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Chlamydia trachomatis Infection Induces Cleavage of the Mitotic Cyclin B1

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The obligate intracellular pathogen *Chlamydia trachomatis* interferes with a number of host cell processes, including cytoskeletal organization, vesicular trafficking, and apoptosis. In this study we report that *C. trachomatis*-infected cells proliferate more slowly than uninfected cells, suggesting that *C. trachomatis* may also manipulate the eukaryotic cell cycle. We further demonstrate that *C. trachomatis* infection destabilizes specific cell cycle proteins involved in the G_2/M transition. *C. trachomatis*-infected cells, compared to uninfected cells, have lower levels of cyclin-dependent kinase 1. Additionally, *C. trachomatis* infection induces an N-terminal truncation of the mitotic cyclin B1. Manipulation of the host cell cycle may represent a strategy used by *C. trachomatis* to ensure a stable environment conducive to bacterial growth and replication.

The obligate intracellular pathogen *Chlamydia trachomatis* has a unique developmental cycle characterized by conversion between two developmental forms, namely, the elementary body (EB) and the reticulate body (RB). The EB is the infectious but metabolically inactive form of the organism. Upon entry into the host cell, EBs rapidly convert to the metabolically active RBs. RBs replicate within a specialized vacuole, known as the inclusion, that does not fuse with the phagolysosome. Approximately 18 to 20 h postinfection (hpi), RBs begin to convert back to EBs. Finally, around 36 to 48 hpi, extensive intracellular replication of *C. trachomatis* leads to lysis of the host cell and the release of EBs. These organisms can then begin new rounds of infection and replication within neighboring host cells (40).

Robust replication of *C. trachomatis* requires optimal conditions within the host cell. Most importantly, the cell must remain viable during infection. *C. trachomatis* has been shown to promote host cell survival by actively inhibiting the mitochondrial pathway of apoptosis, in part by triggering proteasomal degradation of proapoptotic factors within the cell (8, 10–12, 41). Even host cell division may pose challenges to *C. trachomatis* replication, since important nutrients may be consumed as the cell divides. In fact, several studies support the idea that *C. trachomatis* may target the host cell cycle to ensure its successful growth and replication (17, 21). One report demonstrated that host cells infected with *C. trachomatis* undergo a lower rate of cell division than their uninfected counterparts (21). A more recent study concluded that *C. trachomatis* infection inhibits host cell cytokinesis (17).

In this study, we have directly tested whether *C. trachomatis* infection alters the host cell cycle by examining individual proteins involved in cell cycle progression. We demonstrate that *C. trachomatis* infection not only leads to a delay in host cell proliferation but also leads to a reduction in levels of cyclin-dependent kinase 1 (cdk1) and cleavage of the mitotic cyclin B1. The altered stability of cdk1 and cyclin B1 following *C. trachomatis* infection may contribute to a slowed progression of infected cells through the later stages of the cell cycle.

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MATERIALS AND METHODS

Cell culture and synchronization. HeLa and CHO-K1 (Chinese hamster ovary) cells were cultured in Dulbecco's modified Eagle medium (DMEM) and in DMEM–F-12 medium (1:1), respectively, supplemented with 10% fetal calf serum, 2 mM L-glutamine, 50 U/ml penicillin G, and 50 μg/ml streptomycin sulfate. McCoy cells were grown in Eagle's minimal essential medium supplemented with 10% fetal calf serum, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, and 1.5 g/liter sodium bicarbonate. Prior to infection with chlamydiae (see below), all cells were synchronized at the G_1/S border by incubation with 10 mM hydroxyurea for 24 h.

***Chlamydia* propagation and infection of cells.** *C. trachomatis* serovar L2 434/Bu was propagated within McCoy cell monolayers. A combination of glass bead disruption and sonication was used to release EBs from infected McCoy cells. EBs were further purified by ultracentrifugation over Renografin gradients as previously described (22). Aliquots of *C. trachomatis* EBs were stored at −80°C in SPG buffer (250 mM sucrose, 10 mM sodium phosphate, and 5 mM L-glutamic acid, pH 7.2). Aliquots of *C. pneumoniae* serovar/strain K6 were generously provided by Benjamin Wizel (University of Texas at Tyler).

Prior to infection with *Chlamydia*, cells were cultured for 12 to 24 h in their respective media minus antibiotics. Cells were infected with *C. trachomatis* or *C. pneumoniae* by adding EBs diluted in SPG buffer to cells and centrifuging at 37°C for 1 h (2,000 × *g*). The multiplicity of infection used for *C. trachomatis* was 10:1 unless otherwise stated and was 30:1 for *C. pneumoniae*. SPG buffer alone was used for mock infection of cells.

For chloramphenicol treatment, cells were first infected with *C. trachomatis* for the various lengths of time specified. Chloramphenicol was then added to the cells at a concentration of 100 μg/ml or 200 μg/ml for the duration of the experiment.

CFSE labeling of cells and flow cytometry. Cells were resuspended in phosphate-buffered saline (PBS)-0.1% bovine serum albumin (BSA) at a concentration of 5×10^7 cells/ml. Carboxyfluorescein diacetate succinimidyl ester (CFSE) was added to the cells to a final concentration of 10 μM, and cells were incubated for 10 min at 37°C with intermittent shaking. After washing in DMEM–F-12 medium (1:1), cells were plated and incubated at 37°C for 8 h to release excess CFSE label. Cells were then infected with *C. trachomatis* or mock infected as described above. To harvest, cells were treated with 0.05% trypsin-EDTA, washed twice in PBS-0.1% BSA, and fixed in 2% paraformaldehyde-0.2% Tween 20. Cells were stained with the mouse anti-major outer membrane protein (MOMP) antibody YVS1651 (Accurate Chemical and Scientific Corporation, Westbury, NY), for 90 min at 37°C and subsequently labeled with a Cy5-conjugated donkey anti-mouse immunoglobulin G antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA). Samples were washed in PBS-0.1% BSA and analyzed using the BD FACSCalibur system (BD Biosciences, San Jose, CA).

Plasmid construction and transfection. The cyclin B1 gene was amplified from CHO-K1 cDNA using the following primers: cyclin B1 forward, 5′ CCGGAAT TCGCCATGGCGCTCAGGGTCACTAGG 3′; and cyclin B1 reverse, 5′ CCG CTCGAGCGCCTTTGCCACAGCCTTGG 3′.

Amplified inserts were cloned into the EcoRI and XhoI sites of pcDNA3.1+, carrying a C-terminal hemagglutinin (HA) tag and a stop codon between the XhoI and XbaI sites (29), to generate cyclin B1-HA. CHO-K1 cells were transiently transfected with Lipofectamine 2000 (Invitrogen Corporation, Carlsbad, CA).

Immunoblot analysis and antibodies. Unless otherwise stated, cells were lysed in radioimmunoprecipitation assay buffer (1% NP-40, 0.1% sodium dodecyl sulfate [SDS], 150 mM NaCl, 50 mM Tris-HCl, pH 8.0, and 0.5% sodium deoxycholate) in the presence of complete protease inhibitor cocktail (Roche Applied Science, Indianapolis, IN). Lysates were normalized for total protein content by measuring absorption at 280 nm. Normalized samples were subjected to SDS-polyacrylamide gel electrophoresis (PAGE) analysis and immunoblotted with the following antibodies: rabbit antiphospho(Tyr15)-cdk1, rabbit anti-cdc25C, mouse anti-cyclin B1 V152 (Cell Signaling Technology, Danvers, MA), mouse anti-cdk1 clone 17, mouse anti-cyclin B1 GNS1 (Santa Cruz Biotechnology, Inc. Santa Cruz, CA), mouse anti-*C. trachomatis* MOMP YVS1651 (Accurate Chemical and Scientific Corporation, Westbury, NY), mouse anti-*C. pneumoniae* clone M73066 (Fitzgerald Industries, Concord MA), mouse anti-HA 12CA5 (Roche Applied Science, Indianapolis, IN), and mouse anti-β-actin (Sigma-Aldrich, St. Louis, MO).

"Mixed lysate" experiment. Lysate from $\sim 2 \times 10^5$ CHO-K1 cells transfected with cyclin B1-HA was mixed with lysate from $\sim 2 \times 10^5$ mock-infected or *C. trachomatis*-infected CHO-K1 cells. For "buffer only" samples, the infected cell lysate was replaced with an equivalent volume of cell lysis buffer. These mixed lysates were incubated at room temperature for 5 h. Approximately 15% of each sample was analyzed by SDS-PAGE and immunoblot analysis with the mouse anti-HA antibody (see above).

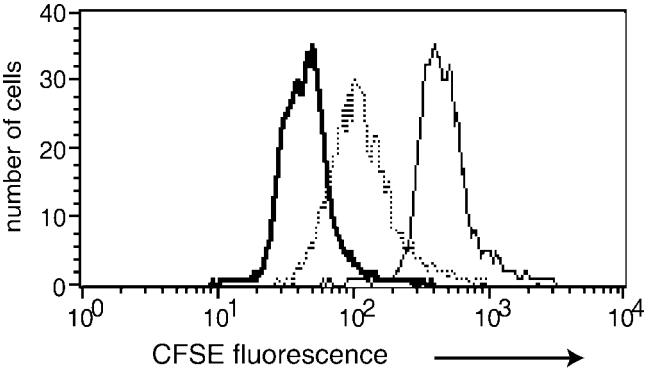

FIG. 1. *C. trachomatis*-infected cells proliferate more slowly than uninfected cells. CHO-K1 cells were labeled with CFSE and subsequently mock infected or infected with *C. trachomatis*. Cells were harvested at either 0 hpi or 40 hpi and then stained with an anti-MOMP antibody (detected with a Cy5-labeled secondary antibody) that specifically distinguishes *C. trachomatis*-infected cells. Samples were subsequently analyzed by flow cytometry. Within the *C. trachomatis*-infected population of cells, only cells that stained $MOMP^+$ and had a side scatter of >200 (indicative of infected cells with large inclusions) were analyzed. Mock-infected cells at 0 hpi are shown with a thin solid line, mock-infected cells at 40 hpi are depicted with a thick solid line, and *C. trachomatis*-infected cells at 40 hpi are represented with the thin dotted line. CFSE fluorescence is measured on the *x* axis, while the number of cells with a particular CFSE fluorescence is plotted on the *y* axis. The data shown are representative of two independent experiments.

RESULTS

***C. trachomatis* infection slows the progression of host cells through the cell cycle.** To determine if *C. trachomatis* infection alters the rate of host cell proliferation, CHO-K1 cells were labeled with CFSE and infected with *C. trachomatis* at a multiplicity of infection of 5:1. CFSE is a fluorescent dye commonly used to monitor cell division (27). When a cell labeled with CFSE divides, the dye is distributed equally to each of the daughter cells such that the more a cell proliferates, the lower its CFSE fluorescence becomes. This change in fluorescence over time can be measured by flow cytometry. As shown in Fig. 1, uninfected cells underwent a reduction in CFSE fluorescence over 40 h that corresponded to approximately three rounds of cell division. In contrast, over this time period *C. trachomatis*-infected cells retained a mean fluorescence intensity higher than that of their uninfected counterparts, indicating that infected cells had undergone fewer than three rounds of cell division after 40 h.

***C. trachomatis* infection affects stability of cell cycle proteins.** Cell cycle progression is controlled by a class of proteins known as cyclins. Cyclins associate with specific partner proteins, known as cdk's, to form active complexes that facilitate the transitions between major stages in the cell cycle. A number of pathogens have evolved strategies to inhibit the host cell cycle by interfering with the levels and/or activation of specific cyclin-cdk complexes (33, 38). We hypothesized that *C. trachomatis* may also target cell cycle proteins as a means of regulating eukaryotic cell proliferation. Because it had previously been shown that eukaryotic DNA synthesis is normal in *C. trachomatis*-infected cells (1, 16), we postulated that *C. trachomatis* may have an effect on the host cell cycle at the later stages of G_2 and M (mitosis).

CHO-K1 cells were infected with *C. trachomatis* for different lengths of time. Cells were then lysed, subjected to SDS-PAGE analysis, and immunoblotted with antibodies against cdk1, cyclin B1 (V152), and cdc25c, which are cellular proteins involved in the G_2/M transition. In comparison to uninfected cells, *C. trachomatis*-infected cells demonstrated significantly lower levels of tyrosine 15-phosphorylated cdk1 (inactive cdk1) over the course of infection (Fig. 2A). This effect was not specific to the phosphorylated form of cdk1, since an antibody against total cdk1 also revealed a decrease in protein levels following *C. trachomatis* infection (Fig. 2B). In contrast, levels of the phosphatase cdc25C were indistinguishable between uninfected and *C. trachomatis*-infected samples at all time points analyzed (Fig. 2C).

Like the levels of cdc25C, the overall levels of cyclin B1 did not change as a result of *C. trachomatis* infection (Fig. 3A). However, we observed that cyclin B1 migrated at differing molecular weights in uninfected versus infected cells (Fig. 3A, lower panel). As early as 14 hpi, cyclin B1 from *C. trachomatis*-infected cells appeared as a protein with a molecular weight lower than that of cyclin B1 detected in uninfected cells. This change in the size of cyclin B1 was observed until at least 24 hpi. Similar results were obtained when cyclin B1 was analyzed in *C. trachomatis*-infected HeLa cells, a human cervical epithelial cell line, and in McCoy cells, a murine fibroblast cell line (data not shown). This modification of cyclin B1 appears to be specific to *C. trachomatis*, since the molecular weight of cyclin B1 in cells infected with *Chlamydia pneumoniae*, a different species of *Chlamydia*, was indistinguishable from that in uninfected cells, even after 72 hpi (Fig. 3B).

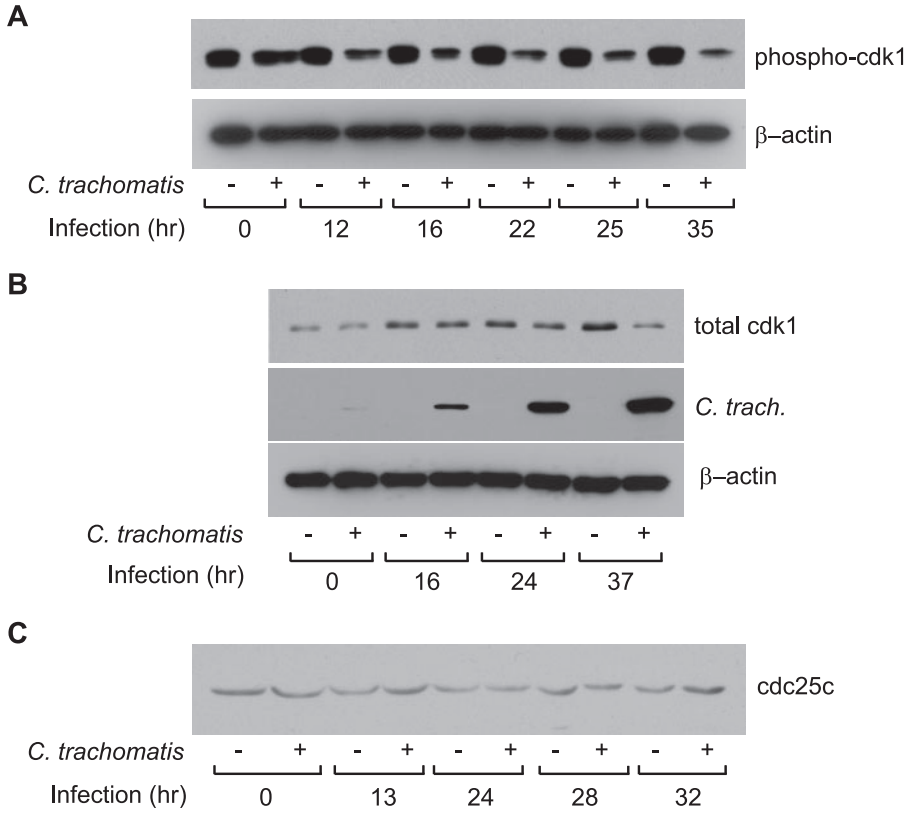

FIG. 2. *C. trachomatis* infection alters the expression level of the cyclin-dependent kinase cdk1. CHO-K1 cells were mock infected or infected with *C. trachomatis*. Samples were harvested at different times postinfection, separated by SDS-PAGE, and then immunoblotted with antibodies that recognize either phosphorylated cdk1 (A), total cdk1 (B), or cdc25c (C). (B) Infection of cells with *C. trachomatis* was confirmed by using an antibody that specifically recognizes the MOMP of *C. trachomatis*. Samples were normalized in each experiment for total protein content by measuring their absorbance at 280 nm. Equivalent protein loading was further confirmed by comparison of β-actin levels across samples. *C. trach*., *C. trachomatis*.

Modification of cyclin B1 depends upon active *C. trachomatis* protein synthesis. To determine whether the modification of cyclin B1 observed for *C. trachomatis*-infected cells requires active bacterial protein synthesis, we treated cells with chloramphenicol, an antibiotic that specifically inhibits bacterial 50S ribosomal subunits. If the reduction in size of cyclin B1 requires metabolically active *Chlamydia*, chloramphenicol treatment of infected cells should prevent the modification of cyclin B1. CHO-K1 cells were infected with *C. trachomatis* for 8 h before the addition of chloramphenicol and then harvested at different times posttreatment. As shown in Fig. 4A, cyclin B1 did not undergo a reduction in size when *C. trachomatis*-infected cells were treated with chloramphenicol. As a control, we harvested cells immediately after the addition of chloramphenicol to exclude the possibility that the mere exposure of cells to *C. trachomatis* infection was sufficient to cause our phenotype. Cumulatively, our results suggest that active protein synthesis by *C. trachomatis* is required for the modification of cyclin B1.

To more precisely define when the bacterial protein responsible for altering cyclin B1 actually becomes active during the *C. trachomatis* developmental cycle, we added chloramphenicol to *C. trachomatis*-infected cells at different times ranging from 0 to 22 hpi. All samples were then harvested at 24 hpi, when cyclin B1 modification is consistently present. When chloramphenicol was added to infected cells after 14 hpi, we observed that cyclin B1 was still modified (Fig. 4B). These data suggest that the bacterial protein which plays a role in modifying cyclin B1 is first active between 14 and 16 h postinfection.

Cyclin B1 undergoes an N-terminal cleavage in the presence of *C. trachomatis* infection. One possible explanation for the change in the size of cyclin B1 is that *C. trachomatis* infection leads to a truncation of the protein. To test whether *C. trachomatis* infection specifically leads to an N-terminal truncation of cyclin B1, we used the anti-cyclin B1 antibody GNS-1, which exclusively recognizes the extreme N terminus of the protein. As shown in Fig. 5A, the smaller forms of cyclin B1 present in *C. trachomatis*-infected cells were not reactive with the GNS-1 antibody, suggesting that *C. trachomatis* infection leads to an N-terminal cleavage of cyclin B1.

We also tested whether cyclin B1 is cleaved at its C terminus following *C. trachomatis* infection. In the absence of a C-terminal antibody against cyclin B1, we generated a plasmid with a HA tag fused to the C terminus of cyclin B1. This cyclin B1-HA construct was transiently transfected into *C. trachomatis*-infected cells for different lengths of time. Similar to the phenotype observed for endogenous cyclin B1, HA-tagged cyclin B1 also underwent a reduction in size in the presence of *C. trachomatis* infection but not with mock infection (Fig. 5B). Moreover, the ability of the anti-HA antibody to recognize the

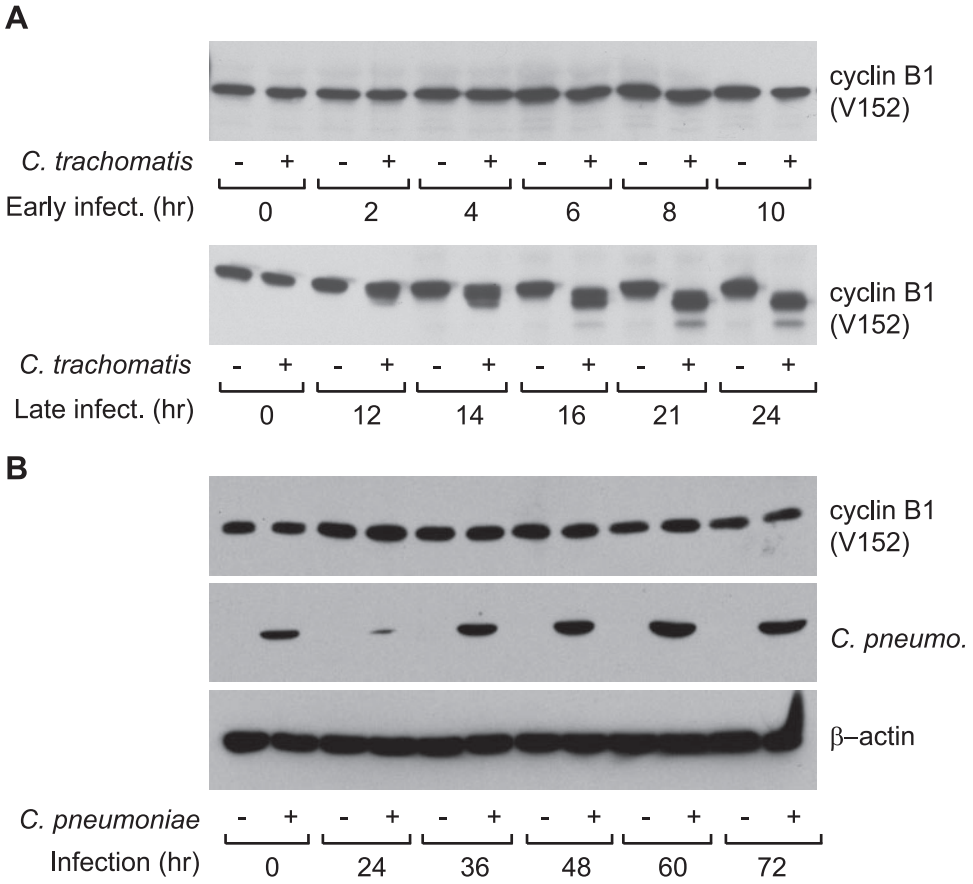

FIG. 3. *C. trachomatis* infection leads to modification of the mitotic cyclin B1 protein. CHO-K1 cells were mock infected or infected with *C. trachomatis* (A) or *C. pneumoniae* (B). Samples were harvested at different times postinfection, separated by SDS-PAGE, and then immunoblotted with antibodies that recognize the anti-cyclin B1 antibody V152. (B) Samples were confirmed to be productively infected with *C. pneumoniae* by performing an immunoblot analysis with an antibody that specifically recognizes *C. pneumoniae*. *C. pneumo*., *C. pneumoniae*; infect., infection.

overexpressed cyclin B1-HA protein within infected cells indicated that *C. trachomatis* infection does not result in a C-terminal truncation of cyclin B1.

Proteolytic activity is preserved in *C. trachomatis*-infected cell lysates. To examine if this *C. trachomatis*-specific proteolytic activity is retained in cell extracts, we performed a mixed lysate experiment. Lysates from *C. trachomatis*-infected cells were mixed with lysates from uninfected cells transfected with cyclin B1-HA. The use of HA-tagged cyclin B1 allowed us to exclusively follow the fate of cyclin B1 that had been added in *trans* to infected extracts. When uninfected cell lysate (Fig. 5C, middle lane) or buffer alone (Fig. 5C, left lane) was added to transfected cell extracts, full-length cyclin B1-HA was detected with the anti-HA antibody. In contrast, *C. trachomatis*-infected cell lysates displayed the ability to cleave the exogenously added cyclin B1-HA (Fig. 5C, right lane). In summary, these data suggest that the bacterial protein responsible for cyclin B1 cleavage remains active in infected cell extracts.

DISCUSSION

In this study, we show that cells infected with *C. trachomatis* proliferate at a lower rate than uninfected cells (Fig. 1). We further demonstrate that levels of cdk1, a cell cycle protein involved in the G_2/M transition, are reduced during *C. trachomatis* infection (Fig. 2). Intriguingly, while cyclin B1 levels remain unchanged following infection of host cells, we show that cyclin B1 undergoes an N-terminal truncation following *C. trachomatis* infection (Fig. 5A) that requires active bacterial protein synthesis (Fig. 4). Notably, not all species of *Chlamydia* have similar effects on cyclin B1. In particular, *C. pneumoniae* infection does not appear to cause cyclin B1 cleavage even at late stages of infection (Fig. 3B). The absence of an effect on cyclin B1 may explain why *C. pneumoniae* infection does not appear to be associated with a delay in host cell cycle progression (5, 28). Rather, evidence points to the tendency of *C. pneumoniae* infection to actually stimulate vascular proliferation and remodeling (20, 37). Perhaps this difference in the effect of *C. trachomatis* infection and that of *C. pneumoniae* infection on host cell cycle progression is partially responsible for the disparity in the types of diseases caused by these two organisms.

Why an intracellular pathogen like *C. trachomatis* would disrupt the host cell cycle is unclear. This question becomes even more complicated when one considers that active infection with the bacteria leads to cell lysis within 36 to 48 h. Perhaps *C. trachomatis* has developed mechanisms to slow down host cell proliferation as a way of conserving cellular

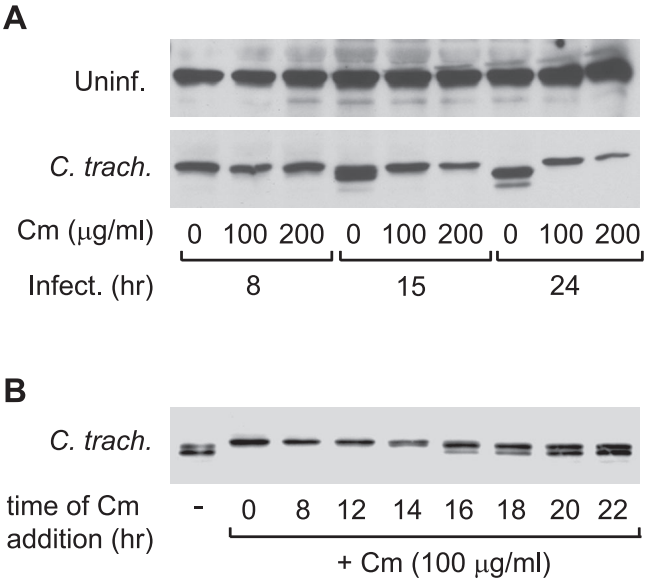

FIG. 4. Modification of cyclin B1 in infected cells requires active *C. trachomatis* protein synthesis. (A) CHO-K1 cells were mock infected or infected with *C. trachomatis* for 8 h and then incubated with various concentrations of chloramphenicol (Cm) to inhibit bacterial protein synthesis. Cells were harvested at 8, 15, or 24 hpi. (B) One hundred micrograms/ml Cm was added to cells that had been infected with *C. trachomatis* for different lengths of time. Regardless of when Cm was added to the cells, all samples were harvested at 24 hpi. Samples shown in panels A and B were analyzed by immunoblotting with the anti-cyclin B1 antibody V152. *C. trach*., *C. trachomatis*; Infect., infection; Uninf., uninfected.

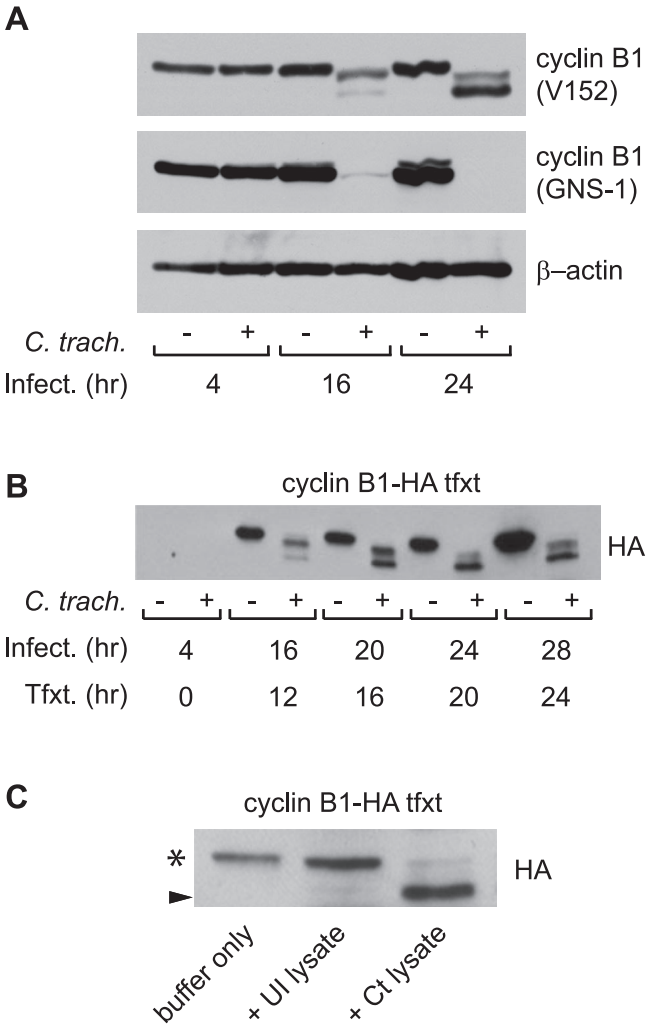

FIG. 5. *C. trachomatis* infection leads to an N-terminal truncation of cyclin B1. (A) CHO-K1 cells infected with *C. trachomatis* or mock infected for 4, 16, or 24 h were lysed and subjected to immunoblot analysis with the anti-cyclin B1 antibody V152 (top panel) or the anti-cyclin B1 antibody GNS-1 (middle panel). GNS-1 specifically recognizes the N-terminal 21 amino acids of cyclin B1. (B) CHO-K1 cells were either mock infected or infected with *C. trachomatis*. Four hours later, cells were transfected with the cyclin B1-HA plasmid that encodes cyclin B1 fused at its C terminus to an HA epitope tag. Cells were harvested at different times postinfection/transfection and subjected to SDS-PAGE and immunoblot analysis using an anti-HA antibody. (C) Uninfected CHO-K1 cells were transiently transfected with cyclin B1-HA for 24 h and then harvested. The transfected cell lysate was then added to the lysate of CHO-K1 cells that had been mock infected (UI; middle lane) or infected with *C. trachomatis* (Ct; right lane) for 24 h. As a control to detect full-length cyclin-HA, transfected cell extract was added to lysate buffer alone (left lane). Following incubation of these mixed lysates, samples were analyzed by immunoblotting with the anti-HA antibody to monitor the cleavage of the exogenous cyclin-HA. Full-length cyclin B1-HA and truncated cyclin B1-HA are designated with an asterisk and an arrowhead, respectively. *C. trach*., *C. trachomatis*; Infect., infection; Tfxt., transfection.

resources to enhance bacterial growth and development. Alternatively, the observation that inclusions are not always transmitted to daughter cells upon cell division (3) suggests that *C. trachomatis* may have evolved a strategy to safeguard large inclusions until host cell lysis. Finally, it is possible that the effect of *C. trachomatis* on the host cell cycle is actually most significant during persistent infection, when the bacteria are residing within the cell for extended periods of time (2, 7, 13, 23).

Altering the eukaryotic cell cycle actually appears to be an emerging theme for pathogens (33, 38). Viruses such as human papillomavirus and human immunodeficiency virus are believed to halt cell cycle progression as a way of enhancing viral DNA replication and virus production (6, 18, 42). Similarly, it has been postulated that bacteria may induce host cell cycle arrest to aid their invasion and/or colonization (33). Interestingly, although examples exist for every stage in the eukaryotic cell cycle, a predominant number of pathogens specifically affect molecules involved in the G_2/M transition (19, 43). A major target appears to be the cdk1/cyclin B1 complex, also known as the mitosis-promoting factor. As its name implies, active mitosis-promoting factor translocates from the cytosol into the nucleus, where it facilitates progression of the cell into mitosis (36). Inhibiting the activation of the cdk1/cyclin B1 complex appears to be a common strategy used by both pathogenic viruses and bacteria to disrupt cell cycle progression (4, 18, 32, 35). Interference with downstream events, including translocation of the active cdk1/cyclin B1 into the nucleus, has also been demonstrated for several viruses (6, 30). Here we demonstrate that *C. trachomatis* may have evolved a separate mechanism to halt cell cycle progression by degrading cdk1 and targeting cyclin B1 for N-terminal truncation. Interest-

ingly, the N-terminal domain of cyclin B1 is not responsible for its activity during G_2/M phase. Rather, it has been shown that the N terminus of cyclin B1 targets the protein for proteasomal degradation at the end of mitosis (15, 24). A mutant of cyclin B1 that lacks its N terminus is more stable than wild-type cyclin B1, and, when overexpressed, can prevent cells from exiting mitosis and entering the next cell cycle (14, 25, 26, 31). Perhaps this is one mechanism whereby *C. trachomatis*-induced cleavage of cyclin B1 slows down host cell cycle progression.

In the course of our studies, we observed that the levels of cyclin B1 cleavage depended upon the nature of the lysis buffer used. Almost all of the cyclin B1 protein within *C. trachomatis*-infected cells underwent a reduction in size when infected cells were lysed in either weak anionic buffers (1% NP-40–0.1% SDS) (Fig. 3A) or nonionic buffers {0.5% TX-100 or 20 mM 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS) } (data not shown). In contrast, infected cells lysed in a strongly anionic buffer (1.0% SDS) displayed a more modest level of cyclin B1 modification (data not shown). Given our findings that the *C. trachomatis*-specific cleavage activity is maintained in infected cell extracts (Fig. 5C), it seems possible that when cells are lysed in a mild detergent, the *C. trachomatis*-specific protease continues to act on cyclin B1 until total stores of cyclin B1 are cleaved. Nevertheless, it has been predicted that once cyclin B1 levels are reduced below a certain threshold, significant effects on the eukaryotic cell cycle can be observed (34). Therefore, although some cyclin B1 may be cleaved following lysis of host cells, it is likely that only moderate levels of cyclin B1 cleavage must occur during *C. trachomatis* infection to alter host cell cycle progression.

While it is possible that a *C. trachomatis* protein directly alters cyclin B1, we also recognize the possibility that cleavage occurs by a host factor that first requires activation by a bacterial factor. Our current efforts are directed towards identifying the *C. trachomatis*-induced protease responsible for cyclin B1 cleavage. Our observation that cleavage activity is maintained in *C. trachomatis*-infected cell extracts (Fig. 5C) provides us with the opportunity to biochemically fractionate the infected cell lysate and use mass spectrometry to identify the protein responsible for modifying cyclin B1. In addition to identifying the *C. trachomatis*-specific protease, it will be important to determine the precise cleavage site within the N terminus of cyclin B1. Knowing the particular consensus sequence recognized by the *C. trachomatis*-induced factor may help in deciphering whether other endogenous targets of this protease exist.

In addition to cyclin B1, several other host proteins have been reported to undergo proteolytic cleavage in response to *C. trachomatis* infection (8, 9, 12, 41, 45, 46). Degradation of the transcription factors RFX5 and USF-1 during *C. trachomatis* infection has been shown to reduce expression of the major histocompatibility complex genes and thereby serve as a putative immune evasion strategy (45, 46). Likewise, degradation of the BH3 (*B*cl-2 *h*omology domain *3*) family of proapoptotic factors protects *C. trachomatis*-infected cells from programmed cell death (8, 12, 41). Finally, *C. trachomatis* also cleaves host keratin 8, a subunit of intermediate filaments (9). Disruption of this component of the host cytoskeletal network has been postulated to allow the *C. trachomatis* vacuole to expand during infection. Proteolysis of several of these proteins has been attributed to the activity of CPAF (*c*hlamydial *p*roteasome-like *a*ctivity *f*actor), a *Chlamydia* protein that is secreted into the host cell cytosol (39, 44). CPAF targets a wide variety host factors for degradation, and it is exciting to speculate that CPAF may also be responsible for the cyclin B1 cleavage observed in *C. trachomatis*-infected cells. However, CPAF is expressed among all *Chlamydia* species, including *C. trachomatis* and *C. pneumoniae*. Therefore, our observation that cyclin B1 cleavage specifically occurs in *C. trachomatis*-infected cells but not *C. pneumoniae*-infected cells suggests that a protease other than CPAF is likely to be responsible for modifying cyclin B1 during *C. trachomatis* infection.

It appears that *C. trachomatis*-mediated degradation of host proteins directly aids the organism by interfering with critical cellular processes such as cell cycle, immune detection, apoptosis, and cytoskeletal rearrangement. However, it has also been suggested that *C. trachomatis* may degrade host proteins to ensure a steady supply of amino acid building blocks for continued synthesis of bacterial proteins during infection (9). Regardless of the purpose, the proteolysis of host factors by *C. trachomatis* appears to promote a safe, nutrient-rich environmental niche for the bacteria to grow and replicate.

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