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# Sensitivity to the Yeast Plasmid 2μ DNA Is Conferred by the Nuclear Allele *nib1*

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Two strains of *Saccharomyces carlsbergensis* that lacked the plasmid 2μ DNA responded differently when the plasmid was introduced into them. In one strain, cells lacking 2μ DNA ("*cir*$^0$") produced the normal "smooth" colony morphology, but cells bearing 2μ DNA ("*cir*$^+$") produced heterogeneous "nibbled" colonies. In the second strain, both *cir*$^+$ and *cir*$^0$ strains exhibited a smooth colony morphology. Crosses between these strains revealed that a single recessive nuclear gene, called *nib1*, conferred the nibbled colony morphology in the presence of 2μ DNA. By a series of backcrosses, *nib1* was introduced into a *Saccharomyces cerevisiae* background. *nib1* caused a nibbled colony morphology in this background just as it did in *S. carlsbergensis*. *nib1* was mapped to the left arm of chromosome XVI. Twelve independent smooth revertants were isolated from two *nib1* [*cir*$^+$] strains. Seven were analyzed, and all were found to be chromosome VII disomes. Chromosome VII disomy and suppression of the nibbled phenotype cosegregated in crosses. Thus, chromosome VII disomy can suppress the nibbled phenotype. The results of other experiments (C. Holm, Cell 29:585–594, 1982) indicate that the nibbled colony morphology is the result of lethal sectoring and that the lethality is caused by a high copy number of 2μ DNA. I suggest, therefore, that the product of the *nib1* gene may play a role in controlling the copy number of 2μ DNA. Possible models for the suppression of the nibbled phenotype by chromosome VII disomy are discussed.

The plasmid 2μ DNA is potentially useful as a model for chromosomal DNA replication in yeast. 2μ DNA is similar to chromosomal DNA in both structure and replication behavior. Gently isolated 2μ DNA molecules have been shown to be complexed with histones (15), and the results of micrococcal nuclease digestion suggest a typical nucleosome conformation (15, 19). 2μ DNA replication occurs during the S phase of the cell cycle (23), and it is controlled by the same cell division cycle genes as is chromosomal DNA (17, 20). Each molecule of 2μ DNA is generally replicated once per cell cycle (23).

The structure of 2μ DNA may be important for its maintenance in the cell. The plasmid is a 6.3-kilobase circle of double-stranded DNA (1, 6, 10, 12), and it is present in 20 to 100 copies in most strains of *Saccharomyces* (7, 9, 10). Two inverted repeats comprise 20% of its length (2, 5, 10, 11, 13, 16), and its sequence contains three long open reading frames (12). One, *FLP*, apparently codes for a product that catalyzes recombination across the inverted repeats of the molecule (4); it has been suggested that this recombination may serve a regulatory role (3, 4). The others, *REP1* and *REP2*, appear to code for products that act to amplify 2μ DNA copy number in cells into which 2μ DNA has been newly introduced (2a, 4).

2μ DNA may be a useful molecule with which to study the chromosomal genes that control DNA replication. The present work identifies a gene, *nib1*, that may play a role in the control of 2μ DNA copy number. The influence of the dosage of chromosome VII on this phenomenon is discussed.

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## MATERIALS AND METHODS

**Yeast strains.** The strains used in this study are listed in Table 1. Petite derivatives were formed by inoculating approximately $10^6$ cells into 10 μg of ethidium bromide per ml in YM-1 medium (12a) and growing them overnight at 30°C.

**Cell growth.** Cells were grown on YEPD medium (18) supplemented with 40 μg of adenine per ml and 40 μg of uracil per ml. Colony morphology was examined after 2 to 3 days of growth at 30°C.

**Genetic techniques.** Standard genetic techniques were used (18).

Because a sectored colony morphology can result from a variety of causes, the *nib1* gene was scored by complementation. The strain to be tested was mated

TABLE 1. Strains used in this study

| Strain | Genotype | Source |
|---|---|---|
| 5D*cyc*-cyto#1, 3, 6, 7, 10, 11 | α *nib1 cyh2* [*cir*$^0$] | D. Livingston |
| 5D*cyc*-cyto#2, 4, 5, 8, 9, 12 | α *nib1 cyh2* [*cir*$^+$] | D. Livingston |
| CB11-cyto#2, 4, 6, 9, 10, 11 | a *ade1 NIB1 cyh2* [*cir*$^0$] | D. Livingston |
| CB11-cyto#1, 3, 5, 7, 8, 12 | a *ade1 NIB1 cyh2* [*cir*$^+$] | D. Livingston |
| 5D*cyc* [*rho*$^0$] | α *cyh2 nib1* [*rho*$^0$] [*cir*$^0$] | D. Livingston |
| 2101-3-3[a] | a *ade1 nib1* [*cir*$^+$] | This study |
| 2103-6-2[a] | α *ade1 nib1* [*cir*$^+$] | This study |
| 2103-5-4 [*rho*$^0$][a] | a *nib1* [*rho*$^0$] [*cir*$^+$] | This study |
| 5D*cyc*-cyto#8 [*rho*$^0$][a] | α *nib1 cyh2* [*rho*$^0$] [*cir*$^+$] | This study |
| A364A | a *ade1 ade2 ura1 lys2 gal1 his7 tyr1* | L. Hartwell |
| 358-2 | a *ade2 ura1 met8 lys1 his4* | L. Hartwell |
| 260-1-3 | a *met2* | L. Hartwell |
| EMS63 | α *his2 gal* | L. Hartwell |
| 4036-11A | α *pet8 ade1 ade2 his5 his6 arg4 trp1 tyr7 gal1 gal2* | S. Dutcher |
| X12-6B | a *rad1-1 ade2-1* | Yeast Stock Center |
| 2130-5-4 | α *ade1 leu1 lys1 met14 NIB1* | This study |
| 2129-6-1 | a *his2 met2 cyh2 NIB1* | This study |
| 2129-6-2 | a *his2 nib1* | This study |
| 2129-6-3 | α *lys1 met2 cyh2 NIB1* | This study |
| 2129-6-4 | α *lys1 nib1* | This study |
| 2129-6-2S10, S17, S27, S36 | a *his2 nib1* (smooth revertants) | This study |
| 2129-6-2N10 | a *his2 nib1* (nibbled parent of above) | This study |
| 2129-6-4S6, S21, S30 | α *lys1 nib1* (smooth revertants) | This study |
| 2129-6-4N6 | α *lys1 nib1* (nibbled parent of above) | This study |

[a] These strains were used for complementation tests.

with a *nib1* strain bearing 2μ DNA and complementing auxotrophies (Table 1). The diploid was selected, and its colony morphology was examined after 2 days of growth on YEPD medium. This method was clearly reliable, because in the diploids, colony morphology almost always segregated 2:2.

**Temperature sensitivity.** The strain to be tested was diluted to a concentration of approximately $5 \times 10^5$ cells per ml. A small drop was placed on each of two YEPD plates. One plate was incubated at 30°C, and the other was incubated at 38°C. After 2 days, the growths produced at the two temperatures were compared.

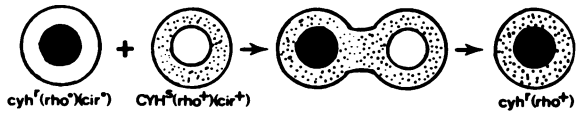
**Chromosome VII disomy assayed by temperature sensitivity.** Chromosome VII disomy caused temperature sensitivity in haploids derived from crosses between strains 2129-6-1, 2129-6-2, 2129-6-3, and 2129-6-4. Temperature sensitivity was shown to be a valid assay for chromosome VII disomy in these strains in two crosses involving a chromosome VII disome and a euploid haploid (2129-6-2S10 × 2129-6-3 and 2129-6-2S17 × 2129-6-3; see Results for evidence that strains 2129-6-2S10 and 2129-6-2S17 are chromosome VII disomes). In these crosses, a total of 30 spores were known to be chromosome VII disomes because they produced cycloheximide-resistant papillae (see Results); these spores were all temperature sensitive. In addition, temperature sensitivity segregated with chromosome VII disomy in seven tetrads in which the chromosome complement of the spores could be deduced from the segregation pattern of *cyh2*. Chromosome VII disomes were temperature sensitive regardless of whether they were *nib1* or *NIB1*.

## RESULTS

**Effect of 2μ DNA on colony morphology.** Livingston (14) produced two groups of isogenic strains that were useful for investigating the biological effects of 2μ DNA. In two separate matings, he crossed a strain lacking 2μ DNA (5D*cyc* [*rho*$^0$] or CB11 [*rho*$^0$]) with a *kar1* strain bearing 2μ DNA (JC K5-25C or JC K5-25D) (Table 2; strains bearing 2μ DNA are designated *cir*$^+$; strains lacking 2μ DNA are designated *cir*$^0$). In each of these crosses, the *kar1* mutation prevented nuclear fusion in the zygotes, and the zygotes produced buds containing intact haploid nuclei. Livingston selected haploid "cytoductants" that contained the cycloheximide resistance gene of the *cir*$^0$ parent and the mitochondria of the *cir*$^+$ parent. In each cross, therefore, all of the cytoductants were isogenic for the nuclear genes of the *cir*$^0$ parent. Among the 12 cytoductants isolated from each cross, Livingston found that 6 contained 2μ DNA and 6 lacked 2μ DNA.

I examined the colony morphology of each of Livingston's cytoductant strains. Strains CB11-cyto#1 to #12 exhibited a normal colony morphology. In strains 5D*cyc*-cyto#1 to #12, however, two distinct colony morphologies were observed: the six strains lacking 2μ DNA produced normal, rounded, "smooth" colonies; the six strains bearing 2μ DNA produced sectored colonies that were heterogeneous in size (a "nibbled" colony morphology) (Fig. 1). These

TABLE 2. Colony morphologies of cytoductants




| | | 2μ DNA Content | Colony Morphology |
|---|---|---|---|
| 5Dcyc(rho)+ JC K5-25C → | 5Dcyc-cyto 1,3,6,7,10,+11 | cir° | Smooth |
| | 5Dcyc-cyto 2,4,5,8,9,+12 | cir+ | Nibbled |
| CB11(rho) + JC K5-25D → | CB11-cyto 2,4,6,9,10,+11 | cir° | Smooth |
| | CB11-cyto 1,3,5,7,8,+12 | cir+ | Smooth |

results suggested that the presence of 2μ DNA caused sectoring in colonies from strains containing a 5D*cyc* nucleus, but not in strains bearing a CB11 nucleus.

**Immunity to effects of 2μ DNA conferred by a single nuclear gene.** I performed a series of crosses between the 5D*cyc* cytoductants and the CB11 cytoductants to investigate the involvement of nuclear genes in the determination of colony morphology (Table 3). In the first two crosses, nibbled strain 5D*cyc*-cyto#8 [*cir*$^+$] was crossed with smooth strains CB11-cyto#5 [*cir*$^+$] and CB11-cyto#2 [*cir*$^0$]. The results of those two crosses were the same. The diploids produced smooth colonies, indicating that the normal colony morphology was dominant. When the diploids were sporulated, the tetrads each produced two nibbled spores and two smooth spores. This 2:2 segregation pattern suggested that a single nuclear gene caused susceptibility to 2μ DNA-associated sectoring. I called this hypothetical gene *nib1*: strains CB11-cyto#1 to #12 were *NIB1*, and strains 5D*cyc*-cyto#1 to #12 were *nib1*. According to my hypothesis, the *NIB1* allele conferred resistance to the effects of 2μ DNA; *NIB1* strains were smooth regardless of their content of 2μ DNA. The *nib1* allele conferred sensitivity to the effects of 2μ DNA; *nib1* [*cir*$^+$] strains were nibbled, and *nib1* [*cir*$^0$] strains were smooth.

Other crosses supported the hypothesis that the combination of the *nib1* allele and 2μ DNA produced the nibbled colony morphology (Table 3). Tetrads obtained from crossing two smooth parents, 5D*cyc*-cyto#1 (*nib1* [*cir*$^0$]) and CB11-cyto#3 (*NIB1* [*cir*$^+$]), showed 2:2 segregation for nibbled versus smooth colonies. Since 2μ DNA is inherited extrachromosomally (14), this result showed that the 2μ DNA contained by CB11 cytoductants could cause the nibbled colony morphology in a *nib1* background. In another cross, spores obtained from crossing two strains lacking 2μ DNA, 5D*cyc*-cyto#1 (*nib1* [*cir*$^0$]) and CB11-cyto#2 (*NIB1* [*cir*$^0$]), were all smooth. Thus, 2μ DNA had to be present in a cross to find 2:2 segregation for colony morphology in the progeny. In a final pair of crosses, the parents were both *nib1* strains: 5D*cyc*-cyto#8 (*nib1* [*cir*$^+$]) was mated with 2101-3-3 (*nib1* [*cir*$^+$]), and 5D*cyc*-cyto#1 (*nib1* [*cir*$^0$]) was also mated with 2101-3-3 (*nib1* [*cir*$^+$]). Identical results were obtained from both crosses; the diploids and the spores they produced were all nibbled. This result shows that the expression of the nibbled phenotype is not limited to haploids.

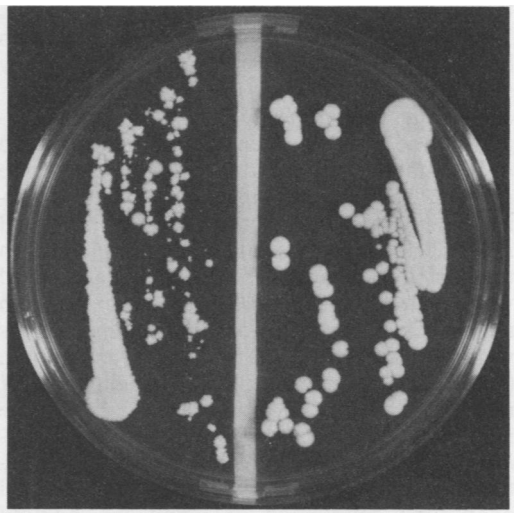


FIG. 1. Nibbled and smooth colony morphologies. Strains 5D*cyc*-cyto#1 [*cir*$^0$] and 5D*cyc*-cyto#8 [*cir*$^+$] were streaked for single colonies on YEPD medium and grown for 3 days at 30°C. (Left) Strain 5D*cyc*-cyto#8, which exhibits a nibbled colony morphology. (Right) Strain 5D*cyc*-cyto#1, which exhibits a smooth colony morphology.

TABLE 3. Crosses between strains sensitive and resistant to 2μ DNA

| Cross no. | Parents[a] | Diploid phenotype | Spore phenotype[b] | No. of tetrads |
|---|---|---|---|---|
| 2101, 2103[c] | 5D*cyc*-cyto#8 [*rho*$^0$] (*nib1* [*cir*$^+$]) × CB11-cyto#5 (*NIB1* [*cir*$^+$]) | Smooth | 2N:2S | 16 |
| 2102, 2104[c] | 5D*cyc*-cyto#8 [*rho*$^0$] (*nib1* [*cir*$^+$]) × CB11-cyto#2 (*NIB1* [*cir*$^0$]) | Smooth | 2N:2S | 21 |
| 2105 | 5D*cyc*-cyto#1 [*rho*$^0$] (*nib1* [*cir*$^0$]) × CB11-cyto#3 (*NIB1* [*cir*$^+$]) | Smooth | 2N:2S | 22 |
| 2106 | 5D*cyc*-cyto#1 [*rho*$^0$] (*nib1* [*cir*$^0$]) × CB11-cyto#2 (*NIB1* [*cir*$^0$]) | Smooth | 0N:4S[d] | 20 |
| 2108 | 5D*cyc*-cyto#8 [*rho*$^0$] (*nib1* [*cir*$^+$]) × 2101-3-3 (*nib1* [*cir*$^+$]) | Nibbled | 4N:0S | 9 |
| 2107 | 5D*cyc*-cyto#1 [*rho*$^0$] (*nib1* [*cir*$^0$]) × 2101-3-3 (*nib1* [*cir*$^+$]) | Nibbled | 4N:0S | 14 |

[a] Genotypes are deduced from the results of these crosses.
[b] N, Nibbled; S, smooth.
[c] These crosses were performed in two ways. In the first case (2101 and 2102), the *nib1* parent was grande, and zygotes were purified by micromanipulation. In the second case, the *nib1* parent was petite. The results of the crosses were identical, and they have been pooled here.
[d] These spores were each tested by complementation for their *nib1* genotype. In each tetrad, two spores were *nib1*, and two were *NIB1*.

***nib1* crossed into a *Saccharomyces cerevisiae* background.** *Saccharomyces carlsbergensis* 5D*cyc* was very clumpy, and it showed poor spore viability when crossed with *S. cerevisiae* strains. These characteristics were problematic for further genetic and physiological studies. Therefore, a series of crosses was undertaken between strain 5D*cyc* and various nonclumpy strains of *S. cerevisiae* (Fig. 2). Genetic differences other than *nib1* affected colony morphology in these crosses; often *nib1* spores appeared to be smooth, and *NIB1* spores appeared to be nibbled. However, complementation tests permitted unambiguous scoring of the *nib1* and *NIB1* alleles regardless of colony morphology in the haploid segregant. The spores in each tetrad were mated with a *nib1* tester strain, and in almost every tetrad two of the resulting diploids were nibbled (*nib1/nib1*) and two were smooth (*NIB1/nib1*). After each cross, a nonclumpy *nib1* nibbled spore was selected to be a parent in the next mating. After six outcrosses to *S. cerevisiae* the resulting diploid produced tetrads containing spores with high viability, the nibbled phenotype segregated 2:2, and the haploid clones were nonclumpy. Four of the spore strains produced by this diploid were used in further studies.

***nib1* mapping.** *nib1* was localized to chromosome XVI by inducing chromosome loss in a diploid heterozygous for *nib1* and for many other markers (J. Wood, Mol. Cell. Biol., in press), and I determined its map position by using standard genetic techniques. The *nib1* gene lies 15 to 20 map units from the centromere of chromosome XVI (Table 4). The segregation pattern of *nib1* relative to *aro7* shows that *nib1* does not lie on the right arm of chromosome XVI: *aro7* lies near the centromere on the right arm of chromosome XVI, and the ratio 6PD:4NPD:23T shows that *nib1* and *aro7* are completely unlinked (Table 4).

The results of the cross 2129-6-4 × X12-6Ba show that *nib1* is closely linked to *rad1*, which is on the left arm of chromosome XVI (Table 4); no recombinants were detected among the viable spores of 46 dissected asci. This tight linkage suggests that *nib1* and *rad1* might be alleles of the same gene. However, *nib1* strains are not unusually UV sensitive (unpublished data), and *rad1* strains are *NIB1*$^+$ in the complementation assay. Thus, it is unlikely that *nib1* and *rad1* are the same gene. Examination of the pattern of spore viability in this cross provided an explanation for the lack of *nib1 rad1* and *NIB1 RAD1* recombinants. Among the 46 tetrads, 37 had four viable spores, 3 had three viable spores, 5 had two viable spores, and 1 had one viable spore. In all five tetrads with two viable spores, one of the spores was *nib1 RAD1*, and the other was *NIB1 rad1*. The probability of getting this result due to random death of the spores is only 3%. It seems

possible, therefore, that a chromosomal aberration is responsible for the lack of recoverable recombinants. It is unlikely that the results are due to the presence of a large inversion or translocation in these strains, because these chromosomal aberrations would have resulted in a great deal more lethality. However, strains 2129-6-2 and X12-6Ba could differ by a small inversion that includes both *nib1* and *rad1*; if this interpretation were correct and each of the five two-spored asci represented a single recombination event between *nib1* and *rad1*, it could be concluded that *nib1* lies within 5 centiMorgans of *rad1*.

**Suppression of nibbled phenotype by chromosome VII disomy.** Spontaneous smooth revertants were isolated by screening the morphology of colonies produced by nibbled strains. Forty nibbled colonies were picked from strain 2129-6-2 (*nib1* [*cir*$^+$]) and strain 2129-6-4 (*nib1* [*cir*$^+$]), and roughly 1,000 viable cells from each colony were spread onto YEPD medium. At least one smooth revertant was produced by cells derived from 12 of the 80 original nibbled colonies. When the revertants were mated with *nib1* [*cir*$^0$] tester strains, all of the resulting diploids were nibbled. This result demonstrated that the smooth revertants contained 2μ DNA and that their 2μ DNA could induce the nibbled colony morphology in an appropriate genetic background. This result also showed that reversion to smooth colony morphology was recessive; smooth revertants,

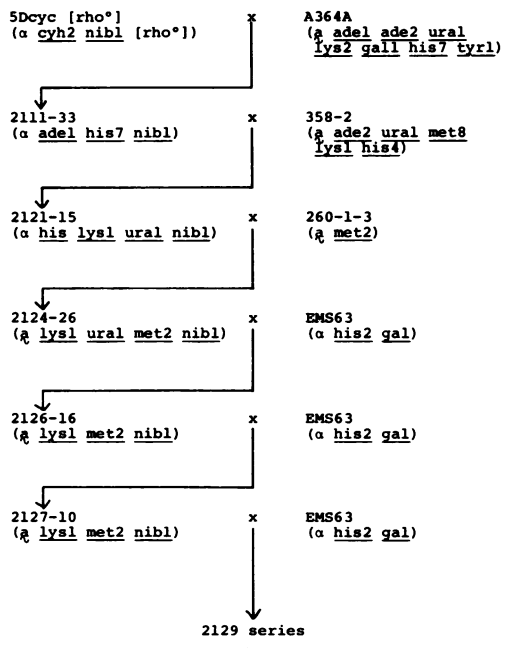



FIG. 2. Construction of *nib1 S. cerevisiae* strains.

TABLE 4. Mapping crosses with *nib1* strains

| Cross | Gene pair | Results[a] | | | Conclusion |
|---|---|---|---|---|---|
| | | PD | NPD | T | |
| 2129-6-2 × 4036-11A | *nib1*, *trp1* | 9 | 3 | 5 | *nib1* is centromere linked |
| 2129-6-2 × 4036-11A | *nib1*, *pet8* | 3 | 6 | 6 | *nib1* is centromere linked |
| 2129-6-2 × 4036-11A | *trp1*, *pet8* | 5 | 10 | 0 | *trp1* and *pet8* segregated normally |
| 2129-6-2 × 4036-11A | *nib1*, *aro7* | 6 | 4 | 23 | *nib1* does not lie on the right arm of chromosome XVI |
| 2129-6-4 × X12-6Ba | *nib1*, *rad1* | 37 | 0 | 0 | *nib1* is closely linked to *rad1* on the left arm of chromosome XVI |

[a] PD, Parental ditype; NPD, Nonparental ditype; T, tetratype.

therefore, had not undergone a mutation from *nib1* to *NIB1*.

The revertants had, however, undergone a single nuclear change. Two representative smooth revertants and their nibbled parent were each crossed with a *nib1* [*cir*$^+$] strain (Table 5). All of the tetrads derived from the cross with the nibbled parent contained four spores that produced nibbled colonies. The tetrads derived from the smooth revertant matings, however, each contained two spores that produced nibbled colonies and two spores that produced smooth colonies. As expected, all of the spores from both crosses were *nib1*: when crossed with a *nib1* [*cir*$^+$] tester strain, they all produced diploids with a nibbled colony morphology. Thus, a single nuclear change in the smooth revertant strains suppressed the nibbled phenotype normally observed in *nib1* [*cir*$^+$] spores.

This single nuclear change proved to be chromosome VII disomy. Seven smooth revertants

TABLE 5. Segregation of the suppression of the nibbled phenotype

| Cross | Tetrad phenotype[a] | |
|---|---|---|
| | 2N:2S | 4N:0S |
| 2129-6-2N10 (*nib1* [*cir*$^+$]) × 2129-6-4 (*nib1* [*cir*$^+$]) | 0 | 4 |
| 2129-6-2S10 (*nib1 sup-nib*$^b$ [*cir*$^+$]) × 2129-6-4 (*nib1* [*cir*$^+$]) | 9 | 0 |
| 2129-6-2S17 (*nib1 sup-nib*$^b$ [*cir*$^+$]) × 2129-6-4 (*nib1* [*cir*$^+$]) | 11 | 0 |

[a] N, Nibbled; S, smooth.
[b] The genotype *sup-nib* is used to indicate the alteration carried by smooth revertant strains.

and their nibbled parents were crossed with strains bearing *cyh*$^r$*2*, which lies on chromosome VII (Table 6). In the crosses involving the nibbled parents, the tetrads each contained two spores resistant to cycloheximide and two spores sensitive to the drug. In the crosses involving the smooth revertants, however, several classes of tetrads were produced: (i) tetrads containing two spores that were sensitive to cycloheximide and two spores that gave cycloheximide-resistant papillae; (ii) tetrads containing one resistant, one papillating, and two sensitive spores; (iii) tetrads containing one resistant and three sensitive spores; and (iv) tetrads containing two resistant and two sensitive spores. (*his2*, *lys1*, *met2*, and mating type all segregated 2:2 in these crosses.) This distribution of tetrad types was consistent with the hypothesis that smooth revertants were chromosome VII disomes. In particular, papillation for cycloheximide resistance is diagnostic of cells carrying two copies of chromosome VII: if one copy bears *cyh*$^r$*2* and the other carries *CYH*$^s$*2*, mitotic recombination produces cells that are *cyh*$^r$*2*/*cyh*$^r$*2*; thus, a sensitive *cyh*$^r$*2*/*CYH*$^s$*2* patch produces resistant *cyh*$^r$*2*/*cyh*$^r$*2* papillations at high frequency. The hypothesis of chromosome VII disomy was confirmed by the results of two crosses with haploid strain 2130-5-4, which carried *leu1* on chromosome VII (Table 7). When this strain was mated with a nibbled parent, *leu1* segregated 2 Leu$^+$:2 Leu$^-$. When it was crossed with a smooth revertant, however, *leu1* segregated both 2 Leu$^+$:2 Leu$^-$ and 4 Leu$^+$:0 Leu$^-$. (Since *leu1* is tightly linked to the centromere of chromosome VII, it is not surprising that tetrads with 3 Leu$^+$:1 Leu$^-$ segregation were not observed in the 13 tetrads examined. *ade1*, *his2*, *lys1*, and *met14* segregated 2:2 in both these crosses.)

By using temperature sensitivity as an assay (see Materials and Methods), I confirmed the hypothesis that chromosome VII disomy suppressed the nibbled phenotype in *nib1* strains. Smooth revertant strain 2129-6-2S17 was crossed with *nib1* nibbled strain 2129-6-4, and the resulting tetrads were tested for temperature sensitivity and colony morphology. Colonies from the 22 *nib1* smooth spores grew poorly at 38°C; the 22 *nib1* nibbled spores grew well at 38°C. Since chromosome VII disomes are temperature sensitive and euploid haploids are not (see above), this result verified that chromosome VII disomy suppressed the nibbled phenotype in *nib1* strains.

TABLE 6. Segregation of cycloheximide resistance in crosses between smooth revertants and strains bearing *cyh*$^r$*2*.

| Cross | Tetrad phenotype[a] | | | |
|---|---|---|---|---|
| | 2P:2S | 1P:1R:2S | 1R:3S | 2R:2S |
| 2129-6-2N10 (*nib1*) × 2129-6-3 | 0 | 0 | 0 | 14 |
| 2129-6-2S10 (*nib1 sup-nib*)[b] × 2129-6-3 | 4 | 11 | 1 | 0 |
| 2129-6-2S17 (*nib1 sup-nib*) × 2129-6-3 | 3 | 5 | 3 | 3 |
| 2129-6-2S27 (*nib1 sup-nib*) × 2129-6-3 | 4 | 1 | 0 | 0 |
| 2129-6-2S36 (*nib1 sup-nib*) × 2129-6-3[c] | 1 | 4 | 2 | 3 |
| 2129-6-4N6 (*nib1*) × 2129-6-1 | 0 | 0 | 0 | 6 |
| 2129-6-4S6 (*nib1 sup-nib*) × 2129-6-1 | 3 | 6 | 1 | 0 |
| 2129-6-4S21 (*nib1 sup-nib*) × 2129-6-1 | 1 | 6 | 1 | 1 |
| 2129-6-4S30 (*nib1 sup-nib*) × 2129-6-1 | 3 | 7 | 1 | 0 |

[a] P, Papillae resistant to cycloheximide appeared after 2 days; R, resistant to cycloheximide; S, sensitive to cycloheximide.

[b] The genotype *sup-nib* is used to indicate the alteration carried by smooth revertant strains.

[c] One tetrad showed 3S:1P segregation.

## DISCUSSION

These results show that *nib1* strains of *Saccharomyces* are sensitive to 2μ DNA: when they contain 2μ DNA, they produce nibbled colonies; when they lack it, they do not. Nibbled colonies are similar in appearance to colonies in which lethal sectors are produced, and other experiments have shown that the nibbled phenotype is in fact due to lethal sectoring (13a). Not surprisingly, the *nib1* allele was found in a strain that originally lacked 2μ DNA; it would have been

TABLE 7. Segregation of *leu1* in a cross with a smooth revertant

| Cross | Tetrad phenotype | |
|---|---|---|
| | 2 Leu$^+$:2 Leu$^-$ | 4 Leu$^+$:0 Leu$^-$ |
| 2129-6-2N10 (*nib1*) × 2130-5-4 (*leu1*) | 12 | 0 |
| 2129-6-2S10 (*nib1 sup-nib*)[a] × 2130-5-4 (*leu1*) | 5 | 8 |

[a] The genotype *sup-nib* indicates the alteration carried by the smooth revertant strain.

selected against in a strain normally containing 2μ DNA. All *cir*$^+$ strains that have been subjected to complementation tests have been found to be *NIB1*$^+$ (unpublished data).

The nibbled phenotype in *nib1* [*cir*$^+$] strains is suppressed by chromosome VII disomy. Since disomy probably arises at a frequency much higher than that of mutation, this source of *nib1* suppressors precludes finding mutations that suppress *nib1*. This problem could be obviated if one could simultaneously select against chromosome VII disomy and select for phenotypic reversion of the nibbled colony morphology. Unfortunately, *nib1* [*cir*$^+$] strains normally exhibit a smooth colony morphology at 36 and 38°C (13a). Thus, although one could select against chromosome VII disomes at 36 and 38°C, it is not possible to screen for smooth revertants at these temperatures. It is interesting that chromosome VII disomy is lethal at the same temperatures at which *nib1* [*cir*$^+$] strains are smooth. The effect of temperature may be coincidental, since the temperature sensitivity of the disomes is not directly related to the *nib1* gene; both *NIB1* and *nib1* chromosome VII disomes are temperature sensitive.

The nibbled colony morphology is caused by the production of sectors of mortal cells, which in turn are caused by a heritable elevation of 2μ DNA copy number (13a). Preliminary experiments have shown that the average copy number of 2μ DNA is reduced in disomic smooth revertant strain 2129-6-2S10 relative to nibbled strain 2129-6-2 (unpublished data). This result is consistent with two hypotheses. First, chromosome VII could carry a gene for a negative regulator of 2μ DNA copy number; its elevated dosage might help keep the copy number of 2μ DNA under control. Second, chromosome VII disomy could hasten the death of cells bearing an elevated amount of 2μ DNA. Lethality in cells bearing excess 2μ DNA may be similar to that seen with runaway mutants of bacterial plasmid R1*drd-19*, where elevated copy number appears to cause cell death by titrating out essential replication products (22). Chromosome VII appears to be the largest of the yeast chromosomes (8), and the extra DNA in chromosome VII disomes may also perturb DNA replication. Thus, disomic cells with high 2μ DNA copy number might die very rapidly; they would then give rise to fewer progeny cells, which would preclude the formation of large sectors of mortal cells bearing a high number of copies of 2μ DNA. If this latter model is correct, it seems odd that other disomies were not found as suppressors of the nibbled phenotype. It is possible, however, that chromosome VII is substantially larger than other yeast chromosomes or markedly more stable in the disomic state.

It is clear that the *nib1* gene plays a role in the regulation of 2μ DNA copy number. Since *NIB1* is the dominant allele, its product probably actively prevents the lethality that can be caused by excess 2μ DNA in *nib1* cells. An attractive possibility for the function of the *NIB1* gene product is the repression of 2μ DNA amplification. Sigurdson et al. (21) have shown that the copy number of 2μ DNA rapidly amplifies from 1 to 30 copies in cells into which the plasmid is newly introduced. However, amplification appears to be repressed in an established *cir*$^+$ culture; in density transfer experiments, Zakian et al. (23) showed that each copy of 2μ DNA replicated only once per cell cycle. (According to its pedigree, the strain examined by Zakian et al. was *NIB1*.) Thus, *NIB1* may code for the repressor of 2μ DNA amplification; *nib1*, then, codes for a product that is at least partially defective in this function.

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