Signal peptides direct the export of secretory proteins from the cytoplasm. After processing by signal peptidase, they are degraded in the membrane and cytoplasm. The resulting fragments can have signaling functions. These observations suggest important roles for signal peptide peptidases. The present studies show that the Gram-positive eubacterium *Bacillus subtilis* contains two genes for proteins, denoted SppA and TepA, with similarity to the signal peptide peptidase A of *Escherichia coli*. Notably, TepA also shows similarity to ClpP proteases. SppA of *B. subtilis* was only required for efficient processing of pre-proteins under conditions of hyper-secretion. In contrast, TepA depletion had a strong effect on pre-protein translocation across the membrane and subsequent processing, not only under conditions of hyper-secretion. Unlike SppA, which is a typical membrane protein, TepA appears to have a cytosolic localization, which is consistent with the observation that TepA is involved in early stages of the secretion process. Our observations demonstrate that SppA and TepA have a role in protein secretion in *B. subtilis*. Based on their similarity to known proteases, it seems likely that SppA and TepA are specifically required for the degradation of proteins or (signal) peptides that are inhibitory to protein translocation.

Signal peptides of secretory proteins seem to serve at least two important biological functions. First, they are required for protein targeting to and translocation across membranes, such as the eubacterial plasma membrane and the endoplasmic reticular membrane of eukaryotes (for reviews, see Refs. 1–4). Second, in addition to their role as determinants for protein targeting and translocation, certain signal peptides have a signaling function. In eukaryotes, signal peptide fragments were shown to be involved in antigen presentation through interaction with the major histocompatibility complex class I molecules (5–8). More recently, it was suggested that fragments of signal peptides of certain cellular and viral proteins interact with cytosolic target molecules. For example, it was shown that signal peptide fragments of preprolactin and human immunodeficiency virus-1 p-gp160 interact in the cytosol with calmodulin (9). In particular the fact that signal peptide fragments are involved in signaling underscores the importance of the proteases involved in signal peptide processing and degradation.

During or shortly after pre-protein translocation, the signal peptide is removed by signal peptidases, which is a prerequisite for the release of secretory proteins from the trans side of the membrane and, in some cases, the post-translational modification of its amino terminus (for reviews, see Refs. 10–13). Proteases, which determine the subsequent fate of cleaved signal peptides were, thus far, only characterized in *Escherichia coli*. In this organism, the signal peptide of the major lipoprotein (Lpp; also known as Braun’s lipoprotein) was shown to be cleaved by signal peptide peptidase (SppA, also known as protease IV), which is an integral membrane protein (14–16). The resulting signal peptide fragments were subsequently degraded in the cytoplasm by the oligopeptidase A (OpdA; Refs. 17 and 18). However, as evidenced by the degradation of signal peptides in a strain lacking SppA other, as yet unidentified, proteases appeared to be involved in this process (19). Even though homologues of *E. coli* SppA have been identified in eubacteria and archaea, and homologues of *E. coli* OpdA in eubacteria, the involvement of none of these enzymes in protein secretion has been documented.

In the present studies, we have investigated the role of two SppA-like proteins of the Gram-positive eubacterium *Bacillus subtilis* in protein secretion. *B. subtilis* is a particularly suitable organism for this purpose, because its capacity for protein secretion can be probed under conditions of hyper-production of secretory pre-proteins (20). The results of our studies show that the absence of *B. subtilis* SppA results in slower processing of secretory pre-proteins without significantly affecting their membrane translocation. In contrast, a second SppA-like protein of *B. subtilis*, denoted TepA, is required for efficient pre-protein translocation across the membrane. Interestingly, TepA seems to be a cytosolic protein, showing sequence similarity to the cytosolic protease ClpP.
Plasmids, Bacterial Strains, and Media—Table I lists the plasmids and bacterial strains used. TY (tryptone/yeast extract) medium contained Bacto-tryptone (1%), Bacto-yeast extract (0.5%), and NaCl (1%). S7 media 1 and 3, for the pulse-labeling of B. subtilis, was obtained by Campbell-type integration of plasmid pMI-trpC2 and transformed into competent B. subtilis 168.

**TABLE I**

| Strains/plasmids | Genotype/properties | Source/Ref. |
|------------------|---------------------|-------------|
| E. coli DH5α     | F80d lacZ-M15 endA1 recA1 hsdR17 (rK− mK−) thi-1 gyrA96 relA1 (lacZYA-argF) U169 | Life Technologies, Inc. |
| B. subtilis 168  | tcrC2; ilepA; pMI-ilepA; tepA-lacZ; EmR | This work |
| ΔtepA           | tcrC2; sppA; pMI-sppA; sppA-lacZ; EmR | This work |
| ΔyhcT           | tcrC2; yhcT; pMI-yhcT; yhcT-lacZ; EmR | This work |
| ΔhscC           | hisA1 glyB133 tcrC2 | This work |
| IH6531          | Like IH6531; pMI-ilepA; EmR | This work |
| HH-ΔtepA       | Like HH6531; pMI-ilepA; yhcT; EmR | This work |
| HH-ΔyhcT       | Like HH6531; yhcT; pMI-yhcT; EmR | This work |
| HH7144         | Derivative of HH6531; hisA1 glyB133 prsA3 | This work |
| IHP-IlepA      | IHT498; like HH7144; pMI-ilepA; EmR | This work |
| IHP-ΔyhcT      | IHT7537; like HH7144; pMI-yhcT; EmR | This work |
| SecDF          | Originally referred to as MIF; derivative of 168; contains an integrated copy of plasmid pMutin2 in the secDF gene; secDF-lacZ; EmR | 36 |

**Plasmids**

- pMutin2: pBR322-based integration vector for B. subtilis; contains a multiple cloning site downstream of the Pspom promoter and a promoterless lacZ gene preceded by the ribosome-binding site of the spoVC gene; ApR; EmR
- pMI-ilepA: pMutin2 derivative; carries 5' sequences of the tepA gene
- pMI-sppA: pMutin2 derivative; carries an internal fragment of the sppA gene
- pMI-yhcT: pMutin2 derivative; carries an internal fragment of the yhcT gene
- pX: Vector for the xylese-inducible transcription of cloned genes by the xyIA promoter; carries the xylR gene; ApR; EmR
- pXm: pX derivative; carries the B. subtilis tepA-myc gene downstream of the xyIA promoter; ApR; EmR
- pM100: Contains the ompA gene under control of the S. hysicus lipase promoter; CmR
- pKTH10: Contains the amyQ gene of B. amyloliquifaciens; KmR
- pKTH10-BT: pKTH10 derivative, encodes an AmyQ-PSB7 fusion protein; KmR

**EXPERIMENTAL PROCEDURES**

**Plasmids, Bacterial Strains, and Media**—Table I lists the plasmids and bacterial strains used. TY (tryptone/yeast extract) medium contained Bacto-tryptone (1%), Bacto-yeast extract (0.5%), and NaCl (1%). S7 media 1 and 3, for the pulse-labeling of B. subtilis, were prepared as described in Ref. 21, with the exception that glucose was replaced by maltose or ribose. Minimal medium (GCHE medium) was prepared as described in Ref. 22. Antibiotics were used in the following concentrations: chloramphenicol, 5 μg/ml; ampicillin, 10 μg/ml; kanamycin, 10 μg/ml; and IPTG1 was used at 1 mM.

**DNA Techniques**—Procedures for DNA purification, restriction, ligation, agarose gel electrophoresis, and transformation of competent E. coli DH5α cells were carried out as described in Ref. 23. Enzymes were from Roche Molecular Biochemicals. B. subtilis was transformed as described in Ref. 22. The nucleotide sequences of primers used for PCR (5’−3’) are listed below; nucleotides identical to genomic template DNA are printed in capital letters and restriction sites used for cloning are underlined.

To construct B. subtilis IlepA, a fragment containing the ribosome-binding site, start codon, and the first 348 nucleotides of the tepA gene, was obtained by Campbell-type integration of plasmid pMutin2 into the chromosome of B. subtilis 168. Correct integration of plasmids in the chromosome of B. subtilis was verified by Southern hybridization.

To construct pEP-IlepA, the entire tepA gene was amplified by PCR with the primers ABp4 (aaggatccACAGAAGAAGAGCGTCC) and ABp4myc (aaggatccGCTTTCCATAATGACG) and cloned into pMutin2, resulting in plasmid pMI-ilepA. B. subtilis IlepA was obtained by Campbell-type integration of plasmid pMI-ilepA into the chromosome of B. subtilis 168.

To construct plasmid pXm, the entire pepA gene was amplified by PCR with primers ABp4 (aaggatccACAGAAGAAGAGCGTCC) and ABp4myc (aaggatccGCTTTCCATAATGACG) and cloned into pMutin2, resulting in plasmid pMI-sppA. B. subtilis ΔsppA was obtained by Campbell-type integration of plasmid pMI-sppA into the chromosome of B. subtilis 168.

**Western Blot Analysis**—Western blotting was performed using a mouse IgG conjugate, using the ECL detection system of Amersham.
Pharmacia Biotech. Streptavidin-horseradish peroxidase conjugate was obtained from Amersham Pharmacia Biotech.

Protein Localization in E. coli—

E. coli cells were grown in TY medium until the end of exponential growth. Cells were collected by centrifugation and spheroplasted as described in Ref. 21. Spheroplasts and periplasmic contents were separated by centrifugation. Subsequently, the spheroplasts were resuspended in buffer (20% sucrose, 50 mM Tris, pH 8.0), and disrupted by French press treatment (3 times at 8000 pounds/square inch). Intact cells and cellular debris were removed from the lysate by centrifugation (15 min at 15,000 × g, 4 °C). Membranes were separated from the cytoplasmic contents by centrifugation (30 min at 300,000 × g, 4 °C) and resuspended in buffer (300 mM NaCl, 50 mM sodium phosphate, pH 8.0, 0.5% Triton X-100).

β-Galactosidase Activity—

Overnight cultures were diluted 100-fold in fresh medium, and samples were taken at hourly intervals for optical density readings at 600 nm and β-galactosidase activity determinations. The β-galactosidase assay and the calculation of β-galactosidase units (per A600) were performed as described in Ref. 27.

RESULTS

Identification of sppA-like Genes of B. subtilis—

The recent completion of the B. subtilis genome sequence (28) allowed us to search for the presence of homologues of SppA of E. coli. Sequence comparisons revealed that the yetl gene, located at 258° on the B. subtilis chromosome, specifies a protein of 335 residues (calculated molecular mass of 36,515) with significant sequence similarity to the SppA protein of E. coli (49% identical residues and conservative replacements; Fig. 1). Even though the YmfB protein showed a lower degree of similarity to SppA of E. coli than SppA of B. subtilis, the z value of the alignment was 30.8. Therefore, we renamed the yetl gene sppA. In addition, the ymfB gene, located at 149.5° on the B. subtilis chromosome, was also shown to specify a protein with sequence similarity to SppA of E. coli (41% identical residues and conservative replacements; Fig. 1).
SppA- and ClpP-like Proteins of B. subtilis

Fig. 2. Patterns of conserved residues in TepA of B. subtilis and ClpP of E. coli. Patterns of conserved amino acids in TepA of B. subtilis (TepA-BSU) and ClpP of E. coli (ClpP-ECO) are numbered I–IV. Identical residues (*) or conservative replacements (†) are marked. The active site residues of ClpP-ECO and the corresponding residues in TepA-BSU are indicated in bold. Residues of E. coli ClpP are numbered on the basis of the 207-residue proprotein, which is autoproteolytically processed to a mature form of 193 residues (51).

Fig. 3. Construction of sppA and tepA mutant strains of B. subtilis. A, schematic presentation of the sppA locus of B. subtilis ΔsppA. By a single crossover event (Campbell-type integration), the sppA gene was disrupted with pMutin2. Simultaneously, the spoVG-lacZ reporter gene of pMutin2 was placed under the transcriptional control of the sppA promoter region. The chromosomal fragment from the sppA region which was amplified by PCR and cloned into pMutin2, is indicated with black bars. Only the restriction sites relevant for the construction are shown (HindIII, B, BamHI). PspA, promoter region of the sppA gene; ori pBR322, replication functions of pBR322; Ap′, ampicillin resistance marker; Em′, erythromycin resistance marker; T1T2, transcriptional terminators on pMutin2; sppA, 3′-truncated sppA gene; sppA, 5′-truncated sppA gene. B, schematic presentation of the tepA locus of B. subtilis ΔtepA. By a single crossover event, the tepA promoter region was replaced with the Pspac promoter of the integrated plasmid pMutin2, which can be repressed by the product of the lacI gene. Simultaneously, the spoVG-lacZ reporter gene of pMutin2 was placed under the transcriptional control of the tepA promoter region. The chromosomal fragment from the tepA region, which was amplified by PCR and cloned into pMutin2, is indicated with black bars. PtepA, promoter region of the tepA gene; tepA, 3′-truncated tepA gene.

YmfB protein TepA (Translocation-enhancing protein). Interestingly, TepA also shows sequence similarity to the known ClpP proteases of E. coli and other organisms, but the similarity between these proteins is limited to four domains, numbered I–IV (Fig. 2). These domains include the conserved residues serine 111 (domain II), histidine 135 (domain III), and aspartic acid 185 (domain IV), which form the active site of ClpP of E. coli (29).

SppA and TepA Are Required for Efficient Processing of α-Amylase—To investigate possible roles for SppA and TepA in protein secretion, two different mutant B. subtilis strains were constructed with the chromosomal integration plasmid pMutin2. In the first strain, denoted B. subtilis ΔsppA, the coding sequence of the sppA gene was disrupted by the integrated pMutin2 (Fig. 3A). In the second strain, denoted B. subtilis ΔtepA, the coding sequence of the tepA gene was left intact, but the tepA promoter was replaced with the IPTG-dependent Pspac promoter, present on the integrated pMutin2 (Fig. 3B). Irrespective of the growth medium used or the presence of IPTG, B. subtilis ΔsppA and B. subtilis ΔtepA showed growth rates comparable to that of the parental strain B. subtilis 168, demonstrating that both SppA and TepA were not essential for growth and viability of the cells, at least under the conditions used. Furthermore, neither SppA nor TepA was required for the development of competence for DNA binding and uptake or sporulation (data not shown).

To test whether SppA or TepA are important for the processing of secretory proteins, the processing kinetics of two secretory pre-proteins were studied by pulse-chase labeling experiments. First, B. subtilis ΔsppA and ΔtepA were transformed with plasmid pKTH10, which results in the hyper-production and hyper-secretion of the α-amyrase AmyQ from Bacillus amyoliqufaciens (∼1–3 gliter; Refs. 20 and 30). As a control, a strain was used in which the yhcT gene was disrupted with pMutin2. This strain, denoted B. subtilis ΔyhcT, was neither affected in growth nor in the secretion of various proteins (data not shown). As shown in Fig. 4, compared with B. subtilis ΔyhcT, processing of pre-AmyQ was affected both in B. subtilis ΔsppA and ΔtepA. The strongest effect was observed in cells depleted of TepA; after a chase of 30 s, ∼12% of the labeled AmyQ was mature in B. subtilis ΔsppA and ΔtepA, whereas under the same conditions, ∼61% of the labeled AmyQ was mature in the control strain ΔyhcT. When B. subtilis ΔtepA was grown in the absence of IPTG, the rate of processing was strongly stimulated, but not completely to wild-type levels. In cells of B. subtilis ΔsppA processing of pre-AmyQ was affected to a much lesser extent (Fig. 4); after 30 s of chase, ∼40% of the labeled AmyQ was mature, which is significantly less than the ∼61% mature AmyQ observed for the control strain. The latter effect of the ΔsppA mutation was observed irrespective of the absence or presence of IPTG (data not shown). Taken together, these findings show that SppA and TepA are required for efficient processing of pre-AmyQ.

Similar to pre-AmyQ, TepA depletion resulted in significantly reduced rates of processing of pre-OmpA from E. coli, as demonstrated with cells of B. subtilis ΔtepA transformed with...
plasmid pJM100 carrying the ompA gene. In addition, the appearance of two stable OmpA degradation products of 16 and 18 kDa, respectively, which are probably formed in the cell wall (31), was strongly affected in TepA-depleted cells (Fig. 5); 60% of the labeled OmpA was still in the precursor form after a chase of 1 min, whereas under the same conditions, 60% of the labeled OmpA was in the precursor form in the control strain. Pre-OmpA processing in B. subtilis ItepA (pJM100) was restored to wild-type levels when the cells were grown in the presence of IPTG. Notably, processing of pre-OmpA was not affected in cells of B. subtilis ΔsppA (data not shown), indicating that TepA is more important for efficient precursor processing than SppA.

TepA Is Required for Efficient Translocation of α-Amylase—To investigate whether the decreased rates of processing of pre-AmyQ in B. subtilis ItepA and ΔsppA would also result in the accumulation of this precursor protein in the cells, Western blotting experiments were performed. As shown in Fig. 6A, only cells depleted of TepA accumulated pre-AmyQ. Upon addition of IPTG to the growth medium, the accumulation of pre-AmyQ in B. subtilis ItepA was reduced to levels comparable with pre-AmyQ levels in the control strain ΔyhcT. In B. subtilis ΔsppA no accumulation of pre-AmyQ was observed (data not shown). Even though processing of pre-AmyQ was slowed down in TepA-depleted cells, no effect was observed on the amounts of mature AmyQ accumulating in the medium (data not shown).

To investigate whether TepA is active before or after PrsA. To this purpose, the accumulation of pre-AmyQ was examined in a tepA-prsA3 double mutant strain. Like the tepA single mutant, the tepA-prsA3 double mutant strain accumulated pre-AmyQ in the absence of IPTG (Fig. 6B). Furthermore, irrespective of the presence or absence of IPTG, strongly reduced levels of mature AmyQ were detectable in cells of the tepA-prsA3 double mutant strain, similar to the yhcT-prsA3 control strain. Consistent with these observations, in the absence of IPTG, 30–40% reduced levels of AmyQ activity were observed in the growth medium of the tepa-prsA3 mutant strain as compared with the yhcT-prsA3 control strain (data not shown). Taken together, these observations showed that TepA acts at an earlier stage in the secretion of pre-AmyQ than PrsA, presumably before or during translocation of pre-AmyQ across the membrane.

To test whether TepA is required for efficient translocation of pre-AmyQ across the membrane, B. subtilis ItepA was transformed with plasmid pKTH10-BT (34). This plasmid specifies a hybrid AmyQ protein containing the biotin-accepting domain (PSBT) of a transcarboxylase from Propionibacterium shermanii (35) fused to its carboxyl terminus. The rationale of this experiment is that pre-AmyQ-PSBT will only be biotinylated by the cytoplasmic biotin ligase if the rate of translocation of pre-AmyQ-PSBT is slowed down to such an extent that the PSBT domain can fold into its native three-dimensional structure and accept biotin before transport across the membrane. As shown in Fig. 7 (A and B), cells depleted of TepA accumulated biotinylated pre-AmyQ-PSBT, whereas no biotinylated
(pre-)AmyQ-PSBT was detected in cells of the parental strain. B. subtilis 168 transformed with pKTH10-BT. Very similar results were obtained using a strain with a disrupted secDF gene (Fig. 7, C and D), which encodes a non-essential component of the pre-protein translocase complex (36). When cells of B. subtilis ItepA were grown in the presence of IPTG, low levels of biotinylated pre-AmyQ-PSBT were observed. In conclusion, these data show that, similar to SecDF (36), TepA is required for the efficient translocation of pre-AmyQ across the membrane. In contrast, no accumulation of (biotinylated) pre-AmyQ-PSBT could be observed in B. subtilis ΔsppA (data not shown), which is consistent with the relatively mild effects of the sppA gene disruption on pre-protein processing as described above.

Localization of SppA and TepA—SppA of E. coli is a membrane protein (16) with three putative membrane-spanning domains, the amino terminus being localized in the cytoplasm (see: Swiss-Prot accession number P08395). The same membrane topology was predicted for SppA of B. subtilis (Fig. 1). In contrast, only one putative membrane-spanning domain was predicted for TepA (Fig. 1). Notably, this hydrophobic domain corresponds partly to the conserved domain II, which is also described above.

Transcription of sppA and tepA—As shown by three lines of evidence, TepA acts at an early stage in the secretion process. First, similar to cells lacking the SecDF protein, TepA-depleted cells accumulated biotinylated pre-AmyQ-PSBT, showing that this precursor was translocated across the membrane at a reduced rate. Second, pre-AmyQ was shown to accumulate in a tepA-prsA3 double mutant, indicating that TepA acts at an earlier stage in the secretion process than PrsA, which is required for the folding of AmyQ as soon as it emerges from the translocation channel. Third, as shown with TepA-Myc, TepA is likely to be a cytosolic protein.

Discussion

In the present studies, we show that B. subtilis has two genes for proteins which are similar to SppA of E. coli. Both proteins, denoted SppA and TepA, are involved in protein secretion by B. subtilis, as pre-AmyQ processing was retarded in sppA and tepA mutant strains. As judged from its sequence similarity to SppA of E. coli, it is conceivable that SppA of B. subtilis has signal peptide peptidase activity. If so, the reduced rates of pre-AmyQ processing in sppA mutant cells secreting high amounts of this protein could be explained by the accumulation of signal peptides in the membrane or the translocation channel. First, these signal peptides could affect the activity of the SecA protein, which was previously shown to be inhibited in vitro by synthetic signal peptides (43). Second, the accumulation of signal peptides could affect the activity of the signal peptidases of B. subtilis, as it was previously shown that, like SecA, the signal peptidase of E. coli was also inhibited in vitro by synthetic signal peptides (44). Compared with the tepA mutation, the effects of the sppA disruption were rather mild, as they could only be shown under conditions of AmyQ hypersecretion. No effects were observed for OmpA of E. coli, which is secreted at moderate levels compared with AmyQ. Interestingly, the transcription of the sppA gene coincides with that of the type I signal peptidase-encoding genes sipS and sipT (40, 41) and the genes for degradative enzymes (45), being low in the exponential growth phase and high in the post-exponential growth phase. The latter observation would be consistent with the hypothesis that SppA has signal peptide peptidase activity, as B. subtilis could thus prevent the accumulation of cleaved signal peptides under conditions of high level synthesis of secretory proteins. It has to be noted, however, that the transcription of sppA was very recently shown to depend on σW (a so-called extracytoplasmic function factor; Ref. 46), whereas the transcription of sipS, sipT, and genes for degradative enzymes depends on the DegS-DegU two-component regulatory system (40, 41).

The second SppA-like protein, TepA, represents a novel determinant for protein secretion in B. subtilis, which also shows similarity to the ClpP family of proteases. Thus far, our data base searches have revealed only one other tepA-like gene in the genomic sequence of the Gram-positive eubacterium Clostridium acetobutylicum, which may suggest that TepA is specific for Gram-positive bacteria.

As shown by three lines of evidence, TepA acts at an early stage in the secretion process. First, similar to cells lacking the SecDF protein, TepA-depleted cells accumulated biotinylated pre-AmyQ-PSBT, showing that this precursor was translocated across the membrane at a reduced rate. Second, pre-AmyQ was shown to accumulate in a tepA-prsA3 double mutant, indicating that TepA acts at an earlier stage in the secretion process than PrsA, which is required for the folding of AmyQ as soon as it emerges from the translocation channel. Third, as shown with TepA-Myc, TepA is likely to be a cytosolic protein.

FIG. 7. Accumulation of biotinylated pre-AmyQ-PSBT. Cells of B. subtilis 168 (parental strain), B. subtilis ItepA, or B. subtilis secDF, transformed with pKTH10-BT, were grown in TY medium with (+) or without (-) 1 mM IPTG until 3 h after the end of exponential growth. Next, cells were collected by centrifugation, and (pre)-AmyQ-PSBT was visualized by SDS-PAGE and Western blotting using a streptavidin-horseradish peroxidase (HRP) conjugate (A and C) or AmyQ-specific antibodies (B and D). p, pre-AmyQ-PSBT; m, mature AmyQ-PSBT.
**Fig. 8. Localization of TepA-Myc in E. coli.** To localize the TepA protein in *E. coli*, cells were transformed with plasmid pXThy5, which carries the tepA-myc gene under control of a xylose-inducible promoter. *E. coli* (pXThy5) was grown in TY medium in the presence (+) or absence (−) of 1% xylose until the end of exponential growth. Subsequently, cells were collected by centrifugation and fractionated as described under “Experimental Procedures.” Samples were used for SDS-PAGE and Western blotting. TepA-Myc was visualized with specific antibodies against the c-Myc epitope. The position of TepA-Myc is indicated.

**Fig. 9. Analysis of tepA and sppA expression with a transcriptional lacZ gene fusion.** The transcriptional tepA-lacZ (□) and sppA-lacZ (○) fusions of *B. subtilis* TepA and *B. subtilis* sppA, respectively, were used to determine the time course of *tepA* and *sppA* expression in cells grown in TY medium. β-Galactosidase activities are indicated in units per A600. Zero time (t = 0) indicates the transition point between the exponential and post-exponential growth phases.

The fact that the three active site residues of ClpP are conserved in TepA suggests that TepA has proteolytic activity. If so, TepA could act in at least two different ways. First, TepA might be involved in the regulation of post-exponential growth phase-specific processes, like ClpP of *B. subtilis*, which was recently shown to have pleiotropic effects on protein secretion, the development of competence for DNA binding and uptake, and sporulation. Most likely, these effects of ClpP are due to the degradation of regulatory proteins in the cytosol (47). No-...
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