Protein Synthesis in Cell-free Extracts of Coxiella burnetii

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Some aspects of protein biosynthesis were investigated in extracts of the obligate intracellular bacterium Coxiella burnetii. Sucrose gradient analysis revealed small quantities of 30S and 50S ribosomal subunits, few 70S ribosomes and no polysomes. Functional endogenous mRNA was not detected. In translation of exogenously added poly(U), extracts required Mg^{2+} (17 mM) and NH_4^+ (60 mM) for optimal polyphenylalanine synthesis; the optimum Mg^{2+} requirement differed from that of Escherichia coli. The translation of coliphage Qβ RNA by C. burnetii extracts required Mg^{2+} (13 mM), NH_4^+ (60 mM) and an energy source for polypeptide synthesis, and was sensitive to chloramphenicol but not to cycloheximide. Under optimal conditions, the translation of Qβ RNA proceeded at a rate and to an extent equal to that obtained in a conventional E. coli system. Electrophoretic analysis of translation products made during incubation of C. burnetii extracts with polycistronic Qβ RNA revealed a major product with a molecular weight of about 14000; this product co-electrophoresed with the coat protein extracted from Qβ phage propagated in E. coli. The results suggested that the extracellular form of the rickettsia-like organism, C. burnetii, possessed the full array of components necessary for the initiation, elongation and termination of polypeptides.

INTRODUCTION

The Q fever bacterium, Coxiella burnetii, is obligately dependent on the intracellular environment of eukaryotic cells for growth. The basis for this host dependency remains unknown. Coxiella burnetii possesses many of the anabolic and catabolic enzyme systems typical of free-living prokaryotes, but demonstration of these has almost always required the use of cell-free extracts (Weiss, 1973). Likewise, it has not been possible to measure protein synthesis in the intact, extracellular organism. Little fundamental knowledge about the development and efficacy of the protein-forming system in these micro-organisms was therefore available.

It was known that extracts from C. burnetii contained DNA-dependent RNA polymerase activity (Jones & Paretsky, 1967; Christian & Paretsky, 1977). Evidence for 23S, 16S and 5S rRNA and for 70S, 50S and 30S ribosome particles has been presented (Thompson et al., 1971; Baca et al., 1973). More recently, Baca (1978) obtained immunochemical evidence for proteins analogous to Escherichia coli elongation factor G (EF-G) and ribosomal proteins L7/L12. Mallavia & Paretsky (1967) found limited incorporation of phenylalanine and leucine into polypeptide material when extracts were used. Baca (1978) demonstrated that extracts possess phenylalanyl-tRNA synthetase as well as ribosomes and associated protein factors capable of some polypeptide elongation in response to a synthetic polynucleotide. Analysis of C. burnetii ribosomal proteins by two-dimensional gel electrophoresis demonstrated a pattern distinct from that of E. coli (Baca, 1978). It was not known whether or not
these partial reactions and associated components were representative of a complete translation apparatus.

In the present work, the polycistronic messenger RNA from Qβ coliphage was used to investigate translational competence in C. burnetii extracts. The advantages of using the well-defined bacteriophage RNAs for translation studies have been stated by Capecchi & Webster (1975). Successful translation required that factors and components responsible for initiation, elongation and termination of polypeptide, as well as for aminoacylation of tRNAs bearing amino acids specified by the mRNA, be present in the obligate parasite extracts. The present paper describes the preparation and characterization of C. burnetii extracts that were completely dependent on the addition of exogenous mRNA for the synthesis of polypeptide. By the use of parallel control experiments consisting of the Qβ RNA and homologous E. coli extract, it was possible to assess the relative efficacy of C. burnetii preparations in several aspects of translation.

METHODS

**Growth of micro-organisms.** Coxiella burnetii, Nine Mile strain, phase I, was obtained from O. G. Baca, University of New Mexico, U.S.A. The organisms were grown in yolk sacs of 6-d-old embryonated chicken eggs (antibiotic-free; Spafus, Norwich, Conn., U.S.A.), harvested, then purified as described by Paretsky et al. (1958). The organisms were subsequently banded by centrifugation through 0.96 to 2.29 m linear sucrose gradients (Thompson et al., 1971). The bands were collected and diluted with phosphate/KCl buffer (0.02 M-potassium phosphate pH 7.4, 0.15 M-KCl). Coxiella burnetii was then pelleted and stored at −80 °C. Escherichia coli Q13, obtained from C. L. Harris, West Virginia University, U.S.A., was grown in Trypticase Soy Broth (BBL). Cultures were harvested in exponential phase when turbidity measured 200 Klett–Summerson units (420 nm). The cells were washed twice in buffer and stored at −80 °C. Escherichia coli C3000 was obtained from the American Type Culture Collection (ATCC 15597) and grown as described below.

**Propagation and purification of bacteriophage Qβ.** Coliphage Qβ (original stock obtained from Miles Laboratories) was propagated essentially as described by Kolakofsky (1971). A mid-exponential phase culture of E. coli C3000 containing 5 × 10^6 cells ml^-1 in ATCC 271 broth at 37 °C was inoculated at a multiplicity of infection of 5 to 10. Incubation was continued until incipient cellular lysis was observed (3 h). Complete cell lysis was then induced by the addition of 50 mg egg white lysozyme dissolved in 0.1 M-Tris/HCl (pH 8.0), 2 ml 0.5 M-Na_2EDTA and 2 ml chloroform. Virus was recovered by adjusting the lysate to 50% saturation with solid (NH_4)SO_4 (300 g l^-1) and subsequently purified by differential and isopycnic centrifugation, as described by Webster et al. (1967).

**Preparation of coliphage RNA.** Deproteinization of virus RNA was achieved by phenol extraction (Kolakofsky, 1971). All glassware and reagents were prepared so that ribonuclease contamination was minimized. Virus particles in 0.15 M-NaCl/0.015 M sodium citrate (SSC) and 0.2% (w/v) sodium dodecyl sulphate (SDS) were shaken vigorously with an equal volume of SSC-saturated, destilled phenol. The aqueous phase was re-extracted with phenol, and a third extraction was performed with an equal volume of chloroform. The aqueous phase was then removed, 0.1 vol. 2 M-sodium acetate (pH 5.5) was added and the RNA was precipitated by the addition of 2 vol. ice-cold 95% ethanol. The RNA was recovered by centrifugation, dissolved in Tris/EDTA buffer (0.001 M-Tris/HCl pH 7.0, 0.001 M-Na_2EDTA) and stored at −80 °C until required. The molecular integrity of the RNA obtained was determined by polyacrylamide gel electrophoresis (Thompson et al., 1971); one symmetrical peak corresponding to a molecular weight of 1.2 × 10^6 was routinely observed.

**Preparation of 14C-labelled virus coat protein.** A culture of E. coli C3000 grown to mid-exponential phase (5 × 10^7 cells ml^-1) in ATCC 271 broth was inoculated with Qβ bacteriophage at a multiplicity of infection of 10. After 5 min, L-[U-14C]lysine [Amersham; 285 mCi mmol^-1 (10.55 GBq mmol^-1)] was added to a final activity of 1 μCi ml^-1 and incubation was continued until incipient cellular lysis was observed (2 to 3 h). Complete cell lysis was induced and the 14C-labelled virus particles were purified as described above and then stored at −80 °C until required for electophoresis.

**Preparation of bacterial cell-free extracts.** Coxiella burnetii or E. coli cell paste, washed once in TMKD buffer (0.01 M-Tris/acetate pH 7.4, 0.01 M-magnesium acetate, 0.05 M-potassium acetate, 0.002 M-dithiothreitol) was re-suspended in twice its wet weight of TMKD buffer. The cell suspension was then subjected to freeze rupture in a Carver Laboratory Press, model B (Fred S. Carver, Summit, N.J., U.S.A.). The preparation of extracts by differential centrifugation was as described by Modolell (1971). Following centrifugation of the freeze-ruptured cell suspension at 30000 g in a Sorvall SS-34 rotor for 30 min at 4 °C, E. coli super-
natant fluid (S30) was dialysed for 4 h against TMKD buffer, divided into samples and stored at −80 °C until required. Coxiella burnetii S30 extracts, centrifuged as above, were not dialysed as this resulted in extracts of irregular activity. Instead they were quickly divided into samples and stored at −80 °C until required. Coxiella burnetii P30 refers to the pellet which resulted from the 30 000 g centrifugation of freeze-ruptured cell suspensions (it contained partially damaged cells, cell debris and approximately 10% intact organisms). The S100 extracts were obtained by centrifuging undialysed E. coli or C. burnetii S30 extracts at 105 000 g in a Spinco type 40 rotor for 4 h at 2 °C. The upper portion of the S100, which was devoid of ribosomes, was removed, dialysed for 4 h against TMKD buffer, divided into samples and stored at −80 °C until required. Protein concentrations in extracts were determined by the Coomassie Brilliant Blue (G250) colorimetric assay of Bradford (1976) using lyophilized E. coli S100 protein as standard.

Preparation of L-[3H]phenylalanyl-tRNA. Escherichia coli Q13 tRNA was prepared by phenol/high salt (1 M-NaCl)/isopropanol extraction and decylated as described by von Ehrenstein (1967). Escherichia coli aminoacyl-tRNA synthetase fractions were prepared by chromatography of S100 fractions on a 2.5 × 20 cm Cellex D (DEAE-cellulose) column (Yamane & Sueoka, 1963). Once unadsorbed protein had been thoroughly washed from the column, the buffer was changed to one containing high salt (0.02 M-potassium phosphate pH 7.7, 0.35 M-NaCl, 0.002 M-dithiothreitol) and fractions were collected and assayed. Fractions containing synthetase were pooled and solid (NH4)2SO4 was added to 50% saturation. The protein precipitate was then collected, dissolved in buffer (0.05 M-Tris/HCl pH 8.0, 0.002 M-dithiothreitol) and dialysed overnight against the same buffer. The L-[3H]phenylalanyl-tRNA was then prepared by incubating the E. coli tRNA, ATP, Mg2+, aminoacyl-tRNA synthetases and L-[2,3-3H]phenylalanine [New England Nuclear; 5 Ci mmol−1 (185 GBq mmol−1)] at 37 °C and subsequently extracting the mixture with phenol as described by Siler & Moldave (1969).

Polyacrylamide gel electrophoresis. The products of translation in vitro were prepared for electrophoresis by incubation of reaction mixtures in 0.33 M-NaOH at 37 °C for 15 min, followed by the addition of 25% (w/v) trichloroacetic acid (TCA). The protein precipitate was then washed twice with 10% (v/v) TCA containing unlabelled leucine or phenylalanine (1 mg ml−1), and once with acetone. The pellet was resuspended in sample buffer (0.0625 M-Tris/HCl pH 6.8) containing 1 mg unlabelled leucine ml−1, and once with acetone. The protein precipitate was then collected, dissolved in buffer (0.05 M-Tris/HCl pH 7.6, 0.002 M-dithiothreitol) and dialysed overnight against the same buffer. The L-[3H]phenylalanyl-tRNA was then prepared by incubating the E. coli tRNA, ATP, Mg2+, aminoacyl-tRNA synthetases and L-[2,3-3H]phenylalanine [New England Nuclear; 5 Ci mmol−1 (185 GBq mmol−1)] at 37 °C and subsequently extracting the mixture with phenol as described by Siler & Moldave (1969).

RESULTS

The ribosome content of an S30 extract from C. burnetii was evaluated by rate-zonal centrifugation. An identically prepared E. coli S30 extract was used as a control with which the C. burnetii S30 extract could be compared. In the E. coli S30 extract most of the A260 material sedimented as 70S ribosomes with a proportionately smaller quantity of A260...
Fig. 1. Sucrose density gradient analysis of ribosomal particles from S30 extracts of *C. burnetii* (a) and *E. coli* (b). Extracts, prepared as described in Methods, were layered on to 15 to 30% (w/v) linear sucrose gradients (containing TMK buffer pH 7.4) and centrifuged at 47000 g (SW27.1 rotor, Spinco) for 15 h at 4 °C. The gradients were then fractionated and the absorbance at 260 nm was measured. The quantities layered were in (a) 10 *A*$_{260}$ units (1.36 mg protein) and in (b) 10 *A*$_{260}$ units (0.98 mg protein).

material sedimenting as 50S and 30S ribosomal subunits (Fig. 1b). By contrast, in the *C. burnetii* S30 extract (Fig. 1a), most of the particulate *A*$_{260}$ material sedimented as 30S and 50S ribosomal subunits, with very little 70S material present. Assuming that the molecular weights of the RNA and protein components of *C. burnetii* and *E. coli* ribosomes are similar, an estimate was made of the quantity of ribosomal particles in *C. burnetii* S30 extract as compared with *E. coli* S30 extract. It was calculated that *C. burnetii* extracts contained 5 pmol 70S ribosomes, 13 pmol 50S subunits and 9 pmol 30S subunits per mg S30 protein, whereas the *E. coli* extracts contained 105 pmol 70S ribosomes, 20 pmol 50S subunits and 14 pmol 30S subunits per mg S30 protein. Although there was a significant difference in 70S ribosome content, the same degree of disparity did not exist in the quantities of 50S and 30S ribosomal subunits present in the two extracts. It was evident, however, that *C. burnetii* S30 extract contained only 20% of the total ribosomal material found in a similar preparation from *E. coli* (Fig. 1). Polyacrylamide gel electrophoresis of the rRNA obtained by phenol extraction of both *C. burnetii* S30 extract and the resultant P30 fraction supported this contention (results not shown).

The Mg$^{2+}$ concentration required for optimal polymerization of phenylalanine, directed by poly(U), was studied in both *C. burnetii* and *E. coli* S30 extracts (Fig. 2). The optimum added Mg$^{2+}$ concentration for polyphenylalanine synthesis in *C. burnetii* S30 extract was 16 to 18 mM, while that for *E. coli* S30 extract was 5 to 6 mM. There was virtually no protein synthesis in *C. burnetii* S30 extract at the Mg$^{2+}$ concentration optimal for polymerization
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Fig. 2. Mg\(^{2+}\) concentration optima for poly(U)-directed polyphenylalanine synthesis in C. burnetii (●) and E. coli (▲) extracts. Each reaction mixture contained C. burnetii or E. coli S30 extract (235 and 246 μg protein, respectively), 25 μg poly(U) and 19.5 μg E. coli tRNA containing 1.06 pmol (14518 d.p.m.) of esterified L-phenylalanine per μg RNA. The final reactant concentrations in each reaction mixture were: 60 mM-Tris/HCl pH 7.6, 0.2 mM-GTP, 2 mM-dithiothreitol, 80 μM-NH\(_4\)Cl and Mg\(^{2+}\) as indicated. The final reaction volume was 0.25 ml. Open symbols (●, C. burnetii; ▲, E. coli) represent controls from which poly(U) was omitted.

Fig. 3. Mg\(^{2+}\) concentration optima for Q\(\beta\) RNA-directed protein synthesis in C. burnetii (●) and E. coli (▲) extracts. Reaction mixture components consisted of the energy system, buffered salts, dithiothreitol and 19 unlabelled amino acids as described in the legend to Table 1, with the following exceptions: Mg\(^{2+}\) concentrations were as indicated and NH\(_4\)Cl concentration was 56 mM for both C. burnetii and E. coli. Each reaction mixture contained C. burnetii or E. coli S30 extract (324 and 246 μg protein, respectively), E. coli S100 (67 μg protein in E. coli system only), 34 μg Q\(\beta\) RNA and 2 μCi [3,4,5-\(^{3}H\)]leucine (New England Nuclear; 145 Ci mmol\(^{-1}\) (5.37 TBq mmol\(^{-1}\)). Open symbols (●, C. burnetii; ▲, E. coli) represent controls from which Q\(\beta\) RNA was omitted.

by E. coli S30 extract. This explains, in part, the low level of poly(U)-dependent phenylalanine incorporation by C. burnetii fractions previously observed (Baca, 1978). However, the difference in the optimal Mg\(^{2+}\) concentration for Q\(\beta\) RNA-directed polypeptide synthesis (Fig. 3) in C. burnetii S30 extract (12 to 14 mM) and E. coli S30 extract (10 to 12 mM) was small. The concentration of NH\(_4\)\(^+\) necessary for optimal peptide synthesis, directed by either poly(U) or Q\(\beta\) RNA, was similar for E. coli and C. burnetii extracts. Effective ranges were 40 to 100 mM in poly(U)-directed synthesis and about 60 mM for Q\(\beta\) RNA-directed synthesis (results not shown). In C. burnetii extracts prepared by sonication, pronounced inhibition of poly(U)-dependent polyphenylalanine synthesis was observed at NH\(_4\)\(^+\) concentrations above 120 mM, whereas extracts prepared by freeze-rupture remained active at much higher NH\(_4\)\(^+\) concentrations (results not shown). At all ionic concentrations and combinations tested, incorporation of amino acids by C. burnetii extracts was completely dependent on addition of mRNA.

Selected characteristics of the C. burnetii S30 protein synthesizing system programmed with Q\(\beta\) RNA are shown in Table 1. The addition of Q\(\beta\) RNA to C. burnetii S30 extract resulted in a 10-fold stimulation of incorporation of [\(^{3}H\)]leucine over the level of incorporation observed in the absence of Q\(\beta\) RNA. The requirement for an energy regeneration system for protein synthesis in undialysed C. burnetii S30 extract reflected a similar requirement found for dialysed E. coli S30 extract. The specific monovalent and divalent cation requirement of the protein synthesis reaction for NH\(_4\)\(^+\) and Mg\(^{2+}\), respectively, was demonstrated
Table 1. Characteristics of the translation of Qβ RNA in C. burnetii S30 extracts

The complete system consisted of C. burnetii S30 extract (324 µg protein), 34 µg Qβ RNA and 2 µCi [3,4,5-3H]leucine [New England Nuclear; 145 Ci mmol⁻¹ (5.37 TBq mmol⁻¹)]. The final reactant concentrations in each reaction mixture were: buffered salts (50 mM-Tris/HCl pH 7-8, 12 mM-Mg²⁺, 60 mM-NH₄⁺); energy system (0-5 mM-ATP, 0-2 mM-GTP, 1 mM-creatine phosphate, 15 µg creatine phosphokinase ml⁻¹); 1 mM-dithiothreitol; 19 unlabelled amino acids (0·05 mM each). The final reaction volume was 0·1 ml.

<table>
<thead>
<tr>
<th>Components</th>
<th>10⁻⁴× Incorporation of [3,4,5-3H]leucine into hot TCA-insoluble material [d.p.m. (mg S30 protein)⁻¹]</th>
</tr>
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<tbody>
<tr>
<td>Complete system</td>
<td>26·7</td>
</tr>
<tr>
<td>Complete system - Qβ RNA</td>
<td>2·7</td>
</tr>
<tr>
<td>Complete system - energy</td>
<td>1·6</td>
</tr>
<tr>
<td>Complete system - NH₄⁺+NaCl (60 mM)</td>
<td>3·7</td>
</tr>
<tr>
<td>Complete system - Mg²⁺+putrescine (12 mM)</td>
<td>2·2</td>
</tr>
<tr>
<td>Complete system + cycloheximide (1 mM)</td>
<td>28·6</td>
</tr>
<tr>
<td>Complete system + chloramphenicol (0·15 mM)</td>
<td>2·1</td>
</tr>
<tr>
<td>Complete system - Qβ RNA + C. burnetii RNA*</td>
<td>3·2</td>
</tr>
<tr>
<td>Complete system - Qβ RNA + globin mRNA†</td>
<td>3·0</td>
</tr>
</tbody>
</table>

* 32 µg purified C. burnetii RNA. † 22 µg rabbit α, β-globin mRNA.

by the inactivity of the extract when Na⁺ was substituted for NH₄⁺, and when putrescine was substituted for Mg²⁺. The prokaryotic nature of the ribosomes was shown by the observation that the protein synthetic reaction was inhibited (92%) by chloramphenicol and was refractory to inhibition by a 10-fold higher concentration of cycloheximide.

Escherichia coli S30 extracts required a preincubation step to effect translation of endogenous mRNA, so that the system would be optimally responsive to the addition of exogenous RNA. In contrast, C. burnetii S30 extracts did not require a preincubation step. Additions of homologous RNA did not stimulate amino acid incorporation when added back to the C. burnetii S30 protein synthesizing system (Table 1). These results suggested a lack of endogenous mRNA activity in extracts prepared from C. burnetii, and offer an explanation for the limited protein synthesis observed in some earlier studies where mRNA was not added to extracts (Mallavia & Paretsky, 1967).

Under the reaction conditions optimal for Qβ RNA-programmed protein synthesis, C. burnetii S30 extracts were inactive in the translation of eukaryotic globin mRNA (Table 1). Also, post-ribosomal supernatant (S100 from C. burnetii, obtained originally from O. G. Baca) was incapable of promoting polyphenylalanine synthesis, directed by poly(U), on mouse liver ribosomes (results not shown). These observations, together with the antibiotic sensitivity data, preclude the possibility of contamination of C. burnetii S30 extracts by host cell cytoplasmic components, and confirm the prokaryotic nature of the translational apparatus under investigation.

The rates of in vitro translation of RNA phage mRNAs by homologous E. coli extracts are known (Capcchi & Webster, 1975). It was of interest to determine how translation rates of extracts from the obligate intracellular bacterium C. burnetii compared when translating an identical message. Therefore, kinetic analysis of Qβ RNA-dependent protein synthesis in C. burnetii and E. coli S30 was performed. In both extracts incorporation of leucine into hot TCA-insoluble material was time-dependent, proceeding almost linearly for at least 15 min (results not shown). It was apparent that both the initial rates and final extents of the reactions were similar in the two extracts. In the absence of Qβ RNA a low level of protein synthesis occurred in the E. coli S30 extract but none was apparent in the C. burnetii S30 extract. Control incubations containing chloramphenicol were uniformly inhibited throughout the entire course of the experiment.
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In order to evaluate the products of Qβ RNA-directed protein synthesis in C. burnetii S30 extracts, SDS-polyacrylamide gel electrophoresis was carried out (Fig. 4). In vitro translation products containing [3H]leucine were co-electrophoresed with 14C-labelled Qβ phage coat protein obtained from purified Qβ phage labelled during in vivo infection of E. coli. The major translation product present in the C. burnetii S30 extract coincided with authentic Qβ phage coat protein. Analysis, in a similar manner, of translation products of an E. coli S30 extract, directed by Qβ RNA, yielded an electrophoretic profile (results not shown) nearly identical to that shown in Fig. 4 for C. burnetii S30 extract. Electrophoresis of control incubations of C. burnetii extract from which Qβ RNA was omitted revealed no radio-labelled peaks (results not shown).

Nascent polypeptide chains would necessarily accumulate to a limited extent at the end of the incubation. The presence of incomplete coat protein molecules in the in vitro translation products could account for the broad leading edge of the coat protein peak observed in Fig. 4. The nature of the series of radiolabelled peaks containing smaller quantities of radioactivity which migrated more slowly than authentic coat protein is unknown.

DISCUSSION

Coxiella burnetii, a host-dependent micro-organism, had previously been shown to contain some of the components necessary for host-independent protein synthesis. However, there was no evidence that the organism was competent in all stages of the translation process. The present report describes the preparation of extracts that are completely dependent on the addition of mRNA for incorporation of amino acids into polypeptides. Under the best conditions established, C. burnetii extracts programmed with Qβ RNA synthesized a polypeptide product that was indistinguishable, in molecular weight, from Qβ phage coat protein synthesized in vivo during infection in E. coli. Translation of Qβ RNA in the E. coli extracts described here also resulted primarily in the synthesis of coat protein. Unlike E. coli extracts (Modolell, 1971), those from C. burnetii contained no detectable endogenous mRNA. Because the present results suggest that addition of highly purified Qβ mRNA to a C. burnetii extract resulted in the initiation and completion of the phage coat protein, C. burnetii probably possesses the required complement of aminoacyl-tRNA synthetases, cognate
tRNAs, ribosomes and factors necessary for the initiation, elongation and termination of authentic polypeptide. No explanation can be offered for the finding that extracts of the parasite are devoid of mRNA activity. One possibility is that rickettsial mRNA is much more labile than the other components of its translational machinery, and that its absence is therefore an artefact of the in vitro preparation procedures. Alternatively, the extracellular organism may not contain mRNA; this explanation would require that new mRNA synthesis occurs after entry into the host cell. The significance of the requirement for much higher quantities of added Mg$^{2+}$ for polyphenylalanine synthesis in C. burnetii extracts is not completely understood. This finding may relate to the previous observation that washing of C. burnetii ribosomes, with Mg$^{2+}$/K$^+$ solutions appropriate for ribosomes from other species, renders these ribosomes inactive in polyphenylalanine synthesis (Baca, 1978).

The Mg$^{2+}$ concentration required for optimal in vitro protein synthesis is dependent both on the species specificity of the ribosomal system employed and the mRNA utilized to programme that system (Stallcup & Rabinowitz, 1973a). This may account in a general way for the demonstrated disparity in Mg$^{2+}$ optima for poly(U)-directed and Qp RNA-directed polypeptide synthesis in C. burnetii and E. coli S30 extracts.

Bacteriophage Qp contains a polycistronic mRNA in which the frequency of initiation at the maturation protein, coat protein and replicase protein cistrons is strictly regulated both in vivo and in vitro. Protein synthesis studies employing extracts of E. coli programmed with coliphage RNA (Qp, MS2, R17, f2) have demonstrated that the coat protein is the predominant translation product (Capecchi & Webster, 1975; Horiuchi et al., 1971; Lodish & Robertson, 1969; Nathans, 1965). The preferential initiation of the coat protein cistron in these bacteriophages is dependent on the specific recognition of RNA primary and secondary structure by the 30S ribosomal subunit (Lodish & Robertson, 1969; Lodish, 1970a, b). In the present study, the predominant product detected in C. burnetii S30 extracts programmed with Qp RNA was a polypeptide identical in molecular weight to the phage coat protein (Fig. 4). This suggests a degree of functional similarity in vitro between 30S ribosomal subunits of C. burnetii and E. coli. It has been demonstrated that ribosomes from different bacterial species exhibit restricted template specificity with regard to their ability to initiate the translation of heterologous mRNA (Leffler & Szer, 1974; Lodish, 1969; Stallcup & Rabinowitz, 1973a, b). Therefore, it is perhaps significant that, first, extracts from the obligate intracellular parasite C. burnetii were capable of translating Qp phage RNA at all and, second, that the specificity of translation was apparently the same as observed in extracts from the free-living bacterium E. coli. However, in this regard, no attempt has yet been made to determine if C. burnetii extracts are also producing the expected low levels of phage replicase protein, maturation protein and coat read-through protein (Weiner & Weber, 1973).

In the extracts obtained for these studies, preparations of C. burnetii contained substantially less ribosomal material than did E. coli preparations. In experiments not reported here, phenol extractions of both S30 and P30 C. burnetii fractions have revealed unusually high tRNA: rRNA ratios (molar ratios of 50:1 to 100:1). The amount of ribosomes found in a bacterial cell is a central aspect of its physiological state (Forchhammer & Kjeldgaard, 1968). More precise work needs to be done before this important aspect of the obligate bacterium's physiology can be defined.

Regardless of ribosome content, C. burnetii S30 extracts are as active in the translation of Qp RNA and poly(U) as E. coli S30 extracts when optimal conditions for each are used. Collectively the results indicate that the protein synthetic activities in C. burnetii and E. coli extracts are similar in relation to total extracted protein or ribosomal subunit content. However, the protein synthetic activity relative to the total ribosome content in the extracts is several times greater in C. burnetii than in E. coli extracts, which have many more 70S ribosomes. Since ribosomal subunits are obligatory for the initiation of protein synthesis (Kaempfer et al., 1968), it is possible that only the subunit pools in both types of extracts

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studied here bind to mRNA and catalyse polypeptide synthesis. The proportion of ribosomes engaged in protein synthesis, the mRNA transit time of ribosomes, and the degree of translational infidelity in the C. burnetii system remain unknown. Thus, it is at present difficult to compare efficiency and fidelity in C. burnetii and E. coli. These aspects require further investigation.

This study demonstrates that the translational system in C. burnetii can function in vitro, and suggests that those factors which dictate the host-dependency of this micro-organism for growth and cell division do not include a basic or intrinsic protein synthetic incompetence in a qualitative sense. However, it is well known that the intact, extracellular organism is metabolically inert, exhibiting metabolic proficiency only in cell-free extracts. Entry into a host cell somehow induces a sufficient level of metabolic activity in C. burnetii to promote growth and cell division. The induction of this metabolic activity may be related to mechanisms for the control of gene expression in C. burnetii. The previous studies by others (Baca et al., 1973; Baca, 1978; Christian & Paretsky, 1977; Jones & Paretsky, 1967; Mallavia & Paretsky, 1967; Thompson et al., 1971) and the development of the protein synthesis system from C. burnetii described here provide important steps toward the construction of a coupled transcription–translation system through which a systematic evaluation of the mechanism of gene expression in this organism may be conducted.

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