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Lyme borreliosis spirochete Erp proteins, their known host ligands, and potential roles in mammalian infection

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Abstract

Lyme borreliae naturally maintain numerous distinct DNA elements of the cp32 family, each of which carries a mono- or bicistronic *erp* locus. The encoded Erp proteins are surface-exposed outer membrane lipoproteins that are produced at high levels during mammalian infection but largely repressed during colonization of vector ticks. Recent studies have revealed that some Erp proteins can serve as bacterial adhesins, binding host proteins such as the complement regulator factor H and the extracellular matrix component laminin. These results suggest that Erp proteins play roles in multiple aspects of mammalian infection.

Keywords

Borrelia burgdorferi; Outer surface protein; Adhesin; Complement; Factor H; Laminin

Introduction

All examined Lyme borreliosis spirochetes contain numerous distinct DNA elements (Casjens et al., 2006). The *Borrelia burgdorferi* type strain, B31, is known to carry at least 25 separate DNA species, ranging from the ~950 kb main chromosome to the ~5 kb plasmid lp5 (Casjens et al., 1997, 2000; Fraser et al., 1997; Miller et al., 2000). Naturally-occurring, infectious isolates contain between 6 and 10 distinct, but homologous, DNA elements called cp32s (Simpson et al., 1990; Porcella et al., 1996; Stevenson et al., 1996, 2001; Zückert and Meyer, 1996; Casjens et al., 1997, 2000, 2006; Akins et al., 1999; Iyer et al., 2003; Stevenson and Miller, 2003). Members of the cp32 family have been identified in all examined Lyme disease-associated spirochetes, including those of the species *B. burgdorferi* sensu stricto, *B. garinii*, *B. spielmanii*, and *B. afzelii* (our unpublished results and Stevenson et al., 2006). Most cp32 elements are circular episomes of approximately 32 kb in size, although some naturally-occurring mutant cp32s have been identified, such as truncated 18 kb plasmids of *B. burgdorferi* strains N40 and 297 and a 56 kb linear cp32-hybrid plasmid of strain B31 (Stevenson et al., 1997; Caimano et al., 2000; Casjens et al., 2000). Several lines of evidence suggests that cp32 elements are bacteriophage genomes, although no one has yet isolated a

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borreliaphage and shown it to be encoded by cp32 genes (Casjens et al., 1997, 2000; Eggers and Samuels, 1999; Damman et al., 2000; Eggers et al., 2001; Zhang and Marconi, 2005).

All members of the cp32 family contain one mono- or bicistronic *erp* locus, the sequences of which generally vary among the different cp32s within an individual bacterium, and also between bacterial strains (Table 1) (Stevenson et al., 2001,2006). All *erp* loci are preceded by nearly identical DNA sequences that include the transcriptional promoter and binding sites for at least three distinct DNA-binding proteins (Marconi et al., 1996;Stevenson et al., 1996, 2001;Babb et al., 2004,2006). As would be expected from the extensive identities of *erp* promoter/operator sequences, almost all analyzed *erp* genes follow the same expression patterns in vitro and in vivo (Stevenson et al., 1995,1998a;El-Hage and Stevenson, 2002;Hefty et al., 2002;Miller et al., 2003). The exceptions to the consensus pattern may reflect promoter mutations among those *erp* loci and/or allelic variations among regulatory factors (Akins et al., 1995;Suk et al., 1995;Hefty et al., 2001;Eggers et al., 2004,2006). The significance of such variations has yet to be determined. In general, however, Erp proteins are synthesized during mammalian infection but repressed during colonization of the vector tick (Das et al., 1997;Gilmore et al., 2001;McDowell et al., 2001;Hefty et al., 2002;Liang et al., 2002;Miller et al., 2003,2005,2006;Miller and Stevenson, 2006).

erp genes and their encoded proteins often differ widely in their sequences (Fig. 1). These differences have led to proposals that this family could be divided into three or more groups, each with a different name (Akins et al., 1999). However, since these genes and proteins share many unifying features, we continue to use the single name *erp*. Please see Stevenson et al. (2006) for a comprehensive review of the similarities and differences between *erp* genes and Erp proteins. Variations among *erp* sequences have proven valuable for studies of *B. burgdorferi* genetic exchange and recombination. An individual spirochete may contain several different cp32 elements that each carry an identical *erp* locus, such as the *erpAB* loci on the cp32-1, cp32-5, and cp32-8 elements of *B. burgdorferi* type strain B31, which are evidence of genetic shuffling within bacteria (Casjens et al., 1997,2000;Stevenson et al., 1998a;Stevenson and Miller, 2003). In addition, some *B. burgdorferi* isolates are genetically distinct at numerous loci, yet contain some identical *erp* genes, indications that cp32s and *erp* genes are naturally transmitted horizontally among different bacteria (Stevenson et al., 1998b;Stevenson and Miller, 2003; Stevenson, Cooley and Woodman, submitted). In all, strain B31 is known to contain 10 distinct cp32 family members, encoding 13 different Erp proteins (Stevenson et al., 1996;Casjens et al., 1997,2000).

Especially relevant to this review, all Erp proteins are surface-exposed outer membrane lipoproteins (Lam et al., 1994; El-Hage et al., 2001; Hefty et al., 2002). These proteins are therefore positioned to interact with the bacteria's environment. As we describe below, the known functions of Erp proteins all involve binding of vertebrate host proteins.

Erp binding of host factor H

As an infected tick feeds on its host, Lyme borreliosis spirochetes are transmitted directly into the blood pool at the tick bite site. Bacteria then spread via the bloodstream and by invasion of host tissues to establish a chronic, disseminated infection (Cassatt et al., 1998; Stanek and Strle, 2003; Wormser, 2006). Spirochetes may later be acquired by additional ticks as they take a blood meal from the infected host. As are many other pathogenic microorganisms, *B. burgdorferi* is naturally resistant to the innate immune system of its hosts. As an example, fewer than 20 bacteria can be sufficient to infect immunocompetent animals (Barthold, 1991). The alternative pathway of complement activation is an important arm of vertebrate innate immunity, which rapidly clears susceptible microorganisms from the host in the absence of antibody. In culture, most infectious isolates of *B. burgdorferi* are resistant to their hosts'

alternative pathway of complement activation (Kochi et al., 1991; Brade et al., 1992; Breitner-Ruddock et al., 1997; van Dam et al., 1997; Kurtenbach et al., 1998, 2002). That characteristic is associated with binding of the host complement regulator factor H, enhanced breakdown of C3b and the C3bBb convertase, and prevention of membrane-attack complex formation (Alitalo et al., 2001; Kraiczy et al., 2001b). Serum-resistant *B. burgdorferi* produce several distinct outer-surface proteins during culture, termed BbCRASPs (*B. burgdorferi* complement regulator-acquiring surface proteins), that can bind host factor H (Kraiczy et al., 2001a, 2001b). The ability of *B. burgdorferi* to bind host factor H to its surface is apparently not the only mechanism by which Lyme borreliosis spirochetes evade host complement in vivo, since mice deficient in factor H can be infected to degrees equal to those of wild-type animals (Woodman et al., 2007).

A number of studies have demonstrated that a specific subset of the Erp family is capable of binding mammalian factor H under physiological conditions. The ErpA, ErpC, and ErpP proteins of type strain B31 exhibit significant affinities for factor H (Hellwage et al., 2001; Alitalo et al., 2002, 2004; Stevenson et al., 2002; Kraiczy et al., 2003, 2004a; Metts et al., 2003). Those proteins are identical to three proteins identified in *B. burgdorferi* strains ZS7 and LW2, named BbCRASP-3 (ErpP), BbCRASP-4 (ErpC), and BbCRASP-5 (ErpA) (Kraiczy et al., 2001a, 2004a). The ErpA, ErpC, and ErpP proteins are very similar to each other, sharing approximately 90% amino acid sequence identities (Fig. 1). Several very similar Erp proteins produced by other *B. burgdorferi* strains are also known to efficiently bind factor H, including the OspE protein of strain N40 and the P21 protein of strain 297 (Fig. 1) (Akins et al., 1999; Hellwage et al., 2001; Alitalo et al., 2002). Strain B31 carries three identical copies of *erpA*, on cp32-1, cp32-5, and cp32-8, and one copy each of *erpC* and *erpP* on cp32-2 and cp32-9, respectively (Stevenson et al., 1996; Casjens et al., 1997, 2000). Other strains of *B. burgdorferi* also carry multiple copies of identical genes that encode factor H-binding Erp proteins, the significance of which has yet to be explored (our unpublished results and Stevenson and Miller, 2003).

Mutagenesis studies have demonstrated that the carboxy-terminus and several internal amino acid motifs of ErpA/ErpC/ErpP/P21/OspE play roles in binding factor H (Alitalo et al., 2002, 2004; Kraiczy et al., 2003; Metts et al., 2003). Whether the identified residues directly interact with factor H or if they are instead required only for correct folding of the Erp proteins remains to be determined. Computer modeling suggested that these Erp proteins may form coiled-coil structures, a prediction that has yet to be tested experimentally (McDowell et al., 2004).

Other members of the Erp protein family also bind factor H in vitro, although those interactions appear to be too weak to be of biological significance (Alitalo et al., 2002; Stevenson et al., 2002; Hovis et al., 2006). In particular, ErpX can bind factor H in vitro, but only at non-physiological pH (Alitalo et al., 2002; Stevenson et al., 2002).

Serum-resistant *B. burgdorferi* produce two additional factor H-binding proteins during cultivation, named BbCRASP-1 and BbCRASP-2. Those outer membrane proteins are encoded by two distinct, unrelated genes named *cspA* (BbCRASP-1) and *cspZ* (BbCRASP-2) (Casjens et al., 2000; Kraiczy et al., 2001a, 2001b, 2002b, 2006; McDowell et al., 2003; Wallich et al., 2005; Hartmann et al., 2006). Factor H consists of 20 repeated motifs, termed short consensus repeats (SCRs) (Zipfel et al., 2002). Erp proteins bind to the carboxy-terminal SCR-20, which is also a major heparin-binding domain of factor H (Hellwage et al., 2001, 2002; Kraiczy et al., 2001a, 2001b, 2002b; Zipfel et al., 2002; Cheng et al., 2006). In contrast, BbCRASPs-1 and -2 both bind primarily to SCR-7 (Kraiczy et al., 2001a, 2001b, 2002b, 2004b; Hartmann et al., 2006). Those different affinities may have important consequences. Factor H in solution folds upon itself, with the carboxy-terminal SCRs exposed but SCR-7 is

apparently buried. However, binding of factor H to adhesins via its carboxy-terminus uncoils the protein, which permits interactions between SCR-7 and its ligands (Aslam and Perkins, 2001; Oppermann et al., 2006). Presumably due to the structure of factor H in solution, the carboxy-terminal SCRs provide initial binding of factor H to mammalian cells (Prodinger et al., 1998; Perkins and Goodship, 2002; Jokiranta et al., 2005; Oppermann et al., 2006; J6szi et al., 2007). By analogy, Erp proteins may provide initial contact between the bacteria and factor H through SCR-20, causing the structure of factor H to open up and permit BbCRASPs-1 and/or -2 to bind the host protein more tightly via SCR-7. Cultured *cspA*[−] *cspZ*⁺ *erp*⁺ *B. burgdorferi* are very sensitive to killing by the alternative complement pathway (Patarakul et al., 1999; Brooks et al., 2005), and complementation of a *cspA*[−] mutant with a copy of the wild-type gene can restore in vitro complement resistance (Brooks et al., 2005). Erp proteins by themselves do not provide complement resistance to cultured *B. burgdorferi*. For examples, a mutant of strain B31 named B31-e2 lacks all BbCRASP-encoding genes except *cspA* plus one copy of *erpA*, but is as resistant to complement as its wild-type parent, while a sibling *cspA*[−] *cspZ*[−] mutant named B313 carries *erpC* and one copy of *erpA*, but is sensitive to in vitro killing by complement (Hartmann et al., 2006, and our unpublished results). Transformation of mutant B313 with a wild-type *cspZ* gene provided resistance to complement in vitro, indicating that BbCRASP-2 can play a role in protecting against complement-mediated killing (Hartmann et al., 2006). However, there are two important caveats to the above-described studies of cultured Lyme borreliosis spirochetes. First, studies have never been performed on *erp*-deficient bacteria to examine the abilities of BbCRASPs-1 or -2 to function in the complete absence of Erp proteins, so the possibility of cooperation between those proteins cannot be ruled out. Second, the relative importance of each gene during infection processes is unknown, since neither *cspA*, *cspZ*, nor all the *erp* genes have been deleted from an otherwise infectious bacterium.

As noted above, mice lacking the factor H gene (*Cfh*^{−/−}) are infected to the same extents as are congenic wild-type mice (Woodman et al., 2007). Those results indicate that the ability of *B. burgdorferi* to bind factor H to its surface is redundant to at least one other mechanism of complement resistance. *B. burgdorferi* appears to synthesize additional substances that protect against complement, such as a putative slime layer (Kraiczy et al., 2000) and a CD59-like protein that inhibits MAC formation (Pausa et al., 2003). Moreover Lyme borreliosis spirochetes are well known to express proteins during mammalian infection that are not produced during laboratory cultivation, so it is quite likely that bacterial factors not yet identified protect borreliae from complement in vivo. Supporting those hypotheses are the isolation of Lyme borreliae that are infectious for humans and other mammals, yet are unable to bind factor H in vitro (Alitalo et al., 2001; Kraiczy et al., 2001b; McDowell et al., 2003; Wallich et al., 2005).

B. burgdorferi may benefit from other functions of factor H. Eukaryotic cells bind factor H to their surfaces through several different specific and non-specific receptors (Avery and Gordon, 1993; DiScipio et al., 1998; Malhotra et al., 1999; Zipfel et al., 2002; Vaziri-Sani et al., 2005). Borrelial binding to factor H may therefore serve as a bridge to facilitate adherence to host cells and tissues (Hammerschmidt et al., 2007).

In addition, the studies of Woodman et al. (2007) indicated that *B. burgdorferi* do not coat themselves with detectable levels of host factor H during transmission from infected mice to feeding larval ticks. Those results suggest that Erp proteins and other CRASPs may bind host components other than factor H, which preclude CRASP-factor H binding (see below, and Hovis et al., 2006; McDowell et al., 2006).

Humans produce an additional serum protein, factor H-like protein 1 (FHL-1), from the same gene as factor H using an alternative mRNA splice site (Misasi et al., 1989; Zipfel et al.,

2002). FHL-1 consists of the first 7 SCRs of factor H, plus a unique 4-amino-acid carboxy terminus. Since FHL-1 lacks the factor H SCR-20, Erp proteins do not bind FHL-1, although BbCRASPs-1 and -2 do bind (Hellwage et al., 2001; Kraiczy et al., 2001a, 2002a, 2002b, 2003, 2004b; Wallich et al., 2005; Hartmann et al., 2006). FHL-1 plays roles in both complement regulation and cell adhesion (Misasi et al., 1989; Hellwage et al., 1997; Friese et al., 1999; Zipfel and Skerka, 1999; Zipfel et al., 2002). However, mice and other rodents do not appear to produce FHL-1 (Stevenson et al., 2002; Zipfel et al., 2002), so while the ability of Lyme borreliæ to bind FHL-1 might have consequences for human disease, that characteristic probably does not contribute to infection of other vertebrates or to the persistence of these spirochetes in nature.

Erp binding of host factor H-related proteins

Vertebrates produce several additional serum proteins known as factor H-related proteins (FHRs), with humans and mice each containing 5 distinct FHR-encoding genes (Zipfel et al., 2002; Hellwage et al., 2006). FHRs are smaller in size than is factor H, being comprised of between 4 and 9 SCRs (Zipfel et al., 2002). Some FHRs bear significant sequence similarities to factor H: for example, SCRs 3, 4, and 5 of human FHR-1 share 100, 100, and 97% identities to human factor H SCRs 18, 19, and 20, respectively (Zipfel et al., 2002). As might be expected from the high degree of similarities between the carboxy-terminal SCRs of factor H and FHR-1, ErpA, ErpC, and ErpP can each bind human FHR-1 (Haupt et al., 2007). Presumably, FHRs are among the unidentified serum proteins that Hovis et al. (2006) showed to bind ErpA and ErpP. The functions of FHR proteins are poorly understood. Some appear to play roles in regulation of the alternative pathway of complement activation (Hellwage et al., 1999, 2002, 2006; McRae et al., 2005; Zipfel et al., 2007). FHRs are also components of a plasma lipoprotein particle of unknown function (Park and Wright, 1996, 2000). Thus, binding of FHRs to the borreliæ outer surface via Erp proteins might help facilitate resistance to complement or have other, unknown consequences.

Erp binding of host laminin

During mammalian infection, Lyme borreliosis spirochetes are frequently found associated with their hosts' extracellular matrices (De Koning et al., 1987; Häupl et al., 1993; Pachner et al., 1995). Several extracellular matrix (ECM) components have been previously identified as potential ligands for *B. burgdorferi* outer surface proteins, including fibronectin and decorin (Guo et al., 1995; Feng et al., 1998; Grab et al., 1998; Hagman et al., 1998; Probert and Johnson, 1998). Laminins are a family of related glycoproteins that constitute major components of vertebrate basement membranes (Colognato and Yurchenco, 2000). Until recently, there had not been any studies on the potential for interactions between that important ECM component and *B. burgdorferi*.

Recent studies by our laboratory and others determined that the pathogenic spirochete *Leptospira interrogans* produces a family of outer surface lipoproteins with varying abilities to bind both factor H and laminin (our unpublished results and Barbosa et al., 2006; Verma et al., 2006). There are no genetic relationships between those leptospiral proteins and borreliæ Erp proteins. However, factor H and laminin share affinities for the synthetic molecule heparin as well as for heparan sulfate and other negatively-charged glycosaminoglycans that are natural components of vertebrate cell surfaces. Most Erp proteins are predicted to have net negative charges, with acidic pI values. As examples, the mature ErpA protein contains 12% glutamate, 6% aspartate, and 14% lysine and has a predicted pI=5.1, while ErpX contains 18% glutamate, 9% aspartate, and 19% lysine residues and has a predicted pI=4.9. Those characteristics, plus the known affinities of some Erp proteins for the heparin-binding domain of factor H, led us to evaluate the abilities of Erp proteins to bind laminin.

Ligand affinity blot analyses of recombinant proteins of *B. burgdorferi* strain B31 revealed that ErpX bound laminin, although no other Erp protein of that strain exhibited affinity for laminin (Fig. 1) (our unpublished results). The *erp26* gene of *B. burgdorferi* strain N40 is the known gene most closely related to *erpX* (Fig. 1), however, recombinant Erp26 protein did not detectably bind laminin (our unpublished results). To date, we have produced several mutant recombinant ErpX proteins, including one that lacks the amino-terminal 30 amino acids and the carboxy-terminal 31 amino acids, but all still bind laminin (our unpublished results). Further studies are ongoing in our laboratory to define the residues responsible for laminin binding by ErpX, discern the mechanism behind those interactions, and evaluate the importance of Erp-laminin binding on *B. burgdorferi* infection processes.

Conclusions and future directions

All Lyme borreliosis spirochetes naturally contain numerous different cp32 elements, each of which carries an *erp* locus. The ubiquity of cp32s is readily explained by the hypothesis of their being bacteriophage genomes: Bacteriophage particles can easily transmit DNA horizontally and result in bacteria carrying multiple compatible prophages, while bacteria that lose a cp32 would be rapidly re-infected by phages produced by neighboring spirochetes. Until recently, the presence of an *erp* locus on each cp32 has been much more difficult to explain. The *erp* locus is outside the apparent operon(s) that encode(s) probable borreliaphage structural and cell lysis proteins (Casjens et al., 2000; Damman et al., 2000; Zhang and Marconi, 2005). The relapsing fever spirochete *B. hermsii* also naturally carries numerous cp32 elements, but none of them contains an *erp* locus (Stevenson et al., 2000). Those data suggest that Erp proteins are not essential for cp32 maintenance within the bacterium or for bacteriophage-specific functions. The discoveries that some Erp proteins can bind host factor H or laminin, and may thereby protect the bacterium from complement-mediated killing or enhance host colonization, point toward functions for Erp proteins that do not directly affect the encoding cp32. It appears that cp32 elements encode Erp proteins to perform functions that benefit their bacterial hosts, and, by enhancing bacterial survival, cp32s also increase their own probabilities of thriving.

The recent discoveries of functions for Erp outer surface proteins raise many new questions. Do the factor H-binding and laminin-binding Erp proteins perform additional functions for the spirochete? Do the other Erp proteins also have functions, and what are they? Do sequence variations among Erp proteins of different bacterial strains affect the affinities of those proteins for their host ligands? Could Erp protein variations affect infectious abilities of the different borreliae? Answering those questions will provide important information on the molecular mechanisms by which Lyme borreliosis spirochetes infect and cause disease in humans and other vertebrate hosts

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Fig. 1.

Unrooted phylogram of predicted amino acid sequences of the Erp proteins encoded by the fully-characterized *B. burgdorferi* strains B31, BL206, N40, Sh-2-82, and 297 (Table 1) and prepared using PAUP* version 4.0b10 (Swofford, 2000). Most of the *erp* genes of strains Sh-2-82 and 297 are completely identical to each other, as are also the *erp* genes of strains B31 and BL206 (our unpublished results and Stevenson and Miller, 2003). A closely-related subset of Erp proteins have been shown to bind mammalian factor H in vitro under physiologically relevant conditions. Of those, the B31/BL206 ErpA, ErpC, and ErpP proteins have also been demonstrated to bind human FHR-1. Only the B31/BL206 ErpX protein is known to bind mammalian laminin. Please see text for details and references. Functions for other Erp proteins have yet to be determined.

Table 1
B. burgdorferi cp32 plasmids and their associated *erp* loci. Various naming schemes have been applied to these genes by their discoverers, resulting in similar genes often having dissimilar names.

Plasmid group ^a	B31	BL206	N40	Sh-2-82	297
cp32-1	<i>erpAB</i>	—	—	<i>erp41, 42</i>	<i>ospE, elpB1</i>
cp32-2/7	<i>erpCD</i> or <i>erpLM</i> ^b	<i>erpCD</i>	<i>ospEF</i>	<i>erp43</i>	<i>elpA2</i>
cp32-3	<i>erpG</i>	<i>erpG</i>	—	<i>erp44</i>	<i>ospF</i>
cp32-4	<i>erpHY</i>	<i>erpHY</i>	<i>erp23, 24</i>	<i>erp45</i>	<i>elpA1</i>
cp32-5	<i>erpAB</i> ^c	<i>erpAB</i>	<i>erp25</i>	<i>erp41, 42</i>	<i>ospE, elpB1</i>
cp32-6	<i>erpK</i>	<i>erpK</i>	—	<i>erp46</i>	<i>bbk2.10</i>
cp32-8	<i>erpAB</i> ^c	—	—	<i>erp50, 51</i>	—
cp32-9	<i>erpPO</i>	<i>erpPO</i>	<i>p21, erp22</i>	<i>erp47, 48</i>	<i>p21, elpB2</i>
cp32-10	<i>erpX</i>	<i>erpX</i>	<i>erp26</i>	—	—
cp32-11	—	<i>erpAB</i>	—	<i>erp49</i>	<i>bbk2.11</i>
cp32-12	—	—	<i>erp27</i>	<i>erp41, 42</i>	<i>ospE, elpB1</i>

— Not detected

^aUnified cp32 nomenclature as previously described (Stevenson et al., 1996, 2001; Casjens et al., 1997, 2006; Stevenson and Miller, 2003). Some earlier descriptions of strain 297 utilized a different naming system (Akins et al., 1999; Caimano et al., 2000; Stevenson et al., 2001).

^bTwo distinct plasmids of group cp32-2 have been identified in strain B31.

^cThe identical *erp* loci of strain B31 cp32-5 and cp32-8 were formerly designated *erpIJ* and *erpNO*, respectively (Casjens et al., 1997, 2000).