Complementation of sporulation and motility defects in a prokaryote by a eukaryotic GTPase

(*Myxococcus xanthus*/gliding/fruiting body morphogenesis/Sar1p/MglA)

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ABSTRACT The complex prokaryote, *Myxococcus xanthus*, undergoes a program of multicellular development when starved for nutrients, culminating in sporulation. *M. xanthus* makes MglA, a 22-kDa, soluble protein that is required for both multicellular development and gliding motility. MglA is similar in sequence to the *Saccharomyces cerevisiae* SAR1 protein, a member of the Ras/Rab/Rho superfamily of small eukaryotic GTPases. The SAR1 gene, when integrated into the *M. xanthus* genome, complements the sporulation defect of a Δ mglA strain. A forward, second-site mutation on the *M. xanthus* chromosome, *rpm*, in combination with SAR1, restores fruiting body morphogenesis and gliding motility to a Δ mglA strain. The result that the *rpm* mutation suppresses the substitution of SAR1 for *mglA* suggests that Sar1p interacts with other *M. xanthus* proteins to control the motility-dependent aggregation of cells during development.

Myxococcus xanthus, a Gram-negative soil bacterium, undergoes multicellular development. When starved for nutrients, groups of $>10^5$ wild-type *M. xanthus* cells aggregate and form fruiting bodies containing heat-resistant spores (1). During the starvation-induced differentiation of *M. xanthus*, like that of the eukaryotic slime mold *Dictyostelium discoideum*, the interaction of multiple cell types (2) is required to produce cells with different terminally differentiated fates. A subset of developing *M. xanthus* cells differentiates into spherical spores that are resistant not only to heat, but also to ultraviolet light and desiccation. Other cells remain rod-shaped and form the base and sheath of the fruiting body (3).

Gliding motility plays a critical role in *M. xanthus* development, because development depends on a critical density of cells achieved by motility-dependent aggregation (4, 5). The genetic basis of gliding motility is complex and involves at least two independent mechanisms, adventurous and social (6, 7). Adventurous gliding enables individual cells to move dependent on interactions of cells with solid surfaces, whereas social gliding enables groups of cells to move dependent on cell-cell interactions (8). Although both mechanisms are required for the normal developmental program, a subset of mutants defective in social gliding can produce heat-resistant spores, and a subset of mutants defective in adventurous gliding can complete the morphogenesis of the fruiting structure (6, 7).

Mutations in only one known gene, *mglA*, affect both adventurous and social gliding mechanisms (6, 9). MglA is a 22-kDa cytoplasmic protein that is transcribed with MglB, an 18-kDa protein that is predicted to interact with and stabilize MglA (10). In the absence of MglB, *M. xanthus* makes normal levels of *mglA* transcript, but reduced levels of MglA protein. Although *mglB* mutants show reduced colony spreading on

growth medium, they exhibit both adventurous and social gliding.

When starved for nutrients, *mglA*[−] strains fail to aggregate or bear fruit and produce less than 0.01% the wild-type complement of spores (11). The defect in sporulation is due, in part, to an inability to mediate the C-signal, an extracellular morphogen essential for sporogenesis during multicellular development (12). In contrast, *mglB*[−] strains aggregate into small, flat mounds and produce a nearly wild-type complement of heat-resistant spores (10). These results suggest that gliding and sporulation require different amounts of MglA protein, or that MglB is dispensable for sporulation.

The critical need for MglA in motility, sporulation, and morphogenesis suggests that MglA plays a central role in signal transduction in *M. xanthus*. MglA shares identity with the small molecular mass eukaryotic GTPases (13) such as Ras, Rab, Rho, Arf, and Sar1p (14). Subgroups of GTPases play critical roles in organelle function, cytoskeletal organization, and the regulation of vesicular trafficking of proteins through the exocytic and endocytic pathways (15). The protooncogene, Ras, controls cellular growth by modulating a mitogen-activated protein kinase signal transduction cascade (16–18). Sar1p has a different, essential function. It is required for protein transport from the endoplasmic reticulum to the Golgi in yeast (19) and mammals (20). Sar1p is regulated by the 70,000 Da integral membrane glycoprotein Sec12p, which is proposed to catalyze guanine-nucleotide exchange and recruit Sar1p to a vesicle formation site on the endoplasmic reticulum membrane (21), and by Sec23p, which activates the GTPase activity of Sar1p (22).

To explore the similarity between MglA and its eukaryotic homologs, Ha-RAS and SAR1 were introduced into a strain carrying a deletion of *mgl*. The yeast GTPase Sar1p is able to complement the sporulation defect of an *mgl* mutant. An allele of SAR1 with an altered GTP consensus fails to complement the sporulation defect. Complementation of the motility defect requires a second-site mutation in combination with SAR1, suggesting that Sar1p interacts with an *M. xanthus* protein to control gliding. These results establish the requirement for a GTPase in motility and development of *M. xanthus*.

MATERIALS AND METHODS

Growth of Bacterial Strains. *M. xanthus* strains DK1622 (motile wild-type), DK3619 (motile, Ω 1901 Tet^R), DK3662 (nonmotile, Ω 1901 Tet^R*mglA*8), DK5208 (*csqA*:: Ω 205 Tet^R), and DK6204 (nonmotile, Δ mglA) used in this study have been described previously (13, 23, 24). *M. xanthus* strains were grown in CTPM medium (1% casitone/10 mM Tris/4 mM MgSO₄/1 mM potassium phosphate, at a final pH of 7.5) at

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Abbreviations: CTPM, 1% casitone/10 mM Tris/4 mM MgSO₄/1 mM potassium phosphate, at a final pH of 7.5; TPM, CTPM with the casitone omitted.

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250 rpm. Magnesium was omitted when tetracycline was added. Solid medium contained 1.5% agar. Phage Mx4 *ts-18 ts-27 htf-1 hrm-1* (25) was used to mediate generalized transduction between strains of *M. xanthus*. *Escherichia coli* strain JM107 (26) was used as the host for construction and maintenance of plasmid DNA. Where applicable, media were supplemented with the antibiotics kanamycin sulfate (40 $\mu\text{g/ml}$), ampicillin (100 $\mu\text{g/ml}$), and/or tetracycline (12.5 $\mu\text{g/ml}$).

Construction of Plasmids. Plasmids pPLH477 and pPLH490 were constructed to express *SAR1* and Ha-*RAS* from the *mgl* promoter. Plasmid pMY3-1 containing the cDNA clone for *SAR1* was obtained from Akihiko Nakano (University of Tokyo, Japan). Plasmid pBR322-c-Ha-*RAS* was from Fuyuhiko Tamanoi (University of California, Los Angeles). The 0.67-kb *SAR1* gene was removed by restriction with *NcoI* and *EcoRI*, purified, and ligated with a 4.4-kb *EcoRI*-*NcoI* fragment from pPLH325 (13) to produce pPLH477. Ha-*RAS* was amplified by the PCR using oligonucleotide 5'-AAGCTTCAATGGCCGAATAC-3' for the 5' end of Ha-*RAS*, which introduces an *NcoI* site, and 5'-GAATTCGTCGACCTATCA-3' for the 3' end of Ha-*RAS*, which introduces a *SalI* site. The 580-bp Ha-*RAS* PCR product was gel purified, digested with *NcoI* and *SalI*, and ligated with a 4.4-kb *SalI*-*NcoI* fragment from pPLH325 to produce pPLH490. The 4.4-kb fragment from plasmid pPLH325 used to construct pPLH477 and pPLH490 contains the ColE1 origin of replication (which does not function in *M. xanthus*), a kanamycin-resistance determinant, and the *mgl* promoter, but lacks all of *mglB* and *mglA*.

To determine the requirement for GTPase activity of *SAR1* in sporulation of *M. xanthus*, lysine 36, a residue in the G1 domain that is essential for GTPase function in Ras (lysine 16) (27) was changed to threonine by site-directed mutagenesis using the PCR overlap method (28). Plasmid pPLH477 was used as template with oligonucleotides 5'-CAATGTGGTG-GTACCGGCATTATCCAA-3' (*SAR1* K36T reverse) and 5'-ATCACAAGCTTAAGAGTCAGGCCCC-3' (outside 1) to produce product A and 5'-TTGGATAATGCCGGTAC-CACCACATTG-3' (*SAR1* K36T forward) and 5'-ATAACA-ATTTCACACAGGAAACA-3' (outside 2) to produce product B. PCR products A and B were purified, annealed, and used as template with the two outside oligonucleotides to amplify *SAR1*K36T and the *mgl* regulatory region. The 940-bp product was purified, digested with *HindIII* and *SalI*, and cloned into pBGS18 to produce pAGS101. The presence of a new *KpnI* site in pAGS101 confirmed that the *SAR1* gene carried the K36T mutation.

Plasmids pPLH477, pPLH490, and pAGS101 were introduced into the chromosome of *M. xanthus* DK6204 (ΔmglA ; ref. 13), by selection for homologous recombination between the *mgl* regulatory region on the plasmid and the homologous region on the chromosome to produce MxH1048, MxH1091, and MxH1116, respectively. Southern analysis (29) was used to confirm that recombinants carry a single, integrated copy of *SAR1* (or the Ha-*RAS*) gene. Chromosomal DNA was prepared as described by Yee and Inouye (30), digested with *EcoRI*, *HindIII*, and *AscI*, separated in 0.7% agarose, and transferred to Nytran. Membranes were probed with ^{32}P -labeled DNA corresponding to *mglA* from pPLH325, *SAR1* from pMY3-1, and the Ha-*RAS* PCR product (data not shown).

Immunoblot Analysis. Anti-Sar1p polyclonal antibody was obtained from Randy Schekman (University of California, Berkeley). Soluble extracts of *M. xanthus* cells were prepared from strains grown in CTPM broth, lysed by passage through a French pressure cell at 20,000 psi, and centrifuged at $200,000 \times g$. Aliquots containing about 15 μg of total protein were separated on 12.5% tricine gels (31), transferred to nitrocellulose, and probed with rabbit anti-Sar1p antibody, diluted 1:2000 in Tris-buffered saline, at pH 7.6 with 0.1%

Tween-20 (32). Antigen-antibody reactions were visualized with ECL (Amersham) according to manufacturer's instructions.

Genetic and Molecular Analysis of Strains. *M. xanthus* strain MxH1056 is a motile derivative of MxH1048 obtained after UV mutagenesis. Cells of MxH1048 were washed with TPM buffer (which is identical to CTPM medium, except that casitone is omitted) and exposed to ultraviolet light in a Stratalinker (Stratagene) for 2 min. Aliquots of cells were then spotted on CTPM agar and incubated at 33°C. MxH1056 was identified as a flare that moved away from the initial spot of growth. To determine if motility of *M. xanthus* MxH1056 was due to a mutation in *SAR1* or in the *mgl* promoter, genetic linkage between the Kan^R determinant that integrated with *SAR1* and the motility phenotype was measured. Mx4 phage lysate of MxH1056 was transduced into the nonmotile parent, DK6204, and Kan^R transductants were selected. Transductants (145) were transferred to CTPM Kan agar, and motility of all 145 was indistinguishable from the parent, MxH1048 ($\Delta\text{mgl SAR1}$). Chromosomal DNA from MxH1056 was digested with *EcoRI* and ligated to recover *SAR1* and the *mgl* promoter for sequencing. Kan^R plasmids were recovered in *E. coli* and sequenced on a LiCor automated sequencer using commercial M13 forward and reverse primers. The sequence of *SAR1* from MxH1056 and the original plasmid pPLH477 is identical to the published sequence of *SAR1* (19). These results show that *rpm* is not linked genetically with *SAR1* and that the gain of motility in MxH1056 is not due to a mutation in *SAR1*.

To determine if *SAR1* and *rpm* both were required for motility, experiments were designed to replace the integrated copy of *SAR1* with other *mgl* alleles or to disrupt the *SAR1* gene. Strains DK3619, DK3662, and MxH1095 carry Tet^R insertions linked with *mglA*⁺, *mglA8*, and ΔmglA , respectively, and were used as donors for introducing *mgl* alleles by Mx4 transduction into *SAR1* backgrounds. MxH1095 was constructed by transduction (DK3619 $\times$ DK6204) with myxophage Mx4; its genotype was confirmed by Southern analysis (not shown). Kan^R recipient strains were MxH1048 ($\Delta\text{mglA SAR1}$) and MxH1056 ($\Delta\text{mglA SAR1 rpm}$). Transductants were selected on CTP agar containing tetracycline. The Tet^R marker is about 1–2 kb from the *mgl* operon, and Tet^R transductants obtain the linked *mgl* allele at a frequency >80% (24). Individual Tet^R transductants then were transferred to plates with tetracycline and kanamycin to determine the number of transductants that carried both markers. Colonies were examined visually for motility.

Upon infection of MxH1056 with phage lysates prepared from the three Ω 1901 Tet^R donor strains, only the *mglA8* donor strain gave rise to Kan^S Tet^R replacements. To confirm that the replacements carried the *mglA8* allele, chromosomal DNA was prepared from eight independent Tet^R Kan^S recombinants for restriction fragment length polymorphism analysis. The *mglA* gene was amplified with oligonucleotides 5'-GCAAAAGCT-TAACCACCCTACTTGAGCT-3' and 5'-ATCACAAGC-CTAAGAGTCAGGCCCC-3' using PCR, and PCR products were purified and restricted with *FokI*, to confirm the loss of a *FokI* site caused by the *mglA8* mutation. PCR and Southern analysis were used to show the absence of *SAR1* in the Kan^S Tet^R strains.

Analysis of Motility and Development. The gliding phenotypes of wild-type and mutant cells were assayed visually, microscopically, and by measuring the spreading areas of 1.5×10^7 cells on 0.3% and 1.5% casitone agar media as described by Shi and Zusman (33). Fruiting body morphogenesis and sporogenesis were compared for mutant $\Delta\text{mglA SAR1}$ cells and otherwise isogenic *mglA*⁺ and ΔmglA strains. To initiate development, cells were grown in CTPM medium to 5×10^8 cells/ml, concentrated by centrifugation at 12,000 $\times g$ for 10 min, suspended in TPM buffer to 5×10^9 cells/ml, spotted (20 μl) on TPM starvation plates and incubated at 33°C. Mor-

phogenesis was monitored microscopically at 6-hr intervals for several days. Events such as rippling, formation of translucent mounds, and progression to dark mounds serve as temporal markers for development, and were monitored visually for 70 hr with a Nikon SMZ-U stereomicroscope. After incubation at 33°C for 5 days, plates were incubated at 50°C for 2 hr to kill undifferentiated cells, and samples were scraped into TPM buffer for spore assays. Samples were sonicated for 5 s at 10% power using a Microson cell disruptor (Heat Systems/Ultrasonics) to uniformly disperse spores, and serial dilutions in TPM buffer were plated on CTPM agar. Colonies formed from germinating spores were counted after incubation at 33°C for 3–4 days.

RESULTS

Eukaryotic GTPases Are Expressed and Stably Maintained in *M. xanthus*. The predicted amino acid sequence of MglA protein shows that it is related to other members of the Ras family of GTPases (13). MglA shares 24% identity and 47% similarity with Sar1p, a member of the Rab subfamily of small GTPases (34) (Fig. 1), and 15% identity (28% similarity) with Ha-Ras (data not shown). To determine if Ha-Ras or Sar1p could complement the defects of a strain with a deletion of *mglA*, the *SAR1* and Ha-*RAS* genes were introduced in single copy in place of the *mglA* gene on the *M. xanthus* genome and expressed under control of the *mgl* promoter. Immunoblots prepared from extracts of strains expressing *SAR1* or Ha-*RAS* were probed with anti-Sar1p antibody. A 21-kDa protein, indistinguishable from Sar1p in yeast, was detected in *M. xanthus* strains expressing an integrated copy of *SAR1* (Fig. 2), or Ha-*RAS* (data not shown).

Sar1p Can Substitute for One Function of MglA, the Production of Heat-Resistant Spores. As shown in Table 1, heat-resistant spores are formed by the Δ *mglA SAR1* strain, MxH1048, with an efficiency close to that of the wild-type (*mglA*⁺) strain and 10⁴-fold higher than that observed for the parental Δ *mglA* strain. Hence, the *SAR1* gene complements the inability of the Δ *mglA* strain to produce heat-resistant spores. The spores produced by MxH1048 are slightly ovoid, whereas the spores produced by the wild type are spherical. The significance of the spore shape is unknown, although variations in spore shape have been observed with other mutants (35). Although Ha-*RAS* encodes a GTPase similar in

MglA	MSFINYSREINCKIVYYGPGLCGKTTLNLQ
	* *
Sar1p	MAGWDIFGWFRDVLASLGLWNKHGKLLFLGLDNAGKTTLH
MglA	YIYNKTAETKGLISLSTETDRTLFFDFLPLSLGEBIRGFKT
	**
Sar1p	MLKNDRLATLQPTWHPTSEELAIG-----NI
	#
MglA	RFHLYTVPQGVFTDASRKLLKGVGVFVAD-SQIERMEAN
	* *** ** * * * * * * * * * *
Sar1p	KFTTFDLGGHIGARRLWKDYFPEVNGIVFLVDAADPERFDEA
MglA	MESLENLRINLAEGQYDLNKPVIQYNNKRDLPNAVTEEMR
	* * * * * * * * * * * * *
Sar1p	RVELDAL-FNIAE----LKDVFFVILGNKIDAPNAVSEAEIR
MglA	KALNHRNPIEQAV-APTGVGVFDTLKAVAKLVLTTELKKG
	* * * * * * * * * * * * *
Sar1p	SALGLLNTTGSQRIGQRPEVFMCSVVMRNGYLEAFQWLSQYI

FIG. 1. MglA shares 24% identity with Sar1p. The complete amino acid sequence for MglA and Sar1p is shown. The primary sequence of the 22-kDa MglA contains 195 amino acids, and the 21-kDa Sar1p contains 190 amino acids. Residues that comprise the conserved GTP consensus are underlined in bold. | indicates identity; * indicates similarity. # indicates the site of the mutation in *mglA*8. The method of Altschul *et al.* (44) was used to align the sequences.

FIG. 2. The 22-kDa yeast protein Sar1p is produced in *M. xanthus*. Immunoblot of extracts from *S. cerevisiae* and *M. xanthus* strains was probed with a 1:2,000 dilution of rabbit anti-Sar1p antibody. Lane 1, Bio-Rad Kaleidoscope prestained standards; carbonic anhydrase (42,600); soybean trypsin inhibitor (29,900) and lysozyme (17,100). Lane 2, *M. xanthus* DK1622 (wild type). Lane 3, *M. xanthus* DK6204 (Δ *mglA*). Lane 4, *M. xanthus* MxH1048 (Δ *mglA SAR1*). Lane 5, *S. cerevisiae*. Lane 6, *M. xanthus* MxH1056 (Δ *mglA SAR1 rpm*). Lane 7, *M. xanthus* MxH1116 (Δ *mglA SAR1 lys36thr*).

size to MglA, it is not able to complement the sporulation defect of the Δ *mglA* mutant.

To determine if the GTPase activity of *SAR1* was required to complement the sporulation defect of the Δ *mgl* mutant, a mutant allele of *SAR1* was constructed and introduced into the *M. xanthus* Δ *mglA* strain. Lysine 36 of *SAR1* is in the G1 domain of the GTP consensus and is predicted to make contact with main-chain oxygens of the phosphate groups of GTP (36). Replacement of lysine 36 with threonine did not affect expression or stability of Sar1p in *M. xanthus* (Fig. 2), but abolished the ability of Sar1p to rescue the sporulation defect of Δ *mglA*. These data show that the GTPase activity of Sar1p is required for complementation of the sporulation defect.

The *SAR1* gene does not complement the defects in motility or fruiting body morphogenesis caused by a Δ *mglA* mutation even though Δ *mglA SAR1* cells can differentiate from rod-shaped vegetative cells into refractile spores. This result shows that gliding motility and the differentiation of heat-resistant spores involve different functions of MglA. Sar1p can complement one, but not both, of these functions.

Sar1p Interacts with Rpm to Complement Motility. Small GTPases like Sar1p, function by interacting with, and modifying the activities of, other proteins including GTPase-activating proteins, guanine-nucleotide exchange factors, and guanine-nucleotide release factors (15). Sar1p complements the sporulation defect caused by a deletion of the *mglA* gene, but it does not complement the motility defect of this mutant. The simplest hypothesis to account for this observation is that Sar1p interacts with a protein that normally interacts with MglA to control sporulation, but cannot interact with a protein that normally interacts with MglA to control motility. To test this hypothesis, a mutation that restores interactions between Sar1p and the target of MglA interaction required for gliding motility was selected. The nonmotile Δ *mgl SAR1* strain, MxH1048, was mutagenized, and a motile derivative was isolated. The spreading ratio for this mutant, designated MxH1056, on 0.3 and 1.5% agar is similar to that of the wild-type strain (ratio = 1.8), which suggests that both adventurous and social modes of gliding have been restored. Microscopic examination of cells at the edges of a colony edges supports this claim. However, because the spreading rate for the motile derivative is only 20% that of the wild-type strain on surfaces of both agar concentrations, MxH1056 is designated Δ *mgl SAR1 rpm* because it restores partial gliding motility (Fig. 3).

Cells of MxH1056 (Δ *mgl SAR1 rpm*) were starved for nutrients to induce fruiting body formation and sporulation. In contrast with cells of the Δ *mglA SAR1* parent, cells of the *rpm* derivative aggregate to form numerous mounds (Fig. 4D), which are smaller and less erect than wild-type mounds (Fig. 4A). The mounds produced by the *rpm* derivative mature to a

Table 1. *SAR1* rescues the sporulation defect of a $\Delta mglA$ strain

Strain	Genotype	Phenotype*		Viable spores, ml	Viable spores†, wild type
		Motility	Fruiting		
DK1622	Wild type	+	+	5×10^8	100.00%
DK6204	$\Delta mglA$	—	—	5×10^4	0.01%
MxH1048	$\Delta mglA$ <i>SAR1</i>	—	—	4.6×10^8	92.00%
MxH1116	Δmgl <i>SAR1</i> K36T	—	—	9×10^4	0.02%
MxH1091	$\Delta mglA$ Ha- <i>RAS</i>	—	—	2×10^5	0.04%

*Assays for motility and morphogenesis are described in *Materials and Methods*.

†Approximately 5-10% of wild-type cells differentiate into heat-resistant spores by day 5.

brown color at the same time as the wild-type cells (30 hr), coincident with their production of spherical, refractile, heat-resistant myxospores.

Mutations in *M. xanthus* that bypass the requirement of MglA for motility have yet to be found. Therefore, the *rpm* mutation is predicted to be in *SAR1* or in a gene encoding a protein that interacts with Sar1p to control motility. To begin to map the *rpm* mutation, the integrated *p_{mgl}SAR1* kanamycin-resistant (Kan^R) plasmid was transduced from the Δmgl *SAR1* *rpm* mutant into *mglA*⁺ and $\Delta mglA$ genetic backgrounds, and Kan^R transductants were selected and scored for motility. Transduction of *p_{mgl}SAR1* into both motile (*mglA*⁺) and nonmotile ($\Delta mglA$) genetic backgrounds yielded only nonmotile recombinants (88/88 and 145/145, respectively). Thus, the *rpm* mutation that confers the gain of motility is not due to a mutation in the integrated *p_{mgl}SAR1* gene, because it is not linked genetically with the Kan^R marker adjacent to *SAR1*.

A series of reciprocal crosses was performed to test whether *SAR1* and *rpm* are required in combination for motility, or if *rpm* bypasses the requirement for Sar1p (and MglA). If *SAR1* and *rpm* are both required for motility, then replacement of *SAR1* with a mutant allele of *mgl* should yield nonmotile recombinants. In these crosses, myxophage Mx4 was grown on donor cells carrying a Tet^R Ω 1901 insertion linked (>80% cotransduction) with *mglA* (24). Tet^R strains that carry Ω 1901 linked with *mglA*⁺, $\Delta mglA$, and *mglA8* were used as donors in these crosses; the Kan^R recipients were the motile Δmgl *SAR1* *rpm* (MxH1056) strain and its nonmotile parent, Δmgl *SAR1* (MxH1048).

As shown in Table 2, replacement of *SAR1* with *mglA8* abolishes gliding motility and shows that Sar1p is required for gliding. Surprisingly, replacement of the *SAR1* gene with *mglA*⁺ or $\Delta mglA$ occurred only in the parental Δmgl *SAR1* strain, and did

not occur in the *rpm* genetic background. Although Tet^R transductants were obtained with Δmgl *SAR1* *rpm* in crosses using *mglA*⁺ and $\Delta mglA$ strains as donors, they retain the integrated *SAR1* Kan^R plasmid. These data suggest that either the *rpm* mutation is synthetic-lethal in combination with the $\Delta mglA$ or *mglA*⁺ alleles, or that the Δmgl *SAR1* *rpm* strain is a poor recipient for generalized transduction. To exclude the latter possibility, a transducing lysate was grown on *M. xanthus* strain DK5208 (provided by L. Shimkets, University of Georgia, Athens) which carries the Tet^R *csgA*:: Ω 205 insertion and is not linked with *mglA*. The numbers of Tet^R Kan^R recombinants arising from crosses with Δmgl *SAR1* and Δmgl *SAR1* *rpm* recipients were similar, 108 and 79, respectively, indicating that the mutant Δmgl *SAR1* *rpm* strain is a good recipient for generalized transduction.

The *mglA8* strain DK3662 carries a missense mutation predicted to result in a conservative amino acid substitution (Leu-93-Phe) in MglA protein (11). Western immunoblot and ELISA assays using anti-MglA antibody show that DK3662 produces a normal level of the inactive, missense MglA8 protein (unpublished results). The Leu-93-Phe mutation is predicted to make MglA8 more like Sar1p, which may account for the ability of MglA8 to substitute for Sar1p in the *rpm* background. Nevertheless, recombinants with the genotype Ω 1901 (Tet^R) *mglA8* *rpm* are not motile and fail to produce heat-resistant spores during starvation-induced development. Therefore, the *rpm* mutation does not suppress the *mglA8* defect. When the *p_{mgl}SAR1* plasmid, pPLH477, is reintroduced and integrated into this host, both motility and sporulation are restored.

FIG. 3. A second-site mutation restores motility to $\Delta mglA$ *SAR1*. Morphology of *M. xanthus* colonies growing on CTPM 1.5% agar plates. (A) *M. xanthus* DK1622. (B) $\Delta mglA$ (DK6204). (C) $\Delta mglA$ *SAR1* (MxH1048). (D) $\Delta mglA$ *SAR1* *rpm* (MxH1056). Photographs were taken with a Nikon SMZ-U stereomicroscope. (Bar = 3 mm.)

FIG. 4. The motile $\Delta mglA$ *SAR1* *rpm* strain forms fruiting aggregates. Morphogenesis of fruiting bodies on TPM starvation medium. (A) *M. xanthus* DK1622. (B) $\Delta mglA$ (DK6204). (C) $\Delta mglA$ *SAR1* (MxH1048). (D) $\Delta mglA$ *SAR1* *rpm* (MxH1056). The parent, $\Delta mglA$ *SAR1*, fails to aggregate (C), although heat-resistant spores are produced. Cells with the $\Delta mglA$ mutation (B) fail to aggregate and fail to differentiate into spores. Photographs were taken 48 hr after the onset of development with a Nikon SMZ-U stereomicroscope. (Bar = 50 mm.)

Table 2. Disruption of *SAR1* in the $\Delta mglA$ *SAR1 rpm* strain abolishes gliding motility

Tet ^R donor	Kan ^R recipient	Total Tet ^R	Tet ^R transductants*			
			Kan ^R		Kan ^S	
			Motile	Nonmotile	Motile	Nonmotile
$\Omega 1901$ <i>mglA</i> ⁺ (motile)	$\Delta mglA$ <i>SAR1</i>	113	0	10	103	0
	$\Delta mglA$ <i>SAR1 rpm</i>	63	63 [†]	0	0	0
$\Omega 1901$ $\Delta mglA$ (nonmotile)	$\Delta mglA$ <i>SAR1</i>	78	0	11	0	67
	$\Delta mglA$ <i>SAR1 rpm</i>	49	49 [†]	0	0	0
$\Omega 1901$ <i>mglA8</i> (nonmotile)	$\Delta mglA$ <i>SAR1</i>	89	0	9	0	80
	$\Delta mglA$ <i>SAR1 rpm</i>	44	12 [†]	0	0	32 [‡]

*Transductants initially were selected on plates with tetracycline and subsequently were scored for resistance to kanamycin as described in *Materials and Methods*.

[†]Motile transductants were identical to the $\Delta mglA$ *SAR1 rpm* recipient and did not carry the donor *mglA* allele.

[‡]DNA from eight transductants was assayed for the presence of the *mglA8* and *SAR1* genes. In all eight, the *mglA8* allele had replaced the integrated copy of *SAR1*.

DISCUSSION

Multiple types of small GTPases have been found in eukaryotic organisms where they are required for a wide range of cellular functions, including signal transduction, cytoskeletal assembly, and transport. Nearly all members of this expanding family of proteins are essential. MglA is not required for growth, but represents a new member of the GTPase family required for motility and sporulation. GTP-binding proteins have been identified in prokaryotic organisms, including EF-Tu (37), Era (38, 39), FtzZ (40), and Obg (41), but they are larger in size and possess extra domains not found in the Ras-like eukaryotic proteins. To date, the *M. xanthus* *mglA* gene encodes the only prokaryotic analog of the lower molecular mass (20–25 kDa) GTPases.

Members of the Ras superfamily have been divided into subgroups based on identity and function. The prototype, Ha-Ras, controls a kinase cascade regulating gene expression and cell morphology. Sar1p, a distant relative of Ha-Ras with only 20–25% identity overall with the Ras and Rab families, lacks the C-terminal cysteine for prenylation and the N-terminal glycine for myristylation, and is essential for vesicular transport. MglA is related to each of these proteins, with 15% and 24% identity with Ras and Sar1p, respectively, and has characteristics of each of these subfamilies. A critical glycine residue in the G1 domain of Ras (G12) is conserved in MglA (G21), whereas Sar1p has an aspartate residue in this position (D32). In contrast, both Sar1p and MglA contain a threonine in the G1 domain, in place of the conserved serine residue in the Ras subfamily.

To confirm the requirement of a small GTPase in sporulation and motility of *M. xanthus*, and to define the subfamily to which MglA belongs, Ha-Ras and Sar1p were substituted for MglA in *M. xanthus*. The strains expressing Ras and Sar1p produced colonies that resembled the $\Delta mglA$ parent on agar and grew at the same rate as the wild-type strain. Hence, neither Ras nor Sar1p complement the motility defect of an *mglA* mutant. However, when cells expressing Sar1p were induced to develop by starvation, the cells differentiated into refractile, heat-resistant spores. Expression of Sar1p having a defective GTPase, or the more distantly related Ha-Ras, failed to complement the sporulation defect. Hence, complementation of the sporulation defect of a $\Delta mglA$ mutant requires a functional GTPase.

The ability of Sar1p, but not Ras, to complement may reflect the closer similarity between MglA and Sar1p than between MglA and Ha-Ras. What particular amino acid residues in the sequences of small GTPases determine their specificity of function, by facilitating interactions with other proteins? Clearly, these residues include Leu-93 in MglA, which is near the active (GTP-binding) site of these effectors. The ability of *mglA8* to replace *SAR1* in the *rpm* strain may be due to the

nature of the *mglA8* mutation, which changes leucine-93 to phenylalanine, making MglA8 protein more like Sar1p.

SAR1 in combination with *rpm* can restore fruiting body morphogenesis and gliding motility to a $\Delta mglA$ host. The interaction of the *rpm* mutation with the *mglA* locus is allele-specific because the *rpm* mutation, which is not linked with the *mglA* locus, is lethal in combination with the $\Delta mglA$ and *mglA*⁺ alleles, but not the *SAR1* or *mglA8* alleles. The genetic specificity of this interaction most likely reflects the specificity of interactions between the small GTPase, MglA, and its GTPase-activating and guanine nucleotide exchange partners, because the activity of Sar1p, like Ras, Rab, and other small GTPases, is modulated through its interactions with other effectors. Sar1p function requires the guanine nucleotide exchange factor Sec12p (42) and the GTPase-activating protein Sec23p (43). The ability of Sar1p to function in *M. xanthus* suggests that functional homologs of these proteins and others may exist in this prokaryote. Work is underway to identify the gene affected by the *rpm* mutation.

The finding that *M. xanthus* encodes a small, nonessential GTPase, a defect in which may be complemented by an essential, eukaryotic GTPase, facilitates the genetic analysis of small GTPase structure and function. This demonstrates that a eukaryotic GTPase can complement the defects of a prokaryotic GTPase. The majority of small eukaryotic GTPases are involved in essential cellular functions. Thus, the complex prokaryote *M. xanthus* provides a system in which mutations that inactivate Sar1p or other GTPases will have a conditional phenotype that is not a conditional-lethal phenotype. Because the small GTPase, MglA, is required for both motility and development, the powerful genetic selections for motility (and for development) may be used to explore the interactions of MglA or its homolog, Sar1p, with other proteins in novel ways.

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