Genetics of Aggregation Pattern Mutations in the Cellular Slime Mould *Dictyostelium discoideum*

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A class of aggregation pattern mutants called 'streamers' have been isolated from *Dictyostelium discoideum* and analysed genetically. The streamer phenotype is the formation of very large streams of centripetally moving amoebae which are collected from abnormally large territories during the aggregation phase of this organism. Such mutants do not show the pleiotropic developmental defects seen with most other classes of aggregation mutants and after the abnormal aggregation phase they develop into normally differentiated stalk cells and spores. Twenty-four haploid streamers were isolated and assigned to seven complementation groups, *stmA* to *stmG*, after selecting diploids formed between pairs of the mutants. The complementation loci were assigned to the following linkage groups using parasexual genetic techniques: *stmA* and *stmF*, linkage group VII; *stmB*, *stmD* and *stmG*, linkage group II; *stmC* and *stmE*, linkage group III. Use was made of a new temperature sensitive for growth marker, *tsgK21*, which was assigned to linkage group VII. The total number of complementation groups giving the streamer phenotype is estimated from statistical calculation, based on the frequency of allelism, to be between seven and nine.

INTRODUCTION

The cellular slime moulds are currently exciting considerable attention from biologists because of their relatively simple programme of differentiation that is triggered by starvation (reviewed by Loomis, 1975) and because of their unusual aggregative phase during which cell communication occurs over large distances by means of a pulsatile chemical signalling system (reviewed by Newell, 1977).

One approach to the study of such a system is to isolate and study developmental mutants. Unfortunately, as for other systems (such as *Bacillus* sporulation; Mandelstam, 1969), the process of development is sequential such that the absence of a particular developmental event usually blocks the normal appearance of all subsequent events. As a consequence, such developmental mutants have pleiotropic defects that are tantalizingly difficult to analyse. It seems unlikely, however, that all enzymes or other proteins formed during development act as inducers whether the programme is a simple linear one as proposed by Loomis *et al.* (1976) or a complex causal net as proposed by Francis (1977). Mutants that affect the pattern of aggregation or later development without causing pleiotropic defects might possess mutations in genes concerned with events on branches off the main regulation sequence. In this class one might expect to find, for example, mutations in those aggregation functions that regulate variables such as the frequency of centre formation, the dimensions of aggregation territories and streams, and the timing and synchronization of aggregational and developmental events.

For this study we chose those mutants that showed abnormally large aggregation streams (hence the name 'streamers') that clearly lacked the normal regulatory system that limited the range of the chemotactic signalling system.

This report describes a genetic study of 24 streamer mutants of *Dictyostelium discoideum* undertaken to ascertain the number of complementation groups that are concerned with this phenotype and to assign them to linkage groups prior to a detailed biochemical investigation. The parasexual genetic techniques employed have recently been reviewed (Newell, 1978).

METHODS

Media and chemicals. The composition and methods of preparation of nutrient (SM) and selective media, salts solution (SS) and the chemicals used were as described previously (Mosses *et al.*, 1975; Ratner & Newell, 1978). For the selective agar plates containing CoCl_2 it was found that a frequently encountered problem of uneven lawns of bacteria could be circumvented by the simple expedient of allowing the CoCl_2 to remain in the autoclaved medium for 15 min before stirring and pouring. Agar plates containing *p*-fluorophenylalanine (PFP) were made by adding 0.1% (w/v) PFP to SM agar before pouring. KK_2 plates were prepared as described by Green & Newell (1975). Dextran (clinical grade) and PFP were obtained from Sigma, *p*-nitrophenyl- α -D-mannopyranoside (A grade) from Calbiochem, and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) from Ralph Emanuel, Wembley.

Strains. *Dictyostelium discoideum* stocks were grown on lawns of the cobalt-resistant mutant of *Klebsiella aerogenes* designated strain OXF1 (Williams & Newell, 1976). All *D. discoideum* strains used are derivatives of NC4 (Raper, 1935). The nomenclature system used is based on that of Demerec *et al.* (1966) (see Kessin *et al.*, 1974). Table 1 shows the origins and genotypes of all haploid strains used. Phenotypes, when given in subsequent tables and in the text, are denoted by the locus symbol with an initial capital (in roman type), the wild type being designated by the superscript +.

Maintenance of stocks. Standard procedures (Williams *et al.*, 1974) were employed for the handling of amoebae and the preservation of spores in silica gel. Some spores were also stored in glycerol as previously described (Ratner & Newell, 1978). This method is not recommended for periods of more than about 6 months. Mutants in use were maintained in association with *K. aerogenes* by cloning at 7 to 10 d intervals.

Mutagenesis. Freshly harvested amoebae were incubated at 10^7 ml^{-1} in SS salts solution for 10 to 45 min with 200 or $500 \mu\text{g NTG ml}^{-1}$ so as to give approximately 10% survival. Mutagenized amoebae were washed three times in SS, plated on KK_2 plates without a bacterial associate and allowed to develop. The resulting spores (approximately 5×10^6 per plate) were harvested and stored in glycerol at -20°C . To screen for mutants, the spores were plated on SM agar at about 50 spores per plate with an inoculum of *K. aerogenes*. Because one division, at most, could have occurred during the development of the spores (Zada-Hames & Ashworth, 1977), it is very unlikely that any of the mutants obtained using this technique had a common origin. Mutants with a stable streamer phenotype occurred in strain XP55 with an approximate frequency of 5×10^{-4} under these conditions.

Formation of diploids. Two haploid strains, one carrying a *tsg* mutation and the other a *bsg* mutation, were incubated for 17 to 22 h as described previously (Newell *et al.*, 1977*a, b*). Diploids were formed at a frequency of about 10^{-5} and were selected by their ability to grow on a *Bacillus subtilis* lawn at 27°C . In all cases two diploids were retained and used in further studies. These are likely to have resulted from independent fusion events.

Segregation of haploids. This was normally done by standard methods (e.g. Williams *et al.*, 1974) using cycloheximide, methanol or CoCl_2 as the selective agent. In some cases, where large numbers of cross-over diploids were formed (homozygous for the selective marker) relative to the frequency of haploidization, the diploid was grown on PFP plates at 25.5°C for 7 d before plating on the selective medium (Coukell & Roxby, 1977). This increased the haploid yield approximately 100-fold.

Determination of segregant phenotypes. The scoring of most phenotypes followed the methods of Ratner & Newell (1978) with the following two modifications. Growth on CoCl_2 -containing plates could be scored at 6 d (rather than 9 d) if even lawns of the bacterial associate were produced by the technique described above. Scoring of growth on plates inoculated with *B. subtilis* was considerably facilitated if the plates spread with the bacteria were incubated for 3 to 6 h at 37°C before transferring the slime mould on to them. This distinguished between haploid and diploid clones by growth rate and virtually eradicated any residual growth of *Bsg* clones such that accurate scoring could be achieved after 4 d.

Table 1. *Genotypes of parental and multiply marked tester strains used in this study*

Symbols: *cyc*, resistance to cycloheximide ($500 \mu\text{g ml}^{-1}$); *spr*, round spores; *acr*, resistance to methanol (2%, v/v); *axe*, ability to grow in axenic medium; *tsg*, temperature sensitive for growth; *bsg*, unable to grow on *Bacillus subtilis*; *bwn*, production of brown pigment; *man*, lack of α -mannosidase; *cob*, resistance to CoCl_2 ($360 \mu\text{g ml}^{-1}$).

Strain	Parents	Genotype										Reference*
		<i>cyc</i>	<i>spr</i>	<i>whi</i>	<i>acr</i>	<i>axe</i>	<i>tsg</i> <i>D12, E13</i>	<i>bsg</i>	<i>bwn</i>	<i>man</i>	<i>cob</i>	
X22	NP14, M28		<i>A1</i>	<i>A1</i>	<i>A1</i>			<i>A5</i>				<i>a</i>
XP55	NP194, X22	<i>A5</i>						<i>A5</i>		<i>A2</i>	<i>A1</i>	<i>b</i>
XP64	XP58, XM101	<i>A1</i> or <i>5</i>							<i>A1</i>	<i>A2</i>	<i>A1</i>	<i>c</i>
XP76	X36, XM101	<i>A1</i>				<i>A1, B1</i>	<i>A1</i>		<i>A1</i>	<i>A2</i>	<i>A1</i>	<i>e</i>
XP86	XP64, XP71		<i>A1</i>	<i>A1</i>	<i>A1</i>	<i>A1</i>		<i>A5</i>	<i>A1</i>	<i>A2</i>	<i>A1</i>	<i>d</i>
XP95	XP64, XP71		<i>A1</i>	<i>A1</i>	<i>A2</i>	<i>A1, B1</i>	<i>A1</i>		<i>A1</i>	<i>A2</i>	<i>A1</i>	<i>c</i>
XP99	XP64, XP71	<i>A1</i> or <i>5</i>						<i>A5</i>	<i>A1</i>	<i>A2</i>	<i>A1</i>	<i>c</i>
NP208		<i>A5</i>					<i>K21</i>					<i>e</i>
NP404		<i>A5</i>			404		<i>K21</i>					<i>e</i>

* *a*, Williams & Newell (1976); *b*, Newell *et al.* (1977*a*); *c*, Ratner & Newell (1978); *d*, D. I. Ratner, unpublished; *e*, P. C. Newell, unpublished.

RESULTS

Isolation of streamer mutants

Using the parental *D. discoideum* strains XP55 and X22, 24 stable streamer mutants were isolated from 10 separate mutagenesis experiments. The streamer phenotype is the abnormal formation of large streams (2.5 to 20 mm in length) leading into the aggregation centre in clones on SM agar with *K. aerogenes* as bacterial associate. Under these conditions the streams of the parental strains are not normally discernible.

All the mutants grew and developed to form fruiting bodies with stalks containing normal spores. (Mutants bearing additional developmental mutations were counter-selected by the isolation procedure as this required all cells to pass through the spore stage for survival.) The size of the aggregates was characteristically different from mutant to mutant, but was invariably larger than those of the parental strains owing to the large territory size from which the amoebae collected. Such aggregates, however, were unable to produce correspondingly large fruiting bodies. The vast mass of cells seemed to be physically unwieldy and in most strains a large number of the cells could not enter the fruiting body but remained as a lump on the agar at the base of one to four short-stalked fruiting bodies that were constructed from each aggregate. In some strains (such as NP368 and NP375), however, the aggregates became completely subdivided thereby giving rise to several normal-sized fruiting bodies close together.

With some of the mutants that formed very large streams (such as NP377 and NP368) the streams appeared to be striped transversely. This phenomenon, which was easily observable with the naked eye in clones of these mutants, has been previously described in the 'Tiger' mutant (mutant M6.63) of Gross *et al.* (1977). Since the stripes moved slowly outward from the centre at a velocity of approximately $40 \mu\text{m min}^{-1}$, they were probably gross manifestations of the pulsatile signalling system.

Complementation analysis

Fusions with strains X22 and XP55. It was deduced that all the streamer mutations were recessive as diploids heterozygous for the streamer character (*stm*/+) were phenotypically wild type. It was therefore possible to carry out complementation tests between the different mutants.

Diploids formed from the fusion of mutants derived from strain X22 (bearing *tsg* mutations) with mutants derived from strain XP55 (bearing the *bsg* mutation) could be selected after the fusion procedure by plating amoebae on agar plates inoculated with *B. subtilis* and incubating for 4 d at 27 °C (the non-permissive temperature) as described previously (Newell *et al.*, 1977a). Using this technique, the three X22-derived mutants were found to be in different complementation groups and to show non-complementation with 13 of the XP55-derived mutants in three groups named *stmA*, *stmB* and *stmD* (Table 2). The remaining eight XP55-derived mutants, however, complemented all three of the X22 mutants.

Replacement of bsgA with tsgA. To select diploids formed from pairs of the remaining eight mutants, their *bsg* mutations had first to be removed and be replaced by a *tsg* mutation or (if this was impracticable) they had to be reverted. All eight mutants were consequently fused to form diploids with strain XP95 bearing *tsgA1* in repulsion to the *bsgA5* marker on linkage group III. *Stm Tsg Bsg*⁺ haploids were selected by segregation of the diploids on methanol- or cycloheximide-containing plates. Using such *tsg* derivatives in complementation tests with the original *bsg*-bearing strains, six of the eight mutants were shown to fall into two distinct complementation groups (*stmF* and *stmG*) and the remaining two mutants (NP383 and NP387), for which no *tsg* derivatives could be found, complemented both of these groups.

Table 2. *Complementation groups determined for the streamer mutants*

The last two columns show derivative strains in which the *bsgA* marker has been removed or reverted.

Com- plementation group	Mutant name	Mutation number	Parental strain	Representative Bsg ⁺ /TsgA derivatives	Bsg ⁺ (revertants or suppressors)/ TsgK derivatives
<i>stmA</i>	NP307	<i>stm-404</i>	X22		
	NP294	<i>stm-400</i>	XP55	XP118	
	NP301	<i>stm-401</i>	XP55		
	NP378	<i>stm-412</i>	XP55		
	NP379	<i>stm-413</i>	XP55	XP162	
	NP382	<i>stm-414</i>	XP55		
	NP399	<i>stm-419</i>	XP55		
	NP400	<i>stm-420</i>	XP55		
<i>stmB</i>	NP306	<i>stm-403</i>	X22		
	NP366	<i>stm-405</i>	XP55		
	NP371	<i>stm-408</i>	XP55	XP180	
	NP391	<i>stm-417</i>	XP55		
<i>stmC</i>	NP383	<i>stm-415</i>	XP55		XP174 XP175
<i>stmD</i>	NP304	<i>stm-402</i>	X22		
	NP370	<i>stm-407</i>	XP55	XP152	
	NP372	<i>stm-409</i>	XP55		
	NP398	<i>stm-418</i>	XP55		
<i>stmE</i>	NP387	<i>stm-416</i>	XP55		XP177 XP178
<i>stmF</i>	NP377	<i>stm-411</i>	XP55	XP181	
	NP401	<i>stm-421</i>	XP55		
<i>stmG</i>	NP368	<i>stm-406</i>	XP55		
	NP375	<i>stm-410</i>	XP55	XP179	
	NP403	<i>stm-422</i>	XP55		
	NP411	<i>stm-423</i>	XP55		

Reversion of bsg mutations. Mutants NP383 and NP387 could not be converted to *bsg*⁺ derivatives because all *bsg*⁺ segregants were *Stm*⁺ (due to their *stm* mutations being on linkage group III, see below). To overcome this problem it was decided to try isolating revertants (or suppressors) of the *bsgA5* mutation. However, in order that such a reverted or suppressed strain could be used for complementation testing, a *tsg* mutation had to be inserted into it *prior* to the *bsg* reversion as without this it would be left without a diploid selector. The mutation *tsgK21* was used for this procedure as it was found to be an excellent diploid selector and was known to be unlinked to the *bsgA* locus. Strain NP404 bearing *tsgK21* was therefore fused to the two *stm* mutants NP383 and NP387 to yield *tsg*/+; +/*bsg* diploids, and Tsg Bsg *Stm* haploid segregants were selected from them by plating the diploids on methanol-containing plates. These haploids were then plated at high density (3×10^6 per plate) on to 15 plates of SM agar inoculated with a dense suspension of *B. subtilis* 36.1. After 7 d a total of two or three Bsg⁺ clones were found growing from each haploid from the 15 plates. Whether these *Stm* Tsg Bsg⁺ derivatives were true *bsg* revertants or suppressors has not been determined, but all those investigated were capable of fusion at the normal frequency with other *bsg*-bearing strains. By fusion of these derivatives to NP383 and NP387 it was shown that the latter mutants bore *stm* mutations in different complementation groups, *stmC* and *stmE* (Table 2).

Linkage of streamer loci

Segregation of diploids. Heterozygous diploids were made from all the *stm* mutants using the appropriate multiply marked tester strains (XP76 or XP99 for X22-derived mutants and XP95 for those derived from XP55; Ratner & Newell, 1978). Haploids were then segregated from representative diploids formed from mutants in each of the complementation groups and tested for the pattern of segregation of markers (Table 3). In most cases the results revealed unambiguous linkage of the *stm* mutations to markers characteristic of particular linkage groups and lack of linkage to all the other groups.

However, with *stmA* and *stmF*, problems were encountered with some mutations in clearly assigning them to a linkage group. Using diploids (such as DP828 and DP832) which were heterozygous for mutation *stmA404*, the pattern of segregation of haploids clearly showed complete linkage of *stmA* to *cobA* on linkage group VII. Using diploids (such as DP817) heterozygous for *stmA400*, however, the pattern initially was difficult to interpret owing to the appearance of a number of non-streamers (*Stm*⁺) that were phenotypically *CobA*⁺ non-parentals. These would not be expected if *stmA* and *cobA* were in repulsion on linkage group VII. Upon further subculture and retesting, this class was rescored as *Stm*⁺*CobA* (as shown in Table 3) and the pattern was thereby resolved. The problem was again encountered with diploids heterozygous for *stmF* (such as DP902 bearing *stmF411*), but in this case even after subculture not all *Stm*⁺*Cob*⁺ segregants were reclassified (see Table 3).

To confirm the location of *stmA* on linkage group VII, diploids heterozygous for *stmA400* were plated on CoCl₂-containing agar and the recombinant diploids (*cobA/cobA*) that grew were selected for further study. (The haploids that also arose were discarded.) When several such independently isolated recombinant diploids were plated on methanol-containing plates to select segregant haploids, no streamer haploids were isolated out of several hundred segregants tested. Controls with the original *cobA/cobA*⁺ diploids plated on methanol plates gave streamers as a very frequent segregant class. The simplest interpretation of such data is that when the cross-over event leading to *cobA/cobA* homozygosity occurred the *stmA*/+ locus was rendered homozygous (+/+) by the recombination. This confirms that *stmA400* is located on linkage group VII and further indicates that *stmA* is probably distal to the *cobA* marker.

Confirmation that *stmA* and *stmF* are on the same linkage group came from an experiment in which they were both placed in the same diploid. Although this diploid, which was *stmA*/+; +/*stmF*, was a non-streamer due to complementation, all haploids that were subsequently segregated from it were found to be streamers, indicating that the two markers were in repulsion on the same linkage group in this diploid.

The tsgK marker. As an independent confirmation of the location of *stmA* and *stmF*, use was made of the temperature sensitive for growth marker *tsgK*, as this had previously been found to show unambiguous linkage to *cobA* on linkage group VII. Representative segregations showing the location of *tsgK* on linkage group VII are shown in Table 4. Diploids were constructed with *tsgK* in repulsion to *stmA* and to *stmF* and haploid segregants were selected on methanol-containing plates. The results (Table 4) clearly confirm that *stmA* and *stmF* are linked to the *tsgK*⁺ allele on linkage group VII.

Deviation from randomness. Although most *stm* loci could be readily located on definite linkage groups, the pattern of segregation seen was at times non-random. For example, all those XP55-derived streamer loci which are located on linkage group II (i.e. *stmB*, *stmD* and *stmG*) tended to produce only three classes of haploid progeny with reference to linkage group III. However, if haploids were selected on cycloheximide plates spread with *B. subtilis*, small numbers of the fourth class, i.e. *B. subtilis*-resistant streamers, were found. These all grew extremely poorly and it appears that there are unidentified mutations on linkage groups II and III which separately are not harmful but in combination are deleterious.

Table 3. Pattern of phenotypes of haploid segregants selected from diploids heterozygous for the streamer mutations in each of the complementation groups

By convention, the recombinant segregants lie on the lower left-upper right diagonal in each set of four figures so that zeros on these diagonals indicate linkage. The zeros in parentheses result from the selection of the *cycA* allele by the haploid selection procedure.

Parental diploid	Parental haploids	Streamer mutation	Stm/Stm ⁺	Linkage group									
				I	II	III	III	III	IV	VI	VII	Cob ⁺	Cob
				Cyc	Act ⁺	Tsg ⁺	Tsg	Bsg	Bwn ⁺	Man ⁺	Cob ⁺		
DP817	{NP294 XP95}	<i>stmA400</i>	Stm	5	0	29	42	20	52		72	0	
			Stm ⁺	2	52	19	32	37	17		0	54	
DP828 and DP832	{NP307 XP76 NP307 XP99}	<i>stmA404</i>	Stm	(0)	74			36	12	86	97	0	
			Stm ⁺	(0)	63			37	46	149	0	188	
DP826 and DP830	{NP306 XP76 NP306 XP99}	<i>stmB403</i>	Stm	0	50			0	2	18	42	8	
			Stm ⁺	7	0			32	121	70	39	40	129
DP963	{NP371 XP95}	<i>stmB408</i>	Stm	21	0	21	0	21	0	20	1	16	5
			Stm ⁺	48	0	23	25	23	25	26	22	17	31
DP916	{NP383 XP95}	<i>stmC415</i>	Stm	77	13	132	0	132	0	84	48	128	4
			Stm ⁺	42	2	0	54	0	54	15	39	45	9
DP891	{NP370 XP95}	<i>stmD407</i>	Stm	21	0	21	0	21	0	17	4	6	15
			Stm ⁺	143	0	118	27	118	27	83	60	83	60
DP917	{NP387 XP95}	<i>stmE416</i>	Stm	75	61	108	0	108	0	64	19	64	42
			Stm ⁺	61	25	0	74	0	74	42	26	44	29
DP902	{NP377 XP95}	<i>stmF411</i>	Stm	24	6	40	6	40	6	17	28	44	0
			Stm ⁺	33	14	25	22	25	22	21	26	16†	31
DP979	{NP368 XP95}	<i>stmG406</i>	Stm	37	0	36	1	36	1	32	5	21	16
			Stm ⁺	70	0	68	58	68	58	103	23	71	77
DP900	{NP375 XP95}	<i>stmG410</i>	Stm	44	0	43	1	43	1	32	12	40	4
			Stm ⁺	72	0	33	61	33	61	60	34	25	59

* Not scored; other experiments showed that the *stm* marker was not linked to these markers.

† An anomalous class of recombinant Cob⁺Stm⁺ segregants, described in the text.

Table 4. Pattern of phenotypes of haploid segregants from diploids heterozygous for *tsgK* showing linkage of this marker to the *cobA*⁺ allele on linkage group VII and confirming the location of *stmA* and *stmF* on linkage group VII

The zeros in parentheses result from the selection of the *cycA*, *acrA* or *cobA* alleles by the haploid selection procedures used.

Diploid	Parental haploid	Tsg/Tsg ⁺	Linkage group						
			I	II	III	IV	VI	VII	
DP783 and DP784	{ NP208 XP86		$\frac{cycA5}{+}$	$\frac{+}{+}$	$\frac{+}{+}$	$\frac{+}{+}$	$\frac{+}{+}$	$\frac{+}{+}$	
			$\frac{Cyc^+}{(0)}$	$\frac{Acr}{124}$	$\frac{Bsg}{120}$	$\frac{Bwn}{27}$	$\frac{Man^+}{15}$	$\frac{Cob^+}{108}$	
DP801	{ NP208 XP64	TsgK	$\frac{Cyc}{(0)}$	$\frac{Acr^+}{(0)}$	$\frac{Bsg}{25}$	$\frac{Bwn}{6}$	$\frac{Man}{2}$	$\frac{Cob}{23}$	
		TsgK ⁺							
DP992	{ NP294 NP404	TsgK	$\frac{cycA5}{cycA5}$	$\frac{+}{*acr-404}$	$\frac{bsgA5}{+}$	$\frac{+}{+}$	$\frac{+}{+}$	$\frac{+}{+}$	
		TsgK ⁺							
DP967	{ NP377 NP404	TsgK	$\frac{cycA5}{cycA5}$	$\frac{+}{*acr-404}$	$\frac{bsgA5}{+}$	$\frac{+}{+}$	$\frac{+}{+}$	$\frac{+}{+}$	
		TsgK ⁺							
		TsgK							

* The identification of this locus as *acrA* has not yet been confirmed.

Table 5. Assignment of complementation groups *stmA* to *stmG* to linkage groups

The assignments are based on the patterns of segregation shown in Tables 3 and 4 and, in the case of *stmA*, on mitotic recombination studies as described in the text.

Complementation group	Linkage group	Markers used to identify linkage groups
<i>stmA</i>	VII	<i>cobA</i> , <i>tsgK</i>
<i>stmB</i>	II	<i>acrA</i>
<i>stmC</i>	III	<i>bsgA</i> , <i>tsgA</i>
<i>stmD</i>	II	<i>acrA</i>
<i>stmE</i>	III	<i>bsgA</i> , <i>tsgA</i>
<i>stmF</i>	VII	<i>cobA</i> , <i>tsgK</i>
<i>stmG</i>	II	<i>acrA</i>

There were also signs of non-randomness with *stmC415* and *stmE416*. Both loci showed clear linkage to markers on linkage group III but they also showed such non-randomness with respect to linkage group VI that the exceptional classes needed careful retesting. This apparent 'near linkage' of groups III and VI was seen in all experiments involving diploids made from XP55 and XP95, but not in any other diploids. It was at first thought that a translocation involving linkage groups III and VI had taken place in one of these strains, probably XP55 as other workers had seen similar effects when using this strain (K. L. Williams, personal communication). However, small numbers of recombinant progeny for linkage group VI and the *stm* markers were always found. It therefore seems that the apparent association between these two linkage groups is something less substantial than a translocation and may be the manifestation of an incompatibility between unknown markers on these linkage groups. In order to confirm the location of *stmC415* and *stmE416* on linkage group III rather than VI, further experiments were performed in which only linkage groups III and VI were scored in larger numbers. The data for these experiments consistently showed no haploids recombinant for linkage group III while haploids recombinant for *stmC* and *stmE* and linkage group VI appeared at a frequency of between 1 and 3%.

The linkage data is summarized in Table 5.

DISCUSSION

The streamer mutants belong to a class of aggregation pattern mutants with an easily recognizable phenotype that enables their isolation and genetic segregation to be readily undertaken. Within this class there are variations in the length of streams formed by the mutants and in the width of growth margins when clones are growing on plates inoculated with bacteria. Detailed comparison of the individual members of the seven complementation groups of streamer mutants showed that considerable variation in clonal phenotype occurs within the groups, particularly between mutants derived from different parental strains or the same mutations inserted into different genetic backgrounds. The streamer phenotype is clearly sensitive to the presence of unrelated minor mutations. The phenotypes shown in Fig. 1 are those most typical of each complementation group and are all of mutations in the parental strain XP55.

Linkage analysis of the mutants revealed that the seven complementation groups are located on groups II, III and VII. Use was made in this study of the multiply marked tester strains constructed in an earlier study (Ratner & Newell, 1978) and these strains greatly facilitated the analysis by allowing a complete linkage test to be performed in one experiment. In a few experiments problems were encountered in scoring cobalt

Fig. 1. Wild type XP55 with streamers representative of each complementation group: *stmA*, NP294; *stmB*, NP371; *stmC*, NP383; *stmD*, NP370; *stmE*, NP387; *stmF*, NP377; *stmG*, NP368. Bar marker represents 0.5 cm.

resistance (*cobA1* on linkage group VII) in segregants selected on methanol- or cycloheximide-containing plates. A class of false recombinant *cobA*⁺ segregants was observed. This class disappeared in most experiments after retesting of the segregants, but it initially cast some doubt on the location of *stmA* and *stmF* on group VII. Independent evidence was sought using a new temperature sensitive for growth marker, *tsgK21*, shown in separate experiments to be on this linkage group. This *tsg* mutation gave unambiguous results in growth tests at the non-permissive temperature and has proved to be a valuable additional marker for linkage group VII.

All of the 24 streamer mutations isolated and used in this study can be placed in seven complementation groups. Two of the groups, however, contain only one member each (see Table 2) so it seems intuitively improbable that all complementation groups have been found. From the complementation data, 47 cases of non-complementation were observed or inferred for the complete cross of the 24 mutants with each other. Statistical analysis of such data using the method of frequency of allelism for a random distribution of mutations over the mutable loci (Lewontin & Prout, 1956) indicates that the most likely number of loci that must be present to give the observed results is six (one less than the actual number found). The simplest explanation of such a result is that the observed distribution of 8, 4, 4, 4, 2, 1, 1 of the mutations over the seven complementation groups is non-random and it seems most likely that it is the *stmA* class with eight mutants that is unbalancing the distribution. If this locus is assumed to represent a mutational 'hot-spot' that is over-represented, then the calculation would suggest an upper estimate of nine for the number of loci able to give the streamer phenotype. Although due care must be taken in the interpretation of such numbers it does seem likely that most of the streamer loci are represented in this genetic study.

A study by Riedel *et al.* (1973) suggested that large aggregation territory size of mutants might be correlated with a deficiency of the enzyme phosphodiesterase which destroys the cyclic AMP used for signalling by *D. discoideum*. Preliminary data from our streamer mutants, however, suggest that such a correlation cannot be made for these mutants. Further biochemical investigations of representatives of the seven known complementation groups is now in progress.

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