Minireview

Obligate intracellular parasites: *Rickettsia prowazekii* and *Chlamydia trachomatis*

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Received 14 April 1999

Abstract Transitions to obligate intracellular parasitism have occurred at numerous times in the evolutionary past. The genome sequences of two obligate intracellular parasites, *Rickettsia prowazekii* and *Chlamydia trachomatis*, were published last year. A comparative analysis of these two genomes has revealed examples of reductive convergent evolution, such as a massive loss of genes involved in biosynthetic functions. In addition, both genomes were found to encode transport systems for ATP and ADP, not otherwise found in bacteria. Here, we discuss adaptations to intracellular habitats by comparing the information obtained from the recently published genome sequences of *R. prowazekii* and *C. trachomatis*.

Key words: Genome evolution; Intracellular parasite; Metabolism; *Rickettsia*; *Chlamydia*

1. Introduction

The inside of a living cell is on the one hand a species-poor, extreme environment, but on the other hand a largely unexploited resource of food. Time after time, distantly related organisms have learnt how to avoid intracellular destruction and multiply in intracellular habitats. These organisms are often difficult to study: they are pathogenic to humans, conditions for cell-free growth have not been developed, host-free purified parasites show limited metabolic capacity and gene transfer systems are normally not available. Nevertheless, the metabolic relationships of obligate intracellular parasites and their hosts are now being elucidated. The recently published genome sequences of the obligate intracellular parasites *Rickettsia prowazekii* and *Chlamydia trachomatis* [1,2] represent an important source of information for future experimental work.

Despite their similarities in life styles, there is no phylogenetic relationship between *Rickettsia* and *Chlamydia*. The genus *Rickettsia* belongs to the α-Proteobacteria [3,4] and consists of two groups: the typhus group (TG) and the spotted fever group (SFG). A majority of the *Rickettsia* species are human pathogens [5], which are spread from one host to another by arthropod vectors. For example, *R. prowazekii* is the causative agent of louse-borne typhus, which has affected millions of people during periods of hunger, flood and wars [6,7]. *Rickettsia* normally multiply directly in the host cell cytoplasm, but some species of the SFG *Rickettsia* are also capable of dividing in the cell nucleus [8].

The genus *Chlamydia* belongs to the order Chlamydiales [9–11] and consists of four species, *C. trachomatis*, *Chlamydia psittaci, Chlamydia pneuomoniae* and *Chlamydia pecorum*. These bacteria are responsible for a variety of diseases in non-human mammals and birds, but are also frequently transmitted to man [12,13]. For example, *C. trachomatis* is the agent of trachoma, a major cause of blindness in Asia and Africa. *Chlamydia* have a unique developmental cycle with two morphologically distinct forms: the elementary body (EB) and the reticulate body (RB). The EB form carries the infection from one host to another, where it reorganizes into the RB form which multiplies by binary fission [14]. The reticulate bodies finally reorganize into a new generation of elementary bodies prior to infecting a new series of host cells, thereby completing the developmental cycle.

Moulder [15] has defined intracellular parasitism as the solution to a set of common problems: "(i) how to get inside the host cell; (ii) how, once inside, to avoid being killed; (iii) how to multiply intracellularly; (iv) how to maintain host functions essential for parasite multiplication; (v) how to get new generations of parasites out of the host cell in which they were made; and, (vi) how to get from old host cells to new ones". Several previous reviews about intracellular parasites have discussed processes such as attachment, internalization, virulence, avoidance of degradation and transmission [5,6,14–16]. The purpose of this review is to discuss general strategies for growth in the intracellular habitat as inferred from a comparative analysis of the 1.1 and 1.0 Mb genomes of *R. prowazekii* and *C. trachomatis*.

2. Functional classification and metabolic profiles

The genomes of *R. prowazekii* and *C. trachomatis* contain a total of 834 and 894 genes, respectively [1,2]. Putative gene functions in *R. prowazekii* and *C. trachomatis* have been inferred on the basis of sequence similarity with genes in other organisms and the chemical reactions identified have been categorized into a set of functional groups which define the metabolic features of these bacteria [1,2]. Can we find evidence for convergent evolution in the metabolic profiles of *Rickettsia* and *Chlamydia*?

2.1. Schematic representation of the functional categories in *Rickettsia and Chlamydia*

The relative fractions of genes allocated to the different functional categories are very similar in *R. prowazekii* and

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PII: S0014-5793(99)00563-3

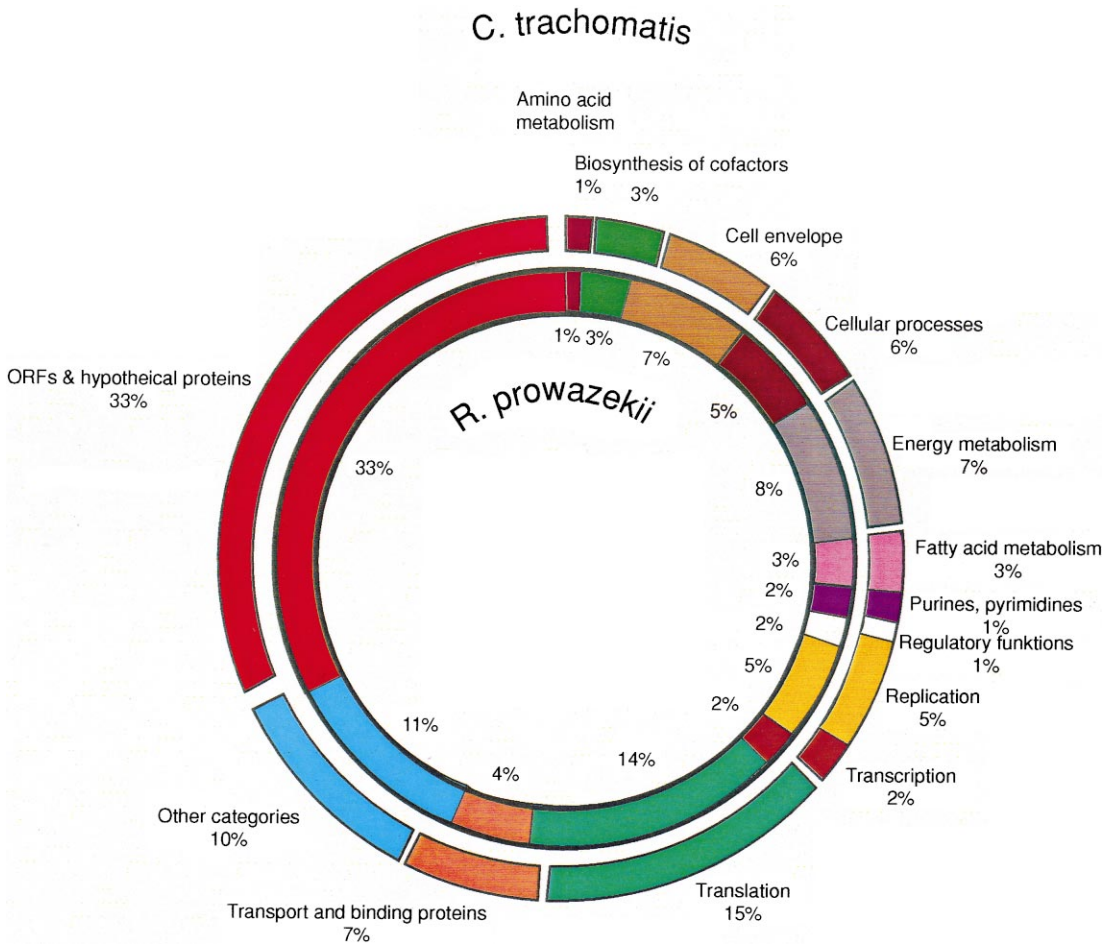

Fig. 1. Schematic representation of the relative fraction of genes in each of the functional categories in *Rickettsia prowazekii* and *Chlamydia trachomatis*.

C. trachomatis (Fig. 1). For most categories, the difference is no more than 1%, with the exception of transport functions which comprise a slightly higher ratio in *C. trachomatis* (7%) than in *R. prowazekii* (4%) (Fig. 1). However, basic information processes (replication, transcription, translation) are encoded by virtually identical gene sets, whereas genes involved in bioenergetic processes are mostly species-specific. To enable a more detailed comparative analysis of these metabolic features, we have identified a set of 325 pairs of genes with mutual best hits in *R. prowazekii* and *C. trachomatis*. The highest fraction of homologs, 31%, was identified in the translational category whereas only 1% of the identified pairs represent categories such as amino acid metabolism and regulatory functions (Fig. 2). Below, we will describe in more detail the identified homologs in a few selected categories which illustrate the gradual evolution of host cell dependence.

3. Metabolic parasitism: reductive convergent evolution

The biosynthesis of essential building blocks imposes a heavy burden on the cell. It is therefore not surprising that bacteria exposed to nutrient-rich environments have tended to dispense with redundant biosynthetic pathways [17]. What can be learnt from the genome sequences about the nature of such imported metabolites?

3.1. Host cell supply of amino acids

It was thought for a long time that intracellular parasites draw on the host cytoplasmic pool for supply of amino acids. Indeed, *R. prowazekii* and *C. trachomatis* have a strongly reduced repertoire of genes involved in amino acid biosynthesis. The gene *glyA*, which encodes serine hydroxymethyltransferase, is the sole amino acid biosynthetic gene present in both genomes. The involvement of the *glyA* gene product in tetrahydrofolate metabolism may explain its universal occurrence in all microbial genomes sequenced so far [1,2,18,19]. *R. prowazekii* and *C. trachomatis* also encode partial pathways leading to the biosynthesis of lysine. However, the gene encoding the final enzyme in this pathway, diaminopimelate decarboxylase, is missing in both genomes and the primary function of the encoded set of enzymes may be in the biosynthesis of diaminopimelate rather than in the biosynthesis of lysine [1,2]. A striking difference between the two genomes is the presence of a partial tryptophan operon in *C. trachomatis*, for which no homologs have been found in *R. prowazekii*. However, these genes are likely to have been acquired by horizontal transfer and may not represent the original gene set [2]. Thus, the biosynthetic genes for most amino acids appear to have been discarded, leading to a direct competition between the parasites and their hosts for the soluble amino acid pool.

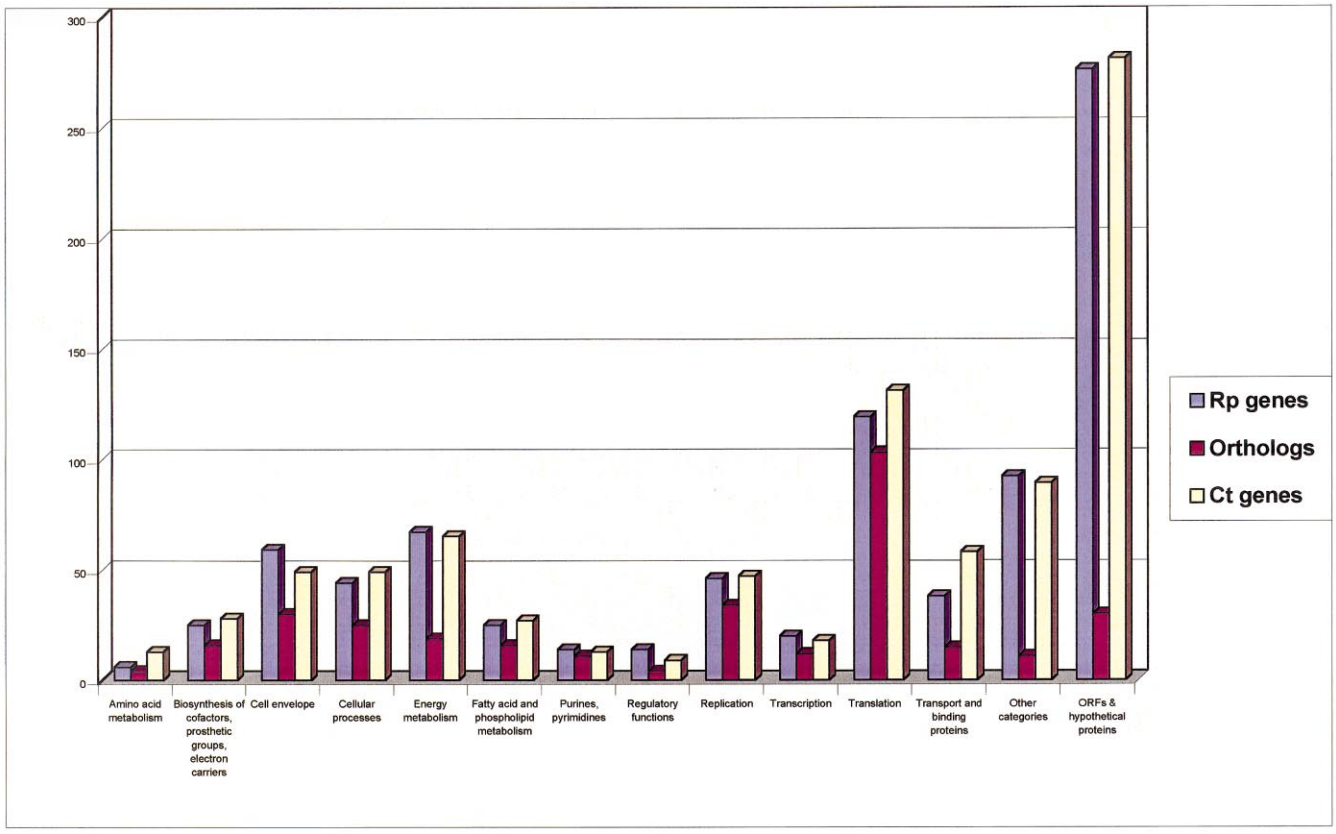

Fig. 2. Schematic representation of the total number of genes in *Rickettsia prowazekii* and *Chlamydia trachomatis* in relation to the number of homologous genes in each of the functional categories. We used the 834 *R. prowazekii* protein sequences to search for sequence similarities in the *C. trachomatis* databases using BLAST [33]. In total, 325 pairs of sequences with mutual best hits ($S > 90$) were identified.

3.2. Host cell supply of nucleoside monophosphates

Not more than about a dozen genes have been allocated to nucleotide metabolism in *R. prowazekii* and *C. trachomatis*, 11 of which are orthologs. It is particularly notable that there is a complete lack of genes encoding enzymes involved in de novo purine or pyrimidine biosynthesis in these two genomes [1,2], although such genes have been identified in all other microbial genomes sequenced to date. This suggests that nucleoside monophosphates may be taken up directly from the host cell cytoplasm. Indeed, several of the few genes identified are involved in the interconversion of nucleoside monophosphates to the essential nucleotides and deoxynucleotides. In addition, a complete thymidylate cycle which converts dCTP to TTP has been identified in *R. prowazekii*, but not in *C. trachomatis*. The missing enzyme in *C. trachomatis* is thymidylate synthase. *R. prowazekii* may have acquired this gene by horizontal transfer, since the highest sequence similarity is to the thymidylate synthase gene product in *Dictyostelium discoideum* [1,2].

4. Loss of biosynthetic gene functions is an ongoing process

Obligate intracellular parasites may in principle be able to dispense with all of the pathways required to make metabolites that are also available in the host cell cytoplasm. Are new metabolic compounds still being explored, or has the process of host cell adaptation and gene loss come to a halt in modern intracellular parasitic genomes?

4.1. Mutational degradation of the metK gene

Recent studies of the *metK* gene, which encodes an enzyme which catalyzes the biosynthesis of *S*-adenosylmethionine (Ado-Met), suggest that gene elimination is an ongoing process, at least in *Rickettsia* [20,21]. Ado-Met is an essential cofactor for a variety of biochemical reactions and the *metK* gene has been identified in all microbial genomes analyzed so far, with the only exception of *C. trachomatis*.

The *metK* gene was found to contain a termination in *R. prowazekii* [20], and degraded remnants of this gene were also identified in seven other *Rickettsia* species [21]. The original open reading frames could be reconstructed by eliminating termination codons and frameshift mutations, which ranged in size from 1 bp to 96 bp [21]. A detailed, comparative analysis of the degraded *metK* gene and another inactivated, nearby gene revealed that deletions strongly predominated over insertions in both genes [21]. This suggests that genetic material will gradually be lost in *Rickettsia* under neutral conditions, which provides an explanation for the small size and the high incidence of pseudogenes in the *R. prowazekii* genome [1]. However, a prerequisite for the inactivation of the *metK* genes in *Rickettsia* and *Chlamydia* must have been the development of appropriate transport systems for Ado-Met. We speculate that *Chlamydia* and *Rickettsia* have learnt how to import Ado-Met from the host cell cytoplasm independently of each other.

5. Energy parasitism: evolution of novel transport systems

The intracellular environment is problematic from the point of view that many metabolites and coenzymes, such as AdoMet, are available from the host cell cytoplasm in forms that require active transport systems. How has the transport machinery in *Rickettsia* and *Chlamydia* responded to the continuous exploitation of new cytosolic compounds?

5.1. The ATP/ADP translocase

The ATP/ADP translocase is a novel, very unusual type of transport system. Bacteria as well as eukaryotes are normally impermeable to nucleotides because they lack suitable transport systems. However, experimental work has shown that both *C. trachomatis* and *R. prowazekii* rely on the host cell for supply of ATP. Indeed, transport systems for ATP and ADP have been identified and experimentally characterized in both *C. trachomatis* and *R. prowazekii* [22,23]. The genome sequence data have confirmed the presence of ATP/ADP transport functions in these two organisms; two genes encoding ATP/ADP translocases have been identified in *C. trachomatis* and as many as five genes were found to encode ATP/ADP translocases in *R. prowazekii* [1,2].

ATP/ADP translocases have also been identified in mitochondria where ATP is exported in exchange for ADP [24,25], as well as in chloroplasts where ATP is imported in exchange for ADP [26]. The lack of a phylogenetic relationship between the mitochondrial and bacterial/chloroplast type of ATP/ADP translocases suggests that the ATP/ADP transport function has originated at least twice in the evolutionary history of bacteria and eukaryotes [27]. The presence of similar types of translocases in the distantly related parasites *Rickettsia* and *Chlamydia* (H. Amiri, R. Podowski, C.G. Kurland and S.G.E. Andersson, unpublished data) is most likely explained by horizontal gene transfer, probably through a eukaryotic intermediate.

6. Energy metabolism: reductive divergent evolution

Chlamydia is considered to be completely dependent on its host for supply of ATP and other energy-rich molecules, whereas *Rickettsia* utilizes cytosolic ATP only during an early phase of the infectious cycle [28]. What can be learnt from the genome sequences about key enzymes and cellular machineries for host-independent ATP production in *Rickettsia* and *Chlamydia*?

6.1. Glycolysis and the TCA cycle

The genome sequence suggests that *C. trachomatis* has an intact glycolytic pathway (although it lacks an identifiable gene for fructose 1,6-diphosphate aldolase), as well as a complete glycogen synthesis and degradation system aldolase [2]. Thus, *Chlamydia* seems to be able to generate at least minimal amounts of ATP by glycolysis. However, the gene encoding hexokinase, which converts glucose to glucose 6-phosphate, could not be identified. This is in accordance with experimental work which suggests that *C. trachomatis* imports glucose 6-phosphate directly from the host cell cytoplasm [14]. In contrast, no glycolytic genes have been identified in *R. prowazekii,* nor have identifiable homologs of the *C. trachomatis* genes for glycogen synthesis and degradation been observed in the genome [1].

Pyruvate is most likely a host-derived intermediate in both parasites. Although there is no experimental evidence which suggests that acetyl CoA can be synthesized from pyruvate in these two organisms, genes encoding pyruvate dehydrogenase, the enzyme complex that converts pyruvate to acetyl CoA, have been identified in both genomes [1,2]. *R. prowazekii* has two genes encoding the E3 component, whereas *C. trachomatis* has a single gene with two regions that are similar to the two genes in *R. prowazekii*. In addition, *C. trachomatis* encodes a large fusion protein which contains all of the three enzymatic functions of the E1, E2 and E3 components of pyruvate dehydrogenase. No corresponding fusion gene has been found in the *R. prowazekii* genome.

A complete set of genes for the TCA cycle has been identified in *R. prowazekii* suggesting that the TCA cycle is functional in this organism, as is also indicated by the purification of several enzymes involved in this pathway [29]. In contrast, the TCA cycle is not complete in *C. trachomatis* and there are no homologs to key enzymes such as aconitate hydratase, citrate synthase or isocitrate dehydrogenase [2].

6.2. Aerobic respiration

Several genes encoding essential functions in aerobic respiration are present in both genomes, however there is no relationship between the two systems [1,2]. The chlamydial NADH-ubiquinone oxidoreductase subunits are orthologs of those found in the marine bacterium *Vibrio alginolyticus* [2]. This particular type of dehydrogenase is coupled with sodium translocation and their primary function may be the translocation of Na^+ across the membrane as observed in *Haemophilus influenzae* [30]. In contrast, the rickettsial NADH dehydrogenases seem to share an evolutionary origin with those in mitochondria [1]. Furthermore, several genes encoding cytochrome oxidase subunits are present in *R. prowazekii* but not in *C. trachomatis*. However, two genes encoding cytochrome oxidase *d* subunit I and II, which may serve a function in the scavenging of oxygen, have been identified in both genomes [2].

Finally, the two obligate intracellular parasites have different systems for ATP synthesis. The *C. trachomatis* genome encodes a V-type archaean-like ATPase present in a few bacterial groups such as the spirochetes and *Thermus* spp., which is thought to have been introduced into the *C. trachomatis* genome by horizontal gene transfer [2]. In contrast, the ATP synthesizing complex in *R. prowazekii* is composed of the F0 and F1 components, and this complex seems to share an origin with the ATP synthesizing complex in mitochondria [1].

Thus, the bioenergetic profiles of *C. trachomatis* and *R. prowazekii* are strikingly different. This reinforces two important concepts built from rRNA sequence data: first that the aerobic respiration system in mitochondria is derived from an intracellular ancestor of the α-Proteobacteria, and second, that *R. prowazekii* and *C. trachomatis* have adapted to the intracellular niches independently of each other [4].

7. Concluding remarks

Transitions to intracellular environments are thought to be associated with massive losses of genetic information. This may not be surprising since the cytoplasm of a eukaryotic host represents an unexploited resource of food which invites

the evolution of metabolic parasitism. Furthermore, obligate intracellular parasites replicate in small isolated populations with little or no contact between variants in the population and transmission from one host to the other frequently involves bottlenecks. Accordingly, deleterious mutations, such as deletions, may accumulate at enhanced fixation rates in these genomes [31,32]. For all of these reasons, a gradual loss of genetic material over time is to be expected for obligate intracellular parasites.

Indeed, both *R. prowazekii* and *C. trachomatis* have a significantly reduced fraction of genes involved in biosynthetic pathways compared to their free-living relatives [1,2,17]. A striking example of this reductive, convergent evolution is the unique absence of genes involved in de novo purine or pyrimidine biosynthesis in *R. prowazekii* and *C. trachomatis*. So far, the data are consistent with our expectations [31,32].

However, an unexpected difference between the two genomes is that the coding content in *R. prowazekii* is only 75.4% as compared to 89.5% in *C. trachomatis*. The coding content of previously sequenced genomes is on average 91%, suggesting that the coding content in *C. trachomatis* is typical of bacterial genomes whereas the high fraction of non-coding DNA in the *R. prowazekii* genome is highly unusual. We have suggested that the relatively large fraction of non-coding DNA in *R. prowazekii* may be mutationally destroyed genes that are in the process of being discarded from the genome [1].

Analyses of the *metK* genes in *Rickettsia* indicate that redundant genes are rapidly eliminated from the genome because of a predominance of deletion mutations under neutral conditions [20,21]. However, such an elimination process is possible only after the invention of efficient transport systems for the corresponding metabolic compounds. The ATP/ADP translocases, which enable cytosolic ATP to be used as a source of energy in *Rickettsia* and *Chlamydia*, demonstrate the capacity for such evolutionary innovations [22,23]. By analogy, it can be speculated that transport systems for Ado-Met have also been invented by these two organisms. However, the presence of such transporters in *Rickettsia* and *Chlamydia* remains to be experimentally determined.

To date, more than 10 pseudogenes have been identified in the *R. prowazekii* genome. Among these are a few short pieces of a gene which show a strong sequence similarity to the *spoT/relA* genes. However, neither the *metK* gene, nor the *spoT/relA* homologs are present in the *C. trachomatis* genome [2]. Thus, it seems as if these two genes, which are pseudogenes in the *R. prowazekii* genome, have already been eliminated from the *C. trachomatis* genome.

This is consistent with three explanations: one, that *C. trachomatis* has a more efficient system for eliminating non-functional gene sequences; two, that the population structures of the two organisms are slightly different; or three, that *C. trachomatis* is an older obligate intracellular parasite than *R. prowazekii*. Comparative analysis of the rates at which deletion events relative to insertion and horizontal transfer events occur may provide further information about the mechanisms of reductive evolutionary processes in organisms growing in isolated, but metabolically rich environments.

Acknowledgements: We thank Charles G. Kurland for comments on the manuscript. The author's work was supported by the Swedish Natural Sciences Research Council, the Knut and Alice Wollenberg Foundation, the Foundation for Strategic Research, and contract BIO4 CT-95-0130 from the European Commission.

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