Sli15 Associates with the Ipl1 Protein Kinase to Promote Proper Chromosome Segregation in *Saccharomyces cerevisiae*

Jae-hyun Kim, Jung-seog Kang, and Clarence S.M. Chan

D. Department of Microbiology and Institute for Cellular and Molecular Biology, The University of Texas, Austin, Texas 78712

**Abstract.** The conserved Ipl1 protein kinase is essential for proper chromosome segregation and thus cell viability in the budding yeast *Saccharomyces cerevisiae*. Its human homologue has been implicated in the tumorigenesis of diverse forms of cancer. We show here that sister chromatids that have separated from each other are not properly segregated to opposite poles of ipl1-2 cells. Failures in chromosome segregation are often associated with abnormal distribution of the spindle pole–associated Nuf2-GFP protein, thus suggesting a link between potential spindle pole defects and chromosome missegregation in ipl1 mutant cells. A small fraction of ipl1-2 cells also appears to be defective in nuclear migration or bipolar spindle formation. Ipl1 associates, probably directly, with the novel and essential Sli15 protein in vivo, and both proteins are localized to the mitotic spindle. Conditional sli15 mutant cells have cytological phenotypes very similar to those of ipl1 cells, and the ipl1-2 mutation exhibits synthetic lethal genetic interaction with sli15 mutations. sli15 mutant phenotype, like ipl1 mutant phenotype, is partially suppressed by perturbations that reduce protein phosphatase 1 function. These genetic and biochemical studies indicate that Sli15 associates with Ipl1 to promote its function in chromosome segregation.

**Key words:** chromosome segregation • Ipl1 • Sli15 • protein kinase • Nuf2

Chromosome segregation is a complicated process that is critical to the proliferation of all cell types. In this process, the functions of many proteins must be activated and inactivated coordinately in a strict temporal and spatial order, thereby leading to an orchestrated series of events that culminate in the segregation of sister chromatids to opposite poles of a mitotic spindle. These events include chromosome condensation, sister chromatid cohesion, bipolar mitotic spindle assembly and elongation, microtubule capture by kinetochores, sister chromatid separation, and kinetochore microtubule-mediated poleward movement of separated sister chromatids (for reviews see references 23, 27, and 43). Protein phosphorylation plays important roles in the control of chromosome segregation. In animal cells, many components of the mitotic spindle apparatus are known to be specifically phosphorylated during M phase (19, 75). In the budding yeast *Saccharomyces cerevisiae*, phosphorylation is known to regulate the function of a number of proteins that play critical roles in chromosome segregation, including those required for kinetochore function and sister chromatid separation (6, 7, 32, 36).

Using a chromosome-gain genetic assay, we have previously identified the Ipl1 protein kinase as being essential for proper chromosome segregation and cell viability in the budding yeast *S. cerevisiae* (8, 13). At the restrictive temperature, most conditional temperature-sensitive (Ts<sup>−</sup>) ipl1-2 mutant cells retain the ability to assemble mitotic spindles that can undergo elongation. However, chromosomes become severely missegregated in the process, resulting in cell death within a single cell cycle. Interestingly, severe chromosome missegregation is not associated with cell cycle arrest, as spindle disassembly and cell division occur in ipl1-2 cells that are destined to be inviable. These results suggest that ipl1-2 mutant cells are defective in the execution of a mitotic process(es) that is not monitored by mitotic spindle checkpoint control (for review see reference 56), or that ipl1 mutant cells are defective in both the mechanics of chromosome segregation and mitotic spindle checkpoint control. However, it is clear that ipl1-2 cells are not totally defective in mitotic spindle checkpoint control, since such cells do become arrested in G2/M phase when exposed to the microtubule-destabilizing drug nocodazole (13). The molecular basis of the chro-

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**Abbreviations used in this paper:**

| Abbreviation | Description |
|--------------|-------------|
| GFP | green fluorescent protein |
| PP1 | protein phosphatase 1 |
| TetR-GFP | Tet repressor-green fluorescent protein |
| Ts<sup>−</sup> | temperature-sensitive |
mosome segregation defect in ipi1 mutant cells has not been well characterized, but genetic analysis has shown that ipi1 mutant phenotypes can be partially suppressed by perturbations that reduce protein phosphatase 1 (PP1) function, thus suggesting that PP1 acts in opposition to the ipi1 protein kinase in vivo (13, 74).

Since our initial report of the Ipl1 protein kinase, several structural homologues of Ipl1 have been described from diverse organisms, including Drosophila (15), X. enopus (49), Caenorhabditis elegans (60, 61), mouse (17, 18, 44, 63, 73, 80), rat (72), and humans (4, 33, 62, 63, 73). Like Ipl1, many of these Ipl1-related protein kinases are known or predicted to play important roles in chromosome segregation. Drosophila cells with mutational alteration of the Ipl1-related A urora kinase are defective in centromere separation and form monopolar spindles (15). C. elegans embryonic cells lacking the A 1-1 kinase, which normally localizes to the centromere, are defective in chromosome segregation (60). E2g, a X enopus homologue of Ipl1, can bind microtubules directly and is associated with the centromere and mitotic spindle in cultured cells. In an in vitro mitotic spindle assembly assay, E2g is required for bipolar spindle assembly (49). Furthermore, E2g also plays a role in the progesterone-activated signaling pathway that triggers oocyte maturation (1). While the biological function of other Ipl1-related kinases is less understood, several are known to be associated with the centromere and/or mitotic spindle (4, 17, 33, 60, 72). Furthermore, a chimera containing the nonkinase domain of Ipl1 and the kinase domain of human A urora2 can partially complement the Ts phenotype of ipi1 mutant cells (4), whereas expression of the murine IA K1 kinase causes lethality in ipi1 mutant but not wild-type yeast cells, possibly because IA K1 may interfere with Ipl1 function by associating nonproductively with normal binding partners or substrates of Ipl1 (17). These observations suggest that at least some members of the Ipl1 family of protein kinases may perform related functions in vivo.

A neuploidy is known to be associated with many forms of human cancer (25). However, relatively few proteins that are involved in chromosome segregation have been found to be mutationally altered in cancer cells. Recently, the gene encoding the Ipl1-related A urora2 kinase was found to be amplified and/or overexpressed in a variety of human tumors, including a significant fraction of colorectal and breast tumors (4, 62). Furthermore, ectopic expression of A urora2 in rodent fibroblasts leads to chromosomal missegregation, centrosome amplification, and cellular transformation, and the transformed cells are tumorigenic in nude mice (4, 81). Thus, the unregulated expression of a human homologue of Ipl1 may be a common contributor to tumorigenesis.

We report here further characterization of the chromosome segregation defect observed in ipi1 mutant cells. In addition, we describe the identification and characterization of Sli15, a regulatory binding partner of Ipl1.

Materials and Methods

Strains, Media, and Genetic Techniques

The yeast strains used in this study are listed in Table I. The diploid strain CBY 1830-53 was constructed by a one-step gene disruption procedure (53), replacing one of the two SL115 genes in DBY 1830 with the sli15-12:: HIS3 allele present on the –4.6-kb PvuII-Sacl fragment of C. Ipl1.

The sli15-3 strain CBY 482-13D–1-1 was constructed by a recombination-mediated two-step gene replacement procedure (58), first by transforming CBY 482-13D to Ura+ with the integrating L EU2-plasmid pCC512 that had been linearized at the unique N del site. The resulting Leu+ transformant was backcrossed to yield CBY 598-49C and CBY 598-52B, which contain a L EU2 marker integrated adjacent to the ipi1-2 locus, were constructed by transforming CBY 405-10B with the integrating L EU2-plasmid pCCS21 that had been linearized at the unique N del site at the C. elegans centromere. The resulting Ura+ transformant was backcrossed to yield CBY 941-2C. CBY 405-10B–2 and CBY 1060-10D–4, which contain a L EU2 marker integrated adjacent to the SL115 and sli15-3 locus, respectively, were constructed by transforming CBY 405-10B and CBY 1060-10D, respectively, to Leu+ with the integrating L EU2-plasmid pCC912 that had been linearized at the unique N del site. The Escherichia coli strain DB1142 (leu pro thr hsdR30 mcrA B lacY1 Met8 galK1 galK2 araD139 galU1 galR1 araC1 endA1 met15 leu2 thi1) was used routinely as a host for plasmids, except in experiments involving recombinant protein expression, where the E. coli strains TO10 (f- mcrA-168 rpsL15 lacI15 lacX74 galU1 galK1 araC1 endA1 met15 leu2 thi1) and strains TOP10 (F mcrA-168 rpsL15 lacI15 lacX74 galU1 galK1 araC1 endA1 met15 leu2 thi1) were used.

Yeast genetic manipulation as well as the preparation of rich medium (YPEF), synthetic complete medium (SC) lacking some amino acids, synthetic minimal medium (SD), and SD with necessary supplements were performed as described (52). 5-fluoroorotic acid (5-FOA; U. S. Biological) was used at 1 mg/ml for plates to be incubated at 26°C and at 0.5 mg/ml for plates to be incubated at 37°C. Yeast cells were grown at 26°C unless otherwise specified.

Identification of Sli15-1 and Sli15-13 Mutants

sli mutants that confer a lethal or very slow-growth phenotype only when combined with the ipi1-2 mutation were identified by a colony sectoring assay (2, 35). The details of this genetic screen will be described elsewhere. In brief, an ade2 ade3 ade4 leu2 ipl1-2 haploid strain that contains IPL1, URA3, and ADE3 on a 2u-plasmid (C. CCY 396-8D) can grow well at 26°C even after spontaneous loss of the plasmid. Thus, it forms sectoring (red and white) colonies on medium containing adenine and uracil, and it is resistant to 5-FOA . This strain was mutagenized by treatment with ethyl methanesulfonate, and mutagenized colonies that were nonsectoring (red and white) colonies on medium containing adenine and uracil, and it is resistant to 5-FOA. This strain was mutagenized by treatment with ethyl methanesulfonate, and mutagenized colonies that were nonsectoring and 5-FOA-sensitive (i.e., could not grow well upon loss of the IPL1-URA3-ADE3-plasmid) at 26°C were identified as potentially containing sli mutations. Standard backcrossing with ade2 ade3 ade4 leu2 ipl1-2 strains that were marked at the ipi1-2 locus with L EU2 (C. CCY 598-49C and C. CCY 598-52B) led to the identification of 23 sli mutant strains, including two that are relevant to this study. A selected three, three ipi1 mutant strains were also identified. The cloned SL115 gene complemented the mutant phenotypes of two of the sli15 mutants (sli15-1 and sli15-13) (C. CCY 757-1D and C. CCY 822-6B), thus suggesting that these two sli15 mutants have mutations in the same gene. This was confirmed by linkage analysis.

Molecular Cloning of SL115 and Construction of Temperature-sensitive sli15 Mutant Alleles

The SL115 gene was cloned by complementation of the nonsectoring and 5-FOA-sensitive phenotypes of ade2 ade3 ade4 leu2 ipl1-2 sli1-1 cells that contained an IPL1-URA3-ADE3-plasmid. Strain C. CCY 822-6B was transformed with plasmid DNA from a yeast genomic library constructed in the L EU2-CEN–plasmid p366 (gift of P. Hieter, University of British Columbia, Vancouver, Canada). Leu+ transformants were selected on SC medium lacking leucine. Sectoring transformant colonies were identified and tested for their ability to grow on supplemented SD medium containing 5-FOA. Plasmids were recovered into E. coli from sectoring colonies that were 5-FOA-resistant. These plasmids were retested for their ability to complement the nonsectoring and 5-FOA-sensitive phenotypes of


| Strain | Genotype |
|--------|----------|
| DBY1830 | ad2/ +lys2-801/ +his3-D200/his3-D200 ura3-52/ura3-52 leu2-3,112/leu2-3,112 trpl-1/htrpl-1 |
| CYB1830-53 | ad2/ +lys2-801/ +his3-D200/his3-D200 ura3-52/ura3-52 leu2-3,112/leu2-3,112 trpl-1/htrpl-1 sli15-Δ2::HIS3/+ |
| CCY2019-1C | a ade2-130 lys2-801 ura3-52 leu2-3,112 his3-D200 trpl-1 [IPL1, URA3, ADE3] (i.e., with the 2μ-plasmid pCC476) |
| CCY396-8D | o ade2-130 lys2-801 ura3-52 leu2-3,112 his3-D200 trpl-1 [IPL1, URA3, ADE3] (i.e., with the 2μ-plasmid pCC476) |
| CCY405-10B | o ade2-130 lys2-801 his3-D200 ura3-52 leu2-3,112 ippl-2 |
| CCY405-10B-2 | o ade2-130 lys2-801 his3-D200 ura3-52 leu2-3,112 ippl-2 LEU2 (at SLI15) |
| CCY482-13D | a ade2-130 his3-D200 ura3-52 leu2-3,112 lys2-Δ101::HIS3::lys2-Δ102 |
| CCY482-13D-1 | a ade2-130 his3-D200 ura3-52 leu2-3,112 lys2-Δ101::HIS3::lys2-Δ102 sli15-3 |
| CCY598-49C | o ade2-130 ura3-52 leu2-3,112 his3-D200 ippl-2 LEU2 (at ippl-2) |
| CCY598-52B | a ade2-130 ura3-52 leu2-3,112 his3-D200 ippl-2 LEU2 (at ippl-2) |
| CCY757-1D | a ade2-130 lys2-801 ura3-52 leu2-3,112 his3-D200 ippl-2 sli15-11 [IPL1, URA3, ADE3] (i.e., with the 2μ-plasmid pCC476) |
| CCY766-9D-1 | a lys2-801 his3-D200 ura3-52 leu2-3,112 trpl-1 NUF2::URA3-NUF2-SGFP |
| CCY822-6B | o ade2-130 ura3-52 leu2-3,112 his3-D200 ippl-2 sli15-11 [IPL1, URA3, ADE3] (i.e., with the 2μ-plasmid pCC476) |
| CCY822-7B | a ade2-130 ura3-52 leu2-3,112 his3-D200 ippl-2 sli15-11 [IPL1, URA3, ADE3] (i.e., with the 2μ-plasmid pCC476) |
| CCY915-2B | a lys2-801 ura3-52 his3-D200 trpl-1 ippl-2 |
| CCY915-13C-5 | a lys2-801 his3-D200 ura3-52 leu2-3,112 trpl-1 ippl-2 NUF2::URA3-NUF2-SGFP |
| CCY941-2C | o lys2-801 ura3-52 leu2-3,112 his3-D200 ippl-2 URA3 (at ippl-2) |
| CCY1022-10C | a lys2-801 his3-D200 ura3-52 leu2-3,112 trpl-1 sli15-Δ2::HIS3::URA3, SLI15] (i.e., with the CEN-plasmid pCC977) |
| CCY1060-1D | a ade2-130 ura3-52 leu2-3,112 his3-D200 trpl-1 [IPL1, URA3, ADE3] (i.e., with the 2μ-plasmid pCC476) |
| CCY1060-1D-4 | a ade2-130 ura3-52 leu2-3,112 his3-D200 trpl-1 [IPL1, URA3, ADE3] (i.e., with the 2μ-plasmid pCC476) |
| CCY1076-25A | o lys2-801 his3 ura3 leu2-3,112 trpl-1 URA3::tetO112 leu2::LEU2-terR-GFP |
| CCY1077-6C | o lys2-801 his3 ura3 leu2-3,112 trpl-1 URA3::tetO112 leu2::LEU2-terR-GFP |
| CCY1078-3B | o lys2-801 his3 ura3 sli15-3 ura3::URA3-terO112::leu2::LEU2-terR-GFP |
| CCY1083-8B | a lys2-801 his3-D200 ura3-52 leu2-3,112 sli15-3 NUF2::URA3-NUF2-SGFP |
| TD4 | a ura3-52 leu2-3,112 trpl-1-289 his5-19 |

Most of the strains were constructed specifically for this study, the exceptions being DBY1830, which is from D. Botstein’s laboratory collection (Stanford University, Palo Alto, CA), and TD4, which is from G. Fink’s laboratory collection (Whitehead Institute, Cambridge, MA). The origin of some of the markers used is indicated in the text. Genes shown in bold type were used as antigen in injections of guinea pigs. Anti-Ipl1 antibodies were affinity-purified with TrpE-Ipl1 that was immobilized on a nitrocellulose membrane.

### Plasmid Constructions

Subcloning experiments were routinely carried out with the high copy number plasmids pSM 217 and pSM 218 (gift of P. Hieter), the low copy number plasmids pRS315 and pRS316 (64), and the integrating plasmids pRS305, pRS306, and YIp5 (58, 64). Plasmids encoding epitope-tagged versions of IPL1 (pCC1128) or Sli15 (pCC1173 and pCC1193) were constructed by inserting a DNA fragment encoding three tandem copies of the HA- or Myc-epitope (from pMY3-3XHA or pMY3-3XMYC [59]) into the coding sequence of IPL1 (after the initiation codon) or Sli15 (before the stop codon). The HA-Ipl1 and Sli15-Myc fusion proteins are functional since they can complement the Ts− phenotype of ippl-2 and sli15-3 cells, respectively (data not shown). Plasmids encoding the GST-Sli15 fusion protein (pCC1061 and pCC1062) were constructed in pEG (KT) (41) (for expression in yeast) and pGEX-ZT (65) (for expression in E. coli). The GST-Sli15 fusion protein is functional since it can complement the viability phenotype of sli15-2 and sli15-3 cells (data not shown).

Mutagenesis of SLI15 was carried out by in vitro error-prone PCR and in vivo gapped-repair as described (42). In brief, T3 and T7 primers (Promega) were used in a PCR reaction with Taq DNA polymerase (Promega) to amplify the SLI15 gene present on the low copy number L E U2-plasmid pCC912, which contained an ~4.8-kb SacI-XhoI yeast genomic DNA fragment derived from the insert present within pCC879, was linearized at the unique Nhel site present within this fragment and used to transform the ade2 ade3 leu2 ura3 ippl-2 strain CCY 405-10B. The resulting Leu+ transformant (CCY 405-10B-2) was mated with an ade2 ade3 leu2 ura3 ippl-2 sli15-1 strain that contained an IPL1-URA3-ADE3-plasmid (CCY 822-7B). Sporulation and tetrad analysis of the resulting diploid revealed absolute linkage between the Leu+ and 5-FOA-sensitive phenotypes. Thus, the insert DNA from pCC879 was derived from the SLI15 locus.

The GST-Sli15 fusion protein is functional since it can complement the viability phenotype of sli15-2 and Sli15 mutant strain (data not shown). Plasmids encoding chimeric proteins containing the green fluorescent protein (GFP) (24) fused to ippl-1 (pCC959) or Sli15 (pCC1060) were constructed by pBR2138 (11). The GFP-Ipl1 and GFP-Sli15 fusion proteins are functional since they can complement the Ts+ phenotype of ippl-2 and the viability phenotype of sli15-2::HIS3 cells, respectively (data not shown). The plasmid encoding His6-Ipl1 (pCC1167), which has six tandem histidine residues fused to the Nhis3 terminus of ippl-1, was constructed with pTrcHis A (Invitrogen Corp.). The plasmid encoding the sli15-Δ2::HIS3 mutant allele (pCC923) was constructed by replacing the DNA sequence between the Aat1 and Nru1 sites present in the low copy number URA3-plasmid pCC883 with the ~1.8-kb Xbal-Smal fragment (containing H Hughes) of plp217 (30). The plasmid encoding the transcript fusion protein (pCC134-16) was constructed by inserting the ~1.5-kb EcoRI DNA fragment of pCC100 (13) into the EcoRI site of pAHT10 (34), resulting in an in-frame fusion between trpE and codon 45 of IPPL1.

### Antibody Production

The TrpE-Ipl1 fusion protein was partially purified from E. coli cells (R R1) harboring pCC134-16 by a previously described method (78) and used as antigen in injections of guinea pigs. Anti-Ipl1 antibodies were affinity-purified with TrpE-Ipl1 that was immobilized on a nitrocellulose membrane (78).
Immunoprecipitation and GST Pulldown Assays

To prepare extracts from yeast cells that coexpressed HA-Ipl1 and GST or HA-Ipl1 and GST-Sli15, a saturated culture of TD4 that contained the plasmids pCC1128 (encoding HA-Ipl1) and pEg(KT) (encoding GST) or the plasmids pCC1128 and pCC1061 (encoding GST-Sli15) was diluted 20-fold into 50 ml of SC medium lacking uracil and leucine and with 2% raffinose (US Biological) instead of glucose as carbon source. A 6-h incubation at 30°C, galactose (US Biological) was added to a final concentration of 4%, and the culture was incubated at 30°C for another 4 h. Cells were harvested and rinsed once with 10 ml of lysis buffer, which consists of 50 mM Hepes-KOH (pH 7.4), 200 mM KCl, 10% glycerol (vol/vol), 1% NP-40 (vol/vol), 1 mM EDTA, 1 mM dithiothreitol, 25 mM NaF, 1 mM NaVO₄, and the following protease inhibitors (Sigma Chemical Co.): 2 μg/ml each of antipain, leupeptin, pepstatin A, chymostatin, and aprotinin; 10 μg/ml of phantethrolidine; 16 μg/ml of benzamidine-HCl; and 1 mM PM SF. Cells were resuspended in 0.45 ml of lysis buffer and aliquoted into three 1.5-ml microcentrifuge tubes. A cis-washed glass beads (425–600-μm diam; Sigma Chemical Co.) were added to each tube to give a final volume of ~0.2 ml. A flter chilling on ice, the tubes were mixed by being vortexed for 1 min. This cycle of chilling and vortexing was repeated six more times. The lysates from the three tubes were combined, followed by a 10-min centrifugation at 20,800 g to remove cell debris. 140-μl aliquots of the resulting supernatant were distributed into three 1.5-ml microcentrifuge tubes, each containing 50 μl of a 50% slurry of glutathione-agarose beads (Sigma Chemical Co.) in equilibration buffer (50 mM Hepes-KOH, pH 7.4, 200 mM KCl, 10% glycerol [vol/vol], 1 mM EDTA). The beads were incubated at 4°C with constant agitation for 2 h. The glutathione-agarose beads were harvested by a 2-min centrifugation at 960 g, followed by one, three, or five 5-min washes with 250 μl of lysis buffer. The proteins bound on the glutathione-agarose beads in each tube were eluted by the addition of 60 μl of sample buffer (50 mM Tris-HCl, pH 6.8, 10 mM dithiothreitol, 2% sodium dodecyl sulfate [wt/vol], 0.1% bromophenol blue [wt/vol], 10% glycerol [vol/vol]).

To prepare extracts from yeast cells that coexpressed HA-Ipl1 and Sli15 or HA-Ipl1 and Sli15-Myc, a saturated culture of TD4 that contained bromophenol blue [wt/vol], 10% glycerol [vol/vol], and the following protease inhibitors (Sigma Chemical Co.): 2 μg/ml each of antipain, leupeptin, pepstatin A, chymostatin, and aprotinin; 10 μg/ml of phantethrolidine; 16 μg/ml of benzamidine-HCl; and 1 mM PM SF. Cells were resuspended in 0.45 ml of lysis buffer and aliquoted into three 1.5-ml microcentrifuge tubes. A cis-washed glass beads (425–600-μm diam; Sigma Chemical Co.) were added to each tube to give a final volume of ~0.2 ml. A flter chilling on ice, the tubes were mixed by being vortexed for 1 min. This cycle of chilling and vortexing was repeated six more times. The lysates from the three tubes were combined, followed by a 10-min centrifugation at 20,800 g to remove cell debris. 140-μl aliquots of the resulting supernatant were distributed into three 1.5-ml microcentrifuge tubes, each containing 50 μl of a 50% slurry of glutathione-agarose beads (Sigma Chemical Co.) in equilibration buffer (50 mM Hepes-KOH, pH 7.4, 200 mM KCl, 10% glycerol [vol/vol], 1 mM EDTA). The tubes were incubated at 4°C with constant agitation for 2 h. The glutathione-agarose beads were harvested by a 2-min centrifugation at 960 g, followed by one, three, or five 5-min washes with 250 μl of lysis buffer. The proteins bound on the glutathione-agarose beads in each tube were eluted by the addition of 60 μl of sample buffer (50 mM Tris-HCl, pH 6.8, 10 mM dithiothreitol, 2% sodium dodecyl sulfate [wt/vol], 0.1% bromophenol blue [wt/vol], 10% glycerol [vol/vol]).

Cytological Techniques

Immunofluorescence staining of yeast cells was carried out as described (47). In experiments that involved immunostaining of microtubules in yeast cells that expressed GFP fusion proteins, cells were fixed in 3.7% formaldehyde at room temperature for 30 min, followed by standard procedures for immunofluorescence staining (47), except that the methanol- and acetone-fixation steps were omitted. In experiments that did not involve immunostaining, GFP fusion proteins were observed either in live yeast cells or in cells that had been fixed for 10 min at room temperature in 3% paraformaldehyde (40). In experiments in which visualization of DNA as well as GFP fusion proteins was desired, 4′,6-diamidino-2-phenylindole (DA PI; A Curate Chemical Co.) was added to the growth medium to a final concentration of 2.5 μg/ml 15 min before preparation of cells for observation.

Results

Seperated Sister Chromatids Are Not Properly Segregated in ipli1 Mutant Cells

We have shown previously that ipl1-2 mutant cells do not have a uniform arrest phenotype at the restrictive temperature of 37°C (13). Instead, they go through the cell cycle, missegregate chromosomes severely, undergo cytokinesis, and become inviable. In a temperature-shift experiment, over 20% of the large-budded cells in an asynchronous culture of ipl1-2 cells that had been incubated at 37°C for 3–4 h had clearly failed to segregate chromosomal DNA evenly to the opposite poles of apparently normal looking mitotic spindles (Fig. 1) (13). The percentage of ipl1-2 cells that had missegregated chromosomes was probably much higher (see below and reference 8), since we were conservative in our scoring of uneven chromosomal DNA masses. To find out whether this failure in chromosome segregation was caused by a failure in sister chromatid separation, we examined the distribution of chromosome V that was marked by the binding of Tet repressor-green fluorescent protein (TetR-GFP) to Tet operator sites located adjacent to the centromere of chromosome V (40). A t both 26°C and after a 2-h incubation at 37°C, ~90% of large-budded wild-type haploid cells that were in early anaphase had clearly separated sister chromatids, as indicated by the presence of two dots of TetR-GFP signal (Fig. 2, a and b, and Fig. 3). A these cells reached late anaphase or early G1, essentially all cells had separated and properly segregated sister chromatids, as indicated by the presence of a single dot of TetR-GFP signal within each of the two evenly segregated chromosomal DNA masses (Fig. 2, c and d, and Fig. 3). In contrast, ipl1-2 cells had properly separated and seggregated sister chromatids at 26 but not 37°C. A flter a 2-h
incubation at 37°C, among ipl1-2 cells that were in early anaphase, ~60% had only a single dot of TetR-GFP signal (Fig. 2, e and f, and Fig. 3), and ~22% had two dots that were located unusually close to each other (Fig. 2, g and h, and Fig. 3). As ipl1-2 cells reached late anaphase or early G1, only ~30% of them appeared to have properly separated and segregated the sister chromatids of chromosome V. Approximately 43% had a single dot of TetR-GFP signal (Fig. 2, i and j, and Fig. 3), and ~27% had two dots that were located within only one of the two (often unevenly segregated) chromosomal DNA masses (Fig. 2, k and l, and Fig. 3). The presence of a single dot of TetR-GFP signal in ipl1-2 cells suggested that sister chromatids had failed to separate, or that sister chromatids had separated but had failed to be properly segregated to opposite poles, thus resulting in the juxtaposition of two dots of TetR-GFP signal that were scored mistakenly as a single dot (70). While we cannot distinguish between these two possibilities, the presence of a large fraction of ipl1-2 cells with two dots of TetR-GFP signal within only one of the two chromosomal DNA masses indicated that sister chromatids had separated in these cells, but these separated sister chromatids had failed to segregate away from each other towards the two opposite poles. Thus, if a defect in sister chromatid separation exists in ipl1-2 cells, it cannot be the only cause of chromosome missegregation in these cells, as sister chromatids that have separated also fail to be properly segregated.

**Mislocalization of Nuf2-GFP in ipl1-2 Cells**

In addition to the most prominent phenotype of uneven chromosome segregation described in the last section, a small fraction of ipl1-2 cells exhibits some other cytological defects. After 3–4 h at 37°C, ~10% of the large-budded cells in an asynchronous culture of ipl1-2 cells exhibited defects in nuclear migration and/or mitotic spindle orientation (Fig. 1). A smaller fraction of ipl1-2 cells appeared to have monopolar spindles (Fig. 1), which are suggestive of defects in spindle pole body duplication or separation. This latter phenotype is more noticeable in ipl1-2 cells that had been presynchronized at G1 before being released into the cell cycle at 37°C (data not shown). To find out whether ipl1-2 cells have normal spindle poles, we examined the localization of Nuf2-GFP in ipl1-2 cells. Previous immunofluorescence and biochemical studies have shown that Nuf2 is associated with the intranuclear region of the
spindle pole body (45, 77), and Nuf2-GFP is a marker commonly used for observing spindle pole dynamics (31). Consistent with previous reports, our immunofluorescence study with wild-type cells showed that at 26°C and after a 2–3 h incubation at 37°C, Nuf2-GFP was found concentrated at the spindle poles. In cells that had a mitotic spindle, the intensities of the Nuf2-GFP signal were even at the two poles (Fig. 4, a–f, and Fig. 5). In contrast, the localization of Nuf2-GFP appeared abnormal in ipl1-2 cells, especially after a 2–3-h incubation at 37°C. First, Nuf2-GFP was no longer restricted to the spindle poles. Instead, it was also found in many ipl1-2 cells as extra dots that colocalized with mitotic spindles that were short to medium in length (Fig. 4, g–i, and Fig. 5). This pattern of Nuf2-GFP was found at much lower frequencies in wild-type cells. A similarly abnormal localization of Nuf2-GFP has been reported in the wild-type cells. A functional Spc42-GFP fusion protein (gift of J. Kilmartin, Medical Research Council, Cambridge, England) was evenly distributed at 26°C (0 h) or for 2 h at 37°C. Uneven segregation of chromosomal DNA masses was indicated by shaded areas of different sizes. For each sample, 100 large-budded cells that were in early anaphase and 100 large-budded cells that were in late anaphase or early G1 were scored. No distinction was made between the mother and bud of these large-budded cells. Whether the centromere region of chromosome V (marked by TetR-GFP) present on sister chromatids appeared separated (sep.) or unseparated (unsep.) was indicated.

To find out whether other spindle pole–associated components may also be mislocalized in ipl1-2 cells, we examined the localization of the spindle pole body central plaque component Spc42 (55). At 26°C, a functional Spc42-GFP fusion protein (gift of J. Kilmartin, Medical Research Council, Cambridge, England) was evenly distributed at opposite spindle poles in 100% of wild-type and ipl1-2 cells. After a 3-h incubation at 37°C, the Spc42-GFP signal at opposite spindle poles was slightly uneven in <3% of wild-type and ipl1-2 cells. Furthermore, Spc42-GFP was never detected along the mitotic spindle in either cell type (data not shown). Thus, Nuf2-GFP, but not Spc42-GFP, becomes distributed abnormally in ipl1-2 cells. This abnormality suggests that potential defects in the spindle poles may contribute to chromosome missegregation in ipl1-2 cells. This subject will be taken up further in the Discussion.
Localization of GFP-Ipl1 to the Mitotic Spindle

To determine the subcellular localization of Ipl1, we generated a fusion gene encoding the GFP and full-length Ipl1. The GFP-IPL1 fusion gene, which was under the control of the ACT1 promoter, was functional (see Materials and Methods). In wild-type yeast cells that carried pCC959, a low level of GFP-Ipl1 signal could be detected in the cytoplasm (Fig. 6). The intensity of this cytoplasmic signal varied somewhat in different cells, perhaps due to the small variations in the copy number of pCC959. GFP-Ipl1 was also found enriched in the nucleus, with special concentration on the mitotic spindle apparatus. In unbudded and small-budded cells, GFP-Ipl1 was sometimes found in a dot-like structure that typically colocalized with the edge of the nucleus (Fig. 6). Immunofluorescent staining of microtubules in these cells indicated that this dot-like structure represented the spindle pole body (data not shown). In large-budded cells that had completed chromosome segregation, GFP-Ipl1 was often found concentrated on elongated or disassembling spindles (Fig. 6 and data not shown). At a lower frequency, GFP-Ipl1 was also found concentrated on mitotic spindles that were short to medium in length. We do not know whether the apparent differences in our ability to detect GFP-Ipl1 on spindles of different lengths were due to cell cycle-specific changes in the localization pattern of GFP-Ipl1, or the possibility that GFP-Ipl1 signal on short/medium-length spindles might be more readily obscured by the overall nuclear signal. We have so far not detected GFP-Ipl1 signal on cytoplasmic microtubules. However, we cannot rule out the possibility that GFP-Ipl1 might be present at low levels on cytoplasmic microtubules.

Mutations in SLI15 Cause ipl1-2 Cells to Be Inviable at 26°C

To identify other proteins that may play a role in the Ipl1-mediated chromosome segregation process, we reasoned that nonlethal mutations that lower or abolish the function of proteins that play a positive role in this process (e.g., as substrates or positive regulators of Ipl1) may be tolerated in wild-type cells but not in mutant cells with reduced Ipl1 function (21). ipl1-2 mutant cells have a normal growth rate at 26°C but they do not have normal Ipl1 protein kinase function, since ipl1-2 cells exhibit an ~10-fold increase in the frequency of chromosome gain at this temperature (8). Thus, we carried out a genetic screen at 26°C for nonlethal sli mutations that confer a lethal or very slow-growth phenotype only when combined with the ipl1-2 mutation (see Materials and Methods). Among 23 sli mu-
tants isolated, two contain recessive mutations in the SLI15 gene.

**SLI15 Is an Essential Protein That Is Localized to the Mitotic Spindle**

The wild-type SLI15 gene was cloned by complementation of the mutant phenotypes of sli15-1 ipl1-2 cells (see Materials and Methods). Subcloning and partial sequencing revealed that SLI15 is identical to YBR156C, which potentially encodes a protein of 698 residues, with a predicted molecular mass of ~79 kD and a predicted pI of ~10. The predicted Sli15 protein sequence is not highly similar to that of any protein listed in the sequence databases at the National Center for Biotechnology Information. A putative nuclear localization signal is present at residues 530–546 (48), and the region (residues 517–565) surrounding this putative localization signal is predicted to have a high probability of adopting a coiled-coil conformation (38).

It has been reported previously that disruption of

|       | WT | ipl1-2 | sli15-3 |
|-------|----|--------|---------|
| 0 h   | 94 | 82     | 95      |
| 3 h   | 84 | 23     | 20      |
|       |    | 13     | 52      |
|       |    | 0      | 20      |
|       |    | 3      | 8       |

The distribution of Nuf2-GFP appeared abnormal is indicated.

**Figure 5. Summary of Nuf2-GFP distribution.** The localization of Nuf2-GFP (dots) and chromosomal DNA masses (shaded) were examined in wild-type (WT) (CCY 766-9D-1), ipl1-2 (CCY 915-13C-5), and sli15-3 (CCY 1083-BB) cells that were incubated at 26°C (0 h) or for 3 h at 37°C. Uneven distribution of Nuf2-GFP was indicated by dots of different sizes within the same cell. Uneven segregation of chromosomal DNA masses was indicated by shaded areas of different sizes. For each sample, 100 large-budded cells that were in preanaphase and 100 large-budded cells that were in late anaphase or early G1 were scored. No distinction was made between the mother and bud of these large-budded cells. Whether the distribution of Nuf2-GFP appeared abnormal is indicated.

**Figure 6. Subcellular localization of GFP-Ipl1 and GFP-Sli15.** The DIC, DAPI-stained, and GFP-fusion protein images were obtained from unfixed wild-type diploid (DBY 1830) cells that carried the low copy number plasmid pCC959 (encoding GFP-Ipl1) or pCC1060 (encoding GFP-Sli15).
**sli15-3 Mutant Cells Have Phenotypes Similar to Those of ipl1-2 Cells**

The sli15-1 and sli15-13 mutants that we identified originally in the synthetic lethal screen do not have major growth phenotypes at 13–37°C. Thus, we carried out in vitro mutagenesis of SLI15 and screened for sli15 mutant alleles that confer a Ts− growth phenotype at 37°C (see Materials and Methods). One such allele, sli15-3, was used to replace the chromosomal copy of the wild-type SLI15 gene in a haploid IPL1 strain (see Materials and Methods). The resulting sli15-3 strain is Ts− for growth at ≥33°C (see Fig. 8). The sli15-3 mutation, like the sli15-1 and sli15-13 mutations, causes a synthetic lethal phenotype at 26°C when combined with the ipl1-2 mutation. Tetrad analysis of a diploid heterozygous for ipl1-2 and sli15-3 (CCY 941-2C × CCY 1060-1D-4) revealed that 0 of 7 ipl1-2 sli15-3 haploid meiotic products were viable at 26°C, whereas 12 of 13 ipl1-2 SLI15 haploids and 13 of 13 IPL1 sli15-3 haploids were viable at this temperature.

To examine the phenotype of sli15-3 cells, we carried out temperature-shift experiments with asynchronous cultures of sli15-3 cells. After a 4-h shift to 37°C, only 15–20% of sli15-3 cells remained viable, and they did not arrest with a uniform cell morphology. Immunofluorescence microscopy showed that >20% of large-budded sli15-3 cells had clearly failed to segregate chromosomal DNA evenly to the opposite poles of apparently normal looking mitotic spindles (Fig. 1); ∼10% of large-budded cells seemed to be defective in nuclear migration and/or mitotic spindle orientation (Fig. 1); and a smaller fraction of large-budded cells appeared to have monopolar spindles (Fig. 1). These phenotypes of sli15-3 cells are very similar to those of ipl1-2 cells.

We have also examined the separation and segregation of sister chromatids in sli15-3 cells, using the TetR-GFP assay described above. After a 2- to 3-h shift to 37°C, among sli15-3 cells that were in early anaphase, ∼53% had only a single dot of TetR-GFP signal (Fig. 2, m and n, and Fig. 3), and ∼25% had two dots that were located unusually close to each other (Fig. 2, o and p, and Fig. 3). As sli15-3 cells reached late anaphase or early G1, only ∼39% of them appeared to separate properly and segregated the sister chromatids of chromosome V. Approximately 32% had a single dot of TetR-GFP signal (Fig. 2, q and r, and Fig. 3), and ∼29% had two dots that were located within only one of the two (often unevenly segregated) chromosome DNA masses (Fig. 2, s and t, and Fig. 3). Thus sli15-3 cells, like ipl1-2 cells, are defective in sister chromatid segregation (and possibly sister chromatid separation).

Finally, we also examined the distribution of Nuf2-GFP in sli15-3 cells. After a 2–3-h shift at 37°C, Nuf2-GFP was no longer restricted to the spindle poles. Instead, it was also found in some sli15-3 cells as extra dots that colocalized with mitotic spindles that were short to medium in length (Fig. 4, p–r, and Fig. 5). Furthermore, the intensities of Nuf2-GFP signal were no longer even at the two poles of a large fraction of mitotic spindles (Fig. 4, s–x, and Fig. 5). Examination of sli15-3 cells that were in late anaphase or early G1 revealed that the pole with greater intensity of Nuf2-GFP signal was mostly associated with a greater-than-normal amount of chromosomal DNA, and in no case was stronger Nuf2-GFP signal associated with a smaller-than-normal amount of chromosomal DNA (Fig. 5). These abnormal patterns of Nuf2-GFP distribution are very similar to those observed in ipl1-2 cells. Furthermore, this abnormality is limited to Nuf2-GFP, as a functional Spc42-GFP fusion protein is evenly distributed to the spindle poles in sli15-3 cells (data not shown).

**Sli15 Binds Ipl1 In Vivo**

The genetic interaction between ipl1-2 and sli15 mutations, the similar phenotypes of ipl1-2 and sli15-3 cells, and the similar subcellular localization of GFP-Ipl1 and GFP-Sli15 suggest that these two proteins may associate with each other. To examine this possibility, we used a high copy number plasmid to express in yeast a functional version of Ipl1 that was fused to the HA-epitope (HA-Ipl1). We coexpressed in the same cells GST or a functional version of Sli15 that was fused to GST (GST-Sli15). The latter proteins were expressed under the control of the GAL1/10 promoter and were thus present in great excess. As shown in Fig. 7 A, affinity purification of GST-Sli15, but not the more abundant GST, led to the copurification of HA-Ipl1. The association between HA-Ipl1 and GST-Sli15 was moderately stable, as repeated rounds of washing led to only partial disruption of this association. Comparison of the level and electrophoretic mobility of HA-Ipl1 in cells that coexpressed GST-Sli15 and in cells that coexpressed GST or GST-Sli15 revealed two interesting features. First, the level of HA-Ipl1 was much higher in cells that coexpressed GST-Sli15, thus suggesting that Sli15 may serve to stabilize Ipl1. Second, in cells that coexpressed GST, HA-Ipl1 could be detected as two forms that differed in electrophoretic mobility, with the slower migrating form being much less abundant (Fig. 7 A). In cells that coexpressed GST-Sli15, the relative abundance...
of the slower-migrating form was increased and this form of HA-Ipl1 appeared to be more readily copurified with GST-Sli15. The slower-migrating form could be converted to the faster-migrating form by endogenous phosphatases that were present in the yeast extract, as the slower-migrating form was detected only when the yeast extract was prepared in the presence of phosphatase inhibitors (Fig. 7 B). These results indicated that the slower-migrating form of HA-Ipl1 is phosphorylated and that GST-Sli15 (and probably Sli15) may promote the phosphorylation of HA-Ipl1.

We have also used high copy number plasmids to coexpress in yeast HA-Ipl1 and Sli15 or a functional version of Sli15 that was fused to the Myc-epitope (Sli15-Myc). As shown in Fig. 7 C, immunoprecipitation of Sli15-Myc from a crude yeast lysate with anti-Myc antibodies led to the coprecipitation of HA-Ipl1. Interestingly, Sli15-Myc appeared as multiple electrophoretic forms, thus suggesting that Sli15 may also be a phosphoprotein.

To find out whether Sli15 binds Ipl1 directly, we expressed GST-Sli15 and His6-Ipl1 in E. coli. As shown in Fig. 7 D, His6-Ipl1 that was present in an E. coli crude extract associated with GST-Sli15, but not GST, that was immobilized on glutathione-agarose beads. The association between GST-Sli15 and His6-Ipl1 was specific, as the control protein His6-NonO-C failed to associate with GST-Sli15. These results together indicate that Sli15 is a binding partner of Ipl1 in vivo and that these two proteins most probably bind to each other directly.

**Suppression of sli15-3 by Increased Dosage of IPL1 or GLC8**

The results described so far strongly suggest that Ipl1 and Sli15 function in a complex to promote proper chromo...
Figure 8. Suppression of slt15-3 by increased dosage of IPL1 and GLC8. Suspensions of slt15-3 mutant cells (CCY 1060-1D) carrying different plasmids were spotted on YEPD and allowed to grow at the indicated temperatures for 1.75 d. The plasmids used were high copy number control plasmid pSM217, low copy number SL115-plasmid pCC977, low copy number IPL-1-plasmid pCC100, high copy number glc7-Δ186-312-plasmid pCC418, and high copy number GLC8-plasmid pCC638.

some segregation. Mutations that affect the function of one component of a protein complex can sometimes be suppressed by overproduction of other components of the complex (22). Thus, we examined whether overproduction of Ipl1 or Sli15 can suppress the slt15-3 or ipl1-2 mutation, respectively. Our results showed that a high copy number SL115-plasmid had no effect on the Ts+ growth phenotype of ipl1-2 cells (data not shown). In contrast, a low copy number IPL-1-plasmid could suppress partially (Fig. 8) and a high copy number IPL-1-plasmid could suppress almost completely (data not shown) the Ts+ phenotype of slt15-3 cells at 37°C. Suppression requires residual Sli15 function, as a high copy number IPL-1-plasmid could not suppress the inviability of slt15-Δ2::HIS3 cells (data not shown). One possible interpretation of these results is that the slt15-3 mutation compromises the ability of mutant Sli15 to associate with Ipl1, whereas the ipl1-2 mutation compromises the catalytic activity of the Ipl1 protein kinase. In fact, the ipl1-2 mutation is known to alter a residue located in the COOH-terminal catalytic domain of Ipl1 (13).

We have shown previously that the Ts+ phenotype of some ipl1 mutants can be suppressed partially by overproduction of a truncated and dominant negative form (Glc7-Δ186-312) of PP1, or by overproduction of Glc8, an inhibitor of PP1, thus suggesting that PP1 acts in opposition to the Ipl1 protein kinase in regulating chromosome segregation (13, 74). If the slt15-3 mutation indeed leads to a reduction in Ipl1 protein kinase function, we might expect the perturbations described above also to result in suppression of slt15-3 mutant phenotype. We found this to be true, as high copy number plasmids carrying either the glc7-Δ186-312 dominant negative allele or the wild-type GLC8 gene could suppress the Ts+ phenotype of slt15-3 cells at 33°C (Fig. 8). Thus, SL115 behaves genetically as a positive regulator of IPL1.

Discussion

Roles of Ipl1 in Chromosome Segregation

The most prominent cytological phenotype of ipl1-2 mutant cells is the uneven segregation of chromosomes to opposite poles of apparently normal looking mitotic spindles that are capable of undergoing elongation and disassembly (13). At the restrictive temperature, sister chromatids of chromosome V often appear unseparated in ipl1-2 cells (Figs. 2 and 3), suggesting that sister chromatid separation has failed, or that sister chromatids that have separated are not properly segregated away from each other to opposite poles of the mitotic spindle. In a separate study, it has been shown that the sister chromatid cohesion protein Mccl/Scc1 (20, 40) dissociates with wild-type kinetics from the chromosomes of ipl1 cells, thus suggesting that sister chromatids probably are separated normally in ipl1 cells (3). Consistent with the idea that ipl1-2 cells are defective in sister chromatid segregation but not separation, sister chromatids of chromosome V are clearly separated in the majority of ipl1-2 cells, although such separated sister chromatids very often are not segregated to opposite poles (Figs. 2 and 3). However, sister chromatid segregation clearly does not fail in all ipl1-2 cells. This observation is consistent with our previous finding that at least some sister chromatids segregate away from each other in ipl1-2 cells, since the mitotic spindle-mediated poleward forces (acting on sister kinetochores) that cause the breakage of topologically intertwined sister chromatids in top2-4 mutant cells (26, 67) also cause chromosome breakage. In top2-4 ipl1-2 cells (12).

Defects in the spindle pole bodies, the kinetochore microtubules, or the kinetochores themselves can all lead to a failure in sister chromatid segregation. Nu f2 is a spindle pole-associated protein that can be copurified with yeast spindle poles (77), and immunofluorescence microscopy has shown that Nu f2 colocalizes with Ndc80 to the intranuclear region of spindle poles (45). Interestingly, the phenotype of ndc80 mutant cells is similar, although not identical, to that of ipl1-2 mutant cells. During mitosis, most of the chromosomal DNA remains at one pole of the elongated mitotic spindle in ndc80-1 cells (77). Immunoelectron microscopy has shown that Ndc80 is associated with spindle microtubules, particularly at regions close to the spindle pole body (54). It was proposed that Ndc80 may be associated specifically with kinetochore microtubules (54, 77). If Nu f2 is localized similarly to the kinetochore microtubules, the observation that uneven amounts of Nu f2-GFP are often found at the spindle poles of ipl1-2 cells (Figs. 4 and 5) would suggest quantitative or qualitative differences between the kinetochore microtubules that emanate from opposite spindle poles, possibly as consequences of defects in spindle pole body. For example, the number of kinetochore microtubules emanating from opposite spindle poles may differ greatly in ipl1-2 cells. We do not favor this idea because kinetochore microtubules make up most of the spindle microtubules (79), and immunofluorescent staining of microtubules has not revealed...
major differences in the intensity of half spindles. A lternatively, the properties of the kinetochore microtubules that emanate from the opposite poles may differ, with those from one pole being less proficient in bringing about sister chromatid segregation to that pole. Such properties may include the ability of kinetochore microtubules to attach to kinetochores and undergo polymerization or depolymerization.

In addition to ndc80 mutants, some ndc10 mutants also have phenotypes that are similar, but not identical, to those of ipi1-2 cells. During mitosis, essentially all chromosomes remain at one pole of the elongated mitotic spindle in ndc10-1 and ndc10-2 cells, and chromosome missegregation is not associated with cell cycle arrest (16, 66). NDC10 encodes an essential component of yeast kinetochores (10, 29). We have examined possible genetic interaction between ipi1 and ndc10 mutations and found that some, but not all, ipi1-2 ndc10-2 double mutants have a restrictive growth temperature lower than that of either single mutant (our unpublished results). Immunofluorescence microscopy has shown that Ndc10 localizes to the spindle pole body region of nearly all cells and also along some short mitotic spindles (16). This observation raises the question whether Nu f2 may actually be associated with yeast kinetochores. We do not favor this idea since Nu f2, but not Ndc10 (or other kinetochore components), is known to be copurified with spindle poles (54, 77).

Furthermore, our preliminary results suggest that whereas Nu f2-GFP is concentrated in a single dot-like structure at the spindle poles of wild-type cells, Ndc10-GFP (gift of J. Kahana, Harvard University Medical School, Boston, MA) is more often found in multiple dots that cluster around the spindle poles and also less frequently along mitotic spindles, including those that are elongated. If Nu f2 is associated with kinetochores, the uneven amounts of Nu f2-GFP found at opposite spindle poles in some ipi1-2 mutant cells may simply reflect unequal numbers of chromosomes (and their kinetochores) that are segregated to the two poles. Furthermore, the presence of Nu f2-GFP in dot-like structures along some mitotic spindles in ipi1-2 cells may reflect a failure of kinetochores to move to opposite spindle poles (Figs. 4 and 5), and it would also suggest that the kinetochores are attached to the kinetochore microtubules in ipi1-2 cells. In a separate study, it has been shown that Ipl1 can phosphorylate Ndc10 in vitro, and that the kinetochores assembled in extracts from ipi1 mutants show altered binding to microtubules, thus suggesting that Ipl1 may affect kinetochore functions (3). A better understanding of the actual site of Nu f2 localization and the quantity and quality of kinetochore microtubules present in ipi1 cells will help us understand to what degree the chromosome missegregation observed in ipi1 cells is due to defects in spindle pole or kinetochore function.

A functional GFP-Ipl1 fusion protein is localized to the entire mitotic spindle (Fig. 6), thus suggesting that Ipl1 may play important roles not only at spindle poles, kinetochore microtubules, or kinetochores. Furthermore, instead of the most prominent phenotype of uneven chromosome segregation, a small fraction of ipi1-2 cells exhibits one of two other phenotypes. First, some ipi1-2 cells exhibit a nuclear migration defect (Fig. 1), which has also been reported for the pac15-1/ipi1 mutant (14). Nuclear migration in yeast is dependent on cytoplasmic microtubules (46, 71) and microtubule-based motor proteins (for review see reference 69). We have not detected GFP-Ipl1 on cytoplasmic microtubules. However, it is possible that spindle pole-associated Ipl1 acts on (motor proteins present at) the minus ends of cytoplasmic microtubules to influence their functions. Second, some ipi1-2 cells appear to have monopolar spindles (Fig. 1), which are suggestive of defects in spindle pole body duplication or separation. This phenotype of ipi1 cells is reminiscent of that of Drosophila aurora mutants, which are defective in centrosome separation and form monopolar spindles due to mutations in the gene encoding a homologue of Ipl1 (15). In budding yeast, spindle pole body separation requires the function of the kinesin-related Cin8 and Kip1 motor proteins (28, 50, 57). Interestingly, the nonessential Cin8 motor protein becomes indispensable in ipi1 mutant cells (14 and our unpublished results), thus suggesting that Ipl1 may act on kinesin-related motor proteins. In this regard, it is interesting to note that microinjection of antibodies against HSeF5, a human homologue of Cin8 and Kip1, leads to the abnormal distribution of some centrosome-associated proteins in HeLa cells (76). Furthermore, C. elegans embryonic cells lacking the centrosome-associated Ipl1-homologue A IR-1 kinase are also defective in the localization of the centrosome-associated protein PIE-1 (60).

Sli5 as a Binding Partner of Ipl1

Several lines of evidence indicate that there is a very close functional relationship between Ipl1 and Sli5. First, non-lethal mutations in SLI15 exacerbate the Ts plus growth phenotype of ipi1-2 cells, leading to cell inviability at 26°C. Second, sli15-3 and ipi1-2 cells have very similar mutant phenotypes, including failure of separated sister chromatids to be properly segregated (Figs. 2 and 3), abnormal distribution of Nu f2-GFP (Figs. 4 and 5), and minor defects in nuclear migration and bipolar spindle formation (Fig. 1). Third, GFP-Ipl1 and GFP-Sli15 are both localized on the mitotic spindle (Fig. 6). Fourth, Ipl1 and Sli15 associate with each other in vivo, most probably through direct binding (Fig. 7). These results suggest that Sli15 may function as a major physiological substrate and/or as a positive regulatory binding partner of Ipl1.

The following observations support the idea that Sli15 functions as a positive regulatory binding partner of Ipl1, although they by no means preclude the possibility that Sli15 may also function as a major physiological substrate of Ipl1. First, the Ts plus phenotype of sli15-3 cells can be suppressed by a small increase in the gene dosage of IPL1 or perturbations that lower the in vivo function of PP1 (Fig. 8). Such perturbations also suppress the Ts plus phenotype of ipi1 mutant cells (13, 74). Second, the sli15-3 mutation exhibits synthetic lethal genetic interaction with the same spectrum of mutations that are synthetic lethal with ipi1-2 (our unpublished results). Third, the abundance of HA-Ipl1 is increased in cells that overexpress GST-Sli15, and the phosphorylation state of HA-Ipl1 is also altered in such cells (Fig. 7). Furthermore, the phosphorylated form of HA-Ipl1 appears to be copurified preferentially with GST-Sli15, thus suggesting that GST-Sli15 may promote the phosphorylation of HA-Ipl1.
As a binding partner of Ipl1, Sli15 potentially may stimulate the protein kinase activity of Ipl1 or it may stabilize Ipl1 and target it to its sites of action. We are currently testing the ability of Sli15 to stimulate the in vitro kinase Ipl1 and target it to its sites of action. We are currently investigating the protein kinase activity of Ipl1 or it may stabilize Ipl1 relative with diverse forms of human cancer.

If such a homologue exists, it will be important to find out whether amplification and/or overexpression of Sli15 is involved in colorectal and breast tumors (4, 62, 81). A human homologue of Sli15 has not yet been identified. If such a homologue exists, it will be important to find out whether amplification and/or overexpression of the gene encoding this Sli15-homologue may also be correlated with diverse forms of human cancer.

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