGTGG-3′.

RESULTS

***In vivo* assays for kinetochore integrity lead to the identification of *s141*:** To identify proteins required for chromosome segregation in *S. cerevisiae*, we adapted two kinetochore integrity assays to screen a subset of 26 *ctf* mutants (from a total of 88 *ctf* mutants). In the first assay (the transcription readthrough assay), we measured the transcriptional readthrough of a *lacZ* reporter gene through an ectopically placed *CENVI* in the genome of the *ctf* mutants (Doheny *et al.* 1993; Basrai *et al.* 1996). Transcription of the lacZ reporter gene through the kinetochore is possible when a mutation *in cis* or *in trans* weakens the *CEN* DNA/protein complex. In the second *in vivo* kinetochore integrity assay (the dicentric stabilization assay), we monitored the stability of a test dicentric chromosome in the *ctf* mutants. It is thought that a dicentric CF can proceed intact to the spindle pole when the spindle forces on a dicentric chromatid are relieved due to a weakened kinetochore (Mann and Davis 1983; Haber and Thorburn 1984; Doheny *et al.* 1993). In the dicentric stabilization assay *CENVI* was integrated into the CF and placed under control of the inducible *GAL* promoter. All strains were maintained on galactose-containing media to induce the *GAL10* promoter and inactivate the conditional *CEN*. This analysis led to the identification of five additional *ctf* mutants (*s138*, *s141*, *s149*, *s145*, and *s151*) as putative determinants of kinetochore function. We have previously shown that one of the mutants, *s138*, is allelic to the *S. cerevisiae SPT4* gene (Basrai *et al.* 1996). One of the mutants, *s141*, which tested positive for both kinetochore integrity assays, was chosen for further studies.

The nucleoporin gene *NUP170* complements the *ctf* defect of *s141*: To isolate the gene that could complement the *Ctf*$^-$ phenotype of the *s141* mutant we transformed *s141* cells with a yeast genomic library (materials and methods). We identified two independent plasmids with nonoverlapping inserts that complemented the *Ctf*$^-$ phenotype of the *s141* mutant. These plasmids contained full-length copies of the nucleoporin genes *NUP170* and

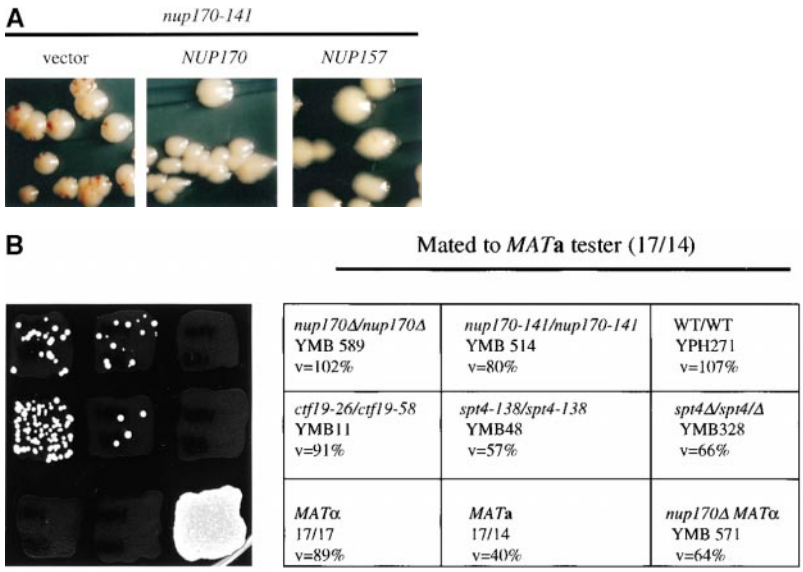

FIGURE 1.—Chromosome-loss phenotypes of *nup170* deletion and *nup170-141* mutants. (A) The *ctf* strain *s141 (nup170-141)* containing a nonessential chromosome fragment was transformed with the following plasmids: *CEN-LEU2* (pRS315, vector alone), *CEN-NUP170* (pMB261), or *CEN-NUP157* (pMB259). Transformants were scored for complementation of the *Ctf*⁻ phenotype after growth for 4–5 days at 30°. A *ctf* mutant gives rise to sectored colonies (red sectors, *ade2-101*) due to loss of the nonessential CF; complementation of the mutant phenotype gives rise to white colonies. (B) A total of 200,000 viable *MAT***a** cells (17/14) were incubated with 50,000 cells of the indicated diploid cells (top and middle rows of patches). As a mating control 17/14 cells were also mated to a *MAT*α *nup170* deletion strain (bottom row). Diploids from the mating of strain 17/17 to strain 17/14 fail to grow on this medium due to lack of complementation of the auxotrophic markers. A total of 500 cells of each strain were independently plated on rich YEPD medium to assess percentage viability (*v*) of the cells in the mating assay.

NUP157 (AITCHISON *et al.* 1995; KENNA *et al.* 1996). The *Ctf*⁻ phenotype of *s141* is complemented by plasmids containing either *NUP170* or *NUP157* in a low-copy *CEN* vector (Figure 1). Subsequently, we showed by integrative mapping that the *s141* mutant is allelic to the *NUP170* gene (see MATERIALS AND METHODS) and not to *NUP157*. These results indicate that *NUP157* is a suppressor of *s141*. We renamed the *s141* strain and henceforth refer to this mutant as *nup170-141*. Nup170p is 42% identical and 61% similar to *S. cerevisiae* Nup157p (AITCHISON *et al.* 1995; KENNA *et al.* 1996; COSTANZO *et al.* 2000). Furthermore, mutations in *NUP170* and *NUP157* are synthetically lethal (AITCHISON *et al.* 1995; KENNA *et al.* 1996). Our finding that a nucleoporin is required for the fidelity of chromosome segregation prompted further analysis for the role of *NUP170* in chromosome segregation and possibly kinetochore function.

Mutation in *NUP170* leads to defects in chromosome segregation and kinetochore integrity: Since the *nup170-141* mutant displays a *Ctf*⁻ phenotype we asked if a deletion of the entire *NUP170* gene could also result in a chromosome-loss phenotype. We found that a deletion of *NUP170* (*nup170*Δ*::HIS3*) like the *nup170-141* mutant leads to loss of the chromosome fragment. *NUP157* functionally complements the *Ctf*⁻ phenotype in both the *nup170-141* (Figure 1A) and *nup170*Δ strains (data not shown).

We examined if the phenotype of CF loss observed for the *nup170* mutants extended to the loss of endogenous chromosomes in the cell. We determined this by assaying the mating ability of *nup170* homozygous diploid strains *(nup170-141/nup170-141; nup170*Δ*/nup170*Δ) and control strains that include a homozygous wild-type strain, haploid strains, and two other homozygous *ctf* mutant strains (*spt4* and *ctf19)*. A mating phenotype of diploid cells suggests loss of chromosome III or homozygosis of the MATα locus on chromosome III by recombination or gene conversion (SPENCER *et al.* 1990). Previous results with the *ctf* mutants *ctf13-30* (DOHENY *et al.* 1993), *ctf18/chl12* (KOUPRINA *et al.* 1994), and *ctf1/chl1* (GERRING *et al.* 1990) have shown that the majority of the triploids arise due to chromosome loss. From our analysis we find that *nup170*, *spt4-138*, and *ctf19* diploids exhibit a mating phenotype (Figure 1B). These data suggest the loss of endogenous chromosome III, implying that other chromosomes may likewise be lost in *nup170* mutants.

We quantitated the CF missegregation defect in homozygous *nup170-141* and *nup170*Δ strains (Table 2). By means of the colony color assay (HIETER *et al.* 1985) we assessed two types of chromosome missegregation events, chromosome loss (1:0 segregation) and chromosome nondisjunction (2:0 segregation). Our results show that chromosome loss in both *nup170* mutants was increased ~100-fold and that nondisjunction was increased by ~10-fold. An increase in chromosome loss (1:0) events for the *nup170* mutants is not inconsistent with a role for Nup170p in chromosome segregation since similar observations have been made for other *ctf* mutants (GERRING *et al.* 1990; DOHENY *et al.* 1993; KOUPRINA *et al.* 1994; BASRAI *et al.* 1996; HYLAND *et al.* 1999). A preponderence of 2:0 events would probably reflect a more direct role for Nup170p in kinetochore function.

We tested if *nup170*Δ*::HIS3*, like a *nup170-141* strain, has a defect in kinetochore integrity using two *in vivo* assays (MATERIALS AND METHODS). In the first assay, the transcriptional readthrough assay, we showed that β-galactosidase activity was increased in the *ctf* mutants,

TABLE 2

Quantitation of chromosome loss in *nup170/nup170* homozygous diploids

Genotype	CF missegregation rate		No. of colonies[a]
	% 1:0 events[b]	% 2:0 events[c]	
NUP170/NUP170	0.03	0.03	29046
nup170-141/nup170-141	2.19	<0.13	776
nup170Δ/nup170Δ	2.53	<0.18	552

The CF missegregation rates [loss (1:0) and nondisjunction (2:0) events] for the non-essential CF were measured as described previously (Gerring *et al.* 1990). The rates for *NUP170/NUP170* wild-type strain YPH279 are from previously reported data (Gerring *et al.* 1990). The CF loss rates for the *nup170* mutants were determined in duplicate for two independent isolates of homozygous *nup170-141/nup170-141* (YMB514) and *nup170Δ::HIS3/nup170Δ::HIS3* (YMB589).

[a] Total number of pink colonies (colonies with one CF).

[b] Number of half-red, half-pink colonies divided by the total number of colonies with one CF.

[c] Number of half-red, half-white colonies divided by the total number of colonies with one CF.

spt4-138, *nup170-141*, and *nup170Δ* strains, and a wild-type strain with a mutant *CEN* [WT (CDEIII 15C)]. The transcriptional readthrough activity was increased ~8- and 50-fold, respectively, for the *nup170Δ::HIS3* (*nup170Δ*) and the *nup170-141* strains compared to a wild-type strain [WT (*CEN*); Figure 2]. In this assay the *nup170-141* mutant displays a more severe phenotype than the *nup170Δ* deletion. A similar observation has been previously described for the *spt4-138* mutant compared to the *spt4Δ::HIS3* strain (Basrai *et al.* 1996) and may be due to the dominant interfering properties of a mutant gene product.

In the second assay, the dicentric stabilization assay, we found that both *nup170-141* and *nup170Δ::HIS3* strains stabilized the dicentric CF, resulting in a sectoring phenotype. Controls included wild-type cells and an uncharacterized *ctf* mutant *s150* (Figure 3B), which showed rapid loss of the dicentric CF, thus giving rise to red colonies. A dicentric chromosome can also be stabilized in wild-type cells when one *CEN* is mutated (CDEIII-15C Figure 3C). These data suggest a deletion of *NUP170* just as the *nup170-141* mutant results in altered kinetochore function as assayed in the tests for kinetochore integrity.

On the basis of the phenotypes of kinetochore integrity, we examined if *nup170* mutants showed genetic interactions with kinetochore mutants. We determined that double mutant strains containing *nup170Δ* and the kinetochore mutations *ctf13-30*, *ndc10-42/ctf14-42*, *skp1-4*, *cbf1Δ*, and *ctf19Δ* do not exhibit synthetic growth phenotypes. Likewise, overexpression of the kineto-

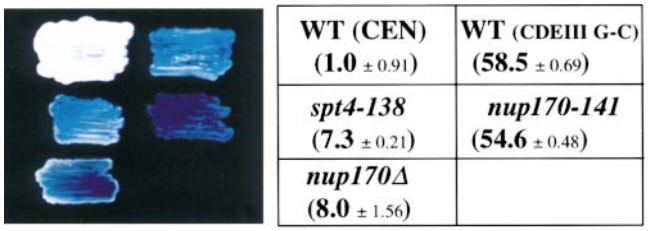

Figure 2.—Nucleoporin mutants *nup170-141* and *nup170Δ::HIS3* allow transcription of the β-galactosidase reporter gene through a wild-type centromere. The reporter construct pMB203 containing a wild-type centromere *(CENVI)* was integrated in the following strains: wild-type (WT) strain (YPH277), *spt4-138*, *nup170Δ* (YMB301), or *nup170-141*. A control included the reporter construct pMB204 with a mutant *CENVI* (CDEIII G-C mutation) in wild-type strain (YPH277). Transformants were patched to medium containing X-Gal and allowed to grow for 5 days at 30°, and results were recorded. β-Galactosidase units were determined by liquid ONPG assays (materials and methods) and all reported values (under strain description) were normalized to the wild-type control strain (β-galactosidase units = 0.23) set at a value of 1.0. Standard errors of measurement are as indicated.

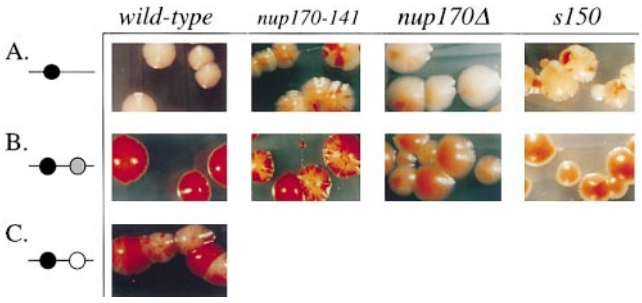

Figure 3.—Nucleoporin mutants *nup170-141* and *nup170Δ::HIS3* missegregate a CF and stabilize a dicentric CF. Wild-type strain (YPH277) and the *ctf* mutants *nup170-141* (*s141*), *nup170Δ* (YMB301), and *s150* containing a monocentric CF (solid circle; A) were assayed for a *Ctf*$^-$ phenotype. A *ctf* mutant produces sectored colonies (red sectors, *ade2-101*) due to loss of the nonessential CF, and wild-type cells produce white colonies. The same strains containing a dicentric CF (solid circle and shaded circle; B) were maintained on galactose-containing media (monocentric, second *CEN* OFF) and plated on glucose-containing media (dicentric, second *CEN* ON). Transformants were scored after 5–6 days at 30°. A control included a wild-type strain (YPH277) containing a dicentric CF with a weak *CEN* (open circle; C). Stabilization of the dicentric CF results in sectored colonies; loss of the dicentric CF leads to the formation of red colonies.

chore protein-encoding genes *CTF13*, *NDC10/CTF14*, *SKP1*, and *CBF1* from 2μ plasmids did not alleviate the *Ctf*⁻ phenotype of *nup170-141* mutants (data not shown).

The majority of Nup170p is distributed across the nuclear envelope throughout the cell cycle: To delineate the potential role of *NUP170* in chromosome segregation we determined if the subcellular localization of Nup170p was dependent on the cell cycle. A *CEN* plasmid containing the *NUP170* gene fused at the C terminus with GFP was transformed into the *nup170Δ::HIS3* strain. The *NUP170-GFP* plasmid complemented both the *nup170Δ::HIS3* growth and the *Ctf*⁻ phenotypes (data not shown). In logarithmically growing cells, Nup170p-GFP stained the nuclear envelope brightly and was distributed across the nuclear envelope independent of cell cycle stage (Figure 4).

We also investigated if Nup170p-GFP colocalized with the SPB in the nuclear envelope. The rationale for this analysis is based on the data that the spindle pole body component, Ndc1p, is a component shared between the NPC and SPB. Furthermore, both Ndc1p and Nup170p are reported to interact with the nucleoporins Nup53p and Nup59p (Chial *et al.* 1998; Marelli *et al.* 1998; Uetz *et al.* 2000). To confirm that the fluorescence pattern for Nup170p-GFP reflects nucleopore association and to explore the possibility that like Ndc1p, Nup170p may be a shared component between NPC and SPB, we did electron microscopy. We determined the localization of Nup170p-GFP in a strain containing an integrated chromosomal version of *NUP170-GFP* as the only source of Nup170p in immunogold-labeling experiments. As previously reported, Nup170p-GFP was associated with nuclear pore structures. We did not find Nup170p-GFP to be colocalized with the SPB (data not shown).

Nup170p does not interact directly with the *CEN* DNA/protein complex: The positive results of our *in vivo* kinetochore integrity assays with *nup170* mutants prompted us to test for specific biochemical kinetochore defects *in vitro*. First, we tested whole-cell protein extracts from a *nup170-141* mutant strain in gel mobility shift assays for binding to *CENIII* probes (CDEI + II or CDEIII). The mobilities and signal intensities of the *CEN* DNA-protein complexes were nearly the same for both wild-type (*WT*) and the mutant strain (*nup170-141*) (Figure 5A).

In a second approach, crosslinked chromatin was immunoprecipitated with anti-HA from a strain expressing either HA-tagged Nup170p or Mcd1p, a cohesin that associates with many chromosomal loci, including centromeres (Megee *et al.* 1999), and assayed for the presence of specific DNA sequences by PCR. We found that amplification with *CENIII*-specific primers results in a robust amplification product in the total chromatin preparations and the precipitated Mcd1p-6HA samples (Figure 5B). Only background bands are visible in the mock precipitation and the Nup170p-HA samples. Thus, our results suggest that Nup170p-HA does not directly associate with *CEN* DNA.

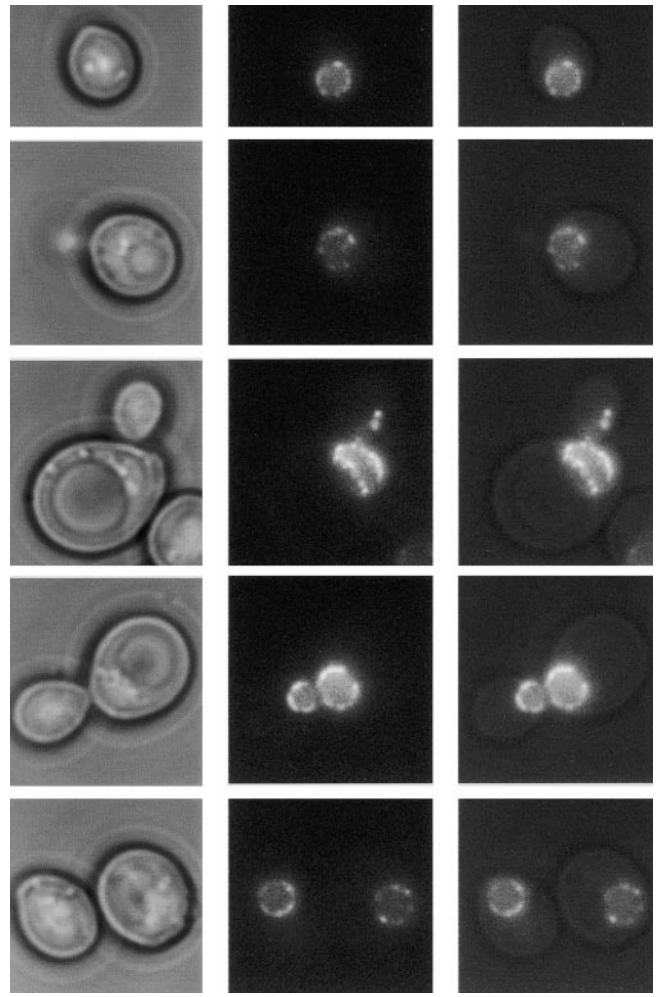

Figure 4.—Nup170p-GFP shows perinuclear staining of the yeast nucleus throughout the cell cycle. Strain *nup170Δ::HIS3* (YMB301) was transformed with *NUP170-GFP* plasmid pOKN170G. Transformants in early logarithmic phase of growth were examined by fluorescence microscopy. Fluorescent (middle column) and DIC (left column) images of representative GFP-stained cells were recorded and overlaid (right column). Perinuclear localization of Nup170p-GFP was confirmed by 4′,6-diamidino-2-phenylindole staining (data not shown).

***NUP170* may be a unique nucleoporin required for chromosome segregation:** We have shown that *NUP157* can suppress the *Ctf*⁻ phenotype of *nup170-141* mutants (Figure 1). Hence, we reasoned that increased expression of other nucleoporins may be able to suppress a *Ctf*⁻ phenotype of *nup170* mutants that may be due to a nuclear import defect. We tested if elevated levels of the nucleoporin-encoding genes, *NUP53* and *NUP59*, and the karyopherins, *SRP1* and *KAP121*, could alleviate the *Ctf*⁻ phenotype of *nup170-141* cells. Nup53p, Nup59p, and Kap121p have been shown to be part of a nuclear pore subcomplex that also contains Nup170p (Marelli

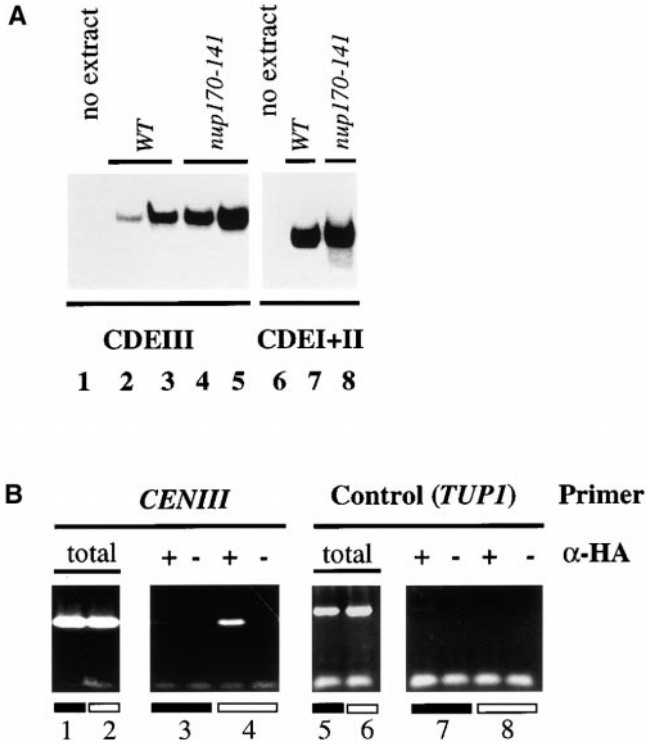

FIGURE 5.—Nup170p does not interact directly with *CEN* DNA/protein complex. (A) *In vitro* CDEIII or CDEI + II binding activities in cell extracts from *nup170-141* and wild-type cells. Whole-cell extracts from wild-type cells and *nup170-141* were prepared from logarithmically growing cells at 30°. Extracts (20 μg, lanes 2, 4, 7, or 40 μg, lanes 3, 5, 8) and labeled CDEIII or CDEI + II fragments were incubated at 30° for 30 min. Shown are the CDEIII/protein complex (lanes 1–5) or the CDEI + II/protein complex (lanes 6–8) after incubation with no extract or with the indicated extracts. Extract from a kinetochore mutant, *ctf13-30* (DOHENY *et al.* 1993), was used as a control for defective CDEIII binding (data not shown). (B) Nup170p-HA tests negative in a chromatin immunoprecipitation (ChIP) assay with *CENIII*-specific primers. ChIP assays were performed on chromatin samples prepared from strain YMB301 expressing Nup170p-HA (solid bars in lanes 1, 3, 5, and 7) and strain 1377A1-4B expressing Mcd1p-6HA (open bars in lanes 2, 4, 6, and 8) (MEGEE *et al.* 1999; MELUH and KOSHLAND 1997). Total chromatin (total), chromatin precipitated with anti-HA antibodies (-HA) (+), or mock-precipitated chromatin (-) were used in PCR amplification reactions. Shown are PCR reactions using centromere-specific primers, PM22 and PM48 *(CENIII)*, or *CEN* unrelated control *TUP1* primers, OMB209 and OMB210 (control).

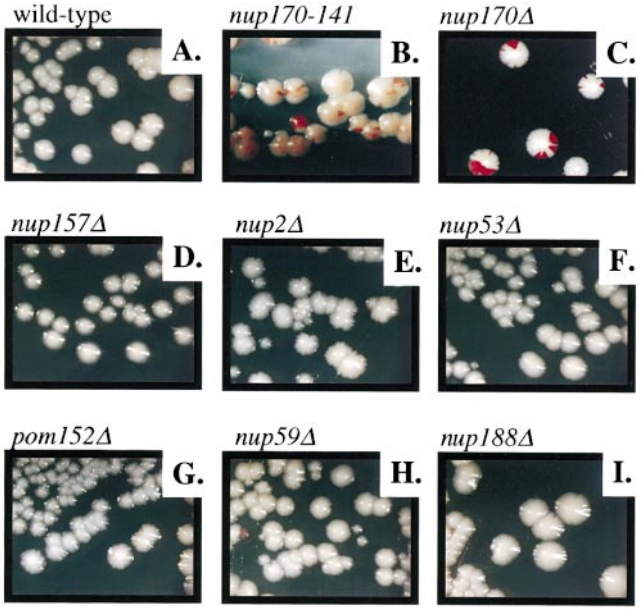

FIGURE 6.—*NUP170* encodes a specialized nucleoporin with a role in chromosome segregation. The nucleoporin genes *NUP170* (C), *NUP157* (D), *NUP2* (E), *NUP53* (F), *POM152* (G), *NUP59* (H), and *NUP188* (I) were deleted in the diploid strain YPH987 containing the nonessential CF (MATERIALS AND METHODS). The strains were sporulated and haploid progeny containing the nucleoporin deletion and a CF were scored for a potential *Ctf*$^-$ phenotype after incubation at 32° for 5 days. Nucleoporin deletion strains harboring the CF were also tested at 25°, 30°, and 37° (data not shown). Controls include a wild-type (A; haploid progeny of strain YPH987) and (B) the *nup170-141* strain.

et al. 1998). Overexpression of *NUP53* and *NUP59* from multicopy plasmids (2μ) did not suppress the *Ctf*$^-$ phenotype of the *nup170-141* mutant (data not shown). Since overexpression of *KAP121* from an inducible *MET* promoter is lethal in a wild-type strain (O. KERSCHER and M. BASRAI, unpublished results), we were unable to assess its overexpression phenotype. However, we found that *CEN* levels of *KAP121* and *CEN*/2μ levels of *SRP1* do not suppress the *Ctf*$^-$ phenotype of *nup170* mutants (data not shown).

NUP170 interacts genetically and physically with several other nuclear pore components. For example, *nup170* deletion mutants are synthetically lethal with the nucleoporin mutants *nup82*, *nup157*, *nup2*, *nup188*, *pom152*, and karyopherin *srp1* (FABRE and HURT 1997). To determine if other nucleoporins were required for chromosome segregation, we constructed and tested deletion strains for *NUP157*, *NUP2*, *NUP53*, *POM152*, *NUP59*, and *NUP188* (Figure 6). A *Ctf*$^-$ phenotype was observed only for the *nup170-141* and *nup170*Δ strains while deletions of other nucleoporins did not affect the loss of the CF. Therefore, our analysis suggests that *NUP170* may be a specialized nucleoporin with a role in chromosome segregation.

DISCUSSION

We have identified Nup170p, a nucleoporin, as a determinant of faithful chromosome segregation and possibly kinetochore function. Our data have shown that *nup170-141*, a *ctf* mutant, tests positive in two *in vivo* kinetochore integrity assays: the *CEN* transcriptional read through and the dicentric stabilization assays. We found that a deletion of *NUP170*, just like the *nup170-141* mutant allele, leads to a *Ctf*$^-$ phenotype and a kinetochore integrity defect. The nucleoporin *NUP157*,

a homolog of *NUP170,* is able to suppress the *Ctf*⁻ phenotype of *nup170* mutants. Unlike *nup170* deletion mutants, however, a deletion of *NUP157* and several other nucleoporins does not result in a *Ctf*⁻ phenotype. Results presented in this study suggest that Nup170p may be a specialized nucleoporin required for chromosome segregation.

From a total of 88 *ctf* mutants that have been screened so far, only 12 mutants have tested positive in the transcriptional readthrough assay. Only 3 of the 12 mutants [*ctf13-30, ctf14-42/ndc10-42* (Doheny *et al.* 1993), and *nup170-141*] have also tested positive in the dicentric stabilization assay for kinetochore integrity. Quantitation of the *Ctf*⁻ phenotype showed that *nup170* mutants exhibit an ~100-fold increase in the loss of a nonessential chromosome fragment comparable to that previously reported for other chromosome segregation mutants (Doheny *et al.* 1993; Connelly and Hieter 1996).

NUP170 and *NUP157* were previously identified in genetic screens for nucleopore function (Aitchison *et al.* 1995; Kenna *et al.* 1996). Nup170p has been shown to be required for maintaining the normal stoichiometry of certain nucleoporins in the nuclear pore complex (Kenna *et al.* 1996), the processing of rRNA in the nucleolus (Moy and Silver 1999), anchoring of other nucleoporins in the NPC (Kenna *et al.* 1996; Ryan and Wente 2000), Ty transposition (Kenna *et al.* 1998), establishment of the functional resting diameter of the NPC (Shulga *et al.* 2000), and the possible regulation of cyclins (Yuste-Rojas and Cross 2000).

How can Nup170p, a nucleopore protein, influence chromosome segregation and kinetochore integrity? We hypothesize that this novel function of Nup170p might be due to one of several possibilities that may not be mutually exclusive. For instance, mutations in *NUP170* might affect the nuclear transport of substrates required for high-fidelity chromosome transmission. It is also possible that absence of Nup170p may alter integrity of the nuclear envelope and nuclear substructures. Alternatively, Nup170p could interact directly with chromosomal subdomains and thus influence chromosome segregation.

Our finding that Nup170p is a determinant of chromosome segregation in yeast is not inconsistent with its identity as a nucleoporin. Substrates required for high-fidelity chromosome transmission may be transported into (and out of) the nucleus via nucleopore complexes and specific transport factors (*e.g.*, karyopherins). In *S. cerevisiae* the nuclear envelope does not assemble and disassemble throughout the cell cycle including mitosis. Thus, it is not surprising that a possible disregulation in nuclear transport may affect the chromosome cycle. A precise role for Nup170p in nuclear transport has not yet been defined (Aitchison *et al.* 1995; Fabre and Hurt 1997; Moy and Silver 1999). There is, however, precedence for the involvement of karyopherins in mitosis. For example, mutations in the karyopherins *SRP1* and *CSE1* lead to defects in mitosis in budding yeast (Xiao *et al.* 1993; Loeb *et al.* 1995). Also, overexpression of *YRB1* and *GSP1*, which are required for Ran mediated transport through the nuclear pore, has been shown to adversely affect chromosome stability in yeast (Ouspenski *et al.* 1999). *YRB1* and *SRP1* have been shown also to genetically interact with *NUP170* (Kenna *et al.* 1996). Unlike these karyopherins and components of the Ran system, nucleoporins have not yet been defined as determinants of chromosome segregation in budding yeast. Therefore, we investigated a potential *Ctf*⁻ phenotype of several other nucleoporins, some of which have previously been linked to nuclear transport and *NUP170*. We found that deletions of the nucleoporin genes *NUP157*, *NUP53*, *NUP59*, *NUP188*, *POM152*, and *NUP2* do not affect the segregation of a nonessential CF. Hence, our findings suggest that Nup170p is a specialized nucleoporin with a role in chromosome transmission fidelity and possibly kinetochore function.

In addition to its potential function in nuclear transport, Nup170p might also play a role in nuclear pore structure and nuclear envelope architecture. Other structurally important nucleoporins, including Nup133p, Nup120p, Nup145p, and Nup159p, are required for the distribution of NPCs across the nuclear envelope (Strambio-De-Castillia *et al.* 1999). Also, a mutation in *NUP145* results in mislocalized telomeres, altered telomere clustering, and mislocalization of the nuclear pore-associated proteins Mlp1 and Mlp2 (Galy *et al.* 2000). We also sought to determine if localization of telomeres was altered in the *nup170* mutants on the basis of visualization of Rap1p-GFP staining in live cells. Our data showed that a mutation in *NUP170* does not result in mislocalized telomeres or altered telomere clustering (O. Kerscher and M. Basrai, unpublished observations). Furthermore, we find that the *nup170* mutants do not have an altered nuclear or spindle morphology and cell cycle defects (O. Kerscher and M. Basrai, unpublished observations). These data are consistent with previous results that a deletion of *NUP170* does not lead to morphological changes of the nuclear envelope (Aitchison *et al.* 1995; Bucci and Wente 1997).

Previously, Nup170p has been found to be required for the normal stoichiometry of other nucleoporins in the NPC (Kenna *et al.* 1996). Therefore, a mutation in *NUP170* could influence NPC substructures or distribution of proteins, such as Ndc1p and other SPB components. Ndc1p, a shared component between the NPC and the SPB, is required for mitosis (Thomas and Botstein 1986; Chial *et al.* 1998; Rout *et al.* 2000). Ndc1p has been found to interact with the nucleoporins Nup53p and Nup59p in a two-hybrid screen (Uetz *et al.* 2000). Both nucleoporins interact directly with Nup170p (Marelli *et al.* 1998). However, a deletion of *NUP53* or *NUP59* does not result in a *Ctf*⁻ phenotype. In summary, potential structural alterations in the architecture of the nuclear pore and/or the nuclear envelope

might play a role in the *Ctf*⁻ and kinetochore integrity phenotype of *nup170* mutants.

We found that GFP-tagged Nup170p is distributed across the nuclear envelope at all stages of the cell cycle. Therefore, the localization of Nup170p is similar to that reported for other nucleoporins. This finding is in contrast to the report that the *Drosophila melanogaster* Nup170p homolog, Nup154p, resides inside the nucleus and overlaps with chromatin in stage 10 egg chambers (Gigliotti *et al.* 1998). The possibility that *S. cerevisiae* Nup170p may also interact with chromosomal DNA is based solely on the result that *nup170* mutants test positive in the kinetochore integrity assay. We were unable to show a direct interaction of Nup170p with *CEN* DNA in gel shift and ChIP assays. However, due to the inherent limitations of these assays we cannot exclude the fact that Nup170p could interact transiently or during specific cell cycle stages with chromosomal DNA.

Nup170p is homologous to another nucleoporin, Nup157p (42% identical and 61% similar), in *S. cerevisiae*. Both nucleoporins were identified by biochemical and genetic approaches and were found to be major constituents of the NPC (Aitchison *et al.* 1995; Kenna *et al.* 1996). Nup170p is evolutionarily conserved with homologs in flies (*NUP154*), rats (*NUP155*), and humans (*NUP155*) (21–22% identical) (Aitchison *et al.* 1995; Zhang *et al.* 1999; Costanzo *et al.* 2000). Rat *NUP155* has been shown to complement the lethal phenotype of *nup170* in a *pom152* strain, suggesting both structural and functional conservation (Aitchison *et al.* 1995). Both the human Nup155p and *D. melanogaster* Nup154p may be required for important developmental processes (Kiger *et al.* 1999; Zhang *et al.* 1999). In our studies, *NUP157* was the only gene that could reproducibly suppress the *Ctf*⁻ phenotype of *nup170* mutants. Future studies will address the molecular role of the evolutionarily conserved Nup170p in chromosome segregation in budding yeast and possibly higher eukaryotes.

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