Erp and Rev adhesins of the Lyme disease spirochete's ubiquitous cp32 prophages assist the bacterium during vertebrate infection

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20 **Abstract**

21 Almost all spirochetes in the genus *Borrelia* (sensu lato) naturally contain multiple
22 variants of closely related prophages. In the Lyme disease borreliae, these prophages are
23 maintained as circular episomes that are called cp32s (circular plasmid 32kb). The cp32s
24 of Lyme agents are particularly unique in that they encode two distinct families of
25 lipoproteins, Erp and Rev, that are expressed on the bacteria's outer surface during
26 infection of vertebrate hosts. All identified functions of those outer surface proteins
27 involve interactions between the spirochetes and host molecules: Erp proteins bind
28 plasmin(ogen), laminin, glycosaminoglycans, and/or components of complement, and
29 Rev proteins bind fibronectin. Thus, cp32 prophages provide their bacterial hosts with
30 surface proteins that can enhance infection processes, thereby facilitating their own
31 survival. Horizontal transfer via bacteriophage particles increases spread of beneficial
32 alleles and creates diversity among Erp and Rev proteins.

33

34 **Introduction**

35 Members of the spirochete genus *Borrelia* (sensu lato) persist through infectious
36 cycles between blood-feeding arthropods and vertebrates, and include agents of
37 infections that plague humans, domestic animals, and wildlife (1, 2). These include Lyme
38 disease, caused by *Borrelia burgdorferi* sensu lato, and tick-borne relapsing fever, caused
39 by *B. hermsii, B. turicatae,* and others. Although a proposal has been raised to divide the
40 genus into *Borrelia* for relapsing fever agents and *Borreliella* for Lyme disease agents, that
41 idea is controversial (3-8). For the purpose of this review, we use the unified genus name
42 *Borrelia*, in part because of the shared property among Lyme disease and relapsing fever
43 spirochetes of near-universal natural colonization with multiple, closely-related cp32
44 prophages (9-13) (Figs. 1 and 2). Of particular significance to this review, the cp32s of
45 both Lyme disease and relapsing fever borreliae carry *mlp* genes, while *erp* and *rev* genes
46 appear to be restricted to the Lyme disease borreliae. The significance of those genes will
47 be discussed further below. For simplicity, we also use the name *Borrelia burgdorferi* to
48 encompass all genospecies of Lyme disease spirochetes, *B. burgdorferi* sensu lato.

49 *Borrelia* species all have multipartite genomes, consisting of a ca. one megabase
50 main chromosome and numerous smaller replicons that are generally referred to as
51 "plasmids" (14-16). Some borrelial "plasmids" carry essential genes, and might be better
52 considered to be additional chromosomes (15-20). Another uncommon feature of
53 borrelial genomes is a preponderance of linear DNAs, with the main chromosome and
54 many of the smaller replicons being linear elements with closed hairpin telomeres (15, 16,
55 18, 21-23). Lyme disease spirochetes and many other borreliae also maintain circular
56 DNA replicons (24-26).

57 During the mid-1990s, four research groups published evidence of multiple,
58 homologous loci in *B. burgdorferi* (26-30). Our group pursued the genomic locations of

59 those loci and discovered that each related locus is carried on a different circular replicon
60 of approximately 32 kb in size, and that a single bacterial cell can contain numerous
61 different variants of those replicons (26). Based on the sizes and circular nature of the
62 replicons, they were designated "cp32"s (circular plasmid 32kb). The majority of
63 nucleotides that comprise *B. burgdorferi* cp32s are identical to each other, differing
64 significantly at only three loci: one that is responsible for plasmid maintenance and two
65 that encode outer-surface lipoproteins (Fig. 1) (12). The highly repetitive nature of cp32s
66 initially prevented assembly of their sequences, such that the initial, 1997 report of the
67 genome of *B. burgdorferi* type strain B31 did not include the native cp32 plasmids (15).
68 Genomics analysts subsequently used the cp32s as learning tools to refine sequence
69 assembly algorithms, and the entire genome sequence of strain B31, including all cp32s
70 and other repetitive sequences, was finally published in 2000 (16).

71 The different cp32s within a single bacterium appear to be compatible with each
72 other due to their encoding two maintenance proteins, ParA and BpaB, that are distinct
73 for each cp32 within a cell (30-35). Yet, *parA-bpaB* locus sequences are conserved between
74 strains (13, 31, 34). Extensive characterization of cp32 sequences from Lyme disease
75 spirochetes that were collected around the world revealed twelve apparent clades of cp32
76 *parA-bpaB* pairings (13, 34, 36). Additionally, some strains contain small plasmids of
77 approximately 9 kb that were derived from cp32s and which carry only a *bpaB* allele (15,
78 16, 37). The absence of a *parA* gene from those cp9 plasmids raises intriguing questions
79 about plasmid maintenance in *Borrelia* species.

80 The conserved size and sequence of *B. burgdorferi* cp32s, along with genes for
81 proteins that resemble bacteriophage portals and holins, led to an early hypothesis that
82 they might be prophage genomes (9, 38). Cultures of *B. burgdorferi* have been observed to
83 clear spontaneously, or when exposed to 1-methyl-3-nitroso-nitroguanadine (MNNG),

84 with production of bacteriophage particles that were named ϕBB1 (10, 39). Molecular
85 analyses of those particles revealed that they contained cp32 DNA, and that they were
86 capable of transducing nucleic acids between *B. burgdorferi* cells (40, 41). A subsequent
87 study revealed that MNNG can induce transcription of a long, multigene cp32 operon
88 that presumably encodes bacteriophage structural proteins (42). Intriguingly, even
89 though MNNG is a mutagen, *B. burgdorferi* lacks an SOS system (15), so the means
90 through which this chemical induces bacteriophage production is not yet known.
91

92 ***B. burgdorferi* cp32-encoded outer-surface Erp lipoproteins.**

93 Two antigenic outer-surface proteins of *B. burgdorferi* strain N40 were reported in
94 1994 and named OspE and OspF (outer surface proteins E and F) (43). Shortly afterward,
95 we found that *B. burgdorferi* N40 expresses different levels of those two proteins under
96 different culture conditions (44). Screening of a lambda library of *B. burgdorferi* type strain
97 B31 DNA revealed several distinct loci in that strain that were closely related to, but
98 distinct from, the strain N40 *ospE-ospF* locus (26). As none of the strain B31 loci could be
99 definitively called "*ospE-ospF*", they were designated "*erp*" (OspEF-related protein).
100 Further analyses revealed that various clonal cultures of *B. burgdorferi* B31 contain up to
101 9 different *erp* loci (9, 16) (Table 1). Strain N40 contains 6 *erp* loci, including the initially-
102 described *ospE-ospF* (13, 34, 36, 43, 45, 46) (Table 1). Researchers who investigated other
103 strains discovered additional *erp* genes, and gave them a variety of names such as *p21,*
104 *bbk2.10, bbk2.11*, *pG* and *elp* (28, 45, 47-49) (Table 1). To avoid unnecessary confusion, we
105 use the names originally applied to *erp* genes and Erp proteins, and urge other authors to
106 also retain names that have been in common use for the past several decades. Structures

107 have been determined for some Erp proteins, primarily alleles that occur in the B31 type
108 strain (50-53).

109 Considering *erp* genes and their proteins as a unified group is supported by their
110 all occupying an allelic location on cp32s, their conserved promoter and operator DNA
111 sequences, simultaneous co-expression in culture and during infection processes,
112 possession of conserved leader polypeptides and initial residues of their mature
113 lipoproteins, and localization of the mature lipoproteins on the outside of the borrelial
114 outer membrane (12, 54). Evolutionary analyses indicated that *erp* genes arose from a
115 single sequence that encodes the leader polypeptide and the 5 amino acid sorting
116 sequence, while the remainder of the open reading frames descended from a large
117 number of independent origins that frequently undergo novel recombination events (34).

118 Dendrograms of known *erp* sequences show at least three branches (31, 34, 55),
119 which led to a suggestion that *erp* genes could be divided and given three different group
120 names: *ospE*, *ospF*, and *elp* (55). In addition to variations in nucleotide sequences, the
121 encoded proteins generally differ considerably in size, with OspE-types being small
122 (approximately 20 kDa), OspF-types being medium sized (approximately 25-30 kDa), and
123 Elp-types being large (approximately 40 kDa) (34). We do not find the OspE/OspF/Elp
124 nomenclature to be particularly helpful, since functions are not always conserved within
125 each group, and there are no consistent differences in gene expression or operon
126 structures. In addition, due to frequent natural recombination events having occurred
127 between *erp* genes, some extended nucleotide sequences are found in members of all three
128 groups (34). For example, some "*elp*" alleles share greater similarities with "*ospF*" genes
129 (as high as 34%) than they do with other "*elp*" genes (as low as 17%) (34). Recombination
130 events have also resulted in alleles that do not fit in the tripartite scheme (34). We
131 previously published extensive analyses of *erp* open reading frames, operon structures,

132 promoter sequences, and both intragenic and intergenic recombination, and refer readers
133 to that publication for deeper evolutionary discussions (34).

134 Erp lipoproteins are localized to the borrelial outer surface (43, 56, 57). The strain
135 B31 Erp proteins are all simultaneously co-expressed, consistent with their genes
136 possessing conserved promoter and operator sequences (58-60). Differences in gene or
137 protein expression have been reported for two other strains, but the reasons for those
138 apparent variations were not investigated (45, 57, 61). The mechanism by which *B.*
139 *burgdorferi* controls transcription of *erp* operons has been well studied, and shown to
140 involve interactions between the BpaB repressor, BpuR co-repressor, and EbfC
141 antirepressor proteins that bind to specific sites adjacent to *erp* promoters. There is
142 evidence that some *erp* operons may be affected by an alternative sigma factor, RpoS (62,
143 63). For an extensive review of the mechanisms by which *erp* transcription is regulated,
144 please see our recent review on that topic (64).

145 *B. burgdorferi* expresses little to none of any Erp protein during colonization of
146 unfed ticks (45, 59, 65). As the tick begins to feed on the blood of a vertebrate host, the
147 bacteria induce production of their entire repertoire of Erp proteins (59). Indirect
148 fluorescence analyses (IFAs) of skin samples at the sites of tick attachment revealed that
149 almost all transmitted *B. burgdorferi* produced detectable levels of every examined Erp
150 protein (59). Quantitative reverse transcription PCR (qRT-PCR) and promoter fusions
151 with *gfp* revealed that *erp* genes remain induced throughout persistent mammalian
152 infection (59, 66, 67). Levels of antibodies against Erp proteins, including IgM, remain
153 elevated during prolonged infection of mice (59, 66-68). The persistence of IgM antibodies
154 against Erp proteins may reflect *B. burgdorferi*'s ability to manipulate host immune
155 responses (69-72). That phenomenon may also explain how *B. burgdorferi* is able to persist
156 extracellularly despite having its outer surface covered with Erp, Rev, and other antigenic

157 proteins. IFAs of naive ticks feeding on infected mice showed that *B. burgdorferi* continue
158 to produce Erp proteins during acquisition by feeding ticks, which ceases soon after
159 detachment (59) (59).

160 Numerous functions have been identified for Erp proteins, all of which involve
161 interactions with vertebrate host tissues:

162 Several Erp proteins, including ErpA, ErpC, and ErpP of strain B31 and OspE of
163 strain N40, exhibit high affinities for complement factor H and the related proteins FHL-
164 1 and the FHR group (73-83). Binding factor H and FHL-1 to *B. burgdorferi* via Erp proteins
165 confers resistance to the alternative pathway of complement activation. The exact
166 functions of FHR proteins are unclear, but they also appear to be involved with
167 complement control (84). Erp proteins with different sequences show varying affinities
168 to factor H molecules of different potential vertebrate host species, and may thus play
169 roles in expanding the host range of Lyme disease spirochetes (85, 86).

170 The ErpB, ErpM and ErpQ proteins of strain B31 (confusingly called ElpB, ElpM
171 and ElpQ in the publications) were recently demonstrated to bind complement factors
172 C1s and C1r (87, 88). Adhesion of those host proteins onto the borrelial surface appears
173 to afford protection from the classical arm of complement.

174 The ErpX protein of *B. burgdorferi* B31 binds human laminin (89). This may help
175 the spirochete target and/or adhere to laminin-rich tissues. The laminin-binding domain
176 of ErpX was mapped to a central, disordered domain that is almost entirely comprised of
177 charged amino acids (89). Of note, other strain B31 Erp proteins with amino acid
178 sequences that are similar to ErpX (others in the "Elp" group: ErpB, ErpM and ErpQ,
179 Table 2) do not detectably bind human laminin (89).

180 The *B. burgdorferi* B31 ErpG and six similar proteins from other strains bind
181 heparan sulfate, and promote attachment to glial cells (90). A phage display library,

182 injected into mice and screened for potential *B. burgdorferi* adhesins, found clones of B31
183 ErpG, ErpK, and ErpL, and strain 297 Bbk2.10 in mouse heart, bladder, and tibiotarsal
184 joint tissues (91). Those four proteins share some sequence similarities (Table 2) (34).

185 The *B. burgdorferi* B31 ErpA, ErpC, and ErpP proteins bind host plasminogen and
186 its proteolytically-active form, plasmin (92). Plasmin bound to Erps and other surface
187 proteins facilitates *B. burgdorferi* migration through solid tissues in vitro, a characteristic
188 that is hypothesized to also enable dissemination during vertebrate infection (93-102). It
189 is notable that the Erp proteins that bind plasmin(ogen) also bind complement factor H
190 (see above).

191 Due to the surface localization and antigenicity of Erp proteins, there have been
192 several investigations into their use as protective vaccines or for serological diagnosis of
193 *B. burgdorferi* infection. However, vaccination of mice with the OspE, OspF, or P21
194 proteins of strain N40 did not provide substantial protection against infection by the same
195 strain (45, 65). Several recombinant Erp proteins have been examined for serological
196 diagnosis of human infection, with mixed results (103-106).

197

198 ***B. burgdorferi* cp32-encoded outer-surface Rev and Mlp lipoproteins.**

199 The cp32 replicons of Lyme disease and relapsing fever borreliae carry a distinct
200 mono- or bicistronic *mlp* (multicopy lipoprotein) operon (Figs. 1 and 2) (11, 16, 29). In the
201 majority of Lyme disease spirochete cp32s, the *mlp* locus is preceded by a distinct gene,
202 *bdr*, which has its own promoter (16, 29) (Fig. 1). The characterized cp32 of the relapsing
203 fever spirochete *B. hermsii* contains a single *mlp* gene, without an adjacent *bdr* (11).
204 Curiously, all cp32s of Lyme disease and relapsing fever borrelia carry a second *bdr* allele
205 adjacent to the episomal replication locus (9, 16, 30) (Fig. 1). Bdr proteins are produced
206 during vertebrate infection, but their function(s) have yet to be discovered (68, 107-115).

207 For unknown reasons, some *B. burgdorferi* cp32s do not contain a *bdr* adjacent to
208 the *mlp* locus, but instead carry a distinct gene named *revA* (so named because such genes
209 are arranged rev<u></u>erse to *mlp*) (16, 29). The *revA* ORF and promoter sequences are very
210 different from those of *mlp* genes, implying that *mlp* and *revA* loci are genetically
211 unrelated (9, 16, 29). In the *B. burgdorferi* type strain, B31, cp32-1 and cp32-6 each contain
212 a *revA* gene adjacent to their *mlp*, while all other B31 cp32s contain only a *mlp* gene (16).
213 Mature RevA is a lipoprotein and localizes to the *B. burgdorferi* outer surface (116).
214 Additionally, some truncated cp32 derivatives, such as B31 cp9, carry a similar gene
215 named *revB* (15, 16, 116). The two *revA* orthologs of strain B31 encode an identical RevA
216 protein, while that strain's RevB protein shares only 28% amino acid identity with RevA.
217 Other strains encode RevA and RevB proteins with varying degrees of differences (29).
218 *B. burgdorferi* in unfed ticks do not produce detectable levels of *revA* mRNA, but
219 do produce elevated transcripts during mouse infection (117). Human patients and
220 experimentally infected mice produce antibodies against RevA (118-120). In culture, *revA*
221 transcription is induced by changes in temperature or pH that correspond to changing
222 conditions encountered by the bacteria during tick feeding and mammalian infection
223 (116). Despite its antigenicity and surface localization, vaccination with RevA did not
224 protect mice from *B. burgdorferi* infection (121).
225 *B. burgdorferi* B31 RevA binds the 70kDa N-terminal region of fibronectin via a
226 domain in the RevA N-terminus (117). This binding was not affected by salt or heparin,
227 suggesting the interaction of these two proteins involves more than ionic interactions.
228 The *B. burgdorferi* RevB protein also binds to fibronectin, despite having limited sequence
229 identity to RevA (117). Polyclonal antibodies against RevA did not block adherence of
230 fibronectin to wild type *B. burgdorferi*, although those antibodies did prevent fibronectin
231 adherence to *B. burgdorferi* that were deleted of another fibronectin-binding protein,

232 BBK32 (122, 123). Similarly, binding of wild-type *B. burgdorferi* to HUVECs or human
233 neuroglial cells was not blocked by anti-RevA monoclonal antibodies (124). Those results
234 underscore the functional redundancy of *B. burgdorferi* fibronectin-binding proteins (125).
235 RevA also binds mammalian laminin, although to a significantly lesser extent than to
236 fibronectin (117).

237 A *B. burgdorferi* mutant unable to produce RevA was found to be defective in
238 colonization of cardiac tissue (123). That mutant also caused increased pathology of
239 tibiotarsal joints and enhanced CCL2 production. Increased inflammation in the absence
240 of RevA protein suggests a role for that surface protein in immune evasion, perhaps by
241 masking a more immunogenic antigen.

242 Precise functions have not yet been ascribed to Mlp proteins. The cp32 prophages
243 of both Lyme disease and relapsing fever *Borrelia* spp. carry *mlp* genes, which suggests
244 that the proteins serve functions that are conserved among borreliae (9, 11, 16, 29, 126)
245 (Fig. 2). As with *erp* and *rev* genes, there is often considerable sequence diversity among
246 *mlp* genes within and between *B. burgdorferi* isolates (16, 126, 127). The patterns of
247 regulated expression and antigenicity of *B. burgdorferi* Mlp proteins imply that they serve
248 functions within vertebrate hosts (127-132). A recombinant Mlp protein was found to
249 adhere to human brain microvascular endothelial cells (133). The structure of the
250 MlpA/P28 protein that is encoded by B31 cp32-1 has been solved, and was noted to
251 contain a structural motif that is similar to one seen in a lipid-binding protein (16, 127,
252 134).

253

254 **Horizontal transfer and recombination**

255 Almost all examined Lyme disease spirochete isolates naturally contain 6-12
256 distinct cp32s, each with an *erp* and a *mlp* / *revA* locus. As noted above, relationships

257 between cp32s can be discriminated by their *parA-bpaB* maintenance loci. Using that as
258 reference, there is clear evidence of recombination having occurred between cp32s, and
259 exchange of cp32 DNA between bacteria (13, 31, 34, 36).

260 *B. burgdorferi* type strain B31 naturally carries three identical *erpAB* operons on
261 cp32-1, cp32-5, and cp32-8 (9, 26). In contrast, those cp32s of other strains possess distinct
262 *erp* operons (31, 32, 34). The cp32-1 and cp32-6 of strain B31 carry *revA* genes that are
263 nearly identical in sequence, and which encode identical RevA proteins, while related
264 plasmids of other strains have different genes at that locus (16, 117, 120). Two other
265 recombination events are also evident in strain B31: cp32-4 has an fragment of a cp32 *sbbP*
266 gene inserted into *erpH*, such that a complete lipoprotein cannot be produced, and cp32-
267 3 has an unrelated gene, *bapA*, inserted 3' of *erpG* (9, 26). No other B31 cp32 carries a *bapA*
268 gene, although paralogous genes, named *eppA*, are found on some cp9 replicons (135-
269 137).

270 Despite cp32s having nearly identical sequences, and multiple cp32s residing
271 within individual bacteria, there are no known examples of recombination occurring
272 during cultivation. Recombination does not take place during mammalian infection, or,
273 at least, not among bacteria of the same strain (138). We hypothesize that genetic transfer
274 and recombination occur when *Borrelia* spirochetes are within ticks.

275 Additional evidence of horizontal transfer of cp32 DNAs in nature was obtained
276 from sequencing genomes of multiple isolates (34). *B. burgdorferi* strains B31 and BL206
277 contain almost identical repertoires of cp32s, the only differences being presence of a
278 cp32-1 in only B31 and a cp32-11 in only BL206. Intriguingly, B31 cp32-1 and BL206 cp32-
279 11 carry an identical *erpAB* locus. Two other strains, 297 and Sh-2-82, contain cp32
280 repertoires that are identical to each other, with the addition of a cp32-8 in only Sh-2-82.
281 The cp32-8 *erp* locus of Sh-2-82 is identical to the cp32-8 *erpAB* locus of strain B31,

282 implying that a cp32-8/ϕBB1 was exchanged between ancestors of those two strains.
283 Strain B31 was isolated from a tick on Shelter Island, New York, in 1981, and Sh-2-82 was
284 isolated from another tick on the same island in 1982 (139, 140). It is also notable that
285 BL206, which is a near clone of B31, was isolated several years later from the blood of a
286 Lyme disease patient in Westchester County, New York (141), and that 297, which is a
287 near clone of Sh-2-82, was isolated from the cerebrospinal fluid of another patient (142).

288 Transduction of cp32 DNA between different strains of *B. burgdorferi* has been
289 observed in culture (40). The mechanisms that induce ϕBB1 particles to form are not yet
290 known. As noted above, *B. burgdorferi* does not possess an SOS system (15). The timing
291 of bacteriophage production is also unknown. All *Borrelia* species are obligate parasites
292 of arthropods and vertebrates, so triggers for ϕBB1 would be limited to conditions
293 experienced within those hosts. Ticks can acquire multiple strains of *B. burgdorferi*, which
294 would reside together in the tick's midgut, leading us to propose that the midguts of
295 feeding ticks are likely locations for DNA exchange. Tick feeding is a time of rapid
296 replication for *B. burgdorferi*, which appears to be used by the spirochetes as an important
297 cue that transmission is imminent (64, 143). That would also be an appropriate time for
298 bacteria to exchange DNA and diversity their repertoires of Erp, Rev, and other surface
299 proteins that interact with vertebrate hosts.

300 At least two advantages appear to be conferred upon *B. burgdorferi* by exchanging
301 and recombining *erp* and *rev* genes. First, both groups of proteins are expressed early
302 during vertebrate infection, are located on the bacterial outer surface, and are antigenic.
303 Reservoir vertebrates are generally fed upon by numerous ticks, all of which might be
304 infected. Thus, Lyme disease spirochetes are under selective pressure to diversify
305 sequences of early, surface-borne antigens, to facilitate infection of animals that have

306 previously been exposed. A second advantage to Erp and Rev variability among
307 individual bacteria stems from their functions as adhesins, and from the fact that the ticks
308 that transmit Lyme borreliae may not be specific in their choices of hosts. For example, in
309 the northeastern USA, *Ixodes scapularis* ticks feed on birds, mice, and other mammals
310 (144). Since a *B. burgdorferi* cannot "know" in advance what species of vertebrate its tick
311 vector will feed upon, it would be advantageous for a bacterium to enter its new host
312 while bristling with a wide variety of adhesins that differ in relative affinities for ligands
313 of different hosts, with the likelihood that some Erp, Rev, and other adhesins will be
314 adequate for the bacteria's survival.
315
316 **Exceptional *Borrelia***
317 To date, only two exceptions are known to the generalization that *Borrelia* species
318 naturally carry cp32s. The agent of avian spirochetosis, *Borrelia anserina*, is the type
319 species of the genus, and lacks cp32 DNA (11). Since Lyme disease and relapsing fever
320 *Borrelia* species carry cp32s, their absence from *B. anserina* suggests that either ϕBB1
321 entered in the genus after the split of *B. anserina* from the other species, *B. anserina* has a
322 means to exclude cp32s/ϕBB1, or cp32s encode properties that are beneficial to Lyme
323 disease and relapsing fever borreliae, but not to avian spirochetosis borreliae. The second
324 known example of a cp32-deficient *Borrelia* is strain Far04, a Lyme disease spirochete that
325 was isolated from a puffin that was collected on the Faroe Islands, Denmark (13, 145, 146).
326 The densely packed puffin nests on those islands are homes to *Ixodes uriae* ticks, which
327 preferentially feed on seabirds. Although Far04 is a single example, it is possible that *I.*
328 *uriae* ticks on the islands primarily feed on puffins, to the extent that some circulating

329 borreliae have lost the need for the variability of adhesin compositions afforded by cp32
330 prophages. Clearly, additional sampling and analyses are needed to test that hypothesis.
331
332 **Future research directions.**
333 Erp and Rev lipoproteins localize to the borrelial outer surface, and are known to
334 interact with a variety of vertebrate host proteins that protect against immune responses
335 and facilitate tissue invasion and colonization. Noting that Erp and Rev proteins were
336 discovered during the mid-1990s, and that adherence of complement C1r and C1s was
337 first reported in 2022, we consider it likely that other functions remain to be discovered.
338 Additional questions include: Why do the cp32s of Lyme disease spirochetes encode Erp
339 and Rev proteins, while those of relapsing fever spirochetes appear to lack those genes?
340 What are the functions of the Mlp outer surface proteins, which are encoded on cp32s of
341 both Lyme disease and relapsing fever borreliae? Why does *B. anserina*, the agent of avian
342 spirochetosis, lack cp32s? When do borreliae produce ϕBB1 bacteriophages during their
343 natural infectious cycles? There is genetic evidence of DNA transfer of other genes
344 encoding borrelial antigens, such as outer surface protein C (OspC) (147, 148) – might
345 that occur by generalized transduction via ϕBB1?
346
347 **Concluding statements**
348 The cp32 prophages / ϕBB1 bacteriophages have evidently co-evolved with many
349 species of *Borrelia*. They have acquired a number of different genes that confer benefits to
350 their bacterial hosts, which thereby enhance their own survival. These bacteriophages
351 have adopted a primarily lysogenic lifestyle, with a limited degree of bacteriophage
352 particle production and host cell lysis. The *parA* and *bpaB* maintenance genes have

353 diversified extensively, to the extent that large numbers of different cp32s can be
354 maintained in a single bacterial cell. This provides both extensive repertoires of *erp*, *rev*,
355 and other genes for horizontal exchange and enables the host bacteria to express wide
356 varieties of surface adhesins during vertebrate infection.
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359
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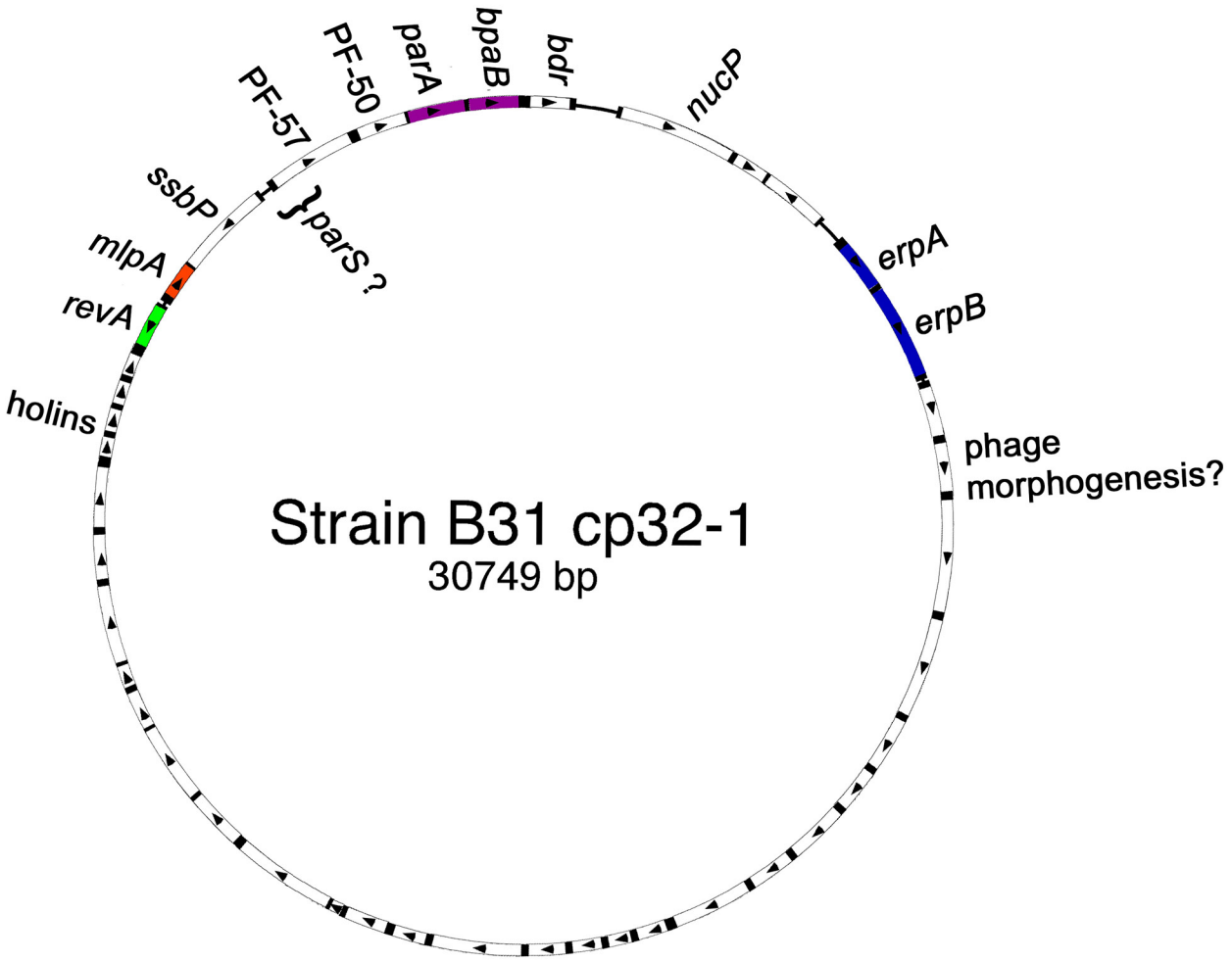
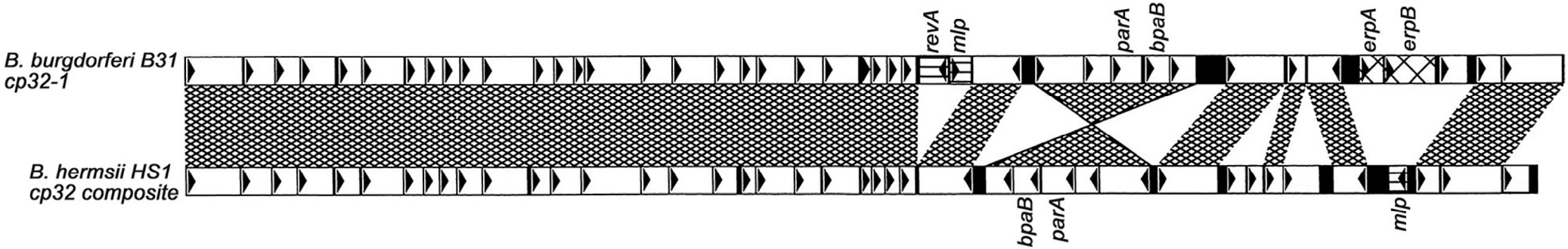
365
366

367 **Figure 1.** Schematic of the open reading frames of a representative *B. burgdorferi* cp32, the
368 cp32-1 of type strain B31. Arrowheads indicate direction of open reading frame
369 transcription. *B. burgdorferi* cp32s contain two loci that encode lipoproteins that are
370 expressed on the bacterial outer surface: a mono- or bicistronic locus that encodes Erp
371 proteins, and a separate locus that consists of either divergently-transcribed *revA* and *mlp*
372 genes, or a *mlp* operon adjacent to a *bdr* gene. B31 cp32-1 and cp32-6 each carry a *revA*
373 gene, while the other cp32s of that strain instead carry a *bdr* gene adjacent to their *mlp*
374 locus. Borreliae are apparently able to maintain numerous, different cp32s in a single cell
375 due to diversity among the *parA-bpaB* (borrelial *parB*-analog) locus. Other open reading
376 frames show up to 100% conservation between different cp32s and *B. burgdorferi* strains,
377 shown as white. Two conserved genes, PF-50 and PF-57, are located adjacent to the *parA-*
378 *bpaB* loci of all cp32s and are necessary for replicon maintenance (149-151). The *parS*
379 segregation site appears to be associated with the 5′ end of *PF-57* (our unpublished results
380 and (149-151). Conserved genes *ssbP* and *nucP* encode a single-stranded DNA binding
381 protein and nuclease, respectively (35). Bacteriophage structural and assembly proteins
382 appear to be encoded from a single operon that extends from 3′ of the *erp* locus to the
383 holin-encoding genes (16, 38, 42, 152).
384
385


bpaB
bdr
nucP
parA
PF-50
PF-57
ssbP
parS ?
mlpA
revA
holins
erpA
erpB
phage
morphogenesis?
Strain B31 cp32-1
30749 bp

386 **Figure 2.** Alignment of the open reading frames of *B. burgdorferi* B31 cp32-1 and a
387 composite cp32 of *B. hermsii* HS-1 that was assembled from sequenced contigs. Directions
388 of transcription are indicated by arrows. The *erp*, *revA* and *mlp* genes are indicated by
389 hatching. ORFs that are nearly identical between cp32s of *B. burgdorferi* and *B. hermsii* are
390 indicated by white boxes. Note that the plasmid replication genes, including *parA* and
391 *bpaB*, are located in reversed directions on the two different cp32 types. Adapted from
392 (11).


B. burgdorferi B31
cp32-1
B. hermsii HS1
cp32 composite
revA
mlp
parA
bpaB
erpA
erpB
bpaB
parA
mlp

393 **Table 1:** The cp32 DNA elements and associated *erp* loci that naturally occur in the three
394 best-characterized Lyme disease spirochete isolates: *B. burgdorferi* strains B31, N40, and
395 297. Plasmid nomenclature is standardized according to segregation locus type, such that
396 all plasmids designated "cp32-1" contain a similar segregation locus, etc. All known cp32
397 segregation loci fall into 12 clades. The first *B. burgdorferi* strain to be completely
398 characterized, B31, contains 2 distinct cp32 prophages that possess identical maintenance
399 loci, cp32-2 and cp32-7, and define segregation locus type 32-2/7. As a result, the
400 numbering scheme for the 12 groups goes up to cp32-13. The published names of *erp*
401 genes are listed. A variety of naming schemes were applied to these genes by their
402 discoverers, particularly for strain 297. Additional *erp* genes of strain N40 that were
403 discovered by our group were given allele numbers only (e.g. *erp22*, *erp23*, etc.). Other
404 strain's *erp* genes have been designated either by an allele number (with a different
405 number for each gene) or by genome sequencing ORF numbers, which are too numerous
406 to list here but examples are described in (34).
407

	B. burgdorferi strain		
cp32 segregation locus type	B31 [a]	N40 [d]	297 [e]
cp32-1	*erpA1*, *erpB1* (or *erpB2*) [b]		*ospE1*, *elpB1-1*
cp32-2/7	*erpC*, *erpD* *erpL*, *erpM* [c]	*ospE*, *ospF*	*elpA2*
cp32-3	*erpG*		*ospF*

cp32-4	*erpH, erpY*	*erp23, erp24*	*elpA1*
cp32-5	*erpA5, erpB5* (previously *erpI, erpJ*)	*erp25*	*ospE5, elpB1-5*
cp32-6	*erpK*		*bbk2.10*
cp32-8	*erpA8, erpB8* (previously *erpN, erpO*)		
cp32-9	*erpP, erpQ* [c]	*p21, erp22*	*p21, elpB2*
cp32-10	*erpX*	*erp26*	
cp32-11			*bbk2.11*
cp32-12		*erp27*	*ospE12, elpB1-12*
cp32-13			

408

409 a. The *erp* loci of strain B31 were first identified and named by the authors and their
410 research colleagues (9, 16, 26). Strain B31 was one of the first organisms to have its
411 genome completely sequenced, but the sequenced culture lacks cp32-2 and cp32-5, so the
412 complete sequences of those prophages remains uncertain. Prophages cp32-1, cp32-5, and
413 cp32-8 carry identical *erp* loci, which are distinguished by allele numbers. The *erp* loci of
414 cp32-1, cp32-5, and cp32-8 encode identical proteins, which are collectively called ErpA
415 and ErpB. At some time in the past, the B31 cp32-10 underwent integration into an
416 unrelated linear replicon, which created an approximately 56kb linear plasmid that is
417 named lp56.

418

419 b. Two alleles of *erpB* have been identified in different cultures of strain B31. The *erp* locus
420 that was first identified in a high-passage, non-infectious culture contains a premature

421 stop codon within the *erpB* gene, and thus encodes a truncated protein (26). Subsequent
422 analyses of infectious cultures of B31 revealed a full-length *erpB* open reading frame,
423 which was designated allele *erpB*2 (9, 16).
424
425 c. Two recent publications designated ErpB as "ElpB", ErpM as "ElpM" and ErpQ as
426 "ElpQ" (87, 88).
427
428 d. Two *erp* loci of strain N40, *ospEF* and *p21*, were named by Fikrig and colleagues (43,
429 45). The remainder of the N40 genes were discovered and named by the authors and their
430 coworkers (34). The cp32-2/7 prophage of strain N40 is naturally truncated to an
431 approximately 18kb circular replicon, cp18 (46).
432
433 e. The *erp* loci of strain 297 were discovered and named by Radolf and colleagues (28, 32,
434 55). Our research group later found that the cp32-1, cp32-5, and cp32-12 prophages each
435 contain an identical *erp* locus, *ospE-elpB1*, which we differentiated by additional allele
436 numbers (34). The cp32-2/7 and cp32-9 plasmids of strain 297 are naturally truncated,
437 and have been named cp18-a and cp18-2, respectively (32).
438
439
440

441 **Table 2.** The Erp proteins of the three best-characterized *B. burgdorferi* strains, B31, N40,
442 and 297, sorted according to the tripartite OspE/OspF/Elp scheme. Please note that even
443 though some proteins of strain 297 proteins were assigned the same names as N40
444 proteins, those proteins are distinct from each other. That is, N40 OspE is not identical to
445 297 OspE, N40 P21 is not identical to 297 P21, and N40 OspF is not identical to 297 OspF.
446 The strain 297 nomenclature is further complicated in that the 297 OspE protein is more
447 similar to the N40 P21 protein than to the N40 OspE protein, and the 297 P21 protein is
448 more similar to the N40 OspE protein (34).

	"OspE" "small" (69% average pairwise identity) [a]	"OspF" "medium" (44% average pairwise identity) [a]	"Elp" "large" (40% average pairwise identity) [a]
B. burgdorferi B31	ErpA ErpC ErpH ErpP	ErpG ErpL ErpK ErpY	ErpB ErpD ErpM ErpQ ErpX
B. burgdorferi N40	OspE P21	OspF Erp23 Erp25 Erp27	Erp22 Erp24 Erp26
B. burgdorferi 297	OspE P21	OspF Bbk2.10 Bbk2.11	ElpA1 ElpA2 ElpB1

			ElpB2

449

450 a. Average pairwise identities of genes and proteins of 21 analyzed Lyme disease *Borrelia*
451 strains (34). That study found that some alleles that fall into the OspF group due to their
452 sizes share greater sequence identity with members of the Elp group than they do with
453 other members of the OspF group, and vice versa. Those similarities and differences
454 appear to be due to frequent recombination between alleles of different groups.

455

456

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