A Dominance Modifier for Cycloheximide Resistance in *Coprinus cinereus*

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Wild and laboratory strains of *Coprinus cinereus* show varying levels of sensitivity to cycloheximide (actidione). Mutants resistant to $1.0\ \mu\text{g}$ cycloheximide ml^{-1} were isolated from UV light-irradiated oidia of two strains unable to grow on more than $0.25\ \mu\text{g}$ cycloheximide ml^{-1} . Of 13 induced mutants, 12 fell into one complementation group, *cy-2*; mutants at this locus were usually recessive, but one of those isolated carried a dominance modifier gene, *modcy*⁺, which led to partial resistance in dikaryons heterozygous for *cy-2*. *Modcy*⁺ was dominant in dikaryons to its non-mutant allele *modcy*⁻, but it had no effect in diploids. However, it affected all *cy-2* alleles tested in dikaryons. Linkage data are presented for *cy-2*, *modcy* and another cycloheximide resistance gene, *cy-1*, which is found in wild strains.

INTRODUCTION

Cycloheximide is a glutarimide antibiotic which is toxic to eukaryotic cells, having its major effect on protein synthesis (Sisler & Siegel, 1967). Mutants resistant to this antibiotic have been isolated in several fungi, including *Neurospora crassa* (Hsu, 1963; Neuhäuser *et al.*, 1970), *Saccharomyces cerevisiae* (Wilkie & Lee, 1965), *Saccharomyces fragilis* (Siegel & Sisler, 1965), *Schizosaccharomyces pombe* (Ibrahim & Coddington, 1976), *Schizophyllum commune* (Pikálek & Necásek, 1978) and *Podospora anserina* (Crouzet *et al.*, 1978). In all these species, except *Schizophyllum commune*, mutants with altered cytoplasmic ribosomes have been identified, although other mechanisms of resistance, for instance detoxification or impermeability, have also been discovered.

Coprinus cinereus is a basidiomycete fungus with a monokaryotic and a dikaryotic stage in its life cycle; stable diploids can be produced artificially (Casselton, 1965). It is therefore possible to study genetic interactions within and between nuclei in this organism: cycloheximide resistance due to an altered ribosomal component could be used as a label for the ribosomes produced by one type of nucleus in a dikaryon.

This paper describes the isolation of cycloheximide-resistant mutants in *C. cinereus* and some of their properties in dikaryons and diploids. A preliminary report of these results has been made (North, 1978).

METHODS

Culture media. These were described by Lewis & North (1974): glucose was sterilized separately and added (at 1%, w/v) after the medium had been autoclaved. Supplements to the medium were added at the usual concentrations (Lewis, 1961). Fruit bodies were grown on agar plates or sterile horse manure.

Materials. Cycloheximide (actidione) was supplied by Koch-Light. Difco Bacto-agar was used throughout. Other materials were purchased from the usual suppliers.

Strains and nomenclature. The strains of *Coprinus cinereus* used in mutagenesis and genetic analysis are listed in Table 1. Apart from the *frt* marker strain ZR197 40/40, the strains were derived from a wild cycloheximide-resistant strain TC4 and the multiply-marked cycloheximide-sensitive strains SR54 and WMR66A, which have a

Table 1. *Strains of C. cinereus used in this investigation*

Strain	Mating type	Markers	Cycloheximide resistance status	Origin
TC4	<i>A</i> ₅ <i>B</i> ₅		<i>cy</i> - <i>I</i> ^r	Wild strain (H series)*
WMR66A	<i>A</i> ₆ <i>B</i> ₆	<i>nic-4 paba-2</i>	<i>cy</i> ^s	Multiple crosses
SR54	<i>A</i> ₆ <i>B</i> ₆	<i>ad-8 me-5 chol-1</i>	<i>cy</i> ^s	Multiple crosses
CY2	<i>A</i> ₅ <i>B</i> ₆	<i>me-5 chol-1</i>	<i>cy</i> - <i>I</i> ^r	Basidiospore progeny of TC4 × SR54
CY3	<i>A</i> ₅ <i>B</i> ₅	<i>me-5 chol-1</i>	<i>cy</i> ^s	
CY5	<i>A</i> ₆ <i>B</i> ₅	<i>ad-8 chol-1</i>	<i>cy</i> - <i>I</i> ^r	
CY6	<i>A</i> ₅ <i>B</i> ₅	<i>nic-4 paba-2</i>	<i>cy</i> ^s	
CY8	<i>A</i> ₆ <i>B</i> ₅	<i>nic-4 paba-2</i>	<i>cy</i> ^s	Basidiospore progeny of TC4 × WMR66A
CY9	<i>A</i> ₆ <i>B</i> ₆	<i>nic-4 paba-2</i>	<i>cy</i> ^s	
CY11	<i>A</i> ₅ <i>B</i> ₆	<i>nic-4 paba-2</i>	<i>cy</i> ^s	
CY13	<i>A</i> ₅ <i>B</i> ₆	<i>me-5 chol-1</i>	<i>cy</i> ^s	Basidiospore progeny of TC4 × SR54
CY14	<i>A</i> ₆ <i>B</i> ₅	<i>ad-8 me-5 chol-1</i>	<i>cy</i> ^s	
ZR197 40/40	<i>A</i> ₄₀ <i>B</i> ₄₀	<i>frt</i>	<i>cy</i> ^s	Basidiospore progeny of mutant and wild strain from Czechoslovakia†

* H series described by Lewis & North (1974).

† Origin described by Moore & Stewart (1971).

complex origin. Basidiospore progeny from crosses of TC4 with SR54 or WMR66A were numbered CY1, CY2 and so on.

Mutants isolated from CY strains were numbered according to their parent strain, for example CY9.23 was derived from CY9. Recombinant progeny from the cross between CY9.23 and CY3 are referred to by spore isolate numbers, e.g. Sp78.

Dikaryons, whose formation is reversible, are described in the same way as a cross: CY9.23 × CY3. Diploids are lettered CYD followed by the numbers of their constituent strains, for instance CYD9/14 was made from CY9 and CY14.

Cycloheximide resistance and sensitivity alleles are represented by *cy*-*I*^r, *cy*-*I*^s, respectively; the presence of an active dominance modifier allele is represented by *modcy*⁺ and its wild-type non-active allele by *modcy*⁻.

Genetic analysis. Dominance and complementation tests in dikaryons and meiotic mapping were carried out as described by Lewis (1961). Diploids were selected by the method of Casselton (1965) and their diploidy confirmed using a dikaryon test, which involves mating to a strain of such genotype that a prototrophic dikaryon can only be formed if the tested strain is a diploid (C. Casselton, personal communication). Mitotic mapping was carried out by using griseofulvin (North, 1977).

Mutagenesis and selection of cycloheximide-resistant mutants. Mutations were induced in oidia of CY9 and CY11 using a Hanovia UV light giving a dose of 0.65 µJ cm⁻². Irradiation of an oidial suspension for 7 min reduced the viability of the spores to about 3% of the unirradiated level; the mutation rate to cycloheximide resistance was 1–2 × 10⁻⁶.

The expression of cycloheximide resistance requires *de novo* synthesis of mutant cellular components (Neuhäuser *et al.*, 1970; Brusick, 1972). In order to allow such expression, mutagenized oidia were plated on complete medium and incubated at 37 °C for 36 h before overlaying with 1% water agar containing 15 µg cycloheximide ml⁻¹. Overlayed plates were incubated for 14–21 d, after which colonies that had grown through to the top were isolated on to complete medium. On testing, the majority of colonies isolated proved to be stable mutants able to grow on at least 1 µg cycloheximide ml⁻¹.

Growth tests. Growth was measured on solid medium after 72 h incubation at 37 °C. Initially, resistance to cycloheximide was scored as the ability to grow on medium containing 1.0 µg cycloheximide ml⁻¹ (haploid and diploid monokaryons) or 0.5 µg cycloheximide ml⁻¹ (dikaryons). Later, more detailed tests were used to determine the maximum concentration of cycloheximide permitting growth. Two or more replicates were set up for each test; tests were repeated several times.

RESULTS

Resistance of wild strain TC4 to cycloheximide

Strains WMR66A and SR54 are both unable to grow on 0.1 µg cycloheximide ml⁻¹. Crosses of these strains to TC4, which can grow on 1.0 µg cycloheximide ml⁻¹, indicated that this level of resistance was due to a single gene, designated *cy*-*I*^r.

Fig. 1. Genetic map of linkage group II of *Coprinus cinereus*. References: *, Senathirajah & Lewis (1975); †, Lewis & North (1974); ‡, this paper; §, Jehan (1979).

Table 2. Growth of strains carrying the *cy-1^r* and *cy-1^s* alleles

Strain	Genotype (<i>cy-1</i>)	Maximum cycloheximide concn. permitting growth ($\mu\text{g ml}^{-1}$)
CY8	<i>s</i>	0.3
CY6	<i>s</i>	0.1
CY2	<i>r</i>	2.0
CY5	<i>r</i>	1.0
TC4	<i>r</i>	3.0
CY2 × CY8	<i>r</i> × <i>s</i> dikaryon	2.0
CYD2/6	<i>r/s</i> diploid	1.0

The *cy-1^r* allele was dominant in dikaryons and diploids. Tests among the progeny of SR54 × TC4 and WMR66A × TC4 showed no segregation of dominance from resistance, indicating that no dominance modifier genes were involved (Table 2).

Analysis of the progeny of ZR197 40/40 and TC4 indicated that *cy-1* is 36.5 ± 4.5 units from *ftr* on linkage group VII.

UV-induced mutants resistant to cycloheximide

Mutants were selected from CY9 and CY11 as described in Methods. Mutants from CY9 were tested for dominance in dikaryons with CY3; those from CY11 were tested with CY14. All the mutants were recessive except for CY9.23, which showed a partial dominant phenotype: the dikaryon CY9.23 × CY3 could grow on minimal medium plus $0.5 \mu\text{g cycloheximide ml}^{-1}$ and could fruit on this medium. This dikaryon was allowed to fruit, and the basidiospores were tested for resistance to $1.0 \mu\text{g cycloheximide ml}^{-1}$ and for dominance of cycloheximide resistance. Certain of the basidiospores were shown to carry cycloheximide resistance which was recessive in the dikaryon, indicating that CY9.23 carried a dominance modifier gene, designated *modcy*⁺. Sensitive progeny carrying the modifier gene were identified by their ability to form partially resistant dikaryons with unmodified resistant strains, indicating that the modifier can act in *trans*. Dikaryons between CY9 and unmodified resistant strains were sensitive, showing that the modifier gene is a new mutation not found in the original CY9 strain, which is therefore *modcy*⁻.

Complementation testing indicated that all except one of the mutants isolated were allelic. There were two cases of apparent interallelic complementation, both involving mutant CY9.24. The two resistance loci identified were designated *cy-2* (12 isolates) and *cy-3* (1 isolate). The only *cy-3* strain isolated was a weak mutant; tests for mitotic and meiotic linkage failed to show association with markers on groups I, II, IV, VI and VII. No further work was carried out with this strain.

Linkage relationships of *cy-2* and *modcy*

cy-2 showed linkage with the *chol-1* locus, which is linked to the *B* mating-type locus on group II (Lewis & North, 1974). Recombination values for crosses involving five *cy-2^r* alleles were statistically homogeneous; the combined data gave the map position of *cy-2* as 27.9 ± 1.8 units distal to *chol-1* (Fig. 1).

Table 3. *Growth on cycloheximide of dikaryons heterozygous for cy-2 alleles with and without modcy⁺*

Key: + + +, no inhibition; + +, good growth; +, poor growth; $\pm$, very slight growth; -, no growth; ND, not determined.

<i>cy-2^r</i> mutant Cycloheximide concn ...	Dikaryon with <i>cy-2^s modcy⁻</i>	Dikaryon with <i>cy-2^s modcy⁺</i>	
	0.5 $\mu\text{g ml}^{-1}$	0.5 $\mu\text{g ml}^{-1}$	1.0 $\mu\text{g ml}^{-1}$
CY9.1	-	+ + +	$\pm$
CY9.21	-	+ +	$\pm$
CY9.24	-	+ +	$\pm$
CY9.25	-	+ +	$\pm$
CY11.30	-	+	$\pm$
CY11.34	-	+	$\pm$
CY11.35	-	+	$\pm$
Sp137 (<i>cy-2^r modcy⁻</i>)*	-	+ +	+
CY9.23 $\times$ CY3	ND	+ +	$\pm$

* Basidiospore progeny from the cross CY9.23 $\times$ CY3.

Analysis of a cross between CY9.23 (*cy-2^r modcy⁺*) and CY3 (*cy-2^s modcy⁻*) indicated that *modcy* is also on group II, 27.0 ± 5.2 units distal to *cy-2*. Senathirajah & Lewis (1975) reported that the *p*-fluorophenylalanine resistance locus *pfp-11^r* and its linked modifier gene are also on group II. A cross between a *cy-2^s pfp-11^r* strain and a *cy-2^r pfp-11^s* strain showed that these two loci recombine freely; the *pfp-11* locus is therefore on the other arm of group II from *chol-1* and *cy-2*. The linkage relationships of the resistance loci reported to be on Group II will be presented in the Discussion.

Specificity of the modcy gene

Strains carrying *modcy⁺* and *cy-2^s* were used to make dikaryons with alleles of *cy-2^r* other than the CY9.23 allele. Dikaryons were tested for their ability to grow on 0.5 and 1.0 μg cycloheximide ml^{-1} . The results, shown in Table 3, indicate that the *modcy⁺* gene confers partial resistance in dikaryons heterozygous for a variety of *cy-2^r* alleles, and it is therefore not allele specific. It is interesting to note that although the mutants used in the test cannot grow on more than 2.0 μg cycloheximide ml^{-1} , the resistance of the modified dikaryons is comparable with CY9.23 $\times$ CY3, where CY 9.23 can grow on 250 μg cycloheximide ml^{-1} .

Effect of the cy-2^r modifier gene in dikaryons and diploids

Basidiospores isolated from the cross CY9.23 $\times$ CY3 were used to construct dikaryons and diploids with various constitutions with respect to the *cy-2^r* allele and its modifier. Because of the linkage of the genes to the *B* mating-type allele and the need for complementary forcing markers to make diploids, some combinations were not possible.

Growth tests were set up on minimal medium for most diploids and dikaryons: those with auxotrophic requirements (denoted ‡ in Table 4) were tested on complete medium, as were the monokaryons, except CY9.23 $\times$ CY3 basidiospore progeny Sp137 and Sp34 that were prototrophs tested on minimal medium.

Table 4 shows the maximum concentration of cycloheximide permitting growth for each genotype. It is clear from these results that there is variation in the level needed to inhibit growth of the strains originally designated sensitive due to their inability to grow on 1.0 μg cycloheximide ml^{-1} . This variation is seen in *cy-2^s modcy⁺* and *cy-2^s modcy⁻* strains; however, the difference between the *cy-2^r* and *cy-2^s* monokaryons is clear. The presence of the modifier gene has no effect on the level of resistance of the *cy-2^r* monokaryons, which also vary in their resistance levels. As indicated by the preliminary results, homozygous resistant dikaryons have a lower tolerance to cycloheximide than resistant haploid monokaryons, but homozygous resistant diploids are similar to resistant haploids.

In sensitive dikaryons, both *cy-2^s $\times$ cy-2^s* and *cy-2^s modcy⁻ $\times$ cy-2^r modcy⁻*, the level of

Table 4. *Properties of monokaryons, dikaryons and diploids with and without cy-2^r and its modifier gene*

Genotype combination*	Component strains†		Maximum cycloheximide tolerance (µg ml ⁻¹)			
			Haploids		Dikaryon	Diploid
<i>s</i> - <i>s</i> -	CY9	CY3	0.250	0.040	0.010	
	CY11	CY14	0.200	0.020	0.015	
	CY9	CY14	0.250	0.020		0.500
<i>s</i> - <i>s</i> +	CY11	Sp24	0.200	0.200	0.250	
	CY6	Sp34	0.100	0.100	0.030	
	CY9	Sp39	0.250	ND		0.050
<i>s</i> + <i>s</i> +	Sp34	Sp135	0.100	0.060	0.050	
<i>s</i> - <i>r</i> -	CY9	Sp137	0.250	60	0.100	
	CY9	Sp20	0.250	50		0.300
<i>s</i> - <i>r</i> +	CY3	CY9.23	0.040	250	1.0	
	CY9	Sp48	0.250	70	3.0‡	
	CY14	Sp138	0.020	50	5.0‡	
	CY14	CY9.23	0.020	250		0.600
<i>s</i> + <i>r</i> -	Sp34	Sp137	0.100	60	3.5	
	Sp78	Sp137	0.400	60	5.0	
	Sp102	Sp137	0.350	60	5.0	
<i>s</i> + <i>r</i> +	Sp78	Sp48	0.400	70	5.0‡	
	Sp34	Sp48	0.100	70	5.0	
	Sp102	Sp53	0.350	70		0.300
<i>r</i> - <i>r</i> -	Sp137	Sp91	60	70	15.0	
	Sp137	Sp98	60	150	20.0	
<i>r</i> - <i>r</i> +	Sp137	CY9.23	60	250	10.0	
	Sp43	CY9.23	70	250		150‡
<i>r</i> + <i>r</i> +	CY9.23	Sp48	250	70	20.0‡	
	Sp138	Sp53	50	70	20.0	
	Sp138	Sp48	50	70		70

ND, Not determined exactly (haploid strain lost); less than 1.0 µg ml⁻¹.

* Genotypes: resistance and sensitivity to cycloheximide (*cy-2* locus) are indicated by *r* and *s* respectively; presence and absence of an active modifier allele (*modcy*) are indicated by + and - respectively.

† Sp indicates basidiospore progeny isolated from CY9.23 × CY3.

‡ Indicates an auxotrophic diploid or dikaryon tested on complete medium.

resistance resembles the more sensitive parent, except for CY6 × Sp34, which is more sensitive. In dikaryons heterozygous for *cy-2^r* the effect of a single or double dose of *modcy*⁺ is to increase the level of resistance 10–200-fold. This effect is not seen in *cy-2^r/cy-2^s* diploids, even when these are homozygous for *modcy*⁺.

DISCUSSION

The cy-2 gene product

Preliminary results indicate that *cy-2^r* strains have cycloheximide-resistant ribosomes (Traynor & North, 1981); this implies that *cy-2* codes for a ribosomal component. As there were examples of interallelic complementation among *cy-2^r* mutants, it is likely that this gene specifies a multimeric protein. Experiments to identify the altered protein are in progress; cycloheximide resistance in other fungi has been shown to be a consequence of such a change (Coddington & Fluri, 1977; Bégueret *et al.*, 1977; Stöcklein & Piepersberg, 1980).

Modification of dominance by modcy⁺

Dominance modifier genes affecting resistance to *p*-fluorophenylalanine and other inhibitors in *C. cinereus* have been described (Senathirajah & Lewis, 1975; Jehan, 1979); in some cases the

mod⁺ genes are present in wild-type strains. In *C. cinereus* dikaryons, nuclear distribution is regular, with one nucleus of each type in each cell; this contrasts with the uneven distribution in heterokaryons (Casselton & Lewis, 1967). Lewis & Vakeria (1977) have suggested that the *pfp-10* locus of *C. cinereus* codes for a multimeric protein. They propose that the dominance modifier *mod-10*⁺ allele favours the formation of *r* homomultimers. The *modcy*⁺ gene, which is present as an induced mutation in CY9.23, is a dominant, allele non-specific enhancer of cycloheximide resistance in dikaryons.

Kacser & Burns (1981) suggest that dominance modifications may be a result of the interaction of proteins involved in a pathway. A protein required for assembly of *Escherichia coli* ribosomes, but not for their activity, has been reported by Nowotny & Nierhaus (1980). I suggest that *modcy* codes for a protein involved in the pathway of ribosome assembly by affecting *cy-2* multimer formation. In the dikaryon, *modcy*⁺ is dominant in *cis* and *trans*, working by facilitating homomultimer assembly in or near its own nucleus, acting in a similar way to the *mod-10*⁺ gene referred to above, which is also dominant in dikaryons and without effect in diploids (Senathirajah & Lewis, 1975). The *modcy* gene product may be a structural part of the ribosome, or its presence may only be necessary during the assembly process, like the bacterial protein mentioned above; its activity may not be locus specific.

Linkage relationships in linkage group II

Senathirajah & Lewis (1975) and Jehan (1979) have reported resistance genes on group II, where *cy-2* and *modcy* are located. *Pfp-11* is not linked to *cy-2*; however, the clustering of these genes on one linkage group is of interest and I have taken this opportunity to collate and show, in Fig. 1, a summary of the present genetic map for this group.

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