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Genetic regulation of differentiation in *Physarum polycephalum*

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SUMMARY

Plasmodial formation in the Myxomycete *Physarum polycephalum* is controlled by a mating type (*mt*) locus. There are a number of different heterothallic *mt* alleles; and also a variant, mt_h, that allows plasmodial formation in pure clones. This paper reports an analysis of this differentiation system. The strain *CL* (mt_h) forms plasmodia in pure clones (i.e. it selfs). *CL* was mutagenized with NMG and 21 mutants unable to differentiate into amoebae were isolated. The mutants, together with *CLd*, fell into two complementation groups, twelve in *difA* and ten in *difB*. Both complementation groups are closely linked or allelic to the *mt* locus. *difA* represents a gene essential for plasmodial formation, but it is suggested that *difB* represents a class of revertants to the mt_2 heterothallic state. A model of the control of plasmodial formation is proposed in which the *mt* locus is suggested to code for the repressor of the *difA* gene. Genetic control is explained in terms of the dilution of allele-specific repressors.

1. INTRODUCTION

The Myxomycete *Physarum polycephalum* is a good model eukaryote system that is amenable to genetic analysis (Anderson & Dee, 1977; Dee, 1975; Wheals, 1970). There are two distinct vegetative phases in the life cycle of *P. polycephalum*: microscopic, uninucleate amoebae and macroscopic, syncytial, multinucleate plasmodia. The development of amoebae into plasmodia has aroused considerable interest and much work has been done on the system.

Before 1970 only heterothallic strains of amoebae were known. In these strains only haploid amoebae possessing different alleles of the mating type (*mt*) locus are able to combine to form diploid plasmodia. A large series of alleles is known at this locus (Dee, 1973). Wheals (1970) characterized the Colonia isolate of *P. polycephalum*, which forms plasmodia in pure clones. Wheals concluded that plasmodial formation is controlled by an allele, mt_h, of the *mt* locus and he described the isolate as homothallic (i.e. syngamy and karyogamy occurs between two mt_h amoebae to form diploid plasmodia). Subsequent evidence, however, indicated that Colonia amoebae and plasmodia are both haploid and plasmodial formation probably occurs by an apogamic mechanism (i.e. amoebal differentiation occurs with neither syngamy nor karyogamy taking place) (Anderson, Cooke & Dee,

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1976; Cooke & Dee, 1974; Mohberg, 1977). Although the apogamic mechanism is probably predominant, appropriate selection procedures can select for homothallic plasmodial formation (Poulter & Honey, 1977). Because Colonia is neither exclusively apogamic nor exclusively homothallic, it is simply referred to in this paper as a 'selfing' isolate.

Cooke & Dee (1975) isolated two derivative strains of Colonia, *CL* (rapid selfing) and *CLd* (delayed selfing). All subsequent work with Colonia has involved the use of these strains. Both the original Colonia isolate and *CLd* have a partial mt_2 specificity. That is, they only cross slowly on a rare basis with mt_2 heterothallic strains, but cross readily with other heterothallic amoebae (Cooke & Dee, 1974; Wheals, 1970).

Once it was found that two Colonia strains could be crossed to form a diploid plasmodium it was meaningful to attempt the analysis of mutants of Colonia by complementation and recombination tests. Wheals (1973) isolated from a rapid selfing strain of Colonia four mutants (designated *apt*$^-$) unable to differentiate from amoebae into plasmodia. They all crossed with each other and one was analysed further and the mutation found to be unlinked to the *mt* locus. Anderson & Dee (1977) isolated thirteen similar differentiation mutants from *CL* (designated *npf*$^-$). They formed three complementation groups: *npfB* and *npfC* closely linked to the *mt* locus and *npfA* unlinked to the *mt* locus. Davidow & Holt (1977) isolated a number of *apt*$^-$ mutants from various selfing strains, but did not completely group them into complementation groups.

We report the isolation of 21 differentiation mutants (designated *dif*$^-$) from *CL* by NMG mutagenesis. *CLd* was considered to be a mutant of the same type and was included in the analysis (Poulter & Honey, 1977). A preliminary report of some aspects of the analysis of eleven of the *dif*$^-$ mutants has been made by Honey, Poulter & Teale (1976). The results were similar to those reported by Anderson & Dee (1977). The data were used to propose a model of the genetic control of amoebal differentiation. This model is based on the concept of allele-specific repression.

2. MATERIALS AND METHODS

(i) *Strains.* The origins of the heterothallic strains *LU648*, *LU688*, and *i* (Cooke & Dee, 1975; Dee, 1966), the selfing strains *CL* and *CLd* (Cooke & Dee, 1975), and the mutant strains *ATP1*, *LU909*, *LU877*, *CL6129*, and *CL6136* (Anderson & Dee, 1977; Wheals, 1973) have all been described previously. The heterothallic strains *OUA9*, *OUD1*, *OUD3*, *OUD7*, and *OUG3* are progeny clones from the cross *LU648* × *i* (Poulter & Honey, 1977). The selfing strain *OUH3* is a progeny clone from the cross *OUG3* × *CLd* (Poulter & Honey, 1977).

(ii) *Loci. mt, mating type.* Heterothallic alleles mt_1 mt_2 (Dee, 1966) and selfing allele mt_h (Wheals, 1970). *fusA, fusB, plasmodial fusion type.* Identity of *fusA* and *fusB* phenotype is a prerequisite for plasmodial fusion. The two alleles $fusA_1$ and $fusA_2$ are codominant, while the allele $fusB_2$ is dominant over $fusB_1$ (Dee, 1973; Poulter, 1969). Thus six fusion phenotypes (known as fusion groups I–VI) result from the possible diploid combinations of alleles at these loci.

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(iii) *Genotypes of amoebal strains. LU648: mt_1 $fusA_1$ $fusB_1$. LU688: mt_2 $fusA_1$ $fusB_1$. i: mt_2 $fusA_2$ $fusB_2$. CL: mt_h $fusA_2$ $fusB_1$. CLd: mt_hd $fusA_2$ $fusB_1$. APT1: mt_h apt1$^-$ $fusA_2$ $fusB_1$. LU909: mt_h npfA$^-$ $fusA_2$ $fusB_1$. LU877: mt_h npfB$^-$ $fusA_2$ $fusB_1$. CL6129: mt_h npfB$^-$ $fusA_2$ $fusB_1$. CL6136: mt_h npfC$^-$ $fusA_2$ $fusB_1$. OUA9: mt_2 $fusA_2$ $fusB_1$. OUD1: mt_2 $fusA_1$ $fusB_2$. OUD3: mt_1 $fusA_2$ $fusB_2$. OUD7: mt_1 $fusA_1$ $fusB_2$. OUG3: mt_1 $fusA_1$ $fusB_2$. OUH3: mt_hd $fusA_1$ $fusB_2$.*

(iv) *Culture conditions.* Plasmodia were maintained at 26 °C on semi-defined medium at pH 4·6 (Dee & Poulter, 1970). Amoebae were maintained in two membered culture with *Escherichia coli* at 26 °C on 5 % SDM at pH 7. Production of spores, spore germination, and the isolation of amoebal progeny clones were carried out as previously described (Wheals, 1970).

(v) *Plasmodial formation.* Plasmodia were formed by crossing the amoebal clones on 5 % SDM, pH 7 already spread with *E. coli*; or by crossing the amoebae on SDM then placing a drop of *E. coli* suspension on top and allowing it to sink into the agar. The second procedure results in a high density of amoebae and was necessary to isolate rare crosses. When plasmodia formed, a block of agar containing the plasmodia was transferred onto 50 % SDM, pH 4·6.

(vi) *Plasmodial fusion tests.* The fusion genotypes of plasmodia not yet classified were determined using the method of Poulter & Dee (1968). The standard plasmodia of known fusion genotype (fusion testers) used in this work were: *LU648* × *LU688* (fusion group I, $fusA_1/fusA_1$ $fusB_1/fusB_1$), *OUD7* × *LU688* (fusion group II, $fusA_1/fusA_1$ $fusB_1/fusB_2$), *LU648* × *CLd* (fusion group III, $fusA_1/fusA_2$ $fusB_1/fusB_1$), *LU648* × *i* (fusion group IV, $fusA_1/fusA_2$ $fusB_1/fusB_2$), *CLd* (selfed) (fusion group V, $fusA_2$ $fusB_1$), *OUD3* × *i* (fusion group VI, $fusA_2/fusA_2$ $fusB_2/fusB_2$). A haploid plasmodium will resemble the corresponding diploid homozygote. For example, a haploid plasmodium with genotype $fusA_1$ $fusB_2$ will fuse with a diploid plasmodium $fusA_1/fusA_1$ $fusB_2/fusB_2$ (and thus also with $fusA_1/fusA_1$ $fusB_1/fusB_2$.

(vii) *Mutagenesis. CL* amoebae were mutagenized with *N*-methyl *N'*-nitro *N*-nitrosoguanidine (NMG). A number of plates of *CL* were subcultured from stock plates with a wire loop and the plates incubated overnight. A 200 μg/ml solution of NMG in sterile distilled water was made and 0·05 ml placed in the centre of each plate of *CL*. The NMG solution was allowed to sink into the agar and the plates were incubated at 26 °C.

(viii) *Selection for* dif$^-$ *mutants.* The non-selfing *dif*$^-$ mutants were selected for from the cultures of *CL* using a modification of the method employed by Anderson & Dee (1977). Each mutagenized culture was allowed to form plasmodia before suspensions of the remaining amoebae were made. Each suspension was divided and replated onto two fresh plates to form duplicate sublines. This duplication was performed as a precaution against bacterial or fungal contamination. The sublines were incubated at 26 °C and the plasmodial formation/washing procedures repeated (up to 10 times) until large plaques that did not form plasmodia were observed. The mutant amoebae (designated *dif*$^-$) were isolated and purified, with no more than one clone being isolated from any pair of duplicate sublines.

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3. RESULTS

(i) *Preliminary observations on* CL

Adler & Holt (1974) found that if *CL* amoebae were incubated at 30 °C the formation of plasmodia was greatly inhibited. *CL* selfs too rapidly at 26 °C to be able to cross with heterothallic amoebae, but at 30 °C selfing is sufficiently delayed that crossing readily occurs. This property was used to investigate whether *CL* has a partial mt_2 specificity. This knowledge was of importance at a later stage of the analysis when the origin of Colonia was considered. *CL* amoebae were crossed

Table 1. *Crosses between* CL *and heterothallic strains at* 30 °C: *number of days for plasmodia to form*

(C: crossed plasmodia detected. S: only selfed plasmodia (fusion class V) detected. X: crossed or selfed nature of plasmodia not determined. –: no plasmodia formed at 7 days. 0 = cross not performed.)

		SDM, pH 7		SDM, acid pH	
Strain	Genotype	Days for plasmodia	Nature of plasmodia	Days for plasmodia	Nature of plasmodia
CL self	$mt_h\ fusA_2\ fusB_1$	4–5	S	—	—
LU648	$mt_1\ fusA_1\ fusB_1$	4	S	0	—
OUD3	$mt_1\ fusA_2\ fusB_2$	3	C	0	—
OUD7	$mt_1\ fusA_1\ fusB_2$	3	C	3	C
LU688	$mt_2\ fusA_1\ fusB_1$	4	S	0	—
OUD1	$mt_2\ fusA_1\ fusB_2$	4	S	4	C
OUA9	$mt_2\ fusA_2\ fusB_1$	4–5	X	3	X
i	$mt_2\ fusA_2\ fusB_2$	5	S	3	C

with a range of heterothallic strains and any resulting plasmodia were analysed for their fusion genotypes, when appropriate fusion alleles were available. Selfing remained a problem and the optimum conditions (with the least selfing) were found to occur when the 5% SDM was kept at an acid pH (between 4·6 and 5·5). The results are summarized in Table 1. They show that *CL* will cross with mt_2, but at a lower frequency and rate than with mt_1. *CL* has a partial mt_2 specificity, similar to *CLd* and the original Colonia isolate.

(ii) *Isolation of mutants*

A total of 115 plates of *CL* were mutagenized as described above; 51 clones showing delayed plasmodial formation were isolated and characterized for their selfing phenotypes. Most formed plasmodia after 4–14 days incubation and some never selfed. Eleven mutants selfed only rarely after seven days or more (similar to *CLd*) and were selected as suitable for further analysis. The other mutants selfed too rapidly to be readily analysed and were discarded. A second series of plates of *CL* were mutagenized and similar results obtained. Ten mutants were isolated, giving a total of 21 suitable *dif*$^-$ mutants.

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(iii) *Incorporation of fusion markers*

The mutant strains *RP1V*, *RP2V*, *RP3V*, *RP4V*, *RP5V*, *RP6V*, *RP7V*, *RP8V*, *RP9V* and *RP10V* (all $fusA_2$ $fusB_1$) were crossed with *D7* ($fusA_1$ $fusB_2$) to form fusion group IV plasmodia and a number of amoebal progeny clones were isolated from each plasmodium. The reason for this was two-fold. Firstly, a range of fusion genotypes was generated for each mutant that was suitable for future analysis. Secondly, it could be determined whether each mutant carried a single nuclear mutation and whether this was linked to the *mt* locus. The analysis of five representative crosses is given in Table 2. Each progeny clone was crossed with *LU648* (mt_1), *LU688* (mt_2), and the parental *dif*$^-$ mutant. The mt_1 progeny only crossed with *LU688* and the parental *dif*$^-$, whereas the mt_h *dif*$^-$ progeny crossed with *LU648* and sometimes with *LU688*. The crosses with *LU648* were fusion tested and scored for their fusion genotypes. No mt_h *dif*$^+$ or mt_1 *dif*$^-$ recombinants were observed.

Table 2. *Analysis of progeny from crosses between* dif$^-$ *mutants and* D7: *number of progeny clones of each genotype*

		Parental *dif*$^-$				
Progeny genotypes		*RP1V*	*RP2V*	*RP3V*	*RP7V*	*RP9V*
mt_1 *dif*$^+$	Fusion class not tested	5	18	13	16	15
mt_h *dif*$^-$	$fusA_1$ $fusB_1$	2	6	1	1	1
	$fusA_1$ $fusB_2$	1	2	1	4	1
	$fusA_2$ $fusB_1$	1	1	2	3	3
	$fusA_2$ $fusB_2$	3	1	1	2	5
	Not tested	0	0	16	4	0
Total no. progeny clones		12	28	34	30	25

The analysis of the ten mutants showed that they all had single mutations closely linked to the *mt* locus and they had all crossed to form diploid plasmodia. Progeny clones with differing fusion genotypes were selected from each mutant and designated by adding appropriate suffixes that indicated the fusion genotypes. For example, *RP1V* ($fusA_2$ $fusB_1$) was used to prepare three derivatives, *RP1I* ($fusA_1 fusB_1$), *RP1II* ($fusA_1 fusB_2$), and *RP1VI* ($fusA_2 fusB_2$). The suffix designates the fusion group of a selfed plasmodium from that clone. The $fusA_1$ $fusB_2$ derivative of *CLd*, *OUH3*, was also included for further analysis.

(iv) *Complementation tests*

Complementation tests were made between the *dif*$^-$ mutants by combining derivatives with suitable fusion markers in pairs, any crossed plasmodia being fusion group IV. The complementation tests were incubated for 21 days and checked daily for the formation of plasmodia before being discarded. Plasmodia usually formed in 5–10 days or not at all. The mutants were also crossed with mt_1 and mt_2 heterothallic amoebae. The mutants, *RP13V*, *RP14V*, *RP16V*, *RP22V*, *RP23V*, *RP24V*, *RP25V*, *RP26V*, *RP27V*, *RP28V*, and *RP29V* were only available with the fusion genotype $fusA_2$ $fusB_1$.

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Table 3. *Complementation tests between* dif⁻ *mutant derivatives of* CL

(++, many plasmodia formed, confirmed crossed; +, rare plasmodia formed, confirmed crossed; *, rare plasmodia formed, not confirmed crossed; −, no crossed plasmodia formed; 0, complementation test not performed. Note. The diagonal divides the results into two mirror images. Only the results above the diagonals, therefore, are given.)

				Dif A⁻											*Dif B⁻*									
				RP											*RP*									
	mt_1	mt_2	*CLd*	1	4	9	10	14	22	23	24	25	27	28	2	3	5	6	7	8	13	16	26	29
CLd	++	+	−	−	−	−	−	0	0	0	0	0	0	0	+	+	−	−	+	+	0	0	0	0
RP 1	++	+	.	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	+	−	−
4	++	+	.	.	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−	−
9	++	+	.	.	.	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−
10	++	+	.	.	.	.	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−	−
14	++	+	.	.	.	.	.	−	−	−	−	−	−	−	+	+	+	+	+	−	−	*	−	−
22	++	+	.	.	.	.	.	.	−	−	−	−	−	−	+	+	+	+	+	−	*	*	*	−
23	++	+	.	.	.	.	.	.	.	−	−	−	−	−	+	+	+	+	+	+	*	*	−	−
24	++	+	.	.	.	.	.	.	.	.	−	−	−	−	+	+	+	+	+	−	*	−	*	−
25	++	+	.	.	.	.	.	.	.	.	.	−	−	−	+	+	+	+	+	−	*	*	*	*
27	++	+	.	.	.	.	.	.	.	.	.	.	−	−	+	+	+	+	+	−	−	−	−	−
28	++	+	.	.	.	.	.	.	.	.	.	.	.	−	+	+	+	+	+	−	−	−	−	−
2	++	−	.	.	.	.	.	.	.	.	.	.	.	.	−	−	−	−	−	−	−	−	−	−
3	++	−	.	.	.	.	.	.	.	.	.	.	.	.	.	−	−	−	−	−	−	−	−	−
5	++	−	.	.	.	.	.	.	.	.	.	.	.	.	.	.	−	−	−	−	−	−	−	−
6	++	−	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	−	−	−	−	−	−	−
7	++	−	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	−	−	−	−	−	−
8	++	−	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	−	−	−	−	−
13	++	−	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	−	−	−	−
16	++	−	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	−	−	−
26	++	−	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	−	−
29	++	−	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	−

The plasmodia arising from tests between these mutants could not, therefore, be checked by fusion tests. The results of the complementation tests are summarized in Table 3. Two distinct complementation groups were observed, designated as *difA* and *difB*. *CLd* is a member of *difA*, supporting the belief that it is a *dif*$^-$ mutant. The failure of some combinations to complement where expected (e.g. *RP1* × *RP13*) is probably due to inappropriate combinations of alleles of secondary loci affecting crossing, similar to the *rac* gene effect reported by Dee (1978). Aston (1978) found evidence supporting the belief that a similar system existed in this analysis.

Twelve mutants fell into the *difA* complementation group. They crossed readily with *D3* (mt_1) and significantly less readily with *i* (mt_2). These mutants therefore retain the partial mt_2 specificity displayed by *CL*. Ten mutants were designated *difB*$^-$. They crossed readily with *D3* but never with *i*. These mutants had therefore gained an apparent full mt_2 specificity and were indistinguishable from mt_2 heterothallic strains.

(v) *Studies of* dif$^-$ *mutants*

Six complementing plasmodia were chosen and sporulated. A number of amoebal progeny clones were isolated from each spore mass and each clone crossed with *RP9I* (*difA*$^-$) and *RP5I* (*difB*$^-$). The progeny could thus be analysed for their *dif*, *fusA*, and *fusB* genotypes, as is summarized in Table 4. The six plasmodia had all been diploid and no recombinants between *difA* and *difB* were observed, indicating that they are closely linked.

Table 4. *Analysis of progeny from* difA$^-$ × difB$^-$ *crosses. Number of clones of each genotype*

	Parental plasmodium					
Progeny genotypes	*RP1II* × *RP3V*	*RP1V* × *RP7II*	*RP4V* × *RP7II*	*RP9V* × *RP3II*	*RP9VI* × *RP6I*	*RP10VI* × *RP5I*
difA$^-$ $fusA_1$ $fusB_1$	3	3	2	3	3	4
$fusA_1$ $fusB_2$	5	1	6	4	4	5
$fusA_2$ $fusB_1$	4	6	3	2	4	5
$fusA_2$ $fusB_2$	3	4	3	8	0	4
difB$^-$ $fusA_1$ $fusB_1$	1	2	5	5	6	2
$fusA_1$ $fusB_2$	1	3	0	5	5	3
$fusA_2$ $fusB_1$	6	2	2	1	0	3
$fusA_2$ $fusB_2$	4	3	3	0	5	2
Total No. of clones	27	24	24	28	27	28

As no recombinants between the *mt*, *difA* and *difB loci* had been detected we decided to investigate this linkage effect in greater detail. The cross *OUG3* (mt_1 *difA*$^+$ *difB*$^+$ $fusA_1$ $fusB_2$) × *CLd* (mt_h *difA*$^-$ *difB*$^+$ $fusA_2$ $fusB_1$) was made and a fusion group IV plasmodium isolated. A large number of amoebal progeny clones were isolated from this cross. Two types of recombinant genotypes were expected: mt_1 *difA*$^-$ and mt_h *difA*$^+$ (rapid selfing). 6911 progeny clones were analysed to

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determine their selfing phenotypes. A single rapid selfing clone (i.e. mt_h *difA*$^+$) was observed. 363 progeny clones were crossed with *LU648* (mt_1), *LU688* (mt_2), *RP7V* (*difB*$^-$), and *RP9V* (*difA*$^-$). Any mt_1 *difA*$^-$ recombinants would be expected to cross only with *LU688* and *RP7V*, but none were observed. 174 of these clones were mt_1 *difA*$^+$ and 189 were mt_h *difA*$^-$. The *mt* and *difA* loci are therefore tightly linked.

Table 5. *Characterization of* APT1 *and* npf$^-$ *mutants with* dif$^-$ *mutants*
(Number of days for crossed plasmodia to form. –: no plasmodia at 14 days.)

Strain	Genotype	Days to self	× *D3* mt_1	× *i* mt_2	× *RP9VI* *difA*$^-$	× *RP5VI* *difB*$^-$
APT1	*apt1*$^-$	–	4	5	5	5
LU909	*npfA*$^-$	5	4	9	5	7
CL6129	*npfB*$^-$	–	4	–	7	–
LU877	*npfB*$^-$	–	4	–	7	–
CL6136	*npfC*$^-$	–	4	7	–	7

A similar analysis was performed with the crossed plasmodium *OUD7* (mt_1 *difB*$^+$) × *RP3V* (mt_h *difB*$^-$). No rapid selfing recombinant clones were observed amongst the 925 progeny clones screened. No mt_1 *difB*$^-$ recombinants were detected amongst the 448 progeny clones analysed as described above. 232 of these clones were mt_1 *difB*$^+$ and 216 were mt_h *difB*$^-$. The *mt* and *difB* loci are therefore closely linked.

Dr Dee supplied representative *npf*$^-$ mutants belonging to the three complementation groups *npfA*, *npfB*, and *npfC*. The mutants were analysed to investigate the possible equivalence of *npf*$^-$ and *dif*$^-$ mutants. The mutants were crossed with *OUD3*, *i*, *RP9VI* and *RP5VI* and the number of days taken for plasmodia to form noted. *APT1* was included in this analysis (Table 5). All crossed plasmodia were checked by fusion tests. The data suggest that the *difA* and *npfC* complementation groups are equivalent, and similarly for the *difB* and *npfB* complementation groups.

4. DISCUSSION

The origin of Colonia is uncertain, but there is an increasing body of evidence that suggests that Colonia was originally derived from the Wisconsin isolate. Colonia has a partial mt_2 specificity, the mt_2 allele being unique to the Wisconsin isolate (Cooke & Dee, 1974; Dee, 1973; Wheals, 1970). Colonia has the *fus* alleles $fusA_2$ $fusB_1$, which are specific to the Wisconsin isolate (Collins & Haskins, 1972; Poulter & Dee, 1968). *CL* and the Wisconsin isolate also have common alleles at the genes controlling the killing reaction that sometimes occurs following the fusion of two plasmodia (unpublished results). If Wisconsin and Indiana strains are crossed to form hybrid progeny clones, significant numbers of aneuploid clones may be formed (e.g. Adler & Holt, 1975). No such problem has been observed in crosses between Colonia and Wisconsin strains (e.g. Cooke & Dee, 1975). There are, therefore, considerable genetic similarities between the Colonia and Wisconsin isolates, suggesting that they are closely related.

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The data reported in this paper indicate the existence of two complementation groups, designated *difA* and *difB*. A complementation group normally indicates the presence of a gene and it was provisionally assumed that *difA* and *difB* represented two differentiation genes. A preliminary report to this effect was made by Honey *et al.* (1976). There are, however, some observations on *difB* that suggest an alternative explanation. Both *CL* and the *difA*$^-$ mutants have a partial mt_2 specificity, but the *difB*$^-$ mutants have gained a full mt_2 specificity. These mutants now resemble mt_2 heterothallic strains in their crossing behaviour, and we suggest that all of the *difB*$^-$ mutants represent revertants to the mt_2 heterothallic state. Subsequent work in our laboratory supports this conclusion (manuscript in preparation), and we shall at present, therefore, assume that a single differentiation gene, *difA*, has been identified in this work.

The data described in this paper were used to propose a hypothesis describing the differentiation of amoebae into plasmodia in *P. polycephalum*. A preliminary description of some aspects of this model has been given by Honey *et al.* (1976). Many heterothallic *mt* alleles have been identified from different geographical isolates, with amoebae of any two mating types being able to cross. It seems unlikely that crossing involves a specific interaction between *mt* alleles. *CL* selfs by an apogamic mechanism without either syngamy or karyogamy taking place. Neither event seems to be an essential requirement for plasmodial formation. No plasmodia are formed in pure heterothallic clones, thus the genes required for plasmodial formation are not expressed. *CL* selfs rapidly, implying that the genes needed are expressed constitutively. The *difA*$^-$ mutants are defective in a gene essential for plasmodial formation that is closely linked to the *mt* locus. There is only one such gene required to initiate plasmodial formation. The *mt* locus is evidently intimately involved in the control of plasmodial formation.

The hypothesis proposes that the *mt* gene codes for a repressor molecule that in heterothallic amoebae binds to an operator site, preventing transcription of the adjacent gene, *difA*. The model further suggests that this interaction is allele-specific. That is, the mt_1 gene product specifically binds to the o_1 receptor site, the mt_2 gene product specifically binds to the o_2 receptor site, etc. A mt_1 repressor cannot bind to an o_2 receptor or to any other allele-specific site, and so on. It is suggested that amoebal cells of any mating type are able to fuse to form transient binucleate cells. If two amoebae of the same mating type fuse there is no effect on the concentration of the allele-specific repressor and plasmodial formation is not initiated. Such a binucleate cell would divide to reform two amoebae of the same mating type. When a mt_1 amoeba fuses with a mt_2 amoeba the concentrations of both the mt_1-specific and mt_2-specific repressors are halved. This is considered sufficient to relieve repression of the transcription of *difA* and so karyogamy and plasmodial formation is initiated. Such an interaction between *mt* alleles would be non-specific, as is required.

The model provides an explanation of the tight linkage between the *mt* and *difA* loci. If recombination between the *mt* and *o* loci occurred, an inappropriate combination of *mt* and *o* alleles would be formed. Repression would become

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ineffective and the recombinant clones would self rapidly. Assuming the organism derives some benefit from the heterothallic sexual cycle, close linkage between the *mt* and *o* loci would be expected in order to reduce the frequency of recombination.

CL selfs rapidly in pure clones, suggesting that there is constitutive expression of *difA*. This could occur by a defect in the repressor system. We suggest that *CL* was originally derived from a mt_2 heterothallic clone by a mutation in the mt_2 gene. This relieved repression of *difA*, but the mutant repressor is suggested to retain some degree of resemblance to the mt_2 repressor. Thus *CL* and its derivatives have a partial mt_2 specificity. Non-selfing derivatives of *CL* can be isolated in either of two ways – by a mutation of the *difA* gene, or by reversion of the defective *mt* gene to the mt_2 heterothallic state.

This model has the clear expectation that it should be possible to mutate heterothallic amoebae and isolate mutants that can form plasmodia in pure clones (i.e. self rapidly, similar to *CL*). A preliminary report confirming this prediction has been made by Poulter, Honey & Teale (1977). These mutants were designated as *het*$^-$. Adler & Holt (1977) isolated similar selfing mutants from a number of heterothallic strains. These results support our model and provide a strong indication that plasmodial formation in heterothallic amoebae is under repressor control.

Only a single differentiation gene, *difA*, was identified in this work although a number are likely to be necessary for plasmodial formation. The selection procedure used probably selected for mutants affected only in the initial step of differentiation, and thus still able to multiply as amoebae. Anderson & Dee (1977) isolated a single mutation in the gene *npfA*, which possibly represents a secondary differentiation gene. The *npfA*$^-$ mutant *CL6111* selfs relatively rapidly after about five days growth. We isolated a number of *dif*$^-$ mutants that resembled *CL6111*, but discarded them as being leaky and unsuitable for genetic analysis. Some of these mutants may have been defective in *npfA* or similar genes.

The concept of allele-specific repressor dilution has some precedent in the prokaryotes. The regulation of the initiation of DNA synthesis during the cell cycle of *E. coli* has been interpreted as involving the dilution during cell growth of a repressor of initiation (Pritchard, Barth & Collins, 1969). DNA synthesis is initiated when the repressor concentration is reduced by 50%. This hypothesis has been extended to cover the phenomenon of plasmid incompatibility in bacteria, and to explain the behaviour of mutant plasmids with altered copy numbers (Cabello, Timmis & Cohen, 1976; Novick & Hoppensteadt, 1978; Uhlin & Nordström, 1975). There are more than twenty plasmid incompatibility groups, each with a group-specific form of the replication repressor. This system therefore involves the concept of control by a repressor that exists in various (allelic) forms.

In conclusion, a model has been proposed to explain the control of the differentiation of amoebae into plasmodia. This hypothesis provides a good explanation of the data described in this paper, and of previously reported observations on mating in *P. polycephalum*. The value of the model is that it can be used to make firm predictions that can readily be tested. In order to test the model, further work

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needs to be performed on the control of amoebal differentiation in *P. polycephalum*. A number of *het*$^-$ mutants need to be isolated and analysed, and *dif*$^-$ mutant derivatives need to be isolated from them. This would then provide an analysis independent of *CL* that might result in further insight into the system. Further reports on this work are currently in preparation.

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