Functional analysis of secA homologues from rickettsiae

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The molecular basis of protein secretion that underlines rickettsial pathogenesis remains unknown. This paper reports the molecular and functional analysis of the putative secA gene, an essential component of the Sec-dependent protein secretion pathway, from Rickettsia rickettsii and Rickettsia typhi, the etiologic agents of Rocky Mountain spotted fever and murine typhus, respectively. The sequence analysis of the cloned secA genes from R. rickettsii and R. typhi show ORFs of 2721 and 2718 nt, respectively. Alignment of the deduced amino acid sequences reveals the presence of highly conserved amino acid residues and motifs considered to be essential for the ATPase activity of SecA in preprotein translocation. Transcription analysis indicates that R. rickettsii secA is expressed monocistronically from the canonical prokaryotic promoter, with a transcriptional start point located 32 nt upstream of the secA initiation codon. Complementation analysis shows that the full-length SecA protein from R. rickettsii and R. typhi fails to restore growth of the temperature-sensitive Escherichia coli strain MM52 secA51(ts) at a non-permissive temperature (42 °C), despite the detection of SecA protein expression by Western blotting. However, the chimeric SecA protein carrying the N-terminal 408 aa of R. rickettsii SecA fused with the C-terminal 480 aa of E. coli SecA restores the growth of E. coli strain MM52 secA51(ts) at the non-permissive temperature (42 °C). These results suggest that the N-terminal ATPase domain is highly conserved, whereas the C-terminal domain appears to be species specific.

INTRODUCTION

The rickettsiae are obligate intracellular, Gram-negative bacteria with a life cycle that involves both vertebrate and invertebrate hosts. Several rickettsial species, including Rickettsia rickettsii, Rickettsia prowazekii and Rickettsia typhi, are the causative agents of severe bacterial diseases of humans, and are widely distributed throughout the world (Azad & Beard, 1998). Rickettsial diseases are often misdiagnosed, or diagnosed too late, due to the absence of specific early symptoms and the inability to culture rickettsiae on microbiological media independent of host cells (Hackstadt, 1996). Currently, no vaccine for the prevention of rickettsial infection is available. Moreover, rickettsial agents such as R. prowazekii and R. rickettsii are gaining notoriety as potential agents of bioterrorism (Azad & Radulovic, 2003).

Protein secretion is a significant aspect of bacterial pathogenesis, and the molecular mechanism of protein secretion in rickettsiae remains an important subject of research. Research on various bacterial pathogens has demonstrated that the majority of virulence factors are either secreted into the extracellular environment or attached to the cell surface (Finlay & Falkow, 1997). The importance of protein export pathways has been demonstrated by the identification of specialized secretion pathways (types I–V) in bacteria (Lee & Schneewind, 2001; Pullen et al., 2003). In bacteria, the majority of extracytoplasmic proteins transported by the specialized secretion pathways (except type III) are synthesized as preproteins with an amino-terminal extension known as the signal or leader peptide, and are translocated by the Sec-dependent protein export pathways (Desvaux et al., 2004). During translocation, the signal or leader sequence is cleaved off by a signal peptidase (Economou, 1999; Pugsley, 1993). These precursor secretory preproteins are selectively targeted to the translocon by interactions with SecB or the signal recognition particle (which is composed of Ffh, 45S RNA and FtsY). These pathways converge at the translocon, which consists of the ATPase SecA, which...
acts as a molecular motor, and the integral membrane proteins (SecD, SecE, SecF, SecG, SecY, YidC and YajC) (Paetzel et al., 2000, 2002). SecA plays a central and essential role in Sec-dependent protein export. It is an ATPase that provides energy for the transmembrane movement of the preprotein. Through cycles of ATP binding and hydrolysis, SecA delivers bound preproteins to the translocase, and undergoes cycles of membrane insertion and retraction to mediate the stepwise translocation of the preprotein (de Keyzer et al., 2003).

In order to elucidate the protein secretion pathways, and their involvement in rickettsial pathogenesis, we set out to characterize the genes involved in rickettsial protein secretion. As in our previous work examining the rickettsial lepB gene encoding signal peptidase I (Rahman et al., 2003), in this paper we report a detailed molecular and functional characterization of the putative secA genes from R. rickettsii and R. typhi.

### METHODS

**Bacterial strains.** R. rickettsii strain Sheila Smith (ATCC VR-149) and R. typhi strain Wilmington (ATCC VR-144) were used in this study. The Escherichia coli strain used for complementation was MM52 secA51(ts), which is a temperature-sensitive derivative of E. coli strain MC4100 (Oliver & Beckwith, 1981; Schmidt et al., 1988).

**Genomic DNA extraction.** African green monkey kidney cells (Vero cells, ATCC CRL-1586) were cultured in Dulbecco’s modified Eagle’s medium with 4-5 g glucose l⁻¹ and glutamine (Biofluids), supplemented with 10 % fetal bovine serum (Gemini). Propagation of R. rickettsii and R. typhi in Vero cells, and their partial purification from the host cells, were previously described (Rahman et al., 2003). Genomic DNA of R. rickettsii and R. typhi was extracted using the Wizard genomic DNA purification kit (Promega,).

**Cloning of the rickettsial secA gene.** Primers were designed based on the published genome sequences of Rickettsia conorii (Ogata et al., 2001) and R. prowazekii (Andersson et al., 1998) and used for cloning the secA genes from R. rickettsii and R. typhi, respectively. Primers used in PCR reactions are shown in Table 1.

### Table 1. Primers used in PCR reactions

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<th>Primer</th>
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*Nucleotides modified to generate restriction sites are indicated in lower case.
†Nucleotide sequence position numbering corresponding to the R. conorii genome sequence under GenBank accession no. AE008642.
‡Nucleotide sequence positions are taken from the E. coli secA operon under GenBank accession no. M20791.
§Nucleotide sequence position numbering corresponding to the R. prowazekii genome sequence under GenBank accession no. A1235272.
||Nucleotide sequence position numbering corresponding to the R. rickettsii secA operon under GenBank accession no. AY312572.
§Nucleotide sequence position numbering corresponding to the R. typhi secA operon under GenBank accession no. AY386402.
The secA gene from *R. rickettsii* was amplified in two different fragments (5’ end and 3’ end) by PCR. The primers AZ1940 and AZ1676 were used to amplify the 1149 bp fragment of the 5’ end of the *secA* gene of *R. rickettsii* (first fragment) using Herculase DNA polymerase (Stratagene). The PCR product (1149 bp) was cloned into the pPCR-Script Amp SK(+) vector (Stratagene), and was transformed into *E. coli* TOP10 cells (Invitrogen Life Technologies). The cloned 5’ end of the *secA* region of *R. rickettsii* was sequenced by the dye termination method, using a model 373 automated fluorescent sequencing system (Applied Biosystems). The 3’ end of the *secA* gene (second fragment of 1785 bp) was PCR amplified from *R. rickettsii* DNA using the primers AZ1668 and AZ1667 by following the same procedure as described for the 5’ end of the *secA* gene (first fragment of 1149 bp). The *R. rickettsii* secA 5’- and 3’-end sequences were combined using MacVector 6.5.3 software (Genetic Computer Group).

The *R. typhi* secA was amplified using primers AZ2123 and AZ2124, and the PCR fragment (2817 bp) was cloned and sequenced as described above.

The sequences of the *secA* genes of *R. rickettsii* and *R. typhi*, and the deduced amino acid sequences, were analysed using MacVector 6.5.3 software. Sequences were compared with those available in GenBank by using BLAST analysis (http://www.ncbi.nlm.nih.gov).

**Isolation of RNA and RT-PCR.** *R. rickettsii* and *R. typhi* were purified from Vero cells (>90% infection), as described above. Total RNA from the partially purified rickettsiae was isolated by use of Trizol reagent (Invitrogen Life Technologies), and treated with RQ1 RNase-free DNase (Promega) by following the manufacturer’s recommendations. RT-PCR was performed with 500 ng total RNA in a 50 μl reaction using SuperScript One-Step RT-PCR with Platinum Taq (Invitrogen Life Technologies). The thermal cycling conditions were one cycle of 45°C for 30 min, 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 48°C for 30 s, 72°C for 3 min, and a final extension of 72°C for 10 min.

**Northern analysis.** Northern blotting of total RNA isolated from *R. rickettsii* was performed by using the NorthernMax kit (Ambion). Total RNA (8 μg) was separated on a 1% agarose gel, and transferred to a positively charged nylon membrane (Ambion). The membrane was hybridized with a radiolabelled probe specific to the *R. rickettsii* secA-coding sequence corresponding to the region from the start codon to the primer AZ1649. The 476 nt probe was prepared by linear amplification with primer AZ1649 in the presence of [α-32P]dATP (Amersham Pharmacia Biotech) using the Strip-EZ PCR probe synthesis kit (Ambion). The hybridized membrane was exposed (16 h) to Kodak Biomax MS film for autoradiography.

**RACE (rapid amplification of cDNA ends).** The transcriptional start point (TSP) of *R. rickettsii* secA gene expression was determined by using the 5’RACE kit (Invitrogen Life Technologies) by following the manufacturer’s instructions. First-strand cDNA was prepared from DNase-treated total RNA (5 μg) of *R. rickettsii* using SuperScript II reverse transcriptase with the secA-gene-specific primer AZ1649. The cDNA was tailcd by adding cytosine residues at the 3’ end using terminal deoxynucleotidyl transferase. The dC-tailed cDNA was PCR amplified with a primer set consisting of a second secA-specific primer AZ1793 and the 5’RACE abridged anchor primer. The primary PCR product was further amplified by nested PCR with the primer AZ1792 and the 5’RACE abridged universal amplification primer. The nested PCR product was cloned and sequenced to determine the transcriptional start point.

**Construction of full-length and chimeric secA genes.** PCR was used to construct plasmids carrying the full-length ORFs of the secA genes from *R. rickettsii*, *R. typhi* and *E. coli* MC4100. To introduce the required restriction sites, nucleotides were changed in the primer design. The primers AZ22116 (BglII) and AZ22115 (EcoRI) were used for cloning the full-length ORF (2729 bp fragment) of *R. rickettsii* secA at the BglII and EcoRI sites of the pTrcHisB vector, which contains an N-terminal His tag under the trc (trp-lac) promoter (Invitrogen Life Technologies), to generate the plasmid pTrcRR73. To clone the full-length ORF (2729 bp fragment) of *R. typhi* secA, a PCR-amplified product obtained by using the primers AZ22454 (BamHI) and AZ22456 (EcoRI) was ligated at the BamHI and EcoRI sites of the pTrcHisB vector to generate the plasmid pTrcKT3. The full-length ORF (2725 bp fragment) of secA from *E. coli* MC4100, amplified by primers AZ2259 (BamHI) and AZ2258 (XhoI), was cloned at the BamHI and XhoI sites into the pTrcHisA vector, which contains an N-terminal His tag under the trc (trp-lac) promoter (Invitrogen Life Technologies), to generate the plasmid pTrcEC85.

Based on strong sequence similarity in the N-terminal ATP binding domains of the SecA protein, the chimeric secA gene was constructed. This gene encoded the N-terminal 408 aa of *R. rickettsii* SecA, and the C-terminal 480 aa of *E. coli* MC4100 SecA. To construct the chimeric secA gene, the third nucleotide of the codon for Leu and Asp residues of *R. rickettsii* SecA protein (chimeric site, shown in Fig. 1) was changed in the primer design, to create a BglII site whilst retaining the Leu and Asp amino acids. *E. coli* secA has a natural BglII site at the same region. The primers AZ22352 (BamHI) and AZ22351 (BglII) were used for PCR amplification of *R. rickettsii* to produce (1250 bp fragment) the 5’ end of the chimeric secA. For the 3’ end of the chimeric secA from *E. coli* MC4100, a 2059 bp fragment (which has a natural BglII site) amplified by primers AZ22349 and AZ22348 (EcoRI) was used for fusion. The 5’ end (BamHI- and BglII-digested 1230 bp fragment) of the chimeric secA from *R. rickettsii*, and the 3’ end (BglII- and EcoRI-digested 1462 bp fragment) of the chimeric secA from *E. coli* MC4100, were ligated together into the BamHI and EcoRI sites of the pTrcHisB vector to generate the plasmid pTrcRE208. The accuracy of the constructed plasmids pTrcRR73, pTrcKT3, pTrcEC85 and pTrcRE208 was checked by sequencing.

**Complementation and expression analysis of rickettsial secA genes.** The constructed plasmids pTrcRR73 (carrying the full-length ORF of *R. rickettsii* secA), pTrcKT3 (carrying the full-length ORF of *R. typhi* secA), pTrcEC85 (carrying the full-length ORF of *E. coli* secA), pTrcRE208 (carrying the chimeric *R. rickettsii*-*E. coli* secA fusion), or the vectors pTrcHisA and pTrcHisB, were transformed into *E. coli* MM52 secA(ts) cells, and selected on Luria–Bertani (LB) agar containing ampicillin (100 μg ml–1) incubated at 30℃ for 20 h. The complementation assay of transformed *E. coli* MM52 was performed by growth curve and c.f.u. assays, as described previously (Rahman et al., 2003). The c.f.u. assay was performed at least three times, and the SD was calculated (shown as ±) using Microsoft Excel software.

The synthesis of the recombinant SecA proteins in the transformed *E. coli* MM52 secA51(ts) was detected by Western blot analysis using a monoclonal antibody to the N-terminal His tag, as described previously (Rahman et al., 2003).

**RESULTS**

**Cloning and sequence analysis of rickettsial secA genes**

The putative secA genes of both *R. rickettsii* and *R. typhi* were cloned and sequenced as described in Methods. The DNA sequence analysis of secA revealed an ORF of 2721 nt from *R. rickettsii*, and an ORF of 2718 nt from *R. typhi*.
Alignment analysis showed that the secA DNA sequences were 89–99% identical among *R. rickettsii*, *R. typhi*, *R. prowazekii*, *R. conorii* and *Rickettsia sibirica* (data not shown).

Likewise the deduced amino acid sequence of secA of *R. rickettsii* showed a very high degree of identity (ranging from 91 to 99%) with other rickettsial species, and the

**Table 1.** Alignment of amino acid sequences deduced from the secA gene of *R. rickettsii* (*Rr*, this work), *R. typhi* (*Rt*, this work), *R. conorii* (*Rc*, GenBank accession no. AE008642), *R. prowazekii* (*Rp*, GenBank accession no. AJ235272), *Rickettsia sibirica* (*Rs*, GenBank accession no. AABW01000001) and *E. coli* (*Ec*, GenBank accession no. M20791). The N-terminal 507 aa of rickettsial SecA, and 520 aa of *E. coli* SecA, are shown in the above alignment. The total number of amino acids (total # a.a.) is given at the end of the sequence of each species. The molecular mass (MW), isoelectric point (pI) and the overall identity with *Rr* SecA protein were computed using MacVector 6.5.3 software. Amino acid residues identical to those of *E. coli* SecA are marked with an asterisk. The conserved amino acid motifs A0, B0, A3 and catalytic carboxylate are shown in bold and underlined. Leu-43 indicates the site of mutation in *E. coli* MM52 conferring temperature-sensitive phenotype. The chimeric site (bold and underlined) indicates the site for the construction of the chimeric *R. rickettsii*–*E. coli* secA gene.

**Fig. 1.** Alignment of amino acid sequences deduced from the secA gene of *R. rickettsii* (*Rr*, this work), *R. typhi* (*Rt*, this work), *R. conorii* (*Rc*, GenBank accession no. AE008642), *R. prowazekii* (*Rp*, GenBank accession no. AJ235272), *Rickettsia sibirica* (*Rs*, GenBank accession no. AABW01000001) and *E. coli* (*Ec*, GenBank accession no. M20791). The N-terminal 507 aa of rickettsial SecA, and 520 aa of *E. coli* SecA, are shown in the above alignment. The total number of amino acids (total # a.a.) is given at the end of the sequence of each species. The molecular mass (MW), isoelectric point (pI) and the overall identity with *Rr* SecA protein were computed using MacVector 6.5.3 software. Amino acid residues identical to those of *E. coli* SecA are marked with an asterisk. The conserved amino acid motifs A0, B0, A3 and catalytic carboxylate are shown in bold and underlined. Leu-43 indicates the site of mutation in *E. coli* MM52 conferring temperature-sensitive phenotype. The chimeric site (bold and underlined) indicates the site for the construction of the chimeric *R. rickettsii*–*E. coli* secA gene.
identity with the secA of E. coli was around 51% (Fig. 1). Alignment of the deduced amino acids of the SecA proteins shown in Fig. 1 revealed highly conserved amino acid motifs considered to be essential for SecA-dependent translocation ATPase activity (de Keyzer et al., 2003). The motifs A0 (residues 102–109; E. coli SecA numbering) and B0 (residues 198–210) are designated high-affinity ATP-binding domains, whereas motif A3 (residues 503–511) is designated the low-affinity ATP-binding domain (Mitchell & Oliver, 1993). The amino acid residues Lys108 (motif A0), Asp133 (catalytic carboxylate) and Asp209 (motif B0), essential for the ATPase activity of SecA preprotein translocase (de Keyzer et al., 2003; Sato et al., 1996), were found to be conserved (Fig. 1) in the rickettsial SecA protein.

Transcriptional analysis of rickettsial secA

Total RNA isolated from R. typhi and R. rickettsii was analysed by RT-PCR to detect the expression of the secA gene. Amplification products (confirmed by sequencing) of 543 bp (lane 1) and 366 bp (lane 2), as shown in Fig. 2, were detected, thereby indicating the expression of secA mRNA in these rickettsial species.

Total RNA isolated from R. rickettsii was analysed by Northern hybridization to assess secA transcript size (data not shown). The hybridization probe specific to the secA-coding sequence detected a band of 2.8 kb, which is comparable with the size of the ORF (2721 nt) of R. rickettsii secA, indicating the monocistronic transcription of secA gene.

The TSP of the R. rickettsii secA gene was determined by 5’RACE as described in Methods. The TSP corresponding to an Ala residue was located 32 nt upstream of the secA start codon (GenBank accession no. AY312572). Analysis of the TSP region revealed the presence of sequences similar to the putative −10 (TATATT) and −35 (TTGATA) consensus sequences separated by 17 nt of the canonical prokaryotic promoter recognized by the general sigma factor (σ70) (Lewin, 1997). The sequence analysis also showed the presence of sequences similar to the putative ribosome-binding site (AAGGA) on the upstream of the secA initiation codon (Lewin, 1997).

Complementation assay with rickettsial secA

The functional activity of rickettsial secA was assayed by genetic complementation in the temperature-sensitive E. coli MM52 secA51(ts). A point mutation (T to C) in the secA gene changing amino acid residue Leu43 to Pro (Fig. 1) renders E. coli MM52 unable to grow at 42°C (Schmidt et al., 1988). Strain MM52 has been used to demonstrate the functional activity of secA homologues from many bacterial species (McNicholas et al., 1995; Owens et al., 2002).

The temperature-sensitive growth of the transformed E. coli MM52 (secA51) was assayed as described in Methods. From the growth curve in Fig. 3, it was observed that E. coli MM52 transformed with plasmid pTrcEC85 (positive...

Fig. 2. Transcription analysis of rickettsial secA gene. RT-PCR analysis of the total RNA isolated from R. typhi and R. rickettsii for secA gene expression. Ethidium-bromide-stained 1% agarose gel in 1× Tris acetate EDTA buffer. Lanes 1 and 3 represent RT-PCR and PCR analysis, respectively, on the total RNA isolated from R. typhi using primers AZ2578 and AZ2579 specific to the secA-coding sequence. Lanes 2 and 4 represent RT-PCR and PCR analysis, respectively, on the total RNA isolated from R. rickettsii using primers AZ2230 and AZ1649 specific to the secA-coding sequence. The control lanes 3 and 4 demonstrate the absence of DNA in the RNA samples. GeneRuler 1 kb DNA ladder (MBI-Fermentas) was used as a DNA size marker (lane M). The identities of the RT-PCR products in lanes 1 and 2 were confirmed by sequencing.

Fig. 3. Growth-curve plot showing the complementation in E. coli MM52 secA51(ts) transformed with appropriate plasmids (as described in Methods). Cultures pregrown at 30°C were diluted 50-fold into LB-ampicillin broth (100 µg ampicillin ml⁻¹; MM52 cells without plasmid were grown in the absence of ampicillin), and incubated with shaking at the non-permissive temperature 42°C.
control, carrying the full-length ORF of *E. coli secA*) grew faster than *E. coli* MM52 with or without control plasmids (pTrcHisA or pTrcHisB) at the non-permissive temperature 42°C. However, *E. coli* MM52 transformed with pTrcRR73 (carrying the full-length ORF of *R. rickettsii secA*) or pTrcRT3 (carrying the full-length ORF of *R. typhi secA*) failed to restore the growth of strain MM52 at 42°C (Fig. 3). For quantitative detection of the growth of the transformed *E. coli* MM52 at the non-permissive temperature, survival was also determined by the c.f.u. assay. In this assay, *E. coli* MM52 showed almost no growth at 42°C, and the control plasmids pTrcHisA and pTrcHisB were unable to restore growth. However, *E. coli* MM52 transformed with the pTrcEC85 (positive control, carrying full-length ORF of *E. coli secA*) gene showed a restoration of growth at 42°C by 99.12±0.96% (compared to that at 30°C). Expression of the recombinant SecA protein was detected in *E. coli* MM52 carrying the positive control plasmid pTrcEC85 (Fig. 4, lane 3, a band of 106 kDa); this was not detected for the negative control (Fig. 4, lanes 1 and 2). *E. coli* MM52 transformed with pTrcRR73 (carrying the full-length ORF of *R. rickettsii secA*) or pTrcRT3 (carrying the full-length ORF of *R. typhi secA*) was still unable to grow at 42°C as judged by the c.f.u. assay, even though the expression of recombinant SecA proteins of *R. rickettsii* and *R. typhi* were detected in *E. coli* by Western blotting as shown in Fig. 4 (lanes 4 and 6, 107 kDa bands). The complementation assay with the full-length ORF of rickettsial secA indicated that the full-length SecA protein from these rickettsial species is not functional in *E. coli* for preprotein translocation.

**Fig. 4.** Western blot analysis of the recombinant SecA protein expression in *E. coli* MM52 secA51(ts). Total proteins from *E. coli* MM52 transformed with the appropriate plasmid were probed with anti His-tag monoclonal antibodies as described in Methods. Lane 1, total proteins from *E. coli* MM52; lane 2, total proteins from *E. coli* MM52–pTrcHisB; lane 3, total proteins from *E. coli* MM52–pTrcEC85; lane 4, total proteins from *E. coli* MM52–pTrcRR73; lane 5, total proteins from *E. coli* MM52–pTrcRE208; lane 6, total proteins from *E. coli* MM52–pTrcRT3; lane M, Bio-Rad Kaleidoscope prestained markers. A band near 39.7 kDa in lanes 1–6 may have resulted from the non-specific binding in the total proteins, and the minor bands in lanes 3–6 could be the degradation products of the recombinant SecA protein.

### Complementation studies with chimeric secA gene

To test the functional activity of the chimeric (*R. rickettsii–E. coli*) SecA protein in the temperature-sensitive *E. coli* MM52 secA51(ts), the chimeric secA gene was constructed as described in Methods. The plasmid pTrcRE208 carries the chimeric secA gene which encodes the N-terminal 408 aa of *R. rickettsii SecA* fused with the C-terminal 480 aa of *E. coli SecA* (chimeric site; Fig. 1).

The functional complementation analysis performed by using the c.f.u. assay in the temperature-sensitive *E. coli* MM52 transformed with pTrcRE208 showed a restoration of growth at 42°C by 97.44±2.16% (compared to that at 30°C). Complementation was also assayed by growth curves. Transformation of *E. coli* MM52 with pTrcRE208 led to an approximately 3±3-fold increase in growth at 42°C after 5±5 h incubation in comparison with *E. coli* MM52 carrying control plasmids pTrcHisA and pTrcHisB (Fig. 3). The expression of the recombinant chimeric SecA protein was detected in *E. coli* MM52 carrying pTrcRE208 by Western blotting, as shown in Fig. 4 (lane 5, a band of 104 kDa).

### DISCUSSION

The genome annotation of *P. prowazekii* (Andersson et al., 1998), *R. conorii* (Ogata et al., 2001) and *R. typhi* (McLeod et al., 2004) revealed the presence of homologues of all the components of the bacterial Sec pathway, with the exception of YajC. In *E. coli*, YajC forms a heterotrimeric complex with SecD and SecF as part of the Sec translocase (Economou, 1999; Paetz et al., 2002). However, disruption of the yajC gene in *E. coli* showed no effect on protein translocation (Pogliano & Beckwith, 1994). As the molecular mechanism of rickettsial protein secretion by the Sec pathway remains unknown, the effect of the absence of yajC in the rickettsial genome is not known. Here, we describe the cloning, sequence analysis, and transcription and functional characterization of the putative secA gene, which encodes the preprotein translocase SecA subunit, from rickettsiae.

The analysis of *E. coli secA* gene expression revealed that secA is the second gene in the polycistronically transcribed secM–secA operon. Translation of secA is coupled to the translation of secM (secretion monitor), since ribosomes translating the distal portion of secM are needed to disrupt the secondary structure of mRNA formed in the secM–secA intergenic region that normally blocks secA translation initiation (Nakatogawa et al., 2004; Sarker & Olver, 2002). However, analysis of the published genome sequences of rickettsiae (Andersson et al., 1998; Ogata et al., 2001; McLeod et al., 2004) showed no SecM homologue. The *R. rickettsii* transcription analysis data presented here show the monocistronic expression of the secA gene with a transcript size of 2-8 kb, and indicate that its translation does not appear to be coupled with any other gene translation.
SecA, an essential component of the general protein translocation pathway, has been found in a wide variety of bacteria (den Blaauwen & Driessen, 1996). Sequence analysis of the deduced amino acids of rickettsial SecA suggests the presence of highly conserved amino acid residues and motifs that are considered to be essential for the ATP catalytic activity of SecA in preprotein translocation (de Keyzer et al., 2003; Mitchell & Oliver, 1993). However, the functional complementation monitored by the c.f.u. assay and the growth curve plot show that the full-length SecA proteins of \( R. \) typhi and \( R. \) rickettsii fail to restore growth in \( E. \) coli MM52 secA51(ts) at the non-permissive temperature (42 °C), despite the expression of recombinant SecA protein, as detected in \( E. \) coli by Western blotting. This failure may explain the inability of recombinant rickettsial SecA to interact with the complex multisubunit translocon of \( E. \) coli cells for \textit{in vivo} preprotein translocation, or it could result from the failure of the recombinant rickettsial SecA to deliver preprotein to the \( E. \) coli Sec pathway for translocation.

The high-affinity ATP binding sites (motifs A0 and B0) confined to the N-terminal domain of SecA function as the ATP hydrolytic site. The low-affinity ATP binding site (motif A3) and the C-terminal domain regulate the ATP hydrolysis at the high-affinity ATP binding site of the SecA preprotein translocase (de Keyzer et al., 2003; Karamanou et al., 1999). Based on strong sequence similarity in the N-terminal ATP binding domains of SecA protein, we constructed a chimeric (\( R. \) rickettsii–\( E. \) coli) secA gene to test the functional complementation in the temperature-sensitive \( E. \) coli strain MM52 secA51. The expression of the chimeric secA gene (encoding the N-terminal 408 aa of \( R. \) rickettsii SecA fused with the C-terminal 480 aa of \( E. \) coli SecA) in \( E. \) coli produced a functional and active preprotein translocase subunit SecA, as demonstrated by a complementation assay. The positive correlation between the functional complementation in the temperature-sensitive secA mutant of \( E. \) coli and the ATP catalytic activity of the amino-terminal domain of SecA has been used to demonstrate \textit{in vivo} preprotein translocase ATPase activity of the putative SecA from other bacteria (McNicholas et al., 1995). Studies with different domains of the SecA indicate that the C-terminal end mediates SecA dimerization, binds with SecB, and interacts with the membrane and the intramolecular regulator of ATP hydrolysis, and, thus, the C-terminal end confers the species-specific characteristic to SecA for preprotein translocation activity (de Keyzer et al., 2003; Karamanou et al., 1999; Owens et al., 2002).

The functionally conserved high-affinity ATP binding sites in the N-terminal end of SecA are the critical regions for functional complementation of the SecA homologue from other bacteria. Our complementation data presented here indicate that \( R. \) rickettsii SecA carries a functional N-terminal ATP catalytic domain for preprotein translocon in \( E. \) coli. Biochemical analysis of rickettsial SecA, and its role in the secretion of virulence factors, are currently under investigation.

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