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

Oliver Kerscher,† Philip Hieter,† Mark Winey‡ and Munira A. Basrai*†

*†Department of Genetics, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892, †Center for Molecular Medicine and Therapeutics, University of British Columbia, Vancouver, British Columbia V5Z4H4 and ‡Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80309

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**ABSTRACT**

We determined that a mutation in the nucleoporin gene NUP170 leads to defects in chromosome transmission fidelity (cf) and kinetochore integrity in *Saccharomyces cerevisiae*. A cf 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 cf mutant involved in the maintenance of kinetochore integrity. We cloned and mapped the gene complementing the cf mutation of s141 and showed that it is identical to the *S. cerevisiae* NUP170 gene. A deletion strain of NUP170 (nup170Δ::HIS3) has a Cf− 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 Cf− phenotype of nup170-141 and nup170Δ::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; Nakienny 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 (Cf−) phenotype has identified several kinetochore proteins, including Ctf13p, Ctf14p/Ndc10p, Ctf19p, Skp1p, Sgr1p, 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 cf 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
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 (Arthchson 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 SpoI-Nof 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 mutant strain (YPH102-s141) 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 CEN 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°C. Plasmid DNA isolated from the two transformants was used to complement the Ctf− phenotype of the s141 mutant. One of the plasmids contained the NUP170 gene (pMB261) and the second contained the NUP157 gene (pMB259) 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 transcripational 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 (Baskai et al. 1996). The two plasmids are identical except that pMB203 has a wild-type centromere (CEN VI) and pMB204 has a mutant CEN VI (CDEIII G to C mutation). The plasmids were digested with NotI 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; Baskai et al. 1996). The color of colonies on X-Gal-containting plates was recorded after incubation at 25°C for 5–6 days. Quantification 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°C 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 Na2CO3, and the absorbance of the reporter allowed transcription of the transcriptional readthrough assay.

**In vivo assay for kinetochore integrity—dicentric stabilization assay:** The reporter constructs (pMB205-wild-type CEN VI and pMB206-mutant CEN VI) were integrated at the MSL1 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°C for 5–6 days.

**Quantitation of chromosome loss phenotype:** Homozygous diploid *nup170Δ::His3/nup170::His3* (YMB589), *nup170-141/nup170-141* (YMB314), and *NUP170/NUP170* (YPH279) strains were used to quantitate the chromosome missegregation rate as previously described (Gerring et al. 1990; Baskai 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 and 2:0 chromosome nondisjunction events [hR/hW]/CF 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 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 described 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 MATA haploid
mating testers and examined the formation of putative triploids. Mating was allowed for 6 hr at 25°C in 75% YEPD medium.

TABLE 1

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CF denotes the nonessential chromosome fragment as described in SPENCER et al. (1990).
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 pRS305 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 nap53Δ::kanMX4 deletion strain (strain no. 10734; Research Genetics, Huntsville, AL). A deletion of NUP188 was constructed as the nap53 deletion, except that strain nap188Δ::HIS3(4-I-l) (provided by R. Wozniak) was used as a template for primers annealing ~200 bp upstream and downstream of nap188Δ::HIS3. NUP59 was deleted by transformation of a HnidIII-linearized construct pBS-Nup592::HIS3 (provided by R. Wozniak) into strain YPH987. NUP2 was deleted by transformation of a BamHI-NotI fragment containing nap2Δ::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°C.

**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′XhoI primer for NUP170 (5′-CCCGTCCGAGTCTAGTTCCTACTCTGG-3′) and 3′NotI primer for NUP170 (5′-ATAGTTAGCCGC GCCCTCTTTTGTAGAAGC-3′). PCR amplification was achieved with Life Technologies (Rockville, MD) Essential enzyme mix. The resulting PCR product was digested with XhoI and NotI and cloned into pBSKII+ (Stratagene, La Jolla, CA) to form pBSN170. The XhoI/NotI NUP170 fragment from pBSN170 was cloned into pAA3 (Sakai 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 XhoI/NotI NUP170 fragment from plasmid pOKN170G in frame with the sequence encoding the hemagglutinin (HA) epitope tag in LEU2/CEN/pamid pAA1 (Sakai and Jensen 1999). The plasmids pOKN170G and pOKN170-HA complemented the nap170 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°C for 30 min and electrophoresed on 4% polyacrylamide gels.

**Chromatin immunoprecipitation:** Chromatin immunoprecipitations (ChIPs) were performed as described previously (Melluh and Koshland 1997; McGee et al. 1999). Briefly, cells expressing NUP170-HA or MCD1-6HA were grown in synthetic dextrose-containing medium to an OD600 of ~1.0 and crosslinked for 2 hr at 25°C 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°C. Precipitated chromatin was extracted with phenol/chloroform and finally analyzed for the enrichment of CENIII and nonspecific sequences with the following primer pairs: (CENIII) PM22, 5′-GATCCGCGCC AAAAAATTTGG-3′; and PM48, 5′-AACCTCCACGTAAG CCGTTCT-3′; (NUP1) OMB209, 5′-CTAGCCGCTGATCTG CATCTGTTG-3′; and OMB210, 5′-GGTGGCCCTGAGACGG CTAAAGTAGTGG-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 the inducible GAL promoter. All strains were maintained on galactose-containing media to induce the GAL10 promoter and inactive 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
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 (nup170Δ::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 nup170Δ 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; nup170Δ/nup170Δ) 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 homoyzogosis of the MATa 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 nup170Δ 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 ∼10-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 preponderance of 2:0 events would probably reflect a more direct role for Nup170p in kinetochore function.

We tested if nup170Δ::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,
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 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 (BASRAS 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, ctf1Δ, and ctf19Δ do not exhibit synthetic growth phenotypes. Likewise, overexpression of the kinetochrome function as assayed in the tests for kinetochore integrity.

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).

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The CF missegregation rates (loss (1:0) and nondisjunction (2:0) events) for the non-essential CF were measured as described previously. Controls included wild-type cells and strains stabilized the dicentric CF, resulting in a sec-

TABLE 2
Quantitation of chromosome loss in nup170/nup170 homozygous diploids

<table>
<thead>
<tr>
<th>Genotype</th>
<th>% 1:0 eventsv</th>
<th>% 2:0 eventsv</th>
<th>No. of coloniesv</th>
</tr>
</thead>
<tbody>
<tr>
<td>NUP170/NUP170</td>
<td>0.03</td>
<td>0.03</td>
<td>29046</td>
</tr>
<tr>
<td>nup170-141/nup170-141</td>
<td>2.19</td>
<td>&lt;0.13</td>
<td>776</td>
</tr>
<tr>
<td>nup170Δ/nup170Δ</td>
<td>2.53</td>
<td>&lt;0.18</td>
<td>552</td>
</tr>
</tbody>
</table>

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).

vNumber of pink colonies (colonies with one CF).
Number of half-red, half-pink colonies divided by the total number of colonies with one CF.
Number of half-red, half-white colonies divided by the total number of colonies with one CF.

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 misregulate 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.
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 nup170D::HIS3 strain. The NUP170-GFP plasmid complemented both the nup170Δ::HIS3 growth and the Ctf2 phenotype (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.

NUP170 may be a unique nucleoporin required for chromosome segregation: We have shown that NUP157 can suppress the Ctf2 phenotype of nup170-141 mutants (Figure 1). Hence, we reasoned that increased expression of other nucleoporins may be able to suppress a Ctf2 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 Ctf2 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 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 encodes a specialized nucleoporin with a role in chromosome segregation. The nucleoporin genes NUP170, NUP157, NUP2, NUP53, POM152, NUP59, and NUP188 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 Ctf2 phenotype after incubation at 32°C for 5 days. Nucleoporin deletion strains harboring the CF were also tested at 25°C, 30°C, and 37°C (data not shown). Controls include a wild-type (A; haploid progeny of strain YPH987) and (B) the nup170-141 strain.

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/2α 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 nup170Δ 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 kinechoire 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 nucleoporine function (ARTCHISON 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 tRNA in the nucleus (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 nucleoporine protein, influence chromosome segregation and kinechoire 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 nucleoporine 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 disruption in nuclear transport may affect the chromosome cycle. A precise role for Nup170p in nuclear transport has not yet been defined (ARTCHISON et al. 1995; FARRE 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 (OUSPENSKA et al. 1999). YRB1 and SRP1 have been shown to act 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 kinechoire 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 Nup135p, Nup120p, Nup145p, and Nup159p, are required for the distribution of NPCs across the nuclear envelope (STRAMBIO-DE-CASSELLIA 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 (ARTCHISON 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 Cif− 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 chromatins 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 Nup170p 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 Cif− 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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