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Adaptive surface variation in mycoplasmas

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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, 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. Many mycoplasma species cause clearly identifiable diseases, which are often chronic in nature and display major elements of immunopathology. How these agents establish and maintain their 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. They completely lack cell walls, and depend on their (host) environment to supply critical nutrients. 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. 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. 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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be widely used in mycoplasmas to tether functional membrane proteins\textsuperscript{14,16-18} (see Fig. 1). Vlps extend from their anchored amino-terminal cysteine residue as extracellular, hydrophilic polypeptides with no predicted secondary structure\textsuperscript{14}. Their exact spatial orientation is not known, but they are accessible to proteases and specific surface-binding monoclonal antibodies (mAbs)\textsuperscript{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 \textit{vlp} genes provides this capability. While only three \textit{vlp} genes were identified in a clonal population reported previously\textsuperscript{14}, indirect evidence\textsuperscript{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\textsuperscript{14,20} and the presence of lysogenic phage reported in this species\textsuperscript{21} offer possible vehicles for mobilizing these genes. However, several features within individual \textit{vlp} genes sequences indicate additional mechanisms for generating alternative sequences\textsuperscript{14} (see Fig. 2). In addition to a conserved signal peptide sequence (region I), all \textit{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 \textit{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\textsuperscript{14} (Fig. 2).

An extraordinary additional feature of all \textit{vlp} genes is the presence of continuous open reading frames in multiple coding registers\textsuperscript{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
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. Each Vlp has been shown to undergo high-frequency phase variation, which occurs independently from other Vlps. The basis of this switch involves a homopolymeric sequence of adenine residues in a conserved promoter region immediately upstream of each vlp gene (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.

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.
REVIEWS

expression 14. sites, upstream -35 sequence and a set of direct containing 17-20 are flanked by a highly conserved promoter region organized as shown 14. A homopolymeric tract Rg. 3. Mutations in promoter elements randomly switch VIp expression on or off. All rip Direct repeats v/p transcriptional start Vlp translational gene, leading to random, and often combinatorial, expression of Vlps on a single cell. Mutations inserting or deleting polyA tract, or within the repetitive region III se-

charged regions to modulate physicochemical surface pitting the range and rates of Vlp variation against specific or generic adhesins or invasins, targeting or many possibilities exist. These proteins may act as tively affect these mutation rates. 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 point21. 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 con-

served prolipoprotein signal peptide motifs17,18 (Fig. 1), suggesting a possible structural theme generally adapted for mycoplasma surface diversification. Another recent study24 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 reported25, 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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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. In general it is believed that T cell responses are more important than antibodies in clearance of viruses from the CNS. 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).

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. 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. 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 \textit{in vitro} and its protective activity \textit{in vivo} has been revealed in many viral diseases. Furthermore, the ability of an antibody to protect \textit{in vivo} cannot be uniformly related to a particular immunoglobulin class. For example, the protective activity of a specific antibody in LCMV infection appears to be related to