# Participation of Bir1p, a member of the inhibitor of apoptosis family, in yeast chromosome segregation events

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Yeast two-hybrid and genetic interaction screens indicate that Bir1p, a yeast protein containing phylogenetically conserved anti-apoptotic repeat domains called baculovirus inhibitor of apoptosis repeats (BIRs), is involved in chromosome segregation events. In the two-hybrid screen, Bir1p specifically interacts with Ndc10p, an essential component of the yeast kinetochore. Although Bir1p carries two BIR motifs in the N-terminal region, the C-terminal third of the protein is sufficient to provide strong interaction with Ndc10p and moderate interaction with Skp1p, another essential component of the yeast kinetochore. In addition, deletion of *BIR1* is synthetically lethal with deletion of *CBF1* or *CTF19*, genes specifying two other components of the yeast kinetochore. Yeast cells deleted of *BIR1* have a chromosome-loss phenotype, which can be completely rescued by elevating *NDC10* dosage. Furthermore, overexpression of either full-length or the C-terminal region of Bir1p can efficiently suppress the chromosome-loss phenotype of both *bir1Δ* null and *skp1-4* mutants. Our data suggest that Bir1p participates in chromosome segregation events, either directly or via interaction with kinetochore proteins, and these effects are apparently not mediated by the BIR domains of Bir1p.

T he yeast (*Saccharomyces cerevisiae*) *BIR1* gene specifies a member of the baculovirus inhibitor of apoptosis repeats (BIR)-containing proteins that are found in viruses, yeast, and metazoans (1). Most mammalian and insect BIR-proteins inhibit cell death when apoptosis is induced by a variety of agents (for recent reviews, see refs. 2 and 3). Human survivin, a particularly interesting example, is abundantly expressed in cancer cells and specifically associates with the mitotic spindle (4). Disruption of the survivin–microtubule interaction results in loss of its antiapoptosis function. *Caenorhabditis elegans* BIR-1 is probably not involved in the general regulation of apoptosis but is required for embryonic cytokinesis (5). Survivin might be functionally similar to the nematode BIR-1, because survivin can partially substitute for BIR-1 in cytokinesis. The yeasts *S. cerevisiae* and *Schizosaccharomyces pombe* each contain single BIR proteins that seem to be involved in some as yet unknown aspect of cell division (6).

One important participant in the cell division process is the centromere kinetochore, a macromolecular structure responsible for accurate chromosome segregation. Budding yeasts, such as *S. cerevisiae*, contain so-called "point" centromeres. In contrast to the large more complex "regional" centromeres found in higher eukaryotes, the minimum DNA sequence required for faithful segregation of the yeast chromosomes consists of only 125 bp (the *CEN* locus), containing three conserved DNA elements, CDEI, CDEII, and CDEIII (reviewed in ref. 7). However, the yeast kinetochore seems to be relatively complex, with 11 protein components identified to date (8, 9). The core centromeric protein complex in yeast (CBF3) consists of four essential subunits: Ndc10p (110 kDa), Cep3p (64 kDa), Ctf13p (58 kDa), and Skp1p (24 kDa; ref. 7). This core complex is thought to be present at the *CEN* locus throughout the yeast cell cycle and serves as a foundation for assembly of a multicomponent complex that interacts with the microtubules during mitosis and meiosis. Herein, we report that Bir1p is involved in the

chromosome segregation process and interacts physically and genetically with several components of the yeast kinetochore. These results extend and confirm previously postulated roles for the BIR proteins in cell division (4, 6).

## Materials and Methods

**Yeast Strains and Cell Culture.** The *S. cerevisiae* strains used in this study are listed in Table 1. The diploid strain YHY399 was constructed by mating haploid strains YPH278 and YPH1015 and subsequently plating on 5-fluoroorotic acid plates to identify colonies that lost CFIII [(*CEN3. L. YPH278*) *URA3 SUP11*] but retained CFIII [(*CEN3. L. YPH983*) *HIS3 SUP11*]. Yeast cultures were grown in yeast extract/peptone/dextrose or, to maintain selection for plasmids, in synthetic minimal medium (SD) supplemented with the appropriate amino acids and/or bases. For tetrad dissection, diploid cells were streaked on sporulation plates and incubated at 25°C for 7–10 days. Media for yeast growth and sporulation have been described (10). For the colony color sectoring assay (11), adenine was added to growth media at 6 mg/liter. Yeast transformations were performed by the lithium acetate method (12).

**Identification of *BIR1* by Two-Hybrid Interaction with Ndc10p.** The C-terminal 850-amino acid region of Ndc10p fused to the Gal4 DNA-binding domain (DBD) was used as bait to screen yeast two-hybrid genomic libraries (13). Among the ≈1 × 10⁶ transformants screened, 24 clones showed reproducible interaction with Ndc10p. The subsequent analysis of the genomic inserts revealed that five clones carried ORFs fused in frame with the Gal4 activation domain (ACD). One plasmid, pACD-Bir1 (C332), contained the C-terminal 332 amino acids of Bir1p fused to ACD. The full-length *BIR1* gene and flanking sequences were isolated from a yeast genomic library constructed in the *CEN* plasmid vector YCp50 (purchased from the American Type Culture Collection; ref. 14). The 4.0-kilobase (kb) *Sna*BI fragment carrying *BIR1* and flanking sequences was introduced into the multicopy vector YEp24 (2 μm, *URA3*) to obtain YEp24-*BIR1*. The entire *BIR1* coding region was subcloned into the *Eco*RI and *Sma*I sites of the pGBD-C3 vector (13) to construct a fusion between DBD and Bir1p. The resulting plasmid was termed pDBD-Bir1 (full).

**Plasmids.** Plasmids carrying either DBD-fused or ACD-fused *CBF1* were made as follows. The *CBF1* ORF was cloned by PCR with yeast genomic DNA and *Eco*RI-linked and *Bgl*II-linked oligonucleotides (5′-CA<u>GAATTC</u>TTAACGATGAACTCTC-TG-3′ and 5′-TT<u>AGATCT</u>CAAGCCTCATGTGGATTA-3′).

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**Table 1. *S. cerevisiae* strains used in this study**

| Strain | Genotype | Source |
|---|---|---|
| YPH278 | *MATα ade2-101 his3-Δ200 leu2-Δ1 lys2-801 ura3-52 CFIII (CEN3.L. YPH278) URA3 SUPP11* | P. Hieter* |
| YPH1015 | *MATa ade2-101 his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ63 ura3-52 CFIII (CEN3.L. YPH983) HIS3 SUPP11* | P. Hieter |
| YPH1161 | *MATa ade2-101 his3-Δ200 leu2-Δ1 lys 2-801 trp1-Δ63 ura3-52 skp1-Δ::TRP1 skp1-4::LEU2 CFIII (CEN3.L. YPH983) HIS3 SUP11* | P. Hieter |
| YPH1317 | *MATa ade2-101 his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ63 ura3-52 ctf19-Δ1::TRP1* | P. Hieter |
| s30 | *MATα ade2-101 his3-Δ200 leu2-Δ1 lys2-801 ura3-52 ctf13-30 CFIII (CEN3.L. YPH278) URA3 SUPP11* | P. Hieter |
| YHY399 | *MATa/Matα ade2-101/ade2-101 his3-Δ200/his3-Δ200 leu2-Δ1/leu2-Δ1 lys-2-801/lys2-801 TRP1/trp1-Δ63 ura3-52/ura3-52 CFIII (CEN3.L. YPH983) HIS3 SUPP11* | This work |
| YHY 400 | *MATa/MATα ade2-101/ade2-101 his3-Δ200/his3-Δ200 leu2-Δ1/leu2-Δ1 lys2-801/lys2-801 TRP1/trp1-Δ63 ura3-52/ura3-52 BIR1/bir1Δ::LEU2 CFIII (CEN3.L. YPH983) HIS3 SUPP11* | This work |
| YHY401 | *MATα ade2-101 his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ63 ura3-52 CFIII (CEN3.L. YPH983) HIS3 SUP11* | This work |
| YHY402 | *MATα ade2-101 his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ63 ura3-52 bir1Δ::LEU2 CFIII (CEN3.L. YPH983) HIS3 SUP11* | This work |
| YHY403 | *MATa ade2-101 his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ63 ura3-52 bir1Δ::LEU2 CFIII (CEN3.L. YPH983) HIS3 SUPP11* | This work |
| YHY13 | *MATa ade2-101 his3-Δ200 leu2-3,112 lys2-801 trp1-Δ63 ura3-52 ndc10-1* | H. Yoon |
| YJL146 | *Mata ade2-101 his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ63 ura3-52 cyh2R mcm21Δ::TRP1* | J. Lechner† |
| YJL158 | *MATa ade2-101 his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ63 ura3-52 cyh2R okp1-5::TRP1* | J. Lechner |
| YSS90 | *MATa ade2-101 leu2-Δ1 lys2-801 ura3-52 cbf1Δ::URA3* | W. Jiang‡ |
| 1cAS281 | *Mata ade2 his3 leu2 lys2 trp1 ura3 cep3-1* | D. Koshland§ |
| PM1002-4c | *MATa ade2 ade3 leu2 ura3 mif2-3* | D. Koshland |
| CSE4-1 (3a) | *MATα ade2 his3 leu2 trp1 ura3 cse4-1* | M. Fitzgerald-Hayes¶ |
| WSCF101 | *MATα ade2-101 his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ63 ura3-52 slk19Δ::HIS3 CFIII (CEN3.L. YPH278) URA3 SUP11* | I. Fitch‖ |

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The PCR product was cut with *Eco*RI and *Bgl*II and subcloned into the *Eco*RI and *Bgl*II sites of pGBD-C3 or pGAD-C3. Plasmids carrying either DBD-fused or ACD-fused Ndc10p, Cep3p, Ctf13p, and Skp1p were provided by J. Lechner. The construction of plasmids carrying truncated Bir1p fragments is described below. The plasmid pDBD-Bir1 (full) was cut with *Cla*I and religated to remove the 3′ 2.2-kb *Cla*I fragment of *BIR1*. The resulting plasmid pDBD-Bir1 (N245) carries the N-terminal 245 amino acids of Bir1p fused to DBD. Plasmids pDBD-Bir1 (N552) and pDBD-Bir1 (N659) were constructed by removing the 3′ 1.3-kb *Bam*HI and 3′ 1.0-kb *Bgl*II fragments, respectively, from pDBD-Bir1 (full). The 3′ 1.3-kb *Bam*HI fragment was subcloned into the *Bam*HI site of the pGBD-C1 vector to obtain the plasmid pDBD-Bir1 (C405), which expresses the C-terminal 405 amino acids of Bir1p fused to DBD. Finally, pDBD-Bir1 (C297) was made by inserting the 3′ 1.0-kb *Bgl*II fragment from pDBD-Bir1 (C405) into the *Bam*HI site of pGBD-C3. The multicopy plasmids YEp24-*NDC10*, YEp24-*CTF13*, and YEp24-*SKP1* carry the 6.6-kb *Bam*HI fragment of *NDC10* derived from pWJ316-110 (15); the 2.2-kb *Xba*I fragment of *CTF13* originated from pKF11 (obtained from P. Hieter), and the 1.7-kb *Spe*I–*Cla*I fragment of *SKP1* originated from pRS316-*SKP1* (P. Hieter). YEp24-*CEP3* is the same as pAS480/1 (from D. Koshland).

**Construction of the *bir1*Δ Null Mutant.** The *BIR1* gene disruption was constructed in diploid strain YHY400 by replacing the internal 1.8-kb *Msc*I–*Bgl*II fragment of *BIR1* with a 2.0-kb *Hpa*I–*Sal*I fragment containing yeast *LEU2*. The *Bgl*II and *Sal*I sites were converted to blunt ends with T4 DNA polymerase before ligation. The resulting diploid strain bearing the minichromosome *CFIII (CEN3.L. YPH983) HIS3 SUP11* and one disrupted copy of *BIR1* was sporulated, and the tetrads were analyzed. Among 30 tetrads, 29 showed four viable spores and

2+:2− segregation of the *LEU2* marker. The *bir1* disruption was verified by genomic Southern blot hybridization with DNA of the haploid strains.

## Results

***BIR1* Interacts with Essential Kinetochore Protein Ndc10p.** In experiments directed toward the isolation of unknown components of the yeast centromere kinetochore, we performed an extensive two-hybrid screen by using Ndc10p fused to the Gal4 DNA-binding protein as bait. A strongly positive clone isolated multiple times in this screen was shown to contain the C-terminal third of the *BIR1* gene product fused to ACD (C332; Fig. 1*A*). This C-terminal region of Bir1p was tested further for two-hybrid interaction with the other CBF3 components—Cep3p, Ctf13p, and Skp1p—and with Cbf1p (16–23). C332 showed moderate interaction with Skp1p and perhaps very weak interaction with Ctf13p and Cep3p (Fig. 1*A*). This C-terminal region of Bir1p had no binding activity toward other known kinetochore proteins, including Mif2p, Cse4p, Ctf19p, Mcm21p, Okp1p, and Slk19p (8, 9, 24–26). Full-length (954 amino acids) *BIR1*-DBD interacted only with Ndc10p among the 11 kinetochore proteins (Fig. 1*B*). To determine the regions of Bir1p interacting with Ndc10p, we generated a series of truncated Bir1p-DBD fusions and tested them for Ndc10p-binding activity in the two-hybrid system. The N-terminal regions bearing the BIR domains (N245, N552, and N659) showed no binding activity. Although the C-terminal 332 amino acids (C332) were sufficient to provide strong interaction with Ndc10p (Fig. 1*C*), the strongest binding activity was observed with the C-terminal 405 amino acids (C405; Fig. 1*C*).

**The *bir1*Δ Null Mutant Has a Chromosome Missegregation Phenotype.** Deletion of BIR1 in haploid yeast did not cause noticeable defects under the various growth conditions that we tested. The
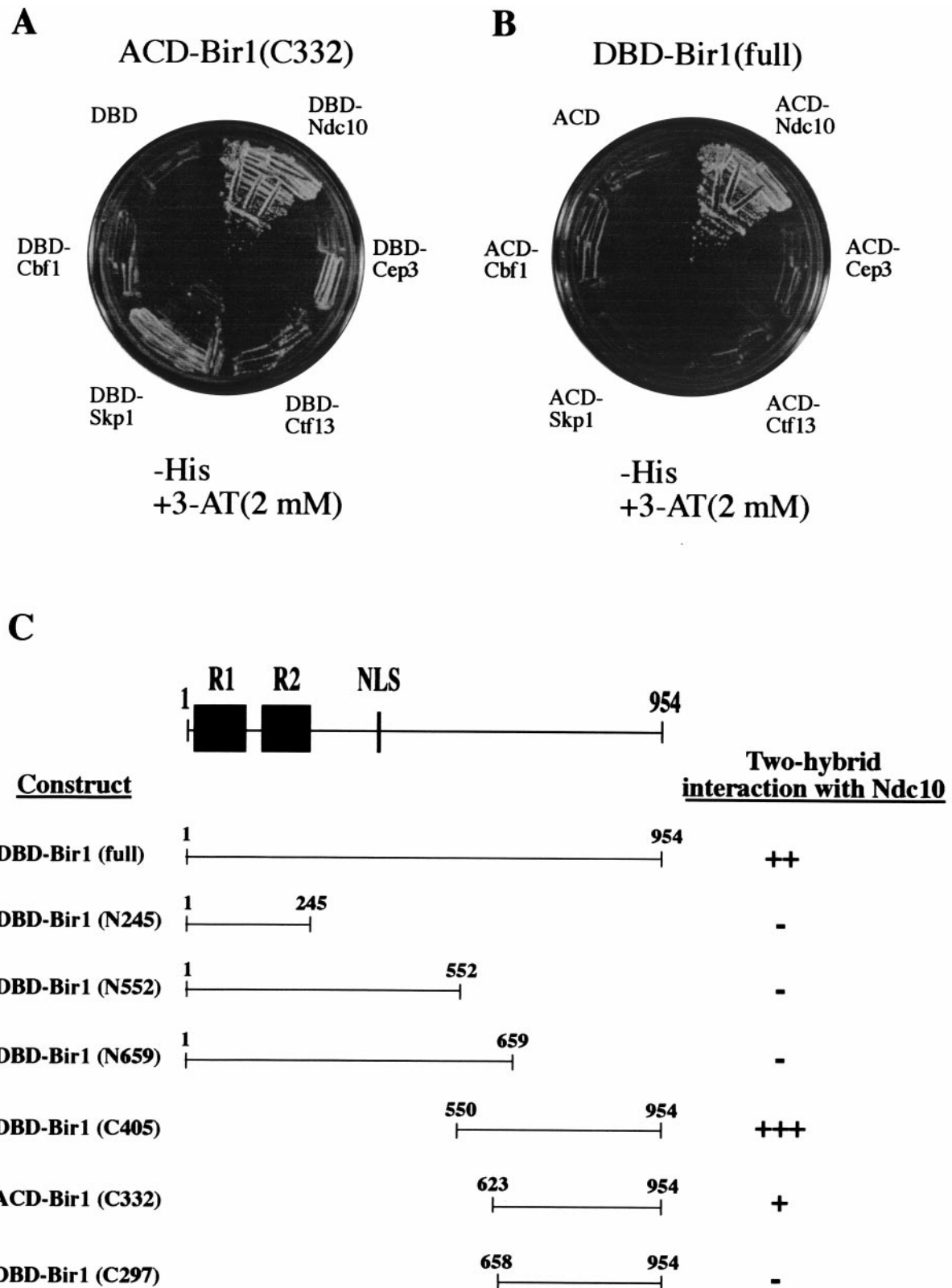
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**Fig. 1.** *In vivo* binding of Bir1p to Ndc10p. (*A*) Two-hybrid interaction between a Bir1p fragment and known kinetochore proteins. A tester strain PJ69–4A (17) carrying the plasmid pACD-Bir1 (C332) was transformed with various plasmids that express DBD alone or DBD-fused Ndc10p, Cep3p, Ctf13p, Skp1p, or Cbf1p. The transformants were streaked on SD/His⁻ medium containing 2 mM 3-AT (3-amino-1 2,4-triazole) to test expression of the *HIS3* reporter gene. (*B*) Two-hybrid assay with strain PJ69-4A carrying pDBD-Bir1 (full). Strain PJ69-4A/pDBD-Bir1 (full) bearing plasmids expressing ACD-fused Mif2p, Cse4p, Ctf19p, Mcm21p, Okp1p, or Slk19p did not grow on minimal salt-dextrose/His⁻ medium containing 2 mM 3-AT (not shown). (*C*) Identification of Bir1p regions interacting with Ndc10p. (*Top*) Schematic view of Bir1p showing the relative locations of BIR domains 1 (R1), 2 (R2), and of nuclear localization signal (NLS), a bipartite nuclear localization signal. Various truncated Bir1p fragments as shown here (See *Materials and Methods*) were tested for two-hybrid interactions with Ndc10p on SD/His⁻ medium containing 2 mM 3-AT.

**Table 2. Rates of minichromosome loss in *bir1Δ* cells**

| Genotype | Red colonies | Half-sectored colonies* | Total colonies | Loss rate/cell division† | Fold increase‡ |
|---|---|---|---|---|---|
| *BIR1* | 6 | 6 | 5,409 | $1.11 \times 10^{-3}$ | 1.0 |
| *bir1Δ* | 134 | 27 | 4,878 | $5.69 \times 10^{-3}$ | 5.1 |

The colony color sectoring assay was performed with isogenic wild-type *BIR1* (YHY401) and *bir1Δ* (YHY402) strains. A single colony from each strain was grown overnight in SD/His⁻ medium and plated for single colonies on yeast extract/peptone/dextrose.
*Includes colonies that contain at least a full half sector. The mother cell underwent a chromosome loss or missegregation event in the first division.
†Loss rate/cell division = half-sectored colonies/(total colonies − red colonies).
‡Fold increase = loss rate/cell division of *bir1Δ* cells divided by loss rate/cell division of *BIR1* cells.

growth of the *bir1Δ* strain was similar to that of the isogenic *BIR1* wild type when subjected to different growth temperatures, carbon sources, metal ions, and mitotic poisons. The spindle and nuclear morphology of *bir1Δ* null cells also seemed normal. However, *bir1Δ* null cells had a chromosome missegregation phenotype, as determined by an accurate and highly reproducible colony color sectoring assay (Table 2). Disruption of *BIR1* led to a 5-fold increase in the marked chromosome fragment loss rate per cell division (Table 2). In addition, *bir1Δ* null cultures grown in media selective for the chromosome fragment contained ≈20 times more segregants lacking the tester chromosome than did the isogenic wild-type cells (Table 2, red colonies).

**Bir1Δ Is Synthetically Lethal with Mutations in the Kinetochore Protein Genes *cbf1* and *ctf19*.** To explore a potential role of Bir1p in kinetochore function, genetic analysis was used to look for synthetic phenotypes revealed by strains carrying *bir1Δ* and mutations in known kinetochore protein genes (Table 3). Crosses were made between a *bir1Δ* null strain and strains bearing mutations in each of the 11 different known kinetochore proteins. Double mutants were isolated after sporulation and tetrad dissection of the resulting heterozygous diploids. We were unable to isolate a *bir1Δ skp1-4* double mutant because of a severe sporulation defect in the corresponding heterozygous diploid. Among the remaining 10 kinetochore protein mutants, only *cbf1Δ* and *ctf19Δ* had synthetic lethal interactions with the *bir1Δ* null (Table 3). Although cells lacking either *CBF1* or *CTF19* alone are viable, the *bir1Δ cbf1Δ* and *bir1Δ ctf19Δ*

**Table 3. Synthetic lethality studies with *bir1Δ* and various kinetochore mutants**

| Double-mutant genotype | Phenotype | Total no. of tetrads |
|---|---|---|
| *bir1Δndc10-1* | Viable | 29 |
| *bir1Δcep3-1* | Viable | 29 |
| *bir1Δctf13-30* | Viable | 34 |
| *bir1Δcbf1Δ* | SL | 65 |
| *bir1Δctf19Δ* | SL | 16 |
| *bir1Δmcm21Δ* | Viable | 36 |
| *bir1Δokp1-5* | Viable | 29 |
| *bir1Δmif2-3* | Viable | 31 |
| *bir1Δcse4-1* | Viable | 33 |
| *bir1Δslk19Δ* | Viable | 39 |

SL, synthetic lethality. Heterozygous diploids containing *bir1Δ* and the various kinetochore protein mutations listed above were sporulated; tetrads were dissected; and the haploid progeny were scored for presence of the double mutations. SL indicates no viable double mutants found in the number of tetrads listed above.

double-mutant strains could not be found among spores germinated at 25°C. Cbf1p and Ctf19p are not absolutely essential for centromere function, but mutations in these genes result in significantly increased chromosome missegregation frequencies (8, 21–23, 26). We also looked for genetic interactions between *bir1Δ* and spindle checkpoint mutants. No synthetically lethal interactions were observed between *bir1Δ* and *mad1*, *mad2*, *mad3*, *bub1*, *bub2*, and *bub3* null mutants (data not shown).

**Overexpression of *NDC10* Reverses the Chromosome-Loss Phenotype of *bir1Δ* Cells.** Direct or indirect *in vivo* interactions between proteins often can be revealed by overexpressing a given wild-type gene in various mutant cells and looking for pleiotropic effects on characteristic mutant phenotype(s). We therefore examined the effects of increasing the dosage of known kinetochore genes on the chromosome-loss phenotype seen in the *bir1Δ* null mutant. As shown in Table 4, overexpression of *NDC10* completely rescued the chromosome-loss phenotype of *bir1Δ* cells. However, overexpressing any of the genes specifying the other three CBF3 subunits (*CEP3*, *CTF13*, and *SKP1*) produced no dosage effects on the *bir1Δ* mutant (Table 4). As a control, overexpression of full-length *BIR1* was shown also to suppress the *bir1Δ* chromosome-loss phenotype. Surprisingly, it was found that overexpression of the 3′ *BIR1* fragment that produces the C-terminal 405 amino acids (C405) lacking the BIR motifs also stabilized the tester minichromosome in *bir1Δ* null cells (Table 4). However, elevated levels of the 5′ *BIR1* fragment that expresses the N-terminal 552 amino acids including both BIR motifs (N552) had little or no effect (Table 4), indicating that the BIR motifs are not significantly involved in Bir1p functions related to chromosome segregation. Overexpressing full-length *BIR1*, N552, or C405 had no effects on minichromosome stability in isogenic wild-type cells.

**High Levels of *BIR1* Increase Chromosome Segregation Fidelity in *skp1-4* Cells.** The reciprocal dosage suppression experiment was performed by overexpressing *BIR1* in various kinetochore mutant strains. The temperature-sensitive *skp1-4* mutant has a pronounced chromosome segregation defect even at the permissive temperature, 25°C. We observed that high levels of *BIR1* fully rescued the severe chromosome-loss phenotype of the *skp1-4* mutant at 25°C (Table 5). Furthermore, overexpressing the 3′ *BIR1* fragment (C405) was as effective as a single gene dosage of *SKP1* (pRS316-*SKP1*) in rescuing tester chromosome stability in *skp1-4* cells. Again, the 5′ *BIR1* fragment (N552) was unable to rescue the *skp1-4* chromosome-loss phenotype (Table 5). However, the temperature-sensitive growth phenotype of *skp1-4* cells was not suppressed by overexpression of *BIR1*. In fact, *BIR1* is not a dosage suppressor of the growth defects seen in any of the known temperature-sensitive kinetochore mutants (data not shown).

**Discussion**

Several lines of evidence point to a role for yeast Bir1p in some aspect of the chromosome segregation process, most likely in kinetochore function. Deletion of *BIR1* is not lethal but does result in a decrease in chromosome segregation fidelity. The *BIR1* gene product interacts *in vivo* either directly or indirectly with four known kinetochore proteins: Ndc10p, Cbf1p, Ctf19p, and Skp1p. Thus, the combined results of two-hybrid and genetic interaction assays suggest that yeast Bir1p participates in chromosome segregation events either directly or via interaction with known kinetochore proteins. However, we do not yet know whether Bir1p is a structural component of the yeast kinetochore. *In vivo* cross-linking and centromere chromatin immunoprecipitation studies failed to identify the presence of Bir1p in the kinetochore. This result is not conclusive, however, because

**Table 4. Dosage effects of kinetochore protein genes on the *bir1Δ* chromosome-loss phenotype**

| Plasmid | Red colonies | Half-sectored colonies | Total colonies | Loss rate/ cell division* | Fold decrease[†] |
|---|---|---|---|---|---|
| YEp24 | 48 | 19 | 4,794 | $4.00 \times 10^{-3}$ | 1.0 |
| YEp24-*BIR1* | 3 | 1 | 13,422 | $7.45 \times 10^{-5}$ | 53.7 |
| YEp24-*BIR1* (N552) | 9 | 4 | 2,848 | $1.41 \times 10^{-3}$ | 2.8 |
| YEp24-BIR1 (C405) | 3 | 1 | 14,108 | $7.09 \times 10^{-5}$ | 56.5 |
| YEp24-*NDC10* | 0 | 1 | 13,540 | $7.39 \times 10^{-5}$ | 54.2 |
| YEp24-*CEP3* | 75 | 3 | 2,955 | $1.04 \times 10^{-3}$ | 3.8 |
| YEP24-*CTF13* | 24 | 6 | 5,200 | $1.16 \times 10^{-3}$ | 3.5 |
| YEP24-*SKP1* | 24 | 12 | 2,279 | $5.32 \times 10^{-3}$ | 0.8 |

The *bir1Δ* mutant (YHY402) was transformed with a high-copy plasmid YEp24 (2 μm, *URA3*) or YEp24 carrying *BIR1, NDC10, CEP3, CTF13,* or *SKP1*. In addition to the complete *BIR1* ORF, a 1.7-kb fragment that expresses the N-terminal 552 amino acids of Bir1p or a 1.2-kb fragment that expreses the C-terminal 405 amino acids of Bir1p was also subcloned into YEp24 and tested for the ability to complement the *bir1* null mutation. The colony color sectoring assay was performed as described in Table 2.
*Loss rate/cell division = half-sectored colonies/(total colonies − red colonies).
[†]Fold decrease = loss rate/cell division of *bir1Δ* cells carrying YEp24 divided by loss rate/cell division of *bir1Δ* cells containing each of the indicated plasmids.

proteins buried internally in the chromatin mass might not be accessible to interaction with antibodies.

Uren *et al.* (6) recently reported that a fusion protein between the N-terminal region of Bir1p (residues 1–474) and green fluorescent protein (GFP) is distributed uniformly through the yeast nucleoplasm, whereas another fusion protein, Bir1p (residues 249–954)-GFP, is associated with a fibrillar structure resembling the mitotic spindle. The latter finding is interesting, because human survivin, another BIR-containing protein, has been localized to the mitotic spindle in mitotic human cells (4). We have made several attempts to localize Bir1p by using full-length Bir1p-GFP and Bir1p-hemagglutinin fusion constructs integrated into the chromosomal *BIR1* locus. Neither GFP fluorescence nor indirect immunofluorescence against GFP or hemagglutinin produced a detectable signal, although both fusion proteins were expressed according to Western blot analysis (H.-J.Y. and J.C., unpublished data). Uren *et al.* (6) also reported that they were unable to localize the plasmid-borne full-length Bir1p-GFP fusion protein.

The presence of the conserved BIR sequence domains in Bir1p suggests a role for this protein in apoptosis. It has been shown previously that apoptosis can be induced in yeast by depletion of glutathione or by low external doses of $H_2O_2$ (27). Yeast can also

be triggered into apoptosis by a specific mutation (S565G) in *CDC48* or by overexpression of mammalian Bax (28–30). In both cases, oxygen radicals accumulate in the cell, and radical depletion or hypoxia prevents apoptosis (27). The yeast genome lacks sequences specifying any components of the established apoptotic death machinery. Bir1p is the only budding yeast protein that belongs to the evolutionarily conserved, cell-death protein family. However, the two BIR domains in Bir1p seem to perform no obvious function in yeast viability, because *bir1Δ* null cells are viable and grow as well as the isogenic wild-type cells. Deletion of *BIR1* becomes essential for cell viability only when combined with disruption of kinetochore protein genes *CBF1* or *CTF19*. The BIR domains also do not seem to play a role in chromosome segregation events. The C-terminal region of Bir1p lacking both BIR domains is sufficient to interact with Ndc10p or Skp1p and to rescue the chromosome-loss phenotype of both *bir1Δ* null and *skp1-4* cells. In addition, our preliminary data suggest that Bir1p is not involved in controlling apoptotic death of *cdc48*-S565G yeast cells or *gsh1Δ* cells depleted of glutathione, because deletion or overexpression of *BIR1* has no effect on these processes. Thus, the function of the BIR domains in Bir1p is still unclear. Previous studies on the yeast and nematode BIR proteins also suggested that they function in cell division and probably not in antiapoptosis (5, 6). Further functional

**Table 5. Dosage effects of *BIR1* expression on the *skp1-4* chromosome-loss phenotype**

| Plasmid | Red colonies | Half-sectored colonies | Total colonies | Loss rate/ cell division* | Fold decrease[†] |
|---|---|---|---|---|---|
| YEp24 | 79 | 55 | 2,418 | $2.35 \times 10^{-2}$ | 1.0 |
| YEp24-*SKP1* | 0 | 1 | 3,577 | $2.80 \times 10^{-4}$ | 84.1 |
| YEp24-*CTF13* | 2 | 1 | 3,567 | $2.81 \times 10^{-4}$ | 83.8 |
| YEp24-*NDC10* | 66 | 36 | 2,356 | $1.57 \times 10^{-2}$ | 1.5 |
| YEP24-*CEP3* | 65 | 42 | 3,404 | $1.26 \times 10^{-2}$ | 1.9 |
| YEp24-*BIR1* | 0 | 1 | 6,933 | $1.44 \times 10^{-4}$ | 163.0 |
| YEP24-*BIR1* (N552) | 63 | 44 | 2,103 | $2.16 \times 10^{-2}$ | 1.1 |
| YEP24-*BIR1* (C405) | 4 | 4 | 2,349 | $1.71 \times 10^{-3}$ | 13.8 |
| pRS316-*SKP1* | 2 | 4 | 2,641 | $1.52 \times 10^{-3}$ | 15.5 |

A high-copy plasmid, YEp24, carrying *SKP1, CTF13, NDC10, CEP3, BIR1,* 5′ 1.7-kb (N552), or 3′ 1.2-kb (C405) fragment of *BIR1* was introduced into *skp1-4* cells, and the colony sectoring assay was performed as described in Table 2, except that the plates were incubated at 25°C for 5 days. The plasmid pRS316 (*CEN6, URA3*) was used to obtain single gene dosage of *SKP1*.
*Loss rate/cell division = half-sectored colonies/(total colonies − red colonies).
[†]Fold decrease = loss rate/cell division of *skp1-4* cells carrying YEp24 divided by loss rate/cell division of *skp1-4* cells containing each of the indicated plasmids.

characterization of yeast Bir1p should aid in unraveling the cellular functions of various BIR proteins.

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