

Research

Dynamic diversity of the tryptophan pathway in chlamydiae: reductive evolution and a novel operon for tryptophan recapture

Gary Xie^{*†}, Carol A Bonner[†] and Roy A Jensen^{*†‡}

Addresses: ^{*}Department of Microbiology and Cell Science, Gainesville, FL 32611, USA. [†]BioScience Division, Los Alamos National Laboratory, Los Alamos, NM 87544, USA. [‡]Department of Chemistry, City College of New York, New York, NY 10031, USA.

Correspondence: Roy A Jensen. E-mail: rjensen@ufl.edu

Published: 29 August 2002

Genome **Biology** 2002, **3**(9):research0051.1–0051.17

The electronic version of this article is the complete one and can be found online at <http://genomebiology.com/2002/3/9/research/0051>

© 2002 Xie et al., licensee BioMed Central Ltd
(Print ISSN 1465-6906; Online ISSN 1465-6914)

Received: 26 April 2002

Revised: 6 May 2002

Accepted: 2 July 2002

Abstract

Background: Complete genomic sequences of closely related organisms, such as the chlamydiae, afford the opportunity to assess significant strain differences against a background of many shared characteristics. The chlamydiae are ubiquitous intracellular parasites that are important pathogens of humans and other organisms. Tryptophan limitation caused by production of interferon- γ by the host and subsequent induction of indoleamine dioxygenase is a key aspect of the host-parasite interaction. It appears that the chlamydiae have learned to recognize tryptophan depletion as a signal for developmental remodeling. The consequent non-cultivable state of persistence can be increasingly equated to chronic disease conditions.

Results: The genes encoding enzymes of tryptophan biosynthesis were the focal point of this study. *Chlamydomphila psittaci* was found to possess a compact operon containing PRPP synthase, kynureninase, and genes encoding all but the first step of tryptophan biosynthesis. All but one of the genes exhibited translational coupling. Other chlamydiae (*Chlamydia trachomatis*, *C. muridarum* and *Chlamydomphila pneumoniae*) lack genes encoding PRPP synthase, kynureninase, and either lack tryptophan-pathway genes altogether or exhibit various stages of reductive loss. The origin of the genes comprising the *trp* operon does not seem to have been from lateral gene transfer.

Conclusions: The factors that accommodate the transition of different chlamydial species to the persistent (chronic) state of pathogenesis include marked differences in strategies deployed to obtain tryptophan from host resources. *C. psittaci* appears to have a novel mechanism for intercepting an early intermediate of tryptophan catabolism and recycling it back to tryptophan. In effect, a host-parasite metabolic mosaic has evolved for tryptophan recycling.

Background

Chlamydiae are obligate intracellular pathogens that infect a variety of host organisms and exhibit individual tissue tropisms within a host species. The availability of complete genomic sequences for different species has provided a contemporary impetus for research to uncover specific relationships of

chlamydial genes with the disease process, an impetus that is particularly welcome because the fastidious growth requirements of the organism have made it relatively intractable to experimentation. A number of analyses dealing with the comparative genomics of the chlamydiae have appeared in the recent literature [1-3].

Chlamydiae all progress through a life cycle that is intimately tied to success as a pathogen. The host is invaded by elementary bodies (EBs), which represent the extracellular infectious stage. The newly established EBs develop into intracellular reticulate bodies (RBs) that replicate in anticipation of maturation to EBs, which then lyse the host cell and initiate a new round of pathogen proliferation. Aggressive progress through repetitions of this cycle characterizes the acute disease process. Distinct changes in cell size, chromatin organization, membrane characteristics and metabolic competence constitute endpoints of a reversible developmental profile. A third metabolic stage called persistence is increasingly recognized to attend the chronic disease process [4]. The persistent state can be induced *in vitro* in tissue culture in response to various environmental cues such as nutrient limitation, antibiotic treatment or presence of interferon- γ (IFN- γ) [5,6]. All of the latter may act to trigger persistence by eliciting a degree of metabolic starvation [7]. In fact, it has been shown [7] that even normal blood plasma concentrations of amino acids are sufficiently limiting to infected cell cultures to promote the persistent state. Cells present in the persistent state have been detected *in vivo*, for example, in the synovial membranes of patients with *Chlamydia*-associated reactive arthritis [8]. Chlamydial cells poised in this state of metabolic latency exist as viable but non-cultivable cells that are 'abnormally' enlarged and which exhibit distinctive morphological characteristics [4].

One of the most prominent host protective responses to chlamydial infections has been the production of the T-cell-derived pro-inflammatory cytokine IFN- γ . This cytokine induces a variety of biochemical changes in host metabolism, apparently designed to thwart the ability of intracellular parasites to gain access to host resources. Relative sensitivity to IFN- γ varies, with *C. muridarum* and *C. psittaci* being relatively resistant compared to *C. pneumoniae* and the various *C. trachomatis* serovars. The relative insensitivity *in vivo* of *C. muridarum*, compared to human strains of *C. trachomatis*, is supported by the results of Cotter *et al.* [9] and Perry *et al.* [10]. Conflicts in the literature about the sensitivities of *C. trachomatis* and *C. muridarum* isolates to IFN- γ -mediated inhibition have been attributed to strain variation [10]. A well documented effect of IFN- γ has been its ability to decrease the availability of L-tryptophan in host cells. (Other anti-chlamydial effects involve the inducible synthesis of nitric oxide and deprivation of iron [11].) Although effective L-tryptophan starvation may resolve an acute infection, a more modulated degree of starvation for L-tryptophan is thought to be intimately involved in the phenomenon of persistence [12]. Thus, tryptophan limitation is increasingly recognized as an important factor in a variety of chronic disease conditions. As initially shown by Byrne *et al.* [13,14] and confirmed by others [15,16], IFN- γ acts by inducing indoleamine 2,3-dioxygenase, a host enzyme that converts L-tryptophan to L-formylkynurenine. (This broad-specificity monomeric enzyme is different from the non-homologous

tryptophan 2,3-dioxygenase in liver [17] and brain tissues [18].) In addition, IFN- γ is a potent inducer of host tryptophanyl-tRNA synthetase [19]. Thus, the host not only decreases the pool of tryptophan available to intracellular parasites, but the remaining tryptophan molecules tend to be increasingly sequestered by the elevated level of host tryptophanyl-tRNA synthetase.

The biological relationship of IFN- γ , indoleamine dioxygenase, tryptophan limitation and the persistent state of intracellular parasitism extends beyond chlamydial parasites, as illustrated by consideration of *Toxoplasma gondii* [20]. This unicellular protozoan is a eukaryotic intracellular parasite that is subject to tryptophan limitation by exactly the same host mechanism used against the chlamydiae. The result is a generally widespread and asymptomatic state of chronic infection. Even extracellular bacteria such as group B streptococci (tryptophan auxotrophs) are inhibited by the IFN- γ mechanism of tryptophan limitation [21]. In this context, it may be no accident that pathogens such as *Enterococcus faecalis*, *Haemophilus ducreyi*, *Clostridium difficile* and *Cl. perfringens* are conspicuous tryptophan auxotrophs that maintain full competence for phenylalanine and tyrosine biosynthesis. The broad biological impact of host tryptophan catabolism is further illustrated by the hypothesis that tryptophan catabolism localized in placental tissue provides a mechanism (suppression of lymphocyte proliferation [22]) to prevent immune rejection of the mammalian fetus [23]. An additional example of the far-reaching consequences of host tryptophan catabolism is illustrated by the potential for persistent immune activation to disrupt the balance between serotonin and kynurenine production from tryptophan, thus linking the immunological network and neuropsychiatric consequences of serotonin imbalance [24].

Results and discussion

Dynamic gene reorganization and gene flux within the chlamydial plasticity zones

Figure 1 shows the gene organization in *C. psittaci* of tryptophan-pathway genes (*trp*), the large toxin gene *lifA*, a perforin-family gene, and a conserved hypothetical gene that is specific to the *C. pneumoniae/C. psittaci* lineage. *lifA*, the *trp* genes, the perforin-encoding gene and a few other genes can be generally recognized as interspecies residents of a 'plasticity zone' located near the terminus of replication [2]. As Read *et al.* [2] pointed out, dynamic events of gene shuffling, gene insertion and gene loss are apparent within this plasticity zone. They discussed the lack of variation in GC content and the absence of evidence for gene transfer, as well as the variation of tryptophan-pathway genes.

Substantial variation is also striking with respect to *lifA*. *C. psittaci* possesses a single copy of *lifA*, *C. muridarum* has three paralog copies, *C. trachomatis* has a single pseudogene with frameshift mutations and *C. pneumoniae* lacks *lifA*

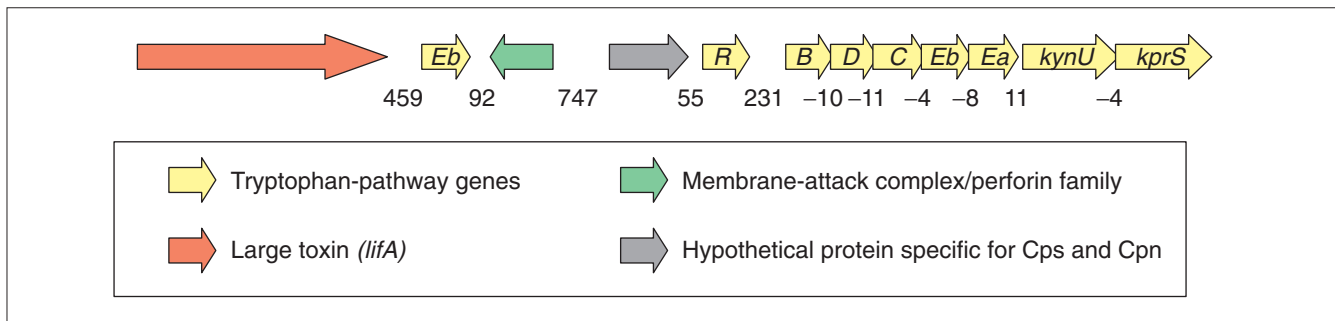


Figure 1

The tryptophan-recapture operon of *Chlamydomophila psittaci* (Cps). Genes are color-coded as shown in the key. Arrows indicate the direction of transcription. The nomenclature for tryptophan-pathway genes is that elaborated by Xie *et al.* [30], whereby genes encoding the five enzymes are named in order of the pathway steps. The two subunits of the first and fifth steps are given additional lower-case identifiers, that is, *trpAa/trpAb* and *trpEa* and *trpEb* (for simplicity, the *trp* genes are labeled without the *trp* identifier). Nucleotide spacing between genes is shown; negative values indicate open reading frames that overlap (translational coupling). The membrane-attack complex/perforin family protein from the chlamydiae, which belongs to PFAM protein family HMM PFO1823, has been discussed by Ponting [59]. The gene encoding this protein in *C. psittaci* appears to have multiple frameshifts, a possible carboxy-terminal truncation, and a possible insertion. Whether sequencing errors might account for this is unknown at the present time. Cpn, *Chlamydomophila pneumoniae*.

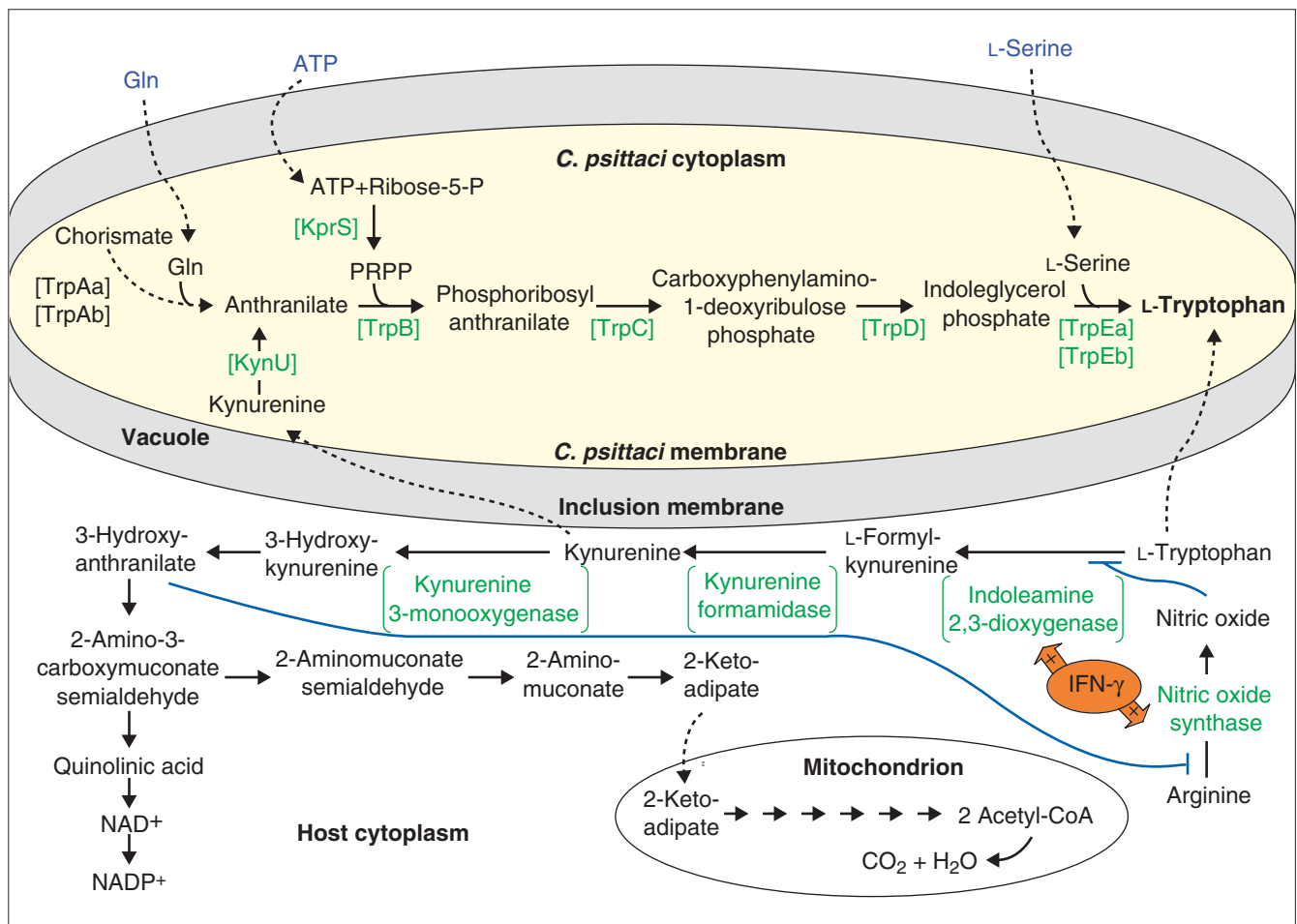
altogether. *lifA* (lymphocyte inhibitory factor) encodes a large toxin that can block production of IFN- γ . It therefore undermines the host's ability to deplete tryptophan through induction of indoleamine dioxygenase. *lifA* is of limited phylogenetic distribution, being present elsewhere only in enteropathogenic strains of *Escherichia coli* [25]. The effect of IFN- γ is dose-dependent [26,27], and different concentration ranges have been shown [28] to allow rapid progress through the acute infection cycle (low IFN- γ concentration), to promote a stable state of persistence (medium IFN- γ concentration), or to completely resolve the infection (high IFN- γ concentration). Thus, to the extent that *lifA* is considered to be a factor, *C. muridarum* presumably has a maximal capability to block induction of IFN- γ , whereas *C. psittaci* has only a partial ability to block IFN- γ induction via *lifA*. The amino-terminal portion of *lifA* (which includes the remnant of *lifA* remaining in *C. trachomatis*) is homologous to the gene encoding a clostridial toxin, and Belland *et al.* [29] have recently demonstrated cytotoxic activities in *C. muridarum* and *C. trachomatis* D that are indistinguishable from those mediated by clostridial toxin B. Cytotoxicity was dose dependent with respect to *lifA* copy number. Belland *et al.* [29] note that if the chlamydial cytotoxin inhibits lymphocyte activation (as does Lifa in *E. coli*), it would provide a mechanism for immune evasion.

Within the plasticity zone shown in Figure 1, *C. psittaci* has an operon arrangement of genes encoding a nearly complete pathway of tryptophan biosynthesis. In contrast, the plasticity zone of *C. trachomatis* has only two structural genes of tryptophan biosynthesis (*trpEb* and *trpEa*), and *C. muridarum* has no tryptophan-pathway genes at all [2]. In addition, both *C. trachomatis* and *C. muridarum* have a *trpC* gene outside of the plasticity zone. *C. pneumoniae* has no tryptophan-pathway genes present anywhere in the genome.

Novelty of the tryptophan operon of *C. psittaci*

The pathway of tryptophan biosynthesis consists of five steps, and we have used the convention [30] of naming the genes in the order of the steps, *trpA*, *trpB*, *trpC*, *trpD* and *trpE*. The α and β subunits of *trpA* are named *trpAa* and *trpAb*, and the α and β subunits of *trpE* are named *trpEa* and *trpEb*. This nomenclature is logical, easy to remember and suited to the modern era of comparative genomics, where gene naming needs to be consistent and to correspond to proteins at the level of catalytic domain (subunit). Thus, *trpAaAbBCDEaEb* corresponds to the conventional *E. coli* designations of *trpEGDFCAB*.

The tryptophan operon in *C. psittaci* is incomplete in that genes encoding the two subunits of anthranilate synthase (*trpAa* and *trpAb*) are absent. These genes are not present elsewhere in the genome. Hence, no biochemical connection with chorismate as a beginning substrate for tryptophan biosynthesis is apparent (Figure 2). The tryptophan operon of *C. psittaci* exhibits further striking aspects of novelty. Not only does it contain genes (*kynU* and *kprS*) that are not components of the classical tryptophan operon, but these genes are not even present in other chlamydiae. *kynU* encoding kynureninase and *kprS* (alternative name: *prsA*) encoding PRPP synthase are located at the 3' end of the *C. psittaci* *trp* operon (see Figure 1). These genes, together with *trpB*, *trpD*, *trpC*, *trpEb* and *trpEa*, comprise a compact operon in which all but one gene overlaps its neighbor in the operon (translational coupling). A regulatory gene, the *trpR* repressor, precedes the tryptophan operon on the amino-terminal side. A second paralog of the tryptophan synthase β subunit (*trpEb-2*) is also present in an extra-operonic location several genes upstream of *trpR*. The possible functional significance of this paralog as serine deaminase has been discussed elsewhere [30].

**Figure 2**

Chlamydia/host tryptophan cycle. Solid arrows indicate conversion by the enzyme in green on the arrow; dotted arrows show movement across membranes. Induction of increased levels of tryptophan dioxygenase by IFN- γ causes flux of the host pool of tryptophan into the kynurenine pathway, causing starvation of *Chlamydia* parasites for tryptophan. Starvation for tryptophan presumably derepresses the *trpR*-regulated operon of tryptophan biosynthesis (*trpBDCEbEa kynU kprS*) in *C. psittaci*. The host-pathway enzymes of interest are located in the cytosol or are located in the mitochondrial compartment. Genes of tryptophan biosynthesis are named in the order of the pathway steps, following the nomenclature used by Xie *et al.* [30]. Thus, *trpAa* and *trpAb* (absent in *C. psittaci*) encode the large (aminase) and small (glutamine-binding) subunits of anthranilate synthase, *trpB* encodes anthranilate phosphoribosyl transferase, *trpC* encodes phosphoribosyl-anthranilate isomerase, *trpD* encodes indoleglycerol phosphate synthase, and *trpEa* and *trpEb* encode the α and β subunits of tryptophan synthase, respectively. *KynU* encodes kynureninase. *kprS* (synonymous with *prsA*) encodes PRPP synthase. Orange arrows indicate induction of indoleamine 2,3-dioxygenase and nitric oxide synthase by IFN- γ . Blue lines indicate inhibition of the latter two enzymes by nitric oxide and 3-hydroxyanthranilate, respectively.

Host-parasite metabolic mosaic for tryptophan cycling

A rationale for inclusion of *kprS* and *kynU* in the *C. psittaci* tryptophan operon can be visualized from an examination of Figure 2. The ability of *C. psittaci* to synthesize L-tryptophan requires an alternative source of anthranilate (other than chorismate) as *C. psittaci* lacks anthranilate synthase. Kynurenine, intercepted from the host stream of catabolism, would satisfy this requirement given the presence of *KynU*. PRPP input is required for the *TrpB*-catalyzed step, and thus it was necessary for *C. psittaci* to recruit PRPP synthase (*kprS*) to the operon. The import of ATP (substrate for PRPP synthase) from the host is also probably needed, and the

presence of ATP translocases in chlamydial genomes has been documented [1-3]. Alternative sources of ATP, for example, utilization of PEP by pyruvate kinase, are not altogether ruled out [31]. Finally, serine import is required for the tryptophan synthase step as chlamydiae are not competent for serine biosynthesis.

Figure 2 illustrates the mammalian 'kynurenine' pathway of tryptophan catabolism, which is prominent in liver and kidney. The initial step is rate limiting and is catalyzed by either of two enzymes: indoleamine 2,3-dioxygenase or tryptophan 2,3-dioxygenase. The latter is the true catabolic entity, has narrow specificity for tryptophan, and is inducible

in the presence of tryptophan, glucocorticoids and heme cofactor [17]. In contrast, indoleamine 2,3-dioxygenase has broad substrate specificity and is capable of depleting low-to-normal concentrations of tryptophan if induced by IFN- γ . The overall host metabolism of tryptophan shown in Figure 2 reflects a general potential that is not necessarily realized in all cell types. The ultimate catabolic process to generate acetyl-CoA is a feature of liver and kidney organ systems. 2-Amino-3-carboxymuconate semialdehyde can be considered to be a branchpoint metabolite that either enters committed catabolism to acetyl-CoA or that enters biosynthesis to NAD⁺/NADP⁺. In the central nervous system, a number of kynurenine-pathway metabolites are neuroactive and appear to be involved in inflammatory neurological diseases [32]. Quinolate can cause excitotoxic neuronal death. Kynurenic acid, derived from kynurenine by transamination, can antagonize the effect of quinolate. 3-Hydroxykynurenine and 3-hydroxyanthranilate have been shown to cause apoptotic or necrotic neuronal death in cell cultures [32]. In glioblastoma cells (and apparently in human fibroblasts) kynurenine is an endpoint of tryptophan catabolism [33]. In human macrophages kynurenine is further metabolized [34]. Indoleamine dioxygenase is a rate-limiting step of tryptophan catabolism, and other steps are not known to be induced by IFN- γ . 3-Hydroxykynurenine is a prominent metabolite in the eye lens (it absorbs UV radiation) and probably supports eye pigmentation in the iris/ciliary body [35].

Hence, in some host tissue types, kynurenine is a largely dead-end product of tryptophan catabolism, whereas it has a variety of metabolic fates in other tissues. In either case, kynurenine generally exhibits a conspicuous pool size [36]. Thus, although *C. psittaci* apparently cannot utilize chorismate (for which it has an intact biosynthetic pathway) as a precursor of L-tryptophan, it has the potential to synthesize its own supply of L-tryptophan from host-generated kynurenine, ATP and L-serine.

It is of interest that the conversion of kynurenine to anthranilate and the conversion of 3-hydroxykynurenine to 3-hydroxyanthranilate are similar hydrolytic reactions. The host enzyme catalyzing the latter reaction is, in fact, a kynureninase which is a homolog of the *C. psittaci* KynU. Kynureninases have been reported to possess a range of substrate specificities that vary between very high specificity for kynurenine and very high specificity for 3-hydroxykynurenine. The kynureninase of rat and human liver has an order-of-magnitude preference for 3-hydroxykynurenine [37], in contrast to a microbial kynureninase that exhibits a very high preference for kynurenine [38]. It seems likely that the *C. psittaci* KynU is specific for kynurenine, whereas the mammalian host may possess isozymes of different substrate specificity in different tissues.

An intriguing possible layer of additional complexity involves a competitive relationship between indoleamine

dioxygenase and nitric oxide (NO) synthase (type II isoform), which are both induced by IFN- γ . In murine macrophages, induction of NO synthase requires at least one additional stimulus (such as bacterial lipopolysaccharide (LPS)) that acts synergistically with IFN- γ ([39] and references therein). Metabolite flow to NAD⁺ is proportional to the input availability of tryptophan in murine macrophages [40]. Under conditions in which there is induction of both indoleamine dioxygenase and NO synthase, an interplay of potential cross-pathway inhibitions are set in motion as illustrated in Figure 2. Nitric oxide inhibits indoleamine dioxygenase [41]. On the other hand, 3-hydroxyanthranilate inhibits both the expression and activity of NO synthase [39]. Thus, small-molecule products of each pathway can be mutually inhibitory.

Consider a scenario where *C. psittaci* has parasitized a host niche where 3-hydroxyanthranilate is a prominent metabolite, as in human macrophages [42]. The withdrawal of kynurenine from this flow route might undermine the levels of 3-hydroxyanthranilate sufficiently to release the restraints on NO synthase. The consequent increase in nitric oxide production might then tend to limit the availability of kynurenine as a result of inhibition of indoleamine dioxygenase. Although this might at first seem an unfavorable outcome for the *C. psittaci* parasitism, a finite but minimal supply of kynurenine might satisfy the highly limited metabolic demands of the persistent state.

Did the *C. psittaci* *trp* operon originate by lateral gene transfer?

The tryptophan operon of *C. psittaci* is probably derived from ancestral chlamydial genes before modern events of gene reduction occurred. However, as the present-day *C. psittaci* operon is unique among chlamydiae, it is also possible that *trp* genes were lost and then reacquired by lateral gene transfer (LGT). As this would have happened recently (after divergence of *C. psittaci* from other chlamydial lineages), one might then expect the genes to have GC contents different from that of the overall *C. psittaci* genome (42%). Table 1 shows that each operon gene, as well as *trpR* and *trpEb-2*, are within the range expected for *C. psittaci*. However, the GC content of the donor genome could have fortuitously been near that of *C. psittaci*. If so, one might expect that the top hits returned from a BLAST search of each *C. psittaci* operon gene would not include other chlamydial genes and would be dominated by one organism having an appropriate GC content. The results (Table 1) show that this expectation was not realized.

It is perhaps intriguing that when *C. psittaci* kynureninase (KynU) was used as a query sequence, the top hits returned from BLAST were the KynU orthologs from man and mouse. Accordingly, the codon usage for the KynU proteins of *C. psittaci* and *Homo sapiens* (left half of Figure 3) was compared with the genomic codon usage of the respective

Table 1**Origin of *trp*-recapture operon by horizontal gene transfer?**

Gene	%GC	First BLAST hit		Second BLAST hit	
		Organism*	% Identity	Organism*	% Identity
<i>trpR</i>	37	<i>Chlamydia trachomatis</i> (42)	61	<i>Vibrio cholerae</i> (48)	37
<i>trpB</i>	40	<i>Arabidopsis thaliana</i> (54)	45	<i>Methanococcus jannaschii</i> (31)	39
<i>trpD</i>	37	<i>Streptomyces coelicolor</i> (72)	43	<i>Mycobacterium leprae</i> (58)	41
<i>trpC</i>	41	<i>Chlamydia trachomatis</i> (42)	47	<i>Chlamydia muridarum</i> (42)	46
<i>trpEb</i>	42	<i>Chlamydia trachomatis</i> (42)	74	<i>Aquifex aeolicus</i> (43)	59
<i>trpEa</i>	39	<i>Chlamydia trachomatis</i> (42)	44	<i>Xylella fastidiosa</i> (54)	40
<i>kynU</i>	40	<i>Homo sapiens</i> (53)	40	<i>Mus musculus</i> (53)	39
<i>kprS</i>	37	<i>Thermotoga maritima</i> (46)	41	<i>Campylobacter jejuni</i> (31)	41
<i>trpEb-2</i> [†]	42	<i>Chlamydia trachomatis</i> (42)	69	<i>Pyrococcus kodakaraensis</i> (55)	56
(<i>C. psittaci</i> genome)	42				

*Organisms having proteins returning the top hits after a BLAST search using the indicated *C. psittaci* genes on the left as query sequences are shown. Overall genomic %GC is given in parentheses. [†]Extra-operonic paralog of *trpEb* (see Figure 1).

organisms (right half of Figure 3). The codon usage for arginine, leucine, proline and valine is distinctive in a comparison of *C. psittaci* and *H. sapiens*. The profile of codon usage for these amino acids by *C. psittaci* KynU clearly matches the genomic codon-usage profile of *C. psittaci*, but not that of *H. sapiens*. Thus, there is no evidence for a recent LGT of *kynU* between *C. psittaci* and *H. sapiens*.

Deterioration of tryptophan-pathway genes in the chlamydiae

Chlamydial species have generally undergone reductive evolution that includes an inability to synthesize tryptophan from chorismate. That the process of reductive evolution is ongoing is suggested by the variability of remaining remnants and by indications that some of these remnants are pseudogenes. At one extreme, *C. pneumoniae* has lost all tryptophan-pathway genes; *C. muridarum* has only one remnant (*trpC*); and *C. trachomatis* has three remnants (*trpC*, *trpEa* and *trpEb*). It appears that *C. psittaci* alone assigns a functional role to tryptophan-pathway genes, but it is a kynurenine-to-tryptophan pathway rather than a chorismate-to-tryptophan pathway. Thus, even *C. psittaci* is dependent upon host resources (that is, kynurenine) for tryptophan.

Figure 4 shows a sequence comparison of TrpC from *E. coli* with those from chlamydial species. Critical residues can be assessed with guidance from X-ray crystallography data (see legend) and invariant residues seen in multiple alignments. Given the presumed lack of selection for function in *C. trachomatis* and *C. muridarum*, it would not be surprising to find evidence of unfavorable mutations. Indeed, the mutations H335 → S335 (*E. coli* numbering) and G385 → E385 in *C. trachomatis* and *C. muridarum*, but not in *C. psittaci*,

probably reflect unfavorable catalytic alterations (see heavy up arrows in Figure 4). Two changes in *C. psittaci*, not present in the other two chlamydial species (V292 → T292 and S429 → T429) are conservative changes that are presumably tolerated.

TrpEa from *C. trachomatis* has clearly accumulated deleterious mutations in contrast to the *C. psittaci* TrpEa (Figure 5). Comparison of these sequences with that of the well studied TrpEa from *Salmonella typhimurium* shows *C. trachomatis* TrpEa (but not *C. psittaci* TrpEa) to have the following changes at critical residues (*S. typhimurium* numbering): G61 → N61, G211 → R211, F/L212 → R212, and G234 → K234. In addition, the intersubunit signaling residue G181 has been changed to A181 in *C. trachomatis*. *C. trachomatis* TrpEa has a four-residue deletion between R192 and K193 that is unique in our comprehensive alignment of TrpEa. Xie *et al.* [30] pointed out that the elongated branch of *C. trachomatis* TrpEa, but not of *C. psittaci* TrpEa, on an unrooted phylogenetic tree of the TrpEa family was consistent with a likely pseudogene status for the former. The rapid deterioration of TrpEa is, in fact, apparent from differences in TrpEa from various serovars of *C. trachomatis* [43]. Thus, serovar B lacks TrpEa altogether, serovars A and C express severely truncated TrpEa proteins, whereas serovars D and L2 express full-length TrpEa proteins (although undoubtedly inactive).

The β subunits of tryptophan synthase in *C. psittaci* (two copies, Figure 1) and *C. trachomatis* appear to have all important residues conserved (Figure 6). This includes conserved catalytic residues and residues that are important for establishing intersubunit and intrasubunit salt bridges needed for formation of the α-β complex of tryptophan synthase. It

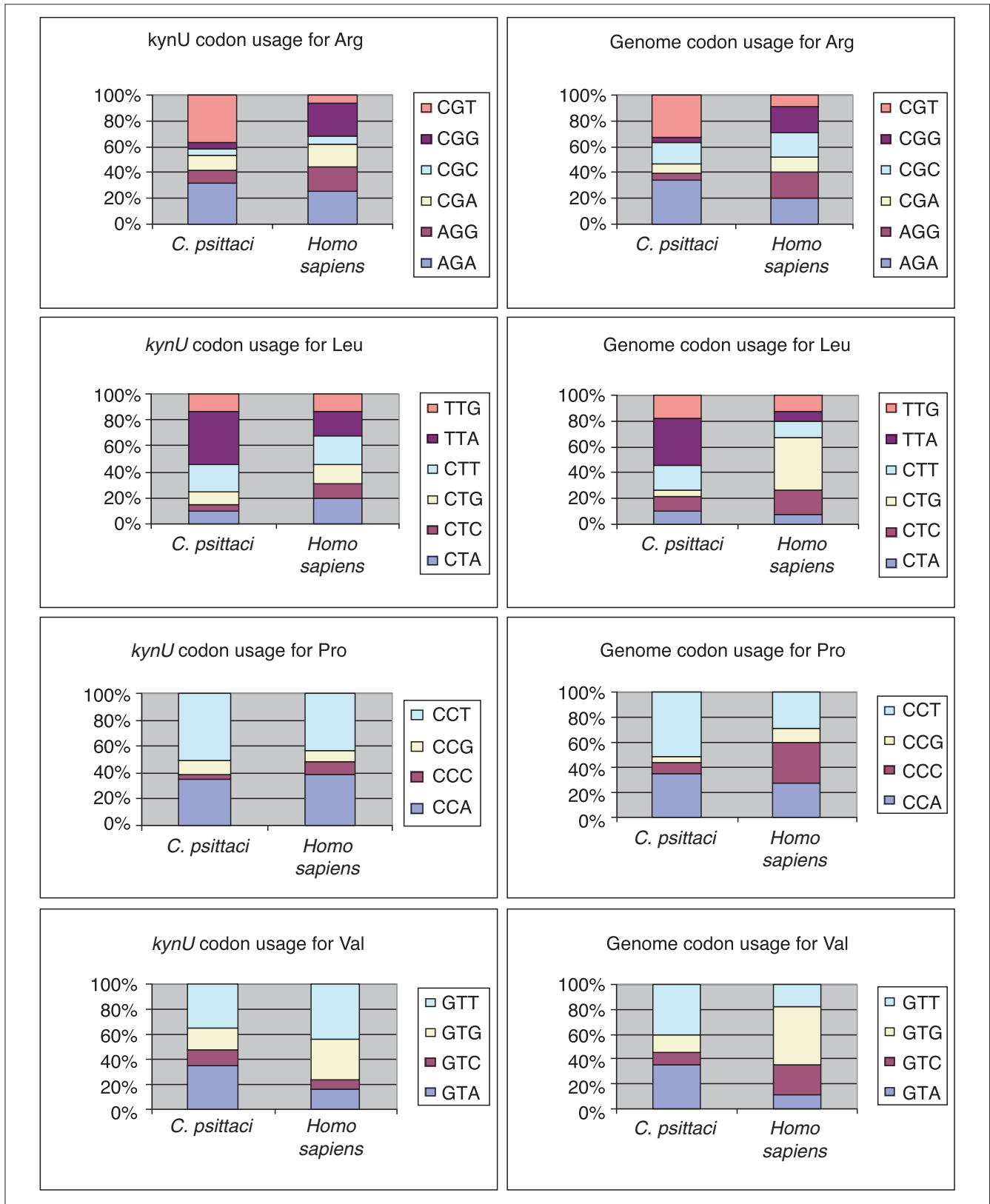
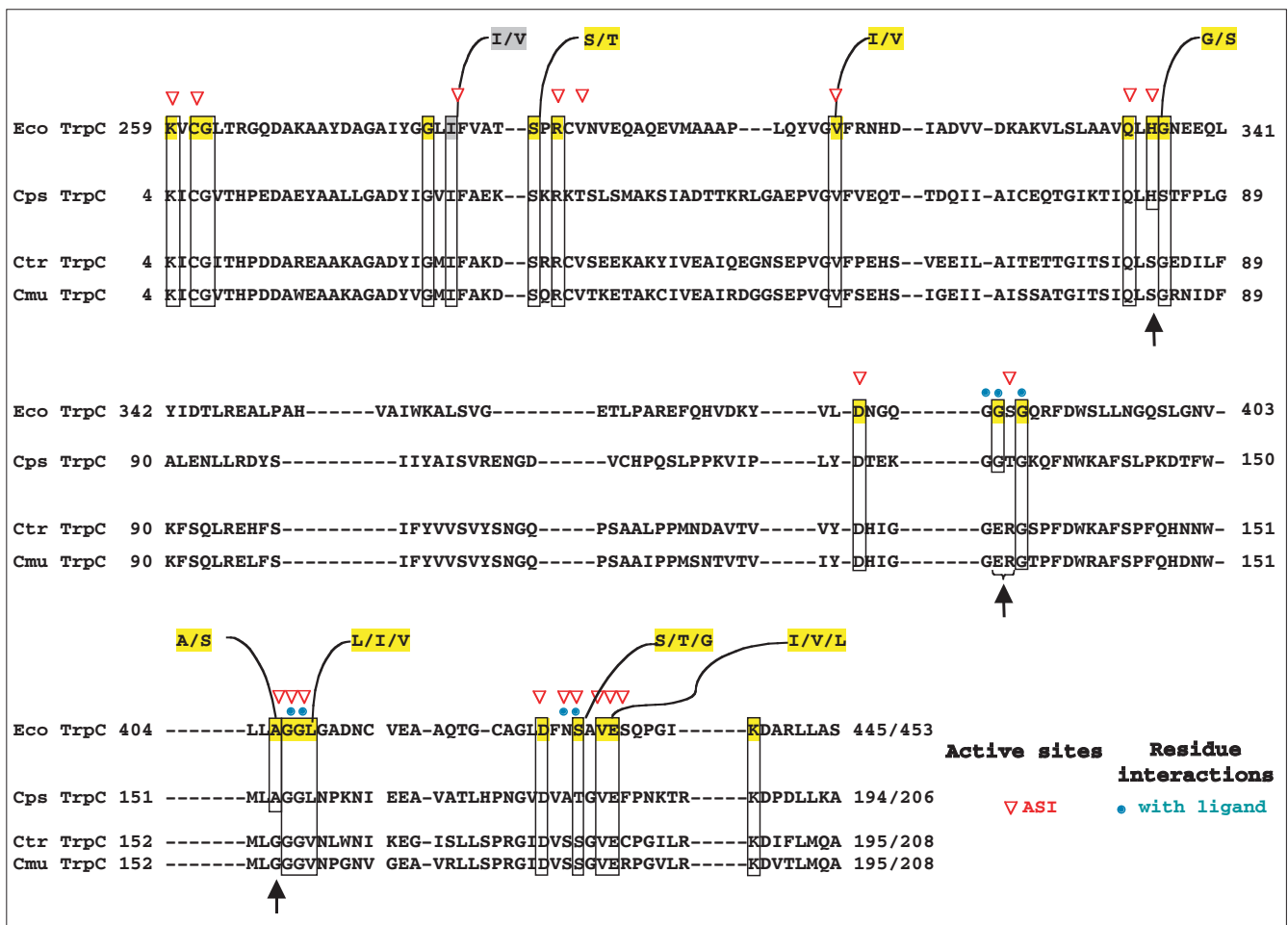


Figure 3
Comparison of codon usage (arginine, leucine, proline and valine) for *kynU* genes from *C. psittaci* and *H. sapiens* with the corresponding whole-genome codon usages.

**Figure 4**

Alignment of *E. coli* (Eco) TrpC from the protein database (PDB code 1PII) with TrpC proteins from the indicated chlamydiae (Cmu, *C. muridarum*; Cps, *C. psittaci*; Ctr, *C. trachomatis*). The TrpC domain shown for Eco TrpC is the carboxy-terminal domain of a fusion protein (TrpD•TrpC) whose amino-terminal domain is TrpD. An extensive multiple alignment of TrpC proteins was carried out, and several potential pseudogenes were excluded. The gaps shown reflect the gaps required to align the Eco•TrpC with other sequences in the latter alignment (available on request). Invariant residues are highlighted yellow, and near-invariant (one exception tolerated) residues are shaded gray. Active-site residues are marked with a red triangle and residues interacting with ligand with blue dots [60]. See Table 2 for gene identification numbers.

appears that there has been rapid deterioration of TrpEa, but not of TrpEb. This might suggest that TrpEb is under positive selection for some functional role other than that of tryptophan synthase. When TrpEb is not complexed with TrpEa, it has substantial activity as serine deaminase. Therefore, present-day TrpEb may function in the chlamydiae as serine deaminase, as has been proposed by Xie *et al.* [30] for some archaea. This is consistent with the total absence of genes in chlamydiae known to encode enzymes with serine deaminase activity. These enzymes include Fe-S serine deaminase (GenBank gi 2501150), PLP-dependent serine deaminase (gi 134387), catabolic threonine deaminase (gi 135723), and biosynthetic threonine deaminase (gi 135720).

A very recent paper by Fehlner-Gardiner *et al.* [44] affirms experimentally the predictions made in this paper that

TrpEb should be functional and TrpEa should not be functional. Fehlner-Gardiner *et al.* [44] sequenced TrpEa and TrpEb from all human serovars of *C. trachomatis*. All of the genital serovars expressed TrpEa and TrpEb proteins, but only TrpEb had catalytic activity. Interestingly, the catalytic indole-utilizing activity of TrpEb required a full-length TrpEa. It appears that these TrpEa proteins, although lacking their own catalytic activity, are still functional in maintaining the TrpEb activity. Even though most of the ocular serovars also had a potentially functional TrpEb, none of them would presumably utilize indole *in vivo* because of the absence of a full-length TrpEa. The authors propose that other bacteria in the microenvironment of genital serovars might be a source of indole, a situation not expected in the microenvironment of ocular serovars. In short, the tissue tropism correlates with ability to convert indole to

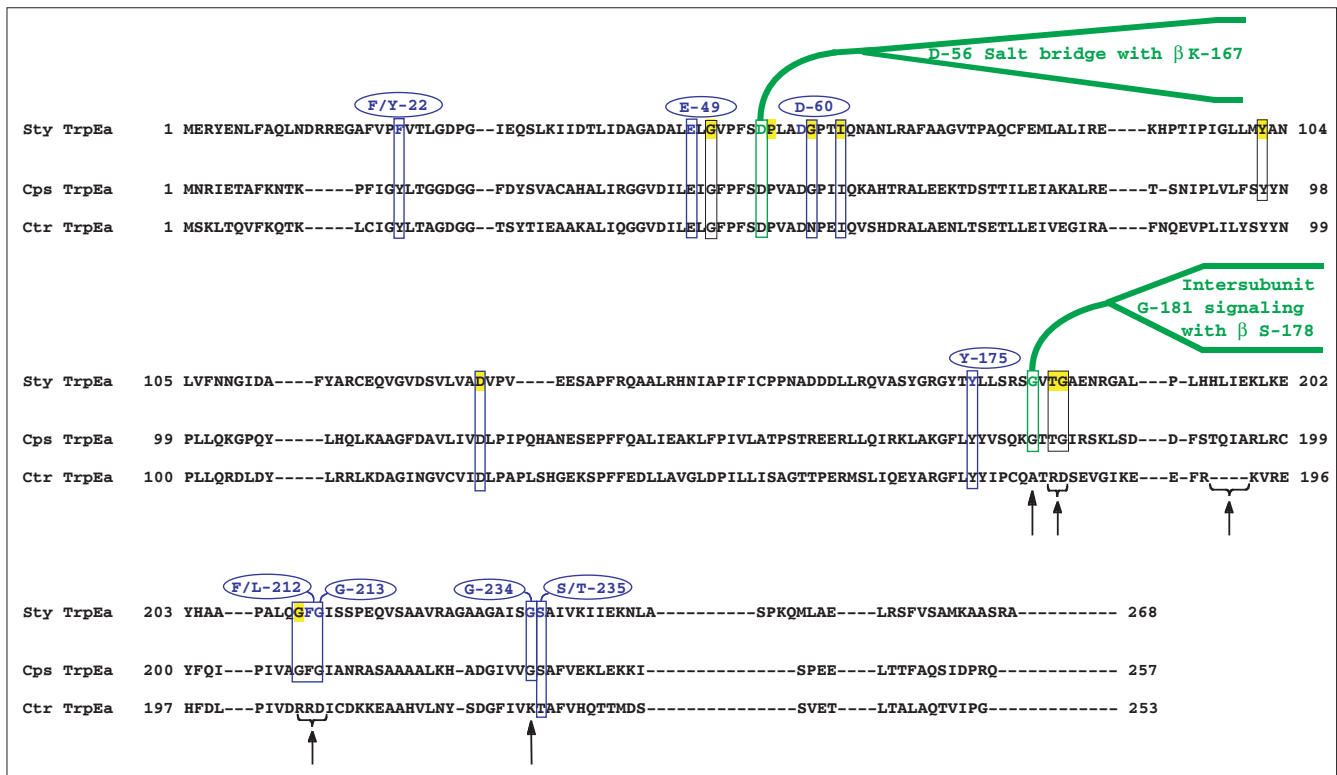


Figure 5

Alignment of TrpEa from *S. typhimurium* (Sty) with the TrpEa proteins of *C. trachomatis* (Ctr) and *C. psittaci* (Cps). The multiple alignment of numerous TrpEa proteins performed as described by Xie *et al.* [30] was used to assess the retention of critical residues by Ctr and Cps. Catalytic residues established in Sty (see [30]) and conserved throughout the multiple alignment are in blue. Residues interacting with the β subunit are in green. Additional residues in yellow are invariant. The gaps represent the gaps obtained in the overall multiple alignment (data not shown). Arrows point to probable inactivating changes in the Ctr sequence. See Table 2 for organism acronyms and gene identification numbers.

L-tryptophan *in vivo*. This seems a reasonable possibility, although it does not explain why most of the ocular serovars have maintained a TrpEb that seems to have resisted reductive evolutionary forces. It would be interesting to know whether the serine deaminase activity of *C. trachomatis* TrpEb (an activity other than indole utilization suggested in this paper) requires full-length TrpEa or not.

Overview of tryptophan-pathway variability in chlamydiae

Shaw *et al.* [43] have correlated the variability in the number of tryptophan-pathway enzymes present in chlamydiae (in particular, the α subunit of tryptophan synthase) with varied sensitivity to IFN- γ treatment [16] and ease of demonstrating the transition to persistence *in vitro*. *C. trachomatis* A, B and C readily develop persistent characteristics following IFN- γ treatment. *C. pneumoniae* also is readily converted to persistence with IFN- γ treatment [45]. Tryptophan has been reported to be essential for growth of serovars A, B and C, but not for growth of *C. psittaci* or serovars D-K and L2 of *C. trachomatis* [45]. Thus, *C. trachomatis* serovars A-C, but not serovars D-K or L1-L3, have been described as 'tryptophan auxotrophs' [43]. Likewise, *C. pneumoniae*, but not

C. psittaci, has been described as a tryptophan auxotroph. Morrison [46] has also discussed the possible relationship of genes present or absent for tryptophan biosynthesis and the differential sensitivities of chlamydiae to inhibitory effects of IFN- γ . Shaw *et al.* [43] imply that *C. trachomatis* D, L2 and *C. psittaci* are competent for tryptophan biosynthesis, unlike *C. trachomatis* A, B, C and *C. pneumoniae*. This correlates nicely with proneness to persistence and the more demonstrable nutritional requirement for tryptophan in the latter strains, but not the former. However, from the current database information available, it appears likely that all chlamydiae, even *C. psittaci*, are tryptophan auxotrophs. As the host itself is incapable of tryptophan biosynthesis, the host is not a credible source of any biosynthetic intermediates. It is also noteworthy that only *C. psittaci* has PRPP synthase, which is needed for provision of PRPP (TrpB step). Thus, there seems to be no basis for the conclusion [43] that variations in *C. trachomatis* serovar pathogenesis can be directly linked to differences in TrpEa, and it therefore seems that the latter differences are coincidental. Differential capabilities for acquisition of the host resources are unknown variables that might distinguish different strains [47]. In this context, it seems possible that variation

Table 2

Key to sequence identifiers		
Genes evaluated	Gene-product acronym	NCBI GI number*
<i>trpC</i> [†]		
<i>Escherichia coli</i>	Eco TrpC	16129223
<i>Chlamydia trachomatis</i> D	Ctra TrpC	7227938
<i>Chlamydia muridarum</i>	Cmu TrpC	8163265
<i>Chlamydophila psittaci</i>	Cpsi TrpC	N/A
<i>Pseudomonas aeruginosa</i>	Pae TrpC	15598309
<i>Neisseria gonorrhoeae</i>	Ngo TrpC	N/A
<i>Neisseria meningitidis</i>	Nme TrpC	15793859
<i>Nitrosomonas europaea</i>	Neu TrpC	N/A
<i>Bordetella pertussis</i>	Bpe TrpC	N/A
<i>Bordetella bronchiseptica</i>	Bbr TrpC	N/A
<i>Xylella fastidiosa</i>	Xfa TrpC	15837975
<i>Thermotoga maritima</i>	Tma TrpC	15642913
<i>Rhodobacter capsulatus</i>	Rca TrpC	N/A
<i>Streptococcus mutans</i>	Smu TrpC	N/A
<i>Aquifex aeolicus</i>	Aae TrpC	15607040
<i>Chlorobium tepidum</i>	Cte TrpC	N/A
<i>Nostoc punctiforme</i>	Npu TrpC	N/A
<i>Streptococcus pneumoniae</i>	Spn TrpC	15901642
<i>Anabaena</i> sp.	Ana TrpC	N/A
<i>Clostridium acetobutylicum</i>	Cac TrpC	15896407
<i>Rhodospseudomonas palustris</i>	Rpa TrpC	N/A
<i>Synechocystis</i> sp.	Syn TrpC	16331130
<i>Haemophilus influenzae</i>	Hin TrpC	16273300
<i>Salmonella typhimurium</i>	Sty TrpC	16765069
<i>Bacillus stearothermophilus</i>	Bst TrpC	N/A
<i>Yersinia pestis</i>	Ype TrpC	16122433
<i>Vibrio cholerae</i>	Vch TrpC	15641184
<i>Pasteurella multocida</i>	Pmu TrpC	15602445
<i>Yersinia pseudotuberculosis</i>	Yps TrpC	N/A
<i>Deinococcus radiodurans</i>	Dra TrpC	15805163
<i>Bacillus subtilis</i>	Bsu TrpC	421536
<i>Escherichia coli</i>	Eco TrpC	16129223
<i>Helicobacter pylori</i>	Hpy TrpC	15645893
<i>Staphylococcus aureus</i>	Sau TrpC	15924361
<i>Bacillus halodurans</i>	Bha TrpC	15614225
<i>Synechococcus</i> sp.	Ssp. TrpC	N/A
<i>Prochlorococcus marinus</i>	Pma TrpC	N/A
<i>Chlamydia trachomatis</i>	Ctr TrpC	15605050
<i>Chlamydia muridarum</i>	Cmu TrpC	15835220
<i>Corynebacterium diphtheriae</i>	Cdi TrpC	N/A
<i>Methanococcus jannaschii</i>	Mja TrpC	15668627
<i>Pyrococcus furiosus</i>	Pfu TrpC	18978079
<i>Archaeoglobus fulgidus</i>	Afu TrpC	11499194
<i>Pyrococcus abyssii</i>	Pab TrpC	N/A
<i>Methanobacterium thermoautotrophicum</i>	Mth TrpC	136346
<i>Methanococcus maripaludis</i>	Mma TrpC	N/A

Table 2 (continued)

Genes evaluated	Gene-product acronym	NCBI GI number*
<i>Ferroplasma acidarmanus</i>	Fac TrpC	N/A
<i>Saccharomyces cerevisiae</i>	Sce TrpC	6320210
<i>Arabidopsis thaliana</i>	Ath TrpC	5031254
<i>trpEa</i> [‡]		
<i>Salmonella typhimurium</i>	Sty TrpEa	16765071
<i>Chlamydia trachomatis</i> D	Ctra TrpEa	15604890
<i>Chlamydophila psittaci</i>	Cpsi TrpEa	N/A
<i>trpEb</i> [§]		
<i>Salmonella typhimurium</i>	Sty TrpEb	136281
<i>Chlamydia trachomatis</i> D	Ctra TrpEb	15604889
<i>Chlamydia psittaci</i>	Cpsi TrpEb1	N/A
<i>Chlamydia psittaci</i>	Cpsi TrpEb2	N/A
Homologs of <i>E. coli</i> TyrP, Mtr and TnaB (Figure 7)		
<i>Chlamydophila psittaci</i>	Cpsi	N/A
<i>Chlamydophila pneumoniae</i> CWL029	Cpn-1	15618876
<i>Chlamydophila pneumoniae</i> CWL029	Cpn-2	15618878
<i>Chlamydia muridarum</i>	Cmu-1	15834824
<i>Chlamydia muridarum</i>	Cmu-2	15834825
<i>Chlamydia trachomatis</i> D	Ctr-1	15605552
<i>Chlamydia trachomatis</i> D	Ctr-2	15605553
<i>Haemophilus ducreyi</i>	Hdu-1	N/A
<i>Haemophilus ducreyi</i>	Hdu-2	N/A
<i>Haemophilus ducreyi</i>	Hdu-3	N/A
<i>Nostoc punctiforme</i>	Npu	N/A
<i>Haemophilus influenzae</i>	Hin-1	16272242
<i>Haemophilus influenzae</i>	Hin-2	16272424
<i>Haemophilus influenzae</i>	Hin-3	16272472
<i>Pasteurella multocida</i>	Pmu-1	15602597
<i>Pasteurella multocida</i>	Pmu-2	15603057
<i>Pasteurella multocida</i>	Pmu-3	15602675
<i>Pasteurella multocida</i>	Pmu-4	15603284
<i>Vibrio cholerae</i>	Vch-1	15601527
<i>Vibrio cholerae</i>	Vch-2	15600930
<i>Escherichia coli</i>	TyrP	16129857
<i>Escherichia coli</i>	Mtr	16131053
<i>Escherichia coli</i>	TnaB	16131577
<i>Yersinia pestis</i>	Ype-1	16121500
<i>Yersinia pestis</i>	Ype-2	16121568
<i>Salmonella typhi</i>	Sty-1	16760884
<i>Salmonella typhi</i>	Sty-2	16762044
<i>Neisseria meningitidis</i>	Nme	15677855
<i>Pseudomonas aeruginosa</i>	Pae-1	15600627
<i>Pseudomonas aeruginosa</i>	Pae-2	15597112
<i>Pseudomonas aeruginosa</i>	Pae-3	15598961
<i>Neisseria gonorrhoeae</i>	Ngo	N/A

Table 2 (continued)

Genes evaluated	Gene-product acronym	NCBI GI number*
Homologs of <i>E. coli</i> SstT and Pgin SstT (Figure 8)		
<i>Chlamydia trachomatis</i> (Group A)	Ctra	gil15605126
<i>Chlamydia muridarum</i> (Group A)	Cmu	gil15835296
<i>Chlamydomphila pneumoniae</i> (Group A)	Cpn	gil15618439
<i>Chlamydia trachomatis</i> (Group B)	Ctra	gil15604951
<i>Chlamydia muridarum</i> (Group B)	Cmu	gil15835119
<i>Chlamydomphila pneumoniae</i> (Group B)	Cpn	gil15618209
<i>Porphyromonas gingivalis</i>	Pgin	N/A
<i>Corynebacterium diphtheriae</i>	Cdip	N/A
<i>Clostridium difficile</i>	Cdi	N/A
<i>Treponema pallidum</i>	Tpa	gil15639544
<i>Vibrio cholerae</i>	Vch	gil15600807
<i>Pseudomonas aeruginosa</i>	Pae	gil15597238
<i>Yersinia pestis</i>	Ype	gil16120913
<i>Escherichia coli</i>	Eco	gil16130984
<i>Yersinia pseudotuberculosis</i>	Ype	N/A
<i>Campylobacter jejuni</i>	Cje	gil15792422
<i>Haemophilus influenzae</i>	Hin	gil16273445
<i>Pasteurella multocida</i>	Pmu	gil15602756
<i>Neisseria meningitidis</i>	Nme	gil15793312
<i>Neisseria gonorrhoeae</i>	Ngo	N/A
<i>Haemophilus ducreyi</i>	Hdu	N/A
<i>Enterococcus faecium</i>	Efa	N/A
<i>Streptococcus pyogenes</i>	Spy	gil15674487
<i>Streptococcus pneumoniae</i>	Spn	gil15903640
<i>Streptococcus equi</i>	Seq	N/A

*N/A applies to genes not yet included in the NCBI database. †Includes in addition to identifiers of the four sequences shown in Figure 4, the sequence identifiers for the sequences used in the multiple alignment that supports the gaps and conserved residues indicated on line 1 of Figure 4. ‡See [30] for the sequence identifiers used in the multiple alignment which supports the gaps and conserved residues indicated on line 1 of Figure 5. §See [29] for the sequence identifiers used in the multiple alignment which support the gaps and conserved residues indicated on line 1 of Figure 6.

in Lifa integrity or copy number could easily explain variation in IFN- γ sensitivity and the nutritional requirement for L-tryptophan *in vitro*.

Except for *C. psittaci*, we may be seeing different strains in varied states of reductive evolution with respect to what remnants of genes for tryptophan biosynthesis remain. *C. pneumoniae* and *C. trachomatis* B lack genes encoding the entire tryptophan pathway. *C. trachomatis* D possesses *trpC*, *trpEa* and *trpEb*; *C. muridarum* possesses only *trpC*; *C. trachomatis* A and C possess *trpEa* and *trpEb*. The truncation of *trpEa* in *C. trachomatis* A and C, the likely pseudo-gene status of *trpEa* in serovar D, and the absence of *trpEa*

altogether in serovar B indicates an active ongoing process of reductive evolution.

Chlamydial transport of tryptophan, kynurenine and serine

The chlamydiae are dependent on host cells for a variety of metabolites that are relevant to the host-parasite relationships of tryptophan metabolism. These include kynurenine, serine, ATP and tryptophan itself. Hence, the nature and variability for transport of these compounds should be of considerable interest.

Species of chlamydiae possess one (*C. psittaci*) or two (*C. muridarum*, *C. trachomatis* and *C. pneumoniae*) homologs of genes encoding the well characterized hydroxy/aromatic amino acid (HAAAP) permease family [48]. Figure 7 presents an unrooted radial tree that shows the chlamydial proteins to comprise a distinct cluster. *E. coli* Mtr (high-affinity tryptophan permease) and TnaB (low-affinity tryptophan permease) comprise one distinct group, and *E. coli* TyrP homologs make up another distinct group. The chlamydial sequences are approximately equidistant from the TyrP/Mtr-TnaB groupings. These chlamydial proteins might be broad-specificity transporters of tryptophan, tyrosine, phenylalanine and perhaps kynurenine as well.

When the Na⁺-coupled serine symporter SdaC from *E. coli* was used as a query against the chlamydial genomes, TyrP was the top hit (22% identity). This reflects the membership of SdaC proteins in the HAAAP family. An alternative query, the *E. coli* CycA serine/alanine/glycine transporter, yielded CT216 as the top hit (only 22% identity). *E. coli* possesses a Na⁺-coupled serine symporter, SstT, which is regulated by tryptophan [49]. Although this is but one of at least five different transporters for serine in *E. coli*, *sstT* encodes the sole serine/threonine transporter in *Porphyromonas gingivalis* [50]. Species of chlamydiae possess two paralogs of SstT, which were judged to be the most likely genes encoding serine transport. Perhaps one favors serine transport and the other threonine transport. Figure 8 shows an unrooted tree of SstT proteins.

The tryptophan repressor

The presence of *trpR* implies that the tryptophan operon is under repression control by L-tryptophan, and some experimental evidence does indeed show derepression under conditions of tryptophan limitation [43]. Starvation for host-derived L-tryptophan, which is initiated by induction of indoleamine dioxygenase by IFN- γ , undoubtedly triggers derepression of the entire tryptophan operon, including the genes encoding PRPP synthase and kynureninase. Kynureninase from *C. psittaci* is a key linker between the anthranilate-utilizing TrpB enzyme that initiates tryptophan biosynthesis in the parasite and the host kynurenine formidase that generates kynurenine. In effect a hybrid host-parasite cycle is generated in which a metabolic stream in

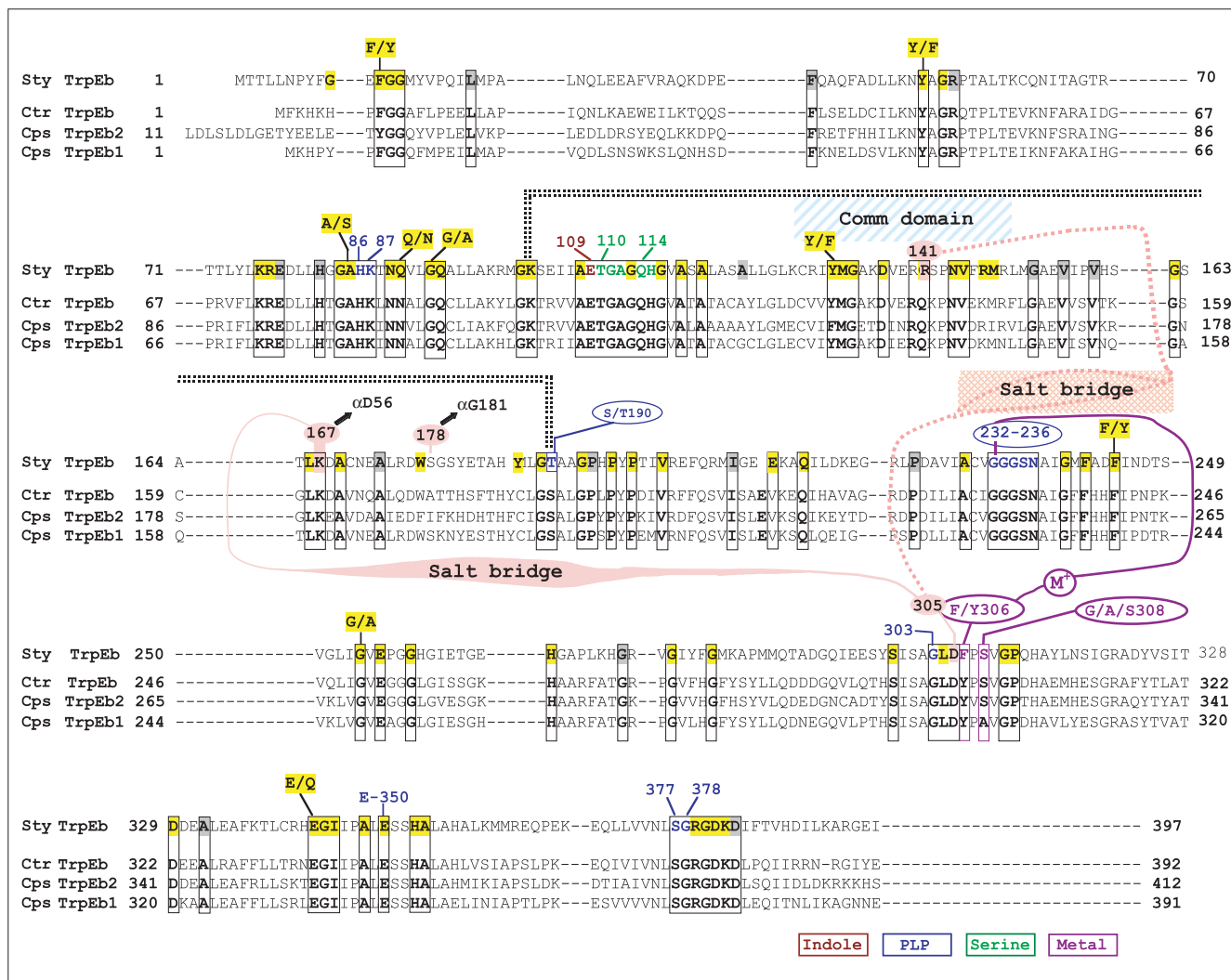


Figure 6

Alignment of TrpEb from *S. typhimurium* (Sty) with the TrpEb proteins from *C. trachomatis* (Ctr) and *C. psittaci* (Cps). Cps TrpEb1 is the paralog within the operon shown in Figure 1. Residues in the Sty sequence that are invariant in an extensive multiple alignment are marked in yellow and near-invariant residues are shaded in gray. Substrate, cofactor and metal-coordinating residues are color-coded as indicated by the key at bottom right. The movable COMM domain that interacts with the α subunit is marked, as well as residues K167 and S178 (not conserved) which contact the indicated residues of the α subunit. Residues critical for formation of internal salt bridges are also shown. See Xie et al. [30] for a detailed analysis. See Table 2 for organism acronyms and gene identification numbers.

the host away from tryptophan is intercepted by a metabolic stream toward tryptophan in *C. psittaci*.

The thoroughly studied repressor protein that regulates tryptophan biosynthesis in *E. coli* is of limited phylogenetic distribution. In fact, *Xylella fastidiosa*, *C. trachomatis* and *C. psittaci* are the only organisms outside the enteric lineage known to possess *trpR*. The GC content of *trpR* was examined for evidence of possible horizontal transfer. Table 3 lists the GC content of *trpR* genes, compared to the GC content of the corresponding genomes. The *trpR* GC content of each organism corresponded relatively well to the genomic GC content, except for *X. fastidiosa* where *trpR* exhibited a low

GC content, more similar to that of the chlamydiae or *H. influenzae*.

Does *C. psittaci* have a regulon controlled by *trpR*?

In *E. coli* the *trp* repressor binds upstream of the *trp* operon and upstream at the *mtr* transport gene in promoter regions where CTCG or CTAG are important for binding [51]. The *sstT* gene of *E. coli* is also subject to repression control by tryptophan and a CTCG upstream region has been proposed to be an additional target region for TrpR [49].

As *C. psittaci* has *trpR*, it seems quite possible that the *trp* operon, *sstT* (for serine transport), and *tyrP* (for tryptophan

Table 3**GC content of *trpR* genes compared to the GC content of the corresponding genomes**

Organism	%GC	
	Genome	<i>trpR</i>
<i>Chlamydia psittaci</i>	42	37
<i>Chlamydia trachomatis D</i>	42	37
<i>Escherichia coli</i>	51	54
<i>Salmonella typhimurium</i>	55	54
<i>Haemophilus influenzae</i>	38	36
<i>Vibrio cholerae</i>	48	50
<i>Enterobacter aerogenes</i>	61	58
<i>Yersinia pestis</i>	50	51
<i>Yersinia pseudotuberculosis</i>	43	50
<i>Pasteurella multocida</i>	38	43
<i>Actinobacillus actinomycetemcomitans</i>	39	44
<i>Xylella fastidiosa</i> *	54	34
<i>Enterobacter cloacae</i>	56	58

**trpR* from *X. fastidiosa* has been found to be part of a low-GC block of genes, for which there is strong evidence for lateral gene transfer (G.X., C.A.B., N. Keyhani, and R.A.J., unpublished observations).

transport) might comprise a regulon controlled by the *trpR* repressor. Indeed, CTAG and/or CTCG motifs were found upstream of all these genes in *C. psittaci*, but the presence of these regions did not exceed random probability sufficiently to justify any concrete assertions. The further use of a computational approach [52] to identify the transcription regulatory pattern was also not illuminating. As chlamydial TrpR proteins are the most divergent of TrpR proteins, the motif pattern for DNA binding may also be divergent.

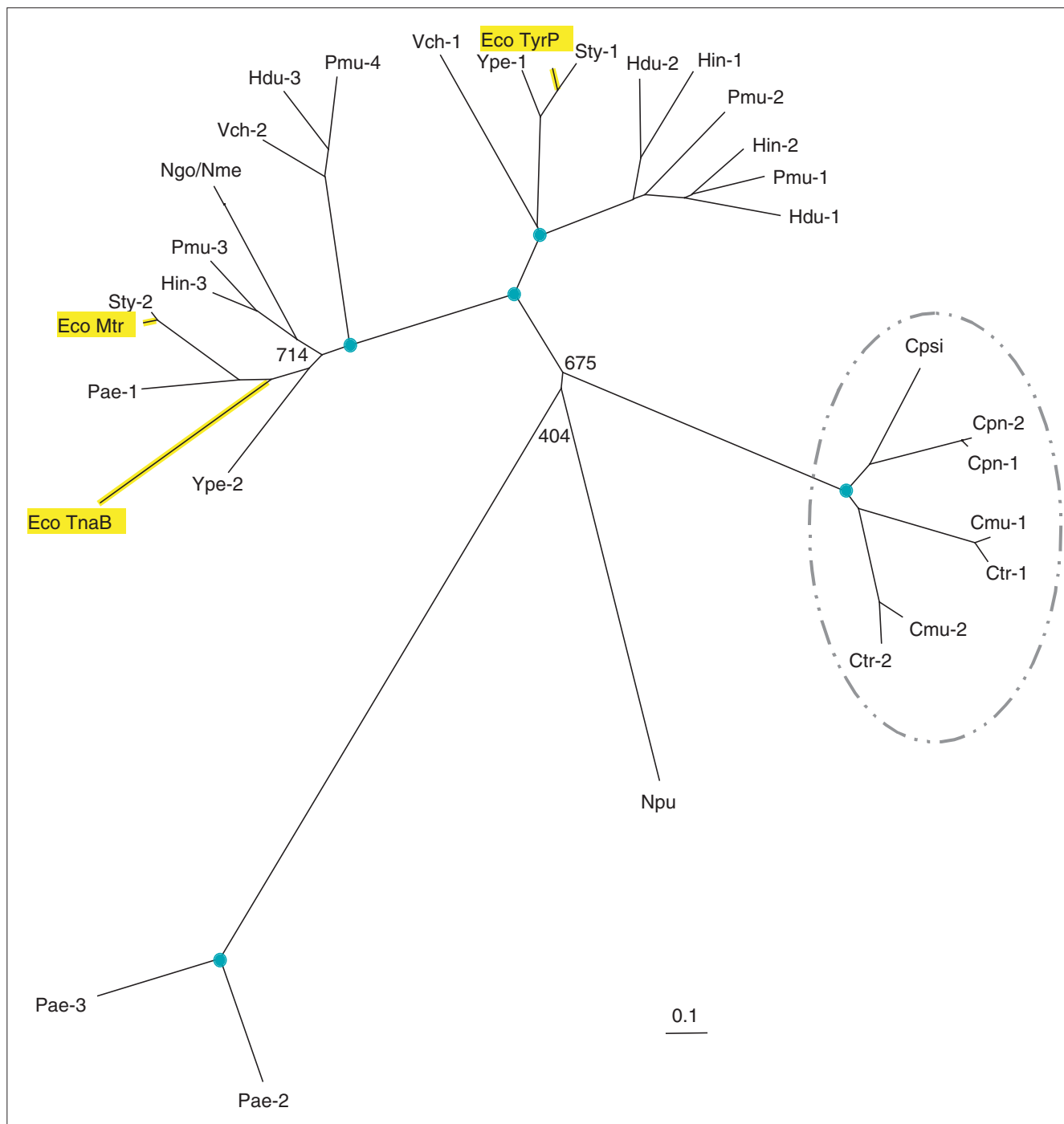
Conclusions

Chlamydial parasites appear to respond to host mechanisms for restriction of tryptophan availability with a diversity of strategies, the exact nature of which we still know very little. One strategy utilized by *C. muridarum* employs a three-copy dose of *lifA*, which could inhibit cytokine proliferation and decrease IFN- γ production by the host (although at least some IFN- γ has been shown to be produced in mice infected with *C. muridarum* [53]). Such a strategy would seem to be consistent with acute infections, which can be a successful mode of pathogenicity in some situations, such as high-density rodent populations. In the case of *C. muridarum*, the mechanism does not necessarily involve blocking the induction of indoleamine dioxygenase and thereby preventing depletion of host tryptophan, in view of some experimental work with the MoPn/mouse model system. The primary effect of the IFN- γ may not be to cause starvation for tryptophan as anti-chlamydial and anti-proliferative activities of murine IFN- γ in mouse cells was not reversible by tryptophan [54]. It has

been concluded in other studies as well [55,56] that host-mediated tryptophan depletion is not an important factor in the MoPn/mouse model system. However, a conflicting conclusion was recently published by Perfettini *et al.* [57] that the inhibitory effect of IFN- γ in mouse cell cultures and genital tracts seems to be due in part to tryptophan depletion (because inhibition was partially reversed by tryptophan). It has been generally observed that in murine systems indoleamine dioxygenase has not been induced by IFN- γ under the same experimental conditions that readily induce synthesis of the enzyme in human systems. However, the potential to induce the dioxygenase in murine systems by IFN- γ exposure has been demonstrated when nitric oxide production is blocked [41]. Thus, it seems that the key difference between the mouse and human systems might lie in the details that dictate the balance between the cross-regulated pathways of tryptophan and arginine metabolism (see Figure 2). If so, in human systems the balance favors domination by the indoleamine dioxygenase route, whereas in murine systems the nitric oxide route is favored. Many individual factors could influence the balance, including differential sensitivities of NO synthase to inhibition by 3-hydroxyanthranilate or of indoleamine dioxygenase to inhibition by nitric oxide. In addition, unidentified cytokine-mediated mechanisms undoubtedly await elucidation.

In any event, for most chlamydiae the successful implementation of tryptophan depletion by the host is closely tied to chlamydial responses that result in a persistent metabolic state and chronic disease. It would appear that chlamydiae have learned to recognize and exploit tryptophan depletion as an environmental cue that initiates a distinct chain of remodeling biochemistry that promotes long-term viability in a latent state.

It is difficult to guess whether the ability of *C. psittaci* to scavenge kynurenine as a source of tryptophan is a recent innovation in this lineage, or whether it represents an ancient chlamydial strategy that has been abandoned by other chlamydiae. Perhaps the strategy is linked to the particular tissue tropism of *C. psittaci*. Because the presence of the tryptophan-pathway enzymes varies so markedly, even between closely related strains, one cannot be certain at this point that the tryptophan-recapture cycle is uniquely characteristic of *C. psittaci*. It cannot be ruled out that other strains of *C. psittaci* might lack the cycle, whereas some strains of *C. trachomatis* or *C. pneumoniae* not yet sequenced might possess it. Different overall host metabolism and different biochemical environments in various tissues undoubtedly influence the availability of kynurenine (as well as other metabolites such as ATP and serine). Hence, tissue tropism must be a crucial factor. It would be interesting to know whether the tryptophan-recapture cycle is capable of providing abundant tryptophan to the parasite, or whether it might be more subtly geared to provision of barely adequate tryptophan for parasites established in the chronic-infection mode.

**Figure 7**

Unrooted phylogenetic tree (radial view) of the TyrP family of transport proteins. A multiple alignment was obtained by input of the indicated sequences into the ClustalW program (version 1.4). Manual alignment adjustments were made with the assistance of the BioEdit multiple alignment tool of Hall [61]. The refined multiple alignment was used as input for generation of a phylogenetic tree using the program packages PHYLIP [62] and PHYLO_WIN [63]. The neighbor-joining and Fitch programs were used to obtain distance-based trees. The distance matrix was obtained using Protodist with a Dayhoff PAM matrix. The Seqboot and Consense programs were then used to assess the statistical strength of the tree using bootstrap resampling. Neighbor-joining (shown) and Fitch trees yielded similar clusters and arrangement of taxa within them. Bootstrap values of 1,000 per 1,000 iterations (indicated with blue circles) supported the major nodes, one of which contains all of the chlamydial proteins. The experimentally documented *E. coli* proteins TyrP (tyrosine transport), Mtr (high-affinity tryptophan transport) and TraB (low-affinity tryptophan transport) are highlighted in yellow. See Table 2 for organism acronyms and gene identification numbers. In contrast to the two paralogs of *tyrP* present in *C. pneumoniae* CWLO29, as shown, some intra-species variation occurs in that *C. pneumoniae* J138 has only a single *tyrP* gene, and a small hypothetical gene is inserted between the two paralog *tyrP* genes of *C. pneumoniae* AR39.

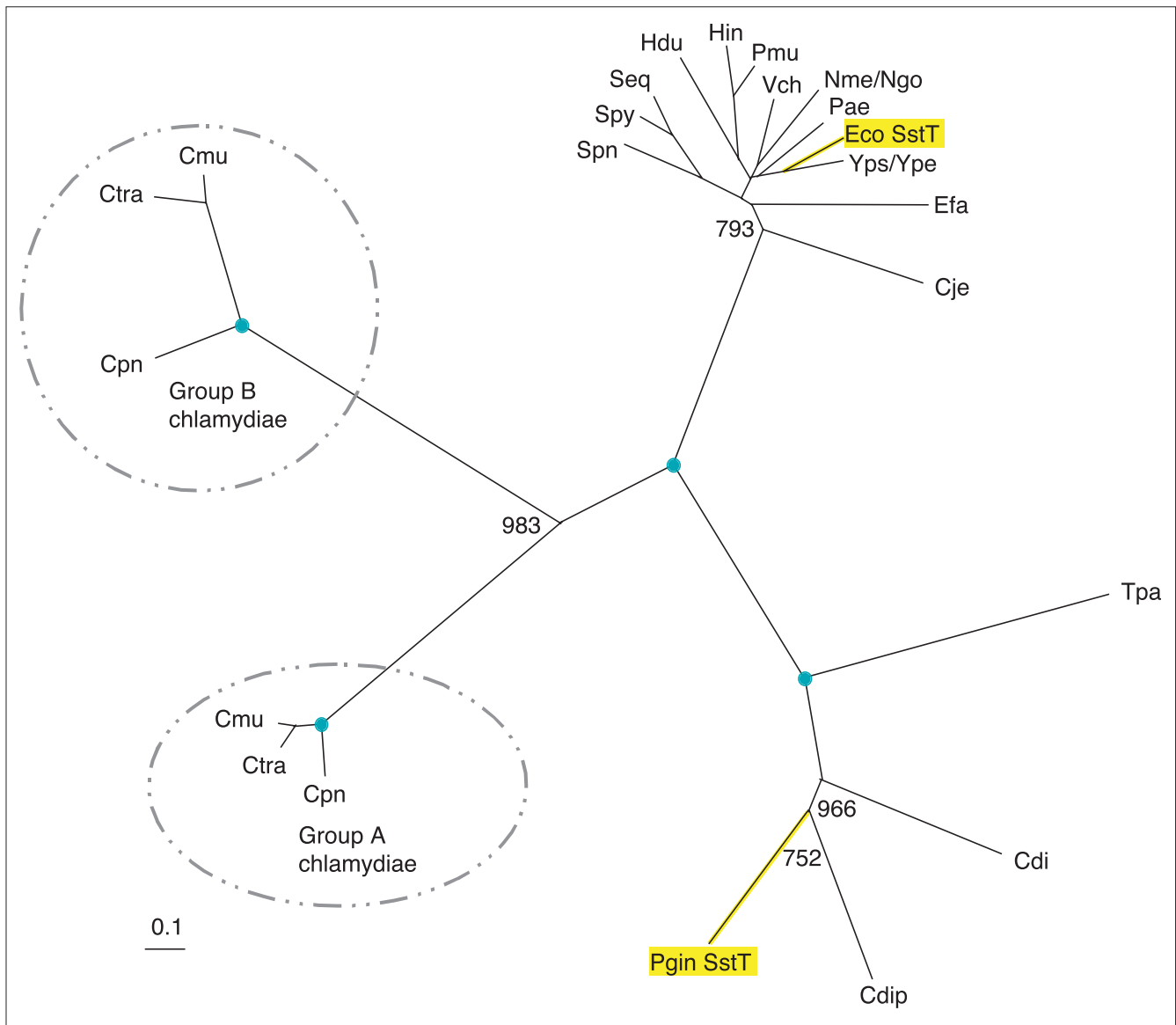


Figure 8
 Unrooted phylogenetic tree (radial view) of the SstT family of transport genes. The data analysis was performed as described in the legend for Figure 7. The experimentally documented SstT proteins of serine transport in *E. coli* (Eco) and *Porphyromonas gingivalis* (Pgin) are highlighted in yellow. Bootstrap values at the major node positions are shown, and those nodes supported by 1,000 of 1,000 iterations are denoted with blue circles. See Table 2 for organism acronyms and gene identification numbers.

Acknowledgements

Preliminary sequence data was obtained from the public website of The Institute for Genomic Research [58].

References

1. Kalman S, Mitchell W, Marathe R, Lammel C, Fan J, Hyman RW, Olinger L, Grimwood J, Davis RW, Stephens RS: **Comparative genomes of *Chlamydia pneumoniae* and *C. trachomatis***. *Nat Genet* 1999, **21**:385-389.
2. Read TD, Brunham RC, Shen C, Gill SR, Heidelberg JF, White O, Hickey EK, Peterson J, Utterback T, Berry K, et al: **Genome sequences of *Chlamydia trachomatis* MoPn and *Chlamydia pneumoniae* AR39**. *Nucleic Acids Res* 2000, **28**:1397-1406.
3. Rockey DD, Lenart J, Stephens RS: **Genome sequencing and our understanding of chlamydiae**. *Infect Immun* 2000, **68**:5473-5479.
4. Beatty WL, Morrison RP, Byrne GI: **Persistent chlamydiae: from cell culture to a paradigm for chlamydial pathogenesis**. *Microbiol Rev* 1994, **58**:686-699.
5. Dreses-Werringloer U, Padubrin I, Jürgens-Saathoff B, Hudson AP, Zeidler H, Köhler L: **Persistence of *Chlamydia trachomatis* is induced by ciprofloxacin and ofloxacin in vitro**. *Antimicrob Agents Chemother* 2000, **44**:3288-3297.
6. Jones ML, Gaston JSH, Pearce JH: **Induction of abnormal *Chlamydia trachomatis* by exposure to interferon- γ or amino acid deprivation and comparative antigenic analysis**. *Microb Pathogen* 2001, **30**:299-309.
7. Harper A, Pogson CI, Jones ML, Pearce JH: **Chlamydial development is adversely affected by minor changes in amino acid**

- supply, blood plasma amino acid levels, and glucose deprivation. *Infect Immun* 2000, **68**:1457-1464.
8. Taylor-Robinson D, Gilroy CB, Thomas BJ, Keat ACS: **Detection of *Chlamydia trachomatis* DNA in joints of reactive arthritis patients by polymerase chain-reaction.** *Lancet* 1992, **340**:81-82.
 9. Cotter TW, Ramsey KH, Miranpuri GS, Poulsen CE, Byrne: **Dissemination of *Chlamydia trachomatis* chronic genital tract infection in gamma interferon gene knock-out mice.** *Infect Immun* 1997, **65**:2145-2152.
 10. Perry LL, Su H, Felizler K, Messer R, Hughes S, Whitmire W, Caldwell HD: **Differential sensitivity of distinct *Chlamydia trachomatis* isolates to IFN- γ -mediated inhibition.** *J Immunol* 1999, **162**:3541-3548.
 11. Igietseme JU, Ananaba GA, Candal DH, Lyn D, Black CM: **Immune control of chlamydia growth in the human epithelial cell line RT4 involves multiple mechanisms that include nitric oxide induction, tryptophan catabolism and iron deprivation.** *Microbial Immunol* 1998, **42**:617-625.
 12. Beatty WI, Belanger TA, Desai AA, Morrison RP, Byrne GI: **Tryptophan depletion as a mechanism of gamma interferon-mediated chlamydial persistence.** *Infect Immun* 1994, **62**:3705-3711.
 13. Byrne GI, Oeyjahkn LK, Landry GJ: **Induction of tryptophan catabolism is the mechanism for gamma-interferon-mediated inhibition of intracellular *Chlamydia psittaci* replication in T24 cells.** *Infect Immun* 1986, **53**:347-351.
 14. Byrne GI, Lehmann LK, Kirschbaum JG, Borden EC, Lee CM, Brown RR: **Induction of tryptophan degradation *in vitro* and *in vivo*: a gamma interferon-stimulated activity.** *J Interferon Res* 1986, **6**:389-396.
 15. Taylor MW, Feng GS: **Relationship between interferon-gamma, indoleamine 2,3-dioxygenase, and tryptophan catabolism.** *FASEB J* 1991, **5**:2516-2522.
 16. Mehta SJ, Miller RD, Ramirez JA, Summersgill JT: **Inhibition of *Chlamydia pneumoniae* replication in HEP-2 cells by interferon-gamma: role of tryptophan catabolism.** *J Infect Dis* 1998, **177**:1326-1331.
 17. Bertazzo A, Ragazzi E, Biasiolo M, Costa CVL, Allergri G: **Enzyme activities involved in tryptophan metabolism along the kynurenine pathway in rabbits.** *Biochim Biophys Acta* 2001, **1527**:167-175.
 18. Haber R, Bessette D, Hulihan-Giblin B, Durcan MJ, Goldman, D: **Identification of tryptophan 2,3-dioxygenase RNA in rodent brain.** *J Neurochem* 1993, **60**:1159-1162.
 19. Fleckner J, Rasmussen HH, Justesen J: **Human interferon γ potentially induces the synthesis of a 55-kDa protein (γ 2) highly homologous to rabbit peptide chain release factor and bovine tryptophanyl-tRNA synthetase.** *Proc Natl Acad Sci USA* 2002, **88**:11520-11524.
 20. Pfefferkorn ER: **Interferon gamma blocks the growth of *Toxoplasma gondii* in human fibroblasts by inducing the host cells to degrade tryptophan.** *Proc Natl Acad Sci USA* 1984, **81**:908-912.
 21. MacKenzie CR, Langen R, Takikawa O, Daubener W: **Inhibition of indoleamine 2,3-dioxygenase in human macrophages inhibits interferon- γ -induced bacteriostasis but does not abrogate toxoplasmatosis.** *Eur J Immunol* 1999, **29**:3254-3261.
 22. Kudo, Y, Boyd CAR, Sargent IL, Redman CWG: **Tryptophan degradation by human placental indoleamine 2,3-dioxygenase regulates lymphocyte proliferation.** *J Physiol* 2001, **535**:207-215.
 23. Munn DH, Zou M, Attwood JT, Bondarev I, Conway SJ, Marshall B, Brown C, Mellor AL: **Prevention of allogeneic fetal rejection by tryptophan catabolism.** *Science* 1998, **281**:1191-1193.
 24. Widner B, Ledochowski M, Fuchs D: **Interferon-gamma-induced tryptophan degradation: neuropsychiatric and immunological consequences.** *Curr Drug Metab* 2000, **1**:193-204.
 25. Klapproth J-M, Scaletsky IC, McNamara BP, Lai L-C, Malstrom C, James SP, Donnenberg MS: **A large toxin from pathogenic *Escherichia coli* strains that inhibits lymphocyte activation.** *Infect Immun* 2000, **68**:2148-2155.
 26. Entrican G, Brown J, Graham S: **Cytokines and the protective host immune response to *Chlamydia psittaci*.** *Comp Immun Microbiol Infect Dis* 1998, **21**:15-26.
 27. Shemer Y, Sarov I: **Inhibition of growth of *Chlamydia trachomatis* by human gamma interferon.** *Infect Immun* 1985, **48**:592-596.
 28. Brown J, Howie SEM, Entrican G: **A role for tryptophan in immune control of chlamydial abortion in sheep.** *Vet Immunol Immunopath* 2001, **82**:107-119.
 29. Belland RJ, Scidmore MA, Crane DD, Hogan DM, Whitmire W, McClarty G, Caldwell HD: ***Chlamydia trachomatis* cytotoxicity associated with complete and partial cytotoxin genes.** *Proc Natl Acad Sci USA* 2001, **98**:13984-13989.
 30. Xie G, Forst C, Bonner C, Jensen RA: **Significance of two distinct types of tryptophan synthase beta chain in Bacteria, Archaea and higher plants.** *Genome Biol* 2001, **3**:research0004.1-0004.13.
 31. Ilife-Lee ER, McClarty G: **Regulation of carbon metabolism in *Chlamydia trachomatis*.** *Mol Microbiol* 2000, **38**:20-30.
 32. Moroni F: **Tryptophan metabolism and brain function: focus on kynurenine and other indole metabolites.** *Eur J Pharmacol* 1999, **375**:87-100.
 33. MacKenzie CR, Willberg CB, Daubener W: **Inhibition of group B streptococcal growth by IFN gamma-activated human glioblastoma cells.** *J Neuroimmunol* 1998, **89**:191-197.
 34. Saito K, Crowley JS, Markey SP, Heyes MP: **A mechanism for increased quinolinic acid formation following acute systemic immune stimulation.** *J Biol Chem* 1993, **268**:15496-15503.
 35. Chiarugi A, Rapizzi E, Moroni F, Moroni F: **The kynurenine metabolic pathway in the eye: studies on 3-hydroxykynurenine, a putative cataractogenic compound.** *FEBS Lett* 1999, **453**:197-200.
 36. Bender, DA: **The kynurenine pathway of tryptophan metabolism.** In *Quinolinic Acid and the Kynurenines*. Edited by Stone TW. New York: CRC Press; 1989: 3-38.
 37. Toma S, Nakamura M, Tone S, Okuno E, Kido R, Breten J, Avanzi N, Cozzi L, Speciale C, Mostardini M, *et al.*: **Cloning and recombinant expression of rat and human kynureninase.** *FEBS Lett* 1997, **408**:5-10.
 38. Koushik SV, Sundararaju B, McGraw RA, Phillips RS: **Cloning, sequence, and expression of kynureninase from *Pseudomonas fluorescens*.** *Arch Biochem Biophys* 1997, **344**:301-308.
 39. Sekkaï D, Guitteï O, Lemaire G, Tenu J-P, Lepoivre M: **Inhibition of nitric oxide synthase expression and activity in macrophages by 3-hydroxyanthranilic acid, a tryptophan metabolite.** *Arch Biochem Biophys* 1997, **340**:117-123.
 40. Grant RS, Passey R, Matanovic G, Smythe G, Kapoor V: **Evidence for increased *de novo* synthesis of NAD in immune-activated RAW264.7 macrophages: a self-protective mechanism?** *Arch Biochem Biophys* 1999, **372**:1-7.
 41. Thomas SR, Mohr D, Stocker R: **Nitric oxide inhibits indoleamine 2,3-dioxygenase activity in interferon-gamma primed mononuclear phagocytes.** *J Biol Chem* 1994, **269**:14457-14464.
 42. Werner-Felmayer G, Werner ER, Fuchs D, Hausen A, Reibnegger G, Wächter H: **Characteristics of interferon induced tryptophan metabolism in human cells *in vitro*.** *Biochim Biophys Acta* 1989, **1012**:140-147.
 43. Shaw AC, Christiansen G, Roepstorff P, Birkelund S: **Genetic differences in the *Chlamydia trachomatis* tryptophan synthase alpha-subunit can explain variations in serovar pathogenesis.** *Microbes Infect* 2000, **2**:581-592.
 44. Fehner-Gardiner C, Roshick C, Carlson JH, Hughes S, Belland RJ, Caldwell HD, McClarty G: **Molecular basis defining human *Chlamydia trachomatis* tissue tropism: a possible role for tryptophan synthase.** *J Biol Chem* 2002, **277**:26893-26903.
 45. Kuo CC, Grayston T: **Amino acid requirements for growth of *Chlamydia pneumoniae* in cell cultures: growth enhancement by lysine or methionine depletion.** *J Clin Microbiol* 1990, **28**:1098-1100.
 46. Morrison RP: **Differential sensitivities of *Chlamydia trachomatis* strains to inhibitory effects of gamma interferon.** *Infect Immun* 2000, **68**:6038-6040.
 47. Kane CD, Vena RM, Ouellette SP, Byrne GI: **Intracellular tryptophan pool sizes may account for differences in gamma interferon-mediated inhibition and persistence of chlamydial growth in polarized and nonpolarized cells.** *Infect Immun* 1999, **67**:1666-1671.
 48. Saier MHJ: **Families of transmembrane transporters selective for amino acids and their derivatives.** *Microbiology* 2000, **146**:1775-1795.
 49. Ogawa W, Kim Y-M, Mizushima T, Tsuchiya T: **Cloning and expression of the gene for the Na⁺-coupled serine transporter from *Escherichia coli* and characteristics of the transporter.** *J Bacteriol* 1998, **180**:6749-6752.

50. Dashper SG, Brownfield L, Slakeski N, Zilm PS, Rogers AH, Reynolds EC: **Sodium ion-driven serine/threonine transport in *Porphyromonas gingivalis***. *J Bacteriol* 2001, **183**:4142-4148.
51. Czernik PJ, Shin DS, Hurlbert BK: **Functional selection and characterization of DNA binding sites for trp repressor of *Escherichia coli***. *J Biol Chem* 1994, **269**:27869-27875.
52. Mironov AA, Koonin EV, Royberg MA, Gelfand MS: **Computer analysis of transcription regulatory patterns in completely sequenced bacterial genomes**. *Nucleic Acids Res* 1999, **27**:2981-2989.
53. Beale AS: **Does *Chlamydia trachomatis* MoPn enter microbologically-inapparent state during experimental infection of the mouse genital tract?** *Microb Pathogen* 1997, **22**:99-112.
54. de la Maza LM, Peterson EM, Gennie CW, Czarniecki CW: **The anti-chlamydial and anti-proliferative activities of recombinant murine interferon- γ are not dependent on tryptophan concentrations**. *J Immunol* 1985, **135**:4198-4200.
55. Ramsey KH, Miranpuri GS, Poulsen CE, Marthakis NB, Braune LM, Byrne GI: **Inducible nitric oxide synthase does not affect resolution of murine chlamydial genital tract infections or eradication of chlamydiae in primary murine cell culture**. *Infect Immun* 1998, **66**:835-838.
56. Ramsey KH, Miranpuri GS, Sigar IM, Ouellette S, Byrne GI: ***Chlamydia trachomatis* persistence in the female mouse genital tract: inducible nitric oxide synthase and infection outcome**. *Infect Immun* 2001, **69**:5131-5137.
57. Perfettini J-L, Darville T, Dautry-Varsat A, Rank RG, Ojcius DM: **Inhibition of apoptosis by gamma interferon in cells and mice infected with *Chlamydia muridarum* (the mouse pneumonitis strain of *Chlamydia trachomatis*)**. *Infect Immun* 2002, **70**:2559-2565.
58. **The Institute of Genomic Research** [<http://www.tigr.org>]
59. Ponting CP: **Chlamydial homologues of the MACPF (MAC/perforin) domain**. *Curr Biol* 1999, **9**:R911-R913.
60. **PDBsum** [<http://www.biochem.ucl.ac.uk/bsm/pdbsum/index.html>]
61. Hall T: *Biological Sequence Alignment Editor for Windows 95/98/NT, 5.09 ed*. Raleigh, NC: North Carolina State University; 1997-2001. [<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>]
62. Felsenstein J: **PHYLIP - phylogeny inference package (version 3.2)**. *Cladistics* 1989, **5**:164-166.
63. Galtier N, Douy M, Gautier C: **SEAVIEW and PHYLO_WIN: two graphic tools for sequence alignment and molecular phylogeny**. *Comput Appl Biosci* 1996, **12**:543-548.