Cell surface differentiation of *Mycoplasma mobile* visualized by surface protein localization

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Mycoplasma mobile has a flask-shaped cell morphology and glides toward its tapered end at a rate of 3–7 cell lengths per s ($2.0\text{--}4.5\ \mu\text{m s}^{-1}$) by an unknown mechanism. Gliding requires that the surface of the cell is in contact with a solid substrate, such as glass or plastic. In order to characterize the nature of the outer surface of *M. mobile*, monoclonal antibodies were raised against intact cells and screened for their ability to recognize surface proteins. Four antibodies were identified and their protein targets were determined. One antibody recognized the Gli349 protein, which is known to be involved in glass binding and gliding. This antibody was also able to displace attached *M. mobile* cells from glass, suggesting that Gli349 is the major adhesion protein in *M. mobile*. The other three antibodies recognized members of the Mvsp family of proteins, which are presumably the major surface antigens of *M. mobile*. Immunofluorescence studies were performed to localize these proteins on the surface of *M. mobile* cells. Gli349 localized to the proximal region of the tapered part of the cell (the 'neck'), while the various Mvsp family members showed several distinct patterns of subcellular localization. MvspN and MvspO localized to the distal end of the tapered part of the cell (the 'head'), MvspK localized to the main part of the cell (the 'body'), and Mvspl localized to both the head and body but not the neck. This analysis shows that *M. mobile* surprisingly expresses multiple versions of its major surface antigen at once but differentiates its surface by differential localization of the various paralogues.

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INTRODUCTION

Mycoplasmas are parasitic or commensal bacteria that lack a peptidoglycan layer and have small genome sizes (Razin *et al.*, 1998). Several mycoplasma species have a membrane protrusion at a cell pole, such as the 'attachment organelle' of *Mycoplasma pneumoniae* and the 'head-like structure' of *Mycoplasma mobile*, and exhibit gliding motility, defined as a smooth translocation of cells on solid surfaces in the direction of the tapered end (Kirchhoff, 1992; Miyata & Seto, 1999; Miyata *et al.*, 2000, 2002). Mycoplasmas have no appendages such as pili or flagella on their cell surface and no homologues of genes that encode pili, flagella or other genes related to bacterial motility; no homologues

of motor proteins that are common in eukaryotic motility have been detected, either (Chambaud *et al.*, 2001; Fraser *et al.*, 1995; Himmelreich *et al.*, 1996; Jaffe *et al.*, 2004b). These observations suggest that mycoplasmas glide by an entirely unknown mechanism, although it is believed to be an ATP-driven process (Jaffe *et al.*, 2004a).

M. mobile, isolated from the gill organ of a fish, has a flask-shaped cell structure (Kirchhoff & Rosengarten, 1984). It typically glides at a rate of 3–7 cell lengths per s ($2.0\text{--}4.5\ \mu\text{m s}^{-1}$) (Kirchhoff, 1992; Miyata *et al.*, 2000, 2002; Rosengarten & Kirchhoff, 1987) in the direction of the tapered end, which is designated the 'head-like' structure, and exerts a maximum force of 27 pN (Miyata *et al.*, 2001, 2002). The gliding behaviour of cells with heads attached to their surfaces and cells with elongated head-like structures has been examined (Miyata *et al.*, 2002; Miyata & Uenoyama, 2002). Rapid-freeze–fracture electron microscopy of actively gliding cells showed 50 nm spikes that stick out from the cell membrane around the head-like structure and bind to the glass surface (Miyata & Petersen, 2004). These observations suggested that the gliding

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machinery is localized at the head-like structure, which might be considered a specialized gliding organelle.

In this study, we isolated antibodies against the mycoplasma cell surface, identified the targets of these antibodies, localized the gliding machinery, and then showed that the cell surface is differentiated into at least three parts.

METHODS

Strains and culture conditions. *M. mobile* strain 163K (ATCC 43663) and its mutants were grown at 25 °C in Aluotto medium (Aluotto *et al.*, 1970; Miyata *et al.*, 2000), consisting of 2.1 % heart infusion broth, 0.56 % yeast extract, 10 % horse serum, 0.025 % thallium acetate and 0.005 % ampicillin. Cells were cultured to reach an optimal density at OD₆₀₀ of 0.07 (corresponding to 7×10^8 c.f.u. ml⁻¹).

Mouse immunization and hybridoma production. These procedures followed the methods previously described, with slight modifications (Harlow & Lane, 1999). Cultured cells of *M. mobile* 163K (ATCC 43663) were collected by centrifugation at 10 000 g for 3 min at room temperature and washed twice with PBS consisting of 75 mM sodium-phosphate buffer (pH 7.3) and 100 mM NaCl. Mycoplasma cells were suspended in PBS and emulsified in complete Freund's adjuvant. Female BALB/c mice were injected intraperitoneally with the emulsion containing 10¹¹ whole *M. mobile* cells. Three weeks after the first injection, a second injection was done with 5×10^{10} whole *M. mobile* cells emulsified with Freund's incomplete adjuvant. Two weeks after the second injection, the mice were injected with 5×10^{10} whole *M. mobile* cells without adjuvant. The mice were sacrificed 3 days after the last boost. The spleens were dissected, and dissociated spleen cells were fused to SP-1 myeloma cells. The hybridomas were cloned and screened three times using an ELISA assay. The class and subclass of the resulting antibodies were determined by the Mouse Immunoglobulin Typing Kit (Wako). mAb9 was typed as IgM and the others were typed as IgG₁.

ELISA and immunoblotting. For the ELISA assay, cultured mycoplasma cells were collected by centrifugation as before. After two washes with PBS, mycoplasma cells were suspended in 10 mM phosphate buffer (pH 7.0) containing 0.1 % SDS and adjusted to 10 µg ml⁻¹ mycoplasma protein solution. A 50 µl aliquot of mycoplasma protein solution was added to each well of a FALCON 96-well Microtitre Plate (Becton Dickinson) and incubated for 2 h. The remainder of the ELISA procedure was performed with a method described by Roggendorf *et al.* (1982).

For immunoblotting, cultured mycoplasma cells were collected by centrifugation, washed with PBS, resuspended in PBS and lysed by addition of sample loading buffer. The lysate containing 5 µg protein for each lane of 7 mm width was fractionated by 5 % and 7 % SDS-PAGE (Sambrook *et al.*, 1989), and analysed by immunoblotting, using a hybridoma medium with appropriate dilutions.

Microscopy. Cultured cells were collected by centrifugation and resuspended at a 30-fold higher concentration in Aluotto medium. Motility was assessed with a 'tunnel slide test' (Uenoyama *et al.*, 2004). Briefly, cells were adhered to a glass coverslip which was assembled into a rudimentary flow chamber constructed with a microscope slide and double-sided tape. Then, antibodies were applied by exchanging the medium in the chamber for medium containing a hybridoma supernatant. Immunofluorescence microscopy without permeabilization was performed as described previously (Seto *et al.*, 2001; Seto & Miyata, 2003; Uenoyama *et al.*, 2004) by using monoclonal hybridoma supernatants at a dilution of 1:100 and polyclonal mouse serum at a dilution of 1:400 with PBS.

Identification of target protein of mAb9. The target protein of mAb9 was purified by successive fractionation of cell lysate using immunoblotting against mAb9 and SDS-PAGE as indicators of specificity and purity. Cells from 3 l of culture were collected by centrifugation at 10 000 g for 10 min at 4 °C, washed twice with 5 ml of buffer consisting of 8 mM HEPES buffer (pH 7.0) and 0.28 M sucrose, and subjected to phase partitioning using 5 ml of a TritonX-114-containing solution (Bordier, 1981). The hydrophobic phase was mixed with 45 ml 50 mM HEPES buffer (pH 7.5) containing 0.1 % Triton X-100, and applied to a HiTrap CM Sepharose FF 1 ml column (Amersham). The flowthrough fraction was loaded onto a HiTrap DEAE Sepharose FF 1 ml column (Amersham). The target protein was retained by the column and eluted by 6 ml 50 mM NaCl. A total of 70 µg of protein was purified by SDS-PAGE following TCA-mediated precipitation, and subjected to in-gel partial digestion, using V8 protease and trypsin (Cleveland *et al.*, 1977). The peptide digests were transferred to a nylon membrane and an N-terminal amino acid sequence was determined by Edman degradation for each preparation. The amino acid sequences N-TTTLTI-C and N-SLITSSRG-C determined for V8 and trypsin digests, respectively, were both found in an ORF in the genome sequence as described above.

Identification of target proteins of mAbs 13 and 14 by mass spectrometry. To identify the target of mAb14, cells from 10 ml culture were washed with PBS, lysed and subjected to 5 % SDS-PAGE. Immunoblots and Coomassie-stained gels showed that the protein band was sufficiently separated from other bands so it was simply excised from the gel and subjected to trypsin digestion for mass spectrometry.

The target of mAb13 was isolated by using antibody-loaded Protein G-conjugated beads (Protein G Sepharose 4 Fast Flow; Amersham Biosciences). Cells from 50 ml of culture were washed and subjected to phase partitioning, whereby whole proteins were fractionated into hydrophobic, hydrophilic and insoluble fractions using Triton X-114 (Bordier, 1981). The hydrophilic fraction was mixed with 10 µl of a slurry of antibody-loaded beads saturated with mAb13 according to the manufacturer's instruction. After mixing at 4 °C for 2 h, the beads were washed three times with PBS and extracted by SDS-PAGE gel-loading buffer (Sambrook *et al.*, 1989). The target proteins were subjected to 10 % SDS-PAGE and excised from the gel after staining with Coomassie blue. A duplicate gel was subjected to immunoblotting to verify that the bands were reactive to the antibodies employed.

The excised bands of target proteins were reduced, alkylated, and digested by trypsin as described by Hanna *et al.* (2000). A nano-spray chromatography column (75 µm ID) was pulled in house and packed with 15 cm of Michrom MAGIC C18AQ media (Michrom). This column was directly interfaced to the mass spectrometer and separations were performed using a linear gradient of 5–40 % acetonitrile/0.1 % formic acid over 100 min at a flow rate of 250 nl min⁻¹. Tandem mass spectra were acquired on an LCQ Deca XP-plus ion-trap mass spectrometer (ThermoFinnigan) using a 'top five' approach, where MS/MS spectra were obtained for the five most abundant peaks in the original MS full (parent) spectrum. Proteins were identified using SEQUEST to search against a database consisting of 6-frame-translated DNA sequences from the *M. mobile* genome (<http://www-genome.wi.mit.edu/annotation/microbes/mycoplasma>) (Eng *et al.*, 1994; Jaffe *et al.*, 2004b). SEQUEST scores > 2.5 for charge state $z=2$ peptides and > 3.75 for charge state $z=3$ peptides were considered valid peptide assignments. ORFs that corresponded to these peptide assignments were identified by comparison against the current *M. mobile* genome annotation (GenBank accession no. AE017308).

Fig. 1. Inhibitory effect of antibodies on glass binding during gliding. Mycoplasma cells bound to the glass surface and floating in medium were seen as black and white dots, respectively. The medium was replaced at time 0 by either a new medium containing a 1 : 10 dilution of hybridoma supernatant including mAb7 or a fresh hybridoma medium for control. Video frames are shown for given time points, as indicated. Bar, 10 μ m.

RESULTS

Establishment of monoclonal antibodies

Hybridomas were made by cell fusion of myeloma and spleen cells derived from four BALB/c mice immunized with whole *M. mobile* cells. After a month, 215 hybridoma cell lines showed positive results for an ELISA assay against *M. mobile* cell lysate. The hybridomas were cloned and screened using the ELISA assay, and ultimately 15 cell lines were established based on the stability for production of antibodies.

Inhibition of gliding

Inhibitory effects of monoclonal antibodies on *M. mobile* attachment and gliding were examined by using the 'tunnel slide test' (Fig. 1). An antibody, designated mAb7, displaced the gliding mycoplasmas from the glass surface within 3 min after addition, while control supernatants (including the other antibodies) did not show any effects. This suggests that the inhibitory effects were specific to mAb7.

Subcellular localization of target proteins

The localization of target proteins on the cell surface was examined by immunofluorescence microscopy (Fig. 2). Fig. 2(a) shows the subcellular localizations of the proteins and Fig. 2(b) shows higher magnification images of them. Polyclonal antibodies against whole *M. mobile* cells obtained from a mouse in our antibody preparation were able to stain the whole cell surface of *M. mobile* ('p' in Fig. 2). Four of 15 antibodies from the established hybridomas showed signals with sufficient intensity to see the localization of their targets. All of the target proteins were found to localize to distinct parts of the cell surface. mAb7

stained only the head-like structure, particularly the middle to basal part of it, showing that this part contains the structures responsible for attachment to glass. We designated this part as the 'neck'. The signal of mAb9 was found only on the main part of the cell below the neck (designated the 'body'). Staining by mAb13 showed a signal mostly at the distal end of the head-like structure (designated the 'head'), but a faint signal could be observed in other regions of the cell. mAb14 stained both the head and the body with only a faint signal in the neck. The signal of mAb14 was sometimes more condensed at the head than the body. These staining patterns are summarized in Table 1.

Identification of target proteins

In immunoblotting monoclonal antibody mAb7 recognized an isolated protein band with an extrapolated molecular mass of 250 kDa. This molecular mass is almost certainly an underestimate as the resolution of the gels at high molecular mass is limited. Immunoblot analysis of the wild-type strain and the gliding mutants previously isolated (Miyata *et al.*, 2000) revealed that the target of mAb7 is the Gli349 protein, which has a predicted molecular mass of 349 kDa and is truncated in a non-binding mutant, m13. Gli349 has been previously implicated in attachment of cells to surfaces and motility (Uenoyama *et al.*, 2004).

mAbs 9, 13 and 14 each recognize a surface structure; their target proteins were identified using a combination of biochemical purification, mass spectrometry and N-terminal Edman sequencing (Table 1). In addition, immunoblots were used to verify the number and molecular mass of the target proteins (Fig. 3). The mAbs recognized various members of a family of related proteins. These proteins are annotated in the genome as the Mvsp proteins, for *m*obile *v*ariable *s*urface protein. The Mvsp proteins were predicted,

Fig. 2. Subcellular localization of target proteins visualized by immunofluorescence microscopy. (a) Fixed mycoplasma cells were stained with the antibodies indicated by the numbers on the left; p indicates polyclonal antibody, i.e. the antiserum from the immunized mouse from which the spleen cells were obtained. Fluorescence (left column) and phase-contrast (middle column) images were taken independently and merged (right column). (b) High magnification images from (a). mAbs used are indicated at the top by the same letter or number as in (a). Bars, 2 μ m.

Table 1. Target structure of antibody

Antibody	Inhibition of gliding by antibody*	Subcellular localization	Gene	Gene locus†	Molecular mass (observed‡/predicted§)	Length of ORF	Motif and other sequence features
mAb7	+	Neck	<i>gli349</i>	MMOB1030	245 000/348 510	3181	None
mAb9	–	Body	<i>mvspK</i>	MMOB3370	74 000/73 710	689	Lipid attachment internal repeat (138–232, 236–330)¶
mAb13	–	Head	<i>mvspN</i>	MMOB6080	47 000/38 372	358	None
		Head	<i>mvspO</i>	MMOB6090	57 500/49 631	458	Internal repeat (110–209, 210–309)¶
mAb14	–	Head and body	<i>mvspI</i>	MMOB3340	191 000/220 992	2002	Lipid attachment internal repeat (989–1076, 1079–1166)¶

*+, Gliding was inhibited by the addition of antibody; –, gliding was not inhibited.

†Gene locus in the database of the whole genome sequence (<http://www-genome.wi.mit.edu/annotation/microbes/mycoplasma>).

‡Molecular mass (in Da) estimated from the band position in SDS-PAGE.

§Molecular mass (in Da) predicted from the amino acid sequence of the ORF.

||Length of ORF measured in amino acids.

¶The positions of repeats are indicated by amino acid numbers.

Fig. 3. Identification of target proteins of mAbs. Whole-cell lysate was separated by 5% (right) and 7% (left) SDS-PAGE, followed by immunoblot analysis. Lanes marked 'P' are blotted nylon membranes stained by Ponceau S. The numbers of the mAbs used for the other lanes are indicated at the top. Molecular mass is indicated on the left.

based on sequence analysis, to be surface exposed, and here we demonstrated (along with immunofluorescence data, Fig. 2) that this prediction was indeed correct. mAb13 recognized two protein bands with estimated molecular masses of 48.0 and 57.5 kDa whose identities are MvspN and MvspO, respectively. mAb9 and mAb14 recognized proteins with estimated molecular masses of 74 and 191 kDa, respectively, whose identities are MvspK and MvspI, respectively.

The expression state of the target proteins of mAbs 9, 13 and 14 was tested in the gliding mutants isolated previously, including m6, 9, 12, 13, 14, 23, 26, 27, 29 and 34, by using immunoblotting and immunofluorescence microscopy (Miyata *et al.*, 2000). The results showed that the target proteins were all expressed in all gliding mutants.

Sequence properties of target proteins

Three of the four surface-reactive antibodies raised for this study recognized proteins in the Mvsp family. This family is composed of 16 members in *M. mobile* and two in *Mycoplasma pulmonis*. The *M. mobile* members of the family share 40–60% similarity at the amino acid level, are tandemly coded in several clusters in the genome, can be aligned to each other in various registers using a multiple sequence alignment program such as CLUSTAL_W, and exhibit sequence features suggestive of transmembrane helices and/or lipid attachment sites (Jaffe *et al.*, 2004b). Here we present a more detailed analysis of the sequence properties of the Mvsp family members detected in this study.

The amino acid sequence of MvspO (the larger target of mAb13) was found to have an internal repeat consisting of a duplication of amino acids 110–209 at positions 210–309. MvspO shares 87.5% identity with the sequence of MvspN (the smaller target of mAb13) if they are compared excluding the second repeat. It is probable that mAb13 recognizes an epitope common to these two proteins. In addition, MvspN is encoded just downstream from MvspO in the genome with respect to the direction of transcription. The observed molecular masses of these proteins are significantly different from those predicted from ORF sequences (Table 1). This disagreement may suggest post-translational modification of these proteins. MvspK and MvspI (the target proteins of mAbs 9 and 14, respectively) also have internal repeats.

mAb7 recognized the Gli349 protein, which has been described elsewhere (Uenoyama *et al.*, 2004). An orthologue of Gli349 was found in the genome of another gliding mycoplasma, *M. pulmonis*. *M. pulmonis* is phylogenetically the closest related mycoplasma to *M. mobile* to have a complete genome sequence (Chambaud *et al.*, 2001; Weisburg *et al.*, 1989).

The Gli349 protein and the Mvsp proteins detected in this study share some common sequence features, which are highlighted in Fig. 4. All of their sequences contain a transmembrane segment near the N-terminus (as predicted by SOSUI; Hirokawa *et al.*, 1998) preceded by a positively charged domain. These features suggest that these proteins may be anchored in the membrane via their N-terminal regions (Navarre & Schneewind, 1999; Pugsley, 1993). In addition, MvspK and MvspI are predicted to have a lipid attachment site by detection of a corresponding PROSITE motif (Hofmann *et al.*, 1999). These proteins might additionally or alternatively be anchored to the membrane by attached lipids.

DISCUSSION

The monoclonal antibody mAb7 isolated in this study displaced gliding *M. mobile* cells from a glass surface. This

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MvspN: M K K A N ----- L F L G V T A G A L A S T V V I V P L S V V A - N Q N T K E V G P V N P
MvspO: M L N M K K V N - L F L A G A A T L V S T V V I V P A S I A A - T K T T P K E V G P
Gli349: M T N K K T K L - I S G I T A T V V V A S A V G I G V G T S F A - I N S E R E V D Q
MvspK:  M I K Q K N K K - I L I G L G L T T A T V V L P I S I V A C A T - N E T N A Q K I T
MvspI:  M L K M K K K - Y L I G I G T L S A I A I L P I T V V A C A T - N T S E N A N N I K
          positively charged                transmembrane

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Fig. 4. Multiple alignment of N-terminal sequences of target proteins. The N-terminal 40 amino acids are shown for each protein indicated on the left. The positively charged region, possible transmembrane segment and additional sequence are presented with connecting gaps to compare the sequences. Lipid attachment motifs and their possible attachment sites are indicated by boxes and underlining, respectively. Positively charged amino acids are marked with dots above the letters.

antibody recognized a 349 kDa protein, Gli349, which is truncated in a non-binding and non-gliding mutant, m13 (Miyata *et al.*, 2000). This observation shows that Gli349 is involved in glass binding during gliding (Uenoyama *et al.*, 2004). Our previous studies suggested that the gliding machinery was localized to the head-like structure, which contains the neck region as discussed above (Miyata *et al.*, 2001, 2002; Miyata & Uenoyama, 2002; Miyata & Petersen, 2004). The current study, aided by the isolation of monoclonal antibodies, establishes that this is indeed true. Our immunofluorescence results allow us to depict the cell surface of *M. mobile* at a higher resolution. Gli349 was localized in the neck region, while the other four target proteins were excluded from the neck, as presented in Fig. 2. These observations are consistent with the hypothesis that the surface components of machinery for gliding motility are located in the neck and not in other part of the cell, as postulated in Fig. 5.

Cell-to-glass binding during gliding was inhibited by mAb7 applied extracellularly, suggesting the existence of surface structures on the cell neck. Recently, we applied rapid freeze and freeze-fracture rotary-shadow electron microscopy to actively gliding mycoplasma cells, and found that a novel 'spike' structure protrudes from the cell membrane and attaches to the glass surface at its end. The spikes had a mean length of 50 nm, a mean diameter of 4 nm, were most abundant around the head and were not observed in a non-binding mutant. Therefore, we concluded that the spike structure is involved in gliding motility (Miyata & Petersen, 2004). It is likely that Gli349, the target protein of mAb7, forms the spike. The dimensions of the spike are consistent with the expected volume of a 349 kDa protein.

The four Mvsp proteins detected in this study are unlikely to be involved in motility or adhesion because antibodies against them did not affect gliding or glass binding. All of the Mvsp proteins localized to regions other than the neck region, as presented in Figs 2 and 5. Interestingly, MvspI

localized to the head and body but specifically not to the neck region. These observations suggest that the neck region is specialized for glass binding and gliding. Further subcellular localization to the head could be seen for MvspN and MvspO, while MvspK localized to the body.

In the process of screening of hybridoma cell lines, we obtained around 20 hybridomas producing antibodies recognizing surface structures; however, only four of them could ultimately be established. In immunofluorescence microscopy all of the antibodies from the precursor hybridoma strains labelled the cells in one of the four patterns shown in Figs 2 and 5 (data not shown). These observations suggest that surface proteins other than those identified here may also be localized in the same patterns.

Genome sequencing revealed 16 highly related proteins that are encoded primarily by two gene clusters and share sequence features indicative of membrane localization. This evidence suggests that they might be involved in antigenic variation in *M. mobile* (Jaffe *et al.*, 2004b). This assumption is supported by the fact that the Mvsp proteins are immunodominant, as Mvsp family members were recognized by three of the four hybridomas established by immunization of mice with whole mycoplasma cells. The *vsa* gene family involved in antigenic variation in *M. pulmonis* has been reported to express only one gene at one time (Bhugra *et al.*, 1995). However, the immunostaining of the Mvsp proteins in this study showed that multiple *mvsp* variants are co-expressed in a *M. mobile* cell, which is consistent with the result of proteomic analysis (Jaffe *et al.*, 2004b). The multiple and localized expression of *mvsp* genes in a *M. mobile* cell suggests that if they are involved in antigenic variation, the mechanism of variation may be different to that in *M. pulmonis*.

We previously quantified the number of Gli349 molecules on the *M. mobile* cell surface and concluded that around 450 molecules exist on a cell (Uenoyama *et al.*, 2004). The 450 units of gliding machinery may form a large cluster, occupy the neck space and exclude other surface proteins from the region. Such a cluster might prevent free diffusion of proteins across this region of the cell membrane and thus provide a basis for differentiation of the cell surface into disparate regions. However, even if this was the case, specific mechanisms must be required for trafficking of newly synthesized proteins to their proper subcellular locations. Analysis of the amino acid sequences near the N-termini of the proteins did not identify obvious signals for protein localization (Fig. 4), but this does not exclude the presence of such signals.

No surface structures specific for the head or body have been found by freeze-fracture or scanning electron microscopy of *M. mobile* (Kirchhoff *et al.*, 1987; Miyata & Petersen, 2004; Rosengarten *et al.*, 1988; Rosengarten & Kirchhoff, 1989). However, sectioned images of chemically fixed cells showed increased electron densities under transmission electron microscopy at the front end of the

Fig. 5. Schematic illustration of three subcellular regions and four patterns of protein localization of *M. mobile*. The direction of gliding is indicated by an open arrow. The proteins are located at the subcellular region indicated. The neck region is specialized for gliding and contains the spike structure which has been observed by rapid-freeze-fracture electron microscopy (Miyata & Petersen, 2004).

head-like structure when it was stained by uranyl acetate (Kirchhoff *et al.*, 1987; Shimizu & Miyata, 2002). The distribution of these increased electron densities may be consistent with the localization of the targets of mAb13, namely MvspN and MvspO.

While subcellular localization in a mycoplasma cell has been reported for several cytoadherence proteins (Krause & Balish, 2004; Uenoyama *et al.*, 2004), localization has not been shown for other classes of proteins. In this study, we have shown subcellular localization of proteins apparently not related to cytoadherence for the first time, and are able to reconstruct a detailed map of the surface differentiation of *M. mobile*. Such surface differentiation might be a feature found in other species of *Mycoplasma* as well.

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