Closing in on *Chlamydia* and its intracellular bag of tricks

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Overview

The genus *Chlamydia* encompasses a unique class of obligate intracellular bacteria, which cause infections in a wide range of animals and contribute to prevalent sexually transmitted, ocular and respiratory tract infectious diseases in humans. Consequences of chronic disease, to which *Chlamydia* owes most of its notoriety, include female infertility, blindness, arthritis and possibly coronary heart disease. In contrast to its pathogenic prominence, relatively little is known about the molecular mechanisms of *Chlamydia* infection, owing mostly to its unique intracellular developmental life style and resistance to genetic manipulation. Recent genomic information, however, in combination with new findings on the molecular and cell biology of infection, have allowed new insights into the biology of these unique organisms. The emerging picture is that of a micro-organism exploiting and subverting host signalling and trafficking pathways to its own developmental and pathogenic ends. To do so, the bacteria express a contact-dependent type III secretion (TTS) pathway, similar to that found in many other bacterial pathogens. Here, we suggest that *Chlamydia* TTS promotes bacterial survival and growth upon translocation of signals across the *Chlamydia* vacuole membrane. We hypothesize that these signals may be responsible for a range of activities, including inhibition of phagolysosome fusion, upregulation of transporters, and diversion of nutrients and constituent molecules from normal eukaryotic trafficking pathways. We further propose that inactivation of TTS upon loss of contact may provide a simple signal for the bacteria to irreversibly engage in late differentiation.

Background

Various species of *Chlamydia* continue to provoke serious infections of humans and animals worldwide, despite extensive work to better characterize the biology of the infection and develop effective vaccines. It is estimated that over 600 million people are infected with *Chlamydia trachomatis*, strains of which include the most common sexually transmitted bacterial pathogens (Gerbase *et al.*, 1998) and also causative agents of conjunctivitis and trachoma. The latter are responsible for between 5 and 6 million current cases of blindness, thus representing the world’s leading cause of infectious blindness (Thylefors *et al.*, 1995). More recently, there has been increasing interest in characterizing infections by *Chlamydia pneumoniae*, which are responsible for approximately a tenth of pneumonia cases in industrialized countries, and especially to determine if there is a link between previous infections with *C. pneumoniae* and increased risk of developing atherosclerosis (Grayston, 1999).

Chlamydiae have a biphasic developmental cycle. The extracellular form, the elementary body (EB), is infectious and is thought to be metabolically inert. The EB are internalized into host epithelial cells via small vacuoles resembling endosomes, most of which avoid fusion with host cell lysosomes. The EB differentiates within the entry vacuole into metabolically active reticulate bodies (RB), which are non-infectious (Moulder, 1991). Until now, efforts to understand molecular aspects of the infection have been hampered by systematic difficulties, including the lack of genetic tools to manipulate *Chlamydia*. In fact, *Chlamydia* is one of the rare human pathogens of any clinical importance that cannot be modified genetically. A second problem arises from the need to grow and extract chlamydiae from cultured eukaryotic cells, which leads to inactivation, alteration and contamination of the organisms. In the face of these difficulties, other lines of investigation, such as genome sequencing, assume disproportionate significance. Indeed, much information on this group of pathogens is currently being gleaned from comparative genomic analyses.
**Comparative genomics**

Extensive comparisons of the *Chlamydia* genomes among themselves and with those of other organisms have recently been published (Kalman *et al*., 1999; Makarova *et al*., 2000; Read *et al*., 2000; Stephens *et al*., 1998; Zomorodipour & Andersson, 1999). From these, a global understanding of chlamydial metabolic and biosynthetic capacities (McClary, 1999; Stephens *et al*., 1998) has been the most tangible benefit. However, relatively little can yet be gleaned from genome comparisons on differential pathogenic properties of chlamydiae. Five of the six fully sequenced genomes are from chlamydiae infecting humans, with the remaining one being from the mouse biovar of *C. trachomatis* (MoPn), which was initially isolated from lungs of serially passaged mice inoculated with throat washings from clinical cases of human influenza (Nigg & Eaton, 1944). Moreover, only limited consideration of the evolutionary path of *Chlamydia* has been possible to date as the genome sample size is still too small (*C. trachomatis* and *C. pneumoniae*). Genome analysis of several members of the more disparate *Chlamydia psittaci* group, with different host specificities, as well as some of the fringe *Chlamydia*-like strains, will probably be necessary before a comprehensive phylogeny can be drawn for the genus. The emerging picture, however, is one of a remarkably stable genome, with relatively few rearrangements and differentially expanded gene families. This suggests that genomic content was established early in chlamydial evolution, possibly prior to committing to intracellular life, and that divergence into different hosts has not been accompanied by major horizontal exchanges as is frequently observed in other, free-living, bacteria.

Based on the comparison of the *C. pneumoniae* and *C. trachomatis* genomes, Read *et al*. (2000) have described a plasticity zone near the origin of replication, which displays much higher genetic reorganization than the rest of the genome. Examples of genetic elements that are found in the plasticity zone are the tryptophan operon, which has been implicated in the development of persistent infection, and genes related to enterohaemorrhagic *Escherichia coli* and clostridial toxins (Burland *et al*., 1998; Makino *et al*., 1998; von Eichel-Streiber *et al*., 1996), in human genital and murine *C. trachomatis*, respectively. A process of reductive evolution, through the gradual dispensing of unessential genes, is likely to have followed the irreversible move of the ancestral form to an obligate intracellular lifestyle. The genomic stability that can be inferred from the sequenced *Chlamydia* genomes is also probably increased by the relative incapacity of the chlamydial genomes to expand through gene duplication. Hence few gene duplicates are found in *Chlamydia*, and the few that are found are mostly tandem duplications (Fig. 1) revealing minimal recombination activity. In the light of the relative ease of genome sequencing in *Chlamydia*, the conclusion that can be drawn at this time is that the genome sequencing effort should be continued to encompass the full spectrum of the genus. The restricted evolutionary path of this group of obligate intracellular micro-organisms, which paradoxically infect a broad range of hosts, can only be unravelled through a comprehensive comparative analysis. Moreover, in the absence of genetic methodologies, expanded comparative genomic and subsequent post-genomic analyses may be most fruitful in identifying the molecular and cellular bases for the array of virulence properties of chlamydiae.

**Chlamydia structure/physiology in the genomic spotlight**

Analysis of *Chlamydia* genome sequences has revealed that *Chlamydia* is, in many respects, closer to other bacteria than previously suspected. In particular, we must readress experimentally the view that *Chlamydia* are Gram-negative bacteria that lack a classical peptidoglycan sacculus (Moulder, 1993) and that they are obligate energy parasites (Moulder, 1991). In fact, *Chlamydia* genomes contain genes necessary for peptidoglycan synthesis and several genes encoding enzymes involved in energy metabolism.

**Peptidoglycan**

Although the existence of a peptidoglycan layer in *Chlamydia* is now suggested by the genes, it is puzzling that its presence should have remained undetected, especially given the inhibitory effect of penicillin on chlamydial growth (Moulder, 1993). This paradox, known as the chlamydial anomaly, is currently being debated in the light of the genomic information. Trace amounts of muramic acid have been detected in only one study involving purified EB of *C. trachomatis* (Su *et al*., 1985). Other previous failed attempts to detect peptidoglycan constituents have all used purified EB, and only one attempt was made to specifically find peptidoglycan in RB (Barbour *et al*., 1982). This raises the possibility that the peptidoglycan is specifically degraded during differentiation of RB into EB by amidases, whose genes are present in the genome (Chopra *et al*., 1998). This hypothesis is consistent with the marked volume reduction during RB to EB differentiation and the presumed lack of structural requirement for peptidoglycan in EB (Hatch, 1996). Chopra *et al*. (1998) have hypothesized that the role of peptidoglycan in RB may then be to substitute for the FtsZ ring-shaped structure involved in cell envelope constriction, predicted to be missing in *Chlamydia* by virtue of the absence of the *ftsZ* gene in the genome. Conflicting with this interpretation, Ghysen & Goffin (1999) predicted that *Chlamydia* synthesize a glycan-less ‘peptidoglycan’, i.e. a polypeptide. This hypothesis is derived from the observation that the *C. trachomatis* genome encodes two high-molecular-mass penicillin-binding proteins (PBPs) of class B, which are devoid of the transglycosylase activity required for glycan chain elongation. They further propose that a weaker glycan-less sacculus might be strengthened by covalently linked lipoproteins, lipopolysaccharides and highly disulfide-cross-linked...
proteins associated with the outer membrane. Hatch (1996) proposed that the cysteine-rich protein layer may of its own be sufficient to provide structural integrity to the cell envelope, consistent with the resistance to detergent solubilization of the ‘chlamydial outer membrane complex’ under non-reducing conditions. Hence the chlamydial anomaly lingers on but on a different level, this regardless of whether or not the hypothetical chlamydial polymer contains glycan. Is there a role for a peptidoglycan/polypeptide layer in cell division? Does synthesis and/or degradation of this layer play a dynamic role in early and late developmental transitions? Does peptidoglycan play a scaffolding role in anchoring cysteine-rich outer-membrane proteins for added EB structural stability?

**Chlamydia antigenic variation**

In *C. trachomatis*, the characterization of *ompA (omp1)*, encoding the major outer-membrane protein (MOMP), from several serovars has revealed sequence variations in four distinct segments that correspond to serotype-, serogroup-, species- and subspecies-specific epitopes (Baehr et al., 1988; Yuan et al., 1989). This variation led Stephens (1989) to propose a role for MOMP in *C. trachomatis* antigenic variation. However, immunological selection of antigenic variants has not been demonstrated and it is also possible that selective pressures linked to adaptation to varied environments are responsible for the MOMP structural variation observed in *C. trachomatis*. Since MOMP variable segments have been implicated in adherence (Su et al., 1990, 1988), it is conceivable that sequence variation occurs in response to different receptor structures, thereby providing a basis for the observed differential tropisms of *C. trachomatis* serovars.

The issue of a MOMP-based mechanism of antigenic variation is further confounded by other factors. MOMP sequence variation has only been found in *C. trachomatis* to date, and *C. psittaci* and *C. pneumoniae* strains that have invariant MOMP sequence (Gaydos et al., 1992; Zhao et al., 1993) can indeed infect susceptible hosts at different anatomical sites, and indeed different hosts (Girjes et al., 1994). More interestingly, genomic sequencing has uncovered gene families encoding high molecular mass (90–180 kDa) polymorphic membrane proteins (Pmps) (also named POMPs or OMPs), some of which have been demonstrated to be exposed at the surface (Knudsen et al., 1999; Longbottom et al., 1998a). Families of 9 and 21 *pmp* genes have been identified in *C. trachomatis* and *C. pneumoniae*, respectively (Kalman et al., 1999; Read et al., 2000; Stephens et al., 1998), and five gene family members have been identified to date in ovine *C. psittaci* (Longbottom et al., 1998b) (K. Laroucau & A. Rodolakis, personal communication). Frameshift mutations are found in some *pmp* genes and *C. pneumoniae* CWL029 *pmp-6* includes a 393 bp segment, which is lost in the AR39 strain. These may represent an underlying mechanism for differential expression of Pmp proteins and/or variation of Pmp protein structure. It is also possible that the makeup of the *pmp* gene families currently derived from genome analysis has been altered through multiple *in vitro* passages of the sequenced strains. In this regard, it may become important to base our interpretation of the function of this intriguing gene family on studies involving fresh clinical isolates.

**Acquisition of energy**

It has long been known that a number of metabolic pathways of prokaryotes—such as protein, RNA,
DNA and phospholipid syntheses – are functional in *Chlamydia*, reaching maximal activity halfway through infection, coincidental with maximal RB replication. Moreover, analysis of the *Chlamydia* genomes has revealed that it is an aerobic microbe, probably utilizing glutamate as a principal source of carbon, and perhaps also glucose and 2-oxoglutarate (Stephens et al., 1998). However, in view of the high number of RB proliferating in infected cells, it is reasonable to ask whether the host cell and/or chlamydial can supply the energy metabolites required to maintain such a large bacterial population.

This issue was addressed by studying the effect of *C. psittaci* strain Guinea Pig Inclusion Conjunctivitis (GPIC) infection on the energy metabolism in infected cells using non-invasive NMR (Ojcius et al., 1998b). The ATP concentration in infected cells increases significantly halfway through infection, decreasing later. Infection also stimulates glutamate synthesis with a similar time-course. The stimulation is caused by an increase in the rate of glucose consumption by the infected cell, which also results in increased lactate production and glycogen accumulation. Finally, infection increases surface expression of a glucose transporter on infected cells, which could thus lead to all the other metabolic modifications observed (Ojcius et al., 1998b). These results may not be surprising, however, as increased host cell glucose metabolism has been reported following infection by viruses (Sorbarra et al., 1996), and may reflect a host stress response. It is expected that infection by other intracellular microbes, including parasites, may lead to similar results.

The *C. trachomatis* genome contains genes that potentially encode enzymes of the glycolytic pathway, with the exception of a gene encoding fructose-1,6-di-phosphate aldolase, which could be replaced by another aldolase encoded by the genome (Stephens et al., 1998). Assuming that these genes express functional proteins, *Chlamydia* could contribute to the elevated ATP concentrations observed during infection. In fact, when four of the genes encoding enzymes involved in producing energy or reducing power were expressed as recombinant proteins in *E. coli*, the recombinant chlamydial enzymes were active (Iliffe-Lee & McClarty, 1999). In addition, RT-PCR analysis demonstrated that the four genes are maximally expressed midway through the infection cycle (Iliffe-Lee & McClarty, 1999), consistent with the highest levels of ATP measured by NMR. The NMR method does not allow one to determine if the additional ATP is produced by chlamydiae or the host cell, but the RT-PCR results suggest that the chlamydiae are probably making at least some of the ATP. Finally, the total level of ATP in infected cells could not increase unless sufficient glucose were available, and the bacteria as well as host cells could use the additional glucose provided by the increased expression of the glucose transporter.

The genome sequences also reveal that the Krebs cycle is incomplete, as genes encoding citrate synthase, aconitase and isocitrate dehydrogenase are absent (Stephens et al., 1998). However, the Krebs cycle could function partially from exogenous glutamate. Genes predicted to encode glutamate transporters exist (Stephens et al., 1998), and the glutamate surplus during infection could be used by the bacteria to make ATP. In addition, the *C. trachomatis* genome contains a gene encoding a dicarboxylate translocator, which could provide the bacteria with 2-oxoglutarate. Both glutamate and 2-oxoglutarate could therefore represent a source of carbon for the partial Krebs cycle as substitutes for acetyl-CoA. Finally, *C. trachomatis* has homologues for all of the enzymes for the pentose phosphate pathway, except for 6-phosphogluconolactonase, which may not be an essential enzyme (McClarty, 1999).

The NMR studies also showed an accumulation of glycogen in host cells during infection with *C. psittaci*. The genome of *C. trachomatis* does in fact possess genes encoding enzymes of glycogen synthesis and degradation (Stephens et al., 1998), suggesting that glycogen degradation could provide energy to *Chlamydia*. However, glycogen accumulation in the inclusion has previously been observed by iodine staining of cells infected by *C. trachomatis* but not of those infected by *C. psittaci* or *C. pneumoniae* (Moulder, 1991). As previously pointed out (McClarty, 1999), the NMR measurements do not distinguish between extra glycogen deposited in chlamydiae, in the *Chlamydia* inclusion or in the host cell cytoplasm. Whilst only a *C. psittaci* genome sequence will reveal whether *C. psittaci* is in fact able to synthesize glycogen (albeit in amounts too small to detect by iodine staining), it is also likely that the host cell synthesizes larger quantities of glycogen, as previously observed for cells overexpressing glucose transporters (Buse et al., 1996; Ren et al., 1993).

Bacteria of the genus *Chlamydia* have been considered as energy parasites because an ATP–ADP exchange activity had been found in *C. psittaci* (Hatch et al., 1982). Genes that encode two transporters, Npt1<sub>Ct</sub> and Npt2<sub>Ct</sub>, homologous to ADP/ATP translocases of *Rickettsia* and mitochondria, are found in the genome (Stephens et al., 1998), and both transporters were active when expressed as recombinant proteins in *E. coli* (Tjadjen et al., 1999). Npt1<sub>Ct</sub> catalysed ATP and ADP transport in an exchange mode, whereas Npt2<sub>Ct</sub> catalysed the net uptake of all four ribonucleoside triphosphates. The presence and expression of the genes involved in ATP biosynthesis thus suggests that chlamydiae are not strict auxotrophs for ATP, whilst the activity of Npt1<sub>Ct</sub> implies that chlamydiae may acquire at least a portion of their ATP from the host cell.

**Interaction of Chlamydia with the host cell cytosol**

To survive within eukaryotic cells, chlamydiae must confront the defence mechanisms of the host cell, and acquire metabolites necessary for bacterial growth. The chlamydial inclusion membrane (CIM), which until recently had not attracted much attention, undoubtedly
plays a major role in the interactions between chlamydiae and the eukaryotic cell. The recent discoveries of genes encoding proteins present in the CIM of C. psittaci GPIC and C. trachomatis (Bannantine & Rockey, 1999; Rockey et al., 1995; Scidmore-Carlson et al., 1999) and genes encoding a TTS system (Hsia et al., 1997; Stephens et al., 1998) represent important developments in this field. Rockey and co-workers have isolated and characterized several RB-specific proteins, named Inc, which are associated with the CIM (Bannantine & Rockey, 1999; Rockey et al., 1995). One of these, IncA of C. psittaci GPIC, faces the cytoplasmic side of the vacuole and is phosphorylated by host cell kinases on serine and threonine residues (Rockey et al., 1997). IncA may therefore subvert signal transduction pathways in the host cell, to the advantage of the pathogen. A recent study has revealed that certain C. trachomatis strains that do not express an IncA homologue also produce uncharacteristic multiple inclusions in single cells (Suchland et al., 2000). Likewise, an IncA-specific monoclonal antibody, when micro-injected into C. trachomatis-infected cells, provokes the development of aberrant multilobed inclusions, similar to those observed in C. psittaci GPIC-infected cells (Hackstadt et al., 1999). These independent findings strongly suggest that one of the functions of the IncA protein is to facilitate homotypic vesicle fusion through IncA intermolecular interactions in C. trachomatis-infected cells (Hackstadt et al., 1999; Suchland et al., 2000).

The Inc proteins are secreted despite the absence of a signal sequence, and as such are candidates for secretion by the TTS pathway. This secretion system has been described in detail in bacterial pathogens of animals and plants, including Salmonella, Shigella, Pseudomonas and Yersinia (Hueck, 1998). Unlike other secretion systems, TTS most often depends on contact between the membranes of the infected eukaryotic cell and the pathogenic bacteria. Contact activates TTS, leading to secretion and simultaneous translocation into the host cell cytoplasm of essential virulence factors. For Chlamydia, it has been proposed that TTS activity is triggered inside the host cell, implying that effector proteins are secreted/translocated either within the inclusion lumen or through the CIM into the host cell cytosol (Hsia et al., 1997). Furthermore, the Chlamydia TTS apparatus may correspond physically to surface projections observed by A. Matsumoto more than twenty years ago (Bavoil & Hsia, 1998). These transmembrane organelles are located to the area of contact between the bacterium and the host-derived CIM and display marked ultrastructural resemblance to TTS organelles of Salmonella (Kubori et al., 1998).

The often observed juxtaposition of replicating RB with the CIM suggests that growth of chlamydiae within the inclusion depends on their interaction with the CIM, and consequently on TTS activity in the area of contact. Contact-dependent RB replication may ultimately lead to physical overcrowding, provoking detachment of weakly attached chlamydiae from the CIM. Detachment from the membrane predictably would result in TTS inactivation, and may coincidentally lead to initiation of late differentiation (Hackstadt et al., 1997; Rockey & Matsumoto, 1999). It is conceivable that physical detachment of the RB and concurrent TTS inactivation/down-regulation constitute a signal for late differentiation of RB towards EB. This hypothesis conflicts with the alternative notion of a temporally regulated developmental cycle, i.e. a developmental clock, which is implied from past circular representations of the developmental cycle. Instead, Chlamydia late differentiation may be regulated by a mechanism based on a ‘developmental compass’, which, upon detachment from the CIM, switches off TTS and switches on late differentiation. Although supporting experimental evidence is fragmented at present, this mechanism is consistent with a long-observed universal phenomenon of chlamydial biology, i.e. that intracellular development within the inclusion, whilst relatively synchronous early on, becomes asynchronistic at later times.

Besides a possible role during Chlamydia development, what may be the direct function(s) of TTS in virulence? This question is at the heart of Chlamydia biology and the answer will undoubtedly require the identification of type-III-secreted effector proteins. Unlike those in other pathogens, TTS genes of Chlamydia are dispersed in small clusters on the genome. In C. trachomatis serovar D, at least four unlinked loci include genes with sequence similarity to TTS genes from other bacteria (Stephens et al., 1998). TTS gene dispersion and the lack of high homology with other type-III-secreted effectors means that identification of the effectors by direct means will be critical to further our understanding of the role of TTS in chlamydial biology. Notwithstanding, a likely role for TTS would be in the intracellular survival of Chlamydia—in the acquisition of nutrients, for example, but especially in the resistance of the bacteria against non-specific defence mechanisms of the infected host, such as acidification of the entry vacuoles and their fusion with lysosomes. An analogy can possibly be made between chlamydial TTS and TTS encoded by Salmonella pathogenicity island 2 (SPI-2). SPI-2 is up-regulated upon internalization by macrophages (Cirillo et al., 1998) and SPI-2 effector proteins are known to promote intra-macrophage survival (Hensel et al., 1998). In this respect, it is also interesting that survival in circulating macrophages is a suspected prerequisite for disseminated C. pneumoniae infection (Gaydos et al., 1996). It is intuitively less attractive to envision a role for Chlamydia TTS in entry, as infectious chlamydiae are metabolically inactive. However, the possibility that surface-associated type-III-secreted effectors produced late in development may play a role in the early steps of infection can not be excluded. Indeed Matsumoto’s surface projections are found in both RB and EB, albeit in lower numbers in the latter, suggesting that ‘pre-loaded’ EB TTS complexes may be poised to unload effector proteins during the earliest steps of infection. This is consistent with a presumed role of TTS in inhibition of phagolysosome fusion (see below), which
takes place earlier than transformation of the internalized EB to RB.

Membrane traffic in infected cells

Although the chlamydial developmental cycle was described long ago, the mechanisms of entry and survival of the pathogen inside host cells remain poorly understood. The entry route used by *Chlamydia* remains controversial, and evidence in favour of both endocytic and phagocytic pathways has been reported. Thus, cytochalasins, which block phagocytosis by acting at the level of the actin cytoskeleton, either inhibit *Chlamydia* infection or have no effect, and a component of budding, coated-pit endosomes, clathrin, either co-localizes with the entry vacuoles or is excluded. Recently, a mutant epithelial cell line deficient in clathrin-dependent endocytosis was infected efficiently with either *C. psittaci* GPIC or the LGV/L2 strain of *C. trachomatis* (Boleti et al., 1999), but other host cell lines and *Chlamydia* strains now need to be evaluated. The apparent contradictions in previous reports, extensively reviewed by Bavoil et al. (1996) and Hackstadt (1999), could be due to different host cell types and/or *Chlamydia* strains or growth conditions used, and suggest that both clathrin-dependent phagocytosis and cytoskeleton-independent endocytosis could be used to internalize chlamydiae. Furthermore, almost all *Chlamydia* laboratories have studied entry in unpolarized cells, which might not reflect accurately the behaviour of the apical surface of polarized cells. When the same epithelial cells were grown as polarized cells or unpolarized cells, *C. trachomatis* entered the polarized cells via endocytosis more often than in the unpolarized cells (Wyrick et al., 1989).

Following internalization, the entry vacuoles inhibit fusion with host cell lysosomes. Undifferentiated EB may secrete via TTS a factor that inhibits the fusion, since blocking chlamydial early transcription or translation with antibiotics causes the entry vacuoles to fuse with lysosomes (Hackstadt et al., 1997). It has also been reported that *C. psittaci* entry vacuoles fuse with lysosomes of dendritic cells (Ojcius et al., 1998a), but this may be due to the zealous proteolytic activity and rapid endosomal/phagosomal maturation in these specialized antigen-presenting cells (Banchereau & Steinman, 1998), which may not allow the chlamydiae sufficient time to inhibit fusion with lysosomes.

Whilst avoiding fusion with lysosomes, the entry vacuoles fuse with sphingomyelin-containing exocytic vesicles of the host cell (Hackstadt et al., 1997). The lipid is derived from the Golgi apparatus of the infected cell, and transfer to the growing *Chlamydia* vacuole begins within a few hours after infection (Hackstadt et al., 1997). Presumably to facilitate lipid transfer, the vacuole is also observed adjacent to the Golgi shortly after infection (Hackstadt et al., 1997). Besides incorporating host-cell sphingomyelins, the *Chlamydia* vacuole acquires glycerophospholipids from the Golgi apparatus, mitochondria and endoplasmic reticulum, which are subsequently modified by the bacteria (Wylie et al., 1997). The chlamydiae thus manage to intercept lipid traffic from several host cell compartments, mimicking in effect the phospholipid composition of the host cell in which the bacteria are grown (Hatch & McClarty, 1998). These results are in line with observations that the phospholipid composition of chlamydiae is closer to that of the eukaryotic host cell than of typical prokaryotes (Newhall, 1988; Wylie et al., 1997). Elucidation of the mechanisms that divert lipids to the vacuole should therefore illuminate our understanding not only of *Chlamydia* infections, but also of normal membrane traffic in uninfected cells.

In addition, infection with *C. trachomatis* leads to phosphorylation of a number of host cell proteins within 15 min after infection (Birkeland et al., 1994; Fawaz et al., 1997). The phosphorylated proteins do not co-localize with *Chlamydia* vacuoles early on, but are found in the vicinity of the growing inclusion at later times. As protein phosphorylation is observed even in cells that had been pretreated with cytochalasin D to prevent *Chlamydia* entry (Birkeland et al., 1994), attachment itself may suffice to induce phosphorylation. This could conceivably occur through TTS, if one assumes that EB are TTS competent by virtue of the presence of premade TTS projections on their surface. However, heparin-coated beads, which are internalized by the same mechanism as chlamydiae, also induce phosphorylation of a similar set of host proteins (Stephens et al., 2000), suggesting that TTS is not required for this host cell response. Interestingly, the bead-containing vacuoles subsequently fuse with lysosomes, implying that chlamydiae must be actively involved in inhibiting fusion with lysosomes.

Perspective

By analogy with other bacterial pathogens, it is likely that TTS plays a central role in the biology and pathogenesis of *Chlamydia*. Fig. 2 summarizes several possible interactions of the nucleus, lysosomal compartment, mitochondria and Golgi apparatus with growing chlamydiae and the chlamydial inclusion. TTS function is probably required for inhibiting fusion with host cell lysosomes and for diverting lipids, while late down-regulation/inactivation of TTS activity is likely associated with RB to EB differentiation. Is TTS also involved in modulating the expression of glucose transporters? Does TTS contribute to the ability of the more virulent *Chlamydia* spp. to survive in macrophages? As tempting as it may be to assign manifold functions to a newly discovered system, one must bear in mind that cells infected with chlamydiae, as with other intracellular microbes, undergo a stress response, which can influence host cell metabolism. Given the limited genomic capacity of *Chlamydia*, the more mundane role played by the host cell in *Chlamydia* infection must also be addressed.

With the advent of genomics methodologies, *Chlamydia* research is now evolving faster than at any other time in recent memory. Although this is not unique to
**Fig. 2.** Schematic representation of the chlamydial developmental cycle and interactions of growing chlamydiae with the host cell. At early times after internalization, there is inhibition of fusion of the initial RB-containing vacuole with lysosomes. During the replication phase, expression of plasma membrane transporters is modified, and lipids, nutrients and metabolites from host cytosol and organelles are diverted to the inclusion. During late phase, there is loss of TTS-mediated contact (represented as ‘?’ at the RB surface), gradual detachment of growing chlamydiae from the inclusion membrane and coupled initiation of late differentiation into initial bodies (IB). Several of these mechanisms may result from TTS-associated activities, from the host stress response or from the interplay of both.

**Chlamydia** pathogenesis

*Chlamydia*, the relative ease of genomic sequencing in this organism (because of its small genome, low G+C content and lack of repeat sequences) suggests that what we are now witnessing is likely to continue and expand. Novel hypotheses, such as those reviewed above pertaining to pathogenicity, have already been formed and are currently being tested. Yet it is safe to assume that the well of genome-derived information will eventually dry up and that ‘old-fashioned’ biology, including genetic analysis, will eventually be required. In this regard, the isolation of plasmid-free chlamydiae (Farencena et al., 1997; Stothing et al., 1998), the discovery of several *Chlamydia* phages (Hsia et al., 2000; Liu et al., 2000; Read et al., 2000; Storey et al., 1989), and promising transformation experiments (O’Connell & Maurelli, 1998) offer opportunities for the development of genetics in *Chlamydia* that should be acted upon as a matter of priority.

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2730


