JOURNAL OF BACTERIOLOGY, Apr. 1985, p. 427–429
0021-9193/85/040427-03$02.00/0

Vol. 162, No. 1

NOTES

Genetic Locus (*stmF*) Associated with Cyclic GMP Phosphodiesterase Activity in *Dictyostelium discoideum* Maps in Linkage Group II

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Received 16 November 1984/Accepted 7 January 1985

Previous attempts to map the *stmF* locus in *Dictyostelium discoideum*, by using only clone morphology as a marker, have led to equivocal results. Since strains carrying mutations at the *stmF* locus possess very low cyclic GMP phosphodiesterase activity, we have remapped this locus using both morphological and biochemical markers. Our results indicate that mutations producing a stable "streamer" phenotype and reduced cyclic GMP phosphodiesterase activity are located in linkage group II, probably centromere distal to *acrA*.

Intracellular cyclic GMP appears to be an important regulator of early development in *Dictyostelium discoideum* (9, 11, 23). Since little cyclic GMP is secreted by the cells (23), intracellular pool levels are probably modulated by the relative activities of the guanylate cyclase (21) and the cyclic GMP-specific phosphodiesterase (cGPD) enzymes (5). This notion is supported by recent studies on a class of chemotaxis mutants called "streamers" (17). For example, cGMP pools in streamer strains carrying mutations at the *stmF* locus are greatly elevated after stimulation by cyclic AMP or folate (18, 19), and these strains appear to have a specific defect in cGPD activity (2, 19). It is not known whether the *stmF* locus is the structural gene of the cGPD or a controlling element. Several attempts have been made to map the *stmF* locus, but its map position is still unclear. Mutations now associated with *stmF* were originally mapped at two different loci: *stm-411* (in strain NP377) at *stmF* in linkage group VII and *stm-406* (in strain NP368) at *stmG* in group II (17). Subsequent complementation studies, however, revealed that these mutations map at the same locus (designated *stmF*) (18), and this site was tentatively assigned to the previously unmarked linkage group V (20). This assignment has not been confirmed, however, and the *stmF* locus was unlisted in a recent genetic map of *D. discoideum* (13). While characterizing a group of revertants of the streamer mutants, we obtained data suggesting that *stmF* is in linkage group II. Therefore, we have examined this possibility further using both morphological (i.e., streamer phenotype) and biochemical (i.e., cGPD activity) markers.

Stm^+ tester strains and *stmF* mutants used in this work are described in Table 1. The genetic nomenclature system employed is based on that of Demerec et al. (4) and Kessin et al. (8). $stm^+/stmF$ diploids were constructed by shaking amoebae of a tester strain with an *stmF* mutant in phosphate buffer overnight at 22°C and then selecting for Tsg^+/Bsg^r clones on *Bacillus subtilis* 36.1 at 26.5°C (14). The resulting diploids were purified by restreaking before they were used in further experiments. To isolate haploid segregants from diploid strains, diploid cells were grown clonally on lawns of *Klebsiella aerogenes* until the cell colonies reached a diameter of 7 to 8 mm. Clones were then excised with a sterile spatula, and the cells were either plated directly with bacteria on standard medium (SM) agar containing 2% methanol (SM-M plates) (3) or diluted in sterile salt solution (1) to ≈100 cells per plate and spread with bacteria on SM agar supplemented with Benlate (Du Pont Co., Wilmington, Del.) at 30 μg ml^{-1} (SM-B plates) (22). The ploidy of the segregants was determined by spore size and shape (7). The phenotype of the haploids was established by picking amoebae onto appropriate indicator plates (3, 14) and assaying the cells for α-mannosidase-1 activity (6). cGPD activity in crude extracts of vegetative and starved (18 h) cells was measured as described (2). One unit of cGPD activity was defined as the amount of enzyme required to hydrolyze 1 nmol of cGMP per min at 30°C. Protein concentration was determined according to the method of Lowry et al. (10).

When clones of $stm^+/stmF$ diploids DC166 (HC91/NP368), DC167 (HC91/NP377), and DC168 (XP95/NP377) were plated on SM-M agar, all of the resulting methanol-resistant haploid segregants were nonstreamers. In contrast, clones of diploid DC177 (NP187/HC325), plated under the same conditions, produced only haploid segregants with a streamer phenotype (Table 2). Haploid segregants were also isolated from diploids DC166, DC167, and DC168 under nonselective conditions on SM-B agar, which contained the haploidizing agent, benlate. Both Stm^+ and Stm^- haploid segregants were obtained from these plates. When the segregants were tested for different genetic markers, all of the nonstreamers were methanol resistant (Stm^+ Acr^-), whereas all of the streamers were methanol sensitive (Stm^- Acr^+) (Table 2). These results suggest that *stmF* is in linkage group II.

To determine whether the very low cGPD activity, which is characteristic of strains NP368 and NP377, always segregated with the streamer phenotype, benlate-induced haploid segregants from DC166, DC167, and DC168 representing different phenotypic classes were assayed for cGPD activity. Since cGPD activity was undetectable in strain NP368 throughout development and maximal in other strains after 16 h (2), segregants of DC166 were starved for 18 h before they were assayed. On the other hand the cGPD activity in starved (18 h) NP377 cells, although always reduced, was

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TABLE 1. Haploid strains of *D. discoideum* used in this study

Strain	Relevant genotype[a] in linkage group:						Reference or source
	I	II	III	IV	VI	VII	
HC91	*tsgE13 sprA1*	*acrA100*		*bwnA1*			2
XP95		*acrA2*	*tsgA1*	*bwnA1*	*manA2*	*cobA1*	16
NP368	*cycA5*	*stmF406*[b]	*bsgA5*				17
NP377	*cycA5*	*stmF411*[b]	*bsgA5*				17
NP187	*cycA5*					*tsgK21*	20
HC325	*cycA5*	*acrA120*[c] *stmF406*[b]	*bsgA5*				Spontaneous methanol-resistant derivative of NP368

[a] Phenotypic traits: *tsg*, unable to grow at 26.5°C; *spr*, produces round spores; *cyc*, resistant to 500 μg of cycloheximide per ml; *acr*, resistant to 2% (vol/vol) methanol; *cob*, resistant to 300 μg of cobaltous chloride per ml; *bwn*, produces brown pigment; *man*, lacks α-mannosidase-1 activity; *bsg*, unable to grow on *B. subtilis*; *stm*, forms long aggregation streams. All markers are recessive except for *sprA*. Only markers used in this study are indicated.

[b] *stmF* was placed in linkage group II based on the results presented in this paper.

[c] The *acr-120* mutation was assigned to locus A because it failed to complement *acrA100* in strain HC91.

often variable. Therefore, to be sure of distinguishing strains with normal and reduced cGPD activities, segregants of strains DC167 and DC168 were assayed as vegetative cells. As shown in Table 3, all Stm^+ (Acr^-) segregants, regardless of the other markers they carried, possessed cGPD activity, whereas none of the Stm^- (Acr^+) strains revealed detectable activity. Hence, the reduced cGPD activity and the streamer phenotype seem to cosegregate.

It is possible to determine the order of markers in a particular linkage group by analyzing haploid segregants from diploids which have undergone mitotic recombination in that group (12, 15). Using this approach, we attempted to establish the position of *stmF* in linkage group II relative to the selective marker, *acrA*. A total of 23 independently isolated methanol-resistant diploids (17 from DC166 and 6 from DC167) were grown clonally on SM plates. Four or more clones of each diploid were cut out; the cells were then diluted and plated with bacteria on SM-B agar at 22°C. After 6 to 8 days, 100 or more clones arising from each diploid were scored for the Stm^+ or Stm^- phenotype. Spores from several dozen clones of each strain were examined to determine their ploidy; virtually all of the clones were haploid. Possible streamer segregants were picked and restreaked onto SM agar to check their clone morphology. Surprisingly, not one stable Stm^- strain was found among segregants of the 23 recombinant diploids, despite the fact that streamers were readily obtained from the parental diploids under the same conditions (Table 2). This result suggests that *stmF* is situated centromere distal to *acrA*. Alternatively, since only a limited number of crossover diploids were analyzed, the *stmF* locus could lie proximal but very close to *acrA*. It should be noted that the failure to isolate streamer segregants from these diploids is further evidence that *stmF* resides in linkage group II; if this locus mapped in any other group, the appearance of streamer segregants would not be affected by crossovers in group II.

TABLE 2. Segregation analysis of different $stm^+/stmF$ diploids

Diploid	Haploid parents	Plated on:	Haploid segregant classes			
			Stm^+ Acr^+	Stm^+ Acr^-	Stm^- Acr^+	Stm^- Acr^-
DC166	HC91 and NP368	SM-M		301		0
		SM-B	0	48	32	0
DC167	HC91 and NP377	SM-M		105		0
		SM-B	0	41	47	0
DC168	XP95 and NP377	SM-M		153		0
		SM-B	0	60	23	0
DC177	NP187 and HC325	SM-M		0		156

TABLE 3. cGPD activity in Stm^+ and Stm^- haploid segregants from SM-B plates

Diploid	Haploid parents	Segregant phenotype	No. assayed	No. with detectable cGPD activity[a]	Range of activity (U mg^{-1} of protein)
DC166[b]	HC91 and NP368	Stm^+	24	24	0.13–2.29
		Stm^-	15	0	<0.01
DC167[c]	HC91 and NP377	Stm^+	10	10	0.04–0.17
		Stm^-	14	0	<0.01
DC168[c]	XP95 and NP377	Stm^+	12	12	0.03–0.21
		Stm^-	6	0	<0.01

[a] The assay can detect cGPD activity >0.01 U mg^{-1} of protein.

[b] cGPD activity was assayed in extracts of cells starved for 18 h on agar plates treated with phosphate-buffered saline (2).

[c] cGPD activity was assayed in extracts of vegetative cells.

Since two other *stm* loci (B and D) have also been mapped in linkage group II (17), strains carrying mutations at these loci (NP371 and NP372, respectively) were assayed to determine whether they have reduced cGPD activity. Vegetative amoebae of both strains had low but detectable activity (≈0.02 U mg^{-1} of protein), whereas cells starved for 18 h exhibited levels of activity comparable with those of wild-type strains (1.5 to 3.0 U mg^{-1} of protein).

Previous attempts to map the *stmF* locus by using only the streamer phenotype as a marker have led to ambiguous results (17, 20). Segregation experiments in these studies might have been complicated by the appearance of a new type of streamer mutant. In a few of our experiments (particularly those involving diploids constructed with XP95), a small number of methanol-resistant segregants were obtained which were scored initially as Stm^-. However, after repicking, these strains usually stopped streaming. Moreover, when assayed for cGPD activity, they invariably exhibited normal levels. Therefore, certain strains used in this work must carry secondary mutations which, in particular combinations, are capable of generating a streamer-like phenotype without altering cGPD activity. The location of these mutations is unknown. However, the mutations which produce a stable streamer phenotype and reduced cGPD activity appear to map at the *stmF* locus in linkage group II.

We thank Fiona Ross and Peter Newell for kindly sending us the streamer strains and Silvia Williams and Colleen Kelly for typing the manuscript.

This work was supported by a grant from the National Sciences and Engineering Research Council of Canada.

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