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The *tgl* Gene: Social Motility and Stimulation in *Myxococcus xanthus*

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Mutations in the *tgl* locus inactivate social gliding motility in *Myxococcus xanthus* and block production of pili. The *tgl* locus is distinctive among the genes for social motility because social gliding and pili can be restored transiently to *tgl* mutant cells by mixing them with *tgl*$^+$ cells, a process known as stimulation. The *tgl* locus was cloned with a linked insertion of transposon Tn*5* by using the kanamycin resistance encoded by that transposon. A 16-kb segment of chromosomal DNA complemented the social motility defect when introduced into *tgl* mutant cells to form a tandem duplication *tgl*$^+$/*tgl* heterozygote. To delimit the autonomous *tgl* transcription unit, subfragments of this 16-kb piece were integrated at the ectopic Mx8 prophage attachment site. A 1.7-kb DNA fragment was identified which, when integrated at the Mx8 site, simultaneously rescued social motility and pilus production. The ability to stimulate *tgl* mutants was also rescued by the 1.7-kb fragment. Because rescue of stimulation from an *mgl*-deficient donor strain which cannot swarm was observed, this demonstrates that a stimulation donor requires a *tgl*$^+$ allele but does not require the capacity to swarm actively. The nucleotide sequence of the 1.7-kb fragment revealed two protein coding regions, open reading frame A and open reading frame B (ORFB). ORFB is the *tgl* gene, because a 613-bp DNA fragment which includes 75% of ORFB rescues *tgl-1*, *-2*, and *-3* mutants and because disruption of ORFB by deletion or insertion of transposon Tn*5lac* constitutes a *tgl* mutation.

Myxococcus xanthus cells are motile by gliding, which allows the cells to spread rapidly over a surface (19, 26, 31, 32). Gliding is regulated by the products of at least 40 genes, recognized by mutations which change the rate of spreading as well as the cell arrangement at the leading edge of the spreading zone. These genes include *mglA* and *mglB* (8, 48), six *frz* loci (53), and two independent motility systems, called adventurous (A) and social (S), each consisting of many genetic loci (14, 15). Either the A system or the S system is required for spreading, though the two normally work together in wild-type cells. Each system responds to the local density of cells, but in different ways (19). Each system is independent of the other and autonomous for spreading in the sense that all A$^-$ S$^-$ mutants, no matter which A-system and which S-system mutations are combined, are nonspreading (13, 14).

Social motility is correlated with pili, long thin hairs in tufts, usually found at one pole of the cell and occasionally at both; the pili are type IV (50). No mutant has been found which lacks pili but has social motility. Adventurous motility, on the other hand, is unrelated to pili. Pili are necessary but not sufficient for social motility in that null mutants of *pilT* retain pili yet have lost social motility (51). Among the social motility genes, *pilA*, *pilB*, *pilC*, *pilD*, *pilR*, and *tgl* mutants lack pili (18, 50, 51). The *tgl* gene is unique among these genes inasmuch as the social motility and pilus defect of a *tgl* mutant can be phenotypically (but not genotypically) rescued by cell contact, a process called stimulation (15). Stimulation of *tgl* mutants can be observed by mixing *tgl* mutant cells (the recipients) with cells which contain a *tgl*$^+$ locus (the donors). It is convenient for such an experiment to work with donor and recipient strains which are nonmotile (or nonspreading), because then the stimulated cells, which transiently acquire social motility, move away from their nonmotile neighbors and are thus easily seen. In the simplest case, the nonmotile (A$^-$ S$^-$) state of the recipient is the result of combining the *tgl* mutation (which is the S$^-$ moiety of the genotype) and any *agl* mutation (which is the A$^-$ moiety). The donor must be *tgl*$^+$ but can have an A$^-$ S$^-$ phenotype as the result of any *agl* mutation (A system) and any *sgl* or *pil* mutation (S system). When donor and recipient cells are mixed and spotted on an agar surface, the spot dries with a sharp edge. After several hours of incubation, flares and rafts of cells moving with characteristic social motility emerge from the edge of the spot (15). The stimulated cells isolated from the spreading zone remain genotypically *tgl* mutants, as has been shown by genetic crosses, yet they spread with a *tgl*$^+$ phenotype; moreover, they assemble typical polar pili (18). The transient quality of stimulation is evident as the stimulated *tgl* cells move away from the donor cells and eventually lose their motility (15). Stimulation evidently occurs without the transfer of stable genetic information since the progeny of the stimulated cells lack pili and have a *tgl* mutant phenotype as well as a *tgl* mutant genotype (15).

To investigate the role of *tgl* in stimulation, social motility, and pilus production, the *tgl* gene was isolated and its sequence was studied. The accompanying paper (34) describes the Tgl protein.

MATERIALS AND METHODS

Bacterial strains, phages, and transposons. The *M. xanthus* strains used are listed in Table 1. The transposons Tn*5*, Tn*5-132*, and Tn*5lac* have been described previously (2, 22). *Escherichia coli* MC1061, JM101, JM107, and DH5α were used for the isolation and growth of plasmids (35). The λgt4Δ211 lysogen, in strain SKB2749, has been described previously (22). The coliphage P1*clr-100cam* was used for the specialized transduction of plasmids from *E. coli* to *M. xanthus* (48). The myxophages Mx4 and Mx8*cp2* were used for generalized transduction of alleles linked to transposon insertions (46).

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Bacterial growth. *M. xanthus* cells were grown in CTT broth at 32°C with gyration (390 rpm) (13). Antibiotic selections were performed on CTT solid media (1.5% Difco-Agar) containing 40 μg of kanamycin per ml or 2.5 μg of oxytetracycline per ml and overlaid 16 to 24 h later with CTT soft agar (0.7% Difco-Agar) to final concentrations of 75 μg of kanamycin per ml or 12.5 μg of oxytetracycline per ml. *E. coli* was grown in Luria-Bertani broth supplemented as needed with carbenicillin (100 μg/ml), kanamycin (50 μg/ml), tetracycline (12.5 μg/ml), or chloramphenicol (12.5 μg/ml).

Tests for social motility and stimulation. The strains used to analyze social motility contained a mutation in the A-system loci. To determine the motility phenotype, cells were spotted or streaked on CTT solid media and the colony edges were inspected (×20 to ×100 magnification) after incubation at 33°C for 1 to 5 days. S^+ colonies had spreading edges with flares of grouped cells. S^- colonies were heaped and had smooth edges. Stimulation was tested by mixing A^- *tgl* mutant recipient cells with phenotypically A^- S^- donor cells with a *tgl*$^+$ allele, as described by Hodgkin and Kaiser (13), except that cells were resuspended at 1×10^9 to 2×10^9 cells/ml.

DNA manipulation. Plasmid and DNA isolation and manipulation were performed by standard methods (35). Restriction enzymes, T4 DNA ligase, calf alkaline phosphatase, exonuclease III, and S1 nuclease were purchased from New England Biolabs or Boehringer Mannheim Biochemicals and were used in buffers similar to those recommended by the manufacturers. *E. coli* strains were made competent as described previously (35). *M. xanthus* chromosomal DNA was purified as described previously (52).

Introduction of plasmids into *M. xanthus*. Plasmids that contained the P1 incompatibility region were transferred from *E. coli* by specialized transduction by using P1*clr-100cam* as described previously (48). Phage lysates for the transduction of pJPR122 and pJPR132 were prepared by thermal induction, and the phage released was concentrated by differential centrifugation (17).

Plasmids pJPR148 and pJPR149 were introduced into *M. xanthus* by electroporation. A colony of DK1250, grown on CTT solid media for 4 to 5 days, was inoculated into 10 ml of CTT broth. Cells were grown to 50 to 80 Klett units, harvested by centrifugation (15,000 × *g*, 10 min, 4°C), and washed twice with 5 ml of ice-cold EPB (272 mM sucrose–5 mM K_2HPO_4–KH_2PO_4 [pH 7.6]–15% glycerol). After resuspension in 1 ml of EPB, the cell suspension (0.5 ml) was transferred into prechilled 1.5-ml Eppendorf tubes and mixed by gentle vortexing with 1 to 10 μg of DNA. Electroporations were performed in 0.4-cm-gap Gene-pulser cuvettes with a Gene-Pulse apparatus (Bio-Rad) set at 2,500 V and 25 μF to deliver a 6.25-kV/cm pulse. The suspension was immediately diluted into 2 ml of CTT at room temperature and plated.

In situ cloning. The method used has been described by Gill et al. (5). Integrants of pREG429 in the two IS*50* elements of Tn*5-132*Ω1935 were isolated in strain DK4162. Strain DK1935 contains Tn5Ω1935, which is 97% cotransducible with the wild-type *tgl* locus (reference 46 and data not shown). Because DK1935 was refractory to infection by P1*clr-100cam*, Tn5Ω1935 was transduced into DK4155 (*mgl-1*) by using Mx4 grown on DK1935, producing DK4160. The allelic replacement of Tn5Ω1935 with Tn*5-132* was carried out in DK4160, as described by Avery and Kaiser (2), to produce strain DK4161. To ensure the isolation of DNA from a motile strain, Tn*5-132*Ω1935 was transduced into the A^+ S^+ strain DK1622 by using Mx8*cp2* grown in DK4161, producing the fully motile strain DK4162. The chromosomal location of Tn*5* and Tn*5-132* in DK1935, DK4161, and DK4162 at the Ω1935 site was confirmed by Southern analysis, following digestion of the chromosomal DNA with *Bam*HI, *Eco*RI, and *Xba*I and probing with ColE1::Tn*5* (data not shown).

pREG429 was integrated at Ω1935 in DK4162 by selection with kanamycin, and 70 colonies obtained in two separate experiments were pooled to ensure integrants at both IS*50* elements. Chromosomal DNA purified from this pool (10 μg) was digested with *Eco*RI or *Bam*HI and ligated at low concentration (400 ng/ml, 18 h, 10°C), and kanamycin-resistant transformants were isolated in *E. coli* MC1061. Digestion with *Eco*RI produced pJPR103 and pJPR105, and digestion with *Bam*HI produced pJPR102. pJPR106 was generated by the digestion of pJPR102 (1 μg) with *Eco*RI and recircularization (100 ng/ml) to delete ~12.8 kb of DNA distal to the *Eco*RI site located 3.2 kb from the IS*50* element (see Fig. 1).

Plasmid constructions. DNA fragments isolated in *E. coli* were introduced into *M. xanthus* with the plasmids pREG422 (42), pREG429 (5), and pDAH160 (16), which integrate by homologous recombination into the *M. xanthus* chromosome. A 12.5-kb DNA region containing the Mx8 prophage attachment site (ATTP) was isolated from pBR328::Mx8ATTP (47). Site-specific integration of pLJS49 into the *M. xanthus* chromosome has been described previously (41).

The limits of the *tgl* transcription unit were experimentally defined within the 3.2-kb DNA fragment contained in plasmid pJPR106 (see Fig. 1) by fragmenting its DNA and testing the ability of the fragments to complement *tgl* mutants. A 3.7-kb fragment, consisting of 3.2 kb of chromosomal DNA and 490 bp from the adjacent IS*50* DNA, was obtained from pJPR106 by digestion with *Eco*RI and *Xho*I and ligated into the *Eco*RI and *Sal*I sites of pUC18 to produce pJPR167. Fragments were obtained from pJPR167 for introduction into *M. xanthus* in two steps. In step one, restriction fragments from the chromosomal insert in pJPR167 were ligated into sites within the multiple-cloning region of pUC18 or pEMBL19 to produce intermediate plasmids in which unique *Eco*RI and *Hin*dIII sites flanked the ends of the chromosomal region. In step two, *Eco*RI-*Hin*dIII fragments isolated from the intermediate plasmids were ligated into the same sites in pDAH160 to produce plasmids for transfer into *M. xanthus* by specialized transduction. To construct the intermediate plasmids pJPR117, pJPR118, pJPR121, and pJPR126, the following fragments were obtained from pJPR167 and ligated into pUC18, digested at the restriction sites indicated: pJPR117, a 2.5-kb *Eco*RI-*Nco*I fragment into the *Eco*RI and *Sma*I sites; pJPR118, a 1.2-kb *Pst*I fragment into the *Pst*I site; and pJPR126, a 2.2-kb *Sma*I-*Sph*I (the *Sph*I site is located in the plasmid multiple-cloning region) fragment into the *Sma*I and *Sph*I sites. To construct plasmids for specialized transduction and integration into the *M. xanthus* chromosome by homologous recombination, pJPR167, pJPR117, pJPR118, and pJPR126 were digested with *Eco*RI and *Hin*dIII to produce fragments which were ligated into pDAH160 digested with *Eco*RI and *Hin*dIII. This produced pJPR119 from pJPR117, pJPR121 from pJPR167, pJPR122 from pJPR118, and pJPR128 from pJPR126 (see Fig. 3). A 2.2-kb *Sma*I-*Hin*dIII fragment from pJPR167 was ligated into pEMBL19 digested with *Hin*dIII and *Hin*cII to produce pJPR271. This plasmid was linearized with *Bam*HI; after partial digestion with *Bal*I, a 6-kb fragment was isolated from 1% SeaPlaque agarose and recircularized to produce pJPR278. A 2.0-kb *Eco*RI-*Hin*dIII fragment from pJPR278 was ligated into pDAH160, predigested with *Eco*RI and *Hin*dIII, to produce pJPR148.

The site-specific recombination plasmids were generated as follows: pJPR121, pJPR122, and pJPR128 (parent plasmids) were digested with *Eco*RI, treated with calf alkaline phosphatase, and ligated to a 12.5-kb DNA *Eco*RI fragment containing the phage Mx8 ATTP region and isolated from pBR328::Mx8ATTP. The parent plasmids and the corresponding ATTP plasmids (pJPR124, pJPR127, and pJPR129) are listed in Table 4. To construct pJPR149, a 2.0-kb *Eco*RI-*Hin*dIII fragment from pJPR278 was ligated into the *Eco*RI and *Hin*dIII sites of pLJS49.

To obtain an internal fragment of *tgl*, pJPR118 was digested with *Hpa*I and *Sph*I, treated with exonuclease III and S1 nuclease, and recircularized (11). A transformant with ≈0.5 kb of *M. xanthus* DNA was selected (pJPR130). A 0.5-kb *Eco*RI-*Hin*dIII fragment was isolated from pJPR130 and ligated into pDAH160 to produce pJPR132. The 3.7-kb *Eco*RI-*Xho*I fragment, obtained from pJPR106, was ligated into pREG422, after digestion with *Eco*RI and *Xho*I, to produce pJPR110.

Isolation of Tn*5lac* transposon insertions. Transpositions of Tn*5lac* were isolated in the 3.2-kb *tgl*$^+$ region of pJPR110 as follows. Plasmid DNA was transformed into *E. coli* SKB2749, which contains a lambda phage lysogen that carries Tn*5lac*. Following selection with kanamycin (300 μg/ml), carbenicillin, and tetracycline, transformants from independent plates were pooled and grown overnight in Luria-Bertani broth supplemented with the same antibiotics. DNA was purified from each pool and transformed into *E. coli* MC1061 by selection with the same antibiotics. The location of the transposon was determined by restriction analysis. Forty-six strains contained independent transpositions, and two of these contained Tn*5lac* within the *M. xanthus* DNA (insertions Ω3950 in pJPR1105 and Ω3951 in pJPR1132; see Fig. 7).

DNA sequencing. Fragments for sequencing were cloned into M13mp18 or -mp19, pEMBL18 or -19 (35), or pKS (43) and sequenced directly or following the generation of a set of overlapping deletions with exonuclease III, as described previously (11). The region between nucleotide positions 1 and 661 (see Fig. 6) was sequenced from one strand by using two oligonucleotide primers complementary to cloned DNA sequences: primer 2Q3, 5′-GAAGCCCCGCACCCGT C-3′, and primer 5, 5′-CCACGAAGGCCAGCGCG-3′. Sequencing reactions were performed by the chain termination method of Sanger et al. (37). The annealing and sequencing reactions with primers 2Q3 and 5 were performed at 60°C. To resolve secondary structure, sequencing reactions were carried out with 7-deaza-2′-dGTP (28) or dITP (35) substituting for dGTP, or the reaction products were electrophoresed in gels containing 40% (vol/vol) formamide and 7.8 M urea (3).

The sequence was compiled with Intellegenetics (Stanford University) programs and analyzed with programs from Intellegenetics and the Genetics Computer Group (University of Wisconsin Biotechnology Center, Madison, Wis.).

Construction of plasmids for the expression of hybrid proteins in *E. coli*. Plasmids for the expression of hybrid proteins consisting of the N terminus of a protein determined by the plasmid and the C terminus determined by *tgl* sequences were constructed (see Fig. 7). The hybrid-protein expression plasmids pATH2, pATH11, and pATH10 (21) and pRIT21, pRIT22, and pRIT23 (obtained from A. Tzagoloff, Department of Biological Sciences, Columbia University) have been described. The reading frames of the predicted fusions are designated so that reading frame 1 (RF1) corresponds to the reading frame of Tgl protein and RF2 and RF3 correspond to the two other reading frames from this region. RF4 corresponds to the reading frame of ORFA, and RF5 corresponds to one of the two other reading frames from the ORFA region. Plasmids pJPR330, pJPR341, pJPR342, pJPR345, and pJPR335 were constructed by the ligation of a 1.1-kb *Pst*I DNA fragment, from pJPR126, encoding the C terminus of the Tgl protein, starting from the nucleotide at position 1067, into the unique *Pst*I sites of the plasmids pATH10, pATH11, pRIT21, pRIT22, and pRIT23. Plasmids pJPR351 and pJPR352 were constructed by digestion of pJPR126 with *Stu*I, producing a blunt end with Klenow, digestion with *Hin*dIII, and isolation of a 1.7-kb fragment that was ligated into pATH11 and pATH2 after they had been linearized with *Sma*I and *Hin*dIII.

Expression of fusion proteins in *E. coli*. TrpE hybrid proteins were expressed in 100-ml cultures of HB101, and inclusion bodies were partially purified as

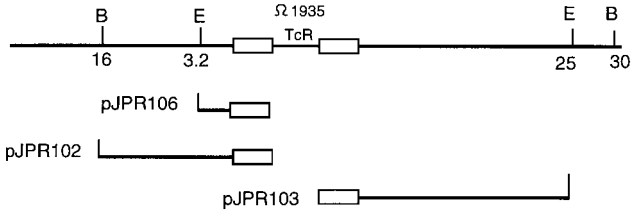

FIG. 1. Restriction map of the *M. xanthus* DNA neighboring Tn*5-132* inserted at the site Ω1935, which is closely linked to the *tgl* locus. Restriction sites flanking Ω1935 are shown above the line. The distance from the end of the transposon is given in kilobases below the line. Open boxes represent the two (oppositely oriented) IS*50* elements of Tn*5-132*. Plasmids that contain DNA segments adjacent to Ω1935 were obtained with the vector plasmid pREG429, which recombines with the IS*50* homologous sequence in the chromosome. TcR, the tetracycline resistance determinant in the internal region of the transposon. Restriction sites: B, *Bam*HI; E, *Eco*RI.

described previously (20). Secreted protein A fusions were harvested from culture supernatants as follows. Overnight cultures of DH5α cells (harboring plasmid pRIT21, pJPR330, pJPR341, or pJPR342) were diluted 100-fold and grown to an optical density at 550 nm of 0.6 to 0.8. Cells were removed by centrifugation (10,000 × *g*, 10 min, 4°C). The supernatants were filtered through 0.45-μm-pore-size filters (Syrfil-MF; Nucleopore), dialyzed in 1 liter of 20 mM Tris (pH 7.6)–5 mM EDTA–0.5 mM benzamidine-HCl–0.5 mM phenylmethylsulfonyl fluoride (16 h, 4°C) in 6- to 8-kDa molecular mass cutoff tubing (Spectrapore 1; Spectrum) and concentrated 10-fold with Centricon-10 filter units (Nucleopore).

Western immunoblot analysis. Protein concentration was determined with the microassay method of Bradford (Bio-Rad) following the manufacturer's recommendations, with bovine gamma globulin as the standard. Approximately 50 μg of protein was boiled for 5 min with the appropriate volume of 5× loading buffer and separated by electrophoresis by the system of Laemmli (23). The transfer of proteins to nitrocellulose, reaction with antibodies, and visualization of enzymatic activity have been described previously (8). The rabbit anti-TrpE antiserum was a gift of W. Talbot and was diluted to 1:2,000. To identify protein A fusions, an anti-β-galactosidase rabbit antibody (Cappel) was diluted to 1:3,000. Goat anti-rabbit antibody conjugated to alkaline phosphatase (Bio-Rad) was used at dilutions of 1:500 to 1:2,000. Alkaline phosphatase activity was visualized with nitroblue tetrazolium (U.S. Biochemicals) and 5-bromo-4-chloro-3-indolyl phosphate (U.S. Biochemicals) following the manufacturer's recommendations.

Nucleotide sequence accession number. The nucleotide sequence of the *tgl* transcription unit has been deposited in GenBank under accession no. U77058.

RESULTS

In situ cloning of the *tgl* locus. Three independent *tgl* mutants, isolated following exposure to UV light, had been mapped to a single locus 97% cotransducible with the transposon Tn*5* insertion Ω1935 (15, 46). The Ω1935 site is outside the *tgl* locus because this Tn*5* insertion itself has no effect on social motility. The recombination frequencies observed suggested the map order *rif*, *tgl*, Tn*5*Ω1935, but the tight linkage between *tgl* and Ω1935 failed to exclude the order *rif*, Tn*5*Ω1935, *tgl*. After confirming the cotransduction of *tgl* with Ω1935, we cut the chromosomal DNA of strains DK1935 and DK4161, which harbor Ω1935 insertions of the transposons Tn*5* or Tn*5-132*, with restriction endonucleases and mapped the neighborhood of Ω1935 with the aid of a Tn*5* DNA probe. *Eco*RI sites were found at 3.2 and 25 kb, and *Bam*HI sites were found at 16 kb and 30 kb on opposite sides of insertion Ω1935 (Fig. 1). Southern hybridization of large chromosomal fragments generated in yeast artificial chromosomes by pJPR167 had shown that *tgl* resides within an approximately 30-kb *Eco*RI fragment (10), in agreement with the mapping around Ω1935. Recent physical mapping has shown that this *Eco*RI fragment is distant from the other genes that are necessary for pilus formation (10, 50). The chromosomal regions to the left and right of insertion Ω1935 were cloned in situ by integration of the vector pREG429 (5). This plasmid vector can be selected by the kanamycin phosphotransferase (Km^r) gene which it carries. When this vector is introduced into *M. xanthus* cells that have Tn*5-132* at Ω1935 (the Tc^r strain DK4162) (Table 1) and these cells are plated on kanamycin agar, recombinants are selected in which the cloning vector pREG429 has integrated by homologous recombination at one or the other of the IS*50* elements of the chromosomal resident Tn*5-132*. DNA isolated from these Km^r P1 transductants was digested with restriction enzymes to produce fragments containing the complete vector and a region of chromosomal DNA adjacent to the transposon insertion.

The two ends of each restriction fragment, having been cut by the same restriction endonuclease, spontaneously join when the DNA is annealed at low concentration. Following ligation, circular plasmid DNAs with their integrated chromosomal DNA fragments are selected by transformation of *E. coli*. Because the integration of pREG429 occurs at either of the two oppositely oriented IS*50* elements at the ends of the transpo-

TABLE 1. *M. xanthus* strains

Strain	Motility genotype	Tn*5* element[a]	Reference or source
DK357	*cglB13 sglA1*		14
DK1218	*cglB2*		15
DK1250	*aglB1 tgl-1*		15
DK1251	*aglJ1 tgl-2*		15
DK1252	*cglF1 tgl-3*		15
DK1622			19
DK1935	*cglF1*	Ω1935 (Tn*5*)	46
DK3642	*mgl-7*	Ω1942 (Tn*5-132*)	K. Stephens
DK4155	*mgl-7*		48
DK4160	*mgl-7*	Ω1935 (Tn*5*)	Mx4(DK1935)xDK4155 (select Km^r)[c]
DK4161	*mgl-1*	Ω1935 (Tn*5-132*)	P1::Tn*5-132*xDK4160 (select Tc^r, screen Km^s)[d]
DK4162		Ω1935 (Tn*5-132*)	Mx8(DK4161)xDK1622 (select Km^r)[c]
DK3915	*aglB1 tgl-1*/ *tgl*$^{+b}$		P1(pJPR167)xDK1250 (select Km^r, screen S^+)[e]
DK3916	*aglJ1 tgl-2*/ *tgl*$^{+b}$		P1(pJPR167)xDK1251 (select Km^r, screen S^+)[e]
DK3917	*cglF1 tgl-3*/ *tgl*$^{+b}$		P1(pJPR167)xDK1252 (select Km^r, screen S^+)[e]
DK3922	*aglB1 mgl-7 tgl-1*/*tgl*$^{+b}$		Mx4(DK3642)xDK3915 (select Km^r, screen S^-)[c]
DK3952	*cglB2 tgl*Ω3950	Ω3950 (Tn*5lac*)	P1(pJPR1105)xDK1218 (select Km^r, screen S^-)[e]

[a] The chromosomal position of transposon insertions is given by Ω followed by an assigned number. The transposon at these positions is shown in parentheses. Tn*5* encodes for kanamycin resistance, and in its derivative, Tn*5-132*, the kanamycin resistance determinant has been replaced by the tetracycline resistance determinant from the transposon Tn*10* (2). Tn*5lac* has been described previously (22). The locations of all transposons at Ω1935 and Ω3950 were confirmed by Southern analysis (data not shown).

[b] Presumed genotype of recipients of cloned DNA fragments. The *tgl* mutant allele harbored by the parent strain is shown at the left of the slash and the wild-type allele carried by the cloned DNA fragment is shown at the right.

[c] The indicated phages were grown in the strain shown in parentheses for transduction into the strain that follows the x. Km^r, kanamycin resistance; Km^s, kanamycin sensitivity.

[d] The use of P1::Tn*5-132* for the allelic replacement of Km^r in Tn*5* by Tc^r (tetracycline resistance) has been described previously (2).

[e] P1*clr-100cm* lysogens were induced in *E. coli* strain MC1061 carrying the plasmid shown in parentheses for the specialized transduction of the plasmid in the *M. xanthus* strain which follows the x.

TABLE 2. Rescue of *tgl* mutants by chromosomal DNA fragments adjacent to Tn*5*Ω1935

Plasmid	% Km^r recipients regaining social motility (n)[a]		
	DK1250	DK1251	DK1252
pJPR102	95 (63)	91 (23)	94 (19)
pJPR106	96 (192)	93 (32)	96 (139)
pJPR103	0 (42)	0 (18)	n.t.

[a] Percentage of Km^r transductants rescued by plasmids carrying the chromosomal DNA fragments shown in Fig. 1. The plasmids integrate into the *M. xanthus* chromosome by homologous recombination. The A$^-$ *tgl* mutant recipient strains are DK1250 (*aglB1 tgl-1*), DK1251 (*aglJ1 tgl-2*), and DK1252 (*cglF1 tgl-3*). The total number of Km^r recombinants tested is shown in parentheses after the frequency. n.t., not tested.

son, both chromosomal regions flanking the transposon insertion are isolated and appear in different *E. coli* transformants. To ensure that both sides were isolated, 70 independent transformants were pooled. From this pool of transformed *E. coli*, plasmid pJPR102, with a unique *Bam*HI site and 16 kb of *Myxococcus* DNA, was obtained from the left of Ω1935. From the right, pJPR103, with 25 kb of *Myxococcus* DNA and a unique *Eco*RI site, was obtained. The relationship of the cloned DNA fragments to Ω1935 is shown in Fig. 1. In addition the pool yielded a second plasmid from the left of Ω1935, with 3.2 kb of *Myxococcus* DNA and a unique *Eco*RI site. However, this initial plasmid from the pool had lost a segment of the vector, and for subsequent work it was replaced by pJPR106, which has the complete vector, including its *Eco*RI site. pJPR106 is shown in Fig. 1.

The S$^+$ phenotype of merodiploids generated by the construction of tandem duplications had shown that *tgl*$^+$ is dominant over the *tgl* mutation (1). Therefore, after introduction into *M. xanthus* and formation of tandem duplications, plasmids carrying *tgl*$^+$ DNA should restore normal social motility to *tgl-1*, *tgl-2*, and *tgl-3* strains (DK1250, DK1251, and DK1252). By this test, the two plasmids pJPR102 and pJPR106, generated from the same region to the left of Ω1935 (Fig. 1), carry *tgl*$^+$. All three *tgl* mutant alleles are rescued by pJPR102 and pJPR106 (Table 2). Rescue of the capacity to spread by pJPR106 is illustrated in the photographs of swarm edges (Fig. 2). The plasmid, pJPR103, containing DNA from the opposite side of Ω1935 failed to rescue these *tgl* mutants (Table 2).

Plasmids containing subfragments of the 3.2-kb region of *Myxococcus* DNA cloned in pJPR106 were generated by means of the restriction sites shown in Fig. 3. The wild-type alleles of *tgl-1*, *tgl-2*, and *tgl-3* were mapped by rescue to the 0.6 kb of *M. xanthus* DNA immediately to the left of Ω1935 (Table 3). The site of the *tgl-1* mutation is probably close to the *Nco*I site at position 0.5 because pJPR119 yielded no S$^+$ rescue among the 67 Km^r transductants of the *tgl-1* recipient examined, although pJPR122 yielded 55% (Table 3). Moreover, the frequency of rescue of *tgl-1* by pJPR122 is significantly less than that of *tgl-2* or *tgl-3*, which may be due to the generation of more *tgl-1*/*tgl-1* gene conversion homozygous tandem duplications for the site

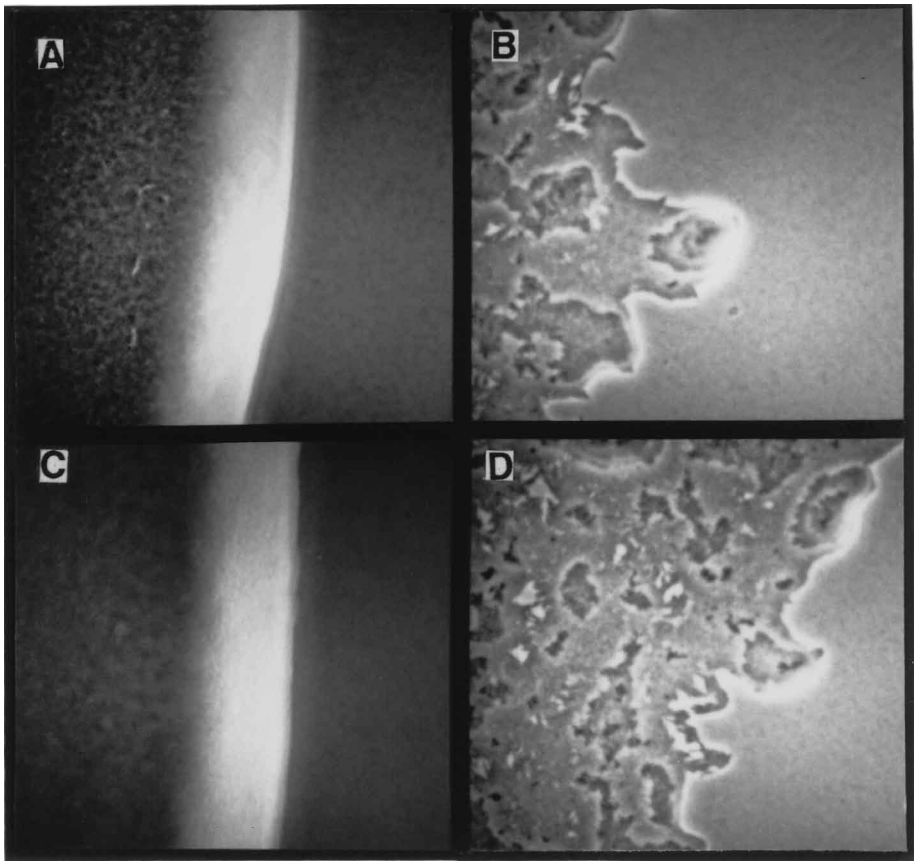

FIG. 2. Rescue of social motility in *tgl* mutants by cloned DNA fragments. DK1250 (*aglB1 tgl-1*) (A) and DK1251 (*aglJ1 tgl-2*) (C) colonies have smooth edges produced by A$^-$ S$^-$ cells. Introduction of pJPR106, carrying a *tgl*$^+$ allele, into DK3915 (*aglB1 tgl-1*/*tgl*$^+$) (B) and DK3916 (*aglJ1 tgl-2*/*tgl*$^+$) (D) results in the outward spreading of peninsulas typical of A$^-$ S$^+$ strains.

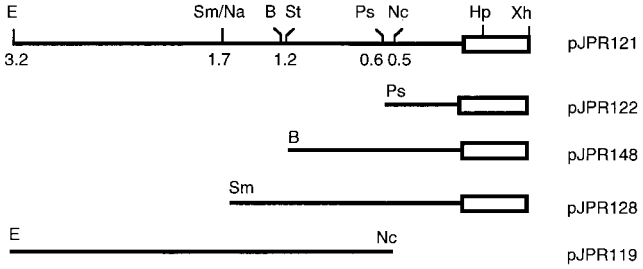

FIG. 3. Subclones generated from the 3.2-kb *tgl*$^+$ fragment. pJPR106 (Fig. 1) was the source of the DNA fragments used to produce the plasmids listed on the right. pJPR121 contains a DNA fragment from the *M. xanthus* chromosome identical to the one present in pJPR106. The isolation of fragments and the generation of intermediate plasmids is detailed in Materials and Methods. Restriction sites are given above the line. The distances in kilobases from the end of the adjacent IS*50* of Tn5Ω1935 are shown below the line. Restriction sites: B, *Bal*I; Hp, *Hpa*I; Nc, *Nco*I; Na, *Nar*I; Ps, *Pst*I; E, *Eco*RI; Sm, *Sma*I; St, *Stu*I; Xh, *Xho*I. (Two additional *Sma*I sites are present between the *Eco*RI and *Sma*I sites shown.)

nearest the end of the fragment. Gene conversion is often associated with such recombination events. Stephens and Kaiser (48) have documented frequent gene conversion in similar crosses involving the *mgl* locus by isolating separately both recombinant copies from a tandem duplication.

Restoration of social motility to *tgl* mutants also restores donor capacity for stimulation. As illustrated by *tgl-1*, *tgl-2*, and *tgl-3* mutants, a mutation in the *tgl* locus simultaneously inactivates social motility, destroys the ability to stimulate another *tgl* mutant, and creates a strain that can be stimulated (15). If stimulation is an inherent function of the product of the *tgl* gene, as this suggests, a cloned DNA fragment which restores social motility to *tgl* mutants is also expected to restore the capacity to stimulate *tgl* mutant cells. To test the stimulation activity of the cells which have become S$^+$ after the integration of *tgl*$^+$ plasmids, it is necessary to mix these cells with *tgl* mutant cells. The technical difficulty of visually observing motility stimulation of *tgl* mutant cells by motile donor cells was overcome by the introduction of an *mgl* allele to render the donor nonspreading. Mutations in *mgl*, which encodes a small ras-like protein (8, 48), are known to be epistatic to mutations in the A and S systems, producing in both cases a sharp, nonspreading colony edge resembling that of an A$^-$ S$^-$ strain, like those shown in Fig. 2A and C (15). An *mgl* mutation linked to Tn*5-132* insertion Ω1942 was transduced into strain DK3915 (*aglB1 tgl-1*/*tgl*$^+$) to produce strain DK3922 (*mgl-7 aglB1 tgl-1*/*tgl*$^+$), as described by Stephens and Kaiser (48). Even though an *mgl* strain fails to spread, it can stimulate the socially motile spreading of a *tgl* mutant recipient strain, provided that *tgl*$^+$ is present in the *mgl* donor (15). When DK3922 was mixed with DK1250 or DK1251, both of which are A$^-$ *tgl* mutants, social motility was observed at the edges of the cell mixture, as illustrated for DK1250 in Fig. 4C. Figure 4A and B show the stimulation recipient and donor strains, respectively. Rescue of the capacity for stimulation of a *tgl* mutant recipient was observed for all four independent S$^+$ recombinants tested.

The unit of *tgl* gene expression. The observed 97% cotransduction between *tgl* and Ω1935 predicted a short physical distance between these markers (46). It is possible, therefore, that a complete unit of *tgl* gene expression lies within the 3.2-kb DNA fragment to the left of Ω1935 (Fig. 3). To define the limits of the *tgl* transcription unit experimentally, the ability of subclones drawn from the 3.2-kb DNA fragment to complement *tgl* mutants (in the sense of providing an active *tgl* gene product) was tested. To ensure that the cloned DNA segment complements, the plasmid was integrated by site-specific recombination at the prophage Mx8 attachment site, thus preventing gene reconstruction, which occurs when homologous recombination generates a tandem duplication at the chromosomal locus. Gene reconstruction is shown in Fig. 5A, which illustrates how a cloned segment lacking an essential 5′ element can regain that element by recombination. A 12.5-kb DNA fragment from the myxophage Mx8 is sufficient to direct site-specific recombination to the Mx8 prophage attachment site (47). This fragment includes the Mx8 DNA segment (ATTP) that recognizes a corresponding segment (ATTB) in the chromosome of *M. xanthus* and also encodes the ATT-specific recombination enzyme(s) necessary to bring about reciprocal recombination between ATTP and ATTB. The plasmids listed in Table 4, whose structures are related to the cloned segments (parent plasmids) shown in Fig. 3, were tested. As shown in Table 4, plasmids pJPR127 and pJPR129 rescue *tgl*$^+$ expression when they are integrated at the Mx8 prophage site. Therefore, a functional *tgl* transcription unit lies within the 1.7 kb-DNA fragment which both pJPR127 and pJPR129 carry and which is adjacent to the Ω1935 insertion site.

In four instances rescue from pJPR124, which carries the 0.6-kb fragment of pJPR122, was observed. This fragment lacks part of the *tgl* gene (see below), but even though a site-specific recombination system is provided in these plasmids, a low frequency of recombination with the chromosomal *tgl* locus through their homologous sequences might be expected. These 4 recombinants, 4 of the 253 total Km^r recombinants tested for all three alleles taken together, are thought to represent events like those depicted in Fig. 5A. This point is taken up in the Discussion.

Nucleotide sequence of *tgl*. The nucleotide sequence of the 1.7-kb DNA fragment that contains the *tgl* transcription unit is shown in Fig. 6. The sequence of IS*50* would begin at nucleotide 1680. Though not shown in Fig. 6, the region was sequenced several times through the IS*50* to locate *tgl* precisely with respect to Tn*5*. Potential translation start and stop codons and the expected strong guanosine-plus-cytosine (G+C) bias for third positions in each codon were used to identify the significant open reading frames in this region. The genetic code limits the nucleotides allowed in the first and second position to a greater extent than those allowed in the third position. Therefore, in G+C-rich organisms, as demonstrated by Bibb et al. (4), the third position and to some extent the first position is biased toward G or C in order to accommodate a high overall G+C content. This approach has been successful in identifying *M. xanthus* open reading frames (40). In *M. xanthus*, with a total genomic G+C content of 67%, a strong third position bias is expected. By using the expected codon

TABLE 3. Rescue of *tgl* mutants by subfragments of the *tgl*$^+$ region

Plasmid	% Recipients regaining social motility (*n*)[a]		
	DK1250	DK1251	DK1252
pJPR121	81 (192)	91 (109)	100 (59)
pJPR128	83 (82)	100 (36)	98 (115)
pJPR122	55 (324)	78 (90)	77 (53)
pJPR148	62 (84)	n.t.	n.t.
pJPR119	<1 (67)	n.t.	n.t.

[a] Frequency of rescue by plasmids carrying the DNA fragments shown in Fig. 3 after integration by homologous recombination into the *M. xanthus* chromosome. Plasmids were introduced into A$^-$ *tgl* mutant strains DK1250 (*aglB1 tgl-1*), DK1251 (*aglJ1 tgl-2*), and DK1252 (*cglF1 tgl-3*). The total number of recombinants analyzed is shown in parentheses. n.t., not tested.

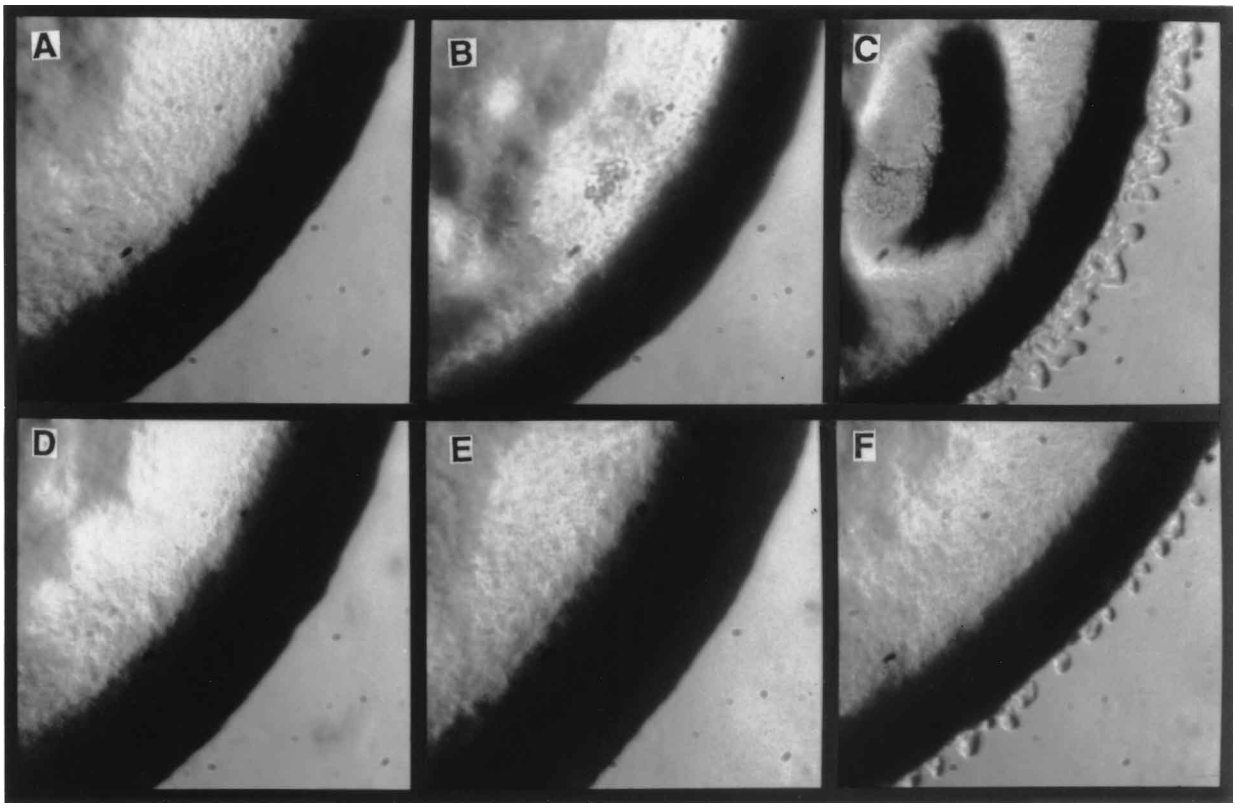

FIG. 4. Rescue of the capacity to be a stimulation donor. Cells of the A^- S^- strains DK1250 (*aglB1 tgl-1*) (A), DK3922 (*aglB1 mgl-7 tgl-1/tgl*$^+$) (B), DK357 (*cglB13 sglA1*) (D), and DK3952 (*cglB2 tgl*Ω3950) (E) have smooth edges. Mixing DK1250 and DK3922 produces flares of socially motile DK1250 cells (C). Motility of DK3952 results from mixing with DK357 (F). (Since DK3922 and DK1250 are both *aglB1* mutants, no stimulation of adventurous motility can occur. Since both DK357 and DK3952 are *cglB* mutants, there is no stimulation of adventurous motility.)

bias, two adjacent open reading frames (ORFA and ORFB) were evident within the sequence shown in Fig. 6. Their observed G+C contents are 92 and 90%, respectively, corresponding with an average of 91% for other known *M. xanthus* genes (40).

The amino acid sequences for ORFA and ORFB, predicted from the nucleotide sequence, are also shown in Fig. 6 (transcription occurs from left to right). ORFA starts with a GUG codon at nucleotide position 399 preceded by a potential Shine-Delgarno sequence (GGAG) at nucleotide positions 389 to 392 (underlined in Fig. 6). This open reading frame is predicted to encode a protein of 155 amino acids with a mass of 16,952 Da. ORFB starts with an AUG codon at nucleotide position 904 and is predicted to encode a protein of 241 amino acids with a mass of 26,921 Da. The start codon is preceded at positions 890 to 894 with the nucleotides GAGAG, which may serve as a Shine-Delgarno sequence for the initiation of translation.

Fusion proteins consisting of N termini from the TrpE protein of *E. coli* or protein A from *Staphylococcus aureus* and carboxy termini from the ORFA and ORFB proteins were constructed to confirm the nucleotide sequence shown (Fig. 7). Stop codons predicted in the three reading frames of ORFB and two reading frames of ORFA were read from the nucleotide sequence. Four plasmids were constructed with the TrpE gene, two from the ORFB region, pJPR345 (encoding TrpE-ORFB) and pJPR335 (TrpE-RF2), and two from the ORFA region, pJPR351 (TrpE-ORFA) and pJPR352 (TrpE-RF5). In the case of pJPR345, 20 kDa from the predicted Tgl protein were fused to TrpE to produce a hybrid protein with a predicted mass of 57 kDa. In the case of pJPR335, the introduction of the ORFB DNA region resulted in the deletion of 11 amino acids from the TrpE protein encoded by pATH10 and the addition of three amino acids. A fusion protein of 36 kDa was predicted; one of 37 kDa was observed. Fusions to the N terminus of protein A were constructed with the three reading frames within the ORFB coding region in the plasmids pRIT21, -22, and -23. In the case of pJPR330, the hybrid protein was predicted to consist of 33 kDa of protein A and 20 kDa of the ORFB protein; a protein of 54 kDa was observed. In the cases of pJPR341 and pJPR342, fusions were constructed of protein A and the proteins encoded by the two other reading frames. Proteins detected by binding of TrpE or β-galactosidase antibody were observed in all cases. The migrations of these proteins in sodium dodecyl sulfate-polyacrylamide gels matched within 3% those predicted from the nucleotide sequences (Fig. 6).

The pattern of complementation by plasmids integrated at the Mx8 prophage attachment site (Table 4) implied that the chromosomal *Pst*I site (between nucleotides 1067 and 1072 in Fig. 6) lies within the *tgl* transcription unit, since pJPR129 was able but pJPR124 was unable to rescue *tgl-1* or *tgl-3* mutants. Moreover, this *Pst*I site is located within ORFB (Fig. 6). To test whether ORFB is the *tgl* gene, it was disrupted while leaving ORFA intact. Integration by homologous recombination of a plasmid carrying a DNA fragment in which both ends of the gene are truncated should result in the inactivation of both copies of the gene in a tandem duplication. This is illustrated in Fig. 5D, which shows the left copy of the gene with a 3′ deletion and the right copy with a 5′ deletion. Starting with

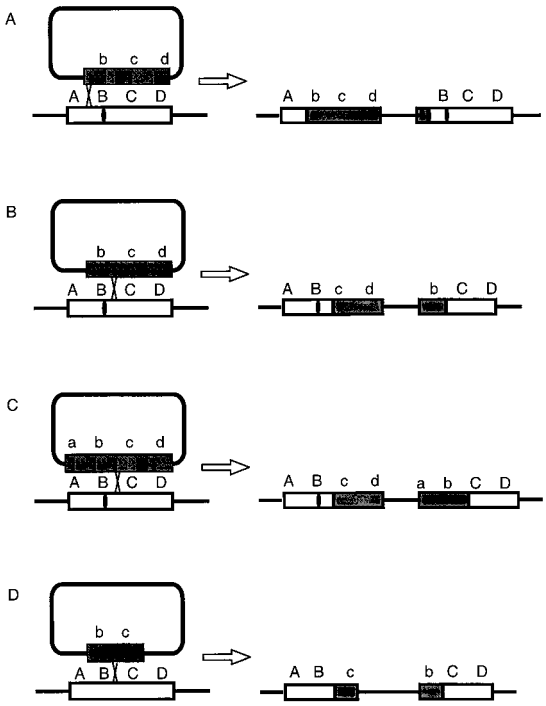

FIG. 5. Integration of DNA fragments carried by plasmids into the chromosome by homologous recombination. The recombination events are described in the text. The open box represents a transcription unit in the chromosome (straight line) with a promoter that produces transcription left to right. The unit consists of four domains, A through D. A mutation in domain B is represented as a black oval. DNA fragments from the transcription units carried by the plasmids are represented as shaded boxes. The domains present in these units are designated a through d. Homologous recombination results in the formation of tandem duplications with the structures shown at the right.

the 0.6-kb DNA *Pst*I fragment, which fails to rescue when integrated at the ATTB site, a 100-bp deletion of *tgl* DNA adjacent to IS*50* was generated by unidirectional deletion with exonuclease III from an *Hpa*I site within the IS*50* DNA. The resulting 500-bp fragment from the *tgl* region was transferred into pDAH160 to generate pJPR132 (Fig. 7) for introduction into *M. xanthus*. All 16 Km^r transformants of pJPR132 into DK1218 lacked social motility (Table 5).

Two insertions of Tn*5lac* were isolated in the 3.2-kb DNA fragment which had been cloned in pJPR106 and pJPR121. Restriction mapping of the plasmids pJPR1105 and pJPR1132 showed that Ω3950 is adjacent to the *Pst*I site within the *tgl* gene and Ω3951 is near the *Eco*RI site that defines the left end of the 3.2-kb fragment (Fig. 7). These two plasmids were introduced into the *tgl* mutant DK1250 to assess the complementation activity of the tgl^+ DNA fragment containing an insertion. While pJPR1132 was able to rescue the *tgl-1* mutant DK1250, pJPR1105 was not (Table 5), implying that it had inactivated *tgl*. Selection for the kanamycin resistance encoded by Tn*5lac* in these experiments ordinarily provides an integration via a single homologous recombination event. However, the less frequent double crossover, with one recombination event on each side of the insertion, provides a gene replacement. To select gene replacements of Ω3950 in the *tgl* region, pJPR1105 was introduced into the $A^- S^+$ strain DK1218 and Km^r transductants were scored visually for the loss of social motility. Among the 40 Km^r transductants obtained, two were $A^- S^-$. As shown in Fig. 4, DK3952, which carries Tn*5lac* at Ω3950, was stimulated when mixed with the tgl^+ $A^- S^-$ strain DK357 (tgl^+ *cglB1 sglA1*), thus confirming that its S^- mutation was the *tgl* mutation (Fig. 4). The transposon insertion Ω3950 was restriction mapped to the interval from bp 1100 to 1300 shown in Fig. 6.

Tgl protein contains TPR units. A search of protein databases with BLAST, BLITZ, and FASTA algorithms revealed regions of significant similarities between the amino acid sequence of the *tgl* gene (ORFB) and the tetratrico peptide repeat (TPR) units in the *Pichia pastoris* peroxisomal targeting signal receptor protein (PAS8) (27), the TPRs of the *Saccharomyces cerevisiae* heat shock protein STI1 (29), and the TPRs of the *S. cerevisiae* PAS10 protein (49). Visual scrutiny of the Tgl protein sequence uncovered six potential TPR units oriented in tandem repeats within the internal residues 32 to 239 (Fig. 8). An alignment of the TPR units from published sequences of seven proteins, SSN6 (39), CDC23 (44), $nuc2^+$ (12), PRP6 (25), MAS70 (9), PAS8 (27), and PAS10 (49), revealed a (degenerate) consensus at 11 positions of amino acid identities or conservative substitutions among this set of reference proteins (Fig. 8). Five of the Tgl repeats have matches of at least six amino acid residues in this consensus; the last repeat in the group has four matches. Among the six Tgl TPRs, at least three matches are present at 8 of the 11 consensus positions. Beyond the matches of Tgl with the (eukaryotic) TPR consensus, there are conserved residues specific to the TPR of Tgl. By using the criterion that at least three of the six Tgl TPRs must match, homology is identified at 10 additional positions (Fig. 8). Over all, an average of 18 matches are found, comparing the six TPR units of Tgl with the consensus of published TPR sequences and with the conserved positions within the six Tgl TPR units.

DISCUSSION

This work describes the identification and isolation of a 1.7-kb DNA fragment that contains an autonomous *tgl* transcription unit. DNA fragments from the chromosomal region of the *tgl* locus were cloned into plasmids that replicate in *E. coli* and could be introduced into *M. xanthus*. Plasmids that carry tgl^+ were identified by rescue of *tgl* mutants after integration into the *M. xanthus* chromosome by homologous recombination to form tandem duplications. The *tgl* mutant phenotype includes both a loss of social motility and a loss of the capacity to stimulate other *tgl* mutant cells (15). Moreover, strains whose social motility had been rescued also regained the capacity to stimulate *tgl* mutant cells. The tgl^+ DNA simul-

TABLE 4. Rescue of *tgl* mutants by plasmids that integrate by site-specific recombination into the *M. xanthus* chromosome

ATTP plasmid (parent)	% Recipients regaining social motility (*n*)[a]		
	DK1250	DK1251	DK1252
pJPR127 (pJPR121)[b]	100 (140)	100 (96)	100 (132)
pJPR129 (pJPR128)	100 (96)	100 (2)	100 (192)
pJPR124 (pJPR122)	<1 (76)	4 (71)	<1 (106)
pJPR149 (pJPR148)	0 (24)	n.t.	n.t.

[a] The DNA fragments from the *tgl* region carried by the parent plasmids are shown in Fig. 3, and the rescue frequencies for the parent plasmids after homologous integration are shown in Table 3. The ATTP plasmids contain equivalent DNA fragments and a segment of the phage Mx8 DNA that mediates site-specific recombination into the prophage attachment site in the *M. xanthus* chromosome. Frequencies and recipient strains are as described in Table 3. n.t., not tested.

[b] The DNA fragment from the *M. xanthus* chromosome contained in pJPR121 is identical to the one present in pJPR106.

```
   1 cccgggcgcctc  acccagcgctac  tggctggtgacg  gagcagacggtg  ccggggatgacg  gggtacgacatc  gacgtgttcatc  aattccacgtgg  96
  97 ctgcgcaagctg  cgaggcaacgag  gaccaggtggtg  gtggacatcacc  cgcaacctgcgg  ccgggggcgaac  aaggtgacgttc  atcgcccggaag 192
 193 ggaaacgccggg  gacgcgcgcagc  agttcgcctgcc  cacgttgtttcg  ggtcatattggc  gagggcaacgag  ggcggcgggaac  gtgatgatcgac 288
 289 aatcccatcctt  cgcttccagaag  acggcggcggac  acgcaggatgcg  acacaggagttc  accctcaccacg  cgctgacgggtg  cggggcttcggg 384
                       1                            10                            20
                     M  F  N  L    D  E  R  Y    R  G  L  P    A  T  R  E    Q  I  L  A    L  H  T  S    L  N  A  P
 385 taacggagacaa  ccgtgttcaacc  tcgacgagcgct  accgcgggctgc  ccgccacgcggg  aacaaatcctgg  cgctgcatacct  ccctcaacgcgc 480
          30                           40                            50                            60
       H  V  A  I    P  G  K  Q    A  G  P  A    Q  A  F  V    V  G  L  R    G  G  Q  G    T  A  A  V    F  V  Y  L
 481 cgcacgtggcca  ttccgggcaagc  aggccggtccgg  cgcaggcctttg  tcgtgggcctcc  ggggagggcagg  ggaccgcggccg  tcttcgtgtacc 576
                       70                            80                            90
       Y  L  A  E    A  A  D  C    A  V  Y  L    S  G  R  R    N  V  S  A    D  E  Y  R    D  D  E  G    D  A  L  A
 577 tgtacctggcgg  aggccgcggact  gcgcggtgtacc  tgtccggccggc  gcaacgtgtcag  cggatgagtacc  gcgacgacgagg  gggacgcgctgg 672
                      100                           110                           120
       F  V  E  S    L  G  F  M    M  D  D  A    N  W  R  A    M  A  P  E    Q  Q  D  E    Q  L  K  T    L  P  V  F
 673 ccttcgtggagt  cgctcgggttca  tgatggacgacg  ccaactggcgcg  cgatggcccccg  agcagcaggacg  agcagctcaaga  cgttgccggtgt 768
                      130                           140                           150
       F  K  D  P    T  L  V  P    A  V  V  A    R  A  E  E    K  K  N  V    T  T  T  L    G  R  F  L    A  A  F  *
 769 tcttcaaggacc  cgacgctcgtgc  ccgctgtggtgg  cgcgcgccgagg  agaagaagaacg  tcaccaccaccc  tgggccgcttcc  tggccgccttct 864
                                                    1                             10
                                                  M  F  R    L  S  T  A    S  C  S  L    A  L  L  L    V  S  S  G
 865 gagtcaccgccc  cctcaccctcgt  cgagagccgctc  cccatgttccgc  ctttccaccgcg  tcctgttcgctc  gcgctgctgctg  gtgtcctccggt 960
       20                            30                            40                            50
       C  S  H  T    P  T  E  K    E  K  R  S    A  E  I  H    Y  D  L  A    L  Q  A  Q    Q  A  G  E    L  Q  D  A
 961 tgctcccacacg  cccacggagaag  gagaagcggagc  gccgagattcac  tacgacctggcg  ttgcaggcccag  caggccggcgag  ctccaggatgcg 1056
                       60                            70                            80
       L  R  E  L    Q  V  S  L    K  N  D  P    D  Y  P  D    A  N  N  A    M  G  I  L    L  H  L  A    F  R  R  P
1057 ctgcgcgagctg  caggtgtcgctg  aagaacgacccg  gactaccccgac  gcgaacaacgcc  atgggcatcctg  ctgcacctggcg  tttcgccgtccc 1152
                       90                           100                           110
       D  E  A  V    K  H  Y  T    K  A  L  E    V  R  P  D    F  S  E  A    R  T  N  L    A  N  V  H    L  D  Q  G
1153 gacgaggccgtc  aagcactacacc  aaggcactggag  gttcgccccgac  ttctccgaggcg  cgcaccaacctc  gccaacgtgcac  ctggaccagggc 1248
                      120                           130                           140
       R  Y  D  D    A  I  K  L    Y  E  L  V    L  N  D  M    L  Y  P  T    P  F  I  A    Q  G  N  L    G  W  A  Y
1249 cgctacgacgac  gccatcaagctc  tatgagctggtc  ctcaatgacatg  ctctacccgacg  cccttcatcgcc  cagggaaacctg  gggtgggcgtac 1344
          150                           160                           170
       Y  K  K  G    E  P  D  R    A  V  E  S    I  K  A  A    V  T  T  N    P  N  F  C    L  G  Y  K    N  L  G  L
1345 tacaagaagggc  gagccggaccgc  gcggtggagagc  atcaaggccgcg  gtgacgaccaac  cccaacttctgt  ttgggctacaag  aacctggggctc 1440
       180                           190                           200                           210
       I  Y  D  E    T  G  R  T    S  E  A  C    R  Q  F  T    H  Y  R  E    N  C  P  D    V  A  E  A    Y  M  R  E
1441 atctacgacgag  acggggcgcacc  tccgaggcgtgc  cgccagttcacg  cactaccgcgag  aattgcccggac  gtggcggaagcg  tacatgcgtgag 1536
                      220                           230                           240
       G  V  C  Q    A  K  L  G    Q  V  D  A    A  K  A  A    F  A  P  V    R  P  R  R    K  L  V  N    R  C  *
1537 ggcgtctgccag  gcgaagctgggg  caggtcgatgcg  gcgaaggcagcc  ttcgcacctgtg  agaccaaggcga  aagctggtgaac  aggtgctgaagg 1632

1633 atgactgccgga  ggctgctggaaa  agctctagcgcg  ttgggtatctg. 1679
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FIG. 6. Nucleotide sequence of the *tgl* transcription unit and its neighborhood. Transcription is predicted to proceed from left to right (see text). The nucleotide at position 1679 corresponds to the nucleotide adjacent to IS*50* from Ω1935, which starts at position 1680 (sequence not shown). Potential ribosomal binding sites are underlined. The predicted amino acid sequence is given above the nucleotide sequence. The first residues for ORFA and ORFB are labeled 1. Stop codons are identified with asterisks. The restriction enzyme recognition sequences are as follows: *Sma*I, nucleotides 1 to 6; *Nar*I, 5 to 10; *Bal*I, 487 to 492; *Stu*I, 520 to 525; *Pst*I, 1066 to 1071; and *Nco*I, 1115 to 1120.

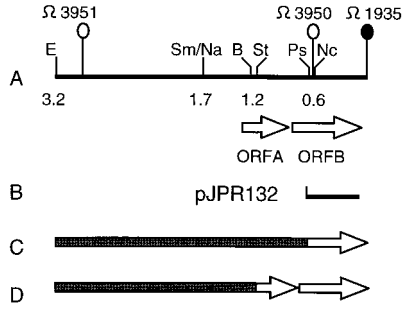

FIG. 7. The *tgl* transcription unit. (A) Map of the 1.7-kb DNA region between the *Sma*I/*Nar*I site and Tn5Ω1935. This region contains the autonomous *tgl* transcription unit, as defined by integration at ATTB. The nucleotide sequence of this region predicts the two open reading frames shown (ORFA and ORFB). Tn*5lac*Ω3950 inactivates the rescue of the *tgl* mutation by plasmids carrying the 3.2-kb DNA fragment shown, while Tn*5lac*Ω3951 does not affect rescue by the *tgl*$^+$ allele. (B) pJPR132, which carries a chromosomal DNA fragment internal to the *tgl* transcription unit. (C and D) The structure of the fusions made by inserting restriction fragments (A) into fusion vector plasmids. The shaded boxes represent DNA from the vector, and the open boxes represent DNA from ORFA and ORFB. The arrows indicate the orientation of transcription for the restriction map (A). Distances from Tn5Ω1935 are given in kilobases below the line. Transposon insertions are shown as lollipops, open for Tn*5lac* and closed for Tn*5*. Restriction sites: B, *Bal*I; E, *Eco*RI; Nc, *Nco*I; Na, *Nar*I; Ps, *Pst*I; Sm, *Sma*I; St, *Stu*I.

taneously rescues the social motility and the stimulation defects produced by a *tgl* mutation.

As shown in Table 3, the plasmids carrying *tgl*$^+$ fall into two classes with respect to frequency of rescue. One class, for which pJPR121 and pJPR128 are examples, produces 83 to 100% rescue. The other class, represented by pJPR122, produces 55 to 78% rescue. This difference in the range of rescue

TABLE 5. Disruption of ORFB by deletion and transposon insertion

Plasmid type	Plasmid	% Recipients regaining social motility (*n*)[a]	
		DK1250	DK1218
Internal fragment	pJPR132	n.t.	0 (16)
Transposon insertion	pJPR1105	0 (136)	95 (40)
Transposon insertion	pJPR1132	100 (43)	n.t.

[a] Frequency of S$^+$ transductants produced after introduction of plasmids and selection for kanamycin resistance in the A$^-$ S$^+$ strain DK1218 (*cglB2*) or the A$^-$ *tgl* mutant strain DK1250 (*aglB1 tgl-1*). The total number of recombinants analyzed is shown in parentheses. pJPR132 inactivates *tgl*$^+$ by a gene interruption that produces two truncated copies of the gene. pJPR1105 and pJPR1132 contain insertions of the transposon Tn*5lac* at Ω3950 and Ω3951 (Fig. 7). Kanamycin resistance is obtained after integration of the plasmid or transposition of Tn*5lac*. pJPR1105 produced two A$^-$ S$^-$ recombinants in DK1218 which were gene replacements at the chromosomal *tgl* region. n.t., not tested.

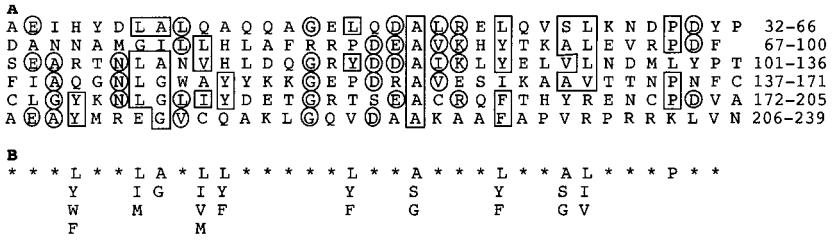

FIG. 8. (A) The predicted amino acid sequence of the Tgl protein from position 32 to 239 is aligned to show six TPR units arranged in tandem. (B) The TPR consensus sequence. Boxes indicate homology between the Tgl TPR units and the consensus sequence. Circles highlight positions at which the Tgl protein TPR units have identical or highly conserved residues in at least three of the six repeats which are not typical of the TPR consensus sequence.

frequency can be explained as the consequence of whether the cloned DNA fragment is a complete copy or a truncated copy of the *tgl* transcription unit and of the position of the *tgl* allele in the recipient chromosome. When a plasmid (like pJPR128) which carries a complete transcription unit integrates, a tandem duplication is produced in which one or the other copy of the duplicated region is wild type, regardless of the site of recombination between donor plasmid and recipient chromosome, as illustrated in Fig. 5C. In contrast, when a plasmid (like pJPR122) which contains a truncated copy of the transcription unit forms a tandem duplication, the formation of a wild-type copy of the transcription unit depends on the site of recombination. As shown in Fig. 5A, when a gene with a deletion at one end recombines upstream of the chromosomal mutation, a wild-type gene is formed. However, when the truncated gene recombines downstream of the chromosomal mutation, one copy of the tandem duplication consists of a transcription unit harboring the chromosomal mutation and the other copy contains the deleted end, as shown in Fig. 5B. Integration of the plasmid in this region fails to generate any intact *tgl* gene. This interpretation of the rescue frequencies was confirmed by modifying the plasmids pJPR128 and pJPR122 to produce pJPR129 and pJPR124, respectively, by the addition of a DNA fragment which mediates the integration of the plasmids into the chromosomal Mx8 prophage attachment site (ATTB). The integration of chromosomal DNA in pJPR124 and pJPR129 at ATTB prevents the reconstruction of an intact transcription unit that may occur when the plasmid integrates by homologous recombination. Therefore, only when a plasmid like pJPR129, which carries the complete transcription unit, integrated at ATTB was the *tgl* mutation rescued.

In four instances pJPR124, which has an incomplete transcription unit, rescued the *tgl* mutation (Table 4). It was suggested in Results that these four events may have arisen by homologous recombination at the *tgl* locus, even though the genes for integration at the Mx8 prophage attachment site were available. To test the plausibility of this suggestion, the relative frequency of homologous recombination (or gene conversion) to site-specific recombination for pJPR124 was estimated. The estimate is based on comparison with pJPR122, which contains the same chromosomal DNA region as pJPR124 but can only integrate by homologous recombination. pJPR122 rescues *tgl-1*, *-2*, and *-3* among 62% of the Km^r transformants, whereas pJPR124 rescued at a frequency of 1.6% (4 of 253). The estimated relative frequency of 1 homologous to 39 site-specific events is compatible with the data of Shimkets and Asher (41) for site-specific versus homologous recombination at another locus, *csgA*.

The fact that the 613-bp chromosomal DNA fragment carried by pJPR122 rescues the *tgl* mutation when the fragment integrates by homologous recombination but fails to rescue when it is integrated as part of pJPR124 into the Mx8 prophage attachment site indicates that the cloned fragment terminates within the *tgl* transcription unit. The boundaries of this fragment are a *Pst*I site on the left and the end of the IS*50* element from the Tn*5-132* insertion at Ω1935 on the right. The transposon insertion at Ω1935 does not produce a *tgl* mutation, even though this insertion and *tgl* alleles are 97% cotransducible (46). Thus, Ω1935 must be outside, albeit very close to, the *tgl* transcription unit. It follows that the truncation in pJPR124 is at the 5′ end and that the *Pst*I site lies within the transcription unit. This deduction was confirmed experimentally by introducing pJPR132 to produce a gene interruption mutation of the *tgl* transcription unit. This plasmid carries a 500-bp fragment whose left end is the *Pst*I site and whose right end was produced by the deletion of approximately 100 bp of DNA adjacent to the IS*50* element. The integration of pJPR132 by homologous recombination resulted in cells that lacked social motility and could be stimulated by a tgl^+ donor to spread with characteristic social motility.

Additional evidence that this region contains the *tgl* transcription unit was obtained by the isolation of the transposon insertion at Ω3950. This insertion (carried by pJPR1105) is located between the *Pst*I site and the end of the IS*50* element (Fig. 7) and produced two effects expected of a transposition into the *tgl* transcription unit. First, it abolished the capacity of pJPR1105 to rescue *tgl* mutants. Second, the introduction of pJPR1105 into recipient strain DK1218 ($A^- S^+$) gave rise to two $A^- S^-$ strains in which homologous recombination events had occurred on both sides of the Tn*5* insertion, resulting in gene replacement. As expected for a *tgl* mutant, stimulation of social motility was observed when these $A^- S^-$ cells were mixed with an $A^- S^-$ tgl^+ donor.

The isolation of chromosomal DNA fragments that rescue the *tgl* mutations confirms that tgl^+ is dominant over the *tgl* mutations, as previously implied by the phenotype produced by tandem duplications of the region adjacent to Ω1935 constructed by generalized transduction (1). The *tgl-1*, *tgl-2*, and *tgl-3* mutations were obtained by UV-induced mutagenesis and, because they are recessive in duplications, are likely to be loss-of-function mutations. Moreover, the *tgl-1*, *tgl-2*, and *tgl-3* mutants have the same phenotype as the *tgl* mutant obtained by the plasmid-mediated gene interruption of Tn*5lac* and the *tgl* mutant obtained by the transposition of Tn*5lac*, both of which are expected to be null mutants. The survival of these *tgl* null mutants indicates that the *tgl* gene is not essential for cell viability.

The nucleotide sequence of the 1.7-kb DNA fragment that contains the functional *tgl* transcription unit contains two substantial protein coding regions (ORFA and ORFB). ORFB corresponds to the *tgl* gene because its coding region includes the 613-bp DNA fragment from nucleotide positions 1066 to

1679, which is sufficient for the rescue of the *tgl* mutations after integration by homologous recombination. In addition, the Tn*5* Ω3950 insertion, which produces a *tgl* mutant phenotype, maps to the region from bp 1100 to 1300. Therefore, ORFB is the *tgl* gene. These sequence data taken with the physical mapping (10) show that the map order is *rif*, *tgl*, Ω1935.

The orientation and proximity of ORFA and ORFB suggest that the two open reading frames might be transcribed together. Deletion of a 490-nucleotide region which includes noncoding sequences upstream of ORFA in pJPR148 (corresponding to the region from the *Sma*I to *Bal*I sites shown in Fig. 3) abolished the ability to rescue *tgl* mutants when the plasmid integrated at the Mx8 prophage attachment site (Table 4). This region may contain the promoter or other sites essential for transcription of *tgl*. The stop codon of ORFA and the start codon of ORFB are separated by 38 nucleotides, and if allowance is made for a ribosome binding site upstream of the ORFB start codon to help set the reading frame, the distance would be further reduced. The space may be insufficient to accommodate a promoter, unless it extends into ORFA. It is possible that *tgl* protein is transcribed as part of a polycistronic message from a promoter upstream of ORFA. The functions of ORFA and the promoter remain to be identified.

tgl mutants have three closely related properties: they lack social motility, they can be stimulated to regain it, and they lack pili (18, 50). When *tgl* mutants are stimulated and become socially motile, they assemble pili (18). The capacity of stimulated cells to assemble pili is transient, in parallel with the stimulation of social motility, and the offspring of the stimulated cells, which remain genotypically *tgl* mutants, lack pili (18). Recently, *pilA*, the gene that encodes *M. xanthus* pilin, the protein subunit of pili, has been identified (50). Known formerly as *sglB3*, *pilA* mutants had been shown by Hodgkin and Kaiser to be unstimulatable (15). Since *tgl* mutants fail to make pili, the *tgl* protein is likely to be a factor required for the assembly of previously synthesized pilin subunits. Whether the *tgl* protein is incorporated into the final structure remains to be determined. Stimulation requires close proximity of donor and recipient cells. When donor and recipient cells are patched as neighbors on an agar surface, there is no stimulation unless the patches intersect (13, 15). Cells spread outward but only from the region where the two cell populations have mixed (13). Because mixing appears to be required, the stimulating factor apparently cannot diffuse far or is unstable. Experiments reported in this paper show that a tgl^+ allele is necessary for the stimulation donor. Two kinds of experiments reported here suggest that expression of Tgl protein may also be sufficient for stimulation. First, it is not necessary that the donor be able to spread, as shown here and previously (13, 15). Second, all the tgl^+ recombinants that had been selected by their recovery of social motility also regained the ability to donate. If the only requirement for a *tgl* donor is that it be able to express tgl^+, the stimulating factor could be the *tgl* protein itself.

The predicted amino acid sequence of the Tgl protein contains six tandem units of a 34-residue-long TPR motif. The TPR motif has been identified in yeast and other organisms in proteins with diverse physiologic functions, including (i) the transport of proteins into organelles (MAS70 and PAS10 in *S. cerevisiae* [29, 49], PAS8 in *P. pastoris* [27], and MOM72 in *Neurospora crassa* [38]); (ii) protein secretion (SEC17 in *S. cerevisiae* [7] and the SNAP proteins in mammalian cells [30]); (iii) transcription regulation (SSN6 [Cyc8] and SKI3 in *S. cerevisiae* [33, 39]), (iv) mitosis (CDC16, CDC26, and CDC27 or SNB1 in *S. cerevisiae* [24] and $nuc2^+$ and $cut9^+$ in *Schizosaccharomyces pombe* [12, 36]); and (v) RNA splicing (PRP6 in *S. cerevisiae* [25]). The TPR motif has also been identified in a stress-induced protein (STI1 in *S. cerevisiae* [29]). To our knowledge, only one prokaryotic member of the TPR protein family has previously been identified, that encoded by an open reading frame of unknown function in *Synechococcus* (45).

Instead of being a predictor of function, we suggest that the Tgl TPR indicates a form of tertiary structure. Each unit of the TPR motif is believed to form an alpha helix. Adjacent units are believed to be separated by a turn (there is a conserved proline shown in Fig. 8), with two helical segments folding against each other to form a single helical coiled coil. The coiled coil would be stabilized by sterically complementary interactions between amino acid side chains in a helix pair. Amino acids with hydrophobic side chains tend to line the interface between the two helical segments, and hydrophobic amino acids with short side chains (principally Gly and Ala) would form a "hole" which is occupied by a "knob" produced by a bulky, uncharged, often aromatic (Tyr or Phe) side chain from an amino acid in the complementary helix (6, 12). Typically, four or more TPR units are found as tandem repeats in a protein, suggesting that they contribute to the tertiary structure of the protein (6, 12, 45). In the case of the 241-amino-acid-long Tgl protein, residues 32 to 239 are all included in six tandem TPR units with no gaps within or between units. Nearly the entire Tgl protein has the TPR structure.

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