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Isolation and Characterization of Chromosome-Gain and Increase-in-Ploidy Mutants in Yeast

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ABSTRACT

We have developed a colony papillation assay for monitoring the copy number of genetically marked chromosomes *II* and *III* in *Saccharomyces cerevisiae*. The unique feature of this assay is that it allows detection of a gain of the marked chromosomes even if there is a gain of the entire set of chromosomes (increase-in-ploidy). This assay was used to screen for chromosome-gain or increase-in-ploidy mutants. Five complementation groups have been defined for recessive mutations that confer an *i*ncrease-in-*pl*oidy (*ipl*) phenotype, which, in each case, cosegregates with a temperature-sensitive growth phenotype. Four new alleles of *CDC31*, which is required for spindle pole body duplication, were also recovered from this screen. Temperature-shift experiments with *ipl1* cells show that they suffer severe nondisjunction at 37°. Similar experiments with *ipl2* cells show that they gain entire sets of chromosomes and become arrested as unbudded cells at 37°. Molecular cloning and genetic mapping show that *IPL1* is a newly identified gene, whereas *IPL2* is allelic to *BEM2*, which is required for normal bud growth.

THE faithful distribution of a complete set of chromosomes to each progeny cell in a eukaryotic cell division cycle requires the precise coordination of a large number of cellular processes. These include a single round of chromosomal DNA replication, mitosis, cell growth and cytokinesis, and, in some cell types, nuclear migration and division. Many supramolecular structures are involved in these processes. They include the mitotic chromosomes themselves, the kinetochores, the cytoplasmic and spindle microtubules, the microtubule organizing centers, and less understood components involved in cell growth and cytokinesis. Defects in the functioning or regulation of these structures may lead to over- or under-replication of chromosomal DNA, failure in chromosome segregation during mitosis, or failure in the co-ordination between chromosome segregation and cell division, thus resulting in cells that contain an abnormal complement of chromosomes.

Genetic means of examining chromosome distribution has been developed in the yeast *Saccharomyces cerevisiae*. Chromosome-loss or -gain assays for chromosomes *III* (Campbell, Fogel and Lusnak 1975), *V* (Wood 1982), *VII* (Esposito *et al.* 1982), and *VIII* (Whittaker *et al.* 1988) are available. These assays were used to show that the spontaneous chromosome-loss or -gain rate of wild-type cells is very low—approximately 1×10^{-5} to 1×10^{-4} losses/chromosome/mitotic division (Esposito *et al.* 1982; Hartwell and Smith 1985; Surosky and Tye 1985). This rate is significantly increased in a number of mutants (Burke, Gasdaska and Hartwell 1989; Hartwell and Smith 1985; Hoyt *et al.* 1992; Hoyt, Stearns and Botstein 1990; Huffaker, Thomas and Botstein 1988; Kouprina *et al.* 1988, 1992; Liras *et al.* 1978; Rockmill and Fogel 1988; Spencer *et al.* 1990). Two sensitive colony color assays have also been developed for monitoring chromosome stability (Hieter *et al.* 1985; Koshland, Kent and Hartwell 1985). Both involve the use of two genetically marked chromosomes; markers on these chromosomes interact in a dosage-dependent manner to produce a characteristic colony color. Changes in the copy number of one genetic marker (*i.e.*, copy number of the marked chromosome) relative to the copy number of the other marker result in colony color changes. These assays have been used to isolate chromosome-loss mutants (Hoyt, Stearns and Botstein 1990; Shero and Hieter 1991; Spencer *et al.* 1990). While these color assays are very sensitive, they only detect changes in the ratio of the copy number of the two marked chromosomes. However, if the copy number for both of these chromosomes is increased to the same degree, there would be no change in the ratio, and thus no detectable color change. This happens when both marked chromosomes, or an entire set of chromosomes, is gained by a cell. In addition, most of the chromosome stability assays mentioned are not applicable to haploid cells that carry only a single copy of each chromosome.

The gain of an entire set of chromosomes (*i.e.*, increase in ploidy) is a known phenotype of some

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mutants. Mutations or deletions in the genes *CDC31* (BYERS 1981; SCHILD, ANANTHASWAMY and MORTIMER 1981), *ESP1* (BAUM *et al.* 1988; MCGREW *et al.* 1992), *SPA1* (SNYDER and DAVIS 1988), *KAR1* (ROSE and FINK 1987; VALLEN *et al.* 1992), *NDC1* (THOMAS and BOTSTEIN 1986), *CHC1* (LEMMON and JONES 1987), *MPS1* and *MPS2* (WINEY *et al.* 1991), as well as treatment of wild-type cells with the microtubule destabilizing drug methyl benzimidazole-2-yl-carbamate (MBC) (WOOD 1982) can lead to an increase in ploidy. *CDC31, MPS1, MPS2* and *NDC1* are essential for the proper functioning of the spindle pole body (the microtubule organizing center of *S. cerevisiae*), whereas *SPA1* and *KAR1* encode components of the spindle pole body itself. Since the increase-in-ploidy phenotype was found already to be associated with so many mutations that affect the mitotic apparatus, we decided it would be fruitful deliberately to isolate new mutants based on this phenotype.

An enrichment procedure for haploid mutants that diploidize at elevated growth temperature was used to screen for the *esp1-1* mutant (BAUM *et al.* 1988). In brief, this procedure is based on the ability of diploid cells to form spores that can be enriched by ether treatment. However, this enrichment/screening procedure is rather tedious and may not be suitable for the isolation of a large number of mutants. A colony papillation assay that allows the distinction between colonies of haploid or diploid cells has also been described (SCHILD, ANANTHASWAMY and MORTIMER 1981). It is based on the observation that haploid cells can be mutagenized by UV irradiation to canavanine resistance much more readily than diploid cells. In this assay, haploid colonies give rise to canavanine-resistant papillae whereas diploid colonies do not do so. However, this semi-quantitative assay is not sensitive enough to distinguish between colonies that contain only haploid cells from those that contain a mixture of haploid and diploid cells. Thus, it cannot be used to screen for haploid mutants that give rise to a mixture of haploid and diploid cells.

Here we describe the development of a new colony papillation assay used to detect yeast cells that have gained a genetically marked chromosome *II* or *III*. The key feature of the assay is that it can detect the gain of the marked chromosome even when there is a gain of an entire set of chromosomes (increase in ploidy). The assay is also suitable for isolating conditional and conditional-lethal mutants. We used the papillation assay to recover mutations in *CDC31*, already known to yield mutations causing ploidy changes (BYERS 1981; SCHILD, ANANTHASWAMY and MORTIMER 1981), as well as mutations that define five additional genes not previously known to affect ploidy or chromosome number. We characterized two of the latter genes (*IPL1* and *IPL2*) further. We found that *IPL1*, which maps to chromosome *XVI*, is an apparently new gene, whereas *IPL2*, which maps to chromosome *V*, is allelic to *BEM2*, a gene required for budding and normal cell growth but heretofore not associated with the maintenance of chromosome number (BENDER and PRINGLE 1991).

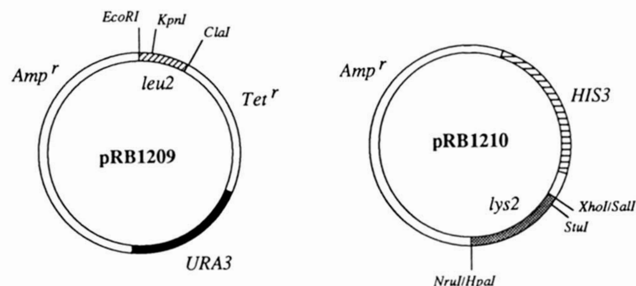


FIGURE 1.—Restriction maps of the integrating plasmids used for marking chromosomes *II* and *III*. Only the relevant restriction sites are shown. The unique *Kpn*I and *Stu*I sites were used for linearizing pRB1209 and pRB1210 for directing plasmid integration into the *LEU2* and *LYS2* loci, respectively.

MATERIALS AND METHODS

Construction of plasmids: The plasmid pRB1209 (Figure 1) was constructed by ligating the 485-bp *Cla*I/*Eco*RI restriction fragment internal to the *LEU2* gene (ANDREADIS *et al.* 1984) into the *Cla*I/*Eco*RI sites of YIp5 (SCHERER and DAVIS 1979). The plasmid pRB1210 (Figure 1) was constructed by ligating the 1.1-kb *Xho*I/*Hpa*I restriction fragment internal to the *LYS2* gene (BARNES and THORNER 1986) into the *Sal*I/*Nru*I sites of pRB328 (SCHATZ, SOLOMON and BOTSTEIN 1986). The plasmid pRB1450 was constructed by ligating the 4.7-kb *Hin*dIII/*Bgl*II restriction fragment (see Figure 7) containing the *IPL1* gene into the *Hin*dIII/*Bam*HI sites of YIp5. The plasmid pRB1451 was constructed by ligating the 6.5-kb *Bam*HI/*Sph*I fragment (Figure 7) located adjacent to the *IPL2* gene into the *Bam*HI/*Sph*I sites of YIp5.

Strains and media: Yeast strains used in this study are listed in Table 1. Haploid yeast strains carrying a genetically marked chromosome *III* (and *II*) were constructed. These strains carry the nonreverting *ura3-52* and *his3-Δ200* mutations. The integrating plasmid pRB1209, carrying a functional *URA3* gene and a 485 bp sequence entirely internal to the *LEU2* gene, was linearized at the unique *Kpn*I site. It was inserted, through transformation (ITO *et al.* 1983) and homologous recombination (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1981), into the wild-type *LEU2* locus present on chromosome *III* of DBY4923 and DBY4924 (see Figure 2). Plasmid integration created two nonfunctional *leu2* genes, one missing its 3′ end (*leu2-Δ101*), the other missing its 5′ end (*leu2-Δ102*). Between the two nonfunctional copies of *leu2*, which contain 485 bp of identical sequence, lies a functional *URA3* gene; these strains (DBY4925 and DBY4926) are thus phenotypically Leu^-Ura^+. To construct a strain that is also marked on chromosome *II*, the integrating plasmid pRB1210, carrying a functional *HIS3* gene and a 1.1-kb sequence entirely internal to the *LYS2* gene, was inserted into the wild-type *LYS2* locus present on chromosome *II* of DBY4925, creating a tandem partial duplication (*lys2-Δ101* and *lys2-Δ102*) comparable to that present on chromosome *III* (Figure 2). The resulting strain (DBY4962) is thus phenotypically $Leu^-Ura^+Lys^-His^+$. The strains DBY4963, DBY4964, and DBY4965 were derived from DBY4962. All the *ipl* mutant yeast strains, with the exception of those used in the complementation analysis, were

TABLE 1

Yeast strains used

Strain	Genotype
DBY1536	*α/α his4-539/HIS4 cry1-51/cry1-51 lys2-801/lys2-801 leu2-3,112/LEU2*
DBY3432	**a** *his3 leu2 trp1 ura3 cin2-7*
DBY4923	**a** *ade2 his3-Δ200 ura3-52*
DBY4924	*α lys2-801 his3-Δ200 ura3-52*
DBY4925	**a** *ade2 his3-Δ200 ura3-52 leu2-Δ101::URA3::leu2-Δ102*
DBY4926	*α lys2-801 his3-Δ200 ura3-52 leu2-Δ101::URA3::leu2-Δ102*
DBY4942	*α lys2-801 his3-Δ200 ura3-52 leu2-Δ101::URA3::leu2-Δ102 ipl1-1*
DBY4946	*α lys2-801 his3-Δ200 ura3-52 leu2-Δ101::URA3::leu2-Δ102 ipl1-2*
DBY4950	**a** *ade2 his3-Δ200 ura3-52 leu2-Δ101::URA3::leu2-Δ102 ipl2-1*
DBY4952	*α lys2-801 his3-Δ200 ura3-52 leu2-Δ101::URA3::leu2-Δ102 ipl2-1*
DBY4962	**a** *ade2 his3-Δ200 ura3-52 leu2-Δ101::URA3::leu2-Δ102 lys2-Δ101::HIS3::lys2-Δ102*
DBY4963	*α ade2 his3-Δ200 ura3-52 leu2-Δ101::URA3::leu2-Δ102 lys2-Δ101::HIS3::lys2-Δ102*
DBY4964	**a** *hom3 his3-Δ200 ura3-52 leu2-Δ101::URA3::leu2-Δ102 lys2-Δ101::HIS3::lys2-Δ102*
DBY4965	*α hom3 his3-200 ura3-52 leu2-Δ101::URA3::leu2-Δ102 lys2-Δ101::HIS3::lys2-Δ102*
DBY5289	**a** *ade2 his3-Δ200 ura3-52 leu2-Δ101::URA3::leu2-Δ102 lys2-Δ101::HIS3::lys2-Δ102 ipl5-1*
DBY5296	**a** *ade2 his3-Δ200 ura3-52 leu2-Δ101::URA3::leu2-Δ102 lys2-Δ101::HIS3::lys2-Δ102 ipl6-1*
DBY5297	**a** *ade2 his3-Δ200 ura3-52 leu2-Δ101::URA3::leu2-Δ102 lys2-Δ101::HIS3::lys2-Δ102 ipl7-1*
DBY5300	*α ade2 his3-Δ200 ura3-52 leu2-Δ101::URA3::leu2-Δ102 lys2-Δ101::HIS3::lys2-Δ102 ipl1-1*
DBY5301	**a** *ade2 his3-Δ200 ura3-52 leu2-Δ101::URA3::leu2-Δ102 lys2-Δ101::HIS3::lys2-Δ102 ipl1-2*
DBY5302	**a** *ade2 his3-Δ200 ura3-52 leu2-Δ101::URA3::leu2-Δ102 lys2-Δ101::HIS3::lys2-Δ102 ipl2-1*
DBY5304	DBY4925 × DBY4926
DBY5359	*α lys2-801 his3-Δ200 ura3-52 ipl2-1 URA3(at SPT2)*
DBH5360	**a** *ade2 his3-Δ200 ura3-52 leu2-3,112 gal4::LEU2*
DBY5361	*α his3-Δ200 ura3 leu2 cin2-7 ipl1-2*
DBY5362	*α lys2-801 his3-Δ200 ura3-52 ipl1-1*
DBY5363	*α lys2-801 his3-Δ200 ura3-52 ipl2-1*
DBY5364	*α lys2-801 his3-Δ200 ura3-52 ipl1-2*
BJ3501	*α pep4::HIS3 prb1-Δ1.6R his3-Δ200 ura3-52 can1*
L2501	**a/a** *his4-Δ5/his4-Δ29 arg11/arg11*

Most of the strains were constructed specifically for this study. The exceptions are L2501, from G. FINK; BJ3501, from E. JONES; and DBY1536, from this laboratory's collection. The origin of some of the markers used is indicated in the text.

backcrossed at least three times with wild-type strains before further use. The strain DBY5359, used for mapping the *IPL2* gene, was derived from a strain carrying a *URA3* gene integrated next to the *SPT2* locus. The *Escherichia coli* strain DB1142 (*leu pro thr hsdR hsdM recA*) was used as a host for plasmids. Rich medium YEPD (with glucose) and YPG (with glycerol), synthetic minimal medium SD, and SD medium with necessary supplements were prepared as described (SHERMAN, FINK and LAWRENCE 1974). Sporulation was done in 1% potassium acetate, pH 6.7. Cells were routinely grown at 26° unless otherwise specified.

Mutagenesis of yeast cells: Yeast cells were grown in liquid YPG medium for 2 days at 30° to stationary phase. Cells were harvested and washed once with 0.1 M sodium phosphate buffer (pH 7.0). They were resuspended in the same buffer to a density of about 4.5×10^8 cells/ml. Ethyl methanesulfonate (EMS), 100 μl, was added to 3.4 ml of the cell suspension. After various time at 30° with rotational agitation, 0.2-ml aliquots were added to 8 ml of freshly made 5% sodium thiosulfate, washed once with the same solution, and once with water. Washed cells were resuspended in water and stored at 26° for a few days (while cell viability was determined) before plating. Mutagenized colonies were recovered on YEPD plates after three days at 26° and screened for mutants as described below.

Genetic analysis of *ipl* mutants: Genetic analysis was performed as described (SHERMAN, FINK and LAWRENCE 1974). For scoring the colony papillation phenotype, spore colonies from each tetrad were suspended in water and transferred to six YEPD plates with a multi-prong inoculating device. Three plates were incubated at 14°, 26°, or 37° to determine temperature or cold sensitivity. The other three plates were incubated at 26° for about 12 hr. One plate was then held at 37° for 3 hr, and another plate was held at 14° for 24 hr. The third plate was left at 26°. All three plates were then incubated at 26° for 2 days more. They were then replica plated onto selective plates lacking leucine and uracil (or lacking histidine and lysine). Papillae were scored on these selective plates after three days at 26° to determine chromosome-gain.

Molecular cloning of the *IPL* genes: The *IPL1* and *IPL2* genes were cloned by complementation of the recessive temperature-sensitive growth phenotype of the corresponding mutants. Strains DBY5362 (*ipl1-1 ura3-52*) and DBY5363 (*ipl2-1 ura3-52*) were transformed by the lithium acetate method (ITO *et al.* 1983) with plasmid DNA from a yeast YCp50 genomic library (ROSE *et al.* 1987). Transformants were selected at 26° on minimal plates lacking uracil. Ura^+ colonies were replica plated to similar plates incubated at 37°. DNA was extracted as described (STRUHL *et al.* 1979), using cultures of colonies that grew at 37°. Complementing plasmids were recovered in *E. coli* strain DB1142 by selecting for the plasmid's ampicillin-resistance gene. Plasmids were retransformed into mutant cells to confirm that they conferred the Ts^+ phenotype.

Measurement of the DNA content of yeast cells: Cellular DNA was stained with propidium iodide as described by HUTTER and EIPEL (1978). For each sample, the DNA content of 15,000 individual cells was measured by flow cytometry using an Ortho Diagnostics System 2151 machine and 488 nm excitation.

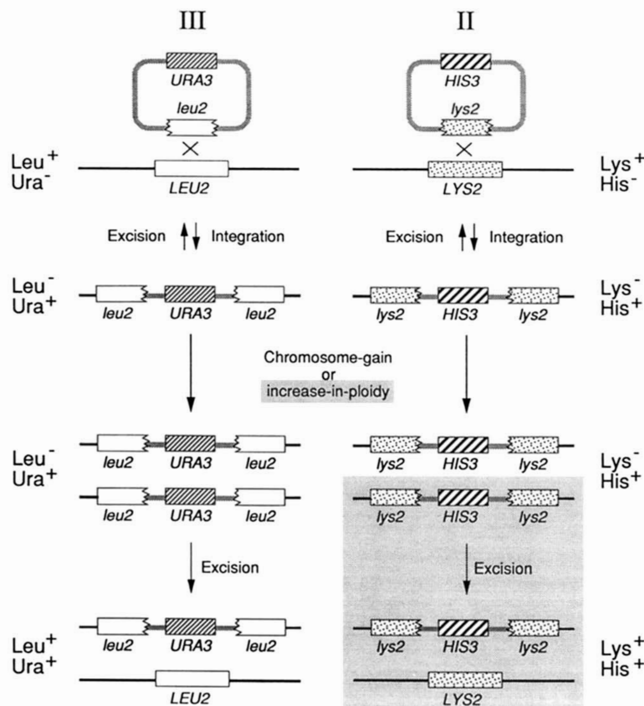


FIGURE 2.—Monitoring of chromosome *II* and *III* copy number. The yeast strains used carry the nonreverting *ura3-52* and *his3-Δ200* mutations.

Morphological observations: Cellular DNA was stained with 4,6-diaminido-2-phenylindole (DAPI) as described by HUFFAKER, THOMAS and BOTSTEIN (1988). Cells were observed under phase contrast or epifluorescence with a Zeiss Axioskop microscope.

RESULTS

We devised a genetic assay that allows one to distinguish, on petri plates, between yeast cells carrying one copy of a genetically marked chromosome and those carrying more than one copy. This assay can be used to screen for haploid mutant cells that become disomic (*e.g.*, due to the gain of a single chromosome) or diploid (due to the gain of an entire set of chromosomes). The principle of the assay depends upon the construction of a mutation in one gene by insertion, through the process of integrative gene disruption, of DNA carrying a functional copy of another. In a haploid, reversion of the insertion mutation in the first gene necessarily involves loss of the inserted copy of the second, functional gene.

Monitoring of chromosome *II* and *III* copy number: The construction we used to monitor the number of copies of chromosome *III* is shown in Figure 2. A haploid yeast strain with partially duplicated but nonfunctional *leu2* genes that are separated by a DNA segment carrying a functional *URA3* gene on chromosome *III* was constructed by the method of integrative gene disruption (SHORTLE, HABER and BOTSTEIN 1982). This strain is phenotypically Leu$^-$Ura$^+$. The method of construction by homologous recombination results in a partial duplication of *LEU2* DNA flanking the *URA3* insertion. Therefore, this haploid strain can readily regain a functional *LEU2* gene and thus become Leu$^+$ through a variety of recombinational events, including plasmid excision and unequal sister chromatid exchange that utilize the homology between the two flanking nonfunctional *leu2* genes (SCHIESTL, IGARASHI and HASTINGS 1988). The single functional *URA3* gene in the haploid genome is lost in these processes, resulting in haploid Leu$^+$ cells that are Ura$^-$. In contrast, the same recombinational processes in a yeast strain with two or more copies of the genetically marked chromosome *III* should result in Leu$^+$Ura$^+$ revertants, because the reversion event will result in the loss of only one of the functional *URA3* genes in the cell. This difference should allow us to detect disomics as well as diploids, because the occurrence of the recombinational processes at the *leu2::URA3::leu2* locus on chromosome *III* should be independent of the copy number of the other 15 yeast chromosomes. We chose chromosome *III* for this assay because it was already known that cells disomic for this chromosome are healthy. The extra chromosome *III* is stable mitotically and does not readily lead to the gain of other chromosomes in the disomic haploid (CAMPBELL, FOGEL and LUSNAK 1975; SHAFFER *et al.* 1971).

This same principle can be applied to other chromosomes. Using a plasmid containing a functional *HIS3* gene and a partial, nonfunctional *lys2* gene, we constructed a Lys$^-$His$^+$ haploid yeast strain that carries a genetically marked chromosome *II* (Figure 2). As with the previously described strain, this Lys$^-$His$^+$ haploid strain should be unable to become Lys$^+$His$^+$ through a single recombination step, whereas a strain with two or more copies of this chromosome *II* should be able to do so.

To show that the genetic assay works as predicted, the frequency of reversion to Leu$^+$(Ura$^+$ or Ura$^-$) was compared to the frequency of reversion to Leu$^+$Ura$^+$ using different Leu$^-$Ura$^+$ strains that carry one or two copies of the marked chromosome *III* (Table 2). Haploid **a** or α and diploid strains exhibited similar frequencies of approximately 3×10^{-4} for the appearance of Leu$^+$ cells. These numbers represent the frequencies of the recombinational events at the *leu2* locus. Leu$^+$Ura$^+$ cells appeared at a much lower frequency (about 9×10^{-7}) in haploid strains. However, there was little difference between the frequencies of occurrence of Leu$^+$ and Leu$^+$Ura$^+$ cells in diploid strains. As predicted, the reversion events that led to Leu$^+$ diploids left a second copy of the functional *URA3* gene intact.

The 150–400-fold difference in the frequencies at which Leu$^+$Ura$^+$ cells appeared in haploid and diploid strains allows us to distinguish between haploid and diploid cells in a plate assay (Figure 3). When patches of diploid cells grown on nonselective medium at 26°

TABLE 2

Frequency of occurrence of Leu⁺ and Leu⁺Ura⁺ cells for wild type haploid and diploid strains with genetically marked chromosome *III*

Strain	Chromosome *III* copy number	Frequency	
		Leu⁺	Leu⁺Ura⁺
Haploid **a**	1	$4.4 \pm 4.0 \times 10^{-4}$	$0.5 \pm 0.2 \times 10^{-6}$
Haploid α	1	$2.8 \pm 0.6 \times 10^{-4}$	$1.2 \pm 0.5 \times 10^{-6}$
Diploid **a**/α	2	$2.4 \pm 1.1 \times 10^{-4}$	$1.8 \pm 1.2 \times 10^{-4}$

The strains used were DBY4925 (**a**), DBY4926 (α) and DBY5304 (**a**/α). Cells were grown to stationary phase in 5 ml YEPD rich medium at 30°. Appropriately diluted and briefly sonicated aliquots were plated out on YEPD, selective medium lacking leucine, and selective medium lacking both leucine and uracil. Colonies were scored after 3 days at 26°. The mean frequency and standard deviation obtained from eight independent cultures of each strain are shown here.

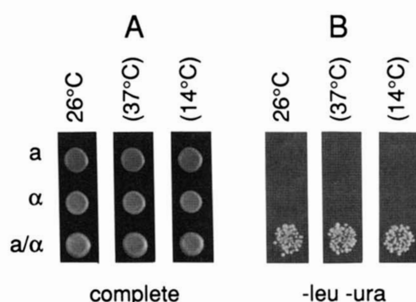


FIGURE 3.—Papillation pattern of haploid and diploid strains. The yeast strains used (DBY4925, DBY4926 and DBY5304) all carry genetically marked chromosome *III*. They were patched onto three YEPD plates and incubated at 26° for about 12 hr. One plate was then incubated at 14° for 24 hr, and another plate was incubated at 37° for 3 hr. All three plates were incubated for another two days at 26°. The patches on these plates (A) were then replica plated onto selective plates (B) lacking leucine and uracil. Papillae were scored after 3 days at 26°C. 14°C = cells that had been incubated at 14°. 26°C = cells that had been incubated only at 26°. 37°C = cells that had been incubated at 37°.

were replica plated onto medium lacking leucine and uracil, they gave rise to dense patches of papillae. In contrast, haploid cells gave rise to few or no papillae. Transient incubation of either haploid or diploid cells at 14° or 37° on the nonselective plate had no effect on the subsequent papillation pattern. Thus, the degree of papillation can be used as a rough indication of the copy number of chromosome *III* if the recombination frequency is constant. In a similar manner, the copy number of chromosome *II* can also be monitored. This plate assay allows us to screen, on the basis of papillation, for haploid colonies that contain, because of mutations and/or environmental manipulations, large numbers of disomic or diploid cells, which give rise to a positive signal of Leu⁺Ura⁺ (or His⁺Lys⁺) papillae. The haploid and diploid papillation patterns of this assay are exactly opposite to those of the canavanine-resistant colony papillation assay described in the Introduction (SCHILD, ANANTHASWAMY and MORTIMER 1981).

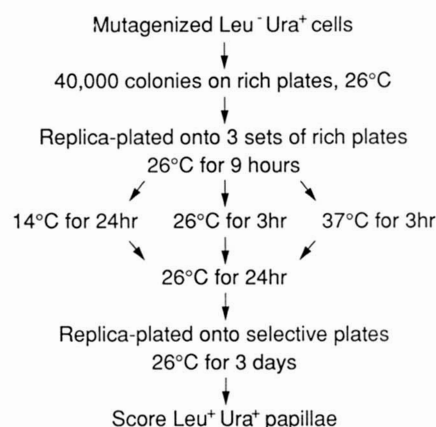


FIGURE 4.—Screening of chromosome-gain or increase-in-ploidy mutants.

Isolation of chromosome-gain or increase-in-ploidy mutants: In the search for chromosome-gain or increase-in-ploidy mutants, we focused on obtaining mutants with extreme defects because these are the most amenable to analysis. However, mutants with severe chromosome-gain or increase-in-ploidy phenotype are likely to have low viability due to the accumulation of aneuploid or polyploid cells, and would therefore be intractable genetically. We thus designed a screen that allowed us to detect and recover mutants that conditionally display severe phenotypes. The papillation assay is well suited for this, because the assay can be applied to colonies that have been exposed transiently to nonpermissive conditions, allowing the easy recognition of heat- or cold-sensitive chromosome-gain or increase-in-ploidy mutants, even those that are unable to grow at the nonpermissive temperature.

Haploid strains DBY4925, DBY4926 (marked on chromosome *III* with the *leu2::URA3::leu2* construction) and DBY4962 (marked on both chromosomes *II* and *III* with the *lys2::HIS3::lys2* and the *leu2::URA3::leu2* constructions, respectively) were mutagenized with ethyl methanesulfonate (EMS) to a viability ranging from 12 to 27% (see MATERIALS AND METHODS). Mutagenized cells were plated onto YEPD medium at 26° (Figure 4). Colonies were replica plated onto three sets of YEPD plates, all of which were incubated at 26° for 9 hr to ensure that the yeast cells were growing exponentially. One set of plates was then held at 37° for 3 hr (about two generations) and another set was held at 14° for 24 hr (about two generations). All plates were then incubated further at 26° for 1–2 days until the colonies were fully grown. These colonies were replica plated onto selective medium lacking leucine and uracil, and the papillation pattern was scored after three days at 26°.

Nonmutagenized wild-type haploid colonies gave few or no papillae on all three sets of selective plates. Nonconditional chromosome-gain or increase-in-

ploidy mutant colonies gave an increased number of papillae on all plates. Colonies of conditional mutants, which gained chromosome *III* or increased in ploidy more frequently at a restrictive temperature, gave increased numbers of papillae if the colonies on the YEPD plates had been incubated transiently at 14° or 37°. These conditional mutants were recovered from YEPD plates that had not been exposed to the restrictive temperature. A total of about 40,000 colonies were screened; more than 200 gave a papillation pattern indistinguishable from that of diploids on all selective plates. These were not analyzed further. Twenty-nine colonies gave intermediate levels of papillation on all three selective plates (*i.e.*, nonconditional). Fourteen colonies gave increased levels of papillation if they had been incubated briefly at 37° (*i.e.*, conditional). These were selected as putative temperature-sensitive chromosome-gain or increase-in-ploidy mutants.

Five nonconditional putative mutants were backcrossed to the strains DBY4925, DBY4926 or DBY4965. The papillation phenotype failed to reappear with two such mutants, and segregated 2:2 with the other three mutants. For one of these latter three, the papillation phenotype, which appeared at all temperatures, nevertheless cosegregated with a recessive temperature-sensitive growth phenotype (Ts^- at 37°). This mutant (*ipl7-1*) was the only nonconditional one we studied further.

All 14 putative conditional (temperature-sensitive) mutants were backcrossed with the strains DBY4925, DBY4926 or DBY4965. The papillation phenotype failed to reappear in five mutants and segregated 2:2 in the other nine. In each of the latter cases, the number of papillae was markedly increased when the cells on the YEPD medium had been incubated briefly at 37°. For each of these nine, the conditional papillation phenotype cosegregated with a recessive Ts^- (at 37°) growth phenotype.

The 10 papillation mutants we studied further are thus Ts^- for growth. Each was backcrossed twice more with appropriate combinations of the strains DBY4925, DBY4926, DBY4962, DBY4963, DBY4964 or DBY4965 to generate strains containing both the *leu2::URA3::leu2* and *lys2::HIS3::lys2* markers to allow us to follow the number of copies of both chromosomes *II* and *III* via the papillation assay. In all these crosses, the Ts^- growth phenotype continued to cosegregate with the papillation phenotype as a single recessive trait.

Complementation analysis of Ts^- mutants: To determine complementation at 37°, the 10 Ts^- mutants isolated in this work were crossed with each other and with previously identified increase-in-ploidy mutants carrying the recessive Ts^- mutations *ndc1-4* (WINEY *et al.* 1993), *esp1-1* (BAUM *et al.* 1988), *cdc31-1* (HARTWELL *et al.* 1973), *mps1-1* and *mps2-1* (WINEY *et al.* 1991). Four of the mutants failed to complement either the *cdc31-1* mutant or each other. Linkage analysis and a complementation test using a plasmid containing *CDC31* (BAUM, FURLONG and BYERS 1986) confirmed that these four mutants are defective in *CDC31*. This result was encouraging because we expected that *cdc31* mutants would be identified in our screen (BYERS 1981; SCHILD, ANANTHASWAMY and MORTIMER 1981).

The remaining six mutants complemented the *ndc1-4*, *esp1-1*, *cdc31-1*, *mps1-1* and *mps2-1* mutants. These six Ts^- mutants fell into five complementation groups that we called *IPL1,2,5,6,7* for *i*ncrease-in-*pl*oidy (see below); only the *IPL1* group contains more than one mutant in our set. Linkage analysis showed that the Ts^- mutations in the two *ipl1* mutants are tightly linked and both mutants could be complemented by the same cloned DNA fragment (see below). Mutations in *CHC1*, *KAR1* and *SPA1* also can lead to increase-in-ploidy (LEMMON and JONES 1987; ROSE and FINK 1987; SNYDER and DAVIS 1988). Single-copy plasmids, each carrying one of these genes, were used to transform the *ipl* mutants; all failed to complement the recessive Ts^- defect. Therefore, the six *ipl* mutants isolated in this work are distinct from *ndc1*, *esp1*, *cdc31*, *mps1*, *mps2*, *chc1*, *kar1* and *spa1* mutants.

Copy number of chromosomes *II* and *III* in *ipl* mutants: To characterize further the Ts^- *ipl* mutants, we measured the extent to which they gained chromosomes *II* and *III* under various conditions. We showed above that there is a 150–400-fold difference between the frequencies of occurrence of Leu^+Ura^+ cells for wild-type haploid and diploid cells carrying one and two copies, respectively, of the *leu2::URA3::leu2* construction on chromosome *III* (Table 2). Thus, for any sample containing a mixture of cells with one or more copies of the marked chromosome *III*, the frequency at which Leu^+Ura^+ cells appear can be used as an indirect measurement of the relative abundance of cells containing an extra copy of chromosome *III* (assuming that recombination frequencies at the *leu2::URA3::leu2* locus are constant).

In a temperature-shift experiment, wild-type haploid cells grown at 26° gave rise to Leu^+Ura^+ cells at a very low frequency, and this increased by fourfold after a 4-hr exposure to 37° (Table 3). The haploid *ipl1-1*, *ipl2-1*, *ipl5-1* and *ipl6-1* mutants also gave rise to Leu^+Ura^+ cells at low frequencies at 26°, indicating that these mutants behaved like wild-type at this temperature. However, these frequencies increased 15–53-fold after exposure to 37° for four hours, suggesting that, at the elevated temperature, these four mutants gave rise to cells containing an extra chromosome *III*. The frequency of Leu^+Ura^+ for the *ipl1-2* mutant was much higher than that of wild-type cells at 26°, but still increased about 17-fold following a

TABLE 3

Frequency of occurrence of Leu^+Ura^+ and Leu^+ cells for wild-type and *ipl* mutant strains

Strain	Leu^+Ura^+ frequency ($\times 10^6$)			Leu^+ frequency ($\times 10^4$)		
	0 hr	4 hr	4 hr/0 hr	0 hr	4 hr	4 hr/0 hr
Wild-type	0.5	2.0	(4.0)	4.7	8.0	(1.7)
ipl1-1	0.4	21.3	(53.3)	3.7	11.9	(3.2)
ipl1-2	9.5	160.0	(16.8)	2.4	9.6	(4.0)
ipl2-1	1.0	37.0	(37.0)	4.8	17.0	(3.5)
ipl5-1	2.1	53.5	(25.5)	20.2	27.1	(1.3)
ipl6-1	1.9	27.5	(14.5)	4.3	6.5	(1.5)
ipl7-1	32.5	275.0	(8.5)	35.0	37.6	(1.1)

The haploid strains used were DBY4962, DBY5289, DBY5296, DBY5297, DBY5300, DBY5301 and DBY5302. Yeast cells were grown at 26° in YEPD rich medium to approximately 5×10^6 cells/ml. They were then shifted to 37° (0 hr). After four hours (4 hr), appropriately diluted and briefly sonicated aliquots were plated onto both YEPD and selective medium lacking leucine and uracil or selective medium lacking leucine. Colonies were scored after 3 days at 26°. Frequencies were calculated as the ratios of the number of colonies on the selective and YEPD plates.

TABLE 4

The fraction of cells with an extra chromosome *III* that also had an extra chromosome *II*

Strain	Time of exposure to 37°	
	0 hr	4 hr
Wild-type	0.85	0.86
ipl1-1	0.31[a]	0.86
ipl1-2	0.15	0.91
ipl2-1	0.45[b]	0.99
ipl5-1	0.47	0.88
ipl6-1	0.65	1.00
ipl7-1	0.35	0.56

Leu^+Ura^+ colonies from the experiment shown in Table 3 were patched onto YEPD-rich plates. After three days at 26°, they were replica plated onto selective plates lacking both lysine and histidine. After 3 days at 26°, the fraction of patches that gave a papillation pattern on the selective plates characteristic of strains carrying two copies of chromosome *II* was scored. Except where indicated, over 50 Leu^+Ura^+ colonies were tested per strain for each time point.

[a] Only 16 colonies were tested.

[b] Only 31 colonies were tested.

temperature shift. It thus appears that the *ipl1-2* mutant is somewhat defective even at 26°, and that the defect is exacerbated at 37°. The *ipl7-1* mutant differs from other *ipl* mutants in that it gave rise to Leu^+Ura^+ cells at a very high frequency at 26°, suggesting that it frequently gained an extra chromosome *III* even at the permissive growth temperature. This observation was to be expected, since this mutant was identified initially as a nonconditional papillation mutant. The Leu^+Ura^+ frequency for *ipl7-1* increased nine-fold after a 4-hr exposure to 37°.

For all six *ipl* mutants studied, the frequencies of appearance of Leu^+Ura^+ cells after exposure to 37° for 4 hr were 10–136-fold higher than that for wild-type cells grown under similar conditions. For the *ipl1*, *ipl2* and *ipl6* mutants, these differences were clearly not due to changes in the recombination frequencies at the *leu2::URA3::leu2* locus because the frequencies of occurrence of Leu^+(Ura^+ or Ura^-) cells for these mutants were very similar to that of wild-type cells (Table 3). For the *ipl5* and *ipl7* mutants, there were small increases in the recombination frequencies at the *leu2::URA3::leu2* locus. However, the small magnitude of such increases cannot account for the large increases in the Leu^+Ura^+ frequencies for these two mutants. Thus the increased frequencies of Leu^+Ura^+ cells in all the *ipl* mutants reflect the increased abundance of cells containing an extra copy of chromosome *III*.

The *ipl* mutants could have gained an extra chromosome *III* alone or together with a number of other chromosomes. In the extreme case, the *ipl* mutants could have gained an entire set of chromosomes. To start studying these different possibilities, we determined whether Leu^+Ura^+ wild-type and mutant cells (presumably with at least two copies of chromosome *III*) could become Lys^+His^+ at high frequencies (meaning they had also acquired at least two copies of chromosome *II*). At 26°, about 85% of Leu^+Ura^+ derivatives of wild-type haploids also became Lys^+His^+ at high frequencies, and exposure to 37° for 4 hr had little effect (Table 4). This suggests that the majority of wild-type cells that gained chromosome *III* also gained chromosome *II* simultaneously. At 26°, the fraction of Leu^+Ura^+ derivatives of *ipl* mutants that also could become Lys^+His^+ was lower than that of wild-type cells. This is especially true for the *ipl1-2* mutant. This difference, and the results from Table 3, suggest that at their permissive growth temperature (26°), the *ipl* mutants may have a relatively simple chromosome-gain defect that ranges from being very mild (*e.g.*, in the *ipl6-1* mutant) to being moderately severe (*e.g.*, in the *ipl1-2* and *ipl7-1* mutants). However, exposure to 37° caused *ipl1*, *ipl2*, *ipl5* and *ipl6* mutant cells to gain chromosomes *III* and *II* simultaneously, as judged from the high fraction of Leu^+Ura^+ derivatives that could become Lys^+His^+ at high frequencies. The *ipl7-1* mutant behaved differently; only about half of the Leu^+Ura^+ (gain of chromosome *III*) derivatives could become Lys^+His^+ at high frequencies, indicating that only about half of them had also gained chromosome *II*. Together, the results in Table 4 suggest that at 37°, *ipl1*, *ipl2*, *ipl5* and *ipl6* mutants mostly gain chromosome *II* and *III* simultaneously, while the *ipl7-1* mutant is equally likely to gain chromosome *III* with or without the concurrent gain of chromosome *III*.

For comparative purposes, we also examined the cold-sensitive *tub2-104* tubulin mutant. Using a chromosome-loss assay for chromosome *V* (WOOD 1982), it was shown that, after exposure to 11° for 24 hr, diploid cells homozygous for the *tub2-104* mutation

lose chromosome *V* at a frequency about 40-fold higher than that of wild-type cells (HUFFAKER, THOMAS and BOTSTEIN 1988). Using the chromosome-gain assay described here, we determined that, after exposure to 11° for 24 hr, haploid *tub2-104* mutant cells gain chromosome *III* at a frequency about 6–9-fold higher than that of wild-type cells (data not shown). Only abut 27% of the *tub2-104* cells that have gained chromosome *III* also have gained chromosome *II*. Thus, at the restrictive temperature, *tub2-104* mutant cells mostly exhibit a relatively simple chromosome-gain defect that is quite distinct from that of the *ipl1*, *ipl2*, *ipl5* and *ipl6* mutants.

Ploidy analysis of *ipl* mutants: The fact that wild-type as well as most of the *ipl* mutant cells tend to gain chromosomes *II* and *III* simultaneously at 37° suggests that these cells may actually gain multiple chromosomes, perhaps as entire or partial genomic sets, thus giving rise to cells that are diploid or disomic for many chromosomes. To determine if the wild-type and mutant cells that had gained additional chromosomes *II* and *III* were true (or near) diploids or simply simultaneously disomic for chromosomes *II* and *III* (and perhaps some other chromosomes), we carried out a mating and sporulation test (THOMAS and BOTSTEIN 1986). This is based on the observation that, upon sporulation, triploid cells give few viable spores, whereas tetraploid cells give four viable spores (ESPOSITO and KLAPHOLZ 1981; MORTIMER and SCHILD 1981). Leu^+Ura^+ cells that became Lys^+His^+ at high frequencies (*i.e.*, those with at least two copies of both chromosomes *II* and *III*) were mated with **a**/**a** or α/α diploid cells. This should result in (near) triploids if the Leu^+Ura^+ cells were aneuploid (*i.e.*, disomic for chromosomes *II* and *III*, and perhaps some other chromosomes), or (near) tetraploids if the Leu^+Ura^+ cells were (near or) true diploids. Most wild-type and mutant Leu^+Ura^+ cells that became Lys^+His^+ at high frequencies, particularly those that had been exposed to 37°, behaved as (near) diploids in this test (Table 5).

These results showed that at 37°, most of the *ipl* haploid mutants give rise at increased frequencies to cells with an extra complete set or close to an extra complete set of chromosomes. By our genetic criteria, these cells appear to have increased in ploidy. Our results also showed that even for wild-type cells, the gain of chromosome *III* is accompanied most of the time by the gain of (almost) a complete set of chromosomes. The *ipl7-1* mutant and, to a lesser extent, the *ipl1-2* mutant are somewhat different in that about half of the cells that contained extra chromosomes *II* and *III* did not behave as diploids. This may mean that these two mutants have two fundamental defects, one resulting in the gain of entire sets of chromosomes and the other resulting in the gain of relatively small numbers of chromosomes. Alternatively, these two mutants may each have a single defect that is manifested to different degrees in different cells, resulting in the gain of a variable number of chromosomes—perhaps ranging from one chromosome to an entire set of chromosomes. We believe that this latter interpretation may explain the phenotype of the *ipl1-2* (see below) and *ipl7-1* mutants. This interpretation may also apply to some of the other *ipl* mutants which appear mostly to gain entire sets of chromosomes at 37° and to gain chromosome *III* without the concurrent gain of chromosome *II* at 26°.

Finally, it is worth noting that all Leu^+Ura^+ cells examined also mated efficiently, thus ruling out homothallic switching and mating as the mechanism of the observed increase in ploidy.

Flow-cytometry analysis of the *ipl1* and *ipl2* mutants: To examine more directly the way in which ploidy or chromosome number increases, we monitored the DNA content of individual *ipl1* and *ipl2* mutant cells. Wild-type and mutant haploid cells growing exponentially at 26° were shifted to 37°. At various time points, the DNA content of individual cells was measured by flow cytometry and the morphology of the cells in these cultures was followed by light microscopy.

Wild-type cells behaved as expected. At 26°, there were roughly equal numbers of unbudded, small-budded and large-budded wild-type cells (Figure 5). About 60% of these cells had replicated their DNA and thus had a $2n$ DNA content. Exposure to 37° had only a moderate effect on these distributions. The behavior of both the *ipl1-2* and *ipl2-1* mutants differed very little from wild type at the permissive growth

TABLE 5

Ploidy analysis of *ipl* mutants

Strain	Time of exposure to 37°: 0 hr, No. tested	0 hr, No. of diploids	4 hr, No. tested	4 hr, No. of diploids
Wild-type	4	4	7	7
ipl1-1	2	2	4	4
ipl1-2	2	0	6	4
ipl2-1	2	2	5	5
ipl5-1	2	2	4	4
ipl6-1	3	2	6	6
ipl7-1	2	1	4	2

Cells from Leu^+Ura^+ colonies (from Table 3) that gave the Lys^+His^+ papillation pattern (Table 4) characteristic of cells that had gained chromosomes *II* and *III* were mated with diploids of the opposite mating type. The diploid strains used were DBY4937 (**a**/**a**) and DBY1536 (α/α). Control crosses were made using strains known to be haploid, **a**/**a** diploid, and α/α diploid. The mating products were sporulated and dissected onto YEPD plates. Spore colonies were scored after four days at 26°. Control triploids gave very few viable spores. Such spores were sick and slow growing. Control tetraploids gave good spore growth and viability. Leu^+Ura^+ colonies that gave good spore growth and viability upon mating as described here were scored as diploids.

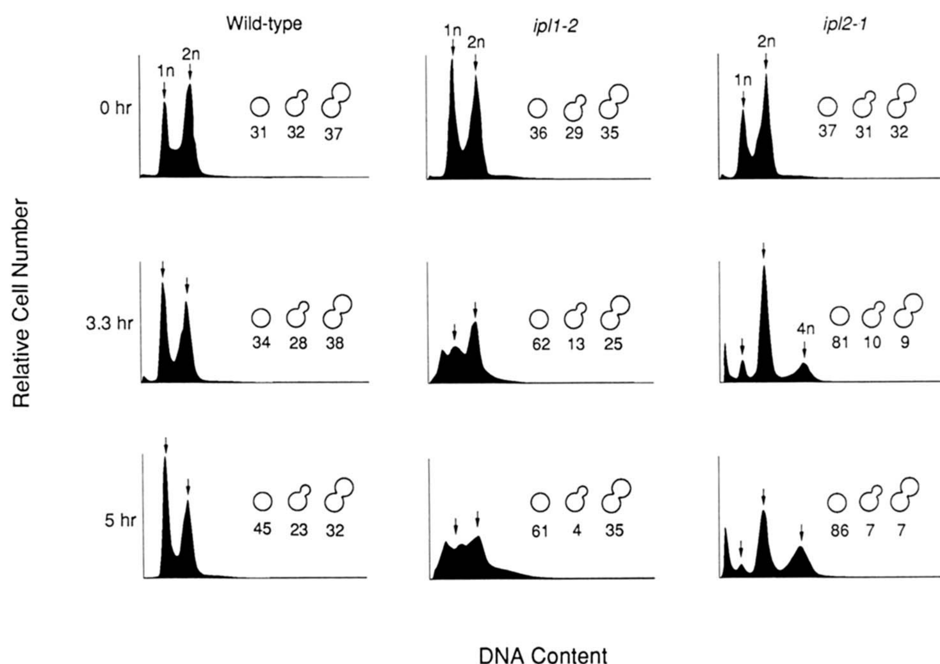


FIGURE 5.—Flow-cytometry analysis of the *ipl1* and *ipl2* mutants. The yeast strains used were DBY4926, DBY4946 and DBY4952. Yeast cells were grown at 26° in liquid YEPD to a cell density of about 4×10^6 cells/ml. They were then shifted to 37°. Aliquots were harvested at the times indicated and processed for flow cytometry. The percentage of cells that were unbudded, small-budded or large-budded are shown. The diameter of the bud on a small-budded cell is less than half of that of the mother cell. The *arrows* indicate the positions of the 1*n*, 2*n* and 4*n* peaks for DNA content.

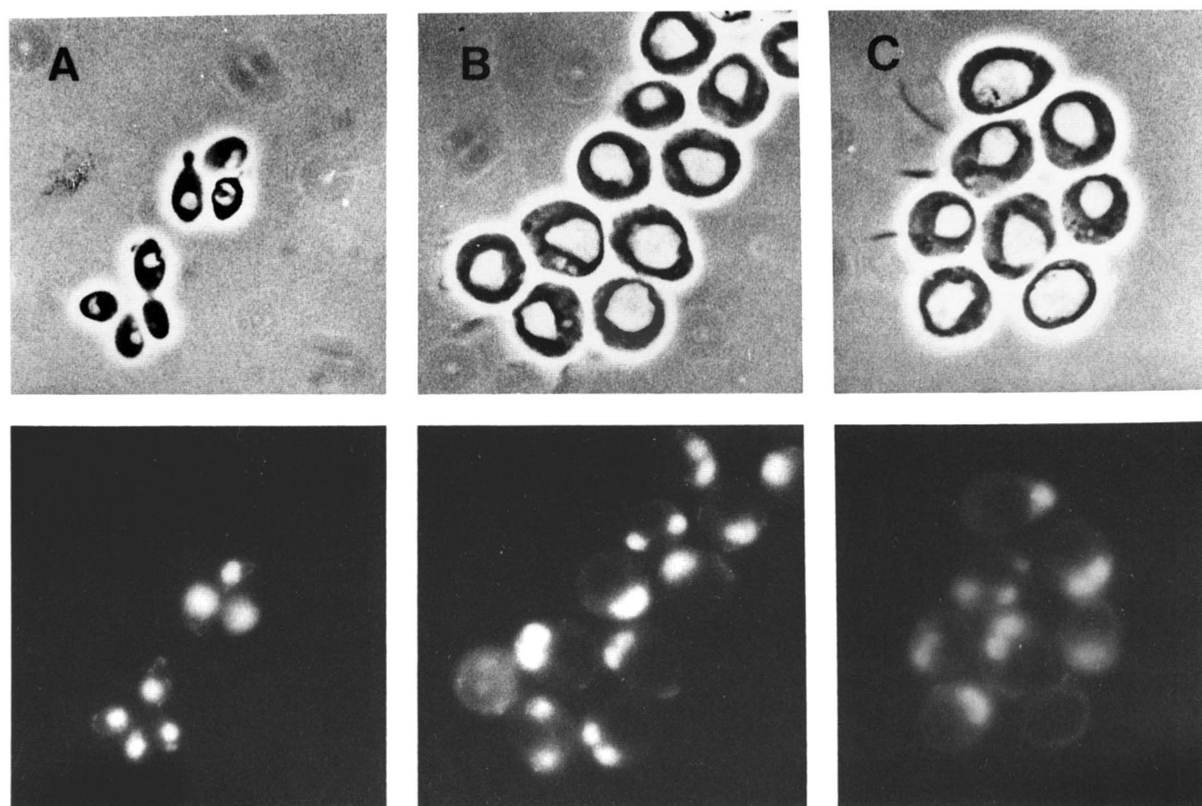


FIGURE 6.—DAPI-staining of wild-type (A) and *ipl2-1* mutant (B and C) diploid cells that had been incubated at 37° for 3.5 hr. All cells are shown at the same magnification. The phase contrast images of the stained cells are shown in the top panels. The wild-type and mutant cells used are (DBY5304) and (DBY4950 × DBY4952), respectively.

temperature (26°). However, the behavior of both these mutants differed markedly from the wild-type controls and from each other during incubation at 37°, the nonpermissive temperature. We describe each mutant in turn.

After 3.3 hr at 37°, *ipl1-2* mutant cells became predominantly unbudded. Substantial numbers of cells had a DNA content of less than 1*n*, between 1*n* and 2*n*, and more than 2*n*. After 5 hr at 37°, it appeared that most of the *ipl1-2* cells had a DNA content that was neither 1*n* nor 2*n*; the definition between the peaks in the distribution of DNA content had largely disappeared and a significant fraction of the cells had a DNA content of less than 1*n* or more

than $2n$. These results, especially the loss of discrete peaks representing euploid sets of chromosomes, suggest severe nondisjunction. The results from flow cytometry must be reconciled with the genetic data (Tables 4 and 5), which suggest that after recovery from a shift to the nonpermissive temperature, over half of the viable *ipl1-2* cells that gained chromosomes *II* and *III* behave as diploids. The explanation may lie in the fact that many of the cells die after the shift to 37°. Indeed, less than 15% of the *ipl1-2* cells remain viable after a 4-hr exposure to this temperature. The genetic tests were applied only to the survivors, whose distribution of DNA content may differ from that of the total population. This subject is taken up further in the DISCUSSION.

Likewise, most *ipl2-1* cells became arrested as unbudded cells when incubated at 37°. However, the distribution of DNA content for these cells changed in a different way, this time apparently consistent with the gain or loss of entire sets of chromosomes. Discrete peaks representing DNA content of $0n$, $1n$, $2n$ and $4n$ appeared after 3.3 hr and remained at 5 hr after the shift to the nonpermissive temperature. After 5 hr at 37°, only 5% of the mutant cells had a DNA content of $1n$. About 15% had a DNA content characteristic of aploid cells (*i.e.*, $0n$), although the possibility of a cell lysis artifact has not been excluded (see below). The presence of aploid cells would suggest an abnormal mitosis in which one of the two daughter cells received none of the duplicated chromosomes. Such an abnormal mitosis occurs in *cdc31* (BYERS 1981; SCHILD, ANANTHASWAMY and MORTIMER 1981), *ndc1* (THOMAS and BOTSTEIN 1986) and possibly *mps1* (WINEY *et al.* 1991) mutants. About 40% of the *ipl2-1* mutant cells had a DNA content of $4n$. Since 86% of the mutant cells were unbudded, it meant that at least some of the unbudded cells had a DNA content of $4n$. Thus, the observed *ipl2-1* phenotype 5 hr after shift to nonpermissive temperature is arrest as unbudded cells with an apparent DNA content of $0n$, $2n$ or $4n$.

Cytological examination of *ipl2-1* mutants: Cells with temperature-sensitive mutations in genes required for bud formation (*CDC24, CDC42, CDC43, MYO2, PFY, BEM1* and *BEM2*) (ADAMS *et al.* 1990; BENDER and PRINGLE 1991; CHANT *et al.* 1991; CHENEVERT *et al.* 1992; HAARER *et al.* 1990; JOHNSTON, PRENDERGAST and SINGER 1991; SLOAT, ADAMS and PRINGLE 1981) become arrested as unbudded cells that are multinucleate at the restrictive temperature because DNA replication and nuclear division continue in the absence of bud formation. Such unbudded cells are expected to have a DNA content that is more than $2n$. To investigate whether the *ipl2-1* mutant cells behave in a similar manner at 37°, we examined the DNA content of these cells microscopically. After 3.5 hr at 37°, most mutant cells became arrested as

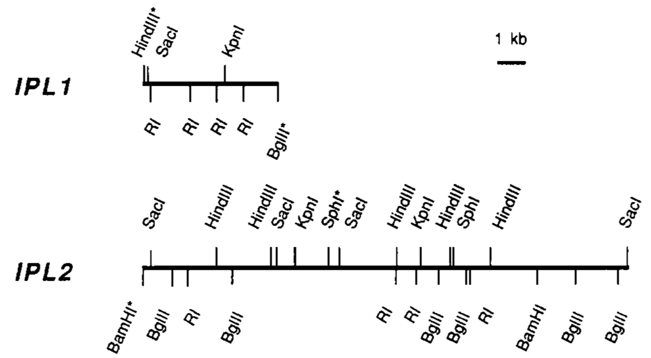
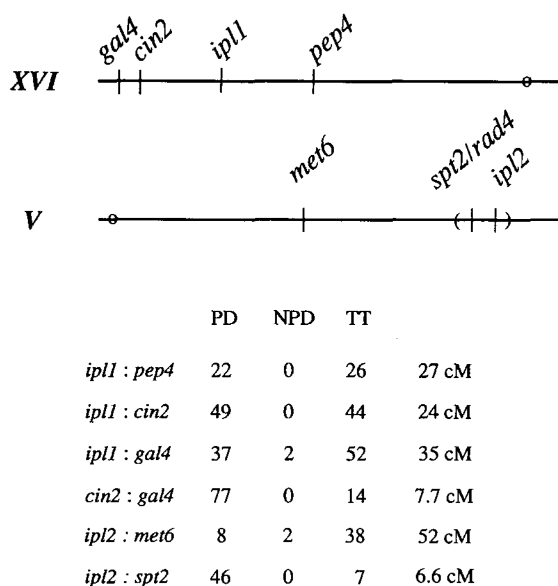


FIGURE 7.—Restriction maps of the *IPL1*- and *IPL2*-containing regions. The asterisks (*) define the restriction fragments inserted into YIp5 to create integrating plasmids.

unbudded cells that were very much enlarged (Figure 6, B and C). A fraction of these unbudded cells were also binucleate. Thus, the *ipl2-1* mutant has a phenotype similar to that of previously characterized bud formation mutants. In addition, a small fraction of the mutant cells appeared to have no nuclear DNA (Figure 6C). However, we could not conclude that these cells all represented truly aploid cells because they often looked abnormal under phase contrast microscopy, some appearing to have especially enlarged vacuoles. It is possible that some of these cells had lysed and lost their nuclei.

Molecular cloning of the *IPL1* and *IPL2* genes: As a first step towards a molecular characterization of the *IPL1* and *IPL2* genes, we cloned these two genes by complementation of the recessive temperature-sensitive growth phenotype of the corresponding mutants, using a library of cloned yeast DNA fragments (ROSE *et al.* 1987) in the low copy-number *URA3* vector YCp50. Ura^+ mutant transformants were screened for their ability to grow at 37°. One plasmid that can confer a Ts^+ phenotype in *ipl1* cells was recovered. Six plasmids, containing two classes of sequences, that can confer a Ts^+ phenotype in *ipl2* cells were recovered. One class of three plasmids contain the *IPL2* gene (see below). Another class of three plasmids contain a common sequence that is unrelated to *IPL2* (L. SALISBURY and C. CHAN, unpublished results).The restriction maps of the *IPL1* and *IPL2* regions are shown in Figure 7.

To show that the plasmids that confer a Ts^+ phenotype actually contain the *IPL1* and *IPL2* genes, subclones were made in an integrating yeast vector (YIp5). The resulting plasmids were used to integrate, by homology, the subclones and their *URA3* marker into the genome. Integration was directed to the *IPL* locus by linearizing the plasmid with an enzyme that cleaved only within the insert DNA. For *IPL1*, the Ts^- strain DBY5364 (*ipl1 ura3*) and the integrating plasmid pRB1450, which contains the *Hin*dIII/*Bgl*II DNA fragment (Figure 7) that complements the Ts^- phenotype, were used. The strain containing the in-



	PD	NPD	TT	
ipl1 : pep4	22	0	26	27 cM
ipl1 : cin2	49	0	44	24 cM
ipl1 : gal4	37	2	52	35 cM
cin2 : gal4	77	0	14	7.7 cM
ipl2 : met6	8	2	38	52 cM
ipl2 : spt2	46	0	7	6.6 cM

FIGURE 8.—Genetic mapping of *IPL1* and *IPL2*. Linkage to various loci was determined by tetrad analysis. The types of tetrad shown are parental ditype (PD), nonparental ditype (NPD) and tetratype (TT). Genetic distances were calculated according to Equation 3 of MORTIMER and SCHILD (1981) and are shown in centimorgans.

tegrated plasmid is thus Ts$^+$Ura$^+$. It was crossed to a strain of genotype *IPL1 ura3* (DBY3432). Tetrad analysis of the resulting diploid showed that Ura$^+$ segregated 2:2 and that all the spores were Ts$^+$. The absence of Ts$^-$ spores showed that the plasmid had integrated at the locus responsible for the Ts$^-$ phenotype. Thus, the insert DNA of pRB1450 carries the *IPL1* gene. For *IPL2*, the Ts$^-$ strain DBY5363 (*ipl2 ura3*) and the integrating plasmid pRB1451, which contains the *Bam*HI/*Sph*I DNA fragment (Figure 7) that does not complement the Ts$^-$ phenotype, were used. The strain with the integrated plasmid is thus Ts$^-$Ura$^+$. It was crossed to a strain of genotype *IPL2 ura3* (DBY4923). Tetrad analysis of the resulting diploid gave 22 parental ditypes, 0 nonparental ditype and 2 tetratypes for the Ura$^+$ and Ts$^-$ phenotypes. This showed that the plasmid had integrated very close to the locus responsible for the Ts$^-$ phenotype. Thus, the insert in pRB1451 came from a clone that carries the *IPL2* gene as part of a bigger insert.

Genetic mapping of the *IPL1* and *IPL2* genes: The cloned *IPL1* and *IPL2* genes were radiolabeled and used to probe a filter containing separated yeast chromosomes (CARLE and OLSON 1985). The results revealed that the *IPL1* gene is located on chromosome *XVI* whereas the *IPL2* gene is located on chromosome *V*.

Tetrad analysis with appropriately marked strains suggested that *IPL1* is located between *PEP4* and *CIN2* on the left arm of chromosome *XVI* (Figure 8). The genes *TPK2* and *RAD53* have also been mapped to this region (MORTIMER *et al.* 1989). From the DNA sequence of *IPL1* (L. SALISBURY and C. CHAN, unpublished results), we know that it is distinct from *TPK2* (TODA *et al.* 1987). Linkage analysis also showed that *IPL1* is distinct from *RAD53* (J. KING, personal communication). Thus, *IPL1* is a newly identified gene that is required for normal chromosome segregation.

Tetrad analysis with appropriately marked strains suggested that *IPL2* is weakly linked to *MET6* on the right arm of chromosome *V* (Figure 8). Further analysis (DBY4923 × DBY5359) showed that *IPL2* is more tightly linked (~7 cM) to *SPT2*. *RAD4* and *BEM2* are the other genes mapped to this region (BENDER and PRINGLE 1991; MORTIMER *et al.* 1989). *IPL2* is most probably distinct from *RAD4* because the latter gene is very tightly linked to *SPT2* (~0.3 cM) (WINSTON *et al.* 1984). Sequencing data (L. SALISBURY and C. CHAN, unpublished results; A. BENDER, personal communication) reveal that *IPL2* is identical (all 2167 amino acid residues predicted are the same) to *BEM2*, a gene previously identified as being involved in bud emergence. This finding is consistent with the budding defect observed in *ipl2-1* mutant cells at the restrictive temperature (Figure 6).

DISCUSSION

We have described above a colony papillation plate assay that detects yeast cells that have gained one or more copies of a chromosome *II* or *III*. This assay is easy to set up; it only requires the use of two cloned genes that encode proteins with selectable functions and a mutation in one of these genes that can be detected on solid medium. The availability of the large number of yeast mutants and their corresponding cloned genes should make it be possible to genetically mark and thereby monitor the copy number of any one of the sixteen yeast chromosomes. The assay should prove useful in cases where particular chromosomes are known to be unstable; for example, α-tubulin mutants often gain an extra copy of chromosome *XIII* (SCHATZ, SOLOMON and BOTSTEIN 1986) and certain β-tubulin mutants often gain an extra copy of chromosome *II* (T. HUFFAKER, personal communication). Chromosome duplications often create a background of false positives in the isolation of pseudorevertants; such false positives can be screened out easily if the duplicated chromosome carries a genetic marker similar to the ones described here.

In principle, our approach also should be applicable to other organisms. Where scorable mutants are not available, a cassette containing a dominant drug resistance marker flanked by two truncated and partially overlapping versions of a different dominant drug resistance marker can be integrated into a chromosome. In this scenario, the assay would simply involve the selection for resistance to one or both drugs. In addition to being useful for monitoring chromosome number, our approach also should be useful for de-

tecting DNA amplification (G. WEINSTOCK, personal communication).

The assay described here is based on the large difference in the frequency of occurrence of Leu^+Ura^+ (or Lys^+His^+) cells between yeast strains carrying one or two copies of the genetically marked chromosome *III* (or *II*). This frequency is actually the product of the recombination frequency at the marked locus and the frequency of occurrence of yeast cells with an extra copy of the marked chromosome. The recombination frequency at the *leu2::URA3::leu2* locus is approximately 3×10^{-4}. The rate of loss for chromosome *III* is about 1×10^{-4} per cell division in a diploid strain (SUROSKY and TYE 1985). If we assume the frequency for a simple gain of chromosome *III* in a haploid strain to be also about 1×10^{-4} and increase-in-ploidy does not occur, a frequency of about 3×10^{-8} for the occurrence of Leu^+Ura^+ cells in a haploid strain would be predicted. This is roughly 30-fold lower than the measured value. This difference, together with our observation that about 85% of the wild-type cells that gain chromosome *III* also gain chromosome *II* and are indeed diploids, suggests that spontaneous increase-in-ploidy occurs in wild-type haploid cells at a frequency (perhaps 6–30-fold) higher than that of the simple gain of chromosome *III*. This means that in the genetic screen described here, simple chromosome-gain mutants can be detected only if they exhibit a relatively large defect. Thus, the assay may favor detection of increase-in-ploidy mutants.

We obtained 10 temperature-sensitive lethal mutants that show greatly increased frequencies of Leu^+Ura^+ cells during mitotic growth. These increases are not due to greatly increased frequencies of recombination at the *leu2* locus, nor are they the result of mating type switching and mating. Four of these mutants have mutations in the *CDC31* gene. The other six mutants belong to five complementation groups (*IPL1,2,5,6* and *7*) that are distinct from *NDC1*, *ESP1*, *CDC31*, *MPS1*, *MPS2*, *KAR1*, *SPA1* and *CHC1*. With the exception of the *ipl7-1* (and to a lesser extent, the *ipl1-2*) mutant, these *ipl* mutants behave rather like wild-type cells at 26°, and they give rise at increased frequencies at 37° to cells that behave as (near) diploids (Tables 3, 4 and 5). The *ipl7-1* (and *ipl1-2*) mutant differs in that it has highly elevated levels of increase-in-ploidy as well as simple chromosome-gain at 26°. As noted earlier, this apparent dual phenotype may simply be caused by a single defect that is manifested to various degrees in different mutant cells.

Among the 5 *IPL* complementation groups, only *IPL1* is represented by more than one mutant. Apart from the four new mutant alleles of *cdc31*, we were somewhat surprised that we did not isolate more alleles of the several other genes known to cause increase-in-ploidy. We even performed a reconstruction experiment with the *ndc1-1* mutant (THOMAS and BOTSTEIN 1986), with the result showing that such a mutant would have been detected readily in our screen. We therefore believe that we are very far from having saturated the genes that can give rise to mutations that can be detected by our assay. So far, we have also limited ourselves mostly to the analysis of mutants that have a conditional papillation phenotype. The nonconditional mutants may also prove to be useful.

The results from flow cytometry suggest that the *ipl1* mutants suffer severe nondisjunction at the restrictive temperature. The majority of the *ipl1* mutant cells also become arrested as unbudded cells at this temperature. This arrest phenotype is interesting because mutants with mitotic defects more typically become arrested as large-budded cells. For example, α- and β-tubulin mutants fail in mitosis because the microtubules are either absent or nonfunctional at the restrictive temperature. They become arrested as large-budded cells (HUFFAKER, THOMAS and BOTSTEIN 1988; SCHATZ, SOLOMON and BOTSTEIN 1988), possibly as the result of a "checkpoint" or feedback control that monitors successful completion of mitotic spindle assembly (HARTWELL and WEINERT 1989; HOYT, TOTIS and ROBERTS 1991; LI and MURRAY 1991). In contrast, *ipl1* mutants seem to undergo and finish mitosis (as well as cytokinesis) even though they are clearly defective in this process at 37°. This observation may be explained in at least two ways. The *ipl1* mutants may have defects in both chromosome segregation and the feedback control of mitosis. Alternatively, the *ipl1* mutants may be defective in a mitotic process that is not monitored by the feedback control system. A number of mutants that have mitotic defects in chromosome segregation and yet do not become arrested at the time when the defect occurs have been described. They include the *ndc1-1* (THOMAS and BOTSTEIN 1986), *mps1-1* (WINEY *et al.* 1991) and *esp1-1* (BAUM *et al.* 1988; MCGREW *et al.* 1992) mutants. These three mutants give rise to grossly aberrant microtubule structures at their restrictive growth temperatures. So far, we have not detected major microtubule defects in *ipl1* mutants. Mutations in the *TOP2* gene, specifying topoisomerase *II* (HOLM *et al.* 1985), also result in mitotic failure but not in arrest with a large bud. In this case, the daughter chromosomes are still entangled when separation by the microtubule apparatus is attempted, resulting in a very slow separation that is accompanied by substantial frequencies of nondisjunction and chromosome breakage (HOLM, STEARNS and BOTSTEIN 1989). So far, we have not detected significant amounts of chromosome breakage in *ipl1* mutants. The exact nature of the *ipl1* mutant defect should be revealed through further study of the cloned *IPL1* gene.

At first glance, our results from flow cytometry, which suggest that *ipl1* mutants suffer severe chromosome nondisjunction at 37°, appear to be inconsistent with our genetic data, which suggest that *ipl1* mutants mostly gain entire sets of chromosomes at this temperature. We think that the apparent discrepancy can be explained as follows. Severe nondisjunction at 37° likely gives rise frequently to *ipl1* mutant cells that have gained or lost multiple chromosomes, resulting in gross chromosomal imbalance and cell death. Only cells that have gained so many chromosomes as to become (almost) diploids are expected to survive. Indeed, less than 15% of *ipl1-2* mutant cells remain viable after a four-hour incubation at 37°. Since the genetic assay employed monitors only living cells, this may explain why most *ipl1* mutants that have gained chromosomes *II* and *III* behave genetically as diploids. Alternatively, the gain of multiple chromosomes may lead to aneuploid cells that are unstable and prone to be converted to diploids (BRUENN and MORTIMER 1970; CAMPBELL *et al.* 1981; PARRY and ZIMMERMAN 1976).

Results from our flow cytometry and cytological studies suggest that the *ipl2-1* mutant has two defects at 37°. One of these is the appearance, at nonpermissive temperature, of discrete classes of cells, some of which have four times the haploid amount of DNA, some with two times the normal amount, and some with virtually no DNA (aploid). The presence of these classes, together with the virtual disappearance of the class containing the haploid ($1n$) amount, suggests that there is a failure in the segregation of euploid sets of chromosomes. Indeed, multinucleate cells are observed, suggesting that nuclear division occurs and possibly the fault is primarily in nuclear migration. The presence of apparently aploid cells might result from a failure in chromosome segregation or nuclear migration. While we have not ruled out cell lysis as the source of the apparently aploid cells, we do not believe that this mechanism can account for all the apparently aploid cells seen because some of them look quite normal when observed in the light microscope.

The other defect observed in the *ipl2-1* mutant is a failure in bud emergence and/or growth. At 37°, most *ipl2-1* mutant cells become arrested as unbudded cells that are enlarged. Many of these cells are also multinucleate. This phenotype is similar to that of many previously identified bud growth mutants. Our genetic mapping (Figure 8) and sequencing (C. CHAN, unpublished results; A. BENDER, personal communication) studies reveal that *IPL2* is identical to *BEM2*, a gene recently identified as being important for bud growth (BENDER and PRINGLE 1991).

Given the phenotype of the *ipl2-1* mutant (failures in bud emergence/growth and possibly chromosome segregation or nuclear migration), we cannot explain definitively the basis of the observed increase in ploidy for the *ipl2-1* mutant after a transient incubation at 37°. While it is easy to imagine how failures in chromosome segregation or nuclear migration can lead to increase-in-ploidy, it is more difficult to imagine how failures in bud growth alone can lead to the same consequence.

An unbudded and binucleate (or multinucleate) *ipl2-1* cell that survives the transient incubation at the nonpermissive temperature conceivably could follow any of three paths upon return to permissive growth temperature at 26°. (1) The survivor cell could continue to grow as a binucleate cell, producing daughter binucleate cells. Such cells should appear as diploids in our colony papillation assay. However, this is unlikely to be the source of our genetically defined diploids (from Table 5): we examined such cells under the microscope and found them not to contain extra nuclei. (2) The two nuclei inside an unbudded *ipl2-1* cell could fuse after a shift to 26°. This seems unlikely since nuclear fusion is not thought to occur efficiently in cells that have not been exposed to mating pheromone (ROSE, PRICE and FINK 1986). (3) The nuclei present in some unbudded *ipl2-1* cells could each contain chromosomes that have been duplicated but not separated (*i.e.*, in the G_2 phase of the nuclear cycle). Upon shifting down to the permissive growth temperature of 26°, these cells might undergo another round of DNA replication in a fashion similar to what was observed in some *Schizosaccharomyces pombe cdc2* mutants (BROEK *et al.* 1991). Segregation of the two nuclei in subsequent cell divisions may result in mononucleate cells that have increased in ploidy.

We do not yet know if any of these alternatives are correct. We favor the third alternative for the reasons given above. It is interesting to note that the *cdc42-1* mutant, which is also defective in bud emergence and/or growth, also appears to gain chromosome *II* when examined by our colony papillation assay (C. CHAN, unpublished results). This result is consistent with the previous observation that most *cdc42-1* cells become arrested with replicated but unseparated chromosomes at the restrictive growth temperature (ADAMS *et al.* 1990). We therefore propose that chromosome-gain and increase-in-ploidy might be a phenotype common to mutants defective in bud emergence and/or growth. This possibility raises intriguing questions concerning the relationship between bud growth and DNA replication, nuclear migration or chromosome segregation. Cytological studies suggest that nuclear migration may involve the interaction of cytoplasmic microtubules with components of the growing bud tip [see, for instance, SNYDER, GEHRUNG and PAGE (1991)]. Further studies of the *ipl2* (and *cdc42*) mutant should help us to understand not only the problem of polarized cell growth, but possibly also how this is

related to DNA replication, nuclear migration and chromosome segregation.

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