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Chromosome Specificity of Polysomy Promotion by Disruptions of the *Saccharomyces cerevisiae RNA1* Gene

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Manuscript received November 4, 1986
Accepted April 3, 1987

ABSTRACT

Previously, we showed that a disruption of the yeast *RNA1* gene with *LEU2* sequences promotes polysomy for chromosome *XIII*. Here we demonstrate that this phenotype is due to sequences specific to the *RNA1* gene and that the disruption allele does not affect nondisjunction of three other chromosomes or polysomy of a minichromosome. Hence polysomy appears to be restricted to chromosome *XIII*.

THE *rna1-1* allele of *Saccharomyces cerevisiae* is a recessive temperature-sensitive lethal mutation affecting the production of mature rRNA, tRNA and mRNA (HUTCHISON, HARTWELL and MCLAUGHLIN 1969; HOPPER, BANKS and EVANGELIDIS 1978; ST. JOHN and DAVIS 1981). *rna1-1* strains grow at 23° but not at temperatures exceeding 30°. Generally, 37° is used as the restrictive temperature.

Previously, we described the construction and phenotype of a recessive lethal mutation of *RNA1*, *rna1::LEU2* [ATKINSON, DUNST and HOPPER 1985 (in this reference this allele was referred to as *RNA1::LEU2*)]. In the *rna1::LEU2* allele the *RNA1* coding region is disrupted approximately 620 nucleotides from the 5′ end of the *RNA1* transcription unit by a 3-kb DNA fragment encoding the yeast *LEU2* gene. This allele produces a truncated transcript and circumstantial evidence suggested that it is translated into a truncated polypeptide (ATKINSON, DUNST and HOPPER 1985). *rna1::LEU2/rna1-1* diploids become polysomic for the chromosome *XIII* bearing the *rna1-1* allele at a frequency of 2–5% (ATKINSON, DUNST and HOPPER 1985). Amplification of the *rna1-1* allele due to polysomy permits growth at 34°, but not at 37°. This phenotype was referred to as partial temperature resistance (PTR). Episomal copies of *rna1::LEU2* also promoted partial temperature resistance (ATKINSON, DUNST and HOPPER 1985).

In order to characterize the promotion of polysomy phenotype associated with *rna1::LEU2* it is necessary to determine: (1) whether this phenotype is the result of *RNA1*-specific sequences or the *LEU2* sequences present in *rna1::LEU2*; and (2) whether polysomy is promoted for chromosomes other than chromosome *XIII*.

If the promotion of polysomy phenotype is specific to *RNA1* sequences and mutated *RNA1* alleles are capable of promoting polysomy for other chromosomes, this could suggest that the *RNA1* gene product is involved in the replication or segregation of chromosomes. Alteration of a gene involved in the maintenance of nuclear structure might be expected to affect nuclear processes as diverse as RNA accumulation and chromosome segregation. Alternatively, if disrupted *RNA1* alleles promote polysomy only for chromosome *XIII*, this could suggest that the truncated *RNA1* product establishes a selection for amplification of the *rna1-1* allele and that this amplification results via polysomy for chromosome *XIII*.

MATERIALS AND METHODS

Yeast strains: All yeast strains used in this study are described in Table 1. 2b×3b, 13d×22c and 3a×9d are diploid strains that are derived from the following strains: 4795303 (a gift from D. KOSHLAND), 21R (JOHNSTON and HOPPER 1985), $\alpha_6$131-20 and EE1b (this laboratory). The strains 2b×3b*rna1::URA3* and 2b×3b*rna1::LEU2* are derived from 2b×3b by gene replacement (ROTHSTEIN 1983). 2b×3b*rna1::LEU2A* and 2b×3b*rna1::LEU2B* are independent isolates of a diploid heterozygous for the *rna1::LEU2* disruption allele (ATKINSON, DUNST and HOPPER 1985).

Construction of YEprna1::URA3: YEp24 was digested with *Eco*RI and overhanging ends were converted to blunt ends. A *Bam*HI linker was inserted at this site by blunt end ligation. This produced plasmid pBR:URA, which is lacking the 2 μ sequences and carries a *URA3* gene flanked by the newly inserted *Bam*HI linker on one side and the *Bam*HI site within the *Escherichia coli* tetracycline gene on the other side. The 1.54-kb *Bam*HI DNA fragment from pBR:URA containing the *URA3* gene was ligated into the *Bam*HI site of the plasmid YEpRNA1(7.7) to yield the plasmid YEprna1::URA3 (Figure 1).

Measurement of the frequency of loss of chromosomal markers: Cells were inoculated into rich medium (YEPD) at a density of 1×10^4 cells per ml with a freshly grown inoculum and were grown for 48 hr at 23° to a density of 1–

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TABLE 1

Genotype of yeast strains

Strain	Genotype
2b×3b	*MAT***a**/*MATα, ura3-52/ura3-52, leu2-3,112/leu2-3,112, rna1-1/rna1-1, ade2/ade2, tyr1/TYR1, his7/HIS7*
2b×3brna1::URA3	*MAT***a**/*MATα, ura3-52/ura3-52, leu2-3,112/leu2-3,112, rna1::URA3/rna1-1, ade2/ade2, tyr1/TYR1, his7/HIS7*
2b×3brna1::LEU2A or B	*MAT***a**/*MATα, ura3-52/ura3-52, leu2-3,112/leu2-3,112, rna1::LEU2/rna1-1, ade2/ade2, tyr1/TYR1, his7/HIS7*
13d×22c	*MAT***a**/*MATα, ura3/ura3, leu2/leu2, rna1-1/rna1-1, ade2/ade2, ade3/ADE3, tyr1/TYR1, his7/HIS7, can1/CAN1*
3a×9d	*MAT***a**/*MATα, ura3/ura3, leu2/leu2, rna1-1/rna1-1, ade2/ade2, ade3/ade3, tyr1/TYR1, his7/his7*
a thr	*MAT***a**, *thr*
α thr	*MATα, thr*

1.7×10^8. One hundred to 400 colony-forming units of these cultures were spread onto YEPD medium and were incubated at 23° for 72 hr. Resulting colonies were replica plated to medium lacking tyrosine or histidine (HOPPER, BANKS and EVANGELIDIS 1978) to monitor the loss of *TYR1* and *HIS7*, respectively (chromosome *II*), or to canavanine-containing medium (medium lacking arginine that contains 80 mg of canavanine sulfate per liter) to monitor chromosome *V*. Loss of a *MAT* allele (chromosome *III*) was scored by replica plating the colonies to freshly prepared lawns of the *MAT***a** and *MATα* mating type tester strains, **a** thr and *α* thr. The replicas were incubated for 24 hr to permit mating and then replica plated to minimal medium. Growth of a colony on minimal medium indicates that cells within the colony have mated with the tester strain. All incubations were performed at 23°. Two controls were used in all experiments: (1) an untransformed isolate of the parent strain and (2) a mock transformed isolate of the parent strain. The loss of genetic markers was used to estimate the loss of the respective chromosome. Transformation was performed as described by DUNN *et al.* (1985).

Measurement of the mitotic segregation of pDK243: An isolate of 3a×9d that carried the minichromosome pDK243 was transformed with YEp24, YEprna1(7.7) or YEprna1::URA3. Cells were maintained on medium that selected for the retention of the plasmids. Three individual clones from each transformant were picked and suspended into YEPD broth and plated onto YEPD medium lacking supplemental adenine. The plates were incubated at 23° for 72–96 hr and then the colonies were scored for coloration.

RESULTS

To determine whether the promotion of polysomy is due to sequences specific to the *RNA1* gene or to the disrupting *LEU2* sequences, we constructed another *RNA1* disruption allele, *rna1::URA3* (see MATERIALS AND METHODS). *rna1::URA3* is disrupted at the same position as in *rna1::LEU2* (Fig. 1). Neither YEprna1(7.7)::LEU2 nor YEprna1::URA3 complements the *rna1-1* mutation.

Gene replacement (ROTHSTEIN 1983) was used to introduce the *rna1::URA3* allele at the *RNA1* locus. A 5.5-kb *Sst*I fragment derived from the plasmid YEprna1::URA3, was used to transform the diploid strain 2b×3b (Table 1) to Ura^+. Tetrad analysis was performed on a stable Ura^+ diploid, 2b×3b rna1:: URA3. Twenty-eight tetrads segregated 2:2 and 3 showed 1:3 segregation for viability to lethality. None of the spores was Ura^+. These data are consistent with the interpretation that the strain 2b×3brna1:: URA3 is heterozygous for a *URA3* disruption at the *RNA1* locus and that this allele behaves as a recessive lethal.

We have previously determined that *rna1::LEU2/rna1-1* diploids give rise to PTR isolates and, using genetic and physical techniques, demonstrated that PTR isolates are polysomic for chromosome *XIII* (ATKINSON, DUNST and HOPPER 1985). We have used this convenient phenotype as an assay for polysomy of chromosome *XIII*. Cells were inoculated in rich medium (YEPD) at a density of 1×10^4 cells per ml with a freshly grown inoculum and were grown for 48 hr at 23° to a density of 1 to 1.7×10^8. One hundred to 400 colony-forming units of these cultures were spread onto YEPD medium and were incubated at 23° for 72 hr. Resulting colonies were replica plated to three YEPD plates. Each replica was incubated for 48 hr at a different temperature, 23°, 34° or 37°, and the frequency of PTR colonies was recorded.

The frequency of PTR isolates was compared between 2b×3brna1::URA3 and two control strains: (1) the untransformed 2b×3b parental strain and (2) mt2b×3b, a mock transformed parental strain. Neither of the control strains produced PTR isolates (827 and 1404 colonies were tested, respectively). The strain 2b×3brna1::URA3 produced 32 PTR isolates out of 434 colonies examined. Experiments of this type were repeated a number of times using two independently derived disruptions and the frequency of PTR colonies was shown to vary between 1.9 and 13.3%. These values are comparable to the frequencies previously reported for the *rna1::LEU2* allele [2–5% (ATKINSON, DUNST and HOPPER 1985)].

Two PTR isolates from 2b×3brna1::URA3 were

grown at 34° and then transferred to 23° for sporulation, dissection and growth of spore clones. Of 46 tetrads from one isolate, the following segregation classes were obtained: 1:3, 2:2, and 3:1 for viability to lethality in the ratio of 17:28:1. Two spores, one from the 3:1 and one from a 2:2 ascus, were Ura$^+$. Thirty-nine tetrads from the other PTR clone segregated 1:3, 2:2 and 3:1 in the ratio of 11:25:2. Eleven (2 from the 3:1, 6 from the 2:2, and 3 from the 1:3 ascus types) of these spores were Ura$^+$. The increased frequency of 1:3 segregation in the PTR isolates compared to the ts isolates is not understood. However, previously we showed that 3:1 and 4:0 segregation classes resulting from the presence of *rna1::LEU2* and suppression of the haplo-lethal phenotype of the disruption were due to chromosome *XIII* polysomy. The 3:1 segregation of viability to lethality and the production of Ura$^+$ meiotic products that carry a gene that is a recessive lethal (*rna1::URA3*) provides evidence that the *rna1::URA3*-induced PTR isolates are also polysomic for chromosome *XIII*. Since the disruption of *RNA1* with either of two unrelated sequences causes the production of PTR colonies and polysomy for chromosome *XIII*, we conclude that the promotion of polysomy is not a consequence of the sequences used to disrupt the gene, but, rather, related to the truncated *RNA1* coding region.

Amplification (polysomy) of minichromosomes occurs primarily as a result of nondisjunction (KOSHLAND, KENT and HARTWELL 1985) and therefore, it is possible that chromosome polysomy results from a nondisjunction event. In order to determine whether the disruption alleles influence the frequency of mitotic nondisjunction, we determined whether the *rna1::LEU2* and *rna1::URA3* alleles enhanced the frequency of nondisjunction and the resultant chromosome loss for three other chromosomes.

The effect of chromosomal disruptions on chromosome loss was analyzed in the strains 2b×3brna1::URA3 and 2b×3brna1::LEU2, which were derived from 2b×3b by gene replacement (Table 1). We have demonstrated that increasing the copy number of the *rna1::LEU2* disruption allele causes an increased frequency of PTR isolates; that is, the frequency of PTR isolates is dependent upon the dosage of *rna1::LEU2* (ATKINSON, DUNST and HOPPER 1985). If the *rna1* disruption alleles promote chromosome polysomy in general, then the frequency of polysomy should increase with increased dosage of the allele. Therefore, we also determined whether a disruption allele carried on a multicopy plasmid [YEprna1::URA3, (Figure 1)] stimulated the frequency of chromosome loss in the strains 2b×3b and 13d×22c.

2b×3b and 13d×22c are heterozygous for three genetic markers: *tyr1/TYR1*, *his7/HIS7* and *MAT***a**/*MAT*α. The *TYR1* and *HIS7* loci are 73 cM apart on the same arm of chromosome *II* and these dominant alleles are in coupling. Loss of the *TYR1* and *HIS7* bearing homologue would "uncover" the recessive *tyr1* and *his7* alleles. Loss of either *MAT* allele on chromosome *III* would enable the strain to mate with haploid tester strains. 13d×22c is also heterozygous for the *can1/CAN1* marker that is located on chromosome *V*. Uncovering of the *can1* allele (loss of *CAN1*) results in colonies that grow on media containing canavanine.

TABLE 2

Frequency of loss of chromosome markers in the derivatives of the strain 2b×3b

Strain	No. of colonies examined	Percent of colonies exhibiting loss of *TYR1* and *HIS7*	Percent of colonies exhibiting loss of *MAT***a** or *MAT*α
2b×3b	827	0	0.36
mt2b×3b	1404	0.07	0
2b×3brna1::URA3	434	1.15	0.92
2b×3brna1::LEU2A	1394	0	0.07
2b×3brna1::LEU2B	515	0	0.39
YEprna1::URA3 in strain 2b×3b	985	0.2	0

Table 2 summarizes the data from derivatives of 2b×3b. Loss of *HIS7* and *TYR1* was concomitant. Since these are unlinked markers on chromosome *II*, we conclude that concomitant loss reflects chromosome loss. In comparison with the controls, the strains 2b×3brna1::LEU2A and 2b×3brna1::LEU2B (two independent isolates that are heterozygous for *rna1::LEU2* and *rna1-1*) did not exhibit an increased frequency of loss of either chromosome *II* or *III*. The 2b×3brna1::URA3 strain showed an increase in loss of chromosome *II*, and to a lesser extent loss of chromosome *III*. The presence of *rna1::URA3* on a multicopy plasmid [5–30 copies per cell (STRUHL *et al.* 1979)] appeared to stimulate loss of chromosome *II* slightly; however, the degree of stimulation was less than that observed with a single copy of *rna1::URA3* and, therefore, was not dosage dependent. The presence of multiple copies of the *rna1* disruption alleles did not cause an increased frequency of loss of the *TYR1*, *HIS7*, *MAT* or *CAN1* loci in strain 13d×22c (Table 3).

To summarize, a slight increase in the frequency of chromosome loss with respect to the controls was observed only for chromosome *II* in the strain 2b×3brna1::URA3. Since neither a chromosomal copy of *rna1::LEU2* nor an episomal copy of *rna1::URA3* stimulated the frequency of loss of chromosomes *II*, *III* or *V*, we conclude that the *rna1* disruption alleles do not enhance the frequency of nondisjunction of these chromosomes.

The disruption alleles did not increase the frequency of polysomy as assayed by chromosome loss.

TABLE 3

Loss of chromosome markers in the strain 13d×22c

Plasmid	*CAN1* Colonies Percent	*CAN1* Colonies Total	*TYR1* or *HIS7* Colonies Percent	*TYR1* or *HIS7* Colonies Total	*MATa* or *MATα* Colonies Percent	*MATa* or *MATα* Colonies Total
Mock transformed	0	832	0.12	832	0.24	832
YEp24	0	657	0	657	0.62	657
YEpRNA1(7.7)	0	3259	0.12	3259	0	3259
YEprna1(7.7)::LEU2	0	493	0	493	0.61	493
YEprna1::URA3	0	1759	0.34	1759	0.46	1759
YCpRNA1(7.7)	0	1510	0	1510	0.2	1510
YCprna1(7.7)::LEU2	0	347	0	347	0	347

The loss of the *CAN1* and *MAT* markers were measured as an estimate of the loss of chromosomes *V* and *III*, respectively. The *TYR1* and *HIS7* markers were measured as an estimate of the loss of chromosome *II*.

However, the loss of these chromosomes may not be well tolerated in these strains or chromosome amplification may occur in the absence of nondisjunction. To assess these possibilities, we investigated the effect of the *RNA1* disruptions upon the segregation of a minichromosome. The minichromosome, pDK243, carries a centromere sequence, *LEU2* and a leaky *ade3 allele. In an ade2 ade3* genetic background it is possible to visually quantitate the minichromosome copy number within a colony (KOSHLAND, KENT and HARTWELL 1985). Cells that carry 0, 1 and 2 copies of the minichromosome produce colonies that are white, pink and red, respectively. The frequency of white colonies reflects the summation of the frequency of simple loss and nondisjunction of the minichromosome. The frequency of the red colonies reflects the summation of the frequency of nondisjunction and oversynthesis of the minichromosomes (KOSHLAND, KENT and HARTWELL 1985). KOSHLAND, KENT and HARTWELL did not observe amplification of the minichromosome in the absence of nondisjunction.

Strain 3a×9d was transformed with pDK243 alone or with pDK243 and one of the following plasmids: YEp24, YEpRNA1(7.7), YEprna1::URA3 (Figure 1). The cells were grown in a medium that selected for retention of the plasmids. Three independent clones from each transformant were assayed for the copy number of pDK243 (Table 4). We did not observe a significant increase in the frequency of white or red colonies associated with the presence of the plasmid YEprna1::URA3. After colony color was scored the colonies were replica plated to media lacking uracil or leucine to detect the presence of the plasmids. No increase in loss of either plasmid was found to be correlated with the presence or absence of a particular *RNA1* allele (data not shown).

DISCUSSION

Our results show that interruption of the *RNA1* coding region at the *Bam*HI site generates *RNA1*

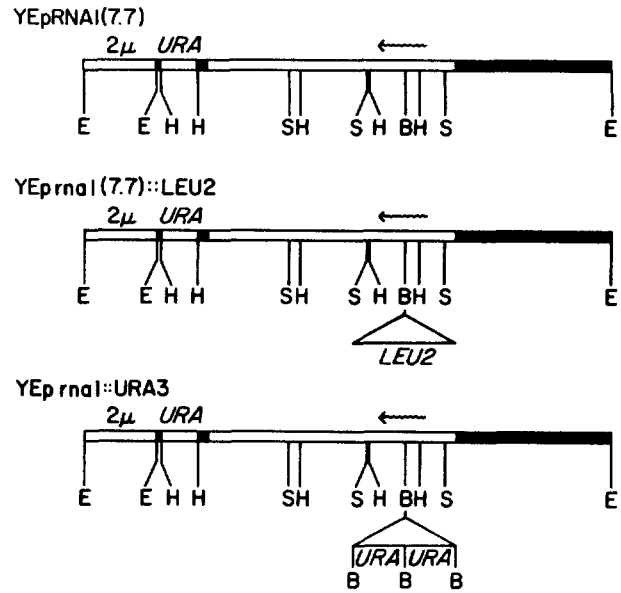

FIGURE 1.—Restriction maps of recombinant plasmids. The darkened regions are pBR322 sequences. The open regions are sequences derived from yeast. The *arrow* represents the *RNA1* transcript and the direction of transcription. Restriction sites and relevant labels: B, *Bam*HI; S, *Sst*I; E, *Eco*RI; H, *Hin*dIII; 2 μ, 2 micron origin of replication; URA, *URA3* gene; LEU2, *LEU2* gene. YEp24 contains yeast 2 μ replicator and the *URA3* gene. YEpRNA1(7.7) was derived from YEp24 by insertion of yeast genomic sequences into the *Bam*HI site of YEp24. Plasmids not shown: YCpRNA1(7.7) and YCprna1(7.7)::LEU2. These plasmids are identical to YEpRNA1(7.7) and YEprna1(7.7)::LEU2, respectively except that the 2 μ sequences have been replaced with *CEN3* and *ARS1*.

TABLE 4

Effect of *rna1* disruptions on mitotic segregation of pDK243

Second plasmid present	Average colonies examined/clone	Mean percent white colonies	Mean percent red colonies
None	610	6.16 (±0.94)[a]	3.67 (±1.75)
YEp24	469	9.24 (±2.84)	4.40 (±5.29)
YEpRNA1(7.7)	472	6.27 (±0.55)	4.09 (±2.59)
YEprna1::URA3	577	6.18 (±0.73)	5.85 (±2.52)

[a] The numbers in parentheses are standard deviations.

alleles that promote polysomy. This phenotype is due to sequences specific to the *RNA1* gene and has been shown not to affect three other chromosomes or a minichromosome. Our hypothesis is that disruption of the *RNA1* coding region at the *Bam*HI site results in a selection for cells that are amplified for the chromosome *XIII* that carries the *rna1-1* allele. We propose that a truncated *RNA1* product encoded by the disruption alleles inhibits an *RNA1*-mediated process and that this inhibition is deleterious to the cell. This inhibition establishes a selection for an increase in the concentration of the intact *rna1-1* gene product in order to compete out the truncated product. This selection is satisfied by *rna1-1* gene amplification mediated via polysomy for chromosome *XIII*. Precedence for the selection of sequence-specific amplification in

eukaryotes has been established for DHFR and PALA-encoding regions (SCHIMKE 1984). In yeast similar iteration of DNA segments surrounding *CUP1* results in resistance to copper (FOGEL and WELCH 1982). Although there are few examples of amplification of entire chromosomes, SCHATZ, SOLOMON and BOTSTEIN (1986) recently reported that a disruption of the yeast *TUB1* gene, which codes for α tubulin, could be suppressed by the gain of an additional copy of the chromosome (*XIII*) that encodes this gene. Since chromosome *XIII* also codes for a second α tubulin gene (*TUB3*), this suppression is probably a result of increased expression of *TUB3*.

We thank RALPH KEIL for critical reading of this manuscript and useful suggestions. This research was supported by a U.S. Public Health Service grant from the National Institutes of Health to A.K.H.

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Communicating editor: E. W. JONES