JOURNAL OF BACTERIOLOGY, Feb. 2007, p. 1158–1162 Vol. 189, No. 3
0021-9193/07/$08.00+0 doi:10.1128/JB.01500-06

SwmB, a 1.12-Megadalton Protein That Is Required for Nonflagellar Swimming Motility in *Synechococcus*[▽]

J. McCarren# and B. Brahamsha*

Marine Biology Research Division, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093-0202

Received 22 September 2006/Accepted 24 November 2006

SwmB is required for swimming motility in *Synechococcus* sp. strain WH8102. This highly repetitive 1.12-MDa polypeptide is associated with the cell surface, where it is arranged in a punctate manner. Inactivation of *swmB* does not affect the localization of SwmA, an S-layer protein also required for swimming.

A monophyletic clade of marine *Synechococcus* (17) exhibits a novel type of motility. These cells are able to swim through their liquid environment in the absence of flagella or any other readily visible organelle (5, 19). While the mechanism of nonflagellar swimming motility in *Synechococcus* is still not understood, three chromosomal regions containing genes required for motility were identified by transposon mutagenesis of *Synechococcus* sp. strain WH8102 (13). One of these regions includes an exceptionally large and repetitive open reading frame (ORF), *swmB*, insertions in which eliminate motility. Here we show that this large ORF is expressed and its product is localized to the cell surface. Furthermore, we show that the absence of SwmB does not affect the localization of SwmA, the S-layer protein that is also required for swimming (4, 14).

Bacterial strains and growth conditions. *Synechococcus* sp. strain WH8102 (19) and its isogenic *swmA* and *swmB* mutant strains S1A1 and Swm-2, respectively (13), were grown as described in reference 14 in SN medium (18) prepared either with local seawater from the Scripps Pier (Scripps Institution of Oceanography, La Jolla, CA) or with synthetic ocean water prepared according to the method of Price et al. (16) except that components were not treated with Chelex.

SwmB contains multiple repeated domains. *swmB* is 32.38 kb in length and encodes a predicted protein of 10,791 amino acids with a molecular mass of 1.126 MDa and a pI of 3.98. SwmB has a highly repetitive primary structure containing four repeat domains, each of which consists of distinct tandem repeats (Fig. 1). Repeats were identified using the MEME/MAST motif discovery and search tool (http://meme.sdsc.edu). Repeat domain A (amino acids 498 to 3819) consists of 28 highly conserved tandem repeats of 117 residues. Domain A repeats can be subdivided into three distinct types of nearly perfect repeats. A_I and A_{II} share 96.6% sequence identity, and these repeats share 71.4% and 70.6% identity, respectively, with type A_{III}. The three subtype repeats within domain A are then built into larger blocks arranged in consecutive order (A_I-A_{II}-A_{III}), and this order itself is repeated multiple times (Fig. 1). The 14th repeat at the middle of this tandem array and the 28th repeat at the end, while still clearly related to the A repeat consensus, are less well conserved. Following domain A there is a short 252-residue region followed by another repeat region, domain B (amino acids 4072 to 6477), which consists of 19 highly conserved tandem repeats of 127 residues each. Domain B repeats are nearly 100% identical with the exception of the first and last repeats, which have 55% and 66% identity, respectively, with the consensus repeat (Fig. 1). While domain A and domain B repeats do not share clear sequence homology, compositional analysis shows that these domains share similarly skewed amino acid usages (Table 1). These regions are especially rich in asparagine and threonine but are deficient in methionine, arginine, and proline (highest-99% quantile and lowest-5% and lowest-1% quantiles in the Swiss-Prot database, respectively, as analyzed by SAPS [6]).

Additional repeats are present towards the C terminus: domain C (amino acids 7947 to 9071) consists of five repeats of approximately 225 amino acids, and domain D (amino acids 10210 to 10423) contains four repeats of approximately 52 amino acids. The repeats within these domains are less well conserved and do not exhibit the near-identical nature seen for domains A and B, but they are similar in that the first and last repeats of each tandem array are more degenerate. Additionally, these repeats show the same distinctive bias in amino acid composition as domains A and B.

Similarity searches were conducted using the entire sequence of *swmB* as well as each domain and each repeat separately. Using BLAST-P analysis (1), no obvious homologs of SwmB were found. Domains A and C of SwmB showed some similarity to RTX exotoxins, which are secreted, calcium-binding proteins that all share a common nonapeptide repeat (20). The sequence of SwmB, however, does not contain this RTX repeat.

In terms of its large size, repetitive architecture, and atypical amino acid usage (Table 1), SwmB resembles certain bacterial cell surface proteins such as *Staphylococcus aureus* Ebh (7, 10), *Pseudomonas fluorescens* LapA (11), and *Rickettsia rickettsii* rOmpA (12).

Motif searches were conducted on the full-length coding sequence of SwmB, on domains, and on individual repeats

---

* Corresponding author. Mailing address: Marine Biology Research Division, 3135 Hubbs Hall, 8750 Biological Grade, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92093-0202. Phone: (858) 534-7505. Fax: (858) 534-7313. E-mail: bbrahamsha@ucsd.edu.

Present address: Massachusetts Institute of Technology, Room 48-336, 15 Vassar Street, Cambridge, MA 02139.

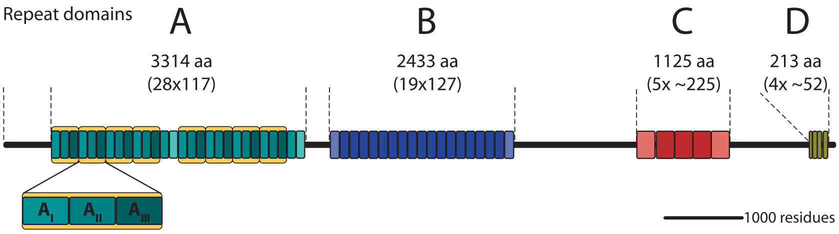

FIG. 1. Diagram of the SwmB primary sequence divided according to repetitive domains A to D. Domain A contains three repeat types sharing over 70% identity that are arranged into a larger unit (A_I-A_{II}-A_{III}) which is itself repeated. The central and C-terminal repeats in domain A as well as both terminal repeats in domains B and C are less well conserved than the central core repeats.

using proteomic and sequence analysis tools available through the ExPASy website (http://us.expasy.org). These searches failed to identify any known prokaryotic motifs, transmembrane helices, or putative secretion signal sequences.

SwmB protein identification. Whole cells, outer membrane (OM) fractions, and proteins concentrated from spent medium were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting. OMs were stripped from cells by treatment with EDTA essentially as described in reference 4. This treatment results in an insoluble OM fraction and a fraction consisting of periplasmic and soluble proteins released from the outer membrane by EDTA (high-speed supernatant). Proteins were separated on NuPAGE Novex Tris-acetate 3 to 8% gradient gels (Invitrogen, Carlsbad, CA) and stained with SYPRO Ruby (Sigma, St. Louis, MO). Rabbit polyclonal antibodies to SwmB obtained from spent medium and gel purified on 3 to 8% gradient gels were raised by Strategic Biosolutions (Newark, DE). Rabbit polyclonal antibodies to SwmA obtained from a high-speed supernatant fraction and gel purified on a 7.5% Tris-glycine SDS gel were raised by HRP, Inc. (now Covance Research Products, Denver, PA).

SDS-PAGE and Western analysis of whole cells, OM fractions, and proteins concentrated from spent media of *Synechococcus* sp. strain WH8102 all show the presence of a high-molecular-mass band of over 1 MDa (Fig. 2A, B, and C). Mass spectrometry analysis of this band identified four unique peptides present within the predicted SwmB sequence, confirming that this band is SwmB (data not shown). Periodic acid-Schiff staining did not detect glycosylation of SwmB (data not shown). Insertional inactivation of *swmB* results in cells that do not produce any detectable SwmB, as observed both on gels (Fig. 2B) and by Western analysis (Fig. 2C). Swm-2 cells do still produce SwmA with wild-type fractionation and abundance (Fig. 2B and D). In addition, strain S1A1, in which the *swmA* gene has been insertionally inactivated, still produces SwmB with wild-type abundance and cellular fractionation, as judged by Western analysis (Fig. 2A and B). Like the S-layer protein SwmA (4), SwmB is removed from cells by treatment with EDTA, which strips off the OM. It does not appear to be an integral outer membrane protein, as it purifies with the soluble fraction of OM (Fig. 2A) preparations. Furthermore, SwmB is found in abundance in spent medium. Sequence similarities of SwmB to RTX proteins, as well as the proximity of the *swmB* ORF to putative transporters (13), suggest how SwmB may be exported to the cell surface and medium. RTX proteins are the prototype substrate for the type I secretion pathway across the gram-negative cell envelope (3), which relies on a multicomponent system comprised of an ABC transporter, a periplasm-spanning membrane fusion protein, and an outer membrane protein. Type I secreted proteins are typically very acidic with a pI around 4 and have very few or no cysteine residues, and many transported proteins that do not contain the actual RTX nonapeptide repeat still contain other types of repeats (8). All of these characteristics apply to SwmB. In addition, an ABC transporter (SYNW0959) of the protein-1 exporter (Prot1E) family (http://www.tcdb.org/tcdb) and a membrane fusion protein (SYNW0958) are present on the low-percent-G+C region containing *swmB* (13).

Immunolocalization of SwmB. In order to localize SwmB, whole cells of wild-type *Synechococcus* sp. strain WH8102 and its isogenic insertional mutant strains S1A1 and Swm-2 were labeled with antibodies raised against SwmB or SwmA followed by a fluorescently labeled secondary antibody and visualized by deconvolution microscopy. Whole cells were fixed directly in SN medium for 30 min with electron microscopy-grade glutaraldehyde (Sigma) at a final concentration of 0.5%. After 30 min of fixation, cells were applied to poly-L-lysine (Sigma)-coated coverslips and incubated for 30 min. Coverslips were washed three times with phosphate-buffered saline (PBS) followed by blocking for 1 h at room temperature with PBS + 1%

TABLE 1. Amino acid usage analysis for several large, repetitive, cell surface prokaryotic proteins[a]

Protein	Amino acid usage in indicated quantile			
	Highest 99%	Highest 95%	Lowest 5%	Lowest 1%
SwmB domain A	N, T	A, D	R	M, P
SwmB domain B	N, S, T		M, P	R
SwmB domain C	T	V, D	K	M, R, H
SwmB domain D	D, V	L	M, R, F	H, G, S
S. aureus Ebh[b] repeats	N	A, Q, T	P, R	F
P. fluorescens LapA domain 2[c]	T	N, V	K, L	H, M, R
P. fluorescens LapA domain 3[c]	T, V			M, R
R. rickettsii rOmpA[d] repeats	N, T, V	A, G	Q, F	E, H, M, P, R, Y
Consensus	N, T, V		M, R, P	

[a] Amino acid usage analysis is based on reference 6.
[b] *Staphylococcus aureus* strain COL Ebh (7).
[c] *Pseudomonas fluorescens* strain WCS365 LapA (11).
[d] *Rickettsia rickettsii* rOmpA (2).

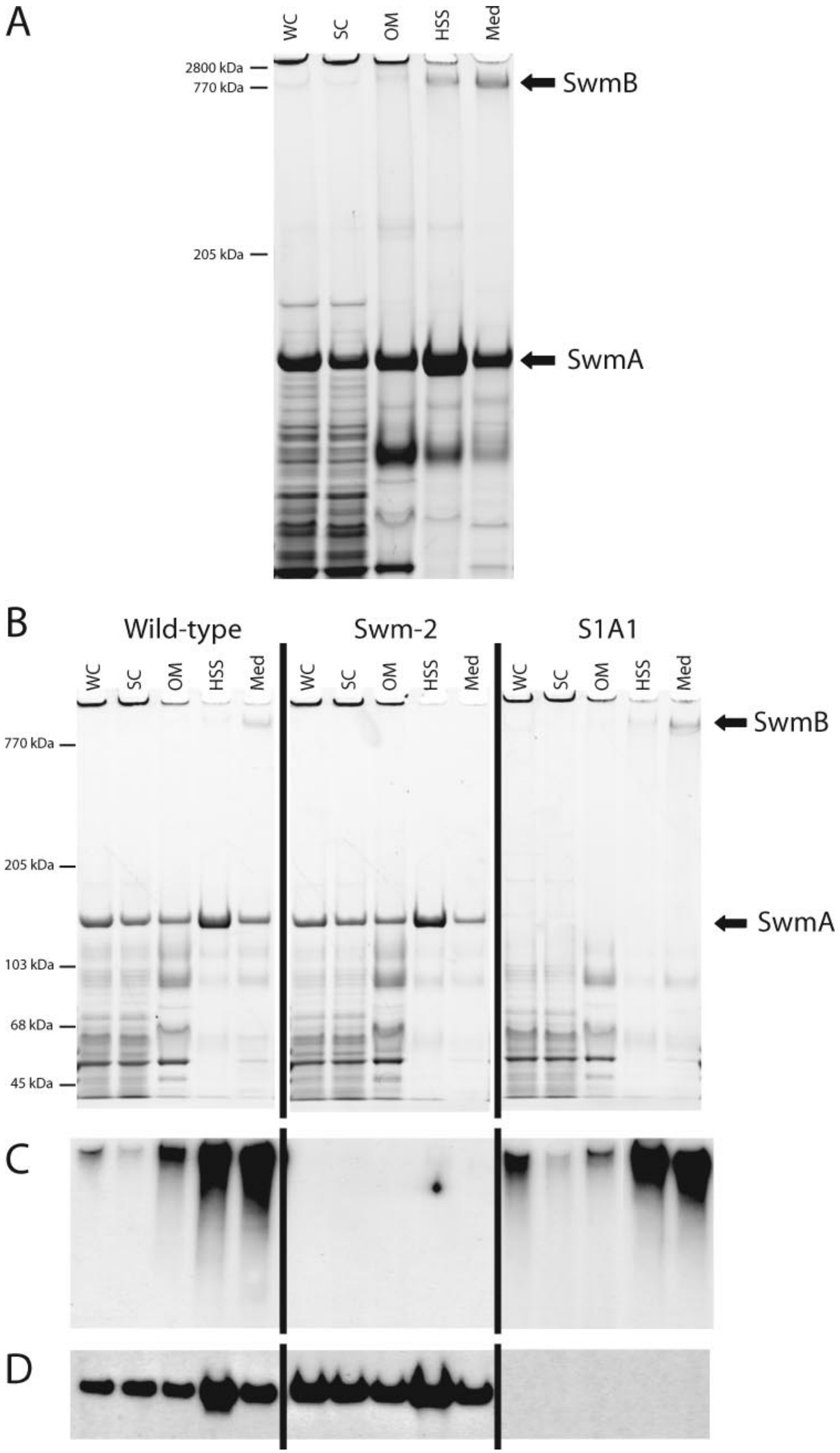

FIG. 2. (A) SYPRO Ruby-stained 3 to 8% gradient SDS-PAGE analysis of cellular fractions from wild-type *Synechococcus* sp. strain WH8102. WC: whole cells; SC, stripped cells; HSS, high-speed supernatant (the soluble fraction released by EDTA stripping); Med, spent culture medium. (B) Comparison of these cellular fractions from wild-type cells as well as from mutants Swm-2 and S1A1, in which *swmB* and *swmA*, respectively, have been insertionally inactivated. The amounts of material loaded for the gels and Westerns (C and D) were normalized to cell number so that equal numbers of cells or material purified from equal numbers of cells or concentrated from an equal volume of spent medium was loaded for all three strains. The migration of molecular mass standards is indicated on the left. Western analysis of cellular fractions using rabbit polyclonal antiserum raised against SwmB (used at 1:50,000) (C) or SwmA (used at 1:500,000) (D).

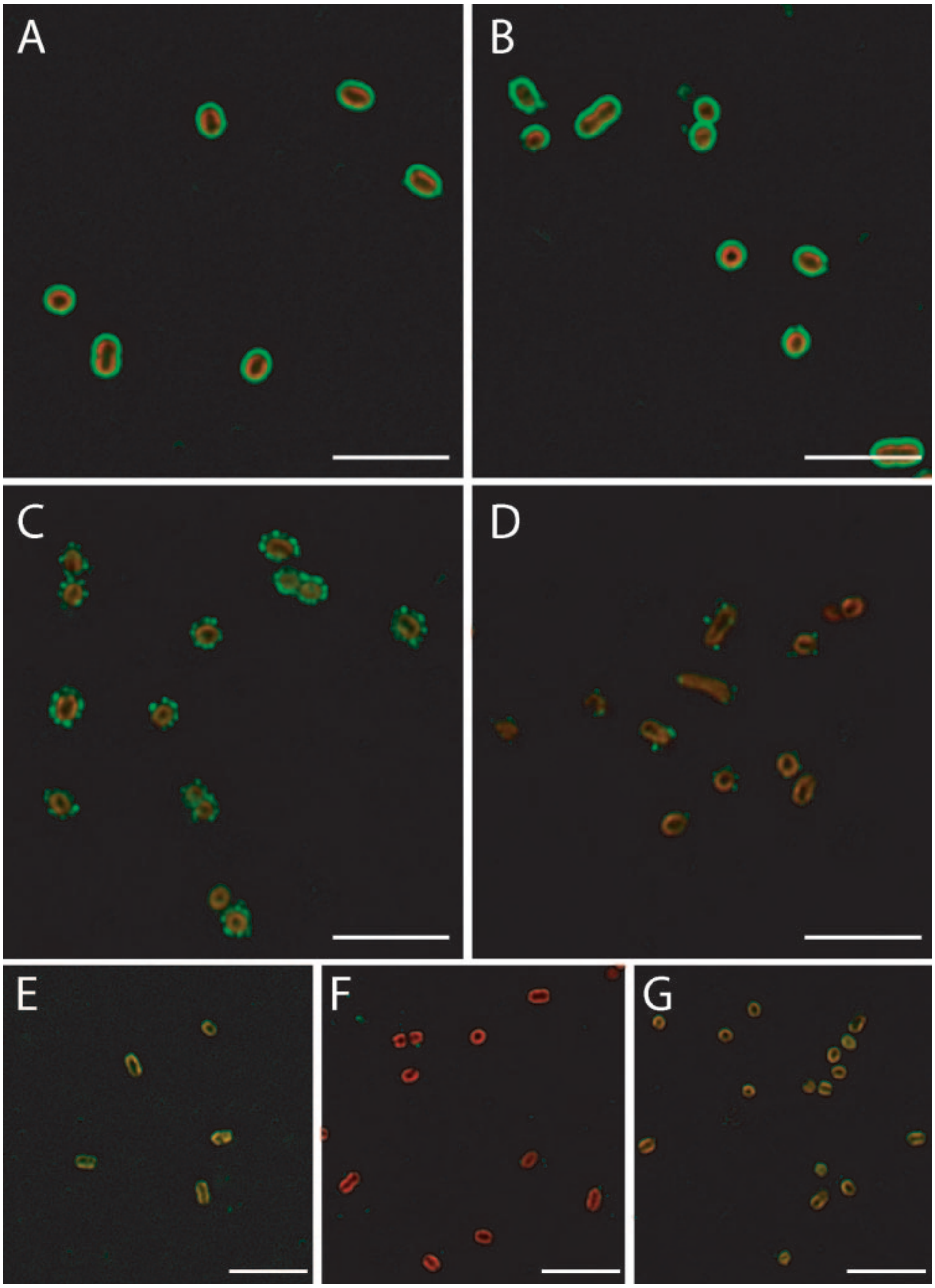

FIG. 3. Immunofluorescent labeling of SwmB and SwmA visualized by deconvolution microscopy. Red autofluorescence is from chlorophyll in the cytoplasmic thylakoid membranes. The green fluorescence is from the AlexaFluor 488-conjugated secondary antibody. SwmA is detected as a bright homogeneous layer in both wild-type (A) and Swm-2 (B) cells, while it is absent from S1A1 (E) cells. Wild-type cells labeled with anti-SwmB reveal the punctate cell surface distribution of SwmB (C), which is less extensive in S1A1 cells (D) and absent from Swm-2 cells (F). Wild-type cells labeled with preimmune antiserum exhibit no labeling (G). Bars, 5 μm (all panels).

gamma globulins (Sigma) + 1% normal goat serum (Sigma). Coverslips were incubated overnight at 4°C with primary antibodies diluted 1:25 in blocking solution. Following primary antibody incubation coverslips were washed nine times with PBS and once with blocking solution. Coverslips were then incubated with AlexaFluor 488-conjugated anti-rabbit immunoglobulin G (Molecular Probes, Carlsbad, CA) diluted 1:50 in blocking solution for 2.5 h at room temperature. Following another eight washes with PBS, samples were equilibrated for 5 min in Slow-Fade Light equilibration buffer (Molecular Probes). Following equilibration, 10 μl Slow-Fade Light (Molecular Probes) was applied to each coverslip prior to mounting. Paired images were collected on an Applied Precision optical sectioning microscope (Issaquah, WA) equipped with a rhodamine filter set (excitation, 555/28; emission, 617/73) to detect fluorescence from chlorophyll and a fluorescein isothiocyanate filter set (excitation, 490/20; emission, 528/38) to detect that from Alexa-488. Images were processed with softWoRx v3.3.6 software. SwmB is found on the cell surface with an irregular, punctate distribution (Fig. 3C). While SwmB has a patchy distribution, it is not localized exclusively to any one part of the cell. The distribution of SwmB revealed by immunofluorescence contrasts with that of SwmA, which appears as a bright homogeneous layer surrounding the cell (Fig. 3A), which is consistent with the fact that SwmA forms an S layer (14). Additionally, immunofluorescent localization reveals that Swm-2 cells possess SwmA with wild-type distribution (Fig. 3B). In order to determine whether the absence of the S-layer protein SwmA affected the localization of SwmB, the anti-SwmB antibody was used to label cells of strain S1A1, which do not produce SwmA. Incubation with the anti-SwmB antibody resulted in labeling similar in patchiness to that of the wild type, but the labeling was not as extensive (Fig. 3D). Because S1A1 cells produce SwmB at levels and with fractionation comparable to those of the wild type (Fig. 2B and C), the immunolabeling results suggest that SwmA may be required for the stable association of SwmB with the cell surface. Whether this is the case for live cells or whether this is due to the fact that in the absence of the SwmA layer SwmB may not be as efficiently cross-linked to the cell by glutaraldehyde during the immunostaining process remains to be determined.

The implication of a second cell surface protein in *Synechococcus* nonflagellar swimming further points to the importance of the cell surface in this still-mysterious phenomenon. Ehlers et al. (9) have proposed a model in which *Synechococcus* moves by generating traveling surface waves by the expansion and contraction of local regions of the outer membrane to create thrust such that the cell moves in the direction of the wave. SwmA appears to constitute the outermost cell layer in *Synechococcus* sp. strain WH8102 and hence may be the layer where such waves travel. The results of immunofluorescence suggest that SwmB is found at distinct foci distributed all over the cell and does not form a complete layer the way SwmA does. Perhaps SwmB somehow connects the SwmA layer to the sites in the outer membrane where the contractions are proposed to occur, or perhaps SwmB itself contracts. SwmB has a highly repetitive structure, which may be important for interaction with the highly repetitive S layer formed by SwmA. If such an interaction occurs, it is conceivable that conformational changes in SwmB could result in structural changes in the S layer (i.e., the mechanical deformations [15] or regions of localized contractions [9] previously proposed). Alternatively, active extrusion of SwmB may be required for swimming, as it is abundant in spent medium. Visualization of the behavior of SwmA and SwmB during swimming should help to address some of these questions.

We are grateful to Kit Pogliano for the use of her microscope for the deconvolution studies and thank both her and Stefan Aung for their advice on this technique. We thank Ross Hoffman and Justin Torpey of the UCSD Biochemistry Department Mass Spectrometry facility for mass spectrometry analysis.

This work was supported by grant DOE DE-FG03-O1ER63148. The Marine Biology Research Division of the Scripps Institution of Oceanography supported the publication costs.

REFERENCES

1. **Altschul, S. F., T. L. Madden, A. A. Schaeffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman.** 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. **25:**3389–3402.
2. **Anderson, B. E., G. A. McDonald, D. C. Jones, and R. L. Regnery.** 1990. A protective protein antigen of *Rickettsia rickettsii* has tandemly repeated, near-identical sequences. Infect. Immun. **58:**2760–2769.
3. **Blight, M. A., C. Chervaux, and I. B. Holland.** 1994. Protein secretion pathways in *Escherichia coli*. Curr. Opin. Biotechnol. **5:**468–474.
4. **Brahamsha, B.** 1996. An abundant cell-surface polypeptide is required for swimming by the nonflagellated marine cyanobacterium *Synechococcus*. Proc. Natl. Acad. Sci. USA **93:**6504–6509.
5. **Brahamsha, B.** 1999. Non-flagellar swimming in marine *Synechococcus*. J. Mol. Microbiol. Biotechnol. **1:**59–62.
6. **Brendel, V., P. Bucher, I. Nourbakhsh, B. Blaisdell, and S. Karlin.** 1992. Methods and algorithms for statistical analysis of protein sequences. Proc. Natl. Acad. Sci. USA **89:**2002–2006.
7. **Clarke, S. R., L. G. Harris, R. G. Richards, and S. J. Foster.** 2002. Analysis of Ebh, a 1.1-megadalton cell wall-associated fibronectin-binding protein of *Staphylococcus aureus*. Infect. Immun. **70:**6680–6687.
8. **Delepelaire, P.** 2004. Type I secretion in gram-negative bacteria. Biochim. Biophys. Acta **1694:**149–161.
9. **Ehlers, K. M., A. D. T. Samuel, H. C. Berg, and R. Montgomery.** 1996. Do cyanobacteria swim using traveling surface waves? Proc. Natl. Acad. Sci. USA **93:**8340–8343.
10. **Foster, T. J., and M. Hook.** 1998. Surface protein adhesins of *Staphylococcus aureus*. Trends Microbiol. **6:**484–488.
11. **Hinsa, S. M., M. Espinosa-Urgel, J. L. Ramos, and G. A. O'Toole.** 2003. Transition from reversible to irreversible attachment during biofilm formation by *Pseudomonas fluorescens* WCS365 requires an ABC transporter and a large secreted protein. Mol. Microbiol. **49:**905–918.
12. **Li, H., and D. H. Walker.** 1998. rOmpA is a critical protein for the adhesion of *Rickettsia rickettsii* to host cells. Microb. Pathog. **24:**289–298.
13. **McCarren, J., and B. Brahamsha.** 2005. Transposon mutagenesis in a marine *Synechococcus* strain: isolation of swimming motility mutants. J. Bacteriol. **187:**4457–4462.
14. **McCarren, J., J. Heuser, R. Roth, N. Yamada, M. Martone, and B. Brahamsha.** 2005. Inactivation of *swmA* results in the loss of an outer cell layer in a swimming *Synechococcus* strain. J. Bacteriol. **187:**224–230.
15. **Pitta, T. P., and H. C. Berg.** 1995. Self-electrophoresis is not the mechanism for motility in swimming cyanobacteria. J. Bacteriol. **177:**5701–5703.
16. **Price, N. M., G. I. Harrison, J. G. Hering, R. J. Hudson, P. M. V. Nirel, B. Palenik, and F. M. M. Morel.** 1988/89. Preparation and chemistry of the artificial algal culture medium Aquil. Biol. Oceanogr. **6:**443–461.
17. **Toledo, G., B. Palenik, and B. Brahamsha.** 1999. Swimming marine *Synechococcus* strains with widely different photosynthetic pigment ratios form a monophyletic group. Appl. Environ. Microbiol. **65:**5247–5251.
18. **Waterbury, J. B., and J. M. Willey.** 1988. Isolation and growth of marine planktonic cyanobacteria. Methods Enzymol. **167:**100–105.
19. **Waterbury, J. B., J. M. Willey, D. G. Franks, F. W. Valois, and S. W. Watson.** 1985. A cyanobacterium capable of swimming motility. Science **230:**74–76.
20. **Welch, R. A., C. Forestier, A. Lobo, S. Pellett, W. Thomas, Jr., and G. Rowe.** 1992. The synthesis and function of the *Escherichia coli* hemolysin and related RTX exotoxins. FEMS Microbiol. Immun. **105:**29–36.