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Cytoplasmic Inheritance of a Cold-sensitive Mutant in *Aspergillus nidulans*

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Cold-sensitive mutants of bacteria are a source of strains defective in the assembly of ribosomes (Guthrie, Nashimoto & Nomura, 1969; Tai, Kessler & Ingraham, 1969). A study of cold-sensitive mutants in *Aspergillus nidulans* was undertaken in the hope that it would provide ribosomal mutants. Cold-sensitive (*cs*) mutants were isolated, and when crossed with wild-type the majority of 32 mutants tested segregated as single-gene mutations.

One mutant, *cs67*, showed non-Mendelian segregation of its cold-sensitive character in such a cross. This paper presents further results demonstrating the extranuclear inheritance of *cs67*.

METHODS

The initial strains of *Aspergillus nidulans* used in this work were translocation-free. R21 (*pabaA1 yA1*) carries the nuclear markers for *p*-aminobenzoic acid requirement and yellow conidia; R153 (*wA3*; *pyroA4*) the nuclear markers for white conidia and pyridoxine requirement.

Conidial suspensions were mutagenized by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, using the method of Alderson & Hartley (1969). Strain *cs67* was isolated from such a suspension of R21 conidia following filtration enrichment (Woodward, De Zeeuw & Srb, 1954). The standard techniques of Pontecorvo *et al.* (1953) were used.

RESULTS

Growth properties of cs67. At 37 °C growth of *cs67* on solid media is indistinguishable from that of wild-type. The mutant forms only small colonies when incubated at 20 °C.

The mutation is not specific to the germination stage since *cs67* fails to grow at 20 °C even after an initial overnight incubation at 37 °C. Mutant *cs67* does not grow at 20 °C in response to nutritional supplements, nor to molar concentrations of KCl or NaCl.

The responses at 37 °C of *cs67* to actidione (either 0·1 mg /ml or 2 mg/ml) or sodium deoxycholate (0·08 %) are the same as wild-type.

Somatic stability of cs67. When conidia of *cs67* were plated and incubated at 37 °C the resulting colonies showed no evidence of vegetative sectoring over several subcultures. Fourteen single-colony isolates from one of these subcultures were tested and all had precisely the same growth properties as the original isolate. Three of these clones (*cs67-1*, *cs67-2* and *cs67-3*) were used in the crosses and heterokaryon tests described below, in addition to the original strain (*cs67-0*).

Crosses between cs67 and wild-type. Progeny from individual hybrid perithecia were tested for the segregation of the four nuclear markers and cold-sensitivity. In all cases the nuclear markers segregated as expected, the ratio of mutant to wild-type alleles being 1:1 ($P > 0{\cdot}05$). However, the progeny from any one perithecium were either all cold-sensitive

Table 1. *Non-Mendelian segregation of cold-sensitivity in different perithecia from crosses between cs67 and wild-type*

	Progeny			
	cs		*cs*$^+$	
Cross	*w*	*w*$^+$	*w*	*w*$^+$
cs67–0 × R153	0	0	56	43
	0	0	42	57
	0	0	51	59
cs67–1 × R153	50	50	0	0
	0	0	46	54
cs67–2 × R153	0	0	17	20
cs67–3 × R153	56	54	0	0
	47	52	0	0
cs58 × R153	27	26	19	28

or all non-cold-sensitive (Table 1). The segregation of the nuclear marker *wA3* in these crosses, and the normal segregation of a nuclear cold-sensitive mutation (*cs58*) in a similar cross, are also shown in Table 1.

Heterokaryon tests. Heterokaryons between *cs67* and R153 were synthesized. After two or three subcultures on minimal medium the heterokaryons were examined by taking samples of conidia with a mounted needle. Suspensions of these conidia were plated at high dilution, and in each case about 100 of the resulting colonies tested for the nuclear markers and cold-sensitivity.

The nuclear markers were found only in the two parental configurations. Generally all the colonies from any one sample lacked the cold-sensitive character, but occasionally a sample gave rise to cold-sensitive colonies only.

DISCUSSION

The mutant *cs67* shows non-Mendelian inheritance in sexual crosses and cold-sensitivity segregates independently of nuclear markers in heterokaryons. Thus *cs67* satisfies the two genetic criteria applicable in *Aspergillus nidulans* amongst those described by Jinks (1964) to establish extranuclear inheritance.

Similar patterns of inheritance have been reported for 'vegetative death' in *Aspergillus amstelodamii* (Handley & Caten, 1973) and one of the 'oligomycin-resistant' mutants of *Aspergillus nidulans* (Rowlands & Turner, 1973). Both of these mutants have altered cytochrome spectra and are considered to be mitochondrial in origin.

G. Turner & R. T. Rowlands (personal communication) have recently shown that *cs67* produces an altered cytochrome spectrum after growth at restrictive temperatures. This result, taken with extranuclear inheritance, strongly indicates that *cs67* is also a mitochondrial mutant. We suggest that selection for cold-sensitivity could be an effective method of enrichment for mitochondrial mutants.

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