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Mapping of *Myxococcus xanthus* Social Motility *dsp* Mutations to the *dif* Genes

Hope Lancero,[1] Jennifer E. Brofft,[2] John Downard,[3] Bruce W. Birren,[4] Chad Nusbaum,[4] Jerome Naylor,[4] Wenyuan Shi,[1] and Lawrence J. Shimkets[2]*

Molecular Biology Institute and School of Dentistry, University of California, Los Angeles, Los Angeles, California 90095-1668[1]; Department of Microbiology, University of Georgia, Athens, Georgia 30602-2605[2]; Department of Botany and Microbiology, University of Oklahoma, Norman, Oklahoma 73019-6131[3]; and Whitehead Institute/MIT Center for Genome Research, Cambridge, Massachusetts 02141[4]

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***Myxococcus xanthus dsp* and *dif* mutants have similar phenotypes in that they are deficient in social motility and fruiting body development. We compared the two loci by genetic mapping, complementation with a cosmid clone, DNA sequencing, and gene disruption and found that 16 of the 18 *dsp* alleles map to the *dif* genes. Another *dsp* allele contains a mutation in the *sglK* gene. About 36.6 kb around the *dsp-dif* locus was sequenced and annotated, and 50% of the genes are novel.**

Many organisms live in biofilms, where they move by surface translocation mechanisms, such as twitching or gliding (28). In *Myxococcus xanthus* there are two different motility systems for surface translocation. Cells can move as individuals using the adventurous (A) motility system (11, 12). Cells can also move in groups using the social (S) motility system, which requires cell-cell contact (3, 13). While the molecular basis of A motility is unknown, S motility, which is analogous to twitching, is dependent on retraction of type IV pili (18, 26, 30). S motility also utilizes a matrix of extracellular protein-associated polysaccharide, known as fibrils, which mediate cell cohesion (1, 2), coordinate cell movement (29), and initiate chemotactic excitation to certain phosphatidylethanolamine species (15, 16).

Physical mapping by cotransduction. One group of S motility mutants is known as *dsp* (dispersed growth) mutants, and the mutations map to a large locus near Tn*5* insertion ΩDK1407 (22, 23). The *dsp* mutants have a distinct phenotype; they lack fibrils and the ability to form fruiting bodies. One of the mutations is a Tn*5* insertion (*dsp-3119*), while the remaining mutations are point mutations generated by chemical and UV mutagenesis. The approximate size of the locus was determined by examining the cotransduction frequency of Ω1407 with each *dsp* allele using bacteriophage Mx8. Cotransduction frequency may be converted to physical distance by using the equation $C = (1 - t)^3$, where C is the cotransduction frequency and t is the fractional physical distance between the markers (27). The length of Mx8 DNA is 56 kb (17), but this length was reduced by the length of Tn*5* (6 kb) to calculate physical distance (27). The predicted physical distances of the *dsp* mutations from Ω1407 range from 1.7 to 11.0 kb for the bulk of the alleles. Allele *dsp-3119* is much farther away, and *dsp-1689* is unlinked (Table 1). These data suggest that the *dsp* locus begins approximately 1.7 kb from Ω1407 and is approximately 9.3 kb long.

Cloning and complementation. Yang et al. discovered mutants with a phenotype similar to that of *dsp* mutants (33). The locus which these authors described is called *dif* (defective in fruiting) and encodes proteins homologous to the Che chemosensory proteins of enteric bacteria. The *dif* locus is also linked to Ω1407 as determined by cotransduction, raising the possibility that *dif* genes are part of the *dsp* locus. To resolve this issue, *M. xanthus* DNA adjacent to Ω1407 was cloned by selecting for kanamycin resistance encoded by the transposon. The clone obtained (pLI209) was used to screen a cosmid library by DNA hybridization (7). A cosmid designated pREG3E1 was isolated and examined to determine whether it complemented each *dsp* allele. In these experiments the cosmid was introduced by specialized transduction with bacteriophage P1, and it integrated into the chromosome by homologous recombination with the native *dsp* locus (25). Transductants were examined for S motility (Table 1). In most cases the frequency of complementation was either 100 or 0%. In a few cases the complementation frequency was 80 to 90%, and in one case the complementation frequency was only 40% (*dsp-2106*). Incomplete complementation is due to gene conversion, which is a consequence of repair of the heteroduplex prior to replication. As a result, some transductants are homozygous merodiploids containing two copies of the mutant allele (25). The frequencies of gene conversion range from 0 to 33% in loci that have been studied in detail (19, 25). With the exception of *dsp-2106*, this range covers all of the crosses reported in Table 1. Thus, in cases in which incomplete complementation was observed, we assumed that the cosmid contained the wild-type allele. The data suggest that all of the *dsp* genes linked to Ω1407 by cotransduction were present on the cosmid. Surprisingly, the 36-kb cosmid also complemented *dsp-3119*, which appeared to be about 33 kb from Ω1407 as determined by cotransduction mapping (Table 1) and about 44 kb from Ω1407 as determined by pulsed-field gel electrophoresis (5). The cosmid did not complement allele *dsp-1689*, which showed no cotransduction with Ω1407 and therefore was assumed to be more than 39 kb from Ω1407.

* Corresponding author. Mailing address: Department of Microbiology, University of Georgia, Athens, GA 30602-2605. Phone: (706) 542-2681. Fax: (706) 542-2674. E-mail: shimkets@arches.uga.edu.

TABLE 1. Complementation and cotransduction of *dsp* mutants

dsp allele[a]	pREG3E1 complementation (no. of transductants complemented/no. of transductants tested)[b]	% Cotransduction with Tn*5* Ω1407[c]	Calculated physical distance (kb)[d]
dsp-1638	24/26	47	11.0
dsp-1639	10/10	53	9.4
dsp-1640	10/10	67	6.1
dsp-1645	10/10	52	9.7
dsp-1660	25/25	88	2.0
dsp-1662	10/10	73	4.9
dsp-1663	NT[e]	56	8.7
dsp-1664	8/10	49	10.4
dsp-1680	28/34	57	8.4
dsp-1689	0/13	<1	>39.1
dsp-1693	NT	78	3.9
dsp-1694	3/3	54	9.2
dsp-2104	10/10	47	11.0
dsp-2105	10/10	90	1.7
dsp-2106	4/10	67	6.1
dsp-2107	9/9	69	5.7
dsp-2160	8/9	88	2.0
dsp-3119	10/10	4	32.7

[a] Most of the alleles are point mutations; the only exception is *dsp-3119*, which was generated by Tn*5* insertion.

[b] pREG3E1 was transduced into each strain with bacteriophage P1, where it integrated by homologous recombination. Each transductant was examined for S motility after 5 days of incubation on Casitone-Tris (CTT) agar at 32°C.

[c] Cotransduction was measured by using bacteriophage Mx8, which contains 56 kb of DNA. Cotransduction frequency was determined by using a minimum of 30 transductants. Each transductant was examined for S motility after 5 days of incubation on CTT agar at 32°C.

[d] Fractional physical distance (t) was calculated from the cotransduction frequency (C) by using the equation $t = (1 - C^{1/3})$ and then was converted to calculated physical distance by assuming that 50 kb is available for recombination.

[e] NT, not tested.

DNA sequence analysis. The *M. xanthus* DNA in pREG3E1 is 36.6 kb long and was annotated with the aid of Artimis (The Sanger Center; http://www.sanger.ac.uk/Software/) and FramePlot 2.3.2 (National Institutes of Health; http://www.nih.go.jp/~jun/cgi-bin/frameplot.pl), which rely on third-position codon bias to identify putative protein-coding regions (14, 20). The *M. xanthus* DNA encodes 29 complete putative protein-coding regions, and the average gene density is about 0.8 kb^{-1}. DNA sequencing of pLI209 was used to identify the insertion site of Ω1407, which follows base 23065 of the insert in pREG3E1 (data not shown). The distance from the insertion to the beginning of the first gene in the *dif* cluster, *difA*, is about 3 kb, and the distance to the end of the last gene in the *dif* cluster, *difE*, is about 10 kb, suggesting that the locus is about 7 kb long. This agrees fairly well with the calculated physical distances based on the cotransduction data for *dsp* (Table 1), suggesting that the *dif* genes are at least part of the *dsp* locus. However, it is formally possible that the *dsp* point mutations and the *dif* genes are located on opposite sides of the Tn*5* insertion. On the side of Ω1407 opposite the *dif* side are several intriguing candidates for motility genes, including an *mglA* homolog and two *pilT* homologs. MglA is a GTPase that is essential for both A motility and S motility (8, 9). PilT is essential for S motility (32).

Gene disruption. Systematic gene disruption was used to determine whether *dsp*-like genes are located on the other side of the transposon insertion or anywhere else on the cosmid. Gene disruption was accomplished by integrating an internal fragment of a gene by homologous recombination into the wild-type (DK1622) genome. Each disruption mutant was confirmed Southern blotting and then examined for agglutination, fruiting body development, sporulation, and S motility, which are uniformly defective in *dsp* strains. Agglutination measures fibril-dependent cohesion and is completely absent in *dsp* strains, which lack fibrils (22). Fruiting body development and sporulation were examined following starvation. The *dsp* mutants failed to form fruiting bodies and produced only 1% of the wild-type levels of spores, as determined by direct counting (23). S motility was assayed on Casitone-yeast extract plates containing 0.3% agar, which favors movement of S^+ strains (21).

The coordinates of the fragments causing gene disruption relative to the cosmid sequence are shown in Table 2, along with the phenotypes of the disrupted mutants. Insertions in each of the *dif* genes generated a *dsp* phenotype, as expected. While the results suggest that all five *dif* genes are essential for S motility, we cannot rule out the possibility that the insertions are polar on *difE*, which is known to be essential for S motility. Disruption of the *mglA* homolog or one of the *pilT* homologs did not have a deleterious effect on motility or development. With the exception of the mutants with *dif* gene disruptions, the disruption mutants were not severely defective in development or S motility. Disruption of open reading frame 3E1-22, which encodes a putative polypeptide with 28% amino acid identity to protein alanine *N*-acetyl transferase, resulted in delayed fruiting body development but normal motility.

Cosmid pREG3E1 complements *dsp-3119*, which was generated by Tn*5* insertion Ω3119. The cotransduction data (Table 1) and physical mapping using pulsed-field gel electrophoresis accurately predicted that this gene is not represented on the cosmid despite the complementation results (5, 10). Complemented cells contain fibrils, as determined by the use of fibril-specific dyes and the agglutination assay (6). To explore this puzzle in more detail, DNA surrounding insertion Ω3119 was cloned into plasmid pLJS107 and sequenced to identify the gene containing the insertion. The insertion is located in *sglK*, a gene that has been described previously, and the phenotype of an *sglK* null mutant is similar to that of a *dsp-3119* mutant in that fibrils are not produced and fruiting body development is inhibited (31, 34). SglK belongs to the DnaK family of chaperone proteins. Although it is possible that a chaperone-like gene on pREG3E1 can substitute for *sglK* at the higher copy number of the merodiploid, none of the putative proteins encoded by the cosmid belong to the DnaK family. Fibrils do not appear to be part of the S motility motor but rather regulate motility and coordinate cell behavior (6). The restoration of both S motility and fibril formation implies that multiple copies of a gene(s) located on the cosmid bypass the mutational block imposed by Ω3119.

Features of the genome. The piece of the DNA sequenced in this study is the largest piece of DNA that has been sequenced for *M. xanthus*, which has one of the largest prokaryotic genomes (the genome of *M. xanthus* is 9.5 Mb long) (4, 24). Perhaps the most striking feature of the cosmid sequence is the number of novel genes (defined by using a cutoff value of 25% amino acid identity), which represent about one-half the total

TABLE 2. Positions of the putative protein-coding regions, their homologies, and the phenotypes of disruption mutants

Open reading frame	Position[a]	Disruption coordinates[b]	Phenotype[c]	Homologue[d]
3E1-1	377–2110	1086–1635	Normal	Unknown
		1606–1975	Normal	
3E1-2	2122–3984	3026–3565	Normal	Unknown
3E1-3	4031–5656	4756–5165	Normal	Unknown
3E1-4	5678–7564	5896–6435	Normal	Unknown
3E1-5	7649–9391	7996–8535	Normal	Unknown
3E1-6	9450–10838	9930–10439	Normal	Histidine kinase (32% identify over 246 amino acids)
3E1-7	comp. 10899–12071	11111–11650	Normal	Hypothetical protein from *Thermatoga maritima* (39% identity over 391 amino acids)
3E1-8	comp. 12077–12829	12276–12650	Normal	Phosphoribosyl amino imidizole carboxylase (46% identity over 247 amino acids)
3E1-9	comp. 12855–13361	12950–13260	Normal	Methyltransferase inhibitor (28% identity over 199 amino acids)
3E1-10	comp. 13454–16015	—[e]	*dsp*	DifE
3E1-11	comp. 16081–16449	16127–16340	*dsp*	DifD
3E1-12	comp. 16520–16942	16640–16826	*dsp*	DifC
3E1-13	comp. 16986–17723	17435–17720	*dsp*	DifB
3E1-14	comp. 17720–18961	—[e]	*dsp*	DifA
3E1-15	comp. 19162–20109	19625–20015	Normal	Thiamine monophosphate kinase (37% identity over 321 amino acids)
3E1-16	20239–21768	20396–21085	Normal	Unknown
3E1-17	comp. 21823–23190	22095–22565	Normal	Unknown
3E1-18	comp. 23288–24118	23536–23805	Normal	Unknown
3E1-19	comp. 24262–24849	24383–24682	Normal	Hypothetical protein from *Mycobacterium tuberculosis* (28% identity over 187 amino acids)
3E1-20	comp. 24919–26427	25466–25985	Normal	Histidine kinase (39% identity over 261 amino acids)
3E1-21	comp. 26424–27269	26646–27080	Normal	MglA (43% identity over 188 amino acids)
3E1-22	27360–27968	27488–27847	Delayed fruiting, not *dsp*, S^+	Protein alanine *N*-acetyl transferase (28% identity over 128 amino acids)
3E1-23	28220–29944	29177–29578	Normal	PilT (43% identity over 342 amino acids)
3E1-24	29946–31055	30465–30815	Normal	PilT (51% identity over 335 amino acids)
3E1-25	31092–31481	—[f]		Unknown
3E1-26	31515–32111	31691–31990	Normal	Unknown
3E1-27	comp. 32144–33601	32356–32895	Normal	Tripeptidyl aminopeptidase (35% identity over 430 amino acids)
3E1-28	comp. 33758–34621	33735–34385	Normal	Unknown
3E1-29	comp. 34618–36363	35476–36015	Normal	Unknown

[a] Coordinates of the putative protein-coding regions as the appear in the GenBank annotation of pREG3E1. comp., complement.
[b] 5′ and 3′ boundaries of the DNA fragment used to disrupt the protein-coding region. All fragments are internal to both ends of the putative protein-coding region and are likely to generate a gene disruption. All DNA insertions were confirmed by Southern blotting.
[c] Mutant cells were examined for fruiting body development and sporulation on MOPS (morpholinepropanesulfonic acid) medium, A motility and S motility were examined on charcoal-yeast extract medium containing 1.5 and 0.3% agar, and agglutination was examined as described by Yang et al. (33, 34). Normal means similar to the wild-type DK1622 phenotype. *dsp* means defective in fruiting body development, sporulation, swarming on 0.3% agar, social motility, and agglutination. The insertion in 3E1-22 delayed fruiting body formation (5 days instead of 24 h) but did not inhibit S motility or agglutination.
[d] Homologies were examined by using BLASTX. In the case of the DifA, DifB, DifC, DifD, and DifE proteins, the matches with known *Myxococcus* sequences were exact. For the remaining proteins, levels of amino acid identity greater than 26% are indicated, while the other levels of homology are listed as unknown.
[e] See reference 35.
[f] The sequence was too small to make an insertion.

number of genes (Table 2). All of the genes, novel or otherwise, have a third-position codon bias typical of high-G+C-content organisms and indicative of *M. xanthus*. Whether these genes were imported from other as-yet-unsequenced genomes or evolved as novel structures in *M. xanthus* should become clearer as more genome sequences are added to the database.

In conclusion, using genetic complementation, DNA sequencing, and systematic mutagenesis, we found that the *dsp* locus is composed of *dif* genes, which are the only S motility genes near Ω1407. Based on these findings, it is likely that all of the *dsp* alleles shown in Table 1 except *dsp-3119* and *dsp-1689* are mutations in *dif* genes. The *dif* genes are chemotaxis homologs located in the cytoplasm and the inner membrane, whereas fibrils are located on the cell surface. Apparently, additional genes are required for the biosynthesis, transport, and assembly of fibrils. The *sglK* gene and the gene represented by the *dsp-1689* allele may be two examples of such genes. It would be interesting to identify the gene products involved in fibril biosynthesis and to study their interaction with the Dif gene products.

The GenBank accession number of the pREG3E1 sequence is AF449411.

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