Structure and Function of Gli123 Involved in *Mycoplasma mobile* Gliding

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ABSTRACT  *Mycoplasma mobile* is a fish pathogen that glides on solid surfaces by means of its own gliding machinery composed of internal and surface structures. In the present study, we focused on the function and structure of Gli123, a surface protein that is essential for the localization of other surface proteins. The amino acid sequence of Gli123, which is 1,128 amino acids long, contains lipoprotein-specific repeats. We isolated the native Gli123 protein from *M. mobile* cells and a recombinant protein, rGli123, from *Escherichia coli*. The isolated rGli123 complemented a nonbinding and nongliding mutant of *M. mobile* that lacked Gli123. Circular dichroism and rotary-shadowing electron microscopy (EM) showed that rGli123 has a structure that is not significantly different from that of the native protein. Rotary-shadowing EM suggested that Gli123 adopts two distinct globular and rod-like structures, depending on the ionic strength of the solution. Negative-staining EM coupled with single-particle analysis revealed that Gli123 forms a globular structure featuring a small protrusion with dimensions of approximately 15.7, 14.7, and 14.1 nm for the “height,” major axis and minor axis, respectively. Small-angle X-ray scattering analyses indicated a rod-like structure composed of several tandem globular domains with total dimensions of approximately 34 nm in length and 6 nm in width. Both molecular structures were suggested to be dimers, based on the predicted molecular size and structure. Gli123 may have evolved by multiplication of repeating lipoprotein units and acquired a role for Gli521 and Gli349 assembly.

IMPORTANCE  Mycoplasmas are pathogenic bacteria that are widespread in animals. They are characterized by small cell and genome sizes but are equipped with unique abilities for infection, such as surface variation and gliding. Here, we focused on a surface-localizing protein named Gli123 that is essential for *Mycoplasma mobile* gliding. This study suggested that Gli123 undergoes drastic conformational changes between its rod-like and globular structures. These changes may be caused by a repetitive structure common in the surface proteins that is responsible for the modulation of the cell surface structure and related to the assembly process for the surface gliding machinery. An evolutionary process for surface proteins essential for this mycoplasma gliding was also suggested in the present study.

KEYWORDS electron microscopy, small-angle X-ray scattering, repeat sequence, single-particle analysis, mollicutes, lipoprotein, conformational change, motility
Class Mollicutes, which includes the genus Mycoplasma, consists of organisms fit to parasitic or commensal life cycles, as represented by their small genome size, lack of peptidoglycan layer, and antigenic modulation (1–3). Interestingly, more than 20 Mycoplasma species are known to glide on solid surfaces, such as animal cells and glass surfaces, through unique mechanisms, and this is thought to be beneficial for infection (4). Mycoplasma gliding mechanisms can be classified into two types, as represented by either Mycoplasma mobile or Mycoplasma pneumoniae (4, 5, 6). Four species are known to perform mobile-type gliding, including M. mobile, Mycoplasma pulmonis, Mycoplasma testudineum, and Mycoplasma agassizii, which serve as pathogens for freshwater fish, mice, turtles, and turtles, respectively (7, 8). The gliding mechanism has been studied with a focus on M. mobile, which glides at speeds of 2.0 to 4.5 μm/s (4, 9). The gliding machinery, which forms as a protrusion, can be divided into two parts: the inside and the surface structures (4, 10, 11). Its surface structure is composed of four proteins, Gli123, Gli349, Gli521, and Gli42. Gli123, Gli349, and Gli521 have been reported as being essential for binding and gliding, based on analyses of mutants missing each of these proteins (12–14). Gli349 is a flexible rod-shaped, 95-nm-long protein with a C-terminal globular domain (12, 15, 16). It catches sialylated oligosaccharides on the surfaces of hosts as a “leg” for gliding and pulls the cell (17, 18). Gli521 is a rather rigid rod-shaped 120-nm-long protein with N-terminal globular and C-terminal hook domains (19, 20) and is suggested to transmit the gliding force generated in an internal motor to Gli349, like a “crank” (5, 19, 20). Gli123 localizes Gli349 and Gli521 correctly to the gliding machinery, as a “mount” (14). Gli349 and Gli521 are conserved in all four species of M. mobile, M. pulmonis, M. testudineum, and M. agassizii, whereas Gli123 and Gli42 are only found in M. mobile and M. pulmonis (21). In this study, we report that incubation of the purified Gli123 protein with a mutant lacking this protein considerably restored the gliding motility of this mutant. We analyzed Gli123 structures using electron microscopy (EM), small-angle X-ray scattering (SAXS), and other biochemical methods, and we suggest a plausible structural model and its conversion, which may be related to the role of the protein in gliding motility.

RESULTS

Gli123 orthologs in the Mycoplasma species. Orthologs of gli123 and gli42 genes are not found in M. agassizii and M. testudineum, unlike the orthologs of nine other genes involved in M. mobile gliding (21). The orthologs of gli349 and gli521 from M. testudineum and M. agassizii have been found to be positioned closer to M. pulmonis than to M. mobile in the phylogenetic tree (21). Therefore, we performed a PSI-BLAST search (22) using the amino acid sequences of Gli123 and Gli42 of M. pulmonis, and we found the orthologs of gli123 and gli42 in the genomes of M. agassizii and M. testudineum (see Table S1 in the supplemental material). The phylogenetic trees constructed for gli123, gli42, gli349, and gli521 genes using the maximum likelihood method shared their topology, as did the phylogenetic tree of 16S ribosomal DNA sequences (Fig. S1) (21). Next, we examined the localization of these genes in the genome. In M. mobile and M. pulmonis, the genes were clustered in one locus (14, 21), while in M. agassizii and M. testudineum, they were split into two loci, gli123-gli42 and gli349-gli521, although the actual distances are unknown in the unassembled genome sequences (Fig. S2).

Sequence analysis of Gli123 and the lipoprotein-17 domain. In this study, we found that the amino acid sequences of Gli123 protein and its orthologs include four to six lipoprotein-17 domains, which are composed of approximately 100 amino acids (Fig. 1A) (23, 24). The lipoprotein-17 domain is found in 233 proteins of 43 bacterial species, mostly in lipoproteins that play a role in antigenic modulation or adhesion. In Mycoplasma, the lipoprotein-17 domain was identified in 221 proteins from 32 species (Table S2). This domain was found in 15 Mvsp (mobile variable surface proteins), Gli123, and Gli349 in M. mobile (24, 25). The Gli123 protein of M. mobile has four lipoprotein-17 domains, while that of M. pulmonis and M. testudineum have five and that of M. agassizii has six (Fig. 1A and Table S3). Mvsp s have a number of lipoprotein-17 domains, up to 16 (Fig. 1A and Table S3). This may suggest that the surface proteins adjust their lengths by means of repetitive fusion of the domain (25–27). We searched
the lipoprotein-17 domain by using a hidden Markov model, because it is not influenced by sequence length. The scores for the lipoprotein-17 domain were distributed between 30 and 70 for the Mvsps repeats and between 10 and 30 for the Gli123 and Gli349 repeats (Fig. 1B).

Mvspl mutant strains of *M. mobile*. As the lipoprotein Mvspl, encoded by MMOB3340, was fractionated with broad distribution in each isolation step, a long process was necessary to remove it from the Gli123 fraction. Therefore, we isolated Gli123 protein from an Mvspl-lacking strain (25). To isolate a spontaneous Mvspl-lacking mutant of *M. mobile*, we screened colonies that were nonreactive to a monoclonal antibody against Mvspl (Fig. S3A). Two colonies that were nonreactive to the antibody were isolated from approximately 10,000 colonies. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of the isolate revealed a lack of Mvspl protein (Fig. S3B). The band intensity of the surface protein MvspF, encoded by MMOB3300, was higher in the isolated mutant than in the wild-type (WT) strain (Fig. S3B). Genome analysis revealed that the mvspl gene of the isolate had a frameshift mutation that resulted in a truncated peptide of 97 amino acids (Table S4). In addition to mutations in the mvspl gene, nine other mutations were
identified in mvspl genes and others (Table S4). This mutant grew constantly through serial cultivation of large volumes, without recovery of Mvspl. The mutant cells glided normally but the culture tended to form clumps, suggesting different surface interactions from those of the WT. This strain was named the Mvspl-lacking mutant strain and used in subsequent experiments.

**Isolation of Gli123 from M. mobile.** We isolated the nGli123 protein from cultured cells of the M. mobile Mvspl-lacking mutant strain (Fig. 2A). The collected cells were suspended in a buffer containing 1% Triton X-100 and treated on ice for 1 h. Following
that, the lysates were separated by means of centrifugation into a precipitate and a soluble fraction containing nGli123, Gli349, Gli521, and MMOB1650, a component of the internal structure of the gliding machinery (28) (Fig. 2B). The soluble fraction was then subjected to anion-exchange chromatography. The nGli123 protein was bound to an anion-exchange column with 20 mM NaCl and eluted using a gradient of NaCl. Gli521 and MMOB1650 were separated from nGli123 by means of this elution process (Fig. 2B). Next, we subjected the fractions containing nGli123 and Gli349 to size exclusion chromatography (SEC). The proteins, which were traced by means of absorbance, were divided into four peaks, other than the void-volume fraction (Fig. 2C). SDS-PAGE and mass spectrometry identified nGli123 in the second peak near the position of the 150-kDa elution (Fig. 2B and C). The Stokes radius of nGli123 was calculated as 5.4 nm from the elution position (Fig. 2C). The final yield of the nGli123 protein was 0.3 mg, isolated from 3 liters of culture. The N-terminal amino acid sequence of the nGli123 protein band collected from the electrophoresis of the whole-cell lysate was analyzed using Edman degradation and determined to be Ala-Ile-Ala-Ile-Gly, thereby showing that the N terminus of 18 amino acids, including the partial transmembrane segment of 11 amino acids, is truncated in the mature protein (Fig. 2A) (14). This result suggested that Gli123 is a peripheral membrane protein rather than a transmembrane protein.

**Expression and purification of recombinant Gli123.** The yield of nGli123 was not high enough to perform various experiments. Therefore, we expressed recombinant Gli123 (rGli123) with a 6×His tag at the C terminus of mature Gli123 in E. coli (Fig. 2A) (14) and purified it using three steps: (i) nickel-nitrilotriacetic acid (Ni2+−NTA) affinity chromatography, (ii) hydrophobic interaction chromatography, and (iii) SEC (Fig. 2B). The purified fraction was recovered by means of SEC as a single peak that included only the rGli123 protein, which was then confirmed using SDS-PAGE and visualized using Coomassie brilliant blue (CBB) staining and mass spectrometry (Fig. 2B and C). The Stokes radius of rGli123 was estimated as 5.4 nm, from the elution position of SEC (Fig. 2C), which agreed with that for nGli123 isolated from M. mobile cells. The final yield of the rGli123 protein was approximately 10 mg per liter of E. coli culture. The circular dichroism spectrum of rGli123 showed secondary structural contents of 14.0% α-helices and 35.1% β-sheets (Fig. 2D) (29, 30), consistent with the predictions from the amino acid sequence using XtalPred, i.e., 14.0% α-helices and 43.0% β-sheets. The purified rGli123 protein was subjected to limited proteolysis with trypsin, to identify the flexible and rigid parts (Fig. 2A). The SDS-PAGE profile of the cleaved fragment showed that the rGli123 protein finally converged into three products through several fragmentation steps (Fig. S4A). Mass spectrometry results showed that the three final fragments (f, g, and h) corresponded at least to the sequences for amino acids 405 to 1128 (+8), 565 to 1128 (+8), and 559 to 789, respectively. These results suggested that some components of the molecule are rigid, as presented in Fig. 2A.

**Complementation of the binding and gliding abilities of the M. mobile mutant with additional Gli123 proteins.** To examine the complementation activity of Gli123 for the Gli123-lacking mutant, m12 (14), we added purified rGli123 protein to the cell suspension and incubated it for 3 h at 25°C. The cells were inserted into the tunnel slides with video recordings of phase-contrast optical microscopy (31). m12 cells were unable to bind to the glass surface (14), whereas cells with rGli123 protein showed binding to the glass surface, indicating that Gli123 contributes to efficient and robust binding of M. mobile to glass surfaces (Fig. 3A). The average number of bound cells in a 100-μm² field on glass was 15.1 ± 3.4 cells (n = 15, mean ± standard deviation), which was 69.1% of that of the WT. A mean of 14.9 ± 8.5% of the bound cells showed unidirectional gliding, similar to the WT strain, with a speed of 1.5 ± 0.8 μm/s (n = 45), comparable with that of the WT strain, 2.7 ± 0.5 μm/s (n = 45) (Fig. 3B and C), suggesting that the rGli123 protein binds and complements the gliding machinery (14). Next, we examined the binding of the rGli123 proteins to the mutant cells. Mutant cells incubated with rGli123 were recovered by means of centrifugation and analyzed using SDS-PAGE (Fig. 3D). The Gli123 band was found in the cell fraction with an intensity...
58% of the WT protein, although rGli123 did not precipitate by centrifugation, showing that rGli123 bound to the mutant cells.

**Conformational changes in Gli123.** We observed the structures of the nGli123 and rGli123 proteins using rotary-shadowing EM. In rotary-shadowing EM, protein molecules sprayed on a rotating mica surface are shadowed by platinum particles from a low angle, to observe their molecular shape (20, 25). We observed particles of similar sizes and shapes in the field of view. The nGli123 and rGli123 proteins showed similar rod-like structures, with lengths of 35.6 ± 5.0 and 35.9 ± 4.1 nm, respectively, under conditions, including 100 mM ammonium acetate (Fig. 4A and B). Next, we observed the nGli123 and rGli123 proteins in the presence of 500 mM ammonium acetate. Interestingly, both proteins showed globular structures with long axes of 21.0 ± 3.2 and 20.1 ± 4.1 nm (Fig. 4A and B). We then examined the molecular ratios of the two structures in 100, 250, and 500 mM ammonium acetate. The proportions of rod-like structures at each concentration were 89.7%, 47.0%, and 5.7%, respectively, and the ratio changed from rod-like to globular structures, depending on the ammonium acetate.
FIG 4  Ionic strength-induced conformational changes in the nGli123 and rGli123 proteins. (A and B) Field images of the nGli123 (A) and rGli123 (B) proteins under the conditions of 100 mM (left) and 500 mM (right) ammonium acetate. Scale bar, 50 nm. The representative particle images are magnified at the bottom. Scale bar, 10 nm. The distributions of diameter and length are shown for the globular and rod-like structures on the right. (C) Ratio of globular and rod-like structures of the rGli123 protein under the conditions of 100, 250, and 500 mM ammonium acetate. (D) Light scattering of rGli123 proteins, as a function of ammonium acetate concentration.
concentration (Fig. 4C). Furthermore, we measured the light scattering of rGli123. Light scattering intensity measured at different concentrations increased with ammonium acetate concentration, in the range of 50 to 650 mM, suggesting that the conformation of rGli123 molecules depended on the concentration of ammonium acetate (Fig. 4D). The 50% effective concentration (EC₅₀) was 216.0 ± 11.3, 238.3 ± 16.1, and 292.9 ± 4.9 mM at protein concentrations of 0.25, 0.75, and 1.23 mg/mL, respectively (Fig. 4D). The changes in the light scattering intensities of rGli123 proteins due to changes in ionic strength were in good agreement with the ratios of globular and rod-like structures measured using rotary-shadowing EM (Fig. 4C and D).

**Globular structure of Gli123.** Negative-staining EM was performed to visualize the three-dimensional structure of globular nGli123 with better resolution. The field images of EM showed uniform globular particles of 15 to 20 nm (Fig. 5A), and their density changed depending on the protein concentration used. In addition, many rod-like structures were observed in the background. We selected globular particles (Fig. 5A) and obtained two-dimensional (2D) averaged images using the structural analysis software EMAN2.91 (Fig. 5B). Based on the averaged image of the Gli123 protein, a 3D image was reconstructed, without (Fig. 5C, C1) and with (C2) the two-rotation symmetry enforcement. Our data
suggested that Gli123 forms a dimeric molecule; however, the image quality was not improved by the symmetry enforcement. This may have been related to the quality of the negative-staining image. The 3D-reconstructed globular structure of Gli123 had dimensions of approximately 15.7, 14.7, and 14.1 nm for the height, major axis, and minor axis, respectively, with a protrusion that was reminiscent of a spinning top (Fig. 5C). Considering the globular molecular shape, the molecular weight of Gli123 was estimated to be 244 kDa from the elution position of SEC, indicating that the protein behaved as a dimer (Fig. 2C). Furthermore, the C2 model fit to the structure of two rGli123 molecules predicted from the amino acid sequence using Robetta (Fig. 5D).

**Rod-like structure of Gli123.** SAXS was used to better obtain the 3D rod-like structure of Gli123. The rGli123 protein under low ionic strength conditions was subjected to SEC-SAXS. The protein showed an elution curve with a single peak (Fig. 6A and Fig. S5A). SAXS frames corresponding to the elution peak were averaged, background subtracted, and used for further processing. We analyzed the scattering curve with a Guinier plot \([\ln I(Q) \text{ versus } Q^2]\), where \(I(Q)\) is the scattering intensity at scattering vector \(Q\), to estimate the size of the rGli123 protein. Guinier approximation is based on the scattering curve at small angles, and the square of the radius of gyration \(R_g\) was determined as the slope of the linear fit in the Guinier region \((R_gQ < 1.3)\) as \(\ln I(Q) = \ln I(0) - R_g^2Q^2/3\), where \(I(0)\) is the zero-angle scattering intensity (32). \(R_g\) of rGli123 was estimated as 8.2 nm from the Guinier approximation (Fig. 6B). This value was unchanged within experimental errors, even when the SAXS frames before and after the elution peak were used (Fig. 5S5B). Furthermore, analysis of each SAXS frame around the elution peak, each containing different concentrations of Gli123, showed that the \(R_g\) was almost independent of the protein concentration (Fig. S5C) and that the \(I(0)\) was proportional to the protein concentration (Fig. 5S5D), indicating the absence of interparticle interference effects and protein aggregation. Next, to discuss the shape of the rGli123 protein, the scattering curve was analyzed using a Kratky plot \([I(Q)Q^2 \text{ versus } Q]\) (Fig. 6C), which showed the characteristics of the protein shape (33). The scattering intensity from a solid body decays at high angles as \(I(Q)\) approaches \(\sim Q^{-4}\), conferring a bell-shaped Kratky plot with a well-defined maximum (34). The plot showed a mountainous curve with a peak around \(Q = 0.1\) Å\(^{-1}\), suggesting that the rGli123 protein was not in a random coil but had a folded structure. For an ideal sphere, the Kratky plot showed a hyperbolic curve with a single peak in the small-angle regions. However, with the present data, the plot showed a shoulder around \(Q = 0.02\) Å\(^{-1}\) (Fig. 6C, marked by an inverted black triangle), suggesting the existence of another hidden peak. The bimodal character of the Kratky plot suggested a dumbbell shape for the protein; the innermost shoulder reflected the globularity of the whole molecule, while the peak reflected that of each domain (35). The pair-distance distribution function, \(P(r)\), which has an almost-Gaussian-like distribution for a spherical protein, showed a skewed distribution for rGli123, suggesting a rod-like structure (31). The \(P(r)\) function also showed that the maximum length \((D_{max})\) of the rGli123 protein was approximately 31.6 nm (Fig. 6D). The 3D structure of the rGli123 protein was modeled from SAXS data using the DAMMIF program for \(ab\) \(initio\) reconstruction from the PrimusQT software (36). The structural model was nod-shaped with total dimensions of approximately 34 nm length and 6 nm width and featured several narrow parts, similar to an articulated robot (Fig. 6E). This structure was consistent with the images obtained using rotary-shadowing EM with low ionic intensity (Fig. 4B). In the cross-section plot \([\ln I(Q)/Q \text{ versus } Q^2]\), which tests the validity of the rod-like structure in the SAXS model (37), the slope of the plot showed high linearity (Fig. 5E). The radius of gyration of cross-section \(R_c\) was 1.14 nm, as calculated using the equation \(R_c = \sqrt{\langle R_g^2 \rangle}\) (38). The radius of a cylinder \(R_y\) (a short-axis radius) was 1.61 nm, as calculated using the equation \(R_y = \langle \sqrt{2}R_g \rangle\) (38). The height of the cylinder, \(L\) (twice the major axis radius), determined from the equation \(R_n^2 = R_y^2/2 + L^2/12\) (38), was 28.2 nm. The \(L\) value obtained from the cross-section plot was consistent with the length \((D_{max} = 31.6\) nm) estimated from the \(P(r)\) function, indicating the high validity of the rod-like shape of the SAXS model. The molecular weight of rGli123 estimated from the Porod volume was 272.8 kDa, which corresponded to the molecular weight of a dimer,
supporting that the rod-like structure is a dimer (Table S5) (32, 39, 40). Furthermore, the SAXS model fit to the structure of two rGli123 molecules predicted from the amino acid sequence using Robetta (Fig. 6F). SAXS measurements were also performed using static cells at three different concentrations of rGli123 (Fig. S7). These results were consistent with those obtained from the SEC-SAXS measurements and supported the absence of aggregation in the SAXS measurements.

**DISCUSSION**

**Surface gliding proteins.** In this study, gli123 and gli42 genes were found in the genomes of *M. testudineum* and *M. agassizii* (Table S3), indicating that the two surface proteins of *M. mobile*, Gli123 and Gli42, are also essential for gliding, similar to Gli349 and Gli521 (21). The topologies of phylogenetic trees were common for all these proteins and 16S rDNA (21), suggesting that these mycoplasmas acquired gliding motility in evolution,
not through horizontal transfer (Fig. S1). In addition, the orthologs of gli521 were encoded just downstream of gli349 orthologs with only 14- to 52-nucleotide gaps in gliding mycoplasmas (Fig. S2) (21). Considering that the cellular localization of Gli349 depends on that of Gli521 (14), these gene alignments suggested that they may form a complex immediately after their synthesis.

**Structure and conformational changes of Gli123.** We isolated the Gli123 protein and found that its molecular structure changed from rod-like to globular depending on the ionic strength. This conformational change may occur in the flexible regions identified through limited proteolysis (Fig. S4). A pair of Gli123 molecules predicted from the amino acid sequence fit into both the rod-like structure obtained using SAXS and the globular structure obtained by means of single-particle analysis, suggesting that these structures of Gli123 are dimers (Fig. 5D and 6F). The structure of the isolated molecule changed drastically with ionic strength; however, *M. mobile* can glide under different ionic strength environments, suggesting that this conformational change is unlikely to occur in the completed machinery (14). We observed that the purified rGli123 protein complemented the gliding of mutant cells lacking Gli123. Conformational changes in the Gli123 molecule may be related to this observation. These three gliding proteins have a localization hierarchy of Gli123-Gli521-Gli349, suggesting a physical interaction between these proteins at the cell surface (14). Gli123 might reach some parts of the target position on the cell surface as a flexible rod and help Gli521 and Gli349 molecules to function after its drastic conformational change, which is induced by the electric fields made by the neighboring proteins, Gli349 and Gli521.

**Roles of the lipoprotein-17 domain.** We found that a common repeat sequence of the lipoprotein-17 domain was shared by the Mvsp and gliding proteins. The relationship between the gliding machinery and Mvsp has been proposed based on the existence of a repeat comprising of approximately 100 amino acids (23, 25). In this study, we succeeded in determining the similarity between the repeat sequences. The existence of the lipoprotein-17 domain in Gli123, Gli349, and their orthologs suggests that they originated from a common surface protein (Fig. 1A and 7A). The structure of the lipoprotein-17 domain in Gli123 and Mvsp predicted using AlphaFold2 featured three β-strands, which was consistent with previously reported structural features determined using nuclear magnetic resonance and crystallography (Fig. S8A and B) (23). The predicted Mvsp structures suggested that an α-helix with a short loop connects the lipoprotein-17 domains to form a flexible chain (Fig. S8C). Although the detailed structures of Gli123 and Gli349 are still unknown, their flexibility may be derived from the structures common with the lipoprotein-17 domain and adjacent regions. Lipoprotein-17 domain may be useful for adjusting molecular length and flexibility (Fig. 7A). It may provide length variation adjustment to Mvsp and Gli349, which are involved in antigenic modulation and the gliding leg, respectively. Flexible and long leg proteins may be advantageous for catching the host cell surface (18). In the case of Gli123, it may provide length and flexibility for its role in localizing other surface gliding proteins on the machinery surface, where as many as 450 gliding units are packed (Fig. 7B). The gliding units fixed by Gli123 can be aligned in an ordered way for smooth and efficient gliding (14, 31).

**MATERIALS AND METHODS**

**Sequence analyses.** The genome sequences of *M. mobile* (163K), *M. pulmonis* (UAB CTIP), *M. agassizii* (PS6), and *M. testudineum* (ATCC 700618) were obtained from the National Center for Biotechnology Information (NCBI) GenBank database (https://www.ncbi.nlm.nih.gov/genome/). A homology search against the NCBI nonredundant protein sequence databank was then performed using PSI-BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi), with an expected threshold of 100. Phylogenetic trees of the proteins were constructed using neighbor-joining and maximum likelihood methods. The reliability of the topology of each phylogenetic tree was examined with 1,000 bootstrap replicates, using the MEGAX package (https://www.megasoftware.net/). The secondary structure of Gli123 was predicted using XtalPred (https://xtalpred.godziklab.org/XtalPred-cgi/xtalpld). Hidden Markov model scores were obtained from Pfam (http://pfam.xfam.org/family/PF04200) and UniProtKB (https://www.uniprot.org/help/uniprotkb), based on the HMMER3 package (http://hmmer.org/).
Strains and culture conditions. *M. mobile* 163K (ATCC 43663) was grown at 25°C in Aluotto medium to an optical density at 600 nm (OD600) of around 0.08, as previously described (41). The *M. mobile* MvspI mutant strain was isolated as follows. Mutants that did not react with a monoclonal antibody against MvspI were selected using colony blotting and amido black staining (42). The colonies were isolated and cultured in Aluotto liquid medium, following which SDS-PAGE analysis of the isolates was carried out, which confirmed the absence of MvspI protein.

*E. coli* DH5α and BL21(DE3) strains were used for DNA recombination and protein expression, respectively.

Isolation of Gli123 from *M. mobile* cells. The nGli123 protein was purified from the *M. mobile* MvspI mutant strain cells. Cells stored at −80°C were precultured and grown in 3 liters of Aluotto liquid medium, dispensed into nine 300-cm² tissue culture flasks, and statically cultured at 25°C until the OD600 reached 0.08. The cells were collected by means of centrifugation at 18,800 × g and 4°C, for 30 min, and washed twice with phosphate-buffered saline (PBS; 58 mM Na₂HPO₄, 17 mM NaH₂PO₄, 68 mM NaCl). The precipitate was suspended in 30 mL of buffer A (20 mM Tris-HCl [pH 8.0], 0.1 mM phenylmethylsulfonyl fluoride) and sonicated on ice for 2 min, at intervals of 5 s, using a sonicator (US-600T, Nippon Seiki Co., Niigata, Japan). The cell lysate was combined with 30 mL of buffer A containing 2% Triton X-100 (vol/vol) and shaken on ice for 2 min, at intervals of 5 s, using a sonicator (US-600T, Nippon Seiki Co., Niigata, Japan). The cell lysate was separated by means of centrifugation at 32,300 × g and 4°C, for 30 min, following which the obtained supernatant was applied to an anion-exchange column (HiTrap Q HP 5 mL; Cytiva, Tokyo, Japan) equilibrated with buffer A. Proteins bound to the column were eluted with an NaCl concentration gradient of 0 to 500 mM, at 4°C. The eluted fraction containing Gli123 was concentrated to a volume of 10 mL and subjected to an SEC column (HiLoad 26/600 Superdex 200 prep grade, Cytiva) equilibrated with SEC buffer (20 mM Tris-HCl [pH 8.0] and 150 mM NaCl), at 4°C. Ferritin (440 kDa), aldolase (158 kDa), conalbumin (75 kDa), and ovalbumin (44 kDa), with Stokes radii of 6.1, 4.81, 3.64, and 3.05 nm, respectively, were used as standards (gel filtration calibration kits, Cytiva). Protein fractions were analyzed using SDS-PAGE with a 10% polyacrylamide gel, followed by CBB staining.

Expression and purification of recombinant Gli123. The N-terminal sequence of Gli123 was determined using Edman degradation, as previously described (12, 13). According to the sequencing results, the DNA sequence encoding the amino acid region from the positions 19 to 1128 was codon-optimized for expression in *E. coli* and inserted between the BamHI and XhoI sites in the multicloning site of the pET21a plasmid (Novagen, Madison, WI). The recombinant protein, rGli123, was expressed in *E. coli* BL21(DE3) and induced using 1 mM isopropylthio-β-galactoside at 15°C, for 18 h. Following that, the cells were collected, suspended in His buffer A (20 mM Tris-HCl [pH 8.0] and 20 mM NaCl), and washed twice. The cells were suspended in 60 mL of His buffer A containing 2% Triton X-100 (vol/vol) and shaken on ice for 1 h, to solubilize the proteins. The cell lysate was separated by means of centrifugation at 32,300 × g and 4°C, for 30 min, following which the obtained supernatant was applied to an anion-exchange column (HiTrap Q HP 5 mL; Cytiva, Tokyo, Japan) equilibrated with buffer A. Proteins bound to the column were eluted with an NaCl concentration gradient of 0 to 500 mM, at 4°C. The eluted fraction containing Gli123 was concentrated to a volume of 10 mL and subjected to an SEC column (HiLoad 26/600 Superdex 200 prep grade, Cytiva) equilibrated with SEC buffer (20 mM Tris-HCl [pH 8.0] and 150 mM NaCl), at 4°C. Ferritin (440 kDa), aldolase (158 kDa), conalbumin (75 kDa), and ovalbumin (44 kDa), with Stokes radii of 6.1, 4.81, 3.64, and 3.05 nm, respectively, were used as standards (gel filtration calibration kits, Cytiva). Protein fractions were analyzed using SDS-PAGE with a 10% polyacrylamide gel, followed by CBB staining.

FIG 7 Role of lipoprotein-17 domain repeats and Gli123. (A) The repeat sequence is possibly used to adjust the length and flexibility of surface molecules that are involved in antigenic modulation (Mvsp) and gliding (Gli349 and Gli123). (B) The flexibility and molecular length of Gli123 allow for conformational changes in it, which facilitate its clamping to other gliding proteins at appropriate positions in the machinery.
chromatography (HisTrap HP 5 mL, Cytiva). Proteins bound to the column were eluted with an imidazole concentration gradient of 0 to 250 mM, at 4°C. The fraction containing rGli123 was adjusted to 20% saturation of ammonium sulfate and kept on ice for 1 h, following which the obtained supernatant was collected after centrifugation at 32,300 × g and 4°C, for 30 min. The supernatant was subjected to a hydrophobic interaction chromatography column (phenyl sepharose HP 1 mL, Cytiva) and eluted in a gradient of 20% to 0% ammonium sulfate. The rGli123 protein was concentrated to 5 mL by means of centrifugation at 5,000 × g and 4°C, using a 50K Amicon tube (Amicon Ultra, Merck, Darmstadt, Germany), and subjected to SEC. The protein concentration was adjusted using a 50K Amicon tube, if necessary, for analyses.

**Optical analyses.** The circular dichroism spectra of the proteins were measured in the range of 200 to 250 nm using a spectropolarimeter (J-805, Jasco International Co., Tokyo, Japan), at 25°C. A quartz cuvette with a 1-mm path length was filled with 1 mg/mL of rGli123 in SEC buffer. The conditions for the measurements have been previously described (43). The measured spectrum was analyzed using BeStSel (30), followed by estimation of the α-helix, β-strand, and turn contents (29). The secondary structure was predicted from the amino acid sequence using XtalPred (44). For light scattering, the rGli123 protein dialyzed against 50 mM ammonium acetate was placed into a 10-mm quartz cell, and the scattered light at the wavelength of 400 nm was measured at 25°C using a spectrofluorometer (FP-6200, Jasco International Co.). The ammonium acetate concentration was adjusted by means of addition of 3 M ammonium acetate solution.

**Optical and electron microscopy.** Rotary-shadowing and negative-staining EM were performed as previously described (11, 20, 25, 45). Protein images were analyzed using the image analysis software EMAN2.91 (https://blake.bcm.edu/emanwiki/EMAN2). The approximately 10,000 particle images were selected and classified into 50 groups, averaged, and then reconstructed into 3D structures from 3,202 particle images. For optical microscopy, M. mobile cells were inserted into a tunnel slide (21) precoated with Aluotto medium, for 60 min. After 1 min, the floating cells were removed. The cells on the glass surface were observed using phase-contrast microscopy and analyzed using ImageJ v1.51w (http://rsb.info.nih.gov/ij/), as previously described (18, 21, 46).

**Assessment of binding of rGli123 to mycoplasma cells.** Cultured M. mobile WT and m12 cells were washed twice and suspended in PBS. The suspended cells were kept in PBS containing 0.5 mg/mL of rGli123 protein for 3 h, following which they were collected by means of centrifugation at 12,000 × g and 25°C, for 5 min, suspended in PBS, sonicated, and analyzed using SDS-PAGE with CBB staining. Protein band intensities were analyzed using ImageJ v1.51w.

**SAXS measurements.** SEC-SAXS measurements were performed at beamline 10C of the Photon Factory of the High Energy Accelerator Research Organization (KEK), Tsukuba, Japan, as described previously (43, 47). Detailed experimental conditions are described in Table S5 (32, 33, 39, 40, 48). The rGli123 protein in SEC buffer, at the concentration of 1 mg/mL, was treated with 0.001-mg/mL trypsin, at 25°C, for various reaction times, following which the reaction was stopped by means of heat treatment at 95°C for 5 min, in SDS-sample solution containing 5% glycerol, 0.025% bromophenol blue, 62.5 mM Tris-HCl (pH 6.8), 2.5% SDS, and 5% β-mercaptoethanol. The protein digests were analyzed using SDS-PAGE with a 10% acrylamide gel and peptide mass fingerprinting, as described previously (20, 45).

**SUPPLEMENTAL MATERIAL**

Supplemental material is available online only.

**SUPPLEMENTAL FILE 1, PDF file, 1.4 MB.**

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