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Expression of Cryptopleurine Resistance in *Saccharomyces cerevisiae*

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An examination of gene expression in diploids may not always be sufficient for determination of the dominant or recessive character of an allele. In *Saccharomyces cerevisiae* resistance to cryptopleurine has been attributed to a single recessive nuclear gene, *cry1*, located on chromosome III. We found, contrary to expectations, that resistance to cryptopleurine is not expressed in diploids that are monosomic for chromosome III. Examination of strains of different ploidy on gradient plates shows that the presence of the sensitive allele in a cell does not affect the level of resistance, but rather the level of resistance is directly related to the ratio of resistant alleles to the number of chromosome sets.

Resistance to the protein synthesis inhibitor cryptopleurine (CRY) in *Saccharomyces cerevisiae* has been reported to be due to a single recessive gene, *cry1*, located on chromosome III, 2.1 map units from the mating type locus (3, 6). However, we found that in diploids that have only one copy of chromosome III (monosomic diploids), CRY resistance was not expressed. Although a recessive gene would show equivalent expession in a monosomic diploid and in a haploid, monosomic diploids containing the CRY-resistant allele were found to be sensitive to CRY.

In this paper we report this experiment and additional experiments on gene dosage effects of CRY resistance and show that sensitivity and resistance cannot be described by a simple Mendelian dominance-recessive relationship.

MATERIALS AND METHODS

Media. The standard culture media used in these experiments have been described previously (4). CRY (Chemsea Manufacturing Pty., Ltd., Peakhurst, New South Wales, Australia) was dissolved in 95% ethanol and added to the autoclaved media. Unless noted otherwise, all media contain CRY at a concentration of 5 μM.

Strains. The strains used in these experiments were derived from strains obtained from R. K. Mortimer (University of California, Berkeley). In the initial experiments, several independent *cry1* isolates were used. For the growth studies and gradient analysis all strains contained the same *cry1* allele; these strains and their genotypes and origins are shown in Table 1. The techniques used to construct higher ploidy strains from haploids, mating diploids, and nonmating diploids have been described elsewhere (M.I. Riley, M.S. thesis, Kansas State University, Manhattan, 1975). For simplicity in describing the CRY genotype of strains, we will use *r* for the resistant (*cry1*) allele, + for the wild-type allele, and *o* for the missing chromosome III in aneuploid strains.

Isolation of mutants. Yeast cells were grown on yeast extract-peptone-adenine-dextrose (YEPAD) slants containing 2 ml of medium for 24 h and then suspended in 2 ml of sterile water. From this suspension, 0.2-ml samples (approximately 2×10^7 cells) were plated on YEPAD plates and irradiated with ultraviolet radiation from a 15-W GE germicidal lamp for 17 s at a distance of 25 cm. (This dose gave approximately 50% survival in haploid strains and 85% in diploid strains.) After incubation for 24 h, to permit expression of the induced mutations, the cells were replica plated to YEPAD + CRY. Resistant colonies were visible in 2 to 4 days. Haploids typically yielded 20 to 200 CRY-resistant clones per plate.

Analysis of CRY resistance on gradient plates. Plates containing a gradient of CRY concentrations were prepared in 90-mm square petri dishes (nominally 100 mm). A 30-ml portion of YEPAD + CRY (5 or 25 μM) was allowed to solidify in plates on a slanted table. An additional 30 ml of YEPAD was added while the plates were horizontal. The plates were prepared 48 h prior to use.

The strains to be tested were diluted, and cell suspensions were spotted at 1-cm intervals across a gradient plate. Each spot contained approximately 20 to 40 cells in approximately 1 to 2 μl. These plates were incubated at 30°C for 4 days. The level of resistance of each strain was recorded on a 0 to 9 scale of increasing concentration according to the highest spot that had growing yeast colonies.

Growth curves and cell volume analysis. Overnight cultures were diluted into YEPAD to a final concentration of approximately 10^6 cells/ml. Fifteen-milliliter cultures in 50-ml Dulong flasks were incubated at 30°C on a rotary shaker at 200 rpm. Cell number was measured by counting samples with a Coulter counter (model Zf, 100-μm aperture) after sonication for 5 s and 50-fold dilution into 0.85%

TABLE 1. *Strain list*

Strain	Genotype[a]	Origin
XP300-29B[b]	a	Parent a strain
XP300-26C[c]	α	Parent α strain
XP300-26C *cry1-7*	*cry1* α	*cry1* mutant isolated from XP300-26C
XJ33-4A	*cry1* a	Spore from XJ33
XJ33-4C	*cry1* α	Spore from XJ33
XP173	α/a	XP300-26C × XP300-29B
XT3428c	*cry1* α/+ a	XP300-26C *cry1-7* × XP300-29B
XT3428c-3	*cry1* α/*cry1* α	Mitotic crossing over in XT3428c
XT3428c-3-I	*cry1* α/o	Non-disjunction in XT3428c-3
XT3428c-48c	*cry1* a/*cry1* a	Mitotic gene conversion and mitotic crossing over in XT3428c
XT3428c-48c-I	*cry1* a/o	Non-disjunction in XT3428c-48c
XJ33	*cry1* α/*cry1* a	Mitotic gene conversion in XT3428c
XJ34	*cry1* α/*cry1* α/*cry1* a	XT3428c-3 × XJ33-4A
XJ35	*cry1* α/*cry1* a/*cry1* a	XT3428c-48c × XJ33-4C
XJ36	*cry1* α/*cry1* α/+ a	XT3428c-3 × XP300-29B
XJ37	+ α/*cry1* a/*cry1* a	XT3428c-48c × XP300-26C
XJ38	*cry1* α/*cry1* a/o	XT3428c-3-I × XJ33-4A
XJ39	*cry1* α/*cry1* a/o	XT3428c-48c-I × XJ33-4C
XJ40	*cry1* α/+ a/o	XT3428c-3-I × XP300-29B
XJ41	+ α/*cry1* a/o	XT3428c-48c-I × XP300-26C
XJ42	*cry1* α/*cry1* α/*cry1* a/*cry1* a	XT3428c-3 × XT3428c-48c
XJ43	*cry1* α/*cry1* α/*cry1* a/o	XT3428c-3 × XT3428c-48c-I
XJ44	*cry1* α/*cry1* a/*cry1* a/o	XT3428c-48c × XT3428c-3-I
XJ45	*cry1* α/*cry1* a/o/o	XT3428c-3-I × XT3428c-48c-I

[a] Only the mating type and *cry1* gene symbols are shown. The *cry1* allele present in these strains is *cry1-7*.

[b] XP300-29B is a *ade 2-1 trp5-18 his6 lys1 gal2*.

[c] XP300-26C is α *thr4 his6 lys1 gal2*.

NaCl. After 4 to 5 h of growth, CRY was added (3 or 6 μM) and we continued monitoring the growth.

Cell volume distributions were determined on the same cultures by analyzing the Coulter counter signals with a Canberra model 8100/e pulse height analyzer (Canberra Industries, Inc., Meriden, Conn.). Ninety-six-channel pulse height distributions were fed into an IBM 370/158 computer for data analysis. The mean and second moment were calculated for each cell volume distribution. Pulse heights were converted to volume (cubic micrometers) by comparison with calibrated polystyrene latex spheres (5.1, 9.53, and 18.04 μm in diameter) obtained from Duke Standards (Palo Alto, Calif.) and Coulter Inc. (Hialeah, Fla.).

RESULTS

Isolation of CRY-resistant mutants. CRY-resistant mutants were isolated from 19 different haploid strains by the procedure described above. An average of 89 resistant colonies per plate was observed when approximately 2×10^7 mutagen-treated cells were plated. When nine normal diploid strains were treated by the same procedure, an average of only 0.7 resistant colonies per plate was observed.

Examination of several CRY-resistant mutants indicated that whereas most were alleles of *cry1*, there appear to be at least two other genes responsible for CRY resistance. Eighteen independent CRY-resistant mutants isolated from 11 different haploid strains were crossed with haploid strains containing the *cry1-7* allele, and the diploids were tested for CRY resistance. We expected that resistant diploids would be formed if both haploids contained alleles of *cry1*. Seventeen of the 18 diploids were resistant and one, contrary to expectation, was sensitive. Nine of the CRY-resistant mutants, including the one that produced a sensitive diploid, were crossed with sensitive test strains and sporulated, and asci were dissected. The results for the eight mutants that are alleles of *cry1* are shown in Table 2. In these mutants, the CRY-resistant alleles map near the *mat1* locus (approximately 4.2 map units). In the nine tetrads where recombination occurred between *cry1* and *mat1*, no recombination was observed between *mat1* and the distal marker *thr4*. Therefore, the gene order is: centromere-*cry1-mat1-thr4*. The mutant that did not appear to contain an allele of *cry1* gave anomalous linkage data. In 59 tetrads in which all four spores germinated, 17 segregated two resistant and two sensitive spores, 36 segregated one resistant and three sensitive spores, and 6 con-

TABLE 2. *cryl tetrad frequencies*

Resistant strain[a]	*cryl-matl*			*cryl-thr4*		
	PD[b]	NPD	T	PD	NPD	T
XP300-26C *cryl-7*	13	0	2	7	0	7
XP300-26C *cryl-10*	6	0	2	4	0	4
XP300-26C *cryl-11*	8	0	1	5	0	3
XP300-29B *cryl-16*	13	0	1	6	0	6
XP300-29B *cryl-17*	13	0	1	8	0	6
XP300-29B *cryl-18*	15	0	0	6	0	8
XT1219-18A *cryl-20*	15	0	0	–	–	–
XT1177s47 *cryl-23*	14	0	2	10	0	5

[a] Strains have been listed according to the strain from which they were isolated and the *cryl* allele they contain.

[b] PD, Parental ditype; NPD, nonparental ditype; T, tetratype.

tained only sensitive spores. Although the CRY-resistant parent was of mating type α, 70% of the resistant progeny were of mating type a. This mutant appears to require two genes for expression of a resistant phenotype; neither gene appears to be linked to *matl*. Therefore, on the basis of map position, among the 24 CRY resistant mutants that have been reported (4 from Skogerson et al. [6], 2 from Grant et al. [3], and 18 in this report), all but one appear to contain alleles of *cryl*.

We attempted to induce mutations to CRY resistance in diploid strains monosomic for chromosome III. Monosomic diploids were expected to mutate to resistance at approximately the same rate as haploids. Twenty-two monosomic strains were treated, and all failed to produce CRY-resistant mutants. Four of these monosomic diploids were subsequently intercrossed, and they gave disomic tetraploid segregation patterns for *matl*, *his4*, and *leu2* (all on chromosome III), showing that the monosomic diploids had not become disomic.

Segregation of CRY resistance in tetraploid strains. Failure to recover CRY-resistant mutants from monosomic diploids could result from the mutations either not being induced or not being expressed. The latter possibility was tested directly using trisomic tetraploid strains homozygous for *cryl*. If *cryl* were expressed in monosomic diploids all spores from a trisomic tetraploid would be resistant. Conversely, if *cryl* were not expressed in monosomic diploids, each ascus would contain two resistant (disomic) and two sensitive (monosomic) spores. Furthermore, if *cryl* is not expressed in monosomic diploids, a tetraploid that is disomic for chromosome III and homozygous for *cryl* should produce only sensitive spores, whereas a complete tetraploid should segregate four resistant spores per ascus.

Disomic, trisomic, and tetrasomic strains were isolated by selecting prototrophs from a mixture of two diploids homozygous for *cryl*. One of these diploids was homozygous for mating type a, whereas the other was heterozygous for mating type; the two diploids carried complementing nutritional markers. Tetraploids were expected to occur in this mixture as a result of matings between homozygous a and α diploids. The α diploids could arise from the heterozygous strain either by mitotic recombination or gene conversion (homozygous α) or by non-disjunction (monosomic α). Formation of disomic tetraploids would require nondisjunction in both strains.

One-hundred and twenty prototrophic, tetraploid colonies were isolated and tested for CRY resistance. All but one (no. 111) were resistant to CRY, and one of these (no. 7) appeared more resistant than any of the others. Three typical clones and these two exceptional ones were sporulated, and asci were dissected and analyzed. The segregation of CRY resistance in these isolates is summarized in Table 3. The number of copies of chromosome III was inferred from the mating type segregation. The three tetraploids with intermediate resistance to CRY (no. 1, 2, and 27) showed typical trisomic segregation patterns for *matl*. These strains segregated two resistant and two sensitive spores per ascus. Furthermore, those spores, which were determined to be monosomic by their *matl* phenotype, were sensitive to CRY and, conversely, disomic spores were resistant. Two asci from clone no. 27 produced four resistant spores, and one of these gave a tetrasomic segregation for *matl*. This clone was apparently contaminated with a few tetrasomic tetraploids.

Clone no. 111, which was sensitive to CRY, segregated four sensitive spores, two of mating

TABLE 3. *Phenotypic classes for tetraploid strains homozygous for CRY resistance*

Copies of chromosome III	Strain no.	CRY (r:+)		
		4:0	2:2	0:4
Tetrasomic	7	10	0	0
Trisomic	1	0	5	0
	2	0	7	0
	27	2	6	0
Disomic	111	0	0	6

type a and two of mating type α. This strain would appear to be a disomic tetraploid. Clone no. 7 showed a tetrasomic segregation pattern for *mat1* and four resistant spores per ascus; it appears to be a complete tetraploid.

Thus, it appears that failure to obtain CRY-resistant mutants from monosomic diploids is due to failure of CRY resistance to be expressed in such strains. Furthermore, the tetraploid strains described above reveal a gradient of resistance that depends on the number of chromosomes carrying the *cry1* allele, with the disomic tetraploid being sensitive, the trisomic of intermediate resistance, and the tetrasomic strain the most resistant.

Quantitative expression of CRY resistance. In addition to the genotype, the media and the density of cells plated affect the apparent level of CRY resistance expressed by a strain. When conventional replica plating is used to transfer cells, inhibition by CRY, even of wild-type strains, is not consistently observed on synthetic media (SC + CRY). However, if the number of cells transferred to the test medium is reduced, by either a second replica plating or spotting a dilute suspension of cells (10^2 to 10^6 cells per plate), inhibition of sensitive strains can be demonstrated on synthetic medium. Mutants isolated as resistant on YEPAD + CRY are also resistant under these conditions on synthetic media. Accordingly, inhibition by CRY is more pronounced at lower cell densities. Although the cell density effect is more apparent on SC than on YEPAD, it is observed on both media. The reason for the greater influence of cell density on SC is not known.

In the tetraploid strains described above, a gradient of resistance was noted among the disomic, trisomic, and tetrasomic strains. When three diploid strains (+/+, r/+, and r/r) and a monosomic diploid (r/o) were replica plated to YEPAD plates containing two different concentrations of CRY (2 and 5 μM), different effects were also noted. On the 2 μM plates only the +/+ strain was sensitive (resistance would appear to be dominant), whereas on the 5 μM plates only the r/r strain was resistant (resistance would appear to be recessive). If the monosomic diploid is not considered, the *cry1* allele would appear to be semidominant. If that were true, then the monosomic diploid should be fully resistant, but this diploid is sensitive. Therefore, it is apparent that the *cry1* allele is not dominant, semidominant, or recessive.

Analysis of CRY resistance on gradient plates. It is evident from the variation between strains of different ploidy and the variation in expression on different levels of CRY that CRY resistance shows a quantitative expression. To provide a uniform genetic background for study of this quantitative resistance, it is necessary that a single *cry1* allele be used in the construction of strains of different ploidy. A series of haploid (+ and r), diploid (+/+, r/o, r/+, and r/r), triploid (r/+/o, r/r/o, r/r/+, and r/r/r), and tetraploid (r/r/o/o, r/r/r/o, r/r/r/r) strains was constructed, all having the same *cry1* allele. The resistance of these strains was measured on YEPAD + CRY gradient plates.

As noted previously, the density of cells influences the apparent level of resistance. To obtain reproducible results, it was necessary to control the number of cells spotted on the gradient plates within a range of 20 to 40 cells per spot.

The relative levels of resistance of different strains are shown in Fig. 1. It should be noted that 6 days elapse between the pouring of the plates and their final examination. Therefore,

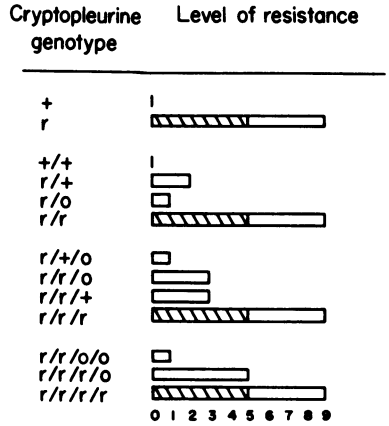

FIG. 1. *Relative levels of resistance of strains of different ploidy. The open bars represent the level of resistance on 0 to 5 μM plates, and the hatched bars represent the level of resistance on 0 to 25 μM plates. See text for explanation of genotypes.*

although we describe the gradients as 0 to 5 and 0 to 25 μM, the end points of the gradient are probably not 0, 5, or 25 throughout the experiment and the gradients may not be linear. These points are reflected by the observation that some of the strains that grow on the 0 to 5 μM plates do not grow at all on the 0 to 25 μM plates. In Fig. 2 we have summarized the data in Fig. 1 so as to emphasize the relationship between the level of resistance and the ratio of resistant alleles to ploidy. From these results, three conclusions are apparent. First, homozygous resistant haploid, diploid, triploid, and tetraploid strains exhibit the same level of resistance. Second, resistance at a given ploidy increases with increasing doses of *cryl*. Third, the sensitive allele does not significantly affect the resistance of a cell, since the same level of resistance is exhibited by *r*/+ and *r*/*o* strains as well as by *r*/*r*/+ and *r*/*r*/*o* strains.

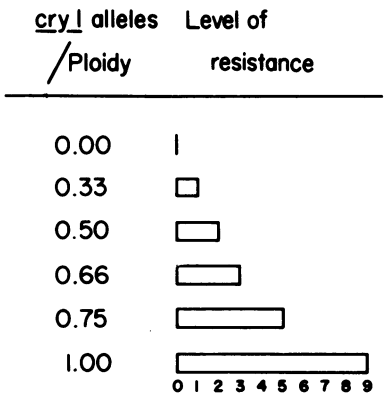

FIG. 2. *Summary of growth on 0 to 5 μM gradient plates. The level of resistance is shown as a function of the ratio of resistant alleles to the ploidy.*

Growth characteristics of strains. As was mentioned above, CRY affects protein synthesis in *S. cerevisiae*. It has been suggested that resistance to CRY may be due to an alteration in the 40S ribosomal subunit (3, 6). Accordingly, an explanation of the above results will likely involve the role of the *cryl* gene product and the influence of aneuploidy for chromosome III on the formation and assembly of ribosomal subunits. To determine whether CRY resistance or aneuploidy affected the growth rate or cell volume of the strains used, we measured these properties in liquid YEPAD. When the exponential doubling time in these cultures had been established, CRY was added and the cell density was followed for 4 to 6 h. The relative levels of resistance observed under these conditions were the same as those observed on gradient plates. Exponential doubling times and cell volumes are shown in Table 4. No systematic differences can be seen in the exponential growth rates among strains of the same ploidy. The mean cell volumes of these strains, which exhibit precise proportionality to ploidy, as previously reported for other ploidy series (5), is also constant. Therefore, there is no effect of the *cryl* mutation or aneuploidy reflected by changes in the normal growth of the cell.

DISCUSSION

The ability of a cell of a given genotype to grow on medium containing CRY is affected by the composition of the medium, the cell density, and the antibiotic concentration. When two of these three variables are held constant, conventional genetic analysis in haploid and diploid strains demonstrates hat the *cryl* allele for resistance to CRY behaves either as a typical Mendelian recessive or as a typical Mendelian

TABLE 4. *Doubling times and mean cell volumes of strains of different ploidy*

Ploidy	Copies of chromosome III	CRY genotype[a]	Doubling time (min)	Mean cell vol/ploidy (μm³)
1n	1	+	90	37
	1	*r*	90	37
2n	2	+/+	80	39.5
	2	*r*/+	80	42.5
	2	*r*/*r*	80	42.0
	1	*r*/*o*	80	43.5
3n	3	*r*/*r*/*r*	88	42.7
	3	*r*/*r*/+	90	42.3
	2	*r*/*r*/*o*	90	43.3
	2	*r*/*r*/*o*	90	40.0
	2	*r*/+/*o*	88	42.7
4n	4	*r*/*r*/*r*/*r*	75	41.3
	3	*r*/*r*/*r*/*o*	75	41.8
	2	*r*/*r*/*o*/*o*	85	40.5
	2	*r*/*r*/*o*/*o*	95	39.5

[a] Each entry represents an independently derived strain.

dominant allele, depending on the concentration of CRY in the medium. However, when resistance is examined as a quantitative character in polyploid strains aneuploid for chromosome III, it is seen that neither traditional description is totally adequate. In strains that are heterozygous, the "sensitive" allele appears to have no effect whatever. The resistant allele does not in itself, however, determine the degree of resistance, but rather the ratio of the number of resistant alleles to the total cell ploidy is the determining factor. This appears to be a unique mode of gene action that has not been described previously.

It has been reported independently by Skogerson et al. (6) and Grant et al. (3) that CRY inhibits protein synthesis in sensitive strains (+ haploids) of yeast. Further, they found that ribosomes from resistant strains (*r* haploids) are less sensitive to CRY inhibition of in vitro polyphenylalanine synthesis than were ribosomes from sensitive strains. They implicated the 40S ribosomal subunit specifically in the resistance. Neither group reported any results for strains that carry both + and *r* alleles, such as a heterozygous diploid. Their results do not distinguish between two possibilities for the formation of resistant ribosomes: the *cryl* locus could code for a component of the 40S subunit or it could code for a ribosome-modifying enzyme.

The simple hypothesis that the *cryl* locus specifies the structure of a ribosomal protein or ribonucleic acid is not adequate. If this were true, then heterozygous strains might be expected to produce both sensitive and resistant (and possibly even hybrid) ribosomes. This would, in principle, predict a gradient of resistance among the +/+, +/*r*, and *r*/*r* diploids. However, it would also predict that monosomic diploids containing the *cryl* allele should be as resistant as a homozygous *cryl* diploid, since only resistant ribosomes would be produced. In our experiments, the monosomic diploids were not as resistant as the homozygous diploid, but rather showed the same level of resistance as the heterozygous diploid. Thus, the gradient of resistance observed in our strains cannot be simply explained by a mixture of sensitive and resistant ribosomes.

How could the gradient of resistance be determined? If the product of *cryl* is a structural component of the ribosome, then the explanation of the quantitative variation in resistance must be found in the regulation of ribosome synthesis. The concentration of CRY that a strain can tolerate in the medium and continue to grow depends on the ratio of the number of *cryl* alleles to the ploidy of the cell (Fig. 2). The influence of the ploidy could be a specific effect reflecting an interaction between the *cryl* gene and one or more other genes not on chromosome III, or it could be a general effect reflecting, for instance, the variation of cell volume with ploidy. It is likely that the number of ribosomes per cell, like the cell volume, is proportional to the ploidy. Accordingly, the ribosome concentration should be the same for cells of any ploidy. If *cryl* or another gene on chromosome III is involved in the coordination of the number of ribosomes with the ploidy, then a monosome could have fewer ribosomes than a full diploid. Such an interaction is plausible, since Gorenstein and Warner (2) have already demonstrated that mutations in either of two genes can cause coordinate inhibition of the synthesis of at least 40 ribosomal proteins. This line of reasoning, combined with the hypothesis discussed in the preceding paragraph, would predict that the level of resistance observed in a strain would be directly proportional to the concentration of resistant ribosomes in a cell. Both a monosomic diploid (*r*/*o*) and a heterozygous diploid (*r*/+) would have the same number of resistant ribosomes: the monosome would produce only half as many ribosomes as normal, but all would be resistant, whereas the heterozygous diploid would have only half of its ribosomes resistant. The alternative possibility, that *cryl* controls the activity of a ribosome-modifying enzyme, leads to entirely different predictions. In this case it is entirely possible that strains having different degrees of resistance contain ribosomes that, in turn, have different degrees of resistance. Modifying activity, determined quantitatively by the *cryl* dosage, could result in either more or less modified ribosomes having graded tolerances for CRY. Again, the results from the monosomic diploid would have to be accounted for by a dosage effect on the level of modifying enzymes in the cell. In this case, there would be no reason to expect the concentration of ribosomes to vary among the different strains.

Our results do not distinguish between the two latter possibilities. However, if the concentration of ribosomes is reduced in aneuploid strains, as required by the former hypothesis, it is not reduced enough to affect their growth rate (Table 4). An understanding of the molecular basis of this phenomenon will require a biochemical characterization of the ribosomes.

The close linkage of *cryl* to *matl* and its drug resistance make it suitable for a number of genetic studies and selection schemes. Additionally, its use in distinguishing monosomic diploids from full diploids makes it valuable as

a genetic marker. A study of chromosome loss such as that by Campbell et al. (1) can be done with much greater ease, since monosomic diploids derived by non-disjunction can be identified from mating diploids derived from mitotic crossing over or gene conversion. Chromosomal gain, previously a difficult phenomenon to detect because homozygous disomes resulting from non-disjunction would appear the same as their monosomic predecessors, are now feasible. Therefore, *cryl* is an exceptionally useful genetic tool, as well as a means for further understanding the biochemistry of ribosomes.

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