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A gene unlinked to mating-type affecting crossing between strains of *Physarum polycephalum*

By JENNIFER DEE

Department of Genetics, University of Leicester, Leicester LE1 7RH, England

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SUMMARY

Two alleles of a gene (*rac*) unlinked to the mating-type locus (*mt*) have been identified in strains of *Physarum polycephalum* from different laboratories. Heterothallic strains differing in *mt* alleles cross more rapidly if they differ also in their *rac* alleles. The recovery of hybrid plasmodia from crosses between apogamic (mt_h) and heterothallic strains is more likely to be achieved if strains of different *rac* genotype are used.

1. INTRODUCTION

The heterothallic life-cycle of the true slime mould *Physarum polycephalum* involves a multiple allelic system at the mating-type locus, *mt*. When two amoebal clones carrying different *mt* alleles are mixed, diploid heterozygous plasmodia are formed. Two heterothallic mating-types (mt_1 and mt_2) were derived originally from a Wisconsin isolate and two (mt_3 and mt_4) from an Indiana isolate (Dee, 1966). Another allele (mt_h) was discovered in a third isolate ('Colonia') of which the origin in nature is unknown (Wheals, 1970). Amoebae carrying mt_h can form haploid plasmodia in clones ('selfing') but can also cross with heterothallic amoebae to form diploid heterozygous plasmodia (Cooke & Dee, 1974). Recent genetic studies are revealing the complex nature of the *mt* locus (Anderson & Dee, 1977; Davidow & Holt, 1977).

Both mt_h and heterothallic strains were obviously valuable for the isolation and analysis of mutants in *P. polycephalum* and since there was a risk that unknown genetic variation between isolates would complicate genetic studies, construction of isogenic strains differing only in *mt* was undertaken. In Professor C. E. Holt's laboratory at Massachusetts Institute of Technology, strains carrying mt_3 and mt_4 in a Colonia genetic background were constructed (Adler & Holt, 1974) while at Leicester University we constructed mt_1 and mt_2 'Colonia-background' strains (Cooke & Dee, 1975). Crossing between mt_h amoebae and mt_3 or mt_4 strains occurred quite readily but with mt_1 and mt_2 strains, crossing was achieved only when an mt_h strain, CLd, showing delayed clonal plasmodium formation, had been isolated (Cooke & Dee, 1975).

The present paper reports the discovery of a locus, *rac*, unlinked to *mt*, which affects plasmodium formation in crosses between strains carrying different *mt* alleles.

2. MATERIALS AND METHODS

(i) *Loci*

mt, amoebal mating-type. *fus*A, plasmodial fusion type. Only plasmodia carrying the same alleles fuse; *fus*A1 and *fus*A2 act codominantly (Poulter and Dee, 1968; Cooke & Dee, 1975). *leu*, leucine requirement (Cooke & Dee, 1975). *hts*, plasmodial temperature-sensitivity. Allele *hts*-1, allowing no plasmodial growth at 31 °C was present in the mutant E27 isolated by Gingold *et al.* (1976). *whi*, plasmodial colour. Plasmodia carrying recessive allele *whi*-1 are white; wild-type colour is yellow (Anderson, in the press).

(ii) *Strains*

LU648 (mt_1; *fus*A1); LU688 (mt_2; *fus*A1); LU860 (mt_1; *fus*A1; *leu*$^-$) and LU640 (mt_h; *fus*A1) are isogenic Colonia-background strains derived from crosses between the Colonia and Wisconsin 1 isolates (Cooke & Dee, 1975). CLd (mt_h; *fus*A2) is a derivative of the Colonia isolate with delayed clonal plasmodium formation (Cooke & Dee, 1975). E27 is a mutant isolated from CLd (Gingold *et al.* 1976). LU202 and LU204 (both mt_1; *fus*A1; *hts*-1; *leu*$^-$) and LU203 (mt_h; *fus*A2; *hts*-1; *leu*$^-$) are progeny of E27 × LU860; all mt_h progeny of this cross showed delayed clonal plasmodium formation as expected in progeny of CLd crosses (Cooke & Dee, 1975). LU862 (mt_3; *fus*A1) is a progeny clone of LU640 × CH188. CH188 (mt_3), supplied to us by Drs P. N. Adler and C. E. Holt, was a Colonia-background strain derived from crosses between the Colonia and Indiana isolates (Adler & Holt, 1974). CH188 carried *fus*C2, an allele of a new locus affecting plasmodial fusion (Adler & Holt, 1974) but all Leicester University strains, including LU862 and CLd, are *fus*C1. LU904 (mt_h; *fus*A2; *whi*-1; *leu*$^-$) is a progeny clone of LU867 × LU896 (Anderson, in the press).

(iii) *Methods*

Methods used for culturing and cloning amoebae on liver infusion agar with *Escherichia coli*, for culturing plasmodia and for performing fusion tests were all as recently described (Anderson & Dee, 1977). For tests of plasmodium formation, amoebae were taken directly from growing cultures incubated at 26 or 22 °C and not from plates stored in the refrigerator. Each test was set up on a separate 50 mm diameter Petri dish containing dilute semi-defined agar medium (Anderson & Dee, 1977), inoculated with a 'puddle' of 0·03–0·05 ml *E. coli* suspension. Mixtures of amoebae were stirred together in the puddle before the fluid soaked into the agar. When a clone was used in a number of tests, it was usually inoculated from a suspension; other clones were usually inoculated with toothpicks. Since both these methods gave repeatable results and allowed a clear discrimination between 'rapid' and 'slow' plasmodium formation, no attempt was made to control inoculum size more accurately. It was estimated that 10^3–10^4 amoebae of each strain were inoculated in each puddle. All tests except those involving

LU904 were incubated at 26 °C. Scoring for plasmodia was done with the aid of a Wild M5 stereo microscope (6× to 50×). Pigmented plasmodia were scored with the naked eye.

3. RESULTS

(i) *Segregation of rapid and slow crossing among* mt_3 *progeny of LU203 × LU862*

The cross LU203 × LU862 was carried out in the course of genetic analysis of a temperature-sensitive mutant (E27). To detect the mutant allele (*hts*-1) in a sample of mt_3 progeny clones, test-crosses were set up between each clone and two mt_1; *hts*-1 strains, LU202 and LU204. Unexpectedly, 8 clones crossed so rapidly with both tester strains that large pigmented plasmodia were present within 5 days. The test crosses with the remaining 10 clones reached the same stage about 5 days later. Since these results suggested the segregation of factors causing fast and slow crossing, all the tests were repeated and 23 additional mt_3 progeny clones from LU203 × LU862 were included. In these tests, 25 clones crossed rapidly with both LU202 and LU204 and 16 clones crossed slowly with both, all repeated tests giving the same results as before. Full genetic analysis of the resulting $mt_1 \times mt_3$ plasmodia showed that 'rapid crossing' recombined with *fus*A, *leu* and *hts* as well as *mt*.

(ii) *Segregation of rapid and slow crossing among progeny of LU860 × LU862*

Since crossing as rapid as that reported in (i) was not normally observed among Leicester University strains, it was reasoned that it may depend on genetic differences between our strains and LU862, which had been derived from an M.I.T. strain. In a preliminary test, LU862 was found to cross rapidly with our strains, LU648, LU688 and LU860, while LU648 × LU688 and LU860 × LU688 crossed slowly. Eighty progeny clones were isolated from the cross LU860 × LU862 and test-crossed with LU860 and LU862. Test-crosses were scored as 'rapid' if numerous plasmodia were present on the third day and in all these crosses large, yellow-pigmented plasmodia were visible by the 5th day. In the 'slow' crosses, no plasmodia were visible on the 3rd day and only a few, if any, by the 5th day. The results (Table 1) indicated segregation of a single pair of factors (designated *rac*-1 and *rac*-2) at a locus unlinked to mating-type.

(iii) *Crossing between progeny of LU860 × LU862*

Four clones of each of the 4 classes shown in Table 1 were test-crossed in all possible $mt_1 \times mt_3$ combinations. Crosses with LU860 and LU862 were also repeated. All the results (Table 2) were consistent with the hypothesis that rapid crossing occurred only between clones which differed in their *rac* alleles as well as in *mt*. LU860 and LU862 were arbitrarily designated *rac*-1 and *rac*-2 respectively and the genotypes of all progeny clones were then deduced (Tables 1 and 2).

Table 1. *Results of crossing 80 progeny clones of LU860 × LU862 with LU860 and LU862*

Class*	No. of clones	Proposed genotype (see Table 2)
1. Rapid crossing × LU860	15	mt_3 *rac*-2
2. Slow crossing × LU860	23	mt_3 *rac*-1
3. Rapid crossing × LU862	22	mt_1 *rac*-1
4. Slow crossing × LU862	20	mt_1 *rac*-2
Total	80	

* No clone crossed with both LU860 and LU862. No clone gave plasmodia in clonal cultures.

Table 2. *Results of crossing LU860 × LU862 progeny clones, representing the classes shown in Table 1, with one another*

		Class 1 (mt_3 *rac*-2)				Class 2 (mt_3 *rac*-1)				LU862
	Clone no.	11	18	25	29	3	4	10	14	—
Class 3 (mt_1 *rac*-1)	Clone no. 2	R	R	R	R	S	S	S	—	R
	5	R	R	R	R	S	S	S	S	R
	7	R	R	R	R	S	S	S	S	R
	9	R	R	R	R	S	S	S	S	R
Class 4 (mt_1 *rac*-2)	6	S	S	S	S	R	R	R	R	S
	13	S	S	S	S	R	R	R	R	S
	28	S	S	S	S	R	R	R	R	S
	33	S	S	S	S	R	R	R	R	S
LU860	—	R	R	R	R	S	S	S	S	R

R = rapid crossing. S = slow crossing.

(iv) *Correlation of factors segregating in LU203 × LU862 and LU860 × LU862*

To determine the genotype of the tester strain LU204, crosses were set up with the 8 mt_3 progeny of LU860 × LU862 shown in Table 2. LU204 crossed rapidly with all 4 *rac*-2 strains and slowly with the 4 *rac*-1 strains, allowing it to be designated *rac*-1. It was concluded that the mt_3 progeny clones of LU203 × LU862 which had crossed rapidly with LU204 were *rac*-2 and the 'slow' clones *rac*-1. These conclusions were checked by test-crosses between representative mt_3 progeny clones of LU203 × LU862 and mt_1 progeny of LU860 × LU862 carrying *rac*-1 and *rac*-2. All results were consistent with the conclusion that the same two *rac* alleles were segregating in both crosses.

(v) *Effect of* rac *on crosses between* mt_h *and heterothallic strains*

When mt_h amoebae are mixed with heterothallic amoebae, haploid 'selfed' plasmodia are usually formed as well as diploid 'crossed' plasmodia. The two types can be distinguished by the use of appropriate genetic markers (see below).

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Table 3. *Results of attempted crosses between LU904 and different* mt rac *genotypes*

Strains mixed with LU904		No. of mixtures	
Genotype	Strain nos.	White plasmodia only	White and yellow* plasmodia
mt_1 *rac*-1	LU860 × LU862 progeny, nos. 2, 5, 7, 9	12	0
	LU860	3	0
	LU204	3	0
mt_1 *rac*-2	LU860 × LU862 progeny, nos. 6, 13, 28, 33	7	5
mt_3 *rac*-1	LU860 × LU862 progeny, nos. 3, 4, 10, 14	12	0
	LU203 × LU862 progeny, nos. 5, 26	6	0
mt_3 *rac*-2	LU860 × LU862 progeny, nos. 11, 18, 25, 29	2	9
	LU203 × LU862 progeny, nos. 24, 56	4	2
	LU862	1	1
Total *rac*-1		36	0
Total *rac*-2		14	17

* Formation of yellow plasmodia was an indication that crossing had occurred.

Using such methods, 3 sets of tests were designed to investigate the effect of *rac* in mt_h strains.

(*a*) Duplicate mixed cultures were set up between each of the 16 progeny clones of LU860 × LU862 shown in Table 2 and CLd. Mixtures were also set up of CLd with LU860 and LU862. In all mixtures of mt_1; *rac*-2 and mt_3; *rac*-2 strains with CLd, numerous plasmodia were present by the 4th day, suggesting that rapid crossing may have occurred. Since all LU860 × LU862 progeny are *fus*A1 and CLd is *fus*A2, crossed plasmodia would have the fusion-type *fus*A1/*fus*A2 and these would remain separate from mt_h selfed plasmodia (fusion-type *fus*A2). In all the *rac*-2 × CLd mixtures, rapid plasmodium formation was followed by plasmodial fusion, resulting in only a single plasmodium on each plate. Thus only one fusion-type was present among the plasmodia formed by each mixture and this was shown to be *fus*A1/*fus*A2 in every case by appropriate fusion-tests. In the *rac*-1 × CLd mixtures, plasmodia were formed no faster than in cultures of CLd alone, few being present even by the 6th day. Since rapid crossing occurred only with the *rac*-2 strains, it was concluded that CLd was *rac*-1.

(*b*) Progeny of the cross LU203 × LU862 included 57 mt_h (delayed) clones which had already been classified for the markers known to be segregating in the cross. A sample of 21 of these clones (all *fus*A2) was now tested for segregation of *rac*-1 and *rac*-2 by test-crossing with LU204, LU860 and LU862. When tests were scored on the 3rd day, clear segregation was observed, numerous plasmodia being formed by each clone *either* with LU862 (*rac*-2) *or* with LU204 and LU860 (both *rac*-1). It was concluded that segregation of *rac*-1 and *rac*-2 was being detected among the mt_h (delayed) progeny. Full classification of all the tested progeny revealed no evidence of linkage among the 5 loci, *mt*, *rac*, *fus*A, *leu*, *hts*.

The 21 mt_h; *fus*A2 and 23 mt_h; *fus*A1 progeny clones were also tested for rapid 'selfing' by inoculating them in puddles alone. Only one culture had formed a few plasmodia by the 4th day and scoring was not continued.

(*c*) To test whether *rac* could influence crossing between heterothallic strains and an mt_h strain which was *not* delayed in clonal plasmodium formation, the strain LU904 (mt_h; *whi*-1; *fus*A2) was used. Test-crosses were set up (in triplicate) between LU904 and progeny clones of LU203 × LU862 and LU860 × LU862 representing the genotypes mt_1; *rac*-1, mt_1; *rac*-2, mt_3; *rac*-1 and mt_3; *rac*-2. All the heterothallic strains were *fus*A1. Rapid 'selfing' of LU904 is expected in all these mixtures, giving rise to white plasmodia of fusion-type *fus*A2 but if crossed plasmodia occur they should remain separate (*fus*A1/*fus*A2) and will be yellow in colour thus being easily detectable. The cultures were incubated at 29 °C to reduce the frequency of selfing (Adler & Holt, 1974). The results (Table 3) indicated that crossed plasmodia were formed only when LU904 was mixed with *rac*-2 strains. Crossing between non-delayed mt_h and mt_1 strains had not previously been observed in this laboratory although numerous attempts had been made to achieve it. It was concluded that *rac* heterozygosity had facilitated crossing between mt_h and heterothallic strains and that LU904 carried *rac*-1.

4. DISCUSSION

Mixtures of amoebae with different heterothallic *mt* alleles yielded plasmodia at an earlier time *and* in greater numbers if the strains also differed in their *rac* alleles. Both these effects could be due to an increase in the number of plasmodia initially formed by matings between amoebae. Cultures containing a larger number of incipient plasmodia yield macroscopic plasmodia sooner because increase in size occurs more rapidly as a result of plasmodial fusion. Discrimination between this and other possible modes of action of *rac* requires the application of an assay for plasmodium formation such as that devised by Youngman *et al.* (1977).

Strains derived from the Wisconsin and Colonia isolates all carried *rac*-1 while *rac*-2 was present in a strain derived from the Indiana isolate. Early crosses between the Wisconsin and Indiana isolates (Dee, 1966) also indicated that genetic differences in addition to *mt* led to rapid crossing between some strains. However, Adler & Holt (1974) failed to detect segregation of 'rapid mating behaviour' during crosses between Colonia and Indiana strains.

Mutant isolation in *P. polycephalum* is normally carried out in mt_h strains and although the 'delayed' strain CLd is preferable for some types of selection, non-delayed amoebae are essential for others. For genetic analysis of mutants, crosses between mt_h and heterothallic strains are required. It has been assumed until now that, crosses of non-delayed mt_h amoebae will be successful with mt_3 and mt_4 but not with mt_1 or mt_2 strains. The results reported in this paper, however, show that it is the *rac* genotype that must be known in order to predict the success of crosses between mt_h and heterothallic strains. This has important practical

implications now that a range of marked strains is being used in several laboratories.

Although the formation of crossed plasmodia between non-delayed mt_h amoebae and mt_1 strains was not detected until strains differing in *rac* were used, it seems more likely that *rac* influences such crosses by increasing either the speed or frequency of mating than by altering mating specificity. If the number of 'crossed' plasmodia present at an early stage is increased, they will have a better chance of competing successfully with the many 'selfed' plasmodia which are rapidly formed by the mt_h amoebae. The effects of such competition between plasmodia were indicated in the tests with CLd. In crosses between mt_1 (*rac*-1) strains and CLd in this laboratory, it has normally been necessary to isolate 10–20 plasmodia from a puddle to find one that is crossed. In the crosses between CLd and mt_1; *rac*-2 strains however, crossing was so rapid that the crossed plasmodia were already large when selfed plasmodia were beginning to appear in puddles of CLd alone; thus any selfed plasmodia arising in the mixed puddles had little chance of growth, and this was probably the reason that none was detected.

An interesting question which remains is whether crossing between mt_h amoebae can be facilitated by using strains differing in *rac*. Although many attempts to isolate diploid, heterozygous plasmodia from mt_h mixtures have failed (Anderson *et al.* 1976), there is now good evidence that they are formed and may be detected if appropriate selective conditions are employed (Poulter & Honey, 1977). Again, this may simply be a problem of detecting a rare type of plasmodium arising among a much greater number of plasmodia formed by apogamy.

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REFERENCES

Adler, P. N. & Holt, C. E. (1974). Genetic analysis in the Colonia strain of *Physarum polycephalum*: heterothallic strains that mate with and are partially isogenic to the Colonia strain. *Genetics* **78**, 1051–1062.

Anderson, R. W. (In the press.) A plasmodial colour mutation in the Myxomycete *Physarum polycephalum*. *Genetical Research*.

Anderson, R. W., Cooke, D. J. & Dee, J. (1976). Apogamic development of plasmodia in the Myxomycete *Physarum polycephalum*: a cinematographic analysis. *Protoplasma* **89**, 29–40.

Anderson, R. W. & Dee, J. (1977). Isolation and analysis of amoebal–plasmodial transition mutants in the Myxomycete *Physarum polycephalum*. *Genetical Research* **29**, 21–34.

Cooke, D. J. & Dee, J. (1974). Plasmodium formation without change in nuclear DNA content in *Physarum polycephalum*. *Genetical Research* **23**, 307–317.

Cooke, D. J. & Dee, J. (1975). Methods for the isolation and analysis of plasmodial mutants in *Physarum polycephalum*. *Genetical Research* **24**, 175–187.

Davidow, L. S. & Holt, C. E. (1977). Mutants with decreased differentiation to plasmodia in *Physarum polycephalum*. *Molecular and General Genetics*, **155**, 291–300.

Dee, J. (1966). Multiple alleles and other factors affecting plasmodium formation in the true slime mould *Physarum polycephalum*. *The Journal of Protozoology* **13**, 610–616.

Gingold, E. C., Grant, W. D., Wheals, A. E. & Wren, M. (1976). Temperature-sensitive mutants of the slime mould *Physarum polycephalum* II. Mutants of the plasmodial phase. *Molecular and General Genetics* **149**, 115–119.

POULTER, R. T. M. & DEE, J. (1968). Segregation of factors controlling fusion between plasmodia of the true slime mould *Physarum polycephalum*. *Genetical Research* **12**, 71–79.

POULTER, R. T. M. & HONEY, N. K. (1977). Genetic analysis of a cross between two homothallic strains of *Physarum polycephalum*. *Genetical Research* **29**, 55–63.

WHEALS, A. E. (1970). A homothallic strain of the Myxomycete *Physarum polycephalum*. *Genetics* **66**, 623–633.

YOUNGMAN, P. J., ADLER, P. N., SHINNICK, T. M. & HOLT, C. E. (1977). An extracellular inducer of asexual plasmodium formation in *Physarum polycephalum*. *Proceedings of the National Academy of Sciences, U.S.A.* **74**, 1120–1124.