SecDF of Bacillus subtilis, a Molecular Siamese Twin Required for the Efficient Secretion of Proteins*

(Received for publication, April 13, 1998, and in revised form, June 10, 1998)

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In the present studies, we show that the SecD and SecF equivalents of the Gram-positive bacterium Bacillus subtilis are jointly present in one polypeptide, denoted SecDF, that is required to maintain a high capacity for protein secretion. Unlike the SecDF subunit of the pre-protein translocase of Escherichia coli, SecDF of B. subtilis was not required for the release of a mature secretory protein from the membrane, indicating that SecDF is involved in earlier translocation steps. Strains lacking intact SecDF showed a cold-sensitive phenotype, which was exacerbated by high level production of secretory proteins, indicating that protein translocation in B. subtilis is intrinsically cold-sensitive. Comparison with SecD and SecF proteins from other organisms revealed the presence of 10 conserved regions in SecDF, some of which appear to be important for SecDF function. Interestingly, the SecDF protein of B. subtilis has 12 putative transmembrane domains. Thus, SecDF does not only show sequence similarity but also structural similarity to secondary solute transporters. Our data suggest that SecDF of B. subtilis represents a novel type of the SecD and SecF proteins, which seems to be present in at least two other organisms.

The translocation of proteins across biological membranes involves the action of complex machineries, consisting of at least two functional units as follows: a force generator (motor) and a translocation channel (1). Escherichia coli is the best characterized of the eubacterial protein translocation machineries. The so-called pre-protein translocase of E. coli consists of at least seven proteins as follows: SecA, SecD, SecE, SecF, SecG, SecY, and YajC. SecA is a peripheral membrane protein, which generates the driving force for the initiation of precursor transport through the translocation channel, of which SecY, SecE, and SecG are the most important components. Recent studies suggest that SecY, SecE, and SecG form a heterotrimeric subcomplex (SecYEG) of a large “holoenzyme,” which also contains a heterotrimeric subcomplex of SecD, SecF, and YajC (SecDFyajC; see Refs. 2 and 3). Once protein translocation is initiated by SecA, further translocation is driven both by SecA and the proton motive force (PMF)1; Refs. 4–6). Current models suggest that SecA drives protein transport through cycles of pre-protein binding, membrane insertion, pre-protein release, and deinsertion from the membrane (7, 8). The cycling of SecA is regulated by ATP binding and hydrolysis, which causes major conformational changes in this protein (9, 10).

The role of SecDFyajC in protein translocation in E. coli is not completely clear, in particular because it is not essential for protein translocation in vitro (11) and because SecD and SecF are required to maintain a stable PMF (12). Moreover, SecDFyajC seems to be involved in at least three aspects of the translocation process: first, the cycling of SecA is modulated by SecDFyajC, which stabilizes the membrane-inserted state of SecA (8, 13–15); second, SecDFyajC seems to stimulate PMF-driven protein translocation (15); and third, SecD appears to be required for the release of mature proteins from the extracytoplasmic side of the membrane (16). Thus, SecDFyajC may have an indirect role in protein translocation in E. coli, for example in the assembly of the protein translocase complex, the gating of the translocation channel (17), or the stimulation of PMF-driven protein translocation (15).

Although homologues of SecD and SecF of E. coli have been identified in several eubacteria and archaea (17), their role in protein translocation has not been addressed experimentally. In the present studies, we have investigated the role of the SecD and SecF equivalents in the Gram-positive bacterium Bacillus subtilis, which is a particularly suitable organism for this purpose: first, B. subtilis secretes proteins into the growth medium directly after translocation across the membrane (see Ref. 18); second, the capacity for protein secretion can be probed under conditions of hypersecretion (19); third, important components of the protein translocation machinery of B. subtilis, such as SecA, SecY, and SecE, have been identified (20–23); and fourth, the gene for a SecF-like protein was recently identified by genome sequencing (24). Interestingly, unlike the secF genes from E. coli and other well characterized microorganisms, the secF-like gene of B. subtilis seemed to represent a natural gene fusion between the equivalents of secD and secF. The results of our studies show that the SecD and SecF equivalents of B. subtilis are indeed jointly present in

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBankTM/EBI Data Bank with accession number(s) AF024506.

Supported by European Union(EU) Biotechnology Grant Bio2-CT93-0254.

Supported by European Union Biotechnology Grants Bio2-CT93-0254 and Bio4-CT96-0097.

Supported by the Dutch Ministry of Economic Affairs through Associatie Biologische Onderzoeksscholen Nederland.

Supported by European Union(EU) Biotechnology Grant Bio2-CT93-0272.

Supported by the Dutch Ministry of Economic Affairs through Associatie Biologische Onderzoeksscholen Nederland.

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1 The abbreviations used are: PMF, proton motive force; IPTG, isopropyl-β-D-thiogalactopyranoside; PCR, polymerase chain reaction; TY, tryptone/yeast extract; PAGE, polyacrylamide gel electrophoresis.
one polypeptide, which is required for efficient translocation of secretory pre-proteins under conditions of hypersecretion.

**EXPERIMENTAL PROCEDURES**

**Plasmids, Bacterial Strains, and Media**—Table I lists the plasmids and bacterial strains used. TY medium and the S7 media 1 and 3, for the pulse-labeling of *B. subtilis*, were prepared as described in Ref. 25, with the exception that in S7 media glucose was replaced by maltose. Minimal medium (GCHIE medium) was prepared as described in Ref. 26. Antibiotics were used in the following concentrations: chloramphenicol, 50 μg/ml; erythromycin, 1 μg/ml; kanamycin, 10 μg/ml; ampicillin, 50 μg/ml. IPTG was used at 1 mM.

**DNA Techniques**—DNA techniques and transformation of competent *E. coli* DH5α cells were carried out as described in Ref. 27. Enzymes were from Boehringer Mannheim (Mannheim, Germany). *B. subtilis* was transformed as described in Ref. 26. The 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. DNA sequences were determined as described in Ref. 24. The incorporation of amyE into the chromosome of *B. subtilis* was confirmed by growing *B. subtilis* XDF-Myc on TY plates containing 1% starch and subsequent exposure of the plates to iodine. As shown by a lack of halo formation, *B. subtilis* XDF-Myc did not secrete active α-amylase.

**Pulse-Chase Protein Labeling, Immunoprecipitation, SDS-PAGE, and Fluorography**—Pulse-chase labeling experiments with *B. subtilis*, immunoprecipitations, and SDS-PAGE were performed as described in Ref. 25. Fluorography was performed with AutoFluor (National Diagnostics, Atlanta, GA). Relative amounts of precursor and mature forms of secreted proteins were estimated by scanning of autoradiographs with a laser densitometer (LKB, Bromma, Sweden).

**Western Blot Analysis**—Western blotting was performed using a semi-dry system as described in Ref. 29. After separation by SDS-PAGE, proteins were transferred to Immobilon-polyvinylidene difluoride membranes (Millipore, Bedford, MA). Proteins were visualized with specific antibodies and horseradish peroxidase anti-rabbit or anti-mouse IgG conjugates, using the ECL detection system of Amersham (Little Chalfont, UK). Streptavidin-horseradish peroxidase conjugate was obtained from Amersham Pharmacia Biotech.

**Protease Accessibility**—Protoplasts were prepared from exponentially growing cells of *B. subtilis*. Cells were concentrated 5-fold in protoplast buffer (20 mM potassium phosphate, pH 7.5; 15 mM MgCl2; 20% sucrose) and incubated for 30 min with 1 mg/ml lysozyme (37 °C). Protoplasts were collected by centrifugation, resuspended in fresh protoplast buffer, and incubated at 37 °C in the presence of 1 mg/ml trypsin (Sigma). The reaction was terminated by the addition of 1.2 mg/ml trypsin inhibitor (Sigma). Finally, protoplasts were collected by centrifugation and analyzed by SDS-PAGE and Western blotting. In parallel, protoplasts were incubated without trypsin, or in the presence of trypsin and 1% Triton X-100. Samples containing Triton X-100 were directly used for SDS-PAGE after the addition of trypsin inhibitor.

**β-Galactosidase Activity**—The β-galactosidase assay and the calculation of β-galactosidase units (per A600) were performed as described in Ref. 30.

**RESULTS**

Identification of the secDF Gene of *B. subtilis*—The remarkable observation that the secD and secF genes of *B. subtilis* might be present in one open reading frame was tested and confirmed to be correct by cloning and resequencing the genomic region comprising the secF-like gene. Therefore, we renamed the secF-like gene secDF. Because a few errors were detected in the published sequence (GenBank™ accession number g2635229; Ref. 24), the corrected sequence was sub-
FIG. 1. SecD and SecF in B. subtilis and E. coli. A, chromosomal organization of the B. subtilis secDF locus (adapted from the Subtilist data base; www.pasteur.fr/Bio/SubtiList.html). B, chromosomal organization of the E. coli secD locus (adapted from Refs. 31 and 32). C, comparison of the deduced amino acid sequences of SecDF (B. subtilis) and SecD (E. coli) or SecF (E. coli) of E. coli. Identical amino acids (*) or conservative replacements (z) are marked. The conserved regions D1–D6 and F1–F4, which are present in all known SecD and SecF proteins/domains are boxed. Conserved residues that are present both in SecD and SecF proteins/domains are marked with black or open bars. Putative membrane-spanning domains (I–XII) are indicated in gray shading. The membrane-spanning domains of SecD (E. coli) and SecF (E. coli) were adapted from Ref. 31 and GenBank™ sequence ID number 134401, respectively. The membrane-spanning domains in SecDF of B. subtilis were predicted using algorithms described by Sipos and von Heijne (37). The point of truncation of the SecDF protein in B. subtilis MIF (for details, see "Experimental Procedures") is indicated with an arrow.
The SecDF protein of B. subtilis specifies a protein of 737 residues (SecDF (B. subtilis)) with a calculated molecular mass of 81,653. An alignment of the deduced amino acid sequences of SecDF (B. subtilis) and the SecD and SecF proteins from E. coli (SecD/F (E. coli)) is shown in Fig. 1C. As inferred from this and other sequence alignments (not shown), the SecDF (B. subtilis) protein has a two-domain structure, consisting of an amino-terminal SecD domain (416 residues) and a carboxyl-terminal SecF domain (291 residues). These domains show significant sequence similarity (about 40–50% identical residues or conservative replacements) to the SecD and SecF proteins from E. coli, Hemophilus influenzae, Helicobacter pylori, Methanothermus jannaschii, Mycobacterium tuberculosis, Rhodobacter capsulatus, Streptomyces coelicolor, and Synechocystis. The stretch of 30 residues that links the SecD and SecF domains of SecDF (B. subtilis) is not conserved in other known SecD or SecF proteins. As previously shown for E. coli SecD and SecF (33, 34), the corresponding domains of SecDF (B. subtilis) also show sequence similarity among themselves, in particular at their carboxyl termini (44% identical residues and conservative replacements) to the SecD and SecF proteins from E. coli, Hemophilus influenzae, Helicobacter pylori, Methanothermus jannaschii, Mycobacterium tuberculosis, Rhodobacter capsulatus, Streptomyces coelicolor, and Synechocystis. The stretch of 30 residues that links the SecD and SecF domains of SecDF (B. subtilis) is not conserved in other known SecD or SecF proteins. As previously shown for E. coli SecD and SecF (33, 34), the corresponding domains of SecDF (B. subtilis) also show sequence similarity among themselves, in particular at their carboxyl termini (44% identical residues and conservative replacements in a stretch of 200 residues; not shown). Finally, like SecD of E. coli (34), SecDF (B. subtilis) shows similarity to solute transporters, such as AcrF of E. coli (42% identical residues and conservative replacements in a stretch of 253 residues; not shown), which is involved in acriflavine resistance (GenBankTM accession number g399429), and ActH3 of S. coelicolor (46% identical residues and conservative replacements in a stretch of 159 residues; not shown), which is involved in the transport of antibiotics (GenBankTM accession number g80715).

Alignment of SecDF (B. subtilis) with the SecD and SecF proteins of the organisms listed in the previous paragraph revealed that these proteins do not show similarity over their entire length. However, 10 short patterns of conserved amino acids were identified, which were named D1–D6 and F1–F4 for the SecD and SecF domains/proteins, respectively (Fig. 1C). Consistent with the observation that the SecD and SecF domains show sequence similarity, some of these conserved regions are (partly) present in both SecD and SecF. This similarity is most obvious for the regions D1 and F1 which, respectively, have the consensus sequences G(L/I)DLRGG and G(D/I)DF(A/T)GG (strictly conserved residues are indicated in bold). Furthermore, parts of the conserved regions D5 and F2 also show similarity (Fig. 1C).

The SecDF Protein—To show that the secDF gene encodes only one protein of approximately 82 kDa, the 3′ end of the secDF gene was extended with 11 codons, specifying the human c-Myc epitope (EQKLISEEDLN; Ref. 35). Next, the myc-tagged secDF gene (secDF-myc) was placed under the transcriptional control of the xylose-inducible xyLA promoter and, subsequently, integrated via a double crossover replacement recombination into the amyE locus of B. subtilis, using the px system developed by Kim et al. (36). The resulting strain was named B. subtilis XDF-Myc. As shown by Western blotting and immunodetection with c-Myc-specific monoclonal antibodies, the SecDF-Myc protein was produced in B. subtilis XDF-Myc cells growing in TY medium with 1% xylose but not in cells growing in TY medium lacking xylose. Similar results were obtained if samples for Western blotting were prepared from intact cells or protoplasts of B. subtilis XDF-Myc (Fig. 2A; only the results for protoplasts are shown). Immunodetection with SecDF-specific antibodies showed that the SecDF-Myc protein was highly overproduced in xylose-induced cells of B. subtilis XDF-Myc, as neither wild-type SecDF nor SecDF-Myc were detectable in uninduced cells (not shown). Judged from its mobility on SDS-PAGE, SecDF-Myc is a protein of about 82 kDa, which is in good agreement with the sequence-based prediction.

Membrane Topology of SecDF—Algorithms of Sipos and von Heijne (37) predict that the SecDF (B. subtilis) protein has 12 membrane-spanning domains, the amino and carboxyl termini being localized in the cytoplasm. Two large extracellular loops are localized between the first and second and the seventh and eighth membrane-spanning domains, respectively (Fig. 3). These predictions are in good agreement with the topology models proposed for SecD and SecF of E. coli, in which both SecD and SecF have six membrane-spanning domains with large periplasmic loops located between the first and second and the seventh and eighth membrane-spanning domains (38). To verify the predicted cytoplasmic localization of the carboxyl terminus of SecDF, we studied the protease accessibility of SecDF-Myc in protoplasts. As shown by Western blotting, two trypsin-resistant SecDF-Myc-derived fragments of about 54 and 23 kDa were detectable upon incubation of intact protoplasts of xylose-induced B. subtilis XDF-Myc cells with trypsin (Fig. 2A). Under the same conditions, the B. subtilis signal peptidase SipS, which is exposed to the external side of the membrane (39), was completely degraded by trypsin (Fig. 2B), whereas the cytoplasmic protein GroEL remained unaffected (Fig. 2C). In contrast, both SecDF-Myc-derived fragments and GroEL were completely de-
The positions of the patterns of conserved residues (I–XII) are indicated in bold. The carboxyl terminus of the truncated SecDF protein of *B. subtilis* MID (for details, see “Experimental Procedures”) is marked with an arrow. N, amino terminus; C, carboxyl terminus.

graded by trypsin when protoplasts were lysed with 1% Triton X-100. Taken together, these findings show that the carboxyl terminus of SecDF-Myc is protected against trypsin in intact protoplasts, suggesting that the carboxyl terminus of SecDF (*B. subtilis*) is localized in the cytoplasm.

To study the kinetics of the formation of the two trypsin-resistant SecDF-Myc-derived fragments, limited proteolysis experiments were performed in which protoplasts of xylose-induced *B. subtilis* XDF-Myc cells were incubated with trypsin for various periods. As shown by Western blotting, the 54-kDa fragment is a transiently existing intermediate product in the degradation of intact SecDF-Myc to the trypsin-resistant 23-kDa fragment (Fig. 2D). As judged from the apparent molecular masses of the trypsin-resistant fragments, it is most likely that trypsin cleavage of SecDF-Myc occurs in the two predicted extracellular domains between the first and second membrane-spanning domains and the seventh and eighth membrane-spanning domains.

Cold-sensitive Growth of *B. subtilis* secDF Mutants—To analyze the effects of SecDF depletion on growth and protein secretion, two mutant *B. subtilis* strains were constructed with the integrative plasmid pMutin2. In the first strain, denoted *B. subtilis* MID, the coding sequence of the secDF gene was left intact, but the secDF promoter was replaced with the IPTG-inducible Pspac promoter, present on pMutin2; in the second strain, denoted *B. subtilis* MIF, the coding sequence of the secDF gene was disrupted with pMutin2 (Fig. 4, A and B, respectively). The point of truncation of the SecDF protein of *B. subtilis* MIF is indicated in Figs. 1 and 3. Irrespective of the growth medium used or the presence of IPTG, both *B. subtilis* MID and MIF showed growth rates at 37 °C similar to that of the parental strain *B. subtilis* 168, demonstrating that under these conditions SecDF was not essential for growth and viability of the cells. By contrast, SecDF was important for growth in TY medium at 15 °C; compared with the parental strain (Fig. 4C, ▼), the growth of *B. subtilis* MID (in the absence of IPTG) and *B. subtilis* MIF was significantly reduced. In fact, the growth rates of the two latter strains were reduced to the same extent (Fig. 4C, ▼ and ▲, respectively), and in addition, the cells of both strains showed a filamentous morphology (not shown). Growth of *B. subtilis* MID at 15 °C could be restored by the addition of IPTG to the medium (Fig. 4C, ▲), although not completely to wild-type levels. Similarly, growth of *B. subtilis* MIF at 15 °C could be restored to a similar level as that of *B. subtilis* MID in the presence of IPTG by introducing the secDF- myc gene in the amyE locus (not shown), indicating that the c-Myc tag did not interfere with SecDF function. Interestingly, the growth defects of *B. subtilis* MID (in the absence of IPTG) and MIF were not observed instantaneously upon incubation at 15 °C, as both strains showed growth rates comparable to those of the parental strain until the mid-exponential growth phase (Δlog = 0.3–0.4; Fig. 4C).

To test whether SecDF might be even more important for growth under conditions of hypersecretion, the *B. subtilis* MID and MIF strains were transformed with plasmid pKTH10, which results in the secretion of the *Bacillus amyloliquefaciens* α-amylase AmyQ at high levels (≈1.3 g/liter; Refs. 19 and 39). Irrespective of the presence of pKTH10, growth of *B. subtilis* MID and MIF at 37 °C was not affected (not shown). In contrast, at 15 °C *B. subtilis* MID (in the absence of IPTG) and MIF cells transformed with pKTH10 completely stopped growing after reaching the mid-exponential growth phase, and subsequently, cells even started to lyse (Fig. 4C, ▲ and ▼, respectively). The latter observation showed that the cold-sensitive phenotype of cells depleted of SecDF was exacerbated by high levels of AmyQ secretion. The presence of pKTH10 did not affect the growth at 15 °C of either the parental strain or *B. subtilis* MID in the presence of IPTG (Fig. 4C, △), showing that high level secretion of AmyQ per se did not affect the growth of *B. subtilis* at low temperature. Taken together, these observations show that the SecDF (*B. subtilis*) protein is required for efficient growth at low temperatures, in particular under conditions of high level protein secretion.

SecDF Is Required for Efficient Secretion of AmyQ—To investigate the importance of SecDF for protein secretion at moderate levels (about 30 mg of protein per liter), the secretion of the neutral protease NprE by *B. subtilis* MIF was analyzed by Western blotting. Interestingly, both at 37 and 15 °C, the absence of SecDF did not result in the accumulation of pre-NprE, and similar amounts of mature NprE were secreted by *B. subtilis* MIF and the parental strain (not shown). To evaluate the importance of SecDF under conditions of hyperseccretion, the secretion of AmyQ into the growth medium was investigated by Western blotting experiments. The results showed that *B. subtilis* MIF (pKTH10) secreted reduced levels of AmyQ into the medium. This was most clearly observed with cells that had been washed and resuspended in fresh medium. If the washed cells were incubated for 1 h at 37 °C, the medium of *B. subtilis* MIF contained about 65 ± 10% of the amount of AmyQ secreted by the parental strain (Fig. 5A). An even more drastic effect was observed at 15 °C; after 16 h of incubation, the medium of *B. subtilis* MIF secreted about 40 ± 10% of the amount of AmyQ secreted by the parental strain (Fig. 5B). The reduced secretion of AmyQ into the medium by *B. subtilis* MIF was paralleled by an increased accumulation of pre-AmyQ in the cells (Fig. 5C). Since the cellular levels of mature AmyQ were not affected in the absence of intact SecDF, these data suggest that SecDF is required for the efficient translocation of pre-AmyQ but not the release of mature AmyQ from the membrane.

To investigate the importance of SecDF for the translocation of pre-AmyQ, *B. subtilis* MIF was transformed with plasmid pKTH10-BT (48) which specifies a hybrid AmyQ protein containing the biotin-accepting domain (PSBT) of a transcarboxylase from *Propionibacterium shermanii* (40) 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 and accept biotin before transport across the membrane. As shown in Fig. 6, cells lacking intact SecDF accumulated biotinylated pre-AmyQ-BT, whereas no biotinylated (pre)-AmyQ-PSBT was detected in cells of the parental strain or *B. subtilis* XDF-Myc, which were transformed with pKTH10-BT. These findings show that the rate of translocation of pre-AmyQ-PSBT is significantly reduced in cells lacking SecDF.

To determine the rate of pre-AmyQ translocation in the
absence of SecDF, the kinetics of pre-AmyQ processing by signal peptidase were studied by pulse-chase labeling of *B. subtilis* MIF containing pKTH10. As shown in Fig. 7A, even at 37 °C the rate of pre-AmyQ processing was clearly decreased in *B. subtilis* MIF, whereas about 59% of the labeled AmyQ was mature in the parental strain. The effects of the absence of intact SecDF were even more pronounced at 23 °C (Fig. 7B); after a chase of 4 min, mature AmyQ was hardly detectable in *B. subtilis* MIF.
and subsequent immunoprecipitation, SDS-PAGE, and fluorography. A Myc) was analyzed by pulse-chase labeling at 37 °C (Fig. 7, A and B). Overproduction of SecDF-Myc did not significantly influence the rate of pre-AmyQ processing, showing that wild-type levels of SecDF are not limiting for the translocation of pre-AmyQ and that overproduction of SecDF-Myc does not interfere with normal SecDF function.

**Growth Phase- and Medium-dependent Transcription of the secDF Gene**—To test whether the transcription of the secDF gene depends on the growth phase or medium composition, as previously shown for the signal peptidase-encoding genes sipS and sipT (30, 41), we made use of the transcriptional secDF-lacZ gene fusions present in B. subtilis MID and MIF (Fig. 4, A and B). Only the results for B. subtilis MIF are documented, as similar results were obtained with B. subtilis MID. B. subtilis MIF was grown in minimal medium, TY, or TY with 1% glucose, and samples withdrawn at hourly intervals were assayed for β-galactosidase activity. Nearly constant levels of β-galactosidase activity were observed during growth in minimal medium, suggesting that the secDF gene was expressed constitutively (Fig. 8, ○). In contrast, cells grown in TY medium showed increasing levels of β-galactosidase activity during exponential growth with a maximum at the beginning of post-exponential growth. The β-galactosidase activity decreased in the post-exponential growth phase (Fig. 8, △), suggesting that secDF promoter activity was highest during the transition from exponential to post-exponential growth. Interestingly, the addition of 1% glucose to TY medium caused a drastic increase in the β-galactosidase levels of cells in the post-exponential growth phase, showing that glucose strongly stimulates the transcription of the secDF gene (Fig. 8, □). Thus, the transcription of the secDF gene depends on the growth phase and growth medium.

**DISCUSSION**

In the present study, we demonstrate that the SecDF protein of B. subtilis is required for efficient translocation and processing of the secretory precursor pre-AmyQ and the secretion of mature AmyQ into the growth medium. The reduced secretion of AmyQ by cells lacking intact SecDF was paralleled by the accumulation of pre-AmyQ. These findings show that SecDF (B. subtilis), like SecD and SecF of E. coli, is required for protein translocation across the cytoplasmic membrane and that the function of SecDF in protein translocation is most likely conserved among eubacteria. Notably, the SecDF-depleted cells did not accumulate mature AmyQ. Thus, it seems that SecDF (B. subtilis), unlike SecD of E. coli (16, 34), is not required for the release of mature proteins from the membrane.

The observation that secDF mutants grow at reduced rates at low temperature indicates that protein translocation in B. subtilis is intrinsically cold-sensitive, as previously suggested for protein translocation in E. coli (42). However, in contrast to E. coli mutants with disrupted secD and/or secF genes, which are cold-sensitive for growth and barely viable at 37 °C (43), B. subtilis strains, which contain a disrupted secD gene (this paper) or which completely lack the secDF gene (not shown), show normal growth and cell viability at 37 °C and do not stop growing at low temperature. SecDF was only essential for growth at 15 °C when AmyQ was overproduced. These findings indicate that SecDF is not strictly required for protein translocation in B. subtilis and that the degree in which SecDF is required depends on the level of synthesis of secretory proteins. The latter view is supported by the observation that the secretion of NprE, which was produced at about 30–50-fold lower levels than AmyQ, was not affected in strains lacking SecDF, even at low temperature (not shown). In addition, even when AmyQ was overproduced, SecDF-depleted cells did not stop to grow instantaneously after a shift from 37 to 15 °C, as growth defects were only observed after cells had entered the mid-exponential growth phase. The latter observation suggests that SecDF is not required for early exponential growth, which is consistent with the fact that exponentially growing cells of B. subtilis secrete only few proteins (see Ref. 44). Although SecDF is involved in protein secretion, which is a post-exponential growth phase-specific phenomenon in B. subtilis, this protein is neither required for the development of competence for DNA binding and uptake nor sporulation (not shown), two post-exponential processes that require the activity of SecA (22).

We have previously shown that the transcription of two signal peptidase-encoding genes, sipS and sipT, is concerted with that of genes for most secretory proteins, which is temporally controlled, being high in the post-exponential growth phase (30, 41). Our present data show that the transcription of secDF is regulated differently as follows: 1) when cells were grown in minimal medium, secDF was transcribed constitutively, whereas the transcription of sipS and sipT was temporally controlled; 2) when cells were grown in TY medium, secDF transcription was maximal in the early post-exponential growth phase, whereas the levels of sipS and sipT transcription...
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Acknowledgments—We thank Dr. M. Sarvas for sera against B. amylophilicae f. a-amydlase; Drs. J. Swaving and A. J. M. Driessen for sera against SecDF (B. subtilis); Drs. L. de Leij and W. Helfrich for c-Myc-specific monoclonal antibodies; Dr. V. Vagner for plasmids pMut and pACR; Drs. T. Wiegert and W. Schumann for plasmids pKTH10-BT and pX; and Drs. H. Tjalsma, J. Jongbloed, and R. Freudt for stimulating discussions. We are indebted to Dr. S. D. Ehrlich for continuous support.

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