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Adaptive surface variation in mycoplasmas

Kim S. Wise

Mycoplasmas excel as infectious agents, despite their very small genomes. In one mycoplasma species, adaptive flexibility is enhanced by an elegant genetic system that diversifies the membrane surface through a set of variable lipoproteins (Vlps). A family of *vlp* genes supplies divergent coding sequences and undergoes high-frequency mutations, thus creating large repertoires of surface mosaics and structural variants.

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Mycoplasmas have long been underestimated in terms of their variety and their distribution in animal hosts. Continued improvements in isolation procedures have now revealed over 90 different species[1], but this number is bound to increase as new technologies and environments are explored. The recent emergence of new species from the immunocompromised human host poignantly illustrates this trend[2]. Many mycoplasma species cause clearly identifiable diseases[3], which are often chronic in nature and display major elements of immunopathology. How these agents establish and maintain the subtle relationship with their hosts, and perturb this relationship as pathogens, is a central theme of current research.

Compared to other bacteria, mycoplasmas might be expected to be severely deficient in adaptive capabilities. Although phylogenetically related to Gram-positive bacteria with a low G+C content, most of these organisms contain less than 25% of the genomic mass of *Escherichia coli*[1]. They completely lack cell walls, and depend on their (host) environment to supply critical nutrients[4]. Evolutionary acquisition of systems to compensate for these limitations may be a *sine qua non* for mycoplasma survival. Some key adaptations are likely to reside in components of the single plasma membrane surrounding these organisms. Their lack of a rigid peptidoglycan cell wall is unique among prokaryotes, yet the exposed membrane effectively mediates all necessary transport, sensory and physical interactions between mycoplasmas and their host environment, including harsh encounters with the immune system[5]. How then do organisms with such limited genetic information and an exposed membrane surface survive? Part of the emerging answer seems to lie in their clever use of genetic information to maintain an imposing membrane surface architecture that is structurally and functionally versatile.

Mycoplasma surface variation as a survival strategy

A formal requirement for any microbial parasite is to survive as a propagating population in the host[6]. The energy expended in achieving this goal is irrelevant, as long as the population can expand, be transmitted and re-establish itself in a new host. Animal hosts provide a vast array of distinct niches, which are subject to temporal changes through several mechanisms including adaptive immunity. One way in which parasites circumvent this problem is by phenotypic variation in the population, so that certain individuals have a selective advantage in a particular microenvironment. Phenotypic variation may be accomplished in two ways: individual organisms may specifically alter their phenotype in response to sensory signals from the changing environment[7]; or, the population as a whole may generate sufficiently large repertoires of genetic variants, at adequate frequencies, to ensure survival of some individuals[8–10]. Importantly, surviving variants that result from the latter strategy must inherit the ability to regenerate large phenotypic repertoires. While random genetic diversification seems demanding, it may not require the complex sets of genes needed for sensory responses to assorted environmental cues[7]. In addition, well-designed diversity-generating systems may provide an added dividend by creating entirely new phenotypes that may exploit novel niches or even alternative hosts. Finally, operation of such systems in a propagating population may provide an effective escape from the diversity-based systems driving immune responses.

While it is possible (although not yet demonstrated) that mycoplasmas may use sensory responses to adapt to some host conditions, they have clearly chosen random genetic variation as a major survival strategy. They effect this by varying membrane proteins, which are encoded in multiple versions and are displayed by indiscriminate expression in various combinations on the organism's surface. Interesting structural variations also occur by a superimposed process that alters the length of specific protein domains (see below). Clues to the existence of these high-frequency phenotypic switching systems emerged from several studies reporting variation in surface membrane proteins among individual isolates or strains of several mycoplasma species (extensively reviewed in Ref. 11). Characteristically,

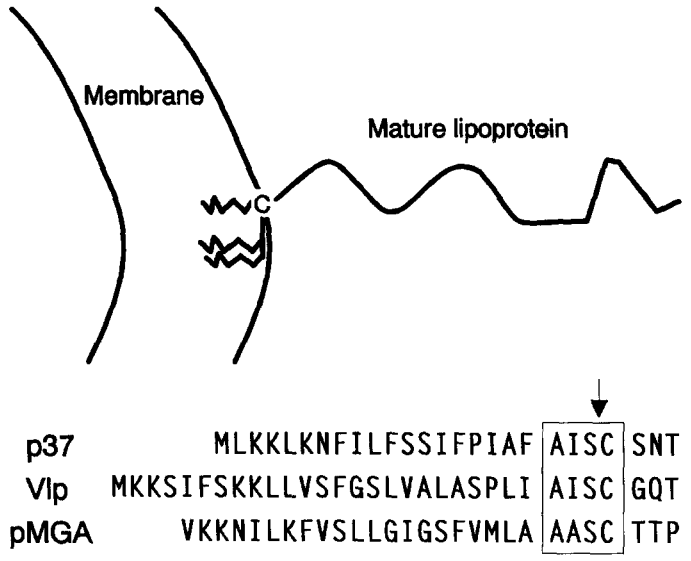

Fig. 1. Processing and anchorage of mycoplasma surface lipoproteins. The figure depicts a mature protein modified by diacylglyceryl- and amide-linked fatty acid chains that are covalently bound to the amino-terminal cysteine (C) residue and integrated into the outer leaflet of the single mycoplasma membrane. The remainder of the protein is predicted to be hydrophilic and external to the membrane surface. The deduced signal peptide sequences of three mycoplasma surface lipoproteins are shown to indicate prolipoprotein acylation and cleavage motifs (boxed, with cleavage site indicated by arrow). The lipoproteins are: p37, a proposed high-affinity substrate-binding protein belonging to a putative transport complex in *M. hyorhinis*[16]; variable lipoproteins (Vlps) of *M. hyorhinis* (see text); and pMGA, a surface hemagglutinin of *M. gallisepticum*[17] which, like Vlps, displays a conserved signal peptide sequence found in multiple copies of otherwise divergent gene coding regions[18].

(1) antibodies to surface epitopes revealed selective expression of corresponding proteins in some populations but not others, (2) variation in the size of proteins bearing the epitopes was observed, often revealing graded 'ladders' of size-variant forms, and (3) multiple antigens with these properties, distinguished by specific antibodies, were concomitantly expressed in some populations. These studies generally used populations propagated in broth culture after standard cloning of isolates, and it was not until isogenic lineages of clonal populations were extensively characterized that the complex basis for these variations was revealed. The population dynamics and relevant genetic mechanisms have been examined in detail[12–14] in *Mycoplasma hyorhinis*, a swine pathogen, and are described here. Other species that have yielded molecular details of analogous systems are also discussed for comparison.

Structural versatility and size variation of Vlp antigens

Mycoplasma hyorhinis displays a set of variable lipoproteins (Vlps) expressed at the membrane surface. These products are extremely abundant, and belong to a general class of lipid-modified proteins that are translocated across the membrane, acylated and anchored by covalently linked fatty acids after processing a specific signal peptide motif found in several prokaryotes[15]. This structural theme seems to be widely used in mycoplasmas to tether functional membrane proteins[14,16–18] (see Fig. 1). Vlps extend from their anchored amino-terminal cysteine residue as extracellular, hydrophilic polypeptides with no predicted secondary structure[14]. Their exact spatial orientation is not known, but they are accessible to proteases and specific surface-binding monoclonal antibodies (mAbs)[12,13]. As exposed surface proteins, Vlps are prime candidates for a variety of functions involving host interactions, and may play a role in immune avoidance.

Phenotypic variation of surface proteins requires a means for displaying alternative sequence variants. A reservoir of alternative coding sequences in clustered chromosomal copies of divergent *vlp* genes provides this capability. While only three *vlp* genes were identified in a clonal population reported previously[14], indirect evidence[12,13,19] suggests that the repertoire may be expanded in other lines of the species, raising the possibility that the gene reservoir is in dynamic flux. Adjacent insertion sequence-like elements[14,20] and the presence of lysogenic phage reported in this species[21] offer possible vehicles for mobilizing these genes. However, several features within individual *vlp* genes sequences indicate additional mechanisms for generating alternative sequences[14] (see Fig. 2). In addition to a conserved signal peptide sequence (region I), all *vlp* genes encode two structural regions, each retaining characteristic features despite the divergence of sequences. An uncharged region (II) contains sequences unique to a particular gene, interspersed with blocks of redundant segments shared by some other *vlp* genes. Exchange or insertion of homologous segments could provide one source of sequence 'scrambling' that would generate coding alternatives in subsequent generations.

A more distal region (III) contains a series of directly repeated sequences that are unique to each Vlp, but encode a highly characteristic repeating charge motif, irrespective of the particular gene sequence employed. Moreover, region III undergoes extensive expansion and contraction (ranging from two to about 30 units) by intragenic insertion and deletion of repeating sequences, which may create radically different surface charge characteristics in proportion to the length of region III expressed. This spontaneous mutational pathway also determines the high-frequency size variation characteristic of Vlps[14] (Fig. 2).

An extraordinary additional feature of all *vlp* genes is the presence of continuous open reading frames in multiple coding registers[14]. These span and are generally limited to regions (II and III) encoding the mature lipoproteins, and occur on both DNA strands. While their full significance is not understood, they could provide a virtually limitless source of mutational diversity, through multiple frameshift mutations bringing random segments of alternative ORFs into the coding register of the lipoprotein. In theory, mutations of this sort occurring in region III could be amplified during expansion of repetitive

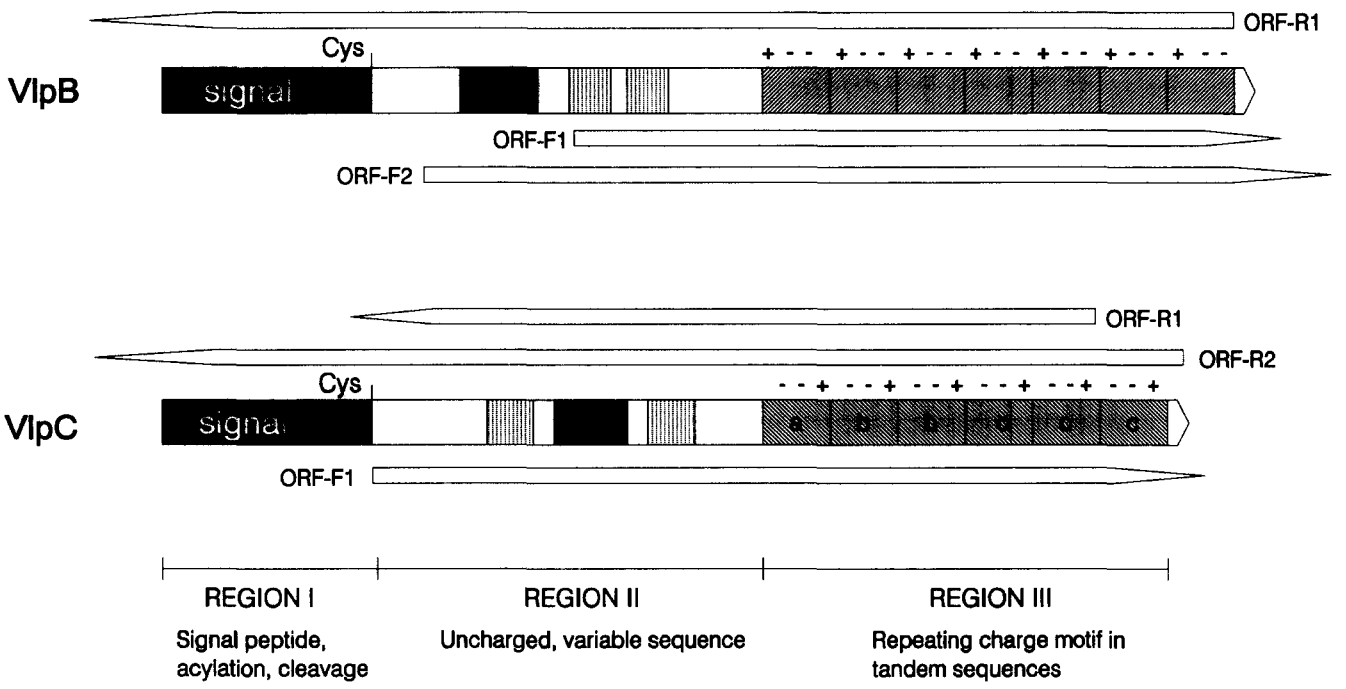

Fig. 2. Variable lipoproteins (Vlps) use a versatile genetic design for sequence and structural diversity. Adjacent, single-copy chromosomal genes encode the two *M. hyorhinis* Vlps shown[14]. These are compared to illustrate key features of Vlp structure. VlpB and VlpC share highly conserved prolipoprotein signal peptides (region I), but diverge in hydrophilic external sequences in the mature protein. An uncharged region (II) includes unique sequences (open) as well as distinctly arranged blocks of similar (shaded) sequences. A charged region (III) contains the motif $(+--)_n$ created by discrete sequences in each protein, which are arranged as tandem copies of identical or nearly identical 12 amino acid segments (a, b and c denote conservative amino acid substitutions among copies within VlpC). Region III expands and contracts by high-frequency mutations which insert or delete precise repeating units in individual *vlp* gene sequences. Remarkably, multiple open reading frames (ORFs) overlap the mature lipoprotein coding regions in all *vlp* genes. This appears to be a highly selected feature and provides an extensive reservoir of potential coding capacity in each gene. Multiple, random insertion/deletion mutations could mobilize this reserve by shifting variable segments of alternative sequence into coding register, but this has not yet been observed in the laboratory, nor in natural infections.

units, thereby propagating a phenotypic alteration throughout this domain. Whether this occurs in mycoplasma populations is not yet known. Overall, however, the potential sequence diversity collectively encoded in *vlp* genes is quite impressive.

Vlp phase variation: which switch is which?

Rapid phenotypic switching among Vlp size variants has been documented and is now explained by spontaneous mutations in region III. However, another spontaneous mutation occurs independently in *vlp* genes, and determines a separate switch parameter affecting the expression of individual genes and their corresponding products[14]. Each Vlp has been shown to undergo high-frequency phase variation, which occurs independently from other Vlps[12,13]. The basis of this switch involves a homopolymeric sequence of adenine residues in a conserved promoter region immediately upstream of each *vlp* gene[14] (see Fig. 3). The polyA sequence is subject to high-frequency mutations that insert or remove adenine residues. Oscillations involving insertions and deletions of single bases correspond precisely to phase transitions between the OFF and ON expression states of the corresponding gene. No other mutations or rearrangements of *vlp* genes occur during phase variation. The effect is provisionally attributed to an altered DNA configuration affecting the spacing or spatial orientation between the polymerase and some interacting factor, currently under investigation[14]. It is interesting to speculate that *vlp* gene expression may be controlled in part by other regulatory factors superimposed on the mutational alterations identified so far. The consequence of these random mutations in *vlp* promoters is the combinatorial expression of Vlp proteins, where any given cell can express the collective products of any genes in the ON configuration. This may reflect single genes or multiple genes, but no variants have been found that fail to express at least one Vlp.

Taking all possibilities together, an individual mycoplasma may draw upon a genetic repertoire supplying alternative proteins, optional sizes and graded charges for each protein, and the collective 'mosaics' created by expressing different combinations of these variant proteins. All of these features have been observed in populations of *M. hyorhinis*.

A comedy of errors?

Heritable phenotypic instability is a hallmark of diversity-generating systems in several microbial pathogens. What is unique about the system revealed in mycoplasmas is the compression of several structural alternatives into limited genomic sequences,

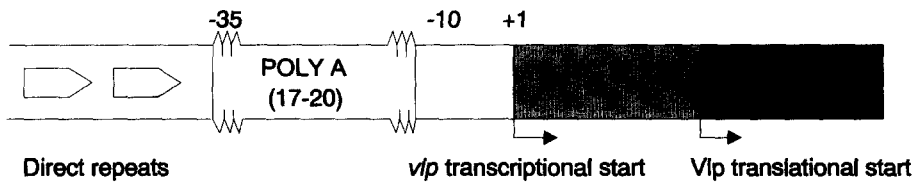

Fig. 3. Mutations in promoter elements randomly switch Vlp expression on or off. All *vlp* genes are flanked by a highly conserved promoter region organized as shown[14]. A homopolymeric tract containing 17–20 adenine residues (polyA) separates the proposed –10 sequence from an upstream –35 sequence and a set of direct repeats resembling known regulator protein binding sites. Mutations inserting or deleting single residues in the polyA tract correspond exactly to the expression state of the corresponding Vlp protein, during high-frequency phase variation in expression[14]. A single mutation appears to determine the independent expression state of each *vlp* gene, leading to random, and often combinatorial, expression of Vlps on a single cell.

combined with an ability to amplify diversity on single organisms by expressing surface mosaics. Perhaps the most imaginative aspect of the system, however, is the use of several distinct mutations that are driven by error-prone processes[11,14]. Indeed, insertions and deletions within the homopolymeric polyA tract, or within the repetitive region III sequences, may both occur by inaccuracies during DNA replication and repair. *Mycoplasma hyorhinis* has been reported to lack repair functions in its DNA polymerase[22], and may exploit this and other deficiencies to create a rather versatile strategy for adaptation. Intracellular influences may also selectively affect these mutation rates.

While the roles of Vlps have yet to be defined, many possibilities exist. These proteins may act as specific or generic adhesins or invasins, targeting or sequestering organisms in specific niches. Vlps could also serve as antigenic decoys for immune avoidance, pitting the range and rates of Vlp variation against the immune recognition system. Vlps may simultaneously act in a completely different role by varying charged regions to modulate physicochemical surface properties affecting selective uptake and transport of metabolites. Finally, through specific or generic interactions with host cell surface components, Vlps could modulate host cell function in ways beneficial to the organism. Host cell modulation could also result in untoward effects that could contribute to pathogenicity. The well-documented pleiomorphic immunomodulatory effect of degraded lipopeptides may be a case in point[23]. Given the apparent ingenuity of mycoplasmas, they may use Vlps or analogous proteins to serve all of these functions.

Vlp analogues in other mycoplasma species

Notable progress in studies with other mycoplasma species suggests that many will be found to possess similar strategies for diversification. Widespread phenotypic variation in size and expression of multiple surface antigens is known to occur in several species (see Ref. 11 for review). Some of these variant proteins appear to be lipid modified. A further pertinent example is the recent description of a family of genes in the avian pathogen *M. gallisepticum* encoding divergent pMGA hemagglutinins with conserved prolipoprotein signal peptide motifs[17,18] (Fig. 1), suggesting a possible structural theme generally adapted for mycoplasma surface diversification. Another recent study[24] indicates that a surface epitope of the human agent, *M. hominis*, is encoded in repetitive sequences found in multiple genomic contexts, although the nature of this reservoir and the expressed gene product are not fully characterized. Finally, the general concept of maintaining genetic reservoirs of segmented coding sequence for expression in alternative surface proteins may be realized in another context. Sequences shared between surface adhesins in two human mycoplasma species have been reported[25], and alternative configurations of these segmented regions have been documented in *M. genitalium* (S.N. Peterson, PhD Dissertation, University of North Carolina at Chapel Hill, 1992). The arrangement and mobilization of coding regions in mycoplasmas will undoubtedly remain an instructive area for research.

Prospects

The molecular basis of variations in surface proteins has been revealed in one pathogenic mycoplasma species, and is under intensive investigation in several others. It is in some ways surprising that such seemingly simple organisms recruit complex, superimposed mutational mechanisms to maintain heritable structural variability. Nevertheless, powerful selective constraints demand efficient mobilization and innovative use of the meager genetic resources available to mycoplasmas. Emerging molecular details from other mycoplasma systems promise equally imaginative applications of genetic information for maintaining phenotypic diversity.

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Antibody-mediated clearance of viruses from the mammalian central nervous system

Bernhard Dietzschold

The novel role of antibody in clearing virus from the central nervous system without the help of other immune effectors is an important phenomenon that has only recently been documented. Possible routes for antibodies across the blood–brain barrier and how they work in the CNS are discussed here.

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The clearance of viruses from infected tissues is thought to depend on several nonspecific and specific immune defenses. In the case of virus infections of the central nervous system (CNS), these defense mechanisms are severely restricted by the immunological privilege of the CNS. The existence of the blood–brain barrier and the lack of essential elements in the CNS that are necessary to produce an effective immune response have important consequences for the immune surveillance of the CNS[1]. In general it is believed that T cell responses are more important than antibodies in clearance of viruses from the CNS[2]. In particular, $CD8^+$ cytotoxic T cells have been shown to be effective in reducing virus titers in the brain after experimental infection with coronavirus, Theiler's virus or lymphocytic choriomeningitis virus (LCMV)[1,3,4].

However, antibodies have also been implicated as major effectors in the control of viral infection of the CNS. For example, antibodies play a major role in the recovery from lethal infection with Theiler's virus and it has been suggested that the antibodies limit viral spread within the CNS by neutralizing extracellular virus[1,4]. Recently a novel function for antibodies in protection against viral CNS infection has been discovered. Two studies have reported that antibodies can mediate complete clearance of virus from the CNS by a mechanism distinct from antibody-dependent cell-mediated cytotoxicity or complement-dependent lysis[5,6]. These findings indicate the great potential of antiviral antibodies as effective therapeutics against viral infections of the CNS. Here, I summarize current information on antibody-mediated viral clearance from the CNS, and discuss parameters that might be involved in the clearance process.

Protection of the CNS against viruses

In recent years the lack of a correlation between an antibody's neutralizing activity *in vitro* and its protective activity *in vivo* has been revealed in many viral diseases[5,7,8]. Furthermore, the ability of an antibody to protect *in vivo* cannot be uniformly related to a particular immunoglobulin class[5,6]. For example, the protective activity of a specific antibody in LCMV infection appears to be related to