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Differential activity of Rickettsia rickettsii ompA and ompB promoter regions in a heterologous reporter gene system

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The outer membrane of the Gram-negative obligate intracellular parasite Rickettsia rickettsii contains two large surface protein antigens with approximate molecular masses of 200 and 135 kDa termed rOmpA and rOmpB, respectively. rOmpB is the most abundant protein in the outer membrane, while rOmpA is a relatively minor constituent. Densitometry of intrinsically radiolabelled protein profiles from R. rickettsii-infected Vero cells indicated a molar ratio of approximately 1:9 between rOmpA and rOmpB. The putative promoter–5′ untranslated regions (5′ UTR) from their recently characterized genes (rompA and rompB) were placed in the promoter assay vector pKK232-8 to test whether these elements conserve aspects of differential expression in a heterologous host–reporter system. Primer extension analysis of RNA from Escherichia coli clones containing the constructs indicated that E. coli RNA polymerase faithfully utilizes mmpA and mmpB transcription start sites identified previously in R. rickettsii. The rompB insert directs 28-fold higher levels of chloramphenicol acetyl transferase activity than the rompA insert.

Keywords: Rickettsia rickettsii, α-proteobacteria, outer membrane proteins, promoter, transcription

INTRODUCTION

Two high molecular mass surface-exposed protein antigens of Rickettsia rickettsii show structural and immunogenic features that warrant further investigation. These proteins have been termed rickettsial outer membrane proteins (rOmp) A and B, respectively (McDonald et al., 1988; Anacker et al., 1984), and the genes encoding them, proposed as ompA and ompB (Gilmore et al., 1991; Hackstadt et al., 1992), are referred to here as rompA and rompB. rOmpB is the predominant protein in R. rickettsii and may play a structural role as an S-layer protein (Palmer et al., 1974; Dasch, 1981). The rompB gene sequence predicts a 168 kDa precursor (Gilmore et al., 1991), while the rompA gene sequence predicts a 217 kDa protein, with a unique pattern of 13 tandemly repeated units of 72–75 amino acids each in the N-terminal end of the protein, and greater than 75% identity between repeat units (Anderson et al., 1990). rOmpA is a minor constituent of the rickettsial cell surface, yet recombinant rOmpA antigen serves as an effective immunogen in mice and guinea pigs against challenge with virulent R. rickettsii (McDonald et al., 1987). Though rOmpB does not possess any sequence similarity to the amino acid repeats found in the rOmpA protein, rOmpA and rOmpB do show homology within their respective C-terminal 320 amino acids (> 36% identity), a region that may include a membrane anchor (Hackstadt et al., 1992) in the last 10 amino acids of each protein. It appears that in the case of rOmpB, a precursor form of the protein is cleaved to yield a 135 kDa N-terminal polypeptide and a 32 kDa C-terminal polypeptide which remain non-covalently associated during radioimmunoprecipitation (Gilmore et al., 1991; Hackstadt et al., 1992).

In this study we investigate possible genetic mechanisms for the large difference in the relative amounts of the rOmpA and rOmpB proteins. This difference could potentially involve promoter recognition by RNA polymerase, half-life of mRNA, ribosome binding and prevention of ribonuclease inactivation, and efficiency of protein processing and maturation under specific growth conditions. This report focuses on the role played by transcription and translation. The rompB gene of R.
**METHODS**

**Bacterial strains and growth conditions.** *E. coli* strain DH3α (Gibco BRL) was used as host for plasmid preparation, for expression of reporter gene function, and as a source of high molecular mass genomic DNA. Yeast tryptone (YT) was used as plating medium and Luria-Bertani (LB) as liquid medium (Maniatis et al., 1982). The *R. rickettsii* strain was grown in Vero cell monolayer culture, isolated and stored frozen. Intrinsic radiolabelling of *R. rickettsii* gene also contains a transcription start site and a putative promoter activity because transcription and clonal isolation of genetically recombined rickettsiae is not feasible at present. Results using this approach indicate these segments of the *rompA* and *rompB* genes do function as promoter elements in *E. coli* and the relative transcriptional activity of these promoters is similar to levels for the intact genes in *R. rickettsii*.

**Intrinsic radiolabelling of *R. rickettsii* and quantitative densitometry.** Monolayers of Vero cells were infected with the *R. rickettsii* and maintained at 34 °C for 5 d. The medium was replaced with RPMI-1640 (Roswell Park Memorial Institute medium 1640) containing 0.1% fetal bovine serum (FBS) and emetine (2.5 μg ml⁻¹), and incubated at 34 °C for 4 h to inhibit host protein synthesis. This medium was replaced with fresh RPMI medium with amino acids, 1% FBS, emetine, plus 1 μCi (37 kBq) ¹⁴C-labelled amino acid mixture ml⁻¹ (New England Nuclear). After 48 h continued incubation at 34 °C, the infected cells were scraped into the medium and pelleted by low-speed centrifugation (1000 r.p.m. for 5 min). All subsequent steps utilized chilled buffers and precautions were taken to keep the materials cold. The supernatant was saved and the cells resuspended in K36 buffer (Weiss, 1965) for disruption by sonication at 40 W for 40 s. The disrupted cells were again centrifuged for 5 min at 1000 r.p.m. and the supernatants pooled. Rickettsiae in the low-speed supernatants were pelleted at 12000 r.p.m. for 15 min. The pellet was resuspended in K36, layered over a 30% (w/v) Renografin (Squibb) pad, and centrifuged at 18000 r.p.m. for 30 min in a Beckman SW 27 rotor. The pellet was washed once with K36 buffer, resuspended in BHI, and samples frozen at -70 °C. Purified organisms were thawed, resuspended after centrifugation in SDS sample buffer and either maintained at 20 °C or heated for 5 min at 100 °C before loading on polyacrylamide gels (Laemmli, 1970). Gels were treated for fluorography, dried as recommended by the manufacturer (Entensify, New England Nuclear) and exposed at -70 °C to Kodak X-omat AR film. Quantitative densitometry was performed on a LKB Ultrascan XL laser densitometer using preflashed (Laskey & Mills, 1975) film.

**Cloning of *rompA* promoter–5' UTR region.** All DNA restriction and modification enzymes were purchased from New England Biolabs and used according to the manufacturer's recommendations. A HindIII digest of 10 μg *R. rickettsii* high molecular mass DNA (Silhavy et al., 1984) was separated by agarose gel electrophoresis, 1.2–1.0 kb fragments were isolated on powdered glass (Geneconic, Bio101), ligated to HindIII-linearized, alkaline phosphatase-treated pUC19, and transformed into competent (Maniatis et al., 1982) *E. coli* cells. This library was screened (Maniatis et al., 1982) with a previously described minus-strand oligonucleotide probe, 155, to nt 34–54 of the putative *rompA* ORF (Policastro et al., 1990), labelled by T4 polynucleotide kinase and [α³²P]ATP [New England Nuclear; 3000 Ci mmol⁻¹, (111 TBq mmol⁻¹)]. A single positive clone, pH5.1, was confirmed by restriction enzyme digest (HindIII, Xbol and PstI) and Southern blot hybridization to contain the 5' HindIII fragment of the *rompA* gene (Anderson et al., 1990; Policastro et al., 1990).

**Amplification of *ompA* gene 5' flanking UTR DNAs and insertion in cat reporter gene vector.** The pH5.1 clone containing the 5' end of the *rompA* gene, a previously described λ-derived phage clone containing the 5' end of the *rompB* gene (Gilmore et al., 1991), and total genomic *E. coli* DNA, prepared as described (Silhavy et al., 1984), were subjected to Taq DNA polymerase amplification of specific segments extending from approximately 120 nt upstream of respective transcription start sites to apparent ribosome binding sites in the 5' UTR of the *R. rickettsii* *rompA*, *rompB* and *E. coli* *ompA* genes, respectively. The *ompA* gene encodes an abundant outer membrane protein, contains one promoter, and has a relatively long 5' UTR of 134 nt (Cole et al., 1982). Oligonucleotides directed to these segments (see Fig. 2a) were synthesized on a Milligen/Biosearch Cyclone Plus DNA synthesizer (Millipore). Upstream, plus strand oligonucleotide primers contained BamHI linker sequences and downstream, minus strand primers contained SalI linker sequences to permit directional ligation. In amplifying the *rompB* gene segment, a BamHI site present in the native sequence was used in the upstream oligonucleotide primer and in the cloning step. PCR products were inserted upstream of the RBS and initiation codon of the cat reporter gene in plasmid pKK232-8 (Brosius, 1984) (Pharmacia). Cloned or total DNA was used as template for amplification (1 or 5 ng), with 20 pmol of each primer and 50 μM dNTPs in a final volume of 200 μl with 5 U Taq DNA polymerase in buffer recommended by the manufacturer (USB), for 30 cycles of 30 s at 94 °C, 30 s at 50 °C, and 1.5 min at 72 °C after an initial denaturation at 94 °C for 5 min in a Perkin Elmer-Cetus Thermal Cycler. Products were extracted with phenol/chloroform, precipitated with 2.5 M ammonium acetate and isopropyl alcohol to recover high molecular mass products, washed with 70% ethanol and digested with BamHI and SalI prior to ligation (King & Blakesley, 1986) to BamHI–SalI-digested pKK232-8. *E. coli* strain DH5α was transformed with ligations and plated on YT containing 250 μg carbenicillin ml⁻¹ and 30 μg chloramphenicol ml⁻¹. Plasmid DNA extracted from clones by miniprep (Maniatis et al., 1982) was analysed by HindIII digestion and agarose gel electrophoresis for the presence of inserts of the expected size. Dideoxynucleotide sequence analysis (Sanger et al., 1977; Sanger & Coulson, 1978) of the plasmid inserts (Sequenase; USB), using a minus strand oligonucleotide (232.2: TCTTTAGATGCCATGTTGGGA) directed against nt 40–59.
of the cat gene ORF, was used to confirm correct orientation of inserts and fidelity to published sequences (Gilmore et al., 1991; Policastro et al., 1990; Anderson et al., 1990; Beck & Bremer, 1980; Movva et al., 1990) and to 5' flanking sequences of the rompA and rompB clones.

Chloramphenicol acetyl transferase (CAT) and β-lactamase (βLA) assays. Overnight cultures of transformants containing vector control or recombinant forms of pKK232-8 grown at 37 °C in LB plus 250 μg carbenicillin ml⁻¹ (vector control) or LB plus 250 μg carbenicillin ml⁻¹ and 30 μg chloramphenicol ml⁻¹ (recombinants) were diluted 1:50 in LB, grown at 30 °C to an OD₆₀₀ of 0.4, and assayed for CAT and βLA activities essentially as described by Tomizawa (1985), except the chloramphenicol concentration in the CAT assay was 0.5 mM rather than 0.1 mM. [1-¹¹C]Acetyl-coenzyme A [Amersham; 5.8 mCi mmol⁻¹ (214.6 MBq mmol⁻¹)] was used as the radio labelled acetyl donor in the CAT assay. Nitrocefin used in the βLA assay was purchased from Becton Dickinson. Units of enzyme activity are expressed as nmol acetylated chloramphenicol formed min⁻¹ (ml cells)⁻¹ at an OD₆₀₀ of 1.0 (CAT assay) and nmol nitrocefin hydrolysed min⁻¹ (ml cells)⁻¹ at an OD₆₀₀ of 1.0 (βLA assay).

RNA extraction and analysis. Samples (8-0 ml) of cells harvested at the same time as the CAT assay samples (above) were chilled on ice, centrifuged and RNA was extracted and treated with DNaseI (Boehringer Mannheim; RNase-free) as described by Emory & Belasco (1990). Electrophoresis in formaldehyde-agarose gels (Fourney et al., 1988), transfer of RNA to nitrocellulose (Thomas, 1980) and hybridization to [α-³²P]dATP (DuPont/NEN)-labelled, randomly primed (Boehringer Mannheim) restriction fragments containing bla and cat gene sequences were performed as described previously (Policastro et al., 1989). The bla gene probe was a 1-kb HinfI-EcoRI fragment from pBR322 and the cat gene probe was a 0.78 kb HindIII fragment from pCM7 (Pharmacia), each isolated on NA45 ion exchange membrane (S克莱彻 and Schuell) after agarose gel electrophoresis. X-omat AR film (Kodak) was exposed to hybridized filters; for quantitative densitometry, preflashed film was exposed with an intensifying screen, and autoradiograms were analysed as described above. Primer extension analysis on total RNA was formed as described (Policastro et al., 1990), except that 2 μg RNA were used and the total reaction volume was reduced to 5.6 μl. The 232.2 minus strand oligonucleotide to the cat gene described above was used as primer in all reactions.

RESULTS

Molar ratio of rOmpA and rOmpB

R. rickettsii purified from Vero cell monolayers after intrinsic labelling by ¹⁴C-amino acids gave the SDS-PAGE profile shown in Fig. 1. Several protein mobilities are altered upon heating of rickettsial particles solubilized in SDS (Anacker et al., 1984): rOmpA migrates near rOmpB in extracts prepared at 20 °C, but migrates at 200 kDa upon heating to 100 °C. Quantitative densitometry (see Methods) on the profile after heating to 100 °C indicates that the rOmpA:rOmpB peak area ratio is 1:6.3. When run in a 7.5% separating gel, the heated samples gave a ratio of 1:5.8, or a mean of 1:6 in the two determinations. To adjust this 1:6 ratio for the estimated mass of each mature protein, we used a value of 135 kDa from relative mobility on SDS-PAGE for rOmpB, and 200 kDa for rOmpA. Using the mature rOmpA:rOmpB molecular mass ratio of 1:48 to adjust the densitometric ratio, we derive a molar ratio of 1:8:8 between rOmpA and rOmpB.

Cloning putative promoter–5' UTR sequences of omp genes

To test whether rompA and rompB sequences upstream of their transcription start sites are recognized in a heterologous host-reporter gene context, we amplified and then inserted elements of these rickettsial omp genes upstream of a cat gene for expression studies in E. coli. The length

Fig. 1. Autoradiogram of an SDS-polyacrylamide gel containing R. rickettsii R intrinsically labelled with a ¹⁴C-amino acid mix. Lanes: left, cells solubilized at 20 °C; right, cells solubilized at 100 °C. Positions of rOmpA and rOmpB proteins and molecular masses of prestained protein standards are shown on the right.
of the segment upstream of the start codon in bacterial mRNAs (5' UTR) has been suggested as an important factor in determining mRNA stability (Emory & Belasco, 1990). Therefore the cloned sequences included approximately 120 nt 5' of the reported transcription start sites and 5' UTRs extending to the nucleotide 5' of the ribosome binding site (RBS) of the rickettsial rompA and rompB genes (Fig. 2a). To compare the relative strength of these foreign omp gene elements to a native E. coli gene, an equivalent segment of the ompA gene of E. coli was also amplified. Target sequences were cloned upstream of the reporter gene in pKK232-8 to yield pRRA, pRRB and pECA, a series of clones containing the R. rickettsii rompA, rompB, and E. coli ompA sequences, respectively (Fig. 2b). This vector contains several transcription terminators flanking the cat gene, and the ColE1 origin of replication and β-lactamase (bla) gene of pBR322. Clones were analysed by restriction endonuclease mapping and DNA sequencing to confirm accurate copies of the target sequences (data not shown).
transcription start sites previously identified for the intact extracts, allowing normalization of CAT activities to an coding sequences, but do not indicate whether specific dependent on inserts placed upstream of the omp-4 gene constructs oligonucleotide annealed to corresponding plasmid DNA that the cloned rickettsia1 sequences contain promoters recognized by the romp promoter-reporter gene constructs. Fig. 3 shows that for each omp construct, a single major primer extension product characterizes the mRNA population encoding the CAT enzyme. No product is observed in the lanes containing RNA from the parental pKK232-8 plasmid. Comparison of these products to the sequence ladders shows that previously identified transcription start sites, as illustrated in Fig. 2, are in fact the major transcription start sites of the recombinant sequences. Specifically, the ompA start site utilized in pECA.1 maps to a G (3’ TGTAAGCGGG 5’) in the antisense strand of the sequencing ladder, corresponding to a C (5’ ACATCGCCA 3’) in the sense strand as reported previously; no evidence was found of transcription from an upstreamompA start site seen in vitro (Cole et al., 1982).

The rompB start site utilized in pRRB.4 maps to a C (5’ GCCCGTAGT 3’) in the sense strand as reported previously (Gilmore et al., 1991). Several additional, minor transcription start sites appear to be utilized in this system that were not observed in the analysis of RNA from R. rickettsii. Alternatively, these may be premature reverse transcriptase products in regions of high thymidine content (Shelness & Williams, 1985). In the case of the rompA construct pRRA.1, the start site maps to an A (5’ TTTTAAGTGA 3’) in the sense strand as identified previously (Policastro et al., 1990). Thus for both romp gene segments, the predominant transcript species initiates at the same site as initially observed in intact rickettsia.

Quantitative analysis of cat transcripts

The CAT activity and primer extension results do not differentiate between promoter strength, message stability and translational efficiency as possible sources of the differences in CAT activity in cell extracts. However, relative intensity of the primer extension products in Fig. 3 suggest that the pRRB and pECA constructs generate

### Table 1. CAT and βLA activities and mRNA levels of reporter plasmid constructs

<table>
<thead>
<tr>
<th>Plasmid construct*</th>
<th>Enzyme activity [nmol min⁻¹ (ml cells)⁻¹]†</th>
<th>mRNA level‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CAT βLA CAT:βLA ratio (mean ± SD)</td>
<td>cat bla cat:bla ratio (mean ± SD)</td>
</tr>
<tr>
<td>pKK232-8</td>
<td>0.035 200.5 ND</td>
<td>0.3 7.05 ND</td>
</tr>
<tr>
<td>pECA (rompA)</td>
<td>25.2 315.0 0.08 ± 0.002</td>
<td>2.25 11.55 0.195 ± 0.007</td>
</tr>
<tr>
<td>pRRA (rompA)</td>
<td>5.5 281 0.02 ± 0.001</td>
<td>2.0 11.33 0.175 ± 0.007</td>
</tr>
<tr>
<td>pRRB (rompB)</td>
<td>19.4 349.5 0.559 ± 0.063</td>
<td>8.7 16.5 0.53 ± 0.085</td>
</tr>
</tbody>
</table>

ND, Not determined.

* DH5α containing plasmid constructs. Measurements made on two independent isolates for each plasmid.

† CAT values are the mean of two separate determinations and βLA values are the mean of three determinations for each isolate. SD for CAT values were < 2% and for βLA, < 10%.

‡ Peak areas for full-length mRNA signals. Values for bla include both mRNAs seen in Fig. 4(b).
higher levels of transcript than the pRRA constructs. To quantify these apparent differences in steady-state mRNA population, a Northern blot of RNA isolated at the same time as clones were extracted for enzyme assays was hybridized to a full-length cat gene cassette probe to compare levels of cat transcripts (Fig. 4a). Prior to this hybridization, the blot was hybridized to a full-length bla gene probe (Fig. 4b) to provide a standard for comparison of cat transcript signals. Some residual bla probe is apparent at a different mobility than the cat transcripts after stripping and re-hybridization (Fig. 4a). The signals indicate that cat mRNA is more abundant in cells containing the pRRB constructs than those with the pECA and pRRA constructs. The slower mobility of the pRRB and pECA cat transcripts relative to the pRRA transcripts results from the longer 5' UTRs in the former mRNAs. Fig. 4(b) shows that bla transcripts are present at approximately equal amounts in all extracts. Two different bla mRNAs are to be expected from this pBR322-derived gene, as previously reported (von Gabain et al., 1983). A densitometric comparison of these steady-state transcript levels is shown in Table 1. The normalized values for cat transcript levels are represented by the ratio of omp-cat to bla mRNA (cat:bla) and indicate that pRRB clones contain approximately twice the level of cat transcript found with pRRA clones or pECA clones. These data, when compared to normalized CAT activities of the cell extracts, show that CAT enzyme levels in these clones are not directly proportional to cat transcript levels. Utilization of mRNA to produce protein may be influenced by the different 5' UTRs of these transcripts, such that the pRRB mRNA generates more CAT enzyme than either of the
other transcripts, and the pECA mRNA generates more than the pRRA mRNA.

**Rickettsial gene comparison to consensus E. coli promoter sequences**

The rompA and rompB gene promoters cannot be identified by classical methods of point mutation and homologous RNA polymerase recognition because transformation of rickettsiae is not feasible at present. However, sequences upstream of their transcription start sites, and comparable sequence from the R. rickettsii 17 kDa genus-specific lipoprotein gene (Anderson et al., 1988), are compared in Fig. 5 to the E. coli promoters and a consensus E. coli RNA polymerase promoter (Hawley & McClure, 1983). The sequence from rompA has an imperfect match to the most conserved (Hawley & McClure, 1983) TGGAC sequence at -35 and TA-A-T sequence at -10. The rompB sequence shows less similarity to the E. coli consensus in both of these regions. The rickettsial 17 kDa lipoprotein gene sequence shows as strong a match to the E. coli consensus -35 and -10 regions as does the rompA gene, but a suboptimal 15 at gap between -35 and -10 regions. These comparisons may explain E. coli RNA polymerase recognition of rickettsial transcription start sites, but the higher CAT activities of the rompB and the ompA constructs relative to the rompA construct appear to be the result of factors in addition to conservation of -35 and -10 sequences. Definitive promoter mapping awaitst introduction of altered rickettsial gene sequences into the cognate species.

**DISCUSSION**

This study began with our interest in understanding the basis for the relative levels of R. rickettsii rOmpA and rOmpB synthesis. The ratio of approximately one rOmpA molecule per nine rOmpB molecules measured late in the rickettsia–host cell interaction (Fig. 1) may not be conserved during all phases of the rickettsial life cycle, and we are presently investigating the dynamics of their relative expression under different culture conditions. Neither of these proteins has a defined role in the colonization by R. rickettsii of tick or vertebrate hosts, but their localization to the rickettsial outer membrane implicates them in the first line of interaction with eukaryotic cell components.

We have demonstrated that the sequences upstream of rompA and rompB serve as accurately recognized targets for expression in E. coli. The primer extension results in Fig. 3 demonstrate that E. coli RNA polymerase is utilizing native rompA, rompB and ompA transcription start sites in the context of the pKK232 multicopy plasmid, and thus represent accurately transcribed mRNAs containing the rickettsial and E. coli 5’ UTRs. Conservation of transcription start site usage has been reported previously for the 17 kDa surface protein of R. rickettsii, expressed in intact rickettsia in Vero cells and in E. coli transformed with the cloned gene (Anderson et al., 1988). It is therefore not surprising that homology exists between E. coli σ^70 promoter regions and sequences upstream of R. rickettsii transcripts (Fig. 5), which may indicate some conservation of RNA polymerase recognition sites between these two species. RNA polymerase holoenzymes extracted from R. prowazekii and from E. coli appear to initiate transcription at identical start sites in two rickettsial genes in vitro (Ding & Winkler, 1993). However, promoter sequence preferences may differ between the genus Rickettsia, part of the α-2 subgroup of purple bacteria, and E. coli, a member of the γ-3 subgroup of the purple bacteria (Weisburg et al., 1985, 1989). Further characterization of rickettsial RNA poly-
merase sequence preferences will be necessary to determine differences in promoter sequence recognition.

In this heterologous system, the relative CAT enzyme activities and cat mRNA levels obtained with rompA and rompB gene sequences show the same trend as rOmpA and rOmpB levels observed in intact rickettsiae. Promoter activity, operationally defined, is at least one factor affecting differential CAT activity from the rickettsial DNA inserts, but the activity of each omp-cat construct in E. coli (Table 1) reflects both production and degradation of transcripts, and efficiency of translation initiation. It must be recognized that the rompA:rompB activity ratio obtained with this reporter gene system may be influenced by host factors specific to E. coli, and cannot be extrapolated to conditions in R. rickettsii. The half-lives of mRNAs for two R. prowazekii proteins in infected mouse L929 cells and in E. coli bearing clones of the genes (Cai & Winkler, 1993) indicate that the E. coli environment may not be comparable to the native rickettsial environment with regard to the relative stability of full-length rickettsial transcripts. The linkage of a reporter gene to the putative rompA and rompB gene promoter segments in the present study allowed us to focus on transcription events. Preliminary data suggest that the half-life of rompB gene transcripts in R. rickettsii is several-fold longer than rompA transcripts, which may be a factor in higher in vivo levels of rOmpB expression (P. Policastro, unpublished). Half-life differences may exist for romp-cat gene fusions in E. coli, since the rompA and rompB 5' UTRs are present in the present study. These segments of untranslated RNA are proposed as determinants of message half-life in several cases, including the E. coli ompA gene (Emery & Belasco, 1990; Movva et al., 1990; Chen et al., 1991).

Genetic manipulation of rickettsiae has been precluded by the difficulty in obtaining directionally mutated variants of organisms that maintain a strictly obligate intracellular life cycle. Analysis of rickettsial gene transcription is limited to cell-free systems or to testing putative regulatory regions of the structural genes in heterologous systems. Likewise, testing specific functional properties of rickettsial gene products requires the expression of these genes in heterologous organisms. This has been possible in E. coli transformants for some rickettsial gene products which complement mutations (Wood et al., 1983) or provide novel catalytic properties (Krause et al., 1985). However, functional expression of outer membrane proteins like the rompA and rompB gene products may require secretory pathway components that are only present in members of the genus Rickettsia. The activity of their putative promoter–5' UTR segments in E. coli provides an explanation for the instability of full-length rompA and rompB genes in this host. In the case of the rompB gene, a 120 kDa product can be expressed in E. coli from a 5' segment of the gene in pUC19 (Gilmore et al., 1989), but the 5' portion of the gene has only been stably maintained as an insert in a lytic bacteriophage λ vector (Gilmore et al., 1991). Transcription from the intact rompB gene in E. coli presumably results in toxic levels of rOmpB, as a result of E. coli recognition of the rickettsial promoter. This information regarding transcription activity will help us to devise alternate strategies for the stable recombinant expression of these important rickettsial antigens.

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