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MicroCommentary

Antigenic variation with a twist – the *Borrelia* story

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Summary

A common mechanism of immune evasion in pathogenic bacteria and protozoa is antigenic variation, in which genetic or epigenetic changes result in rapid, sequential shifts in a surface-exposed antigen. In this issue of *Molecular Microbiology*, Dai *et al*. provide the most complete description to date of the *vlp*/*vsp* antigenic variation system of the relapsing fever spirochaete, *Borrelia hermsii*. This elaborate, plasmid-encoded system involves an expression site that can acquire either variable large protein (*vlp*) or variable small protein (*vsp*) surface lipoprotein genes from 59 different archival copies. The archival *vlp* and *vsp* genes are arranged in clusters on at least five different plasmids. Gene conversion occurs through recombination events at upstream homology sequences (UHS) found in each gene copy, and at downstream homology sequences (DHS) found periodically among the *vlp*/*vsp* archival genes. Previous studies have shown that antigenic variation in relapsing fever *Borrelia* not only permits the evasion of host antibody responses, but can also result in changes in neurotropism and other pathogenic properties. The *vlsE* antigenic variation locus of Lyme disease spirochaetes, although similar in sequence to the relapsing fever *vlp* genes, has evolved a completely different antigenic variation mechanism involving segmental recombination from a contiguous array of *vls* silent cassettes. These two systems thus appear to represent divergence from a common precursor followed by functional convergence to create two distinct antigenic variation processes.

Many, if not most, microbial pathogens have developed mechanisms for evading the immune response, including the invasion of immunoprivileged sites, the inhibition of immune responses, blocking of engulfment or intracellular killing by phagocytes, masking of surface antigens, phase variation and antigenic variation. Phase variation and antigenic variation are perhaps the most complex, involving genetic or epigenetic changes that occur more frequently than the basal mutation rate. Many of these systems are mediated by specialized *cis*-acting elements and *trans*-acting factors. Phase variation refers to a switch between two phenotypes, i.e. turning gene expression on or off (as exemplified by the surface protein gene *opa* of *Neisseria gonorrhoeae*) or toggling between the production of two different gene products (such as the switching between H1 and H2 flagellar antigen types in *Salmonella typhimurium*). Antigenic variation involves the sequential expression of multiple different forms of an antigenic surface protein, permitting the organism to keep one step ahead of immune response. A large number of phase variation and antigenic variation systems have been described in bacterial and protozoal pathogens. Indeed, a possible point of view is that if one of these systems has not been found in a persistent pathogen, it is because we have not looked hard enough. In this issue of *Molecular Microbiology*, Dai *et al*. (2006) provide a detailed analysis of the *vlp*/*vsp* antigenic variation system of the relapsing fever spirochaete, *Borrelia hermsii*, one of the prime examples of bacterial antigenic variation.

Relapsing fever is an arthropod-transmitted bacterial infection caused by any of a number of *Borrelia* species. The epidemic form of relapsing fever is caused by *Borrelia recurrentis* and is transmitted by the human body louse, *Pudiculus humanus*. Found in areas with crowding and poor sanitation, epidemic relapsing fever caused outbreaks involving hundreds of thousands of soldiers and civilians during World War I and after World War II. Soft-bodied ticks of the genus Ornithodoros transmit the endemic form of relapsing fever. Sporadic human cases of this epizootic disease result from exposure to infected ticks acquired from animals. There is host specificity between the *Borrelia* species and the tick; for example, *B. hermsii* is transmitted by *Ornithodoros hermsi*. Relapsing fever is characterized by several spikes of high temperature accompanied by phases of bacteraemia, in which the number of *Borrelia* in blood can reach 10^8 ml^{-1} (Fig. 1A). Each cycle of infection is caused by spirocha-

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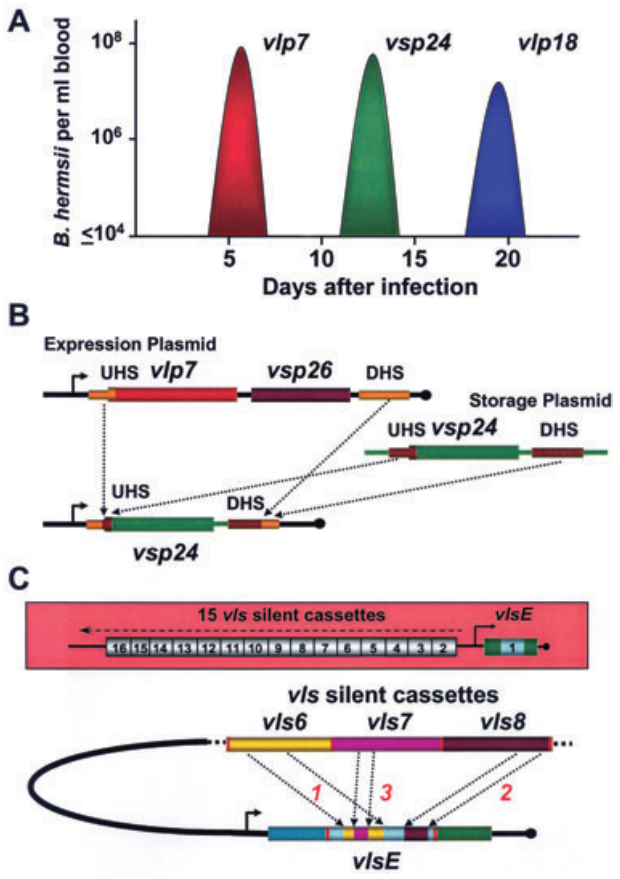

Fig. 1. Antigenic variation mechanisms in relapsing fever and Lyme disease *Borrelia*.
A. Pattern of infection antigenic variation during mammalian infection with the relapsing fever spirochaete, *Borrelia hermsii*. During the initial phase of infection, a predominant serotype (in this case, Vlp7) is expressed. Concentrations of organisms in the blood can reach 10^8 ml^{-1}. The first serotype is rapidly eliminated by serotype-specific antibodies, and subsequent relapses are caused by spirochaetes that have undergone antigenic variation to different Vlp or Vsp serotypes (e.g. Vsp24 and Vlp18).
B. The principal mechanism of antigenic variation in *B. hermsii* is gene conversion involving recombination at the upstream homology sequences (UHS) and the downstream homology sequences (DHS), resulting in replacement of the *vlp* or *vsp* gene at the expression site.
C. Antigenic variation in the Lyme disease spirochaete *Borrelia burgdorferi* utilizes a different mechanism, in which segments within the *vlsE* cassette region are replaced by sections of varied length and location from the silent cassettes. In this hypothetical example, *vlsE* has undergone three sequential gene conversion events with silent cassettes *vls6*, *vls8* and *vls7*. In contrast to relapsing fever, mammals infected with Lyme disease *Borrelia* appear to harbour thousands of different VlsE variants rather than a single predominant serotype.

etes producing a different form of the Vlp or Vsp surface lipoprotein (Fig. 1B). Clearance occurs when the host produces sufficient antibodies specific for that serotype to result in elimination. *vlp*/*vsp* variants occur at high frequency (10^{-4} to 10^{-3} recombination events per cell per generation), and each new wave of infection is predominated by a bacterial clone producing one particular form of Vlp or Vsp. Bacteraemia in animals is thereby able to persist long enough to permit transmission to feeding ticks, thus completing the tenuous infection cycle.

Lyme disease spirochaetes (e.g. *Borrelia burgdorferi*, *B. garinii* and *B. afzelii*) are transmitted by hard-bodied ticks of the genus *Ixodes*. Lyme disease is characterized by a localized infection (erythema migrans) at the site of the tick bite, followed by disseminated and chronic stages with neurological, cardiological, dermatological and arthritic manifestations. In contrast to relapsing fever, Lyme disease *Borrelia* never achieve high blood concentrations but, instead, establish low levels of infection in multiple tissues that can persist for months to years. Lyme disease *Borrelia* possess the *vlsE* antigenic variation system (Zhang *et al*., 1997), in which segmental recombination results in the establishment of a large repertoire of variants in the surface lipoprotein VlsE (Fig. 1C). The degree of variation is so high that each clone examined from a single mouse skin biopsy 28 days post infection had a different *vlsE* sequence (Zhang and Norris, 1998). Thus, the relapsing fever system favours the predominance of a single Vlp/Vsp variant at any one time, whereas Lyme disease infection results in a mosaic of bacterial clones each producing a different VlsE variant.

Both relapsing fever and Lyme disease *Borrelia* have a large number of linear and circular plasmids, representing about a third of the genome. By sequencing ~445 kb of the *B. hermsii* HS1 plasmids, Dai *et al*. (2006) found that the genome contains at least 21 *vsp* and 38 *vlp* silent gene segments, each of which comprises a complete open reading frame (ORF) and ribosome binding site without a promoter. The *vlp* and *vsp* sequences and encoded proteins have no homology to one another (other than a shared sequence in the 5′ region; see below) and, thus, appear to have evolved separately. The *vlp* alleles are subdivided into α, β, γ and δ groups based on the ≥60% sequence identity within each group. Amplification of *vlp* and *vsp* sequences using specific primers indicated that the 59 alleles identified represent ~90% of the total number present. The *vlp* and *vsp* alleles are arranged together in 10 clusters containing 2–14 gene segments each. Each *vlp* and *vsp* allele contains a ~60 bp upstream homology sequence (UHS) that comprises the region immediately upstream of the ORF and the first 27 nt of the ORF; divergence at two central nucleotides provided a marker for the location of recombination cross-over events. Thirteen copies of a 214 bp downstream homology sequence (DHS) are interspersed among the variable gene segment alleles. The expression site with a promoter sequence is at one end of a 28 kb linear plasmid, lp28-1.

The recombination events occurring during relapses were then determined by characterizing 83 variants that arose following infection of mice with *B. hermsii* HS1 expressing either *vlp7* or *vlp17*. In all cases, the newly expressed sequences could be attributed to a gene con-

version event involving 17 of the 59 available *vlp* and *vsp* silent (archival) sequences. In 68 of the 83 variants, both upstream and downstream sequences were available to evaluate the locations of recombination. The 5′ end of the recombination events occurred primarily within the 3′ half of the UHS, as determined by examining the polymorphisms occurring in this region. Only one of these 5′ recombinations occurred beyond the UHS, resulting in a chimeric *vlp7*/*vlp18* gene. With few exceptions, the 3′ recombination event could be traced to a pairing of DHS regions, with 83% of the recombinations occurring within the distal half of the DHS. In a few examples where more than one DHS was available downstream of the archival gene, the most distant DHS was selected. Overall, the vast majority of relapse serotypes resulted from recombination at the UHS and DHS sites, replacing the intervening DNA with the archival 'donor' sequence (Fig. 1B); the remaining scenarios are presented in detail by Dai *et al*. (2006). The most frequently used archival sequences (α*vlp18*, *vsp24*, *vsp2*, *vsp1* and *vsp6*) all have a DHS immediately downstream of the silent gene segment, indicating that this arrangement is favourable to gene conversion. Interestingly, a small number of variants utilized archival sequences that lacked a DHS, implicating other sequences in the downstream cross-over event. The frequencies of archival *vls* and *vlp* gene utilization will be addressed in a separate article (A.G. Barbour, Q. Dai, B.I. Restrepo and S.A. Frank, submitted).

In Lyme disease *Borrelia*, antigenic variation involves segmental gene conversion between the *vlsE* expression site and one of a series of *vls* silent cassettes corresponding to the middle region of *vlsE* (Fig. 1C). In *B. burgdorferi* B31, *vlsE* and the contiguous array of *vls* silent cassettes are located adjacent to one another in the linear plasmid lp28-1 (Zhang *et al*., 1997). The *vls* silent cassettes are arranged in a similar manner in the other *B. burgdorferi*, *B. garinii* and *B. afzelii* strains examined to date (Kawabata *et al*., 1998; Wang *et al*., 2001; 2003; Glöckner *et al*., 2004). Gene conversion events involve replacement of random, variable length segments of the central cassette region of *vlsE* with corresponding regions from any of the silent cassettes. The recombination events have been detected as early as 4 days after infection of mice and appear to occur continuously during infection. As a result, mammals could harbour thousands of different variants at any one time, resulting in altered epitopes (McDowell *et al*., 2002) and confounding efforts by the immune response to keep up with the sequence variation. The sequence changes primarily affect six variable regions that are localized in the most exposed, membrane distal portion of VlsE (Eicken *et al*., 2002). Interestingly, a strong antibody response is mounted against conserved regions (particularly invariant region 6) of VlsE, and VlsE-based recombinant proteins or synthetic peptides are now being used for the immunodiagnosis of Lyme disease (Bacon *et al*., 2003; Schulte-Spechtel *et al*., 2003). These highly antigenic regions are 'buried' in subsurface regions of VlsE and are apparently inaccessible to antibodies. Variation in *vlsE* has not been detected during *in vitro* culture, in ticks, or even in dialysis membrane chambers implanted in animals, indicating that as yet uncharacterized host factor(s) trigger the recombination process.

Although the functions of Vlp, Vsp and VlsE lipoproteins are not known, there are indications that they are involved in host–pathogen interactions independent of their antigenic variation properties. Their production is highly regulated, resulting in higher abundance during mammalian infection. Production of the *B. hermsii* 'bloodstream' proteins Vlp or Vsp in mammals alternates with the induced production of the 'tick' protein Vtp (also called Vsp33) in ticks and *in vitro* culture (Schwan and Hinnebusch, 1998; Barbour, 2003; Porcella *et al*., 2005). Vtp is closely related to the Vsps. In addition, the Lyme disease *Borrelia* protein OspC is homologous to the Vsps, is induced during tick feeding and is vital to transmission of *B. burgdorferi* from ticks to mammals (Grimm *et al*., 2004; Pal *et al*., 2004). Variants of the relapsing fever organism *Borrelia turicatae* producing two different Vsp proteins, VspA and VspB, differ markedly in their pattern of pathogenesis in mice: VspA production results in infection of the central nervous system, whereas VspB is associated with 10-fold higher levels in the blood, severe arthritis, myocarditis and vestibular dysfunction (Pennington *et al*., 1997; Cadavid *et al*., 2001). The involvement of Vlps and the related Lyme disease protein VlsE in pathogenesis has not been studied in great detail, but a study by Crother *et al*. (2003) indicates that VlsE levels are higher in bacteria in the joint and skin tissue than in heart tissue of *B. burgdorferi*-infected mice. Taken together, these results point towards a role of the *Borrelia* antigenic variation proteins in tissue tropism and varied patterns of pathogenesis.

In summary, the relapsing fever and Lyme disease *Borrelia* have evolved complex, yet distinct, antigenic variation systems that are based on two discrete gene families encoding Vlp and Vsp homologues. Although these two branches of the *Borrelia* genus are closely related (i.e. are about as similar as are *Escherichia coli* and *S. typhimurium*), it is likely that they diverged a million or more years ago. The common *Borrelia* progenitor carried *vlp* and *vsp* precursors that underwent duplication and divergence in sequence, expression patterns and function. A chicken or egg (or mammal or tick) question is whether an antigenic variation system already existed in this common precursor, or the *vlp*/*vsp* and *vlsE* variation systems evolved after the split between relapsing fever and Lyme borreliosis organisms occurred. Most of the evidence points to the latter possibility. In the relapsing fever *vlp*/*vls* system, recombination occurs predominantly

within the intragenic UHS and extragenic DHS sequences, and results in replacement of the entire gene (except for part of the UHS), whereas the Lyme borreliosis system results in the replacement of random, seemingly unanchored segments within the central cassette region of *vlsE*. In this respect, the relapsing fever system most closely resembles gene conversion in trypanosomes, while the Lyme borreliosis mechanism is similar to the *N. gonorrhoeae pilE* system (Criss *et al*., 2005). The promoterless gene copies are loosely arranged in 10 clusters in the *B. hermsii* system; the *vls* silent cassettes in the Lyme disease strains examined comprise only the central region of the expression site and are packed cheek to jowl in a contiguous array. Finally, the relapsing fever system has the unique ability to alternate between two distinct gene families (expressing Vlps and Vsps). Thus, these remarkable antigenic variation systems, further revealed by the work of Dai *et al*. (2006), may represent a rare example of divergent evolution leading to convergent function in terms of antigenic variation and immune evasion.

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