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REVIEWS

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Effector Proteins of Chlamydiae

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Abstract—This review summarizes the recently published data on the molecular mechanisms of Chlamydiae–host cell interaction, first of all, on chlamydial effector proteins. Such proteins, along with type III transport system proteins, which transfer many effector proteins into the host cytoplasm, are attractive targets for drug therapy of chlamydial infections. The majority of the data concerns two species, *Chlamydia trachomatis* and *Chlamydophila pneumoniae*. The *C. trachomatis* protein TARP, which is presynthesized in elementary bodies, plays an essential role in the initial stages of infection. The pathogen proteins that are involved in the next stage, which is the intracellular inclusion traffic to the centrosome, are *C. trachomatis* CT229 and *C. pneumoniae* Cpn0585, which interact with cell Rab GTPases. In *C. trachomatis*, IncA plays a key role in the fusion of chlamydial inclusions, CT847 modulates the life cycle of the host cell, and LDA3 is essential for the acquisition of nutrients. The protease CPAF and the inclusion membrane proteins IncG and CADD are involved in suppressing apoptosis of infected cells. The proteases CPAF and CT441 and the deubiquitinating protein ChlaDub1 help the pathogen to evade the immune response.

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Key words: chlamydial effector proteins, inclusion membrane proteins, type III transport system, *Chlamydia trachomatis, Chlamydia pneumoniae*

INTRODUCTION

Chlamydiae belong to a group of obligate intracellular bacteria with a characteristic developmental cycle, which involves two stages differing in morphological and biological properties. Several species of Chlamydiae are known to infect humans, but *Chlamydia trachomatis* and *Chlamydophila pneumoniae* are most clinically significant as primary human pathogens. Chlamydiae may cause both acute and chronic infections. The latter present the most pressing problem, since the resulting pathologies are severe and there is no means to efficiently control their spread. Modern medicine still lacks effective drugs to treat chronic chlamydioses. The classical approaches, which are based on antibiotic treatments and stimulation of the patients' immunity, are ineffective in many cases. Hence, it is necessary to develop new drugs for treating chronic chlamydial infections.

Modern strategies to design therapeutics and preventive drugs require that adequate drug targets be selected at the initial stage. In the case of intracellular bacteria, such as Chlamydiae, such targets may include the proteins that mediate the interaction of Chlamydiae with host cells. This review focuses on the effector proteins of various species of Chlamydiae.

LIFE CYCLE OF CHLAMYDIAE

Chlamydiae occur as two cell forms, infective extracellular elementary bodies (EBs), which are metabolically inactive, and vegetative intracellular reticular bodies (RBs) (for a review, see [1]; Fig. 1). To infect cells of the host organism, EBs bind to and enter them, e.g., via phagocytosis. Chlamydiae infect cells of the nonciliated cylindrical or cubical epithelium of mucous membranes (conjunctiva, urethra, cervical canal, endometrium, and uterine tubes), visceral epithelial cells, reticuloendothelial cells, leukocytes, monocytes, and macrophages. Chlamydiae are not degraded in the phagosome of the host cell, but use the phagosome as a niche to implement their life strategy.

Within the cell, chlamydial EBs are transformed into RBs after 6–8 h and occur in a cytoplasmic vacuole (a chlamydial inclusion), forming a microcolony. Several EBs may simultaneously enter one host cell to produce several chlamydial inclusions, which then may fuse together. Compared with EBs, RBs are approximately threefold larger in size, are metaboli-

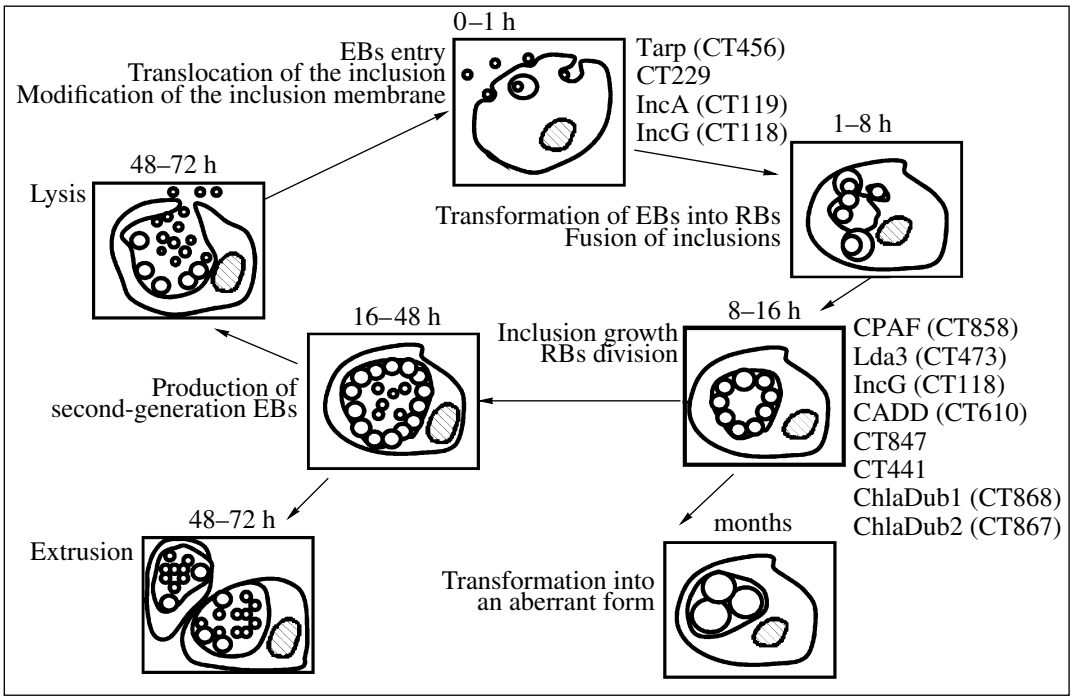

Fig. 1. Life cycle of Chlamydiae. EBs is elementary body and RBs is reticular body.

cally active, and are capable of division. RBs divide for 8–24 h and undergo 8–12 division cycles. Some of the new RBs decrease in size, become denser, and are transformed into second-generation EBs. Chamydial inclusions become easily distinguishable under a light microscope 24–36 h after the start of infection. After 48–72 h, the cell membrane is disrupted, and several hundreds of chlamydial EBs and RBs are released into the intercellular space. Then, EBs again enter cells via phagocytosis, and a new chlamydial life cycle begins. What as known as extrusion occurs in place of cell lysis in some cases; i.e., a part of a chlamydial inclusion, surrounded by the cell membrane, is budded off the cell [2]. The cell still contains chlamydial inclusions but does not die.

In acute infection, the chlamydial life cycle usually takes 36–72 h. Various stress factors, such as cytokines, antibiotics, and starvation, may cause deviations from the typical life cycle. In particular, the formation of infective EBs is terminated, and aberrant persisting forms of Chlamydiae survive for a long time in the eukaryotic cell. The persisting forms can be defined as viable, metabolically active, nonculturable forms that have alterations not only in morphology, but also in the expression of key chlamydial genes. Such forms are resistant to many antibacterial drugs, may cause pathological immune conditions in host cells, and may reverse to infective forms.

Below we consider the stages of the life cycle when Chlamydiae interact with the host, the host systems and mechanisms that interact with Chlamydiae, and the groups of proteins that are involved in this interaction (Fig. 2).

THE INTERACTION WITH THE IMMUNE SYSTEM OF THE HOST ORGANISM

The immune response to *C. trachomatis* infection involves a coordinate activation of B cells and CD4+ and CD8+ T cells. The sera of infected patients react with many individual chlamydial proteins, such as the major outer membrane protein (MOMP), ompA, or CT681 [3, 4], the heat shock proteins GroEL (CT110, also known as GroL, HybB, or MopA) and GroES (CT111, also known as GroS, HypA, or MopB) [5], and the inclusion membrane (Inc) proteins, which are located on the membrane of chlamydial inclusions (table) [6]. The levels of antibodies to chlamydial protease/proteasome-like activity factor (CPAF, CT858) in the sera of patients diagnosed with *C. trachomatis* cervicitis are higher than the levels of antibodies against MOMP and the heat shock proteins [7].

A certain role in the interaction with the host immune system is played by the highly polymorphic membrane proteins (Pmp). The chlamydial genomes encode a great variety of Pmp proteins. At least one of them, PmpD (CT812) was experimentally demonstrated to induce neutralizing antibodies [8]. The *C. trachomatis* NrdB protein (CT828, ribonucleoside diphosphate reductase subunit β [EC 1.17.4.1]) acts as an antigen that induces the CD4+ T-cell response [9].

Properties of the putative *C. trachomatis* Inc proteins

Gene	ORF	Annotation[a]	Location [6][b]	Experimental localization[c]	In silico prediction[d]		Interaction with antisera [6][e]	Relative immunodominance of the N- and C-terminal regions [6][f]
					[72]	[73]		
lcrE/copN	CT089	Low Calcium Response E	IM	IM	–	–	ID	C
incD	CT115	Inc. membrane protein D	IM	IM	+	+	ID	C
incE	CT116	Inc. membrane protein E	IM	IM	+	+	ID	C
incF	CT117	Inc. membrane protein F	IM	IM	+	+	L	
incG	CT118	Inc. membrane protein G	IM	IM	+	+	ID	C
incA	CT119	Inc. membrane protein A	IM	IM	+	+	ID	C
	CT147	PUP	IM	IM	–	+	ID	C
	CT223	PUP	IM	IM	+	+	ID	N
	CT225	PUP	IM	–	+	+	ID	
	CT226	PUP	IM	IM	+	+	ID	C
	CT228	PUP	IM	–	+	+	ID	C
	CT229	PUP	IM	IM	+	+	ID	C
incB	CT232	Inc. Membrane Protein B	IM	IM	+	+	L	
incC	CT233	Inc. Membrane Protein C	IM	IM	+	+	L	
	CT249	PUP	IM	IM	–	+	L	
	CT288	PUP	IM	IM	+	+	L	
	CT358	PUP	IM	–	+	+	M	
	CT440	PUP	IM	–	+	+	L	
crpA	CT442	Cysteine-rich protein A	IM	IM	+	–	ID	C
cap1	CT529	Class I accessible protein 1	IM	IM	–	–	ID	N
	CT618	PUP	IM	IM	+	–	ID	N
	CT813	PUP	IM	IM	+	+	ID	C
pls1	CT049	Pmp-like secreted protein 1		II, IM	–	–		
pls2	CT050	Pmp-like secreted protein 2		II, IM	–	–		
	CT058	PUP	II	–	+	+	M	
	CT192	PUP	II	–	+	+	M	
	CT195	PUP	II	–	+	+	M	
	CT383	PUP	II	–	+	+	M	
	CT484	PUP	II	II	+	+	ID	C
	CT565	PUP	II	–	+	–	M	
	CT850	PUP	II	–	+	–	ID	
	CT005	PUP	U	–	+	–	L	
	CT006	PUP	U	–	+	–	M	
	CT036	PUP	U	–	+	+	L	
	CT101	PUP	U	–	+	–	L	
	CT134	PUP	U	–	+	–	M	
	CT135	PUP	U	–	+	–	M	
	CT164	PUP	U	–	+	–	M	
	CT179	PUP	U	–	–	+	M	
	CT196	PUP	U	–	+	+	M	

Table (Contd.)

Gene	ORF	Annotation[a]	Location [6][b]	Experimental localization[c]	In silico prediction[d]		Interaction with antisera [6][e]	Relative immunodominance of the N- and C-terminal regions [6][f]
					[72]	[73]		
	CT214	PUP	U	–	+	+	L	
	CT222	PUP	U	–	–	+	L	
	CT224	PUP	U	–	+	+	M	
	CT227	PUP	U	–	+	+	M	
	CT300	PUP	U	–	+	+	M	
	CT345	PUP	U	–	+	+	M	
	CT357	PUP	U	–	+	+	–	
	CT365	PUP	U	–	–	+	M	
	CT449	PUP	U	–	+	+	M	
	CT483	PUP	U	–	+	+	M	
	CT728	PUP	U	–	+	–	M	
	CT789	PUP	U	–	+	–	M	

[a] PUP, putative uncharacterized protein.

[b] Location: IM, inclusion membrane; II, intrainclusion; U, undetermined.

[c] The intracellular location was characterized for the *C. trachomatis* proteins CT049, CT050 [146], CT089 [130], CT115–119 [147], CT147 [136], CT223, CT229, CT233, CT288, CT442, CT484 [72], CT226 [148], CT229 [57], CT232 (IncB), CT233 (IncC) [149], CT249 [115], CT442 [13], CT529 [14], CT618 [150], and CT813 [151]. Location: IM, inclusion membrane; II, intrainclusion; (–), data unavailable.

[d] The presence (+) or absence (–) in the inclusion membrane was predicted.

[e] The proteins were tested for the interaction with 17 sera from patients. ID are immunodominant antigens that interacted with eight or more sera, M are antigens that interacted with three to seven sera, L are antigens that interacted with one or two sera, (–) are proteins that did not interact with any serum.

[f] Relative immunodominance of the N- and C-terminal regions was estimated only for immunodominant proteins.

Several CD+ T-cell antigens are known in Chlamydiae: MOMP [10], the 60-kDa cysteine-rich OMP protein (OmcB, Omc2, or Omc2B; CT443), PmpI (CT874) [12], cysteine-rich protein A (CrpA, CT442) [13], and class I accessible protein 1 (Cap1, CT529) [14]. The last two proteins occur on the membrane of chlamydial inclusions (table) and act as immunodominant antigens, which interact with a substantial number of sera from patients infected with *C. trachomatis.* The above proteins can be considered as promising antigens in designing preventive drugs and diagnostic tests.

Cell infection with *C. trachomatis* is accompanied by the degradation of the eukaryotic transcription factors RFX5 and USF-1 in the host-cell cytoplasm [15, 16]. The former serves as an activator of the promoters of the class I major histocompatibility complex (MHC) genes and the β2-microglobulin gene, while USF-1 activates the interferon γ-induced expression of class II MHC genes. The degradation of these factors alters the presentation of intracellular bacterial antigens on the cell surface. CPAF (CT858) was found to be responsible for the degradation of the transcription factors [17]. The same function is characteristic of *C. pneumoniae* CPAFpn, whose amino acid sequence has only a 48% similarity to the amino acid sequence of its *C. trachomatis* counterpart [18].

Cells infected with *C. trachomatis* display a lower surface exposure of CD1d, which is an MHC-like glycoprotein that presents lipid antigen to natural killer T (NKT) cells [19]. CD1d plays an important role in both innate and acquired immunity to various bacteria, viruses, fungi, and parasites. A decrease in CD1d content on the outer cell membrane involves CPAF. As the β2-microglobulin content in the cell is decreased by CPAF, incompletely modified CD1d is retained in the endoplasmic reticulum (before it is transferred in the Golgi complex). CPAF binds to the cytoplasmic domain of CD1d, which is followed by the ubiquitination of either CPAF (when CD1d is incompletely glycosylated) or CD1d (when its glycosylation is complete), and the CPAF-bound CD1d is transferred into the cytoplasm. CD1d is degraded by the cell proteasome or via a proteasome-independent mechanism due to CPAF proteolytic activity [19].

Another way to evade the immune system is based on the effect on the transcription factor NF-κB. This factor plays a key role in the inflammatory response to

bacterial infection in eukaryotes. However, although purified chlamydial lipopolysaccharide activates NF-κB [20, 21], an appreciable NF-κB activation is not observed in chlamydial infection [22]. NF-κB is a heterodimer of two subunits, p50/NF-κB1 and p65/RelA; inactive NF-κB occurs in the cytoplasm in complex with its inhibitor I-κB. Normally, I-κB is rapidly degraded in response to bacterial infection, inflammatory cytokines, and lipopolysaccharides and, consequently, NF-κB is transferred into the nucleus and activates the transcription of certain genes. In the case of *C. trachomatis* infection, I-κB is not degraded, nor is p65/RelA transferred into the nucleus. Moreover, it is p65/RelA that undergoes specific proteolytic degradation. The so-called tail-specific protease CT441 is responsible for this degradation [23, 24]. Chlamydiae overcome the inflammatory response of the host in this way, which may explain why chlamydial infection is asymptomatic in many cases.

The ability of Chlamydia to proteolytically degrade eukaryotic p65 is host cell specific. For instance, various *C. trachomatis* serovars hydrolyze human p65 but not its mouse counterpart. This fact may account for the difference in the course of chlamydial infection between humans and mice. In mice, infection spontaneously resolves within several weeks in most cases and induces long-term protective immunity. In humans, infection often becomes chronic when untreated, and induction of the protective immune response is poor. It is known that activation of the NF-κB-dependent pathway is a key step in triggering the mechanisms of innate and adaptive immunity [25–27]. Hence, a mechanism allowing chlamydial infection to become chronic and a poor immune response are possibly associated with disruption of the NF-κB-depending signaling pathway by chlamydial CT441.

Chlamydiae have other means to inhibit the NF-κB-dependent signaling. For instance, *C. trachomatis* codes for two deubiquitinating proteins, *Chla*Dub1 (CT868) and *Chla*Dub2 (CT867), which hydrolyze the thioester bond between ubiquitin or the ubiquitin-like protein NEDD8 and other proteins in vitro [28]. Transfection experiments showed that *Chla*Dub1 suppresses the NF-κB activation induced by several inflammatory stimuli, including cytokines and Toll-like receptor 4 (TLR4) ligands. To exert its inhibitory effect, *Chla*Dub1 binds and deubiquitinates I-κBα (a protein of the the NF-κB inhibitor complex), thus preventing its degradation in the cell [29].

CHLAMYDIAL ENTRY INTO HOST CELLS

Chlamydiae utilize several mechanisms to enter cells of the host organism at the EB stage. One of the mechanisms is receptor-mediated endocytosis, which is characteristic of polarized epithelial cells and involves specific edged clathrin-coated pits [30]. In addition, Chlamydiae enter cells via pinocytosis [31] and phagocytosis [32]. The entry strategy probably depends on the type of cells interacting with Chlamydiae.

It is thought that EBs form a contact with the host cytoplasm via a type III transport system as early as the entry stage (see Type III Transport System: Export of Chlamydial Proteins into the Host-Cell Cytoplasm). When a contact with the cell is formed, chlamydial outer protein N/low calcium response E (CopN/LcrE, CT089), which is a putative regulator of the injectisome channel, initiates the transfer of effector proteins into the cell. The effector proteins modulate the signaling system to allow intracellular movements of chlamydial inclusions and to block caspase-3 activation in order to prevent apoptosis [34].

EB binding is followed by the local activation of Rho-GTPase Rac1, leading to a reorganization of actin filaments and internalization of EBs [35]. The EB-associated translocated actin recruiting phosphoprotein (TARP, CT456) is most likely a key protein factor that initiates and organizes the reorganization of the actin cytoskeleton when *C. trachomatis* enters the cell. It is thought that TARP, which is contained in EBs, is transferred into the cytoplasm of eukaryotic cells via the type III transport system immediately after Chlamydiae have attached to the cell surface. TARP is phorphorylated by cell kinases within the cell [36]. This modification is characteristic of many effector proteins of pathogenic bacteria [37], in particular, of the enteropathogenic *Escherichia coli* Tir protein, which is incorporated into the plasma membrane and interacts with intimin (a *E. coli* surface adhesin) [38]. TARP contains two tyrosine-rich regions. The regions were found to harbor sites recognizable by Src and Abl kinases. TARP is phorphorylated by purified kinases p60-src, Yes, Fyn, and Abelson (Abl) [39]. Phosphorylated TARP triggers a cascade of protein–protein interactions [40–42]. The interaction with TARP involves the so-called guanine nucleotide exchange factors (GEFs)—Vav2 and a complex of Sos1, Eps8, and Abi1—which activate the Rho-GTPase Rac1. In turn, Rac1 triggers Arp2/3-dependent actin polymerization at the attachment sites of Chlamydia.

Early *C. trachomatis* infection is accompanied by phosphorylation of the human Abl and Pvr (PDGF- and VEGF-receptor related) kinases; the latter is homologous to the platelet-derived growth factor receptor (PDGFR) and is also known as PDGFRβ [42]. The two kinases are recruited to the bacterial attachment site. PDGFRβ may function as a receptor of Chlamydiae, since its inhibition with the use of antibodies or RNA interference substantially decreases its binding with bacteria, while Abl inhibition has no effect on binding. The internalization of bacteria is possibly mediated by the activation of

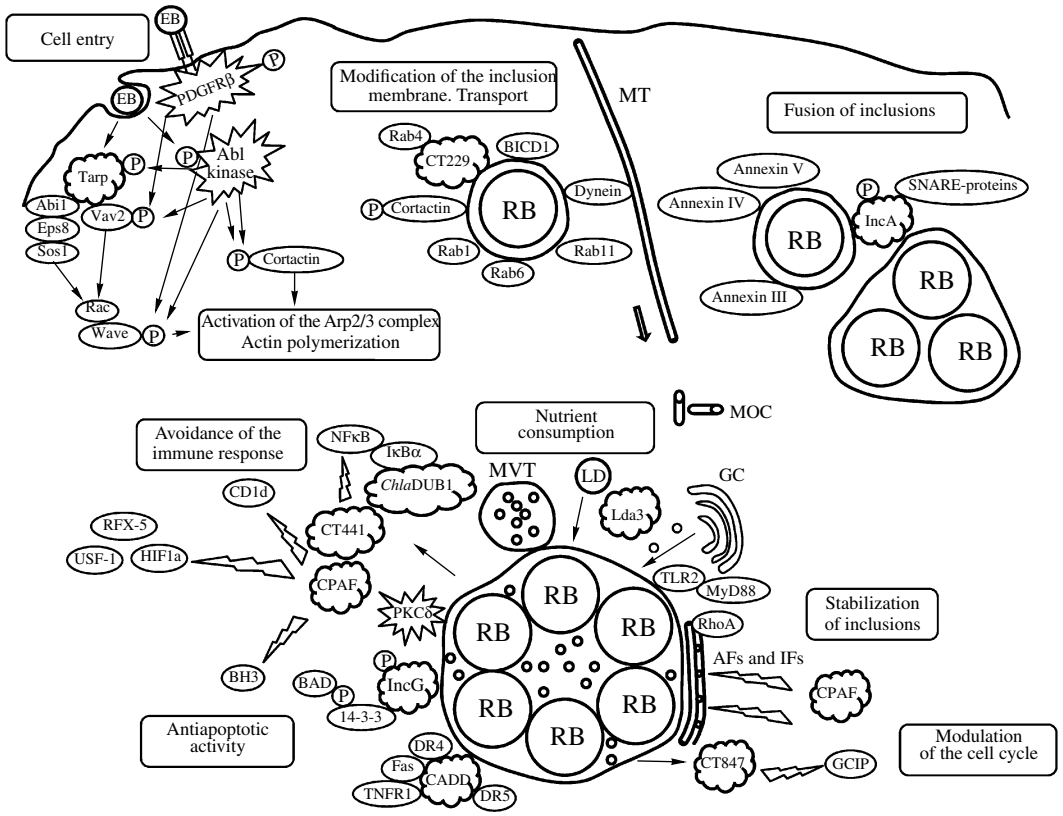

Fig. 2. Interaction of Chlamydiae with the host cell. Cell proteins are shown in open ovals, *C. trachomatis* proteins are outlined with wavy lines, and protein phosphokinases are outlined with broken lines. MT are microtubules, MVT is microvesicular body, LD is lipid droplet, GC is the Golgi complex, AFs are actin filaments, IFs are intermediate filaments, and MOC is the microtubule organization center (centrosome).

PDGFRβ or, independently, Abl, which leads to the phosphorylation of chlamydial TARP and human proteins, including Rac GEF, Vav2, and two proteins involved in actin polymerization, WAVE2 and cortactin [42] (Fig. 2).

A certain role in the entry into the cell is ascribed to the chlamydial Pmp proteins [43, 44]. The Pmp polypeptide chains include repetitive motifs of four amino acid residues, GGAI and FxxN, which occur in bacterial adhesins, for instance, rOpmA of obligate intracellular bacteria *Rickettsia* spp. [45]. The Pmp proteins are similar in amino acid sequence to autotransporters of Gram-negative bacteria [45, 46]. The autotransporters play different roles, but are similar in some structural and functional features [46]. They are transferred across the outer cell membrane via a Sec-dependent mechanism, have a C-terminal translocation domain and the N-terminal domain, which is secreted onto the bacterial cell surface. The translocation domain forms a β-barrel, and the N-terminal domain is transferred outward through this structure with or without proteolytic hydrolysis [47].

The *C. trachomatis* genome contains nine genes coding for Pmp proteins: PmpA (CT412), PmpB (CT413), PmpC (CT414), PmpD (CT812), PmpE (CT869), PmpF (CT870), PmpG (CT871), PmpH (CT872), and PmpI (CT874). All of these genes are expressed [48]. The *C. pneumoniae* genome has genes homologous to *C. trachomatis pmp.* There are 21 such genes, which form six families: A, B/C, D, E/F, and H [45]. Of all *C. trachomatis* Pmp proteins, PmpD varies to the lowest extent among different strains and species. This is important to note because less variable proteins often play more important functions.

EARLY STAGES OF INFECTION: MODIFICATION OF THE MEMBRANE AND HOMOTYPIC FUSION OF CHLAMYDIAL INCLUSIONS

After the entry into the host cell, an EB occurs within a vesicle (early endosome). Maturation of an endosome usually leads to its gradual transformation into a lysosome, but endosomes containing chlamydial EBs are not transformed into lysosomes. The endosome containing chlamydial EBs initially has plasma membrane markers, which are lost within the first 30 min after the entry into the cell [49]. The contents of the endosome have a neutral pH at the moment

of its formation; pH decreases to 6.2 and then increases to 6.6, possibly, due to a permanent function of endosomal Na^+/K^+-ATPase [50]. Certain cell proteins, such as actin-binding cortactin, are phosphorylated [51], which induces a rearrangement of the host-cell cytoskeleton. Phosphorylated cell proteins, including cortactin, occur on the membrane of chlamydial inclusions [51]. This is followed by a local accumulation of F-actin and clathrin [52], which provide a framework for the transfer of EB-containing endosomes into the perinuclear region. The transfer involves dynein [53], which is a eukaryotic locomotor protein and is responsible for the transfer of vacuoles containing Chlamydiae along microtubules [54] to their center [55]. Several Rab-GTPases, which act as central regulators of membrane transport, are recruited at the same time to the inclusion membrane [56]. These proteins presumably allow chlamydial inclusions to selectively interact with host-cell organelles.

The *C. trachomatis* protein CT229, which belongs to the so-called Inc proteins, was found to interact with cell Rab-GTPase (Rab4A) in a guanine-dependent manner [57]. It is thought that CT229 mimics the Rab4A effector and thus recruits Rab4A to the inclusion membrane. The N-terminal region of the *C. pneumoniae* Inc protein Cpn0585 (Cpn0585(102–651)) interacts with cell Rab1, Rab10, and Rab11 [58]. It is of interest that the interaction of the Rab proteins with the inclusion membrane is species specific: Rab1, Rab4, and Rab11 are associated with the inclusion membrane of all Chlamydiae, while Rab6 and Rab10 display such an association in the case of *C. trachomatis* and *C. pneumoniae*, respectively [56]. Bicaudal D1 (BICD1), which interacts with Rab6 in eukaryotic cells, is recruited to the chlamydial inclusion membrane independently of Rab6 [59], suggesting its direct interaction with certain surface structures of the inclusion membrane.

In early infection, chlamydial inclusions are colocalized with cell annexins III, IV, and V, which are capable of binding to membrane phospholipids and facilitate membrane fusion [60, 61]. Annexins of these types are thought to initiate a homotypic fusion of chlamydial inclusions (i.e., a fusion of inclusions with each other rather than with different membrane vesicles of the cell).

The fusion of endosomes containing chlamydial EBs involves Chlamydia-specific factors as well, because only endosomes containing EBs of the same species can fuse. The possibility of fusion was experimentally demonstrated for endosomes containing EBs of different *C. trachomatis* serovars [62]. Such a fusion can facilitate the intraspecific exchange of genetic information.

A chlamydial protein involved in endosome fusion was identified as IncA (CT119), which is capable of oligomerization and belongs to the Inc proteins (surface proteins of the inclusion membrane) [63–65]. The examination of 11400 *C. trachomatis* isolates showed that 176 isolates were capable of producing multiple inclusions, but did not have IncA on the inclusion membrane [66]. A sequencing of their *incA* revealed a deletion that eliminated two amino acid residues from the so-called hydrophobic domain, which is characteristic of all Inc proteins. IncA is phosphorylated by eukaryotic cell enzymes [67, 68].

The IncA capabilities of oligomerization and regulation of the homotypic fusion of chlamydial inclusions are presumably due to the so-called SNARE (soluble NSF (N-ethylmaleimide-sensitive factor) attachment protein receptors) motif, which is found in many eukaryotic proteins that mediate membrane fusion in various cell compartments (endosomes, vesicles of the Golgi complex, membranes of the endothelial reticulum, etc.). IncA can form complexes with the cell SNARE proteins Vamp3, Vamp7, and Vamp8 [69]. In this case, the bacterial protein possibly mimics the eukaryotic motif that performs a specific function in the cell. The SNARE motif was predicted for IncA homologs from six other strains of Chlamydiae with the exception of *C. pneumoniae* and for the *C. trachomatis* Inc proteins CT813 and CT223 and their *C. muridarium* orthologs. A second SNARE-like motif is predicted to occur in the N-terminal region of *C. trachomatis* IncA and is responsible for its oligomerization [63]. Thus, the Inc-mediated interactions with the cell SNARE proteins are possibly one of the key mechanisms regulating the fusion of chlamydial inclusions in infected cells.

In addition, *C. trachomatis* IncA plays a role in the generation of long membrane processes during the formation of secondary inclusions [70]. The transfection of eukaryotic cells with IncA blocks the development of chlamydial infection in transfected cells [63, 71].

The modification of the membrane of chlamydial inclusions involves certain genes that are expressed within the first few hours of chlamydial infection; many of these genes code for Inc proteins. In total, 22 *C. trachomatis* proteins were localized in the membrane of chlamydial inclusions (see the table for proteins and references). Seven proteins were localized within inclusions. The table, which characterizes the properties of putative Inc proteins, includes the data on their interactions with sera from female patients with *C. trachomatis* urogenital infections and shows the C- or N-terminal localization of their immunodominant regions. Interactions with antibodies are mostly observed for inclusion membrane proteins [6]. The Inc proteins predicted in silico [72, 73] include almost all proteins that were experimentally localized to the inclusion membrane. However, since the in silico predictions do not fully match the experimental findings, it is necessary to further identify the Inc proteins of Chlamydiae.

In addition to IncA and CT229, *C. trachomatis* IncG (CT118) is involved in modifying the inclusion membrane and recruiting cell proteins and their complexes. IncG binds the phosphoserin-binding protein 14-3-3β of the host cell and recruits it to the inclusion surface [74]. In turn, 14-3-3β is capable of binding the phosphorylated form of Bad, one of the key regulators of apoptosis. This is one of the mechanisms that prevent apoptosis in the infected host cell (see below) [75]. IncG interacts with and is phosphorylated by Pkn1, one of the two *C. trachomatis* Ser/Thr kinases [76].

Two cell proteins involved in signal transduction upon cell contact with bacterial lipopolysaccharides, Toll-like receptor 2 (TLR2) and the adaptor protein MyD88, are associated with chlamydial inclusion membrane in cells infected with *C. trachomatis* [77]. This finding makes it possible to assume that Chlamydiae utilize the TLR2-dependent pathway to transmit signals in the cell. These eukaryotic proteins are possibly recruited to the membrane of chlamydial inclusions via their fusion with vesicles budding from the Golgi complex.

GROWTH OF CHLAMYDIAL INCLUSIONS

Effects on the Cell Cytoskeleton

The cytoskeleton of eukaryotic cells is formed by microfilaments, microtubules, and intermediate filaments, which consist, respectively, of proteins of the actin–myosin system, the tubulin–dynein system, and keratins. Microfilaments are mostly concentrated close to the plasma membrane. They are responsible for cell shape, produce surface protrusions (pseudopodia and microvilli), are involved in intercellular interactions and signal transduction, and play a role in muscle contractions along with myosin. Microtubules play a key role in intracellular transport, serve as rails to allow movements of the molecular motors kinesin and dynein, and are involved in cell division. Intermediate filaments, which consist of keratin polymers, are the most dynamic part of the cytoskeleton. Their main function is to maintain cell integrity. Keratin 8, which is synthesized in epithelial cells, is one of the major components of intermediate filaments. Secreted *C. trachomatis* CPAF (CT858) is capable of hydrolyzing keratin 8 in the cytoplasm of epithelial cells [78]. It is thought that this activity serves to make the cytoskeleton less rigid and to allow the growth of chlamydial inclusions within the cell.

Approximately 20 h after entering the cell, chlamydial inclusions are covered with a network of F-actin filaments and intermediate filaments [79], which form a dynamic structure that stabilizes growing inclusions and maintains their integrity. The formation of F-actin filaments on the inclusion surface is regulated by RhoA GTPase. A chlamydial factor that recruits RhoA to the inclusion surface and the mechanism of RhoA activation are unknown. It is thought that intermediate filaments are attached to F-actin filaments via plakin-like proteins. In the absence of RhoA, the network of intermediate filaments grows looser, and chlamydial cells are released into the cytoplasm to activate innate immunity. CPAF plays a role in processing the intermediate filaments on the inclusion surface by cleaving sites between the N-terminal and central regions of microfilament proteins. In vitro, CPAF hydrolyzes not only keratin 8, but also cytokeratin 18 and vimentin [79]. This activity may modify the structure of intermediate filaments to make them more agile and to allow the grown of inclusions without completely distorting the properties of microfilaments that play a framework function in the cell.

Utilization of Host-cell Resources for the Growth of Chlamydial Inclusions

Approximately 6 h after the start of infection, EB transformation into RBs is complete, and chlamydial microcolonies start to exponentially grow with a doubling time of approximately 2.5 h. The growth continues for 24–40 h, depending on the strain. The chlamydial genome lacks a gene for a homolog of FtsZ [80], which is an important component of the system ensuring binary divisions of bacterial cells. Homologs of the FtsZ gene are found in almost all eubacteria and archaea and in eukaryotic plastids. FtsZ forms the so-called Z-ring, which provides a framework for accessory cell division proteins. The resulting structure is responsible for the formation of a septum. The lack of a FtsZ homolog may explain the mode of division where one large RB divides simultaneously into several small RBs [80].

The growth of chlamydial microcolonies must be accompanied by a growth of the membrane of chlamydial inclusions. The inclusions, which are on the way to endocytosis 2 h after the start of infection, grow their membrane by incorporating the vesicles that separate from the Golgi complex and move toward the surface of the epithelial cell [81, 82]. As inclusions fuse with vesicles, sphingomyelin occurring on the vesicle surface is relocated to the outer inclusion membrane and then transferred onto the membrane of RBs attached to the outer membrane. The outer membrane of inclusions captures phosphatidylcholine and phosphatidylinositol, which are contained in membranes of the endoplasmic reticulum; cardiolipin, which is contained in mitochondria; and cholesterol [83]. Then, Chlamydiae modify nonbranched fatty acids, which are characteristic of eukaryotes, into branched fatty acids, which are characteristic of prokaryotes.

The mechanism sustaining the translocation of lipid droplets from the host-cell cytoplasm into the lumen of chlamydial inclusions was studied by fluo-

rescence and electron microscopy [84]. Lipid droplets are organelles that accumulate and store neutral lipids in the cell. They are surrounded by a phospholipid monolayer with specific surface proteins, which prevent lipid droplets from degradation (lipolysis). When lipid droplets come close to the inclusion membrane, they produce vesicles that occur within the membrane and are in immediate contact with RBs. This is probably followed by a detachment of the vesicles from the inclusion membrane and lipolysis. IncA (but not the other Inc proteins) occurs in the same fraction with lipid droplets upon fractionation and accumulates simultaneously with droplets in the inclusion lumen. The transfer of lipid droplets presumably takes place in IncA-rich subdomains of chlamydial inclusions, but IncA was not demonstrated to be essential for the assimilation of lipid droplets by inclusions. It is possible that secreted *C. trachomatis* Lda3 (CT473) is involved in the attachment of cytoplasmic lipid droplets to the inclusion membrane and the elimination of the protective adipocyte differentiation-related protein (ADRP), which covers lipid droplets, to facilitate the initiation of lipolysis [84]. Mass transfer of lipid droplets from the host-cell cytoplasm into chlamydial inclusions is a unique adaptive mechanism that allows Chlamydiae to utilize whole cell organelles for their needs. Four proteins distinctly showed affinity for lipid droplets when chlamydial genes were expressed in yeast cells. These lipid droplet-associated (Lda) proteins are Lda1 (CT156), Lda2 (CT163), Lda3 (CT473), and Lda4 (CT257) [85]. Lda1 and Lda2 are characteristic only of *C. trachomatis.*

Use of Energy and Metabolic Resources of the Eukaryotic Cell

Actively growing RBs occur at the periphery of an inclusion, while newly formed EBs occur in its central region. It is clear that RBs utilize host-cell energy and nutrients to grow. There is experimental evidence for this assumption.

First, interferon γ induces a decrease of the tryptophan level in the host cell. This results in abnormally large and metabolically altered RBs (aberrant RBs), which are incapable of transformation into EBs [86]. Such slow metabolizing (persisting) RBs may occur in this state for a long time without being affected by antibiotics used to treat chlamydioses.

Second, like in other bacteria, iron deficiency induces the synthesis of specific chlamydial proteins, including the *hsp60* product. Electron microscopy reveals a layer of electron-dense material, which is presumably ferritin, around chlamydial inclusions. It is thought that Chlamydiae mimic mitochondria in order to obtain iron in the form of ATP-Fe(III) from ferritin [87].

Third, passive diffusion of molecules through the inclusion membrane was assumed to explain how nutrients penetrate into chlamydial inclusions. However, this hypothesis is probably invalid, because experiments with microinjections of labeled 520-Da molecules into the cytoplasm did not detect an accumulation of the molecules in chlamydial inclusions [88]. There are data that chlamydial inclusions interact with multivesicular bodies, which are organelles that mediate protein and lipid transport in the cell. The contents of microvesicular bodies, in particular, the CD63 marker protein, are detectable within inclusions [89]. However, CD63 is not essential for the above interaction [90].

Fourth, it was believed for a long time that Chlamydia act as energy parasites. More recent experimental studies showed that the *C. trachomatis* strain LGV has active enzymatic systems that produce ATP via oxidative phosphorylation [91]. The presence of such enzymes in this and other strains of Chlamydiae were confirmed by genome analysis. At the same time, Chlamydiae were found to have genes for ATP/ADP translocases (*tlcA* (CT065) and *tlcB* (CT495) in *C. trachomatis*), which transport ATP into the bacterial cell in exchange for ADP. It is likely that chlamydial species vary in how they use energy resources of the host cell. For instance, several features were observed for *C. psittaci.* Mitochondria are in the immediate vicinity of isolated inclusions. ATP/ADP translocase is present. ATP transport was demonstrated and proved to involve MOMP, which acts as a porin [92]. It is thought that the multiplicity of *C. psittaci* inclusions serves to increase the inclusion surface area and, consequently, to intensify the exchange of energy resources and metabolites with the host cell.

INTERACTION OF CHLAMYDIAE WITH CELL APOPTOSIS SYSTEMS

Apoptosis of the host cell can be induced via two main pathways, internal and external. In the former case, internal stimuli induce mitochondria to release proapoptotic factors, such as cytochrome *c*, which are involved in the formation of an apoptosome and thus lead to the activation of effector caspases 3, 6, and 7. Caspases hydrolyze various proteins and activate endonucleases, eventually causing apoptosis.

The external apoptotic pathway is triggered as the so-called death receptors interact with their ligands on the cell surface. The interaction yields signaling complexes, which activate caspases, in particular, caspase 8. In turn, caspase 8 activates effector caspases, which trigger the terminal steps of apoptosis by inducing the release of cytochrome *c* from mitochondria.

The release of proapoptotic factors, in particular, cytochrome *c*, from mitochondria is regulated by proteins of the Bcl-2 family, which includes several do-

zens of proteins. The proteins share at least one domain, which is known as the Bcl-2 homology (BH) domain. Bcl-2 has four BH domains, which occur in the order of BH4, BH3, BH1, and BH2 from the N to the C end.

The family is divided into three subfamilies according to the structural and functional characteristics of their members [93, 94]. Antiapoptotic Bcl-2 subfamily proteins (Mcl-1, Bcl-2, and Bcl-xL) have three out of the four BH domains. Proapoptotic multidomain Bcl-2 subfamily proteins (Bax and Bak) have more than one BH domain. Their activation can induce the release of mitochondrial cytochrome *c*, triggering apoptosis [95]. Proapoptotic proteins of the BH3-only subfamily (Bid, Bad, Bik, Puma, Bim, Bmf, Noxa, and Hrk) possess only one BH3 domain [96].

The protein–protein interactions of the Bcl-2 family proteins in regulating the cytochrome *c* release from mitochondria are not completely understood. It is thought that the BH3-only proteins associated with intracellular organelles, which perceive the signals from proteins of the external (Bid) or internal (Bim, Bmf, Puma, and Bik) apoptotic pathways, undergo posttranslational modification and are transferred to mitochondria. These BH3-only proteins may transmit the death signal to mitochondria by inhibiting antiapoptotic proteins and activating proapoptotic proteins, such as Bax and Bak [93, 96].

Chlamydiae block apoptosis in both productive [34] and persistent [97] infection. Cell infection with *C. trachomatis* activates the Raf/MEK/ERK and phosphatidylinositol-3 kinase (PI3K)/AKT signaling pathways. These pathways mediate, respectively, a higher production and stabilization of the antiapoptotic protein Mcl-1, which belongs to the Bcl-2 family. A decrease in Mcl-1 sensitizes cells to apoptosis, which may be induced via the TNF receptor or by DNA damage, granzyme B, or stress. An increase in Mcl-1 is thought to be a key step in triggering the processes that render *C. trachomatis*-infected cells resistant to apoptosis [98].

There is evidence that an increase in the cellular inhibitor of apoptosis (cIAP) and the formation of IAP–IAP heteromeric complexes play an important role in the resistance of cells infected with *C. trachomatis* to TNF-induced apoptosis [99].

At the same time, contradictory data were obtained in experiments with mouse embryo fibroblasts knocked out in the cIAP1, cIAP2, cIAP1/cIAP2, XIAP, or Mcl-1 genes [100]. Infection with *C. trachomatis* equally protected all of these cells from apoptosis induced by TNF/cycloheximide (IAP knockout cells) or staurosporine (Mcl-1 knockout cells). Thus, the IAP proteins and Mcl-1 are probably inessential for preventing host cell apoptosis in *C. trachomatis* infection.

Studies showed that antiapoptotic activity of Chlamydiae correlates with a block of cytochrome *c* release from mitochondria [34], suppressed activation of the Bax and Bak proapoptotic proteins [96], and degradation of the BH3-only proteins [101–103]. The BH3-only proteins are degraded by chlamydial CPAF (CT858) [104].

To prevent apoptosis of the host cell, *C. trachomatis* utilizes not only proteolytic degradation of the BH3-only proteins, but also another strategy, which is based on the fact that the phosphorylated form of the Bad BH3-only protein binds to the surface of chlamydial inclusions [75]. A key role is played by the human adaptor protein 14-3-3β, which occurs in the cytoplasm of noninfected cells. In cells infected with *C. trachomatis*, the 14-3-3β protein is located on the surface of the inclusion membrane as a result of its interaction with chlamydial IncG (CT118) [74]. In noninfected cells, the 14-3-3β protein binds with phosphorylated Bad in the presence of survival factors and thus sequesters Bad, preventing its binding with its targets Bax and Bac on mitochondria. In the absence of survival factors, Bad is dephosphorylated and, when associated with mitochondria, facilitates the cytochrome *c* release and cell death. Bad is phosphorylated by AKT/protein kinase B (PKB), which is activated by PI3K in the presence of survival factors. Infection with *C. trachomatis* activates the PI3K-dependent pathway; consequently, Bad is phosphorylated and fixed on the surface of chlamydial inclusions as a result of its complexation with 14-3-3β and IncG [75]. PI3K inhibition sensitizes cells infected with *C. trachomatis* to staurosporine-induced apoptosis. A decrease in AKT via RNA interference restores the apoptosis resistance in infected cells. The PI3K-dependent pathway is not involved in the prevention of apoptosis in the absence of phosphorylated Bad or in cells infected with *C. pneumoniae*, which lacks an IncG analog in contrast to *C. trachomatis.*

Another way to prevent apoptosis of infected cells is to fix the proapoptotic kinase PKCδ on the membrane of chlamydial inclusions [105]. This enzyme binds to diacylglycerol contained in the inclusion membrane via its diacylglycerol-binding (C1) domain. The intracellular PKCδ pool is almost totally associated with chlamydial inclusions in infected cells. This prevents PKCδ from activating apoptosis on mitochondria.

Certain data implicate the chlamydial protein associating with death domains (CADD, CT610) in modulating apoptosis of cells infected with *C. trachomatis* [106]. CADD induces apoptosis in mammalian cells upon transient transfection with its gene. In vitro, CADD interacts with the death domain of the TNF family receptors TNFR1, Fas, DR4, and DR5. CADD colocalizes with DR5 on the perimeter of chlamydial inclusions in infected epithelial cells. It was assumed,

to explain the antiapoptotic effect, that the receptor proteins, which are involved in transmitting signals that trigger apoptosis, are recruited to chlamydial inclusions and thus removed from the signal transduction pathway.

EFFECTS OF CHLAMYDIAE ON THE CELL CYCLE

Cell division may interfere with the intracellular life of Chlamydiae by consuming a substantial portion of cell nutrient reserves. Cells infected with *C. trachomatis* divide at a lower rate as compared with noninfected cells [107, 108]. Cytokinesis (cell division) is suppressed, while the mitosis rate remains almost unchanged; this results in cells with two or more nuclei [108]. These data are directly related to the finding that chlamydial inclusions are tightly associated with the cell centrosomes [109]. The association is mediated by cell dynein [55]. The interaction of inclusions with centrosomes substantially increases the centrosome number, distorts chromosome segregation, and causes chromosome abnormalities in cells infected with Chlamydiae [109].

Infection with *C. trachomatis* decreases the level of cyclin-dependent kinase 1 (CDK1) and leads to cyclin B1 hydrolysis [110]. The changes in CDK1 and cyclin B1 stability may cause infected cells to more slowly proceed through the cell cycle. This is explained by the role of cyclin B1 in cell division. Cyclin B1 concentration starts to increase in the late S and through G2 phases and reaches its maximum in mitosis, allowing cyclins B1 and B2 to replace cyclin A in complex with CDK1. Yet, this is insufficient for complete CDK1 inactivation, which is achieved as CDK1 is phosphorylated and dephosphorylated at specific amino acid residues. When these processes are complete, the cell enters mitosis. The factors that reduce the levels of CDK1 and cyclin B1 are unknown.

Some other data [111] contradict the above strategy. It was demonstrated that *C. trachomatis* CT847 is a substrate of the type III transport system. Experiments with the yeast two-hybrid system identified the human Grap2 cyclin D-interacting protein (GCIP) as an interaction partner of CT847. The GCIP level in the cell starts to decrease as CT847 production starts 8–12 h after infection. After 24 h, GCIP becomes completely undetectable. It is thought that GCIP is directed to the proteasome as a result of its interaction with CT847, since the GCIP level does not decrease in the presence of proteasome inhibitors.

GCIP is multifunctional. In particular, GCIP is capable of interacting with cyclin D1 and modulating cell proliferation. A decrease in GCIP stimulates cell proliferation by facilitating cell progress through the G1/S checkpoint. It is unclear how GCIP degradation and stimulation of the G1/S transition help *C. trachomatis* infection to develop. There is evidence, however, that cell treatment with small interfering RNAs (siRNAs) to reduce the GCIP level before infection allows a more rapid progress of infection [111]. It is of interest that the CT847 ortholog of *C. pneumoniae* (Cpn1004) does not interact with GCIP in the yeast two-hybrid system. This finding indicates that, in contrast to *C. trachomatis, C. pneumoniae*, does not use a strategy based on the effects on the host cell cycle, which is supported by the fact that the CDK1 and cyclin B1 levels do not decrease in cells infected with *C. pneumoniae.*

PROTECTION FROM TNF-α

Infection with *C. trachomatis* reduces the level of TNFR1 on the cell surface [112], probably because TNFR1 is captured by chlamydial inclusions. This phenomenon takes place when the host cell contains metabolically active growing bacteria. The effect is observed in cells of various types, including epithelial and endothelial cells, monocytes, and T cells, thus reflecting a general mechanism. CADD is a possible candidate protein that retains TNFR1 on the surface of chlamydial inclusions, since CADD interacts in vitro with CD95, TNFR1, TRAIL-R4 (DR4), and TRAIL-R2 (DR5) [106]. However, this assumption is questionable because the CD95 (Fas/Apo1) concentration on the cell surface does not decrease in chlamydial infection.

The general production of TNFR1 increases in cells infected with Chlamydiae [113]. However, a substantial portion of TNFR1 is concentrated close to chlamydial inclusions and trans-Golgi regions and is associated with lipid rafts [114, 115], which are cholesterol- and sphingolipid-rich microdomains of the membrane lipid bilayer [116] and are abundant in the membrane of chlamydial inclusions [117]. It is possible that TNFR1 is transferred from the Golgi complex to chlamydial inclusions as cholesterol and sphingolipids are captured. A higher expression of TNFR1 is controlled via the MEK–ERK pathway, since MEK1/2 inhibition decreases the TNFR1 concentration.

The ERK pathway is activated in chlamydial infection [118, 119]. The TNFR1 release involves matrix metalloproteinase TACE [120], since it is blocked by TACE inhibitor TAPI-1 in a concentration-dependent manner. TACE phosphorylation is driven by enzymes of the ERK pathway and is essential for the transfer of TACE to the cell surface [121]. TACE is incorporated in lipid rafts when transferred through the Golgi complex [122] and, consequently, may be recruited (along with TNFR1) to chlamydial inclusions simultaneously with cholesterol and sphingolipids. TNFR1 released from infected cells may bind bactericidal TNF-α in the intercellular space. Other pathogens, such as the Epstein–Barr virus, cytomegalovirus, and

Shigella, also have strategies to reduce the expression or surface concentration of TNFR1 [123–135]. Thus, a decrease in surface TNFR1 is one of the strategies used by Chlamydiae to evade the effect of antibacterial cytokines of the host organism.

RELEASE FROM THE HOST CELL

The signals responsible for a completion of the chlamydial life cycle are still poorly understood. The process is probably initiated when nutrient reserves of the host cell are depleted. The depletion possibly induces a detachment of RBs from the inner surface of the inclusion membrane and triggers the synthesis of histones, which regulate the expression of specific genes responsible for the last steps of the life cycle and DNA condensation [126, 127].

Chlamydiae may be released from the host cell in several ways. As the inclusion membrane is disrupted, EBs are released into the cytoplasm. This allows EBs to be laterally transferred into neighbor cells and to infect them. Microimaging showed that chlamydial inclusions intensely rotate first counterclockwise and then clockwise; consequently, the cell is disrupted and the inclusion contents are released within several minutes. It was also observed that chlamydial inclusions move toward the eukaryotic cell surface as in exocytosis and that the inclusion membrane then fuses with the apical or basal membrane of the cell [128]. EBs appearing in the extracellular space infect new cells, while RBs, which are sensitive to osmotic shock, are disrupted and die.

Recent data demonstrate that Chlamydiae leave the host cell via extrusion in half of the cases; i.e., a part of a chlamydial inclusion surrounded by the cell plasma membrane buds from the cell [2]. The cell containing the other part of the inclusion is not degraded. The fate of the budding part of the inclusion is unclear. It is possible that such structures infect new cells by fusing with their membranes or are captured by macrophages, which are consequently infected.

TYPE III TRANSPORT SYSTEM: EXPORT OF CHLAMYDIAL PROTEINS INTO THE HOST-CELL CYTOPLASM

Chlamydial effector proteins are actively transferred into the host-cell cytoplasm by the type III transport system. The system consists of an injectisome (or a molecular nanosyringe), which is a special structure of 20–25 proteins, and a chaperone system. The sequenced chlamydial genomes contain a set of highly conserved determinants of the type III secretion system, which are distributed through several chromosome regions. The structural and regulatory proteins of the system and the chaperones have homologs in *Yersinia, Shigella, E. coli*, and Gram-negative plant pathogens [129], while homology is not observed for secreted effector proteins. This is characteristic of all known type III secretion systems. Figure 3 shows the putative structure assumed for the chlamydial type III transport system by homology of transport system proteins between Chlamydiae and other Gram-negative bacteria.

The substrates of the *C. trachomatis* type III transport system probably include the putative external transport system components CopN [130], CopB [131], and CopD [132]; the Inc membrane-associated proteins [133, 134]; TARP, which is involved in invasion [36]; Pkn5, which is a Ser/Thr kinase whose gene cluster together with the type III transport system genes immediately upstream of the CdsC (contact-dependent secretion C) gene [132]; CT847, which interacts with the cell cycle modulator GCIP [111]; and some other proteins that still lack functional annotation (CT671, CT652.1, CT718, and CT848) [132, 135].

CopN, which is homologous to the *Y. pestis* YopN regulator, presumably regulates the channel of the type III transport system and determines the detachment of the injectisome from the inclusion membrane at the step of RB differentiation into EBs. This agrees with the fact that CopN is synthesized at the late stages of infection [136, 137]. It is thought that YopN blocks the channel of the type III transport system when interacting with its chaperone [138]. A contact with the cell abolishes this blockage. CopN possibly plays a similar role in Chlamydiae.

In the *C. pneumoniae* strain MoPn, TC0044 is a potential effector protein. TC0044 has a conserved serine–threonine motif, and its gene is within cluster II of type III transport system genes. Other possible effectors are encoded by *TC0042, TC0867*, and *TC0868*, which are also close to a cluster of type III transport system genes [129].

Substrates are exported through a secretion pore. In *C. trachomatis*, the pore consists of the secretin-like outer membrane protein CdsC (CT674), the periplasmic lipoprotein CdsJ (CT559), and many structural proteins of the inner RB membrane (SdsR (CT562), CdsS (CT563), CdsT (CT564), CdsU (CT091), and CdsV (CT090) [130]). In addition, CdsJ probably interacts with the inner membrane protein CdsD (CT664), which is found on the surface of *C. trachomatis* EBs [139]. CdsN (CT669) acts as an ATPase and appears to supply energy for secretion [130]. By homology with its *Yersinia* counterpart, CdsL (CT561) is possibly associated with secretion complex proteins of the inner membrane on the cytoplasmic side and interacts with the protein product of *CT669*.

By amino acid sequence similarities with *Yersinia* proteins, Scc1 (CT088), Scc2 (CT576), and Scc3 (CT862) act as chaperones for substrates of the type

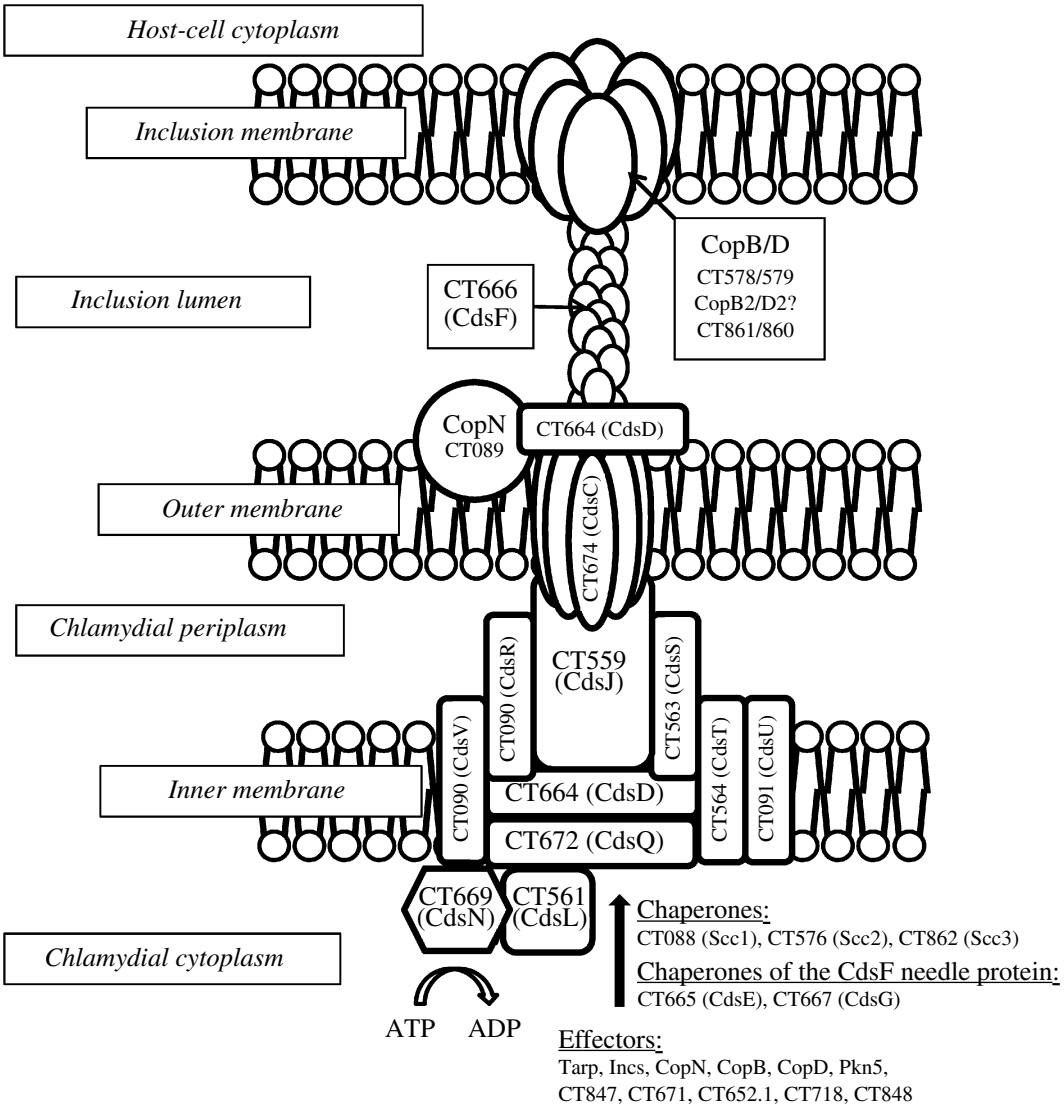

Fig. 3. Type III secretion system of Chlamydiae. Chlamydial proteins are designated as accepted for the ORFs of the *C. trachomatis* serovar D genome.

III transport system. The CT666 ORF seems to code for a structural component of the "needle" of the CdsF nanosyringe. CdsE (CT665) and CdsG (CT667) act as chaperones for CdsF [140]. By homology to *Yersinia* YscE/YscG, these proteins probably form a heterodimer, which forms a complex with CdsF. By homology to the YopB family proteins, CopB (CT578) and CopB2 (CT861) probably act as translocation proteins of the type III transport system and transfer the effector proteins across the bacterial plasma membrane [131]. However, while CopB occurs in the inclusion membrane, CopB2 is found in the host-cell cytoplasm [131], suggesting another function for this YopB homolog in Chlamydiae. CopD/D2 (CT579/860) also belong to translocation proteins according to their homologies [141].

Homologs were studied for YscD, YscQ, and YscL, which are components of the *Yersinia pestis* type III transport system. The *C. pneumoniae* homologs are Cpn0712 (CdsD), Cpn0704 (CdsQ), and Cpn0826 (CdsL). The three proteins interact both in vitro and in vivo [142]. CdsD occurs in both RB and EB lysates and is located within inclusions. It is of interest to note that CdsD is phosphorylated by the chlamydial kinase PknD at serine and tyrosine, while PknD is autophosphorylated at threonine and tyrosine [142]. PknD provides the first known example of a bacterial kinase that phosphorylates both serine/threonine and tyrosine.

The chlamydial genomes have a set of genes homologous to the genes that regulate the movements of bacterial flagella (flagellin system), although Chlamydiae are not motile. The flagellar genes *fihA*

(CT060), *fliF* (CT719), and *fli1* (CT717) are homologous to the so-called *cds* genes and form two genomic clusters in all genomes of the family Chlamydiaceae. All of these genes are expressed at the middle steps of chlamydial infection [136], but their functions are obscure, since they code for only some proteins of the flagellar basal body. It is possible that the genes code for some proteins of hypothetical flagella, which ensure chlamydial motility within an inclusion, interact with the injectisome of the type III transport system, or play another unidentified role [141].

CONCLUSIONS

Over millions of years of their intracellular life, Chlamydiae developed many adaptive mechanisms to efficiently utilize eukaryotic cells as habitats and to successfully evade the host mechanisms aimed at eliminating infection. Many functions are redundant and concurrent at almost all steps of the interaction of Chlamydiae with the host cell.

A typical example is provided by the *C. trachomatis–host* cell interactions that render infected cells resistant to apoptosis induced by external factors. Three chlamydial protein factors—cytoplasmic CPAF and the IncG and CADD proteins associated on the inclusion membrane—are involved in inhibiting or binding the proapoptotic factors of the cell. CPAF cleaves the BH3-only proteins, which play a role in the mitochondrial apoptotic pathway. IncG acts through the eukaryotic 14-3-3β protein and binds the phosphorylated form of Bad, one of the key components of the mitochondrial apoptotic pathway. CADD facilitates the localization close to the inclusion membrane for TNFR1, DR4, DR5, and FasR, which transmit the external signals that trigger apoptosis. The pool of the cell proapoptotic kinase PKCδ is almost totally bound on the inclusion membrane via interactions with diacylglycerol to further contribute to the antiapoptotic phenotype of infected cells.

The chlamydial systems that prevent the immune response to infection are as redundant. CPAF hydrolyzes the host transcription factors responsible for the production of MHCI and MHCII components, which present chlamydial antigens on the surface of infected cells. In addition, CPAF degrades the MHC-like surface glycoprotein CD1d, which presents lipid antigens to NKT cells. Protease CT441 specifically degrades the p65/RelA subunit of the transcription factor NF-κB, thus preventing NF-κB from entering the nucleus to initiate transcription of its target genes and, eventually, to induce an inflammatory response. ChlaDub1 suppresses the activation of NF-κB by deubiquitinating its inhibitor I-κBα and consequently preventing its degradation.

Another mechanism to evade the immune response is based on the role that CPAF plays at the late stages of infection. At these stages, inclusions are large, contain a huge number of RBs and second-generation EBs, and need additional stabilization. Components of the cytoskeleton are utilized for such stabilization: inclusions are covered with a network of actin fibrils and fibrils of intermediate filaments. The resulting rigid structure is modified and becomes more elastic as CPAF partly hydrolyzes the fibrils of intermediate filaments. When the supporting structures of inclusions are disrupted, some of the chlamydial cells are released into the host cytoplasm and degraded; consequently, chlamydial antigens may be presented on the cell surface, and the infected cell may die.

Parallel processes are also observed when Chlamydiae enter the cell, triggering actin polymerization. This involves the cell kinases PDGFRβ and Abl, which phosphorylate several proteins and trigger a cascade of protein–protein interactions. Chlamydial TARP plays a key role in this process.

Further processes involve the transport of chlamydial inclusions into the perinuclear region to their final location at the centrosomes (microtubule organization centers), in the immediate vicinity of the Golgi complex. These processes concurrently involve many cell factors, such as Rab-GTPases, cortactin, BICD1, and dynein.

Rab-GTPases are associated with inclusions via chlamydial protein factors, CT229 in *C. trachomatis* and Cpn0585 in *C. pneumoniae*. The homotypic fusion of chlamydial inclusions is also facilitated by numerous protein factors. The key role is played by IncA, which has the SNARE domain and interacts with the SNARE proteins of the cell. A certain role is probably played by host-cell annexins.

Chlamydia trachomatis modulates the life cycle of the host cell by inducing cyclin B1 and GCIP degradation. The latter effect is mediated by chlamydial CT847, which interacts with GCIP to cause its transfer into the proteasome.

The chlamydial effector factors include both proteins characteristic of all Chlamydiae (CPAF and IncA) and species-specific proteins (CT229 and IncG in *C. trachomatis*). The latter may perform functions that are conserved among Chlamydiae, e.g., preventing apoptosis or recruiting Rab-GTPases to the inclusion surface. This effect probably prevents the host cell from developing resistance to Chlamydiae [144].

The effector proteins influence the cell systems in various ways, e.g., via proteolysis, protein–protein interactions, phosphorylation, and ubiquitination/deubiquitination. One of the main roles in the interaction with the host cell is played by CPAF, whose known targets are continuously increasing in number.

Studies of the multiple effects of chlamydial infection explain the association of chlamydioses with other human disorders. Cyclin B1 degradation,

delayed cytokinesis, and an increased centrosome number in the infected cell may destabilize the genome. Given the antiapoptotic activity of Chlamydiae, the above effects may be responsible for the observed association between *C. trachomatis* infection and cervical cancer [145].

Chlamydiae are characterized by a cyclic development and a lack of metabolic activity in EBs. However, as early as the first steps of their interaction with the eukaryotic cell, Chlamydiae use specific protein factors, such as TARP and CopN. These proteins are synthesized at later stages and accumulate in EBs, possibly, in an association with the type III transport system to initiate the internalization of Chlamydiae upon their contact with the target cell.

Thus, Chlamydiae acquired a complex adaptive system during their intracellular life, and the multiplicity of the mechanisms involved ensures the stability of the system and allows Chlamydiae to develop in cells of various species and to withstand various stress factors. The system provides a unique example of a contact and interplay between the prokaryotic and eukaryotic domains of life. Further investigation of the molecular mechanisms that sustain the interaction of Chlamydiae with their eukaryotic environment has both theoretical and applied significance, making it possible to identify the key protein targets for drug therapy of chlamydioses.

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