Characterization of a Novel Transferrin Receptor in Bovine Strains of Pasteurella multocida

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Analysis of bovine respiratory isolates of Pasteurella multocida demonstrated that six of nine strains tested were capable of growth dependent upon bovine transferrin and of specifically binding ruminant transferrins. A single 82-kDa protein was affinity isolated from the P. multocida strains with immobilized bovine transferrin. In contrast to what has been observed in other species, binding of this protein to immobilized transferrin was specifically blocked by the N-lobe subfragment of bovine transferrin. A single gene encoding the 82-kDa protein was flanked by a leucyl-tRNA synthetase gene and an IS1060 element, in contrast to other species where genes encoding the two receptor proteins (TbpB and TbpA) are found in an operon arrangement. A similar gene arrangement was observed in all of the receptor-positive strains, in spite of the observation that they belonged to different genomic groups. Analysis of the deduced amino acid sequence of the receptor protein indicated that it is a member of the TonB-dependent outer membrane receptor family, and although it is related to transferrin and lactoferrin receptor proteins (TbpAs and LbpAs) from other species, it differs substantially from other members of this group. Amino acid alignments suggest that the reduced size (20 kDa smaller) of the P. multocida TbpA is primarily due to the absence of larger predicted external loops. Collectively these results suggest that P. multocida has a single, novel receptor protein (TbpA) that is capable of efficiently mediating iron acquisition from bovine transferrin without the involvement of a second receptor protein (TbpB).

Pasteurella multocida is a gram-negative bacterial species isolated from a variety of wild and domesticated animals as well as from humans. The bacterium is associated with a variety of specific diseases of domestic animals, such as pneumatic pasteurellosis in cattle, fowl cholera in poultry, atrophic rhinitis in pigs, and hemorrhagic septicemia in cattle and buffalo. This species has been subgrouped on the basis of capsular serotype, providing some correlation with disease manifestation (type A with pneumatic pasteurellosis in cattle and fowl cholera, type D with atrophic rhinitis in pigs, and types B and E with hemorrhagic septicemia). However, it is evident that further understanding of the taxonomy and phylogeny of this species is needed (4, 18) to provide a better appreciation of the host-pathogen relationship and factors influencing disease causation. Of particular note is that, in contrast to P. multocida, other pathogenic species in the Pasteurellaceae, such as Mannheimia (Pasteurella) haemolytica, Actinobacillus pleuropneumoniae, Haemophilus somnus, and Haemophilus influenzae, are restricted to a specific host species.

In North American cattle, P. multocida serogroup A is associated mainly with bronchopneumonia (enzootic pneumonia) in young calves and to a lesser extent with fibronous pneumonia (shipping fever) of feedlot cattle (8). The increasing incidence of P. multocida isolation from cases of pneumatic pasteurellosis (28) has led to a renewed interest in this pathogen and in the development of vaccines for prevention of this infection. The currently available vaccines, bacterins and modified live vaccines (6), have limited efficacy, thus prompting consideration of subunit vaccines based on individual antigens. However, important immunogens for P. multocida infection in cattle have not been well characterized (8). Cattle have not been readily protected following immunization with lipopolysaccharide. Interpretation of the immunogenic potentials of outer membrane proteins (OMPs) as vaccine antigens in this animal species have been limited by lipopolysaccharide and capsular contamination of the OMPs (8).

One vaccine strategy that has been adopted in several other bacterial species in the Pasteurellaceae is to target the surface proteins involved in acquisition of iron in the host as vaccine antigens (20, 26, 30). The rationale for targeting these antigens is that they are essential for overcoming the iron restriction imposed by the host iron binding protein transferrin (Tf) and are accessible at the cell surface.

The Tf receptor, which mediates the first step in iron acquisition from Tf, is composed of two distinct Tf binding proteins (Tbps), TbpA and TbpB (15). The genes encoding TbpA and TbpB (tbpA and tbpB) are in an operonic arrangement, with tbpB preceding tbpA and putative regulatory and promoter sequences upstream of the tbpB gene (13, 14, 24). TbpB is a large surface-exposed lipoprotein, capable of independently binding Tf and participating in the iron acquisition process, but it is not absolutely essential for iron acquisition in vitro (14). This protein ranges in size from 60 to 90 kDa in different strains and species (15). Experimental studies support the use of Tbps as a vaccine antigen (20, 26, 30). TbpA is an integral, TonB-dependent OMP proposed to mediate transport of iron across the outer membrane (32). TbpAs are approximately 100 kDa, significantly larger than the related siderophore receptor proteins that have been more extensively characterized (5, 12, 19). Although TbpA is absolutely essential for the iron acquisition process (14), there is currently limited evidence to sup-

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port the use of the intact protein as a vaccine candidate (20, 26).

Previous studies have reported the presence of Tf receptors in bovine *P. multocida* strains associated with pneumonia (21) and hemorrhagic septicaemia (33). Attempts to identify the receptor proteins by affinity isolation with immobilized bovine Tf (bTf) yielded a single 82-kDa protein (21, 33), but it was unclear whether this represented either the TbpA or TbpB protein present in receptors from other species. The present study was established to more fully characterize the bTf receptor in this species.

**MATERIALS AND METHODS**

**Bacterial strains and growth conditions.** *P. multocida* strains h48 and h77 and the bovine clinical isolate *M. haemolytica* h44 have all been previously reported (21, 22). *P. multocida* strains h241 through h247 were clinical isolates from cases of bovine pneumonic pasteurellosis and were generously donated by Andrew Potter, Veterinary Infectious Diseases Organization (VIDO), Saskatoon, Saskatchewan, Canada. Preparation of media and growth under iron-deficient conditions were as described previously (21, 22).

**Preparation and use of Tfs.** Purification of commercial bTf by concanavalin A affinity chromatography to remove a species of bTf incapable of binding concanavalin A, preparation of individual N and C lobes by proteolytic digestion of the purified bTf with protease K, and the use of bTf and its subfragments in competitive solid-phase binding assays were essentially as described previously (34). The ability of bovine strains of *P. multocida* to acquire iron from iron-bound Tf was tested by a previously described disk diffusion method (22).

**Affinity purification of receptor proteins.** For analytical affinity experiments, crude membranes (1 to 2 mg of protein) from iron-deficient cells were solubilized in a 50 mM Tris-HCl buffer containing 1.0 M NaCl, 0.05% sarcosyl, and 5 mM EDTA. Membrane debris was removed by centrifugation at 13,000 × g for 10 min. The supernatant containing the Tf receptor was applied to a bTf-Sepharose column prepared from CNBr-activated Sepharose. After a series of washing min. The supernatant containing the Tf receptor was applied to a bTf-Sepharose column prepared from CNBr-activated Sepharose. After a series of washing steps with Tris-NaCl buffer to remove contaminating proteins, the receptor was eluted from the ligand by boiling in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer, followed by SDS-PAGE. After electrophoresis, proteins were fixed and stained with Coomassie blue.

**CNBr cleavage and N-terminal amino acid sequence determination.** N-terminal amino acid analysis was performed as previously described (24). Essentially, samples of affinity-purified Tbp from *P. multocida* strain h48 were subjected to SDS-PAGE in duplicate. One gel was stained with Coomassie blue and the other stained from the N-terminal amino acid sequence of the intact receptor protein, were used in PCR amplifications from the N-terminal amino acid sequence of the 60-kDa CNBr subfragment, YGSGLGSGTVEFT. The underlined amino acids were used for design of the primer.

**RESULTS**

**Prevalence and properties of the Tf receptor in *P. multocida* isolates.** A collection of nine clinical isolates of *P. multocida* from cattle with respiratory infections were obtained for analysis. The strains were grown in iron-limiting media and tested for their ability to use bTf as a source of iron for growth. Cells harvested from the iron-limited media were also tested for their ability to bind labeled bTf. Six of the nine strains were capable of utilizing bTf for growth and of binding labeled bTf (Fig. 1). The genomic structures of the nine clinical isolates and a larger collection of *P. multocida* strains were compared by pulsed-field gel electrophoretic analysis (18). This analysis revealed that receptor-positive strains were from several different genomic groups, indicating that they do not represent a single lineage (data not shown).

In order to identify the receptor protein(s), we employed an affinity isolation procedure using immobilized bTf. A single 82-kDa protein was isolated from each of the strains of *P. multocida* that were positive for growth and bTf binding (Fig. 1). This contrasts with the receptor from the control *M. haemolytica* strain (h44), which consists of two proteins of 100 and 63 kDa (Fig. 1). The 82-kDa protein was not readily detected when membranes from iron-sufficient cells were used in the experiment (data not shown). The additional band of 39 kDa that was common to the receptor-positive strains, the receptor-negative strain, and *M. haemolytica* was also isolated with Sepharose alone, and thus it does not represent a bTf binding receptor protein.

Our prior experience with isolating receptor proteins from *M. haemolytica* demonstrated that the conditions could affect whether one or two receptor proteins were identified (22, 24). Thus, we attempted to isolate receptor proteins from the receptor-positive strains of *P. multocida* using different condi-

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* Direction relative to the orientation of the *tbpA* gene.

<sup>a</sup> Based on the N-terminal amino acid sequence of the intact receptor protein, DxDDSKTTPNKA. The underlined amino acids were used for design of the primer.

<sup>b</sup> Based on the N-terminal amino acid sequence of the 69-kDa CNBr subfragment, YGSGLGSGTVEFT. The underlined amino acids were used for design of the primer.

<sup>c</sup> leu-tRNA synthetase.

<sup>d</sup> leucyl-tRNA synthetase.
Affinity isolation of Tf receptor proteins. Membranes from iron-deficient cells of M. haemolytica (h044) or P. multocida (h048 to h247) were subjected to an affinity isolation procedure with immobilized bTf, and the eluted proteins were analyzed by SDS-PAGE. The arrowhead indicates the position of the receptor protein in P. multocida isolates. Numbers refer to molecular weights, in thousands, of protein standards. A plus sign indicates growth on iron-deficient media with bTf as an iron source or binding of labeled bTf by iron-deficient cells in a solid-phase binding assay as described in Materials and Methods. A minus sign indicates lack of growth or binding activity.

To compare the species specificity of the receptor from P. multocida with that of receptors from other bovine pathogens, labeled Tfs from cattle, sheep, and goats were used in binding studies with membranes from P. multocida strain h48 and M. haemolytica strain h196. The membranes from iron-limited P. multocida were capable of binding Tf from all three ruminants but not the control labeled human or porcine Tf (data not shown), a pattern similar to that observed for M. haemolytica.

Tf is a bilobed protein with two structurally equivalent lobes that are each capable of binding a single ferric ion. To localize the primary receptor-binding determinants on bTf, we performed competitive binding studies using preparations of isolated N-lobe and C-lobe subfragments of bTf (Fig. 2). The results demonstrate that the receptor from P. multocida was effectively blocked by intact bTf and the bTf N lobe but not by the bTf C lobe. These results contrast with what was observed with M. haemolytica, in which the C-lobe subfragment but not the N-lobe subfragment blocked binding to the receptor. Competitive affinity isolation experiments were performed in which intact bTf or its subfragments were mixed with solubilized membranes prior to exposure to immobilized bTf. The results from these competitive affinity isolation experiments essentially paralleled those of the binding experiments in that pre-binding of intact bTf or its N-lobe subfragment inhibited isolation of the 82-kDa protein from the P. multocida strains (data not shown). In contrast, affinity isolations of both TbpA (100 kDa) and TbpB (63 kDa) were inhibited by intact bTf and the C lobe but not by the N lobe in M. haemolytica. These results suggest that regions on the N lobe of bTf were primarily involved in binding to the 82-kDa receptor protein from P. multocida.

Cloning of the Tf receptor gene. A rapid PCR-based approach (23) was used in preliminary attempts at cloning the Tf receptor gene from P. multocida. However, PCRs with the degenerate primers failed to produce the appropriately sized PCR product from the bacteria, even though this approach had been successful with all other species known to produce bacterial Tf and lactoferrin receptors. These results suggested that either there was no TbpA homologue present in P. multocida or it was lacking one of the TbpA signature sequences used to identify TbpAs from other species.

To clone the gene encoding the receptor protein from P. multocida, we adopted an alternate PCR-based approach that involved determining the N-terminal amino acid sequence of the receptor protein and receptor protein subfragments. The receptor from strain h48 was purified and subjected to proteolytic cleavage analysis. Since proteolytic cleavage did not yield stable subfragments, we performed CNBr cleavage experiments. Using conditions favoring partial cleavage, two main subfragments of 60 and 45 kDa were obtained. The N-terminal amino acid sequence of the intact protein and the 60-kDa subfragment yielded 12 and 13 readable amino acids, respectively (Table 1). Two oligonucleotide primers were designed for amplifying the DNA region encoding the portion of the receptor protein between the start of the intact protein and the beginning of the CNBr-derived internal fragment. Forward primer 569, based on the sequence of the last 8 of the 12 N-terminal amino acids of the intact protein, was used in combination with reverse primer 570, based on the last 7 of the 13 N-terminal amino acids of the 60-kDa protein. The 410-bp product resulting from the PCR amplification was cloned and sequenced. Analysis of the sequence confirmed that it was the correct PCR product since it encoded the first six amino acids (YGSGAL) of the 60-kDa subfragment. In addition, the sequence analysis revealed that the protein was a homologue of TbpAs in other species, although it clearly had distinct features.

Mapping of the P. multocida tbpA region was achieved by using the 410-bp fragment as a probe in a Southern blot analysis of digests of P. multocida h48 chromosomal DNA. Using
FIG. 3. Genomic map of the *P. multocida* tbpA region. Chromosomal DNA is represented by the stippled bar. Below the bar, the genes and regions are represented by solid lines with arrowheads representing the direction of transcription (genes) or the orientation of inverted repeats (IS1016). Above the bar, oligonucleotide primers are represented by small arrowheads indicating their directions. The sequence between the start of the tbpA gene and the leucyl-tRNA synthetase synthetase gene is provided in the expanded section, with the two opposing start codons in bold.

this information, inverse PCR was performed with primers from the cloned region to amplify upstream and downstream regions as described in Materials and Methods. The PCR-amplified regions were sequenced and additional sets of primers were used to amplify overlapping PCR products from this chromosomal region. Several independently amplified PCR products were used to determine the sequence of the tbpA region, in order to eliminate PCR amplification as a source of sequencing errors.

**Characterization of the chromosomal locus.** In contrast to *tbpA* genes from other species, the *P. multocida* *tbpA* gene was not preceded by a *tbpB* gene. Immediately upstream of the *tbpA* gene is an open reading frame (ORF) in the opposite orientation, encoding a leucyl-tRNA synthetase homologue (Fig. 3) separated from the 5′ start of the *tbpA* gene by a 250-bp intergenic region. Presumably this region contains promoter sites for both the *tbpA* and leucyl-tRNA synthetase genes and possibly a *Fur* binding site for regulating the *tbpA* promoter. The lack of strong matches with consensus promoter regions or with the *Fur* box regulatory region indicates that direct experimentation will be required to identify these components. Notably, there were no sequences with evident homology to the flanking regions of insertional elements, such as inverted repeats, in this region.

The region immediately (at 150 bp) downstream of the 3′ end of the *tbpA* gene contains an IS-like element with a core 19-bp repeat of GGCCGTAGTGATTGC. The insertion element has an 90% homology in the core region with IS1060 elements in other species (*H. influenzae*, *Neisseria meningitidis*, and *Haemophilus paragallinarum*) and exhibits complete identity in the 19-bp repeats (17). Within the IS element is a region encoding a putative transposase. This region contains two stop codons resulting in a 370-bp ORF. In contrast to other IS1060-like elements (17), a long ORF was not found on the opposite strand.

The rather unusual organization of the *tbpA* locus in *P. multocida* strain h48 prompted us to investigate whether this configuration was unique to the strain or whether it occurred in other isolates. To test the organization of this locus in other strains, colony PCR was performed with oligonucleotide primers from the upstream or downstream regions in combination with primers from the *tbpA* gene. PCR products were amplified regions were sequenced and additional sets of primers were used to amplify overlapping PCR products from this chromosomal region. Several independently amplified PCR products were used to determine the sequence of the *tbpA* region, in order to eliminate PCR amplification as a source of sequencing errors.

**Characterization of the Tf receptor gene.** The predicted amino acid sequence of *P. multocida* TbpA shows homology with TbpAs from other species and with a variety of other TonB-dependent receptor proteins, indicating that it belongs to this family of outer membrane receptors. BlastX analyses revealed identities ranging from 22 to 29% with receptors involved in iron acquisition from heme (e.g., HmbR and HpuB from *N. meningitidis*), Tf (e.g., TbpA from *N. meningitidis* and *A. pleuropneumoniae*), and lactoferrin (e.g., LbpA from *N. meningitidis* and *Moraxella catarrhalis*). The amino acid identity among TbpAs from other species ranges from 45 to 50%, suggesting that the *P. multocida* TbpA is quite distinct.

There is a notable cluster of identity in the N-terminal portion of the protein, a region that has recently been shown to be a globular plug in the siderophore receptors FhuA and FepA (5, 12, 19). BlastX analyses using the plug region from *P. multocida* yielded identities of up to 50% (for TbpA from *M. haemolytica*) with other receptors involved in iron acquisition. There is substantial identity in the C-terminal portion of the plug region just prior to the plug-barrel junction (Fig. 4) that encompasses two of the core beta strands of the plug region. This supports the concept that the plug region from TbpAs may have a structure similar overall to that of the siderophore receptors and suggests that the plug diameter may be similar.

The C-terminal (beta-barrel) portion of *P. multocida* TbpA is substantially smaller (by 150 to 200 amino acids) than that of...
other TbpAs. It is likely that this is primarily due to differences in the sizes of the external loops, as the beta barrels in different TbpAs are probably of similar sizes and internal diameters to accommodate the N-terminal plug region. Alignment of the P. multocida TbpA with TbpAs from other species reveals substantial gaps in the P. multocida sequence (Fig. 5) which are localized to presumed external loop regions, particularly loops 2 and 3. In this alignment, the locations of the predicted beta strands are based on a previous model for LbpA (25). The locations of several of these loops have been confirmed in

FIG. 4. Alignment of the N-terminal plug regions. The predicted amino acid (AA) sequences for the N-terminal plug region of Escherichia coli FepA (FepA), P. multocida TbpA (PmTbpA), and M. haemolytica TbpA (PhtTbpA) were aligned with the Clustal alignment algorithm using Gene Inspector (Textco Inc.). Some adjustments were made to align the TonB box regions and to include amino acid identities identified in BlastX analyses. Amino acids which are identical in PmTbpA and FepA and in PmTbpA and PhTbpA are indicated by single underline. The predicted TonB box is indicated by double underline. The secondary structure of the FepA plug region (2Str) is indicated by S (beta sheet) or H (alpha helix).

FIG. 5. Alignment of the beta-barrel regions. The predicted amino acid (AA) sequences for the C-terminal barrel regions of P. multocida TbpA (PmTbpA) and M. haemolytica TbpA (PhTbpA) were initially aligned with the Clustal alignment algorithm using Gene Inspector (Textco Inc.). Amino acids which are identical in PhTbpA and PmTbpA are indicated by asterisks. The topology of these proteins was inferred by a combination of multiple alignments, comparisons with a recent topology model for LbpA (27), and the known structures of the FepA and FhuA siderophore receptors (5, 12, 19). External loops are indicated by single underline, and the numbers below indicate the numbers of the external loops (numbered from the N end to the C end of the polypeptide). Cysteines are in small letters to facilitate recognition of the two potentially pairing cysteines in individual external loops. Internal (periplasmic) loops are indicated by double underline. Numbers on the left indicate residue numbers of the C-terminal region. A strikethrough indicates the approximate region of peptides of LbpA that were reactive with antisera in intact cells.
LbpA from N. meningitidis by reactivity of antipeptide antisera against intact cells (27) (Fig. 5).

**DISCUSSION**

Bacteria have developed high-affinity iron acquisition systems in order to maintain growth in iron-limited environments. A versatile and common mechanism found in many gram-negative species involves the synthesis and secretion of small iron-chelating molecules, siderophores, and the subsequent uptake of the iron-siderophore complex (10). The binding and subsequent transport of the iron-siderophore complex across the outer membrane are mediated by a TonB-dependent integral OMP. The structures of two siderophore receptors have recently been determined (5, 12, 19), providing insights into the potential mechanism of transport across the outer membrane. After transport across the outer membrane, the iron-siderophore complex is subsequently bound by a binding protein in the periplasm, whose structure has also recently been determined (7). The periplasmic siderophore-binding protein shuttles the iron-siderophore complex to an inner membrane transport complex.

Pathogenic bacterial species in the *Pasteurellaceae* and *Neisseriaceae* utilize a different strategy for dealing with the iron-limited environment of the host by using surface receptors that directly bind and acquire iron from the host iron binding protein Tf (15). In contrast to siderophore receptors that consist of a single receptor protein, the Tf receptors in various bacterial species have been shown to consist of two proteins (15). TbpA, a transmembrane protein that is a homologue of the siderophore receptor proteins, mediates the transport of iron across the outer membrane. This likely occurs by a mechanism similar to that of the siderophore receptor proteins. Thus, mutants deficient in TbpA are completely deficient in iron uptake and Tf-dependent growth (9, 14, 16). The Tf receptor is confronted with an additional task, removal of iron from the high-affinity site on Tf, a process in which TbpB, a surface-exposed lipoprotein, probably plays a significant role. Evidence suggests that there is an extensive interaction between TbpB and Tf (29), presumably to facilitate the iron removal process. Thus, mutants deficient in TbpB are substantially impaired in their ability to use Tf as a source of iron for growth (3, 14, 16).

In this study we present biochemical (Fig. 1) and genetic (Fig. 3) evidence indicating that the Tf receptor in bovine strains of *P. multocida* consists of a single receptor protein, TbpA. The failure to identify a second receptor protein by affinity methods does not necessarily exclude the possibility that a second receptor protein exists, as exemplified by the identification of a single receptor protein in preliminary studies with *M. haemolytica* (22). However, we tested a variety of different expression conditions and affinity isolation procedures that led to successful identification of the second receptor protein in *M. haemolytica* (34). The bipartite receptors are usually encoded by an operon consisting of adjacent tbpB and tbpA genes (15, 24). Therefore, the absence of a tbpB gene adjacent to tbpA in *P. multocida* (Fig. 3) supports the hypothesis of a single receptor protein. In addition, attempts to detect a tbpB gene by Southern analysis or by PCR with degenerate oligonucleotide primers (23) were unsuccessful (data not shown). Collectively these results lead to the conclusion that the *P. multocida* receptor consists solely of TbpA.

The *P. multocida* receptor protein clearly falls within the superfamily of TonB-dependent receptor proteins. It is most closely related to the receptors involved in iron acquisition from heme, Tf, and lactoferrin, but it does not exhibit the degree of identity found among TbpAs from other bacterial species. It is considerably smaller (by 20 kDa) than previously characterized TbpAs, which is likely due to a dramatic reduction in size of several of the predicted external loops (Fig. 5). Since there is no TbpB present in *P. multocida*, these results could indicate that portions of these loops in other TbpAs are required for interactions with TbpB.

Another striking contrast is that the *P. multocida* TbpA primarily recognizes regions of the bTf N lobe (Fig. 2), whereas TbpAs from all other species studied to date bind to the C lobe (1, 2, 34). In spite of the marked difference in binding, the results do not necessarily indicate that the mechanism of iron removal is fundamentally different. Iron removal may be mediated by conformational changes in Tf (32), and the differences may primarily reside in which lobe of Tf is involved. The predominant binding of the N lobe of bTf by *P. multocida* TbpA (Fig. 2) might suggest that iron would be preferentially removed from this lobe, which is interesting in light of the observation that monoferric N-lobe bTf is the predominant form of bTf in bovine serum. Clearly, studies directed at monitoring the release from the individual lobes of Tf would provide useful insights into the mechanism of iron removal, and the presence of a single receptor protein in *P. multocida* makes it an obvious candidate for this type of study.

The fact that isolates of *P. multocida* appear as effective as *M. haemolytica* in using bovine Tf as a source of iron for growth (data not shown) suggests that efficient iron removal and uptake do not require a second receptor protein. This leads to the obvious question as to why a more complex bipartite receptor developed in other bacterial species. It is possible that TbpB is required for iron removal under particular conditions or that TbpB performs an additional role unrelated to iron acquisition. It is also possible that TbpB confers the ability to remove iron from both lobes of Tf, which might have an advantage in specific niches or during certain stages of the pathogenic process.

The genetic locus for the Tf receptor in *P. multocida* is unique in that it contains only a tbpA gene and is flanked by an IS1060 element (Fig. 3). This gene arrangement was confirmed in all six strains of *P. multocida*, which came from different geographical locations within Canada and which all expressed the bovine Tf receptor activity and possessed the 82-kDa receptor. These strains were from several different genomic groups (18), suggesting that the acquisition of the Tf receptor gene preceded the genomic rearrangements or that this entire region was a component of a larger mobile genetic element that has been transmitted among different genomic lineages.

A previous report (33) showed that *P. multocida* serotype B:2,5 strains associated with clinical signs of hemorrhagic septicemia in buffalo and cattle expressed an 82-kDa Tf binding protein, while serotype B:3,4 strains associated with wound infection in these animals failed to express this protein. It was suggested by the authors that the ability to use Tf as an iron source might be partially responsible for the virulence of se-
P. multocida strains might represent the pneumonia-associated strains, while the receptor-negative strains might either be commensals or be associated with some other clinical manifestations in cattle. It would be interesting to determine whether the gene encoding the 82-kDa TF receptor in the hemorrhagic septicemia isolates would be interesting to determine whether the gene encoding the 82-kDa TF receptor in the hemorrhagic septicemia isolates is sufficiently different from other TbpAs to possibly represent a separate subfamily of the TonB-dependent receptors, and this opens the question as to the prevalence of this gene in other gram-negative bacteria. Since some of our current methods for detecting TF receptor genes (23) failed to identify the receptor in P. multocida, caution is needed in interpreting data from screening experiments. Current methods have detected TF receptors only in bacterial species from the Pasteurellaceae and Neisseriaceae families (15), but this mechanism of iron acquisition may be more widely used in gram-negative bacteria than is currently appreciated.

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