Presence of Ribosomal Ribonucleic Acid in the Rickettsia *Coxiella burneti*

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(Received 16 August 1971)

The rickettsial agent *Coxiella burneti*, which is a prokaryotic organism, is an intracellular obligate parasite with a broad host range. Although the organisms incorporate amino acids (Mallavia & Paretsky, 1967), and electron microscopy shows structures similar to ribosomes (Burton, Kordova & Paretsky, 1971), there has been no direct demonstration of their presence. We now present evidence for the existence of rickettsial ribosomes.

Coxiella burneti, Nine Mile strain, phase I, was propagated in embryonated eggs and purified (Paretsky, Downs, Consigli & Joyce, 1958). Subsequently, 40–50 mg of the resultant suspension was layered on 28 ml 30–60% (w/w) linear sucrose gradients (ribonuclease-free sucrose; Mann Research Laboratories, New York, N.Y., U.S.A.) in 0.2 M-potassium phosphate buffer, pH 7.4, and centrifuged in a Spinco SW 25.1 rotor at 20 000 rev./min for 90 min. The organisms and residual host material formed three separate bands and a pellet, with the rickettsiae in the band above the pellet. The rickettsiae were removed with a syringe and bent needle and were then pelleted by centrifuging at 14 000 g for 60 min. The pellet was washed twice in water and collected by centrifugation at 14 000 g for 30 min. The preparation was examined for purity by the Giménez stain and by electron microscopy. This established rickettsial homogeneity and the absence of detectable host material. The purified rickettsiae were stored at -70°C .

Rickettsial RNA was extracted by a modified phenol method (Pace, Peterson & Pace, 1970). Purified rickettsiae (60–120 mg wet weight) were suspended in 4.25 ml of ice-cold 1 M-magnesium chloride–10 mM-tris–HCl buffer, pH 7.3. Egg-white lysozyme (3 × recrystallized, A grade; Calbiochem, Los Angeles, Calif., U.S.A.) and deoxyribonuclease (electrophoretically pure, ribonuclease-free; Worthington Biochemical Corp., Freehold, N.J., U.S.A.) were added to the rickettsial suspension to final concentrations of 1 mg/ml and 10 µg/ml respectively, in a final volume of 5.0 ml. The suspension was rapidly frozen and thawed three times, and sodium dodecyl sulphate was added to a concentration of 1%, and EDTA to 5 mM. The suspension was extracted in the cold with water-saturated phenol, with vigorous shaking, twice for 20 min and

once for 15 min. The final aqueous phase was treated with 2 vol. of ethanol. After storage at -20°C overnight, the nucleic acids that had precipitated were collected by centrifugation, dissolved in 0.1 M-sodium chloride–1 mM-EDTA–0.01 M-sodium acetate buffer, pH 5.1, to a concentration of about 1 mg of nucleic acid/ml, and stored at -70°C until used. Reference marker rRNA species were prepared from chick yolk sac and *Escherichia coli* B, by the same procedures. All preparations had an E_{260}/E_{280} ratio of 1.9–2.1. Polyacrylamide-gel electrophoresis was performed in 10 cm tubes as described by Loening (1967), except that polymerized gels were removed from the tubes, transferred to electrophoresis buffer and left for at least 72 h before being run (Bishop, Claybrook & Spiegelman, 1967). Gels were pre-run at 3 mA/gel for 1 h; then 10–35 µl of sample in 20% (w/v) sucrose was overlaid on the gels, and electrophoresis was resumed. Gels were scanned at 260 nm in a linear transport accessory attached to a Gilford 2400 recording spectrophotometer.

When *C. burneti* RNA was subjected to electrophoresis, three major peaks were obtained (Fig. 1a). The low-molecular-weight material migrated coincidentally with *E. coli* tRNA^{Met} (given by Dr G. D. Novelli) and yolk-sac 4–5 S RNA. This is suggestive of tRNA species in *C. burneti*. Electrophoreses of yolk-sac RNA, *C. burneti* RNA and a mixture of the two were simultaneously performed in the same chamber (Fig. 1). The yolk-sac rRNA species (Figs. 1b and 1c) migrated more slowly than those of *C. burneti*. The patterns rule out the possibility that the major peaks shown in the *C. burneti* RNA preparation are yolk-sac contaminants.

Molecular weights of the species were estimated by using the inverse relationship of electrophoretic mobility to the logarithm of the molecular weight (Bishop *et al.* 1967). *E. coli* B rRNA was used as a standard reference marker, by using molecular-weight values of 1.07×10^6 and 0.56×10^6 for the 23S and 16S species (Loening, 1968; Stanley & Bock, 1965). By use of internal markers the molecular weights of 28S and 18S yolk-sac RNA were determined to be 1.67×10^6 (s.e.m. $\pm 0.015 \times 10^6$; five determinations) and 0.65×10^6 (s.e.m. $\pm 0.006 \times 10^6$;

Fig. 1. Electrophoresis of *Coxiella burnetii* and yolk-sac RNA. Gels prepared from the same batch of 2.4% acrylamide and 0.12% bisacrylamide in buffered sodium dodecyl sulphate were overlaid with 30 μ g of *C. burnetii* RNA (a), 20 μ g of yolk-sac RNA (b), and a mixture of 22 μ g of *C. burnetii* RNA + 14 μ g of yolk-sac RNA (c). The buffer contained 40 mM-tris, 20 mM-sodium acetate, 2 mM-EDTA and 0.1% sodium dodecyl sulphate, adjusted to pH 7.8 with acetic acid. Electrophoresis was performed for 3 h at 3 mA/gel at 25–27°C. Y, yolk-sac RNA; C, *C. burnetii* RNA.

five determinations). The molecular weights of *C. burnetii* rRNA species calculated from electrophoresis of mixtures of yolk-sac and *C. burnetii* RNA were 1.05×10^6 (s.e.m. $\pm 0.01 \times 10^6$; five determinations) and 0.57×10^6 (s.e.m. $\pm 0.009 \times 10^6$; four determinations). *E. coli* RNA and *C. burnetii* RNA preparations were subjected to electrophoresis together and showed a nearly coincidental pattern of migration, confirming the calculated molecular weights of *C. burnetii* rRNA.

The 23S- and 16S-like RNA species observed for *C. burnetii* indicate the presence of ribosomes in the obligate intracellular rickettsial agent *C. burnetii*, and are consistent with observations of host-cell-independent synthesis of protein (Mallavia & Paretsky, 1967) and electron photomicrographs of *C. burnetii* (Burton *et al.* 1971). The mechanisms by which rickettsiae obtain their protein have long been uncertain. The present report suggests that rickettsial synthesis of protein may be comparable with that found in other prokaryotic living forms.

This work was supported by National Science Foundation research grant GB-3704 and U.S. Public Health Service predoctoral training grant GM-703 from the National Institute of General Medical Sciences.

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