Infections with the obligate intracellular pathogen *Chlamydia trachomatis* are among the most common sexually transmitted diseases worldwide, with approximately 1.5 million reported cases in the United States in 2012 (1). While most of the acute infections of the lower urogenital tract are asymptomatic and remain unrecognized by the affected people, ascending infections in females often result in severe chronic sequelae, such as pelvic inflammatory disease, ectopic pregnancy, and infertility (2). Despite its clinical relevance, many aspects of the underlying virulence mechanisms have not been elucidated so far.

As for other pathogens, infectivity and the propensity to manipulate host immune responses largely depend on the repertoire of pathogenicity factors. The most extensively studied effector protein in *Chlamydia* research is CPAF (chlamydial protease-like activity factor), which has been reported to degrade a broad spectrum of host cell proteins (3). However, it has been shown that the observed degradation of many previously identified CPAF substrates is an artifact of the protein isolation process (4), and thus whether CPAF actively degrades host cell proteins during the intracellular developmental cycle is a controversial subject of discussion.

A second chlamydial protease, designated CT441 for *C. trachomatis*, shares significant amino acid sequence similarity with tail-specific proteases (Tsp) from other species (e.g., 25% identity with Tsp from *Escherichia coli*) and was first proposed by Lad et al. to interfere with host antimicrobial and inflammatory responses (5, 6); however, in later reports, a role of CT441 and CPAF in the cleavage of NF-κB during the chlamydial infection has been put into question (4, 7). Unique regions that show no similarities to any characterized domain are present at the N terminus of Tsp proteins. *E. coli* Tsp cleaves substrate proteins labeled with a C-terminal ssrA-encoded peptide tag (small stable RNA A) and is involved in protein quality control in the periplasm (8). Borth et al. observed that CT441 modulates the estrogen signaling pathway of the host cell by interaction with host-derived SRAP1 (steroid receptor RNA activator protein 1), a coactivator of estrogen receptor α (ERα) (9). The interaction between CT441 and SRAP1, mediated via the PDZ domain of CT441, was confirmed by glutathione S-transferase (GST) pulldown and intracellular colocalization experiments. Lysates of eukaryotic cells transfected with CT441 showed proteolytic cleavage of endogenous p65, yet no degradation of SRAP1 was observed. However, when analyzed in a yeast system, coactivation of ERα by SRAP1 was strongly diminished in the presence of CT441 or its isolated PDZ domain (9).

To elucidate the role of CT441 in *C. trachomatis* infections, we combined analysis of the protein structure using X-ray crystallography with functional assays on protein-protein interactions and CT441 biological activities. While the protease activity of recombinant CT441 in vitro could not be confirmed during the intracellular *C. trachomatis* developmental cycle, a completely novel chaperone function for CT441 was detected.

### MATERIALS AND METHODS

**Protein production and purification of CT441 proteins.** Details on recombinant production and purification of CT441 from *C. trachomatis* L2/Bu/434 will be given in a future publication. Briefly, N-terminally His-tagged CT441 proteins lacking the signal sequence were produced in E. coli C43(DE3) cells, purified by nickel affinity and size exclusion chromatography (SEC), and concentrated to 2.5 to 10 mg/ml in 20 mM Tris–150 mM NaCl, pH 7.4. For crystallization, a proteolytically inactive variant was used (CT441_{sSsA} [CT441°]; the His tag was removed by human trypsin. Additional details are given in the Supplemental Material.
rhinovirus 3C protease cleavage. Site-directed mutagenesis (for CT441\(^+\), CT441\(^{K481A}\), CT441\(^{Q465A}\), and CT441\(^{D258W}\)) was performed using the QuikChange kit (Stratagene); domain variants (CT441\(^{DUF340}\), CT441\(^{NITD\&PDZ}\), and CT441\(^{NITD}\)) were generated using standard PCR-based cloning techniques (see Table S2 in the supplemental material).

**Production and purification of SRAP1.** N-terminally His-tagged SRAP1 was produced in *E. coli* BL21(DE3) CodonPlus-RIL (Stratagene) and purified as described for CT441. After removal of the His tag and SEC, SRAP1-containing fractions were concentrated to 2.5 mg/ml in 20 mM Tris-150 mM NaCl, pH 7.4. Note that the C terminus of our SRAP1 construct deviates from that used by Borth et al. (9) to reflect the updated sequence (AF293026.1) at NCBI.

**Crystallography, diffraction data collection, and structure determination.** Equal volumes (5 \(\mu l\)) of protein (2.5 mg/ml) and crystallization solution (100 mM morpholineethanesulfonic acid (MES) [pH 6.0], 100 mM MnSO\(_4\), 5% [vol/vol] polyethylene glycol [PEG] 6000, and 34.5% [vol/vol] ethylene glycol) were mixed and equilibrated against 500 \(\mu\)l reservoir solution (1.5 M NaCl). Crystals grew within 2 to 4 weeks at 20°C to a final size of 0.13 mm by 0.11 mm by 0.08 mm. Prior to diffraction experiments, crystals were directly transferred into cryoprotection solution (70 mM MES [pH 6.0], 140 mM MnSO\(_4\), 3.5% [vol/vol] PEG 6000, and 34.5% [vol/vol] ethylene glycol), mounted in CryoLoops (Hampton Research), and flash-cooled in liquid nitrogen. For single anomalous dispersion (SAD) experiments, crystals were soaked in solutions containing 500 mM NaI or Ta\(_6\)Br\(_{12}\) (Jena Bioscience) according to the manufacturer's protocol for 1 h to 24 h at 4°C. X-ray diffraction data were collected at BESSY (Berlin, Germany), integrated with the MOSFELM (1O) or XDS (11) software program, and scaled and merged with the program SCALA (12).

**RESULTS**

**CT441 is a serine protease with a catalytic triad comprising three distinct domains.** To gain detailed insights into the structural organization and its catalytic mechanism, the three-dimensional structure of CT441 was determined by X-ray crystallography to a resolution of 3.0 \(\AA\) (see Table S1 in the supplemental material). CT441 has a modular domain organization comprising an N-terminal domain (NTD) (residues 22 to 242), a PDZ domain (residues 243 to 341), and a C-terminal protease domain (CTD) (residues 342 to 649), which harbors the catalytic residues S455 and K481 (Fig. 1A). The NTD and CTD are well defined by electron density in all three CT441 molecules of the asymmetric unit, although average temperature factors for atoms of the NTD in molecules A and C indicate a high degree of flexibility (see Table S1). No electron density was observed for the PDZ domain, since it is loosely attached to the NTD and the CTD by long flexible loops which allow for multiple positions of the domain in the crystal lattice. SDS-PAGE analysis of dissolved CT441 crystals confirmed that the PDZ domain was not degraded or removed during the crystallization process (see Fig. S1).

The NTD displays a novel fold consisting of 10 \(\alpha\)-helices (A to J) and a short \(\beta\)-strand (B\(_1\)). Helices B to F form a parallel helix-bundle-like structure which packs against helices A and I on one end and against helix G on the other end. Helix J and \(\beta\)-strand 1 are located in the interface region between the NTD and the CTD (Fig. 1B and C). A DALI search (19) revealed no structural homologs of the NTD. The CTD of CT441 contains 7 \(\alpha\)-helices (K to Q) and 10 \(\beta\)-strands (2 to 11), forming two \(\beta\)-sheets. Whereas
one β-sheet (strands 1, 5, 6, 9, and 10) establishes a stable but flexible connection to the NTD, the second β-sheet (strands 2, 3, 4, 7, 8, and 11) provides a scaffold against which the helices K, L, M, O, and Q are stacked (Fig. 1B and C). A DALI search identified the photosystem II protease D1P (root mean square deviation [RMSD], 2.0 Å for 178 Cα atoms) (20), the signaling peptidase CtpB from B. subtilis (RMSD, 2.7 Å for 200 Cα atoms) (21), the chlamydial protease CPAF (RMSD, 2.7 Å for 199 Cα atoms) (22), and two hypothetical bacterial peptidases from Bacteroides unifor-
mis (PDB code 4GHN; RMSD, 2.4 Å for 182 Cα atoms) and Para-
bacteroides merdae (PDB code 4L8K; RMSD, 2.8 Å for 194 Cα
atoms) as harboring domains with structural homology to the CTD. A superimposition showed that the core of the CTD is well conserved within this group of proteins, whereas helices N, O, and P appear to be unique to CT441 (see Fig. S2 in the supplemental material). Residues 528 to 644 (including helices N, O, P, and Q) previously annotated as DUF3340 (domain of unknown function) are part of the CTD (Fig. 1B). This region is of critical importance for substrate processing, since a truncated CT441 variant (CT441ΔDUF3340) is unable to cleave a fluorogenic reporter peptide (Fig. 1E).

The active-site residues S455 and K481 are located in the deep
crevice between the NTD and the CTD (Fig. 1B). Although the proteolytically inactive S455A variant (CT441°) was used for crystallization, side chain positions indicate that K481-Nζ can accept a proton from S455-Oη and thus acts as a general base during catalysis (Fig. 1D). Interestingly, in CT441, a hydrogen bond between the side chains of K481 and Q485 secures an optimal positioning of the general base (Fig. 1D). This suggests that Q485 has a function similar to that of the aspartate residue in the catalytic triad of classical serine proteases. Indeed, the replacement of either S455, K481, or Q485 by alanine prohibits proteolytic activity, corroborating that CT441 utilizes a catalytic triad for substrate cleavage (Fig. 1E). The active-site cleft of CT441 is rather shallow. With the exception of a deep, mainly hydrophobic S1 pocket, it contains surfaces rather than pronounced depressions to accommodate amino acid side chains of substrate molecules (see Fig. S3 in the supplemental material). It cannot be excluded that the PDZ domain, not visible in our structure, participates in the binding of protein-protein interactions (24). The interface, which includes numerous hydrogen bonds, consists of helices O, Q, and loop β2-3 of one molecule and corresponding regions of a second molecule (Fig. 2B). Since identical dimers were also observed in a second crystal form of CT441 (space group C222₁), it cannot be excluded that this assembly has physiological relevance.

**CT441 is able to degrade SRAP1 in vitro.** It has been proposed that after the infection of human host cells with *C. trachomatis*, CT441 interacts with SRAP1 to modulate the estrogen signaling pathway (9). To analyze this interaction in vitro, SRAP1 was reconstitutively produced in *E. coli*, purified, and incubated with lysates of *C. trachomatis*-infected HEK293 cells. Western blot analysis revealed that lysates collected 24 to 48 h postinfection (p.i.) effectively degraded recombinant SRAP1 (Fig. 3A). Lysates from uninfected cells or cells collected 8 h p.i. did not show any proteolytic activity toward SRAP1. It is conceivable that the un-specific chlamydial protease CPAF cleaves SRAP1 under these conditions. Indeed, assays performed with a CPAF-deficient *C. trachomatis* strain (25) confirmed this notion (see Fig. S4 in the supplemental material).

To specifically analyze the interaction between CT441 and SRAP1, both proteins were purified and coincubated in vitro. Interestingly, CT441 efficiently hydrolyzed SRAP1 with almost complete substrate turnover within 1 h, whereas CT441° did not show any proteolytic activity even after 4 h of incubation time (Fig. 3B). Similar results were obtained using SRAP1 produced in HEK293 cells (Fig. 3C). Lowering the reaction temperature to 4°C

FIG 2 Homodimer formation of CT441. (A) CT441 homodimer colored as in Fig. 1B, with the second protomer in a lighter shade. The dashed line indicates the 2-fold symmetry of the homodimer. (B) Homodimerization interface within the CTD. Structural elements of one CT441 molecule (helices αO, αQ, and loop β2-3; purple) interact with corresponding elements of an adjacent molecule (helices αO’, αQ’, and loop β2-3’; green) to form a symmetric interface.

FIG 3 CT441 is able to degrade SRAP1. (A) HEK293 cells were lysed at indicated time points after infection with *C. trachomatis* (C.t.), lysates collected 24 h p.i. or later show pronounced proteolytic activity against purified SRAP1 recombinantly produced in *E. coli*. In contrast, lysates of uninfected HEK293 cells show no proteolytic activity. (B) CT441 was incubated with SRAP1 produced in *E. coli* for indicated periods of time. (C) CT441 was incubated with SRAP1 produced in HEK293 cells. In both cases, CT441 was able to degrade SRAP1, whereas proteolytically incompetent CT441° shows no SRAP1 cleavage. All samples were analyzed by Western blotting using a commercial anti-SRAP1 antibody; a commercial anti-β-actin antibody was used for detection of β-actin as a loading control.

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FIG 4 SRAP1 degradation in host cells is detectable only at late stages of the infection. (A) HEK293 cells overexpressing cytosolic SRAP1 were lysed at indicated time points after infection with C. trachomatis (C.t.). To prevent ongoing proteolysis during lysis preparation, cells were harvested in the presence of a strongly denaturing buffer containing 8 M urea. Although lysates collected 32 h.p.i. or later show some proteolytic activity, the bulk of SRAP1 remains unaffected. Lysates of uninfected HEK293 cells show no proteolytic activity against SRAP1. A commercial anti-β-actin antibody was used for detection of β-actin as a loading control. (B) Supernatants of HEK293 cells were analyzed for lactate dehydrogenase (LDH) activity at indicated time points. -infected HEK293 cells (+ C. t) showed an increase in LDH activity, indicating the disruption of host cells by C. trachomatis. A statistically significant increase of LDH release due to disruption of host cells by C. trachomatis was observed at 32 h and 48 h.p.i. (indicated by asterisks).

allowed us to isolate distinct SRAP1 degradation intermediates, which were subsequently subjected to N-terminal amino acid sequencing and identification of two primary cleavage sites between Ala₁₄-Glu₁₅ and Tyr₃₅-Gly₃₆ (see Fig. S5 in the supplemental material). Several weaker degradation bands of lower molecular mass (<25 kDa) could not be successfully sequenced. Based on these results, we propose that CT441 initiates the degradation of SRAP1 by cleaving peptide bonds in the N-terminal region of SRAP1, which then leads to a rapid processing of SRAP1 into small fragments.

Host cells infected with C. trachomatis do not show significantly reduced SRAP1 levels. Our experiments clearly show that CT441 and CPAF both have the capacity to cleave SRAP1 in vitro. To address the question of whether CT441 or other chlamydial proteases interfere with cytoplasmic SRAP1 levels during intracellular chlamydial development, lysates of C. trachomatis-infected host cells were analyzed. To overcome low inherent SRAP1 levels, SRAP1-overexpressing HEK293 cells (HEK293SRAP1⁺) were generated. HEK293SRAP1⁺ cells infected with C. trachomatis were harvested and lysed in the presence of a strongly denaturing buffer containing 8 M urea. Under these conditions, no significant SRAP1 degradation was observed up to 24 h.p.i. (Fig. 4A). Even at late stages of the infection (32 h and 48 h.p.i.), the bulk of the cytosolic SRAP1 appeared to be intact, with only minor degradation bands detectable by Western blotting (Fig. 4A). Host cell viability was analyzed by monitoring lactate dehydrogenase activity in the cell culture medium. C. trachomatis-induced disruption of the host cell plasma membrane was detected 32 h.p.i., and more than 90% of the host cells were lysed 48 h.p.i. (Fig. 4B). Interestingly, the first appearance of SRAP1 degradation bands (Fig. 4A) coincided with the disruption of the plasma membrane (Fig. 4B) and release of infectious chlamydial elementary bodies from the host cell (see Fig. S6 in the supplemental material).

CT441 and SRAP1 colocalize only after disruption of the inclusion membrane. To investigate whether CT441-mediated degradation of SRAP1 occurs in intact C. trachomatis-infected cells or as a consequence of cellular disruption at later stages of the infection, we analyzed SRAP1 and CT441 expression in C. trachomatis-infected HEK293SRAP1⁺ cells by immunofluorescence confocal microscopy. Within the first 32 h.p.i., SRAP1 was detected mainly in the cytosol of the transfected host cells, whereas CT441 staining was restricted to the chlamydial inclusion (Fig. 5, upper panel). Quantification of the fluorescence signal across the interface between the cytosol and the inclusion revealed no overlap between the signals for CT441 and SRAP1 in intact cells. However, in some cells, a partial overlap of the fluorescence signals for CT441 and SRAP1 in the vicinity of the inclusion was observed 32 p.i. (Fig. 5, middle panel). Since these cells are rounded, it is likely that they belong to the population of dying cells with partially disrupted cellular membranes observed 32 h.p.i. (Fig. 4B). Immunofluorescence images taken 48 h.p.i. showed an almost complete overlap of signals for CT441 and SRAP1 in cells with abrogated cellular compartmentalization and completely disrupted chlamydial inclusions (Fig. 5, lower panel). These results indicate that CT441 and SRAP1 colocalize only at very late stages of the infection.

CT441 is a bifunctional enzyme with chaperone and protease activities. In contrast to findings for eukaryotes, PDZ-containing proteins are relatively scarce in prokaryotes (26). Whereas eukaryotic PDZ domains mostly serve as protein-protein interaction modules, their prokaryotic counterparts are often involved in substrate binding or regulatory processes (23). The role of PDZ domains is well understood in bacterial HtrA (high temperature requirement A) proteases, which are prominent protein quality control factors in the bacterial periplasm (27, 28). Interestingly, several HtrA proteins are bifunctional enzymes with tightly regulated protease and chaperone activities, facilitating degradation or refolding of misfolded periplasmatic proteins. Since CT441 homologues such as E. coli Tsp are also involved in protein quality control processes (8), we tested if CT441 possesses a chaperone-like activity as reported for the HtrA proteins DegP and DegQ (28). Using a chaperone assay based on the heat-induced denaturation of citrate synthase, we found that CT441 has a pronounced protective effect (Fig. 6). Comparable results were obtained for the inactive variant CT441A and for CT441ΔDUF3340, indicating independent chaperone and protease functions. In contrast, a truncation of the protease domain (CT441NTD&PDZ, comprising residues 22 to 341) or of the protease along with the PDZ domain (CT441NTD, comprising residues 22 to 242) resulted in reduced chaperone activity. Taken together, these results indicate that CT441 exhibits pronounced chaperone activity that depends on the presence of all three domains.

DISCUSSION

To survive in the hostile environment inside the host cell, C. trachomatis has developed sophisticated molecular mechanisms, including the remodeling of intracellular vacuoles and modulation of the host cell immune response. CT441 has been reported to act as a chlamydial effector protein that interacts with SRAP1 and partially alleviates estrogen signaling pathways (9). In contrast to
previous results, we show that CT441 is able to cleave SRAP1. These conflicting findings are most likely due to differences in the protein variants (N-terminal (HA)$_2$ tag in CT441, different isoform of SRAP1) and the experimental setup (coexpression of CT441 and SRAP1 in the cytoplasm of HEK293 cells) used by Borth et al. (9). However, most importantly our results show no significant SRAP1 degradation during the intracellular developmental cycle of C. trachomatis. Furthermore, immunofluorescence images did not provide any evidence for the colocalization of CT441 and SRAP1 prior to the disruption of the inclusion membrane at the end of the infection cycle (Fig. 4A and 5). This is in line with findings from others who also could not detect CT441 outside the chlamydial inclusion (29, 30). Given the detection limits of immunofluorescence imaging, a direct interaction of CT441 with SRAP1 cannot be completely ruled out. Our data, however, strongly indicate that CT441 does not result in extensive SRAP1 degradation in intact cells with maintained inclusion morphology. Colocalization of CT441 and SRAP1 could be detected only at very late stages of the infection, when the inclusion membrane starts to disrupt and infectious chlamydiae are released from the host cell (31–33). The liberation of CT441 might therefore play a role, e.g., by degrading SRAP1 or interacting with other chlamydial or host cell proteins in the extracellular phase of the chlamydial developmental cycle.

To provide a framework for a detailed analysis of its molecular function, we have determined the three-dimensional structure of CT441. The NTD of CT441 displays a novel fold, with no structural homologues present in the PDB. According to sequence analysis and secondary structure prediction (see Fig. S7 in the supplemental material), many Tsp proteins include an NTD structurally very similar to that of CT441. In CT441, the NTD is crucial for folding and/or solubility, since CT441 variants lacking the NTD tend to aggregate and could not be purified. In addition, the NTD is important for chaperone activity of CT441 (see below). In contrast to the NTD, the core region of the CTD is structurally well conserved among Tsp homologues from prokaryotes (3DOR, 4L8K, 4GHN, and 4C2E) (21, 22) and eukaryotes (1FC6) (20). The CTD of CT441 mediates the formation of homodimers and harbors the active-site residues. It is easily possible that dimerization is needed for proteolytic activity and that the disruption of the dimerization interface in CT441/ΔDUF3340 is respon-

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**FIG 5** CT441 colocalizes with SRAP1 only after disruption of the chlamydial inclusion. Infected HEK293 cells overexpressing SRAP1 were stained with antibodies against SRAP1 (green) and CT441 (red), DNA was stained with 4',6-diamidino-2-phenylindole (DAPI) (blue). Representative confocal images 32 h or 48 h p.i. are shown (bar = 5 μm). The right column displays fluorescence distribution profiles along broken lines in the merged confocal images. Most cells imaged 32 h p.i. do not show overlapping profiles of SRAP1 and CT441 (upper panel). However, in some cells, overlapping fluorescence profiles were observed as early as 32 h p.i. (middle panel), and in most cells, this was the case at 48 h p.i. (lower panel). This indicates a progressing colocalization of SRAP1 and CT441 at late stages of the infection, most likely due to the disruption of the inclusion membrane before egress of C. trachomatis from the host cell.

**FIG 6** CT441 has chaperone activity. Citrate synthase (CS) was heat inactivated for the indicated period of time in the presence of CT441 proteins or bovine serum albumin (BSA), and residual CS activity was determined. CT441, CT441*, and CT441*ΔDUF3340 show pronounced chaperone activity ($P < 0.05$ between 2 min and 8 min); the truncated variant CT441NTD&PDZ ($P < 0.01$ at 2 min), lacking protease or protease and the PDZ domain, respectively, display a reduced protective effect. CS without any additional protein was used as a control. As expected, BSA did not affect CS activity. For statistical analysis, the 2-way ANOVA method based on results from three independent measurements was used.
sible for its inability to cleave peptide substrates (Fig. 1E). However, the exact role of dimer formation in CT441 has to be addressed in future experiments. Our combined structural and mutational analysis revealed that CT441 harbors a catalytic triad composed of S455, K481, and Q485. A comparison of CT441 active-site residues with homologous structures of CPAF (22), D1P (20), and CtpB (21) revealed an equivalent positioning of the nucleophile (for CT441, S455; for CPAF, S499; for D1P, S372; for CtpB, S309) and the general base (for CT441, K481; for CPAF, H105; for D1P, K397; for CtpB, K334) (see Fig. S8A). Whereas in CT441, Q485 is crucial for the correct positioning of the general base (Fig. 1D and E), a water-mediated hydrogen bond to E558 fulfills this function in CPAF. Interestingly, Q401 of D1P corresponds to Q485 of CT441. Although in the D1P structure, which displays an inactive state of the enzyme, Q401 is not in a position to contact the general base (distance from K397-N to Q401-Ot1, 6.5 Å) (20), it is likely that a hydrogen bond between the two residues is formed in the active conformation of the enzyme. Indeed, it has been proposed that many Ser/Lys proteases use a third residue for the positioning of the catalytic lysine (34). Q485 is essential for proteolytic activity of CT441 and is highly conserved among related proteins from many bacteria, higher plants, and algae (35, 36) (see Fig. S8B). It is therefore easily possible that in many if not all TspS and related proteases, a Gln residue complements the prototypical Ser/Lys dyad to form a catalytic triad, as observed in CT441. Very recently, a Ser/Lys/Gln catalytic triad has also been identified in CtpB from Bacillus subtilis (21). Due to high flexibility of loop regions connecting the PDZ domain to the NTD and CTD, the PDZ domain of CT441 is not defined in the crystal structure. Highly flexible interdomain loops have also been observed for D1P (20), and a repositioning of the PDZ domain is important for transforming CtpB into its active state (21). It is therefore likely that for substrate binding and/or catalysis, a repositioning of the PDZ domain is important for Tsp proteins in general. PDZ domains typically bind the C terminus of substrate molecules (23); however, in some cases internal peptides are recognized (37). Our mutational analysis revealed that in CT441, the integrity of the conserved substrate recognition motif within the PDZ domain is critical for proteolytic activity (Fig. 1E). Therefore, several modes of action for the PDZ domain during catalysis are conceivable: (i) the PDZ domain recognizes internal residues of the substrate close to the cleavage site and modulates binding specificity of CT441; (ii) an interaction of the PDZ domain with the substrate has regulatory functions, e.g., by allosterically controlling processing of substrates, as reported for HtrA family proteases (28) and CtpB (21); or (iii) the PDZ domain secures a substrate protein to allow for efficient processing, e.g., by using a hold-and-bite mechanism (38). For shorter substrates, such as our reporter peptide, allosteric regulation or a hold-and-bite mechanism is unlikely, because the rather bulky C-terminal AMC residue of the peptide should prevent recognition by the PDZ in the first place. However, it cannot be excluded that such mechanisms are relevant for the processing of larger protein substrates. CT441 can process SRAP1 in vitro and has been reported to specifically interact with SRAP1 via its PDZ domain (9). It is interesting to note that SRAP1 contains a sequence in the C-terminal region with similarity to the SsrA degradation tag, a molecular label that is found on dysfunctional cytoplasmatic and periplasmatic proteins in prokaryotes (SsrA, AANDENYALAA; SsrA-like sequence in SRAP1, 213AANEKSAATA223). The SsrA tag is recognized by the PDZ domain of E. coli Tsp and other proteases of the protein quality control system to facilitate efficient degradation of the labeled protein substrate (39).

Our results on structure, function, and intracellular localization of CT441 are compatible with a role in chlamydial protein quality control. Interestingly, other prokaryotic PDZ proteins have also been implicated to counteract protein folding stress, with the HtrA family members DegP and DegQ representing prominent examples. Our analysis revealed that apart from its proteolytic function, CT441 can also act as a chaperone. This novel activity is independent of a functional protease active site. Although the isolated NTD shows some protective effect against heat-induced denaturation of substrates, the presence of the PDZ and protease domains is needed for full chaperone activity. In contrast to the well-characterized proteolytic function of HtrA proteins, the chaperone function is still not well understood. Results from cryo-electron microscopy (cryo-EM) studies of DegQ from E. coli showed that the chaperone function is most likely dependent on the formation of large, higher-order protein complexes consisting of at least 12 DegQ molecules (40). However, the chaperone activity of DegQ from Legionella fallonii seems to be independent of the assembly of large complexes (41). For CT441, oligomerization is not necessary, since a protein variant lacking the C-terminal DUF3340 domain, including the dimerization interface, exhibits full chaperone activity (Fig. 6). Since CT441 shares an identical domain organization and significant sequence similarity with Tsp proteins from other organisms, the chaperone activity might also be a common feature of these proteins.

The establishment of genetic modification tools has dramatically advanced the field of Chlamydia research in the last 3 years (42, 43). Having these new techniques at hand, it is now possible to directly target chlamydial factors of interest for the detailed analysis of host-pathogen interactions. With information on molecular structure and catalytic function available, CT441 will be a very exciting target for future research into chlamydial pathogenicity mechanisms and protein quality control.

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mydial protease TT441 interacts with SRAP1 co-activator of estrogen recep-


