Supporting Information

Quantitative proteomics reveals metabolic and pathogenic properties of *Chlamydia trachomatis* developmental forms.

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Figure S1

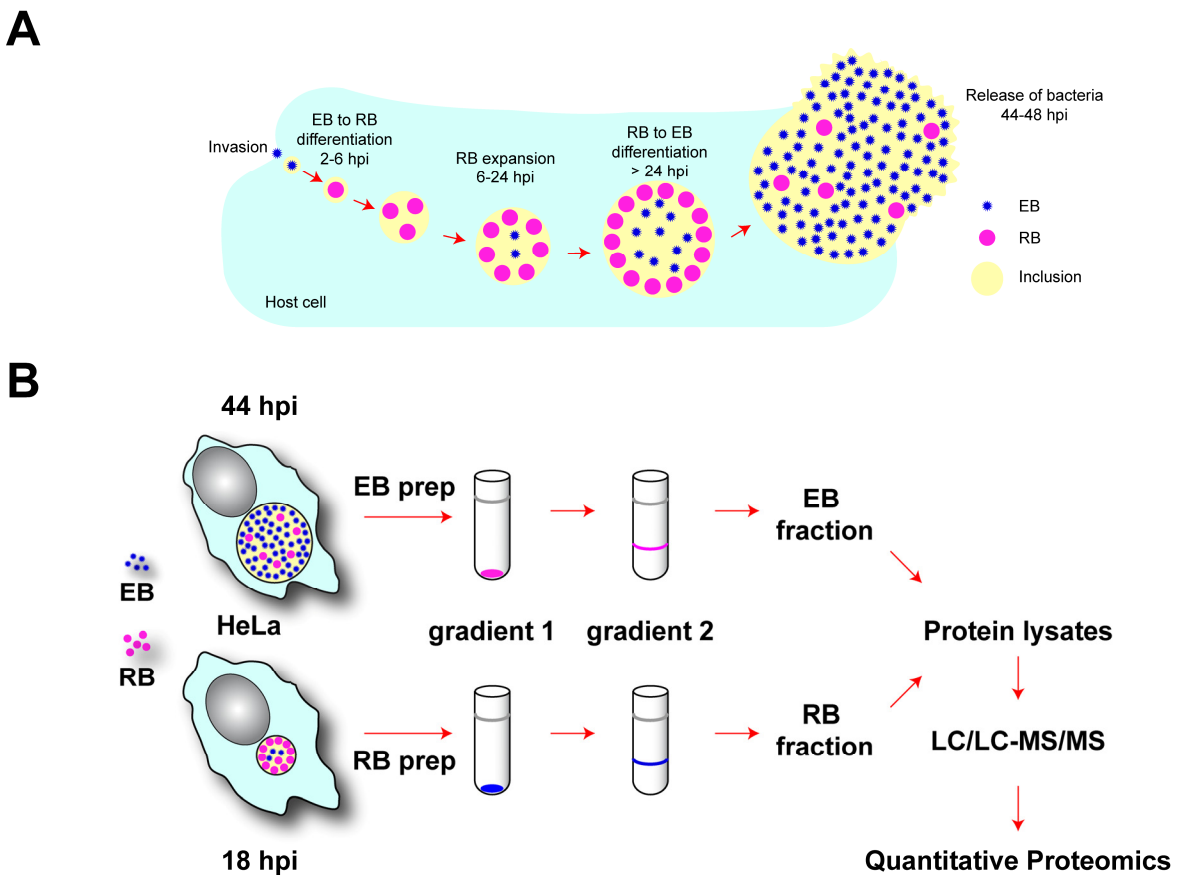

A
Invasion
EB to RB differentiation 2-6 hpi
RB expansion 6-24 hpi
RB to EB differentiation > 24 hpi
Release of bacteria 44-48 hpi
Host cell
EB
RB
Inclusion
B
44 hpi
EB
RB
HeLa
18 hpi
EB prep
RB prep
gradient 1
gradient 2
EB fraction
RB fraction
Protein lysates
LC/LC-MS/MS
Quantitative Proteomics
C
DIC
Bodipy TR
MOMP
EB
RB
D
M
EB
RB
kDa:
200
150
100
75
50
37
25
20
15

Figure S2

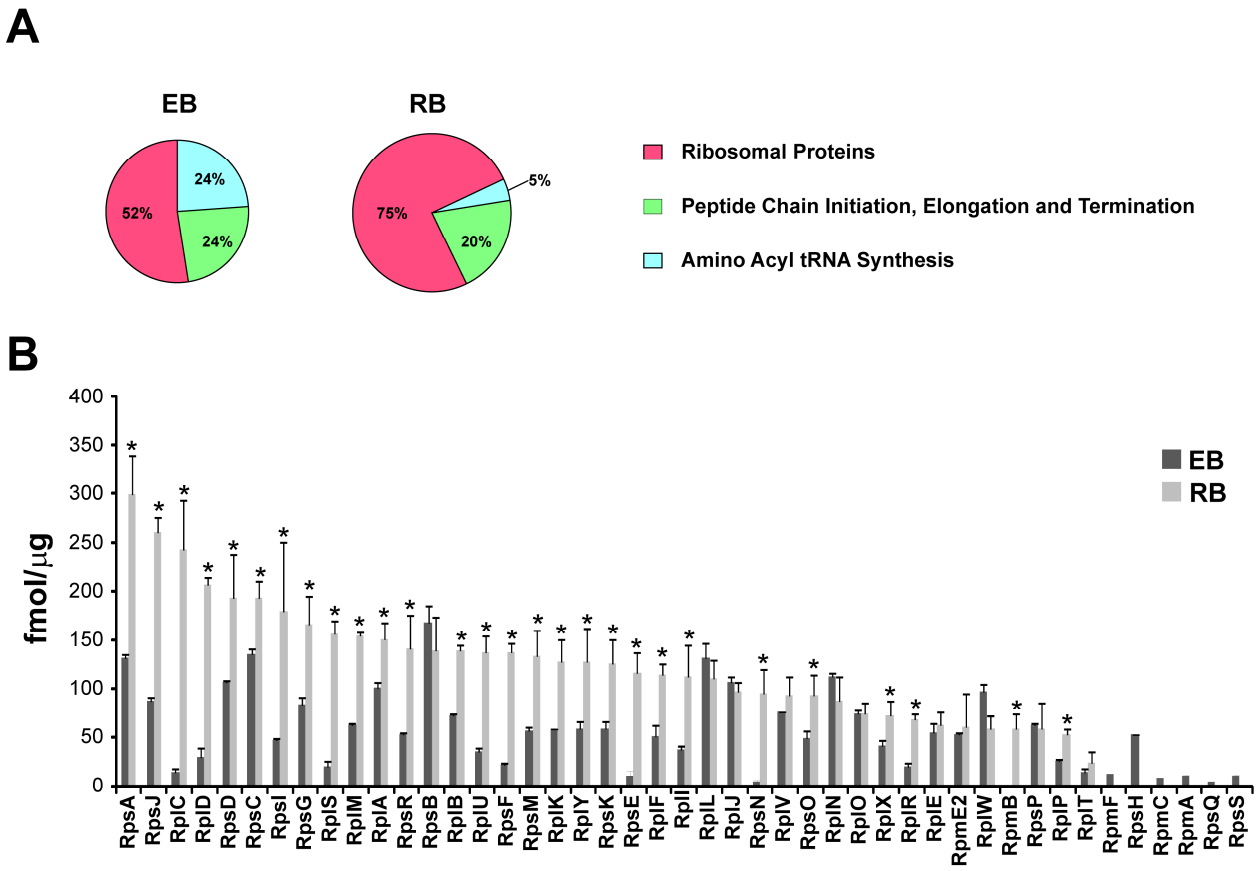
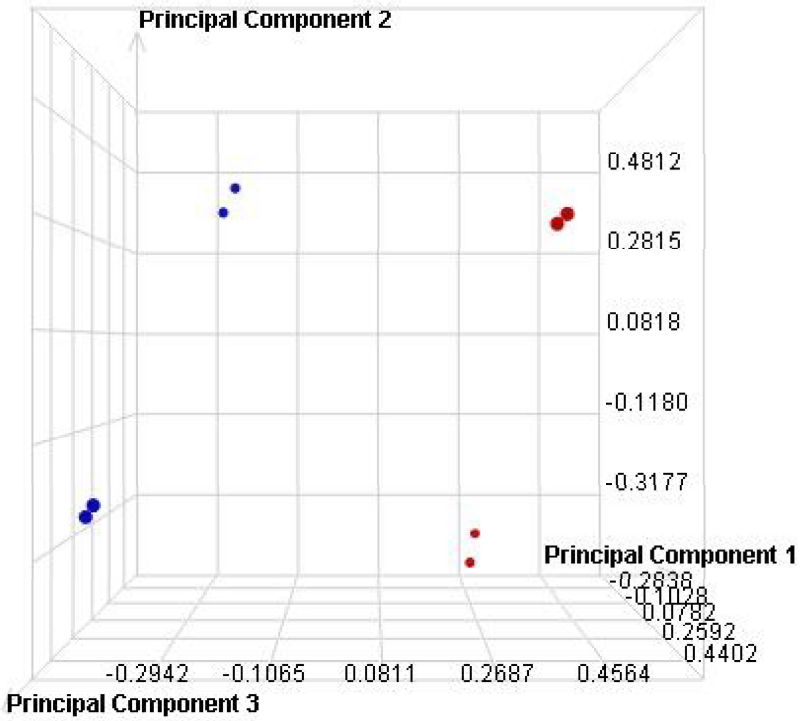

A
EB
RB
24%
52%
24%
75%
20%
5%
Ribosomal Proteins
Peptide Chain Initiation, Elongation and Termination
Amino Acyl tRNA Synthesis
B
fmol/µg
EB
RB
0
50
100
150
200
250
300
350
400
RpsA
RpsJ
RplC
RplD
RpsD
RpsC
RpsI
RpsG
RplS
RplM
RplA
RpsR
RpsB
RplB
RplU
RpsF
RpsM
RplK
RplY
RpsK
RpsE
RplF
RplI
RplL
RplJ
RpsN
RplV
RpsO
RplN
RplO
RplX
RplR
RplE
RpmE2
RplW
RpmB
RpsP
RplP
RplT
RpmF
RpsH
RpmC
RpmA
RpsQ
RpsS

Figure S3


Principal Component 2
0.4812
0.2815
0.0818
-0.1180
-0.3177
Principal Component 1
-0.2838
-0.1028
0.0782
0.2592
0.4402
-0.2942
-0.1065
0.0811
0.2687
0.4564
Principal Component 3

Supporting figures legends

Figure S1. Quantitative proteomics of *C. trachomatis* developmental forms.

(A) Graphic representation of the developmental cycle of *C. trachomatis* serovar LGV-L2. Upon invasion of epithelial host cells, infectious EBs differentiate to metabolically active RBs, which replicate by binary fission within the pathogenic vacuole (inclusion). Approximately 24 hours post-infection (hpi), RBs have already begun to differentiate back into EBs in an asynchronous process. Release of infectious EB progeny occurs at 44-48 hpi. **(B)** Flowchart for the preparation of *C. trachomatis* protein samples used for label-free quantitative proteomic analysis. To enhance purity, *C. trachomatis* developmental forms were purified from infected HeLa cells by two sequential rounds of density gradient centrifugation. Trypsin-digested protein lysates from EBs and RBs were subjected to reverse phase two-dimensional liquid chromatography followed by LC/LC-MS/MS. **(C)** Mount preparations of EBs and RBs used for proteomics analysis were stained with Bodipy TR C_5 Ceramide (Bodipy TR) and imaged by fluorescence microscopy and differential interference contrast microscopy (DIC) (*left panel*) or immunostained for the *C. trachomatis*-specific major outer membrane protein (MOMP) (*right panel*). Note morphological differences among the highly enriched EB and RB preparations. Scale bars represent 1 µm. **(D)** Band patterns correspond to EB and RB protein lysates used for proteomics analysis. Proteins were separated in SDS-PAGE 4-15% gradient gels and stained with Sypro Orange.

Figure S2. Increased levels of ribosomal proteins in RBs. (A) Relative contribution of sub-categories within the “Translation” category is shown and expressed as percentage. **(B)** Expression levels for individual ribosomal proteins are represented and expressed as the mean (fmol/µg) from four independent MS-based determinations. Error bars indicate the standard deviation. Asterisks indicate statistically significant differences ($p<0.05$, Alternate Welch T-test not assuming equal standard deviation).

Figure S3. Principal Component Analysis (PCA) of biological and technical replicates. EB and RB quantitative data was analyzed by principal components analysis (PCA) for each independent sample measurement. The data was first summed to the protein expression level for each LC/LC-MS/MS run and z-score normalized, then 3 principal components were calculated for each of the 8 analyses (4 EB and 4 RB). The PCA plot indicates that EB (blue) and RB (red) samples separated along principal component 1, as expected. Additionally, the duplicate analyses of each sample showed excellent reproducibility, as indicated by the tight grouping of the replicates.

Supporting tables legends

(supporting tables are provided as separate excel files)

Table S1. Comprehensive list of proteins detected and/or quantified by proteomics analysis (LC/LC-MS/MS) of *C. trachomatis* L2 EBs and RBs. A total of 495 chlamydial proteins were detected. The primary locus encoding each protein is indicated according to the gene nomenclature for *C. trachomatis* L2 434/Bu strain (434/Bu column). The corresponding ortholog for *C. trachomatis* serovar D UW-3/CX strain is also indicated (UW-3/CX column). The abbreviated gene name, when available, was also included. GI identifiers (assigned by NCBI) and protein names are indicated. Functional groups were assigned as described in Experimental procedures. Quantification of proteins (fmol/µg) for each EB and RB replicate (2 biological replicates processed by MSE and DDA) as well as the mean ± standard deviation are shown.

Table S2. Evidence of proteolytic processing of Polymorphic membrane proteins (Pmp) by semitryptic peptides analysis. Semitryptic peptides -those for which only one end, either C-terminus or N-terminus, has been cleaved after lysine (K) or arginine (R)- matching Pmps in

C. trachomatis L2 EB and RB forms were identified and potential cleavage sites were mapped. GI identifiers (assigned by NCBI), primary locus based on ORF-assigned number for *C. trachomatis* L2 434/Bu and their corresponding orthologs in serovar D UW-3/CX strain are indicated. Cleavage sites corresponding to the signal peptide were obtained by using SignalP 3.0 (http://www.cbs.dtu.dk/services/SignalP/) trained for Gram negative bacterial species. Amino acids where tryptic cleavage occurred are indicated in bold. Amino acids previous and next to N-terminal and C-terminal extremes of the peptides are indicated, along with cleavage motifs.

Table S3. The *Chlamydia* outer membrane complex (COMC). All proteins enriched in the *C. trachomatis* L2 COMC as determined in a previous proteomics study by Liu *et al.* (Liu et al., 2010) are listed. Primary locus based on ORF-assigned number for *C. trachomatis* L2 434/Bu and their corresponding orthologs in serovar D UW-3/CX strain, gene abbreviated names (when available), GI identifiers (assigned by NCBI), functional groups and protein names are indicated. Quantitative values in EB and RB samples are specified (fmol/µg, mean $\pm$ standard deviation, extracted from Table S1).