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# CLONING AND EXPRESSION IN *ESCHERICHIA COLI* OF THE 37-, 14-, AND/OR 16-KILODALTON ANTIGENS GENES FROM *RICKETTSIA PROWAZEKII* STRAIN E

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*Summary.* - A gene bank of *Rickettsia prowazekii* strain E constructed in the phage vector λEMBL4 was screened for antigen production with anti-*R. prowazekii* serum. One of the immunoreactive clones, grown at 37 °C exhibited the expression of at least two antigens of molecular weight ($M_r$) 37 kD and 14 kD. Subcloning and further analysis revealed that the antigens (polypeptides) of $M_r$ 37, 14, and/or 16 kD apparently represent structural units of the 138 kD complex antigen. Assembly of the above mentioned polypeptides was found to be thermosensitive as it took place at 30 °C but not at 37 °C and resulted in an oligomeric structure of $M_r$ 138 kD. The nucleotide sequence of the gene coding for a precursor of the mature polypeptides of $M_r$ 14 and/or 16 kD was determined.

*Key words: Rickettsia prowazekii; cloning; antigen; nucleotide sequence*

## *Introduction*

The obligate intracellular parasite *Rickettsia prowazekii* is the aetiologic agent of epidemic typhus and Brill-Zinsser disease. The mechanisms of immune response to this pathogen are still poorly understood (Winkler and Turco, 1988). The role and nature of rickettsial antigens responsible for eliciting the immune response during infection are not enough clarified due to difficulties connected with obtaining of sufficient quantities of purified proteins for such studies.

Molecular cloning has proved helpful in the study of rickettsial proteins. The following genes of *R. prowazekii* protein antigens have been cloned and expressed in *E. coli;* the 51 kD protein gene (Krause *et al.,* 1985), the 17 kD protein gene (Anderson and Tzianabos, 1989) and the 138 kD protein gene (Ching *et al.,* 1990). This paper describes the cloning and expression in *E. coli* of the 37-, 14-, and/or 16 kD protein antigen genes from *R. prowazekii* strain E.

The polypeptides of $M_r$ 37 kD, 14 kD, and/or 16 kD seem to represent structural units of the 138 kD oligomeric protein of *R. prowazekii* strain E.

## *Materials and Methods*

*Bacterial strains, bacteriophage, plasmids, and growth conditions. R. prowazekii* strain E was cultured in yolk sacs of embryonated hen eggs and purified as described (Aniskovich *et al.,* 1989).

*E. coli* strains QD5003 (sup E), Q359 (Karn *et al.,* 1980), XL1-Blue (Bullock *et al.,* 1987), and K38 (Russel and Model, 1984) were used in this study. *E. coli* strain K38 containing the plasmid pGP1-2 (Tabor and Richardson, 1985) was kindly provided by dr. Tabor.

Bacteriophage $\lambda$ replacement vector EMBL4 (Frischauf *et al.,* 1983), plasmid vectors pUC19 (Yanisch-Perron *et al.,* 1985) and pBluescript SK+ (Mead *et al.,* 1985) were used as described.

*E. coli* strain K38/pGP1-2 was grown at 30 °C, but all other strains were cultured at 37 °C.

*DNA technology.* Chromosomal DNA of *R. prowazekii* was extracted according to Maniatis *et al.* (1982) and digested with *Sau*3A under conditions which optimized the yield of DNA fragments in the 9- to 22-kb range. *Sau*3A fragments were then dephosphorylated by treatment with alkaline phosphatase from *E. coli* (Pharmacia), and a bank was generated by ligation into $\lambda$EMBL4 arms (Amersham). Ligation mixtures were packaged *in vitro* using prepared packaging extracts (Amersham). The infectious phage particles then were used to infect *E. coli* strains QD5003 and Q359.

*The isolation of recombinant phage and plasmid DNA,* restriction endonuclease mapping, ligation, agarose gel electrophoresis, transformation, Southern blotting, and DNA hybridization were performed by standard procedures (Maniatis *et al.,* 1982).

*SDS-PAGE.* Sodium dodecyl sulphate-polyacrylamide gel electrophoresis of polypeptides was carried out according to Laemmli (1970) by using a 3 % stacking gel and a 12 % running gel.

*Analysis of polypeptides encoding the recombinant plasmid.* The exclusive labelling of plasmid proteins was carried out using the T7 RNA polymerase/promoter system according to Tabor and Richardson (1985).

An *in vitro* transcription/translation system that utilizes purified plasmid DNA was used according to the instructions of the manufacturer (Amersham).

*The antiserum* to the large-molecular-weight protein antigens of *R. prowazekii* strain E was prepared. Following separation of *R. prowazekii* proteins by SDS-PAGE, the gel part containing the 110-150 kD proteins was cut out and homogenized in a buffered saline. This antigen was used for repeated subcutaneous immunization of chinchilla rabbits. The primary dose was 100 $\mu$g of protein. For subsequent immunization 100, 200, and 400 $\mu$g of the antigen, respectively, were injected on days 14, 28, and 56. The animals were bled on day 70 after first antigen injection.

*Preparation of anti-R. prowazekii E serum* was previously described (Aniskovich *et al.,* 1989). Monoclonal antibody to species-specific heat-sensitive epitope of the 138 kD *R. prowazekii* proteins were kindly provided by Drobyshevskaya and Nedyalkov (1990).

*For immunoscreening of recombinant phage plaques,* the latter were transferred to nitrocellulose paper by overlaying agar plates with nitrocellulose disks (HATF, Millipore, 0.45 $\mu$m) for 2 hr at 4 °C. The following stages were carried out according to the schedule of Tsang *et al.* (1983) developed for Western blot analysis.

To determine the specificity of the antibody and to characterize the recombinant clones, Western blots were prepared by the method of Tsang *et al.* (1983) using nitrocellulose paper (HAWP, Millipore, 0.45 $\mu$m). Peroxidase-conjugated goat anti-rabbit immunoglobulin G (Bio-Rad) was used as secondary antibody.

To determine heat-sensitive (protective) epitope on the product encoded by the cloned sequence, dot immunoblot (Bio-Rad) was prepared using monoclonal antibody. $^{125}$I-conjugated protein A (Amersham) was used for the detection of the antibody bound.

*DNA nucleotide sequence.* The DNA nucleotide sequence was determined by the method of Sanger *et al.* (1977). The sequencing was performed with T7 sequencing kit (Pharmacia).

Sequence analyses were performed with the DNASIS and PROSIS software packages (Pharmacia).

## *Results and Discussion*

A gene bank of *R. prowazekii* strain E was prepared in the phage vector λEMBL4 in *E. coli* QD5003. The packaging efficiency was 4500 PFU of *R. prowazekii* DNA per μg. After plating of the gene bank on *E. coli* Q359 (P2 lysogen), 95 % of phages were found to have phenotype Spi⁻ (red⁻gam⁻Chi+).

Immunoscreening of 600 phage plaques was performed with anti-*R. prowazekii* E serum. As a result, an immunoreactive clone was selected and designated λLPA120. Subsequent Western blot analysis of clone λLPA120 revealed the production of at least two antigens of $M_r$ 37 kD and 14 kD (Fig. 1).

To localize more precisely the gene(s) encoding the 37- and 14-kD antigens, a restriction map of recombinant phage λLPA120 was generated (Fig. 2A). The size of the phage λLPA120 insert was found to be 16.5 kilobase (kb). Southern blot analysis revealed that the insert of phage λLPA120 is a hybrid of rickettsial and phage DNAs. The internal part of the insert represents a central fragment of λEMBL4 without the 0.4-kb *Sal*I fragment and with modified position of restriction site for *Pst*I. The lack of the 0.4-kb *Sal*I fragment containing the part of gene gam (Frischauf *et al.*, 1983), was apparently responsible for Spi⁻ phenotype expressed by λLPA120. The fragments adjacent to phage arms belong to

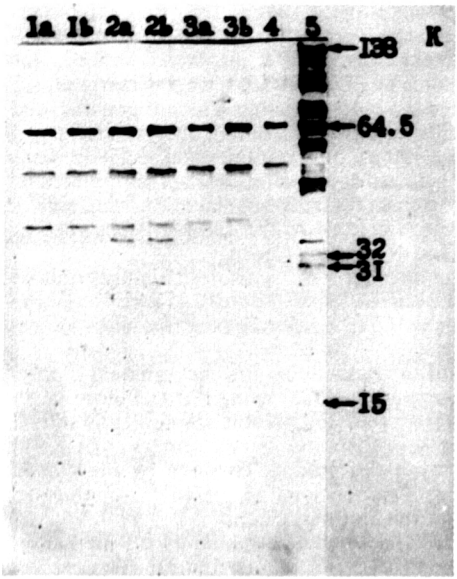


**Fig. 1**
Western blot analysis of protein from *R. prowazekii,* and *E. coli* QD5003 phage clones positive (λLPA120) or negative (λLPA9, λLPA28, λEMBL4) by the primary screening for antigen expression Lanes: 1a-1b, λLPA9 lysate; 2a-2b, λLPA120 lysate; 3a-3b, λLPA28 lysate; 4, λEMBL4 lysate; 5, 10-μg sample of *R. prowazekii.*. Three-fold increase of the volume of sample loaded into a gel allows to reveal also the 14 kD antigen expression in λLPA120 (data not shown). Numbers on the right indicate molecular weights of *R. prowazekii* major proteins determined by Oaks *et al.* (1981).

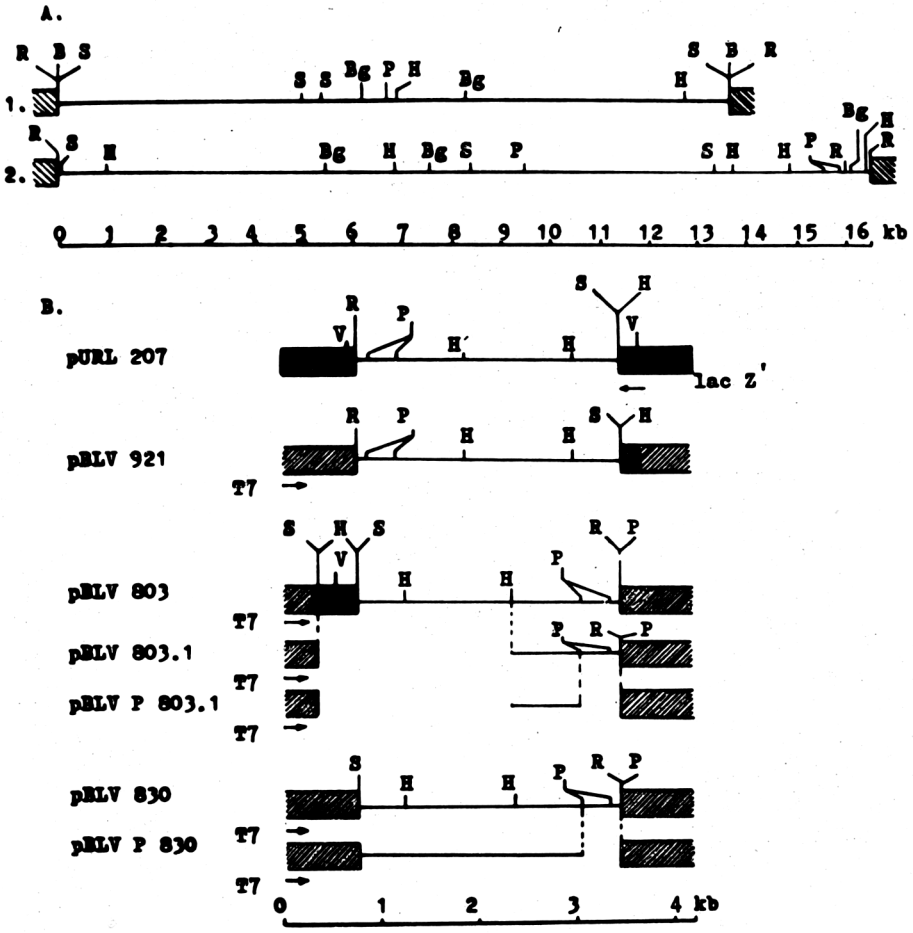


Fig. 2

A. Restriction endonuclease maps of the λEMBL4 central fragment (1) and the λLPA120 insert (2). The crosshatched parts represent λEMBL4 vector arms. B. Restriction maps of recombinant plasmids obtained in this study

Plasmid pURL207 is the result of subcloning of the 2.7-kb *Sal*I-*Eco*RI fragment of λLPA120 in pUC19. Plasmid series pBLV is the result of subcloning of the following fragments of pURL207 in pBluescript SK+: the 2.88-kb *Eco*RI-*Pvu*II fragment (pBLV921 insert); the 3.06-kb *Eco*RI-*Sal*I fragment (pBLV803 insert) consisting of the 2.88-kb *Eco*RI-*Pvu*II fragment and the 0.18-kb *Pvu*II-*Sal*I fragment; the 2.7-kb *Eco*RI-*Sal*I fragment (pBLV830 insert). Plasmids pBLV803.1 and pBLVP803.1 are the pBLV803 deletion derivatives. Plasmid pBLVP830 is the pBLV830 deletion derivative. The fully shaded boxes represent pUC19 DNA sequences, and partially shaded boxes represent pBluescript SK+ DNA sequences.

Restriction endonuclease abbreviations: R, *Eco*RI; B, *Bam*HI; S *Sal*I; Bg, *Bgl*II; H, *Hind*III; P, *Pst*I; V, *Pvu*II.

rickettsial DNA. One of these fragments (2.7-kb *Eco*RI-*Sal*I) was subcloned into vector pUC19. The resulting plasmid was designated pURL207 (Fig. 2B). *E. coli* XL1-Blue/pURL207 was tested for antigen expression by Western blot analysis. As a result of this analysis, the evidence was obtained of the presence of gene(s), encoding for the 37- and 14 kD proteins, in rickettsial 2.7-kb *Eco*RI-*Sal*I fragment (not shown).

To analyse the mature polypeptides encoded by rickettsial DNA in recombinant plasmids, we employed the T7 RNA polymerase/promoter system (Tabor and Richardson, 1985). For this purpose in vector pBluescript SK+, containing bacteriophage T7 promoter, various parts of the 3-kb *Pvu*II fragments of plasmid pURL207 were cloned and recombinant plasmids pBLV921, pBLV803, and pBLV830 were obtained (Fig. 2B). The obtained recombinant plasmids

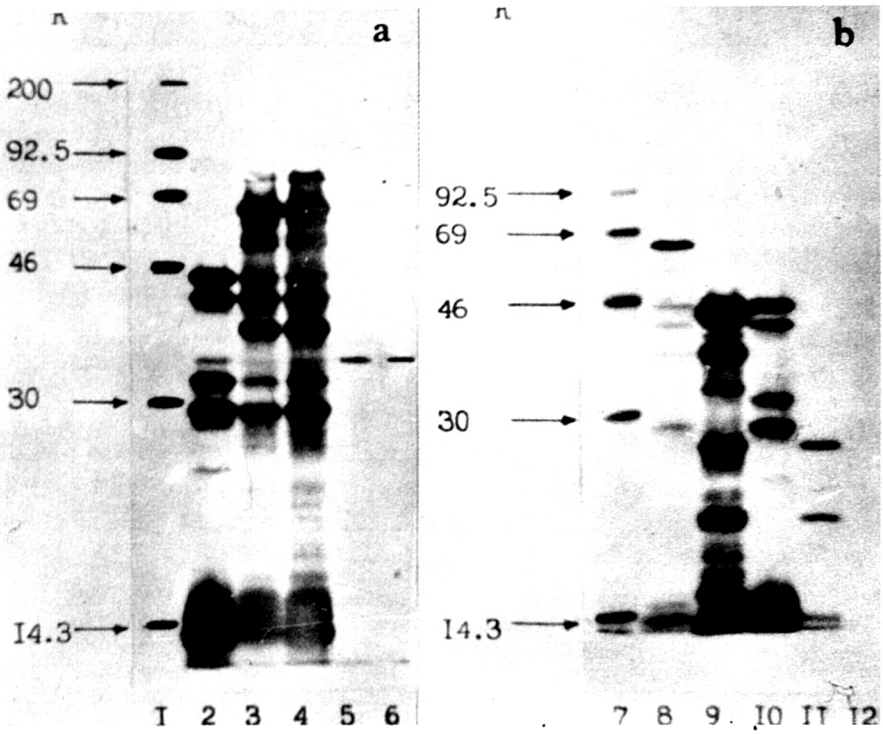


**Fig. 3**

SDS-PAGE of radiolabelled plasmid-encoded proteins. The exclusive labelling of plasmid proteins was carried out by the T7 RNA polymerase/promoter system

The samples used were *E. coli* K38/pGP1-2 containing the following plasmids: pBLV803.1 (lanes 2 and 10), pBLV830 (lanes 3 and 8), pBLV803 (lane 4), pBLV921 (lane 5), pBluescript SK+ (lanes 6 and 12), pBLVP830 (lane 9), pBLVP803.1 (lane 11), $^{14}$C-labelled molecular mass markers (Amersham) are shown (lanes 1 and 7).

were transformed into *E. coli* K38, bearing pGP1-2 plasmid providing expression of T7 RNA polymerase. Analysis of the peptides encoded by the inserts of the above plasmids and processed in *E. coli* (Fig. 3, lines 3-6) demonstrated that there was a production of a whole number of identical polypeptides, including those of $M_r$ 37 kD and 14 kD, observed in *E. coli* containing plasmids pBLV830 or pBLV803.

In order to find out the reason for the multiplicity of forms of rickettsial polypeptides produced by *E. coli* containing the plasmids pBLV830 or pBLV803, the deletion derivatives of these plasmids were obtained (Fig. 2B) and in addition to the T7 RNA polymerase/promoter system, an *in vitro* transcription/ /translation system was also employed. An *in vitro* translation system which contains functions necessary for transcription and translation of the genes encoded by exogenous DNA molecules is used to determine the size of the primary translation product. In both systems containing plasmids pBLV830 or pBLV803, apart from vector-encoded polypeptide, the 64.5 kD polypeptide was also produced, whereas plasmid pBLV921 was responsible for production of the 60 kD polypeptide (Fig. 4, lines 2-4).

The discrepancies in size of the primary translation products encoded by plasmids pBLV921 and pBLV803(830) became explicable after the nucleotide sequence had been determined (Fig. 5). It was found that one of the open reading frames (ORF2) in plasmid pBLV803 extends beyond the limits of the *Eco*RI site located at the joint position of rickettsial and vector DNAs. ORF2 was capable of encoding a fusion polypeptide of calculated $M_r$ 25 kD. In case of

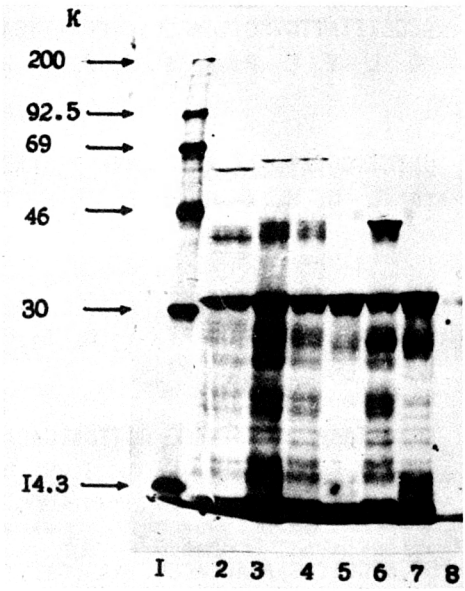


**Fig. 4**
SDS-PAGE of plasmid-encoded gene products synthesized *in vitro*
Plasmid DNA was used as a substrate for coupled *in vitro* transcription/translation, incorporating ($^{35}$S) methionine. The following plasmid DNA templates were used: lane 2, pBLV921; lane 3, pBLV803; lane 4, pBLV830; lane 5, pBLV803.1; lane 6, pBLVP830; lane 7, pBLVP803.1; lane 8, pBluescript SK+. Radiolabelled molecular weight standards are shown (lane 1).

A.

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CGTTCTTGATCGTGCATTACTGCGTCCTGGTAGATTTGATCGTCAAATTGCTGTTGCAAA      60

CCCTGATATAAATGGTCGTGAGCAAATTCTAAAAGTACATTTAAAAAAAATTAAATATAA     120
                                                -10     +1

TAGTACGGTACTAGCACGAATTATTGCTCGTGGAACTCCTGGTTTCTCCGGTGCTGAACT     180

TGCTAATTTAGTTAATGAACTGCGCTTATTGCTGCGAGGCTTGGTAAAAAAGAAGTAGAT     240
                                                          RBS

ATGCACGATATGGAAGAAGCAAAAGATAAGGTTTTGATGGGTGTTGTGCGTCGCTCTATT     300
_        M  E  E  A  K  D  K  V  L  M  G  V  V  R  R  S  I       17

GCAATGTCAGAGAAGGAGAAAAGATTAACTGCGTATCATGAAGGAGGACACGCATTAGTC     360
 A  M  S  E  K  E  K  R  L  T  A  Y  H  E  G  G  H  A  L  V      37

            Pst I
GGGCTTTATTGTCCTGCAGCCTCGCCTATTCATAAAGCTACGATTATACCACGTGGTAAT     420
 G  L  Y  C  P  A  A  S  P  I  H  K  A  T  I  I  P  R  G  N      57

GCTCTTGGTATGGTACAAAGACTTCCTGAAACTGATGAATATCCTCAGAATCGTGAACAG     480
 A  L  G  M  V  Q  R  L  P  E  T  D  E  Y  P  Q  N  R  E  Q      77

ATGGAATCATCTATAGCAGTTTATATGGCAGGAAGAGTAGCAGAAGAAATTATTTTTGGT     540
 M  E  S  S  I  A  V  Y  M  A  G  R  V  A  E  E  I  I  F  G      97

AGAAATAAAGTAACATCAGGAGCTTCATCAGATATAAAAGGTGCAACTAATATTGCAAGG     600
 R  N  K  V  T  S  G  A  S  S  D  I  K  G  A  T  N  I  A  R     117

GCGATGGTTACAAAAGCAGGTTTAAGTGATTTAATAGGACCAATATTCCACGGTTCAAAC     660
 A  M  V  T  K  A  G  L  S  D  L  I  G  P  I  F  H  G  S  N     137
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                                                    Pst I
AGTGATGATATGTATGGTAGACAATCAAGTAATGAAATTTCGGAAGCTACTGCAGAGTTA    720
 S  D  D  M  Y  G  R  Q  S  S  N  E  I  S  E  A  T  A  E  L    157

                                          EcoR I
ATTGATGCTGAAGTGAAAAGAATTATTACGCAAGGTTATGAATTCCTGCAGCCCGGGGGA    780
 I  D  A  E  V  K  R  I  I  T  Q  G  Y  E  F  L  Q  P  G  G    177

TCCACTAGTTCTAGAGCGGCCGCCACCGCGGTGGAGCTCCAGCTTTTGTTCCCTTTAGTG    840
 S  T  S  S  R  A  A  A  T  A  V  E  L  Q  L  L  F  P  L  V    197

AGGGTTAATTGCGCGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTA    900
 R  V  N  C  A  L  G  V  I  M  V  I  A  V  S  C  V  K  L  L    217

TCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGC    960
 S  A  H  N  S  T  Q  H  T  S  R  K  H  K  V  *
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B.

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                                          EcoR I
.....GAATTCGATATCAAGCTTATC    780
      E  F  D  I  K  L  I    177

GATACCGTCGACCTCGAGGGGGGGCCCGGTACCCAATTCGCCCTATAGTGAGTCGTATTA    840
 D  T  V  D  L  E  G  G  P  G  T  Q  F  A  L  *  *
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**Fig. 5**

**Nucleotide sequence of the 25 kD (A) and 20.7 kD (B) fusion polypeptide genes of plasmids pBLV830 (803) and pBLV921, respectively**

**The deduced amino acid sequence is shown. The presumed -10 promoter sequence, the start site for transcription (+1), potential ribosome-binding site (RBS) are underlined. The *PstI* and *Eco*RI sites are indicated.**

reverse orientation of the insert in plasmid pBLV921, termination of translation of the fusion polypeptide occurred in another region of the vector. As a result, in the latter case it appears that the 20.7 kD (the calculated $M_r$) was translated. However, the molecular weights of polypeptides encoded by these fusion genes, turned out to be smaller by 39.5 kD than $M_r$ of the proteins detected by the *in vitro* transcription/translation system (Fig. 4). This discrepancy may be explained by the fact that two additional ORFs terminating at 68 or 235 positions were detected upstream to ORF2. It is apparent that one of the frames (ORF1) encodes the 39.5 kD polypeptide. The 64.5 kD proteins observed (Fig. 4) may result from association of two primary translation products of $M_r$ 39.5 kD and 25 kD, whereas that of $M_r$ 60 kD was from association of the 39.5- and 20.7 kD polypeptides. Such behaviour of the polypeptides may serve as evidence to their functional relation. Since the genes encoding the functionally related polypeptides are usually included in the single operon, we suggested that the genes, cloned in our work, also are the constituent parts of the single (bicystronal) operon. This suggestion is supported by the lack of products encoded by the insert of plasmid pBLV803.1 in the *in vitro* transcription/translation system. The plasmid is a derivative of pBLV803 plasmid with deletion of the region which probably encodes N-terminal part of the 39.5 kD polypeptide. The computer analysis data on intergenic region are also in accord with this assumption. Although upstream to ORF2 a number of sequences resembling the -10 regions of promoters described for *E. coli* were detected, none of these sequences was preceded by regions with any significant homologies to the -35 consensus sequence and only one of them - AAAAAA - having 50 % homology to the -10 consensus sequence (TATAAT) was located at the position from -11 to -5 from the presumed +1 region (position 116). It was also revealed that the presumed start codon ATG at position 250 to 252 is preceded by region AGTAGATA at position 234 to 241 which is a potential prokaryotic ribosome binding site.

As it has been already noted, the 39.5 kD and 25 kD polypeptides encoded by genes of the presumed bicystronal operon are the primary translation products. The primary translation products are known to be frequently liable to covalent modification in the cell (Wu, 1987). While comparing the mature polypeptides encoded by the inserts of plasmids pBLV830 and pBLV803 and their deletion derivatives (Fig. 3), one may conclude that the 37 kD polypeptide is a processed from of the primary translation product of $M_r$ 39.5 kD, whereas the primary translation product of $M_r$ 25 kD is responsible for formation of the 14 kD and/or 16 kD mature forms capable to assemble into di- and trimers (proteins of $M_r$ 28 kD, 32 kD, 42 kD, and 45 kD). The 4.5 kD polypeptide encoded by the insert of pBLVP803.1 and being the N-terminal part of the 25 kD polypeptide is able to form tetra-, pepta-, and hexameres (proteins of $M_r$ 18 kD, 22 kD, and 27 kD), probably due to elimination of some steric limitation (Fig. 3, lines 9 and 11). Thus it is apparent that one of the fragments of the 14- and/or 16 kD monomers responsible for the assemblage into polymer structure is their N-terminal

fragment. As it was mentioned above, the polymers (for example, proteins of $M_r$ 51 and 53 kD) may probably be also formed from various monomer types of $M_r$ 37, 14, and/or 16 kD.

To determine the types of covalent modifications involved in formation of the monomers of $M_r$ 14 kD and 16 kD from their 25 kD precursor, the deduced amino acid sequence of the 25 kD polypeptide was analysed. It was found that the polypeptide has a C-terminal prokaryotic secretory signal sequence. The cleavage of this sequence may result in formation of the 19.5 kD polypeptide. What other types of the covalent modification of proteins may form monomers of $M_r$ 14 kD and 16 kD is still to be determined.

To test the antigenic activity of the polypeptides produced in *E. coli* K38/pGP1-2 which contains plasmids pBLV830 or pBLV803.1, we employed Western blot analysis of the lysates with anti-*R. prowazekii* E serum. As a result it was revealed that in contrast to *E. coli* K38/pGP1-2/pBLV803.1, one of the products of *E. coli* K38/pGP1-2/pBLV830 is an antigen comigrating with the

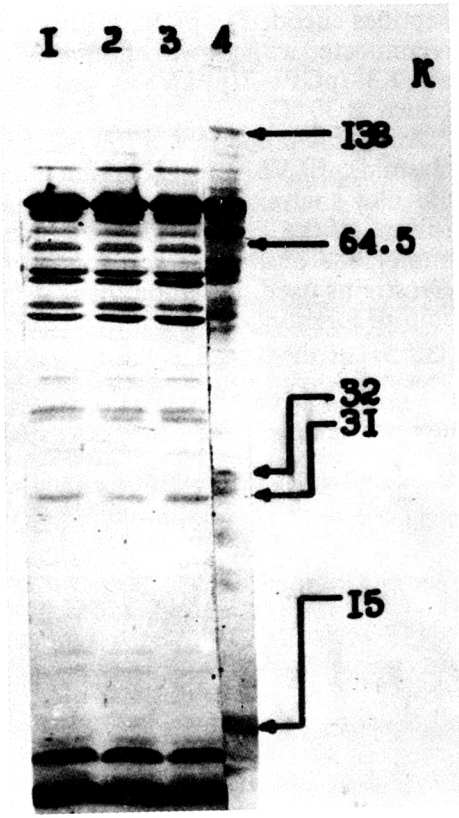


**Fig. 6**
Western blot analysis of protein from *R. prowazekii* (lane 4), and *E. coli* K38/pGP1-2 containing the following plasmids: pBLV803.1 (lane 1), pBLV830 (lane 2), pBluescript SK+ (lane 3)
Numbers on the right indicate molecular weights of *R. prowazekii* E major proteins determined by Oaks *et al.* (1981).

138 kD *R. prowazekii* antigen (Fig. 6). The 138 kD protein produced by *E. coli* K38/pGP1-2/pBLV830 also was found to react with the antiserum to the large-molecular-weight protein antigen of *R. prowazekii* E (data not shown).

As described before, *E. coli* XL1-Blue harboring the plasmid pURL207 synthesized the recombinant protein antigens of $M_r$ 37 kD and 14 kD which did not form the 138 kD oligomeric structure. On the contrary, *E. coli* K38/pGP1-2 with the plasmid pBLV830 containing the insert identical to that of plasmid pURL207 produced this 138 kD oligomeric protein. Comparing the above mentioned data with the results of Oaks (personal communication), we tried to explain the observed inconformity. Following the effect of incubation temperature on polypeptide composition of *R. prowazekii* (Breinl and E strains), Oaks observed lower quantities of the 138 kD polypeptide in *R. prowazekii* strain E grown at 37 °C than in rickettsia grown at 29 °C. Moreover, at 37 °C there appeared minor polypeptides of $M_r$ 36.5, 42.5, 49, and 91.2 kD not seen at 29 °C. Having compared the results obtained by Oaks with those presented in this paper, we suggest that the minor polypeptides observed by Oaks were not assembled parts of the 138 kD oligomer protein. Disability of the mature polypeptides encoded by pURL207 to assemble into the 138 kD oligomer structure is connected with growth of *E. coli* XL1-Blue at 37 °C. It should be noted that *E. coli* K38/pGP1-2/pBLV830, producing the 138 kD oligomeric protein, was grown at 30 °C.

Antigenic properties of the polypeptides of *E. coli* K38/pGP1-2 containing plasmids pBLV830, pBLV803 and their deletion derivatives, were also tested in dot blot analysis with monoclonal antibodies to heat-sensitive (protective) epitope of the 138 kD protein antigens of *R. prowazekii* (Fig. 7). As a result, monoclonal antibodies were found to react with recombinant products of all *E. coli* strains used. Based on these data one may conclude that the binding site epitope for monoclonal antibodies of this series is formed by amino acids of the

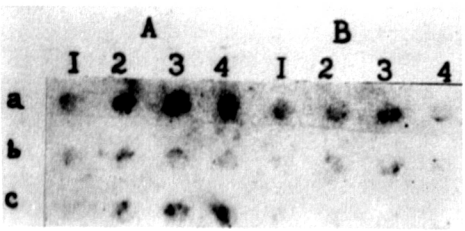


**Fig. 7**
Dot blot analysis demonstrating the presence of a heat-sensitive epitope on the cloned product
The used samples were the sonic lysates of *E. coli* K38/pGP1-2 harbouring the following plasmids; pBluescript SK+ (row 1), pBLV830 (row 2), pBLV803.1 (row 3), pBLVP803.1 (row 4). The samples were loaded in protein amounts of: 1 μg (row a), 0.5 μg (row b), 0.25 μg (row c). At location A is the result of analysis of the sonic lysates held at room temperature prior to loading; at location B is the result of analysis of the sonic lysates heated in a boiling water bath for 5 min before loading.

N-terminal fragment of the 14 and/or 16 kD monomers. Computer hydropathy analysis of the 25 kD fused polypeptide supported these results. The peak of hydrophilicity that has its centre near the residue 24 might represent an antigenic determinant exposed at the surface (Hopp and Woods, 1981). Moreover, the sequence from 25 to 29 amino acid residues might represent the T-cell epitope predicted by the model of Rothbard and Taylor (1988).

We conclude that the 138 kD antigen produced by *E. coli* K38/pGP1-2/pBLV830: 1) corresponds to the antigen of *R. prowazekii* E with identical molecular weight, 2) has an epitope cross-reacting with the species-specific epitope of the 138 kD *R. prowazekii* protein antigen, 3) apparently consists of different protein subunits of $M_r$ 37 and 14 and/or 16 kD. In accordance with the revealed properties of the expressed in *E. coli* K38/pGP1-2/pBLV830 antigen, it probably might be considered to belong to the family of high-molecular-weight species-specific (serotype) protein antigens (SPAs) of typhus rickettsiae (Ching *et al.*, 1990).

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