Supporting Information

Omsland et al. 10.1073/pnas.1212831109

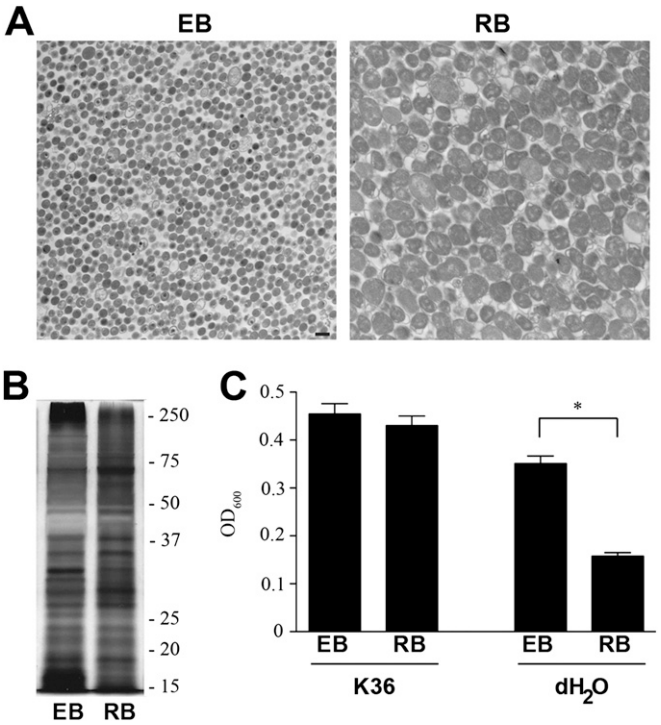

Fig. S1. Characterization of enriched *C. trachomatis* EB and RB populations. EBs and RBs purified by density gradient centrifugation were characterized to facilitate cell form-specific analyses. (*A*) Ultrastructural analysis of EBs (*Left*) and RBs (*Right*) by TEM show prototypical small cells (~0.3 μm in diameter) with condensed chromatin or large cells (~1 μm in diameter) with relaxed chromatin, respectively. A representative image is shown. (Scale bar, 500 nm.) (*B*) SDS/PAGE and silver staining of EB and RB whole-cell lysates show cell form-specific protein expression patterns. Molecular weight markers are in kilodaltons. (*C*) Exposure of EBs and RBs to hypoosmotic stress reveal tolerance by EBs, but not RBs, as measured by optical density. Data are expressed as the mean (n = 6) ± SEM.

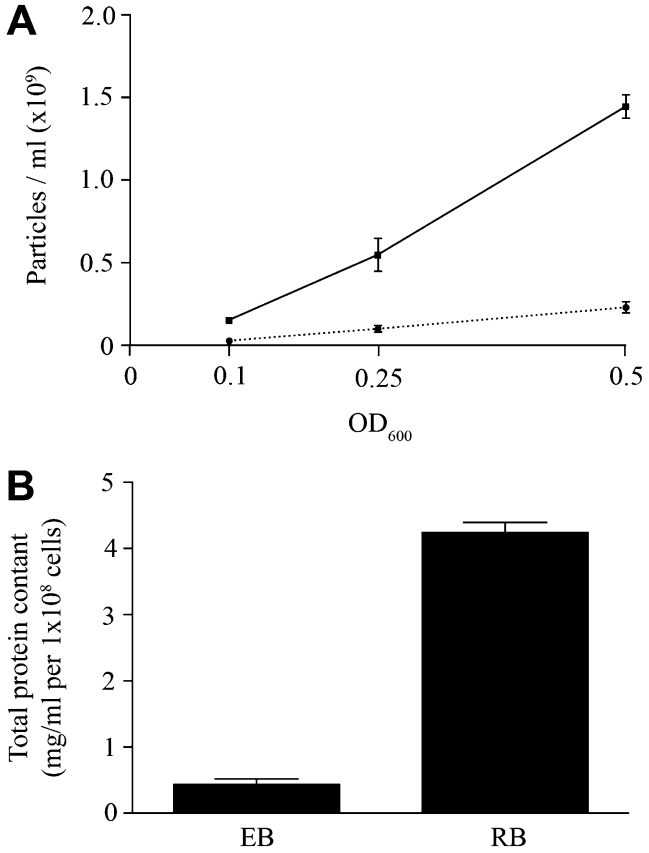

Fig. S2. Normalization of EB and RB populations. EBs and RBs were normalized according to direct particle count and total protein content. (*A*) Comparison of EB and RB particle densities was performed by measuring the direct particle counts of fixed bacterial suspensions adjusted to OD_{600} of 0.1, 0.25, and 0.5. The EB (solid line) direct particle count was approximately fivefold higher than the corresponding value for RBs (hatched line). (*B*) EB and RB total protein content was compared by measuring protein levels in whole-cell lysates from cells in 100-μL bacterial suspensions normalized to $OD_{600} = 0.5$ (i.e., EB = 1.4×10^8; RB = 2.2×10^7). The cellular protein density of EBs was ~10-fold lower than that observed for RBs. Data are expressed as the mean ($n = 4$) ± SEM.

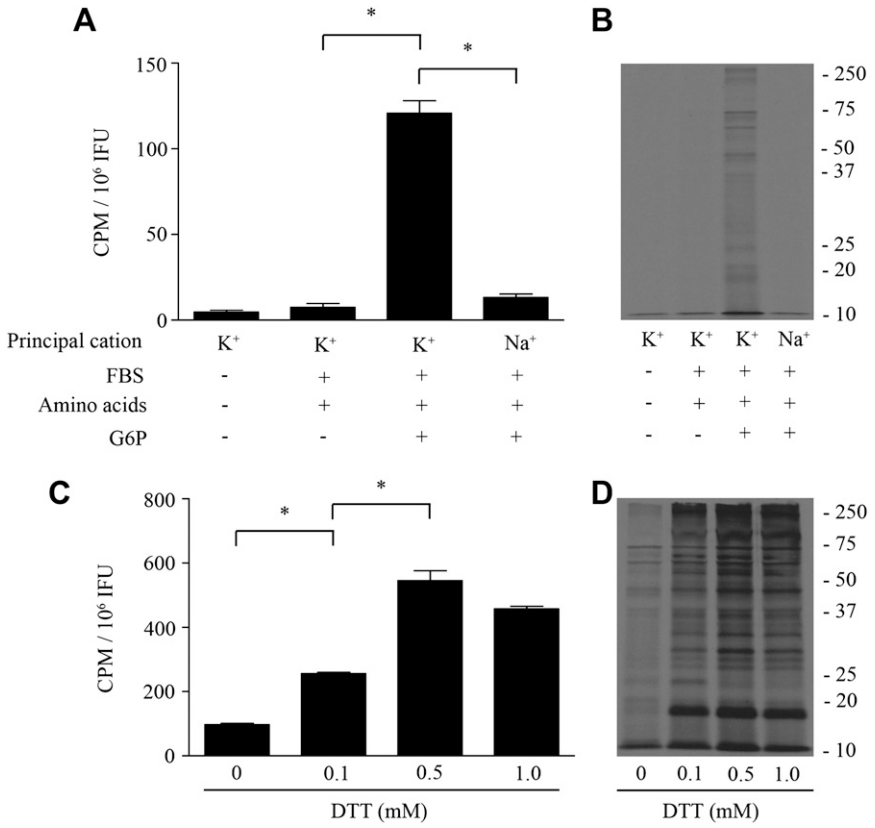

Fig. S3. Development of a permissive nutrient medium. Metabolic activity by enriched EBs under different nutritional conditions was assessed by radiolabel incorporation via scintillation counting of bacterial lysates and autoradiography after a 6-h incubation. (*A* and *B*) Incubation of EBs in buffer alone, buffer supplemented with nutrients (FBS and amino acids), or buffer containing nutrients and G6P show a stepwise increase in radiolabel incorporation. Radiolabel incorporation was dependent on potassium. (*C* and *D*) Supplementation of medium containing nutrients with DTT resulted in a dose-dependent increase in radiolabel incorporation. Data are expressed as the mean ($n = 6$) ± SEM. The asterisk indicates statistical significance ($P < 0.05$). Background signal measured from incubation with heat-inactivated organisms was subtracted from all samples. Autoradiographs from representative experiments are shown. Molecular weight markers are in kilodaltons.

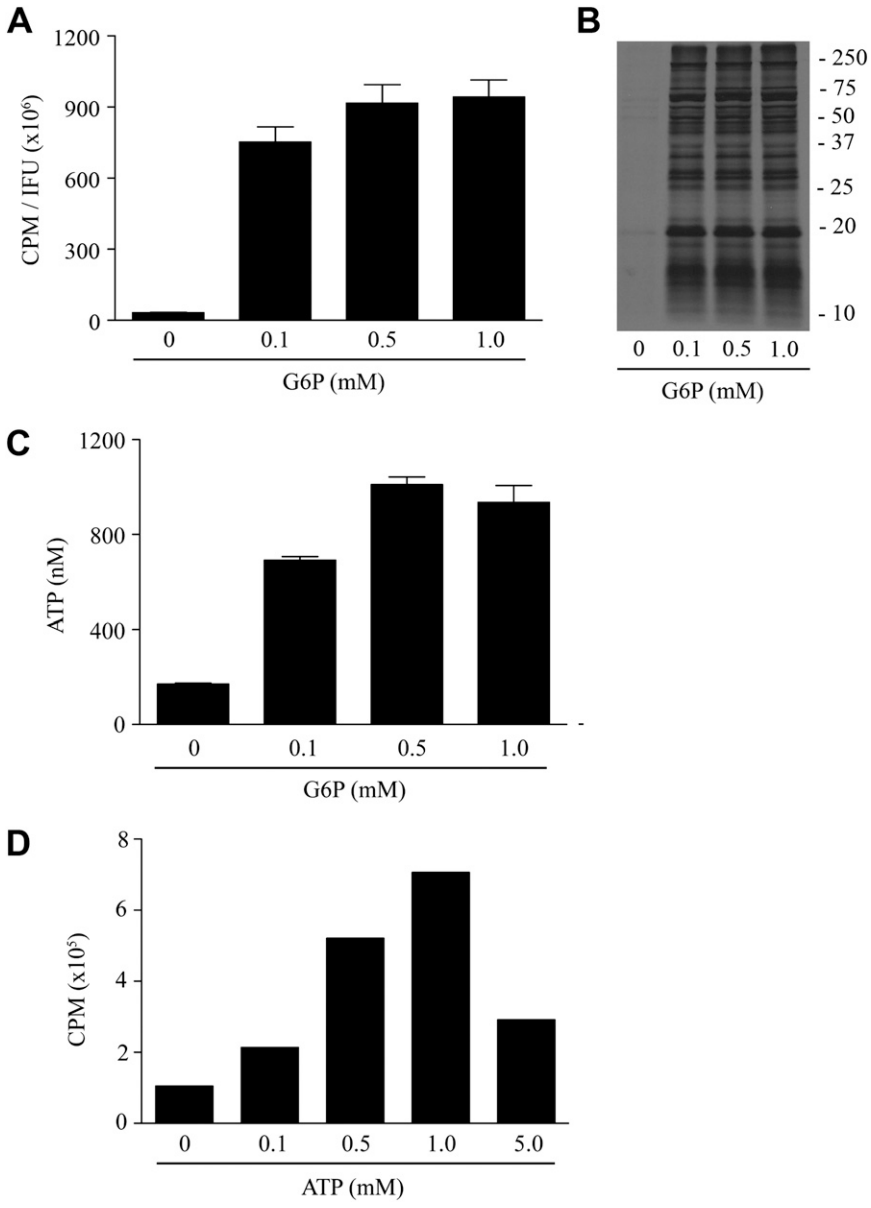

Fig. S4. EB utilization of G6P for ATP production. Utilization of G6P by EBs was characterized by incubating enriched EBs in medium containing a concentration gradient of G6P. (*A* and *B*) Scintillation counting and autoradiography show dose-dependent incorporation of [^{35}S]Cys-Met into bacterial proteins. (*C*) Measurement of EB ATP pool following incubation in medium containing different concentrations of G6P shows dose-dependent increase in the bacterial ATP pool with increased G6P availability. Data are expressed as the mean ($n = 6$) ± SEM. The asterisk indicates statistical significance ($P < 0.05$). (*D*) Dose-dependent incorporation of [^{35}S]Cys-Met in RBs in response to ATP. Data from a representative experiment are shown. Background signal measured from incubation with heat-inactivated organisms was subtracted from all samples. An autoradiograph from a representative experiment is shown. Molecular weight markers are in kilodaltons.

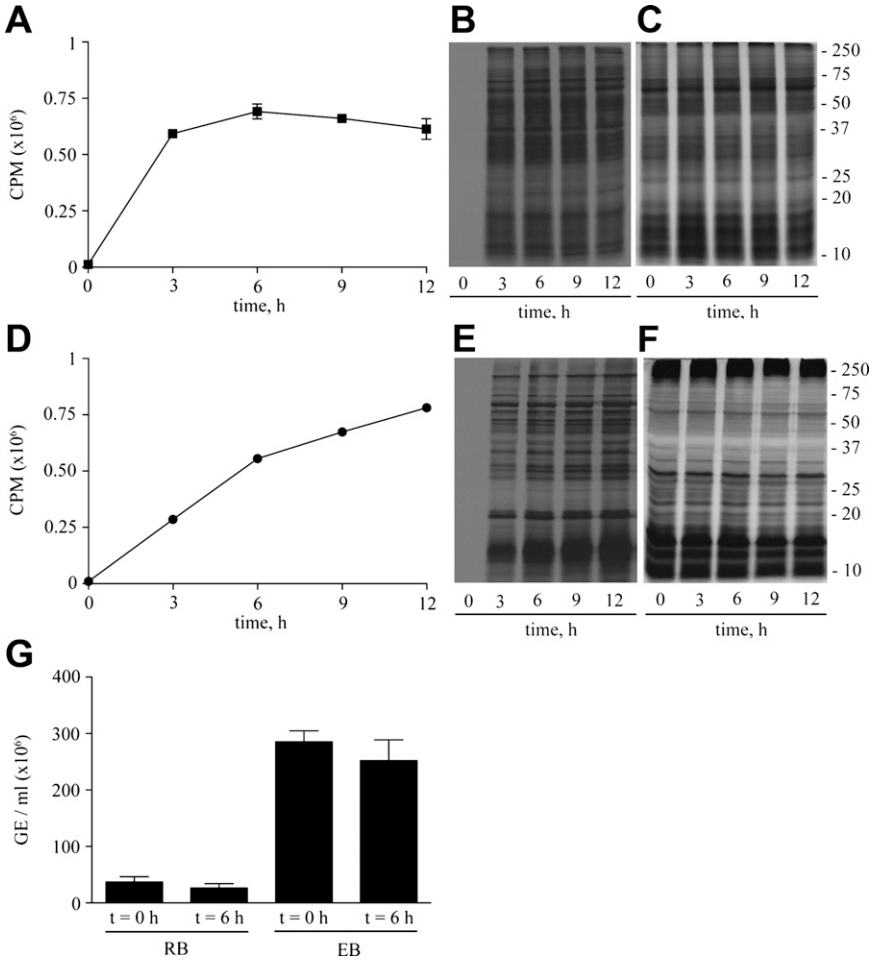

Fig. S5. [^{35}S]Cys-Met incorporation into EBs or RBs over time. Purified EBs or RBs were incubated in CIP-1 supplemented with [^{35}S]Cys-Met in a 2.5% oxygen environment, and incorporation was measured every 3 h for 12 h. (*A*) Scintillation counting and (*B*) autoradiography showed increases in radiolabel incorporation in RBs for 6 h. Data are expressed as the mean (n = 2) ± SEM. (*C*) Silver staining confirms equivalent loading of lanes. An autoradiograph from a representative experiment is shown. Molecular weight markers are in kilodaltons. (*D*) Scintillation counting and (*E*) autoradiography showed increases in radiolabel incorporation in EBs for at least 12 h. Data are expressed as the mean (n = 3) ± SEM. (*F*) Silver staining confirms equivalent loading of lanes. In a parallel experiment, (*G*) genome equivalents (GE) measured by TaqMan quantitative PCR using a primer and probe set to the chlamydial Hc1 gene. A standard curve for chlamydial GE was prepared using a characterized stock of density gradient purified *C. trachomatis*. No increase in number of genomes was detected over the 6-h duration of the experiment. Data are expressed as the mean (n = 3) ± SEM.

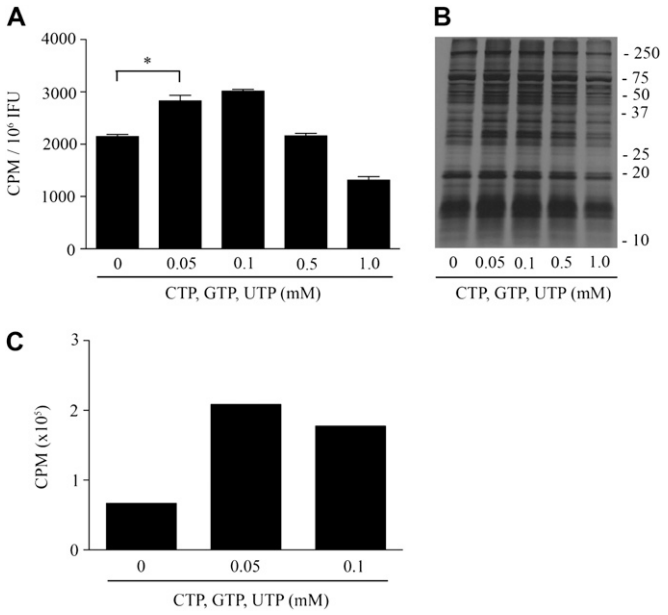

Fig. S6. Effect of ribonucleotide availability on bacterial axenic protein synthesis. The ribonucleotides CTP, GTP, and UTP were added to IPB also supplemented with DTT, G6P, ATP, FBS, and amino acids, and EB protein synthesis was measured by incorporation of [^{35}S]Cys-Met following 6 h of incubation. (*A* and *B*) Scintillation counting and autoradiography show enhanced protein synthesis in the presence of ribonucleotides. Data are expressed as the mean (n = 4) ± SEM. The asterisk indicates statistical significance ($P < 0.05$). (*C*) Dose-dependent incorporation of [^{35}S]Cys-Met in RBs in response to CTP, GTP, and UTP. Data from a representative experiment are shown. Background signal measured from incubation with heat-inactivated organisms was subtracted from all samples. An autoradiograph from a representative experiment is shown. Molecular weight markers are in kilodaltons.

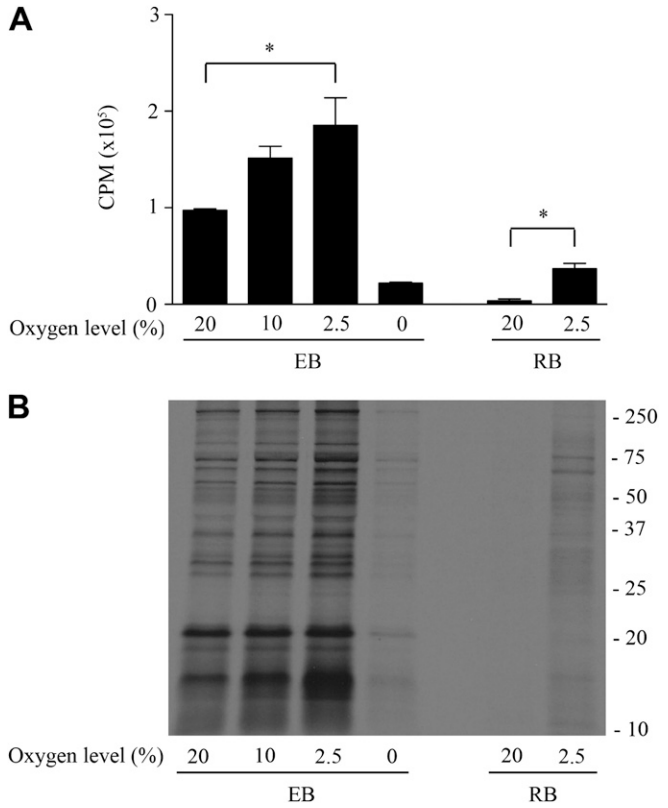

Fig. S7. Effect of reduced oxygen tension on EB and RB metabolic activity. Maintenance of bacterial metabolic activity was determined by preincubating EBs and RBs in CIP-1 under defined oxygen conditions for 24 h followed by a 6-h incubation in medium containing [^{35}S]Cys-Met. (*A* and *B*) Scintillation counting and autoradiography show enhanced radiolabel incorporation by bacteria preincubated under microaerobic conditions. Data are expressed as the mean (n = 4) ± SEM. The asterisk indicates statistical significance ($P < 0.05$). Background signal measured from incubation with heat-inactivated organisms was subtracted from all samples. An autoradiograph from a representative experiment is shown. Molecular weight markers are in kilodaltons.

Other Supporting Information Files

Table S1 (DOCX)
Table S2 (DOCX)