The Genetic Basis of the Incompatibility Reaction following Plasmodial Fusion between Different Strains of the Myxomycete *Physarum polycephalum*

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SUMMARY

Post-fusion incompatibility among plasmodia derived from *Physarum polycephalum* strain 29 is controlled by interactions between alleles at three loci, two of which are linked. A reaction will occur between a plasmodium which is heterozygous or a homozygous dominant at a locus, and one which is a homozygous recessive at the same locus. Depending on genotype with respect to the three loci, incompatibility reactions among plasmodia can be absent, unilateral or bilateral.

INTRODUCTION

Carlile & Dee (1967) found that a lethal reaction sometimes followed plasmodial fusion between different strains of the myxomycete *Physarum polycephalum* Schweinitz. Carlile (1972) carried out a study of this lethal interaction with plasmodial strains 15 and 29, and found that the reaction was unilateral, strain 29 being sensitive. Under conditions of sub-optimal nutrition, no lethal reaction occurred but elimination of strain 29 still took place. Border & Carlile (1974) found that nuclear damage occurred early in the lethal reaction, and that the damaged nuclei were mostly derived from strain 29. It was therefore suggested that a major feature of the incompatibility reaction was the elimination of the nuclei of the sensitive strain. Further it was suggested (Carlile, 1973) that the lethal effects which occur under rich nutritional conditions might be uncommon in nature, since large plasmodia will only make contact if stimulated to migrate by starvation. The lethal reaction, however, is useful to the investigator, as it permits detection of incompatibility without the need to resort to subsequent genetic analysis. The present work extends the above studies to other pairs of strains and elucidates the genetic basis of the reaction.

METHODS

Origin and maintenance of strains. All the amoeboid strains employed, with the exception of strain i (Dee, 1962), were the progeny of the plasmodial strain 29, full designation i × A7029 (Carlile, 1972). Plasmodial strains were obtained by carrying out matings among these amoeboid strains. Strain 29 has the advantage of being homozygous with respect to the *fusA* and *fusB* loci which control plasmodial fusion (Cooke & Dee, 1975), so fusion occurs readily among all plasmodial progeny.

Amoeboid strains were maintained in two-membered culture with *Escherichia coli* in Petri dishes of half-strength cornmeal (CM/2) agar (Gray & Alexopoulos, 1968) with monthly

subculture, and on slopes of 0.1 % liver extract (LE) agar (Dee, 1962) with twice-yearly subculture. Plastic vials were used for slope cultures as the rubber washers of glass bottles were found to have adverse effects on amoeboid growth. Strains were also stored under sterile mineral oil (Dee, 1966) in plastic vials and remained viable for at least two years.

Plasmodia were maintained by weekly subculture on SD (St) agar, which was the semi-defined (SD) agar medium of Carlile (1972) but with soluble starch replacing glucose. Strain 29 was also maintained in shaken liquid SD medium with twice-weekly subculture (Carlile, 1971) and in ampoules stored in liquid nitrogen (Boder & Johnson, 1967).

Production of new strains. Sporulation of strain 29 was brought about by exposing fully grown plasmodia on SD (St) agar to daylight. Amoeboid strains were obtained by spreading serial dilutions of spores on CM/2 agar already spread with *E. coli*, and recloning the strains thus isolated by spreading serial dilutions of encysted amoebae. Mating of amoebae to yield plasmodia was carried out by mixing suspensions of the amoeboid strains and spreading on CM/2 agar buffered at pH 6.0 (Collins & Tang, 1973). Plasmodia appeared within two weeks, were fed with sterile oatflakes, and freed from *E. coli* by migration on plain agar.

Incompatibility tests. Tests were carried out on SD (St) agar in 9 cm diam Petri dishes at 24 °C. Large centrally-placed inocula were employed so that 24 h growth yielded a plasmodium about 4 cm in diameter. A portion of plasmodium with underlying agar was then excised from the margin of one strain (donor) and placed in an inverted position on a second (recipient) strain near the margin of the plasmodium. A reciprocal test was carried out in which the first strain was the recipient and the second the donor. Rapid fusion between the two strains occurs in these tests, and the plasmodium soon vacates the relatively anaerobic region beneath the excised block of agar. The two strains mingle and become indistinguishable, so that any resulting reactions occur in a heterokaryon which in one test contains an excess of the nuclei and cytoplasm of one strain and in its reciprocal a corresponding excess of the other. The results of the transfers were observed at 6 h and 1, 2 and 3 days after transfer. Control cultures of both strains were also examined to discriminate between the degenerative changes which occur with repeated subculturing of some strains and incompatibility reactions. The transfer method of initiating incompatibility reactions had previously been used in studying the reaction between strains 15 and 29 (Border & Carlile, 1974).

RESULTS

Spores from strain 29 were germinated to yield amoeboid strains 29.01 to 29.20, hereafter referred to as .01 to .20. Preliminary experiments suggested that four incompatibility genotypes occurred among the amoebae. Four amoeboid strains of one mating type, which represented the four incompatibility genotypes, were selected and mated in all possible combinations with four amoeboid strains which also represented the four genotypes but which were of the other mating type, to yield 16 plasmodial strains. Incompatibility tests were then carried out between pairs of these strains in all possible combinations, with each strain serving in turn as donor and as recipient (Table 1). Some tests never gave any incompatibility reaction. Other tests sometimes gave no reaction and on other occasions gave a weak reaction with effects visible only with the microscope, such as the discharge at the edge of the plasmodium of particles resembling nuclei or of more extensive debris, usually within 6 h. Yet other tests commonly resulted in a strong reaction – usually a widespread lethal effect visible to the naked eye, similar to that described by Carlile (1972) and commencing within 6 h, but occasionally taking the form of cessation of growth, contraction of the plasmodium, massive production of debris and death in about 3 days.

Table 1. Reactions resulting from fusion of plasmodia derived by sib matings of progeny of strain 29

Reaction class	I			II			III			IV			V	VI
	ABC			AbC			AbC			aBC			Abc	aBc
Proposed phenotype	AaBbCC			AaBbCc			Abc			aBC			Abc	aBc
Proposed genotype	AaBbCC			AaBbCc			Abc			aBC			Abc	aBc
Recipient strains	...			...			...			...			...	...
Donor strains	.02 × .08	0	0	0	0	0	.03	.02	.04	.12	.03	.02	.12	.03
	.04 × .01	0	0	0	0	0	.03	.02	.04	.12	.03	.02	.12	.03
	.03 × .08	0	0	0	0	0	.03	.02	.04	.12	.03	.02	.12	.03
	.02 × .14	0	0	0	0	0	.03	.02	.04	.12	.03	.02	.12	.03
	.04 × .19	0	0	0	0	0	.03	.02	.04	.12	.03	.02	.12	.03
	.12 × .01	0	0	0	0	0	.03	.02	.04	.12	.03	.02	.12	.03
	.03 × .14	W	0	W	0	W	.03	.02	.04	.12	.03	.02	.12	.03
	.12 × .19	W	0	W	0	W	.03	.02	.04	.12	.03	.02	.12	.03
	.04 × .08	0	0	0	0	0	.03	.02	.04	.12	.03	.02	.12	.03
	.04 × .14	0	0	0	0	0	.03	.02	.04	.12	.03	.02	.12	.03
	.12 × .08	0	0	0	0	0	.03	.02	.04	.12	.03	.02	.12	.03
	.02 × .01	0	0	0	0	0	.03	.02	.04	.12	.03	.02	.12	.03
	.03 × .01	W	W	0	0	0	.03	.02	.04	.12	.03	.02	.12	.03
	.02 × .19	0	0	0	0	0	.03	.02	.04	.12	.03	.02	.12	.03
	.12 × .14	0	0	0	0	0	.03	.02	.04	.12	.03	.02	.12	.03
	.03 × .19	W	0	0	0	0	.03	.02	.04	.12	.03	.02	.12	.03

o, No reaction; w, weak reaction, i.e. detectable only with microscope and consisting of nuclear debris; s, strong reaction, detectable with naked eye.

Table 2. *Proposed genotype of amoeboid strains*

Incompatibility type	Mating type	
	<i>mt</i> ₁	<i>mt</i> ₂
<i>AbC</i>	.07, .08, A7029*	.04
<i>aBC</i>	.01, .16	.02, .05, .09
<i>Abc</i>	.10, .11, .14, .17	.12
<i>aBc</i>	.15, .19	.03, i

* Not available for testing but deduced: i × A7029 must be heterozygous for all three loci and i acts as *aBc*.

Sometimes, however, only microscopic debris was observed or no reaction was detected. Such reaction failures where an extensive reaction had been expected occurred most frequently when the recipient strain was one that showed poor growth or abnormal morphology. The results recorded in Table 1 are the severest reactions observed between each pair of strains.

The plasmodial strains fell into six classes. Within each class, fusion between strains resulted in no perceptible adverse effects, whereas fusion between strains from different classes commonly did so. Plasmodia of class I almost always produced severe reactions in plasmodia of other classes when used as donor, but only microscopic effects (limited amounts of nuclear debris presumably derived from the other strain) were seen when they were used as the recipient. Such reactions can be regarded as unilateral, with the class I plasmodium as killer and plasmodia of other classes as sensitive, since a small amount of a class I plasmodium produced a lethal effect in plasmodia of other classes that were many times larger. Reactions between other classes were in some instances unilateral and in some bilateral, a severe reaction being obtained regardless of which strain was recipient. For example, plasmodia of class II gave unilateral reactions with classes V and VI but bilateral reactions with classes III and IV. Tests on strain 29 showed it to behave as a member of class I.

The most simple hypothesis consistent with the results (Table 1) is that strain 29 is heterozygous for three incompatibility genes *A*, *B* and *C* and their recessive alleles *a*, *b* and *c*, and that the eight strains of amoeba employed, which will be haploid since meiosis occurs during sporulation, have the genotypes indicated in Table 2. The mating of these amoebae will then produce the six reaction classes demonstrated in Table 1, provided that a reaction occurs between plasmodia which carry at least one dominant allele and those that carry two recessive alleles. The occurrence of unilateral reactions is explicable if a dominant incompatibility gene is able to cause damage in a plasmodium lacking it, but a recessive incompatibility gene is unable to cause damage in a plasmodium containing the dominant gene.

On the above hypothesis one would expect the occurrence among the progeny of strain 29 of four further incompatibility genotypes among the amoebae (*ABC*, *ABc*, *abC* and *abc*) and two further incompatibility classes among plasmodia, corresponding to plasmodial genotypes *aabbCC* or *aabbCc*, and *aabbcc*. Eight further amoeboid strains were therefore mated with amoebae of strains .12 or .14 (postulated genotype *Abc*) and .03 or .19 (postulated genotype *aBc*) to yield plasmodia, the incompatibility classes of which were determined by fusion with plasmodia of known class. The reaction classes of the two plasmodial strains obtained by mating an unknown amoeboid strain with amoebae of the genotypes indicated above enable an unambiguous determination to be made of the genotype of the unknown

Table 3. Tests for determining the genotypes of amoeboid strains

Tester genotype*	Reaction class of plasmodium							
	I	II	III	I	V	II	III	V
<i>Abc</i>	I	II	III	I	V	II	III	V
<i>aBc</i>	I	II	I	IV	II	VI	IV	VI
Deduced genotype ...	<i>ABC</i>	<i>ABc</i>	<i>AbC</i>	<i>aBC</i>	<i>Abc</i>	<i>aBc</i>	<i>abc</i>	<i>abc</i>

* The genotype of the amoeboid strains with which amoebae were mated to yield plasmodia for testing.

amoeboid strain (Table 3). The genotypes of all eight strains determined in this way proved to belong to the four amoeba incompatibility genotypes already known (Table 2). This result is explicable if in strain 29 the *A* and *B* loci are linked with the alleles in the *trans* arrangement and that the linkage was unbroken in the production of the 16 progeny amoebae tested. The genotype of strain i was determined as above to be *aBc* and hence A7029, no longer available, would have been *AbC*. On the basis of the postulated genotypes of strains i and A7029 and unbroken linkage between *A* and *B* loci, the expected ratios among the 16 progeny amoebae would be *AbC*, 4:*aBC*, 4:*Abc*, 4:*aBc*, 4. The ratios obtained (3:5:5:3) are similar to the expected ones.

DISCUSSION

The interactions among plasmodia derived from the progeny of strain 29 have been interpreted above in terms of three genes, *A*, *B* and *C*, giving lethal interactions with their recessive alleles *a*, *b* and *c*. This interpretation is consistent with the widely distributed phenomenon of allelic heterogenic incompatibility (Esser & Blaich, 1973). Since recombination between loci *A* and *B* was not observed, it is possible to interpret the results in terms of two genes only, one with alleles corresponding to genotypes *Ab* and *aB*, and the other to alleles *C* and *c*. This, however, is inadvisable, since to obtain the full pattern of interactions of Table 1 would require subsidiary hypotheses to account for the direction of the interactions which are not readily consistent with the known attributes of allelic incompatibility.

Plasmodial incompatibility having a similar genetic basis – a dominant allele in one plasmodium causing a lethal interaction following fusion with a plasmodium which is homozygous recessive with respect to that locus – has been demonstrated in another myxomycete, *Didymium iridis*, by Clark & Collins (1973), Ling & Ling (1974) and Ling & Upadhyaya (1974). Post-fusion incompatibility between strains has also been observed in another myxomycete, *Badhamia utricularis* (Carlile, 1974), but the genetic basis has not been elucidated.

The present study demonstrates the occurrence of three genes in the progeny of *Physarum polycephalum* strain i × A7029 capable of causing post-fusion lethal interactions between plasmodia. On the basis of the terminology proposed by D. J. Cooke, R. T. Poulter and A. E. Wheals (personal communication) it is suggested that they should be known as *let* (for lethal) *A*, *letB* and *letC*, and their recessive alleles as *leta*, *letb* and *letc*. Strain i × A7029 is already inbred, and it is likely that many more genes capable of causing the lethal interaction occur in *P. polycephalum*. It is, indeed, necessary to postulate a fourth to account for the unilateral lethal interaction (Carlile, 1972) between strain 15 (killer) and strain 29 (sensitive).

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