Transcriptomic analysis of plasmid and plasmid-related chromosomal ORFs in *C. trachomatis* strains with difference cell-appetence

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Introduction

Despite the undergoing chromosomal size-reduction of *Chlamydia trachomatis*, almost all strains maintain the conserved 7.5kb plasmid. It has been recently considered a virulence factor, as plasmid-bearing strains evidenced a higher ability to successfully colonize epithelial cells and sustain infection than plasmidless strains. More, 22 chromosomal genes were predicted to be regulated by the plasmid [1]. However, the biological role of the eight plasmid genes as well as their impact on chromosomal genes remain poorly characterized.

Objectives

• To evaluate if *C. trachomatis* regulates the number of plasmids according to the developmental stage.

• To evaluate the relative expression of both the eight plasmid ORFs and a pool of 25 chromosomal genes (22 that seem to be regulated by the plasmid [1] and three with similar function or with homology to the plasmid partitioning proteins).

• To correlate the expression of each plasmid ORFs with the number of plasmids *per* genome as well as with the expression of the chromosomal genes.

• To compare the expression data of clinical isolates with the prototype strains of the same serovar.

Methodology

• HeLa 229 cells monolayers were independently inoculated with the *C. trachomatis* prototype strains C/TW3, E/Bour and L2/434, and a current circulating strain CS19/08 (L2b genotype).

• The infectious cycle was interrupted at 4, 12, 20, 30 and 42 h post-infection (pi) for DNA and RNA extraction protocols (see ref. [2] and poster B8).

• The DNA from each time-point was used for the quantification of both the plasmid and genome copy number [2] through absolute real-time quantitative PCR (qPCR).

• The generated cDNA from each time-point was used in the relative quantification assay of transcripts of all eight plasmid genes and the plasmid-related chromosomal genes (Table 1). *16SrRNA* was used as endogenous control gene as it was previously validated (see ref. [2] and poster B8).

(1) Results

Number of plasmids *per* genome

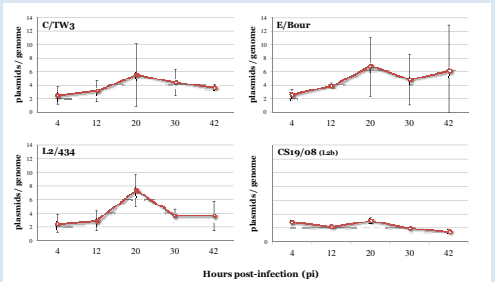

Fig. 1. Number of plasmids *per* genome during the infectious cycle for the strains C/TW3, E/Bour, L2/434 and clinical isolate CS19/08 (L2b).

• For all prototype strains, the lower and the higher number of plasmids *per* genome were detected at 4 h (pi) and 20 h (pi), respectively, ranging from **2.48** (SD ±1.28) to **5.53** (SD ±4.65) for C/TW3, **2.6** (SD ±0.79) to **6.73** (SD ± 4.36) for E/Bour and **2.53** (SD ±1.35) to **7.4** (SD ±2.34) for L2/434.

• The clinical isolate (L2b) showed a lower number of plasmids *per* genome than the LGV prototype strain with a mean value of **2.3** (SD ±0.66). The plasmids/genomes ratio ranged from **1.39** (SD ±0.35) at 42 h (pi) to **3.03** (SD ±0.44) at 20 h (pi).

References

1. Carlson JH, Whitmire WM, Crane DD, Wicke L, Virtaneva K, Sturdevant DE, Kupko JJ 3rd, Porcella SF, Martinez-Orengo N, Heinzen RA, Kari L, Caldwell HD. 2008. The *Chlamydia trachomatis* plasmid is a transcriptional regulator of chromosomal genes and a virulence factor. Infect Immun. 76, 2273-83.
2. Borges V, Ferreira R, Nunes A, Nogueira P, Borrego MJ, Gomes JP. 2010. Normalization strategies for real-time expression data in *Chlamydia trachomatis*. J Microbiol Methods. 82, 256-264.
3. Thomas NS, Lusher M, Storey CC, Clarke IN. 1997. Plasmid diversity in Chlamydia. Microbiology. 143, 1847-1854.
4. Ricci S, Cevenini R, Cocco E, Comanducci M, Ratti G, Scarlato V. 1993. Transcriptional analysis of the *Chlamydia trachomatis* plasmid pCT identifies temporally regulated transcripts, anti-sense RNA and σ^{70}-selected promoters. Mol Gen Genet. 237, 318-326.
5. Albrecht M, Sharma CM, Reinhardt R, Vogel J, Rudel T. 2010. Deep sequencing-based discovery of the Chlamydia trachomatis transcriptome. Nucleic Acids Res. 38, 868-877.

(2) Results

Fig. 2. Schematic representation of the *C. trachomatis* plasmid. ORFs designation is based on Thomas *et al*, *1997* [3].

Plasmid genes expression overview

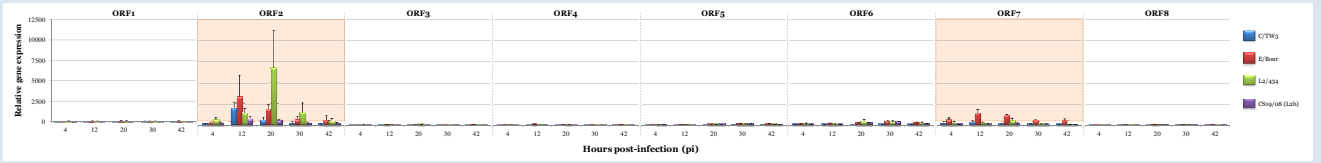

Fig. 3. Comparison of the gene expression levels of the eight plasmid genes during the infectious cycle of the *C. trachomatis*. All the expression values were obtained through the normalization of the RT-qPCR raw data against the copy number of *16SrRNA* transcripts. The expression values are based on three independent experiments for the prototype strains whereas for the clinical isolate only a single assay is represented.

• The plasmid gene encoding the **major replication protein** (ORF2) was the **most expressed** (Fig. 3) throughout the infectious cycle (especially in the replicative stage). For instance, its expression level for L2/434 was 10 to 84-fold higher than that detected for the other plasmid genes. More, its expression level increased about 9 times from 4 to 20 h (pi).

• The ORF 7 (coding for a **partioning protein**) was generally the **second most expressed** gene (Fig. 3).

Expression of the plasmid genes with unknown function

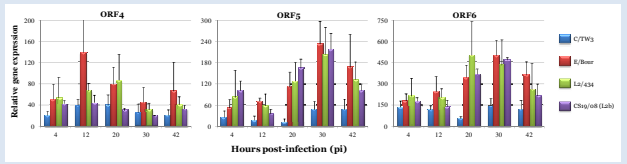

Fig. 4. Gene expression of the three plasmid genes with no predicted function throughout the developmental cycle of the *C. trachomatis*. All the expression values were obtained through the normalization of the RT-qPCR raw data against the copy number of *16SrRNA* transcripts. The expression values are based on three independent experiments for the prototype strains whereas for the clinical isolate only a single assay is represented.

• **No major differences** were observed in the **expression patterns of the plasmid genes** between strains with different cell-appetence. The overall expression levels of **E/Bour** and **L2/434** were about **two-fold higher** than those of C/TW3 (Fig. 4).

• The clinical isolate **CS19/08 (L2b)** and the prototype strain **L2/434** showed **similar expression patterns and levels** (Fig. 4).

Plasmid and plasmid-related chromosomal genes

Table 1. Plasmid and plasmid-related chromosomal genes under evaluation.

ORF	Gene	Product Description	Expression in this study	Putative plasmid regulator
Plasmid				
ORF1	Hyp	Replication protein (pGP7-D)	Mid	-
ORF2	Hyp	Major replication protein (pGP6-D)	Mid	-
ORF3	*draB_2*	Replicative DNA helicase	Early/Mid	-
ORF4	Hyp	Hypothetical protein (pGP3-D)	Early/Mid	-
ORF5	Hyp	Hypothetical protein (pGP3-D)	Mid/Late	-
ORF6	Hyp	Hypothetical protein (pGP4-D)	Mid/Late	-
ORF7	Hyp	Partitioning protein (pGP5-D)	Early/Mid	-
ORF8	Hyp	Partitioning protein (pGP8-D)	Mid/Late	-
Chromosome				
CT017	Hyp	-	ND	
CT063	*gnd*	6-phosphogluconate dehydrogenase	Mid	
CT121	Hyp	-	Mid/Late	ORF5/ORF6
CT180	*tauB*	Nitrate/iron ABC transporter ATPase	Early/Mid	ORF4
CT248	*glgP*	Glycogen phosphorylase	Mid/Late	ORF5/ORF6
CT304	*sodM*	Superoxide dismutase	Early/Mid	ORF4
CT330	Hyp	-	Early/Mid	ORF4
CT335	Hyp	-	Under evaluation	
CT361	*dapA*	Dihydrodipicolinate synthase	Mid	
CT364	*dapB*	Dihydrodipicolinate reductase	Mid	
CT378	*pgi*	Glucose-6-phosphate isomerase	Mid	
CT382.1	Hyp	-	ND	
CT500	*ndk*	Nucleoside diphosphate kinase	Early/Mid	ORF4
CT533	*lpxC*	UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase	Mid	
CT565	Hyp	-	Under evaluation	
CT582*	*minD*	Chromosome partitioning ATPase-CHLTR plasmid protein homolog GP3D	Under evaluation	
CT583*	*gpt6D*	Virulence plasmid protein pGP6-D-related protein	Under evaluation	
CT688*	*parB*	Chromosome partitioning protein	Under evaluation	
CT702	Hyp	-	-	
CT775	Hyp	1-acyl-sn-glycerol-3-phosphate acyltransferase	Under evaluation	
CT798	*glgA*	Glycogen synthase	Under evaluation	
CT815	*glmM*	Phosphoglucosamine mutase	-	
CT849.1	Hyp	-	-	
CT858	*cpn*	Chlamydia protease/proteosome-like activity factor	Under evaluation	
CT866	*glgB*	Glycogen branching enzyme	Under evaluation	

Hyp – hypothetical protein; ND – not defined; a – chromosomal genes with similar function/homology to plasmid partitioning proteins. The expression data of the plasmid genes is based on three independent experiments whereas for the chromosomal genes only the data of one preliminary assay is shown.

• The **chromosomal genes** were up to **10-fold more expressed** than the **plasmid genes** (except for ORF2) (data not shown).

• The expression patterns of chromosomal genes did not seem to vary between strains with distinct cell-appetence.

Conclusions/Discussion

• Small fluctuations in the number of plasmids *per* genome were observed throughout the development cycle of *C. trachomatis*, however the highest ratios were observed in the replicative phase (20 h pi) for all strains. Also, for the three ORFs not associated with plasmid replication and partitioning, the higher expression levels were obtained at time points other than 20 h (pi). Thus, a higher number of plasmids seems to be strictly associated with their transmission to the daughter-cells.

• Comparing to other bacteria the number of plasmids *per* genome in *C. trachomatis* is unusually low. However, *C. trachomatis* seems to be fully dedicated to plasmid maintenance. In fact, ORF2 (major replication protein) showed to be the most expressed plasmid gene (up to 84-fold than the other plasmid genes for L2/434) and it is apparently object of tight regulation by several antisense small RNAs [4, 5].

• Considering that it was previously shown that the presence/absence of the plasmid directly affects the transcription of a specific pool of 22 chromosomal genes [1], and that five of the eight plasmid genes are implicated in both plasmid replication and partitioning, we speculate that the three other plasmid ORFs (ORF4, ORF5 and ORF6) encoding proteins with unknown function are likely the ones that regulate the chromosomal set. Assuming that the transcriptional regulator and its target are expressed at approximately the same developmental stage we speculate that: *i*) ORF4 (early/mid expressed gene) may potentially regulate the expression of the genes *tauB*, *sodM*, CT330 and *ndk;* and *ii*) ORF5 and ORF6 (mid/late) may be putative transcriptional regulators of the genes CT142 and *glgP*. Further, experiments will be needed to confirm this hypothesis.

Acknowledgements

This work was supported by a grant from Fundação para a Ciência e a Tecnologia (FCT) (PTDC/BIA-BCM/71117/2006) (to JPG) which funds the fellowship of RF. VB and AN are the recipients of a Ph.D. grant (SFRH/BD/68527/2010) and a Post Ph.D. grant (SFRH/BPD/75295/2010), respectively, from FCT.