GENERAL INTRODUCTION

Proteins synthesized on ribosomes in the cytosol must be targeted to the right compartment in order to fulfil their specific function, because a protein function depends critically on its correct subcelluar location or exterior. To achieve this, many proteins are usually synthesized as precursors with an amino-terminal, transient "zip code" (signal peptide), which is recognized and deciphered by a cellular sorting and translocation machinery (von Heijne, 1990a, 1990b, 1998). The precursors of exported proteins contain signal peptides. Signal peptides consist of short stretches of amino acids which, after protein delivery to the correct subcelluar compartment, are frequently removed by specialized signal peptidases. In general, a secretory preprotein is first recognized by soluble targeting factors for its transport to the target membrane, where the protein becomes associated with translocation machinery. Next, the polypeptide chain is transported through a proteinaceous channel. In most cases this transport process is driven by a translocation motor that binds and hydrolyzes nucleoside triphosphates. Finally, the signal peptide is removed, resulting in release of the mature protein from the translocase. If the protein is translocated in an unfolded conformation, the mature protein will fold into its native conformation shortly after release from the translocase. Notably, several integral membrane proteins retain their signal-like peptides and diffuse from the translocase laterally. These basic principles of protein transport across membranes apply to most eukaryotic and prokaryotic organisms (Economou, 1998; Pohlschröder, 1997; Riezman, 1997; Schatz, 1996).

In eukaryotes, many organelles including the nucleus, mitochondria, chroloplast, endoplasmic reticulum (ER), Golgi apparatus and so on are existed in the cell which are separated by the membrane (Fig. 1). Thereby, the protein localization pathways in eukaryotic cells are complex and many factors involved in protein localization have been identified and characterized. In prokaryotes, the localization of proteins is very simple. The proteins which are synthesized within the cytosol are divided into cytoplasm, cytoplasmic membrane, extracellular

medium, moreover endospore in the spore forming cell e.g. *Bacillus subtilis* (Fig. 2). It is thought that the sporulation of *B. subtilis* is the simplest model of differentiation of cell structure and function (Fig. 3). In response to starvation, *B. subtilis* constructs a highly resistant endospore during a process called sporulation. The general pattern of endospore formation has been divided, on the basis of electron microscopical studies, into several successive morphological stages (Ryter, 1965) (Fig. 3). An asymmetric cell division septum (stage II) is the first visible sign that a cell has entered the developmental pathway of sporulation. The membranes of the asymmetric septum then engulf the smaller prespore compartment, given rise to the forespore, a spore protoplast enclosed within the mother cell (stage III). A layer of peptidoglycan, known as the cortex, is laid down between the membranes of the forespore (stage IV), and the protein layers of the spore coat are then deposited on the outer membrane (stage V). In its final act, the mother cell lyses, releasing the now mature spore into the environment. During this process, the forespore and the mother-cell contains a chromosome and engages in a specific genetic program governed by four σ factor; σ^F and σ^G in the forespore, σ^E and σ^K in the mother-cell (Fig. 4). σ factor activation, which appears to be co-ordinated with the progression of the morphological stages, is organized in a cascade triggered by activation of σ^F and followed in a cascade triggered by activation of σ^F and followed by activation of σ^E, then σ^G and finally σ^K (Haldenwang, 1995; Stragier and Losick, 1996). In addition, to produce matured spores, spore proteins, which are another gene products of σ factors also are transported between mother cell and forespore (or prespore). In the assembly of the cortex or coat, a number of small polypeptides are synthesized within the mother cell chamber and deposited on the developing forespore. This strategy of forming a spore entirely within a mother cell is a distinctive feature of sporulation in *B. subtilis*.

In eukaryotic cells, secretory proteins pass through the ER membrane, and are secreted into extracellular space through Golgi apparatus and secretion granules. In contrast, proteins are directly secreted from the cytoplasm to extracellular space (or periplasmic space) across the cytoplasmic membrane in prokaryotes. However, the initial steps of protein secretion in these organisms have several features in common. The protein secretion requires many other factors in

both organisms and these have been supposed to be different from each other. In prokaryotes, the protein secretion has been extensively studied using *Escherichia coli* by both genetic and biochemical means (Fig. 5). In this pathway, cytosolic molecular chaperones like SecB function to maintain the translocation competence of secretory proteins in the cytosol (Kumamoto, 1991). Then SecA recognizes and binds to the signal peptide (Akita *et al.*, 1990; Joly and Whickner, 1993). When SecA interacts with the cytoplasmic membrane and the signal peptide, the ATPase activity of it is activated (Lill *et al.*, 1989; Schiebel *et al.*, 1991). Using the energy produced by ATP hydrolysis, the translocation of the secretory protein is commenced and progressed pass through the translocation channel consists of SecY, SecE and SecG complex (Hartl *et al.*, 1990; Nishiyama *et al.*, 1994). *B. subtilis* is a gram-positive bacterium and is genetically well characterized in prokaryotes. In *B. subtilis*, the *secY*, *secA* and *secE* gene have been cloned (Nakamura *et al.*, 1990a; Saḍaie *et al.*, 1991; Jeong *et al.*, 1993). *B. subtilis secY* and *secE* gene complements *E. coli secY* and *secE* mutant, respectively (Nakamura *et al.*, 1990b; Jeong *et al.*, 1993). *B. subtilis secA341* (*div-341*) mutation causes defects in cell growth, protein secretion, cell division, and competent cell formation etc. at nonpermissive temperatures (Miyakawa and Komano, 1981; Sadaie and Kada, 1985). Therefore, it has been used as a marker to investigate cell division and sporulation mechanisms, though its wild-type gene and the molecular mechanism causing the pleiotropic phenotype had been unknown. The pulse-chase experiment evaluating the processing of presecretory proteins indicated that *secA341* mutant has a defect in the protein secretion machinery and that *secA341* can be classified into the "sec" mutation first identified in *B. subtilis* (Takamatsu *et al.*, 1992). Therefore, it is suggested that the process of the protein secretion proposed in *E. coli* is in common with *B. subtilis*.

In eukaryotic cells, targeting of most proteins to the ER membrane is mediated by the signal recognition particle (SRP) which consists of one RNA molecule (7S RNA) and six polypeptides of 9, 14, 19, 54, 68 and 72 kDa (Walter and Blobel, 1982; Siegel and Walter, 1988) (Fig. 6). The SRP binds via its 54 kDa subunit (SRP54) to the signal sequence of nascent polypeptide chain on translating ribosome and blocks its translation (Walter and Blobel, 1981;

Walter *et al.*, 1981). SRP54 protein has a structure consisting of an N-terminal G-domain, which contains a conserved GTP-binding motif, and C-terminal M-domain, which is rich in methionine residues and contains four predicted amphipathic α-helices (Bernstein *et al.*, 1989). The G-domain may play an essential role in mediating the interaction between SRP and SRP receptor (Zopf *et al.*, 1993). The M-domain is a binding site for the signal sequence and SRP RNA (High and Dobberstein, 1991; Lütcke *et al.*, 1992; Römisch *et al.*, 1990; Zopf *et al.*, 1990). The complex of the ribosome, nascent chain and SRP is targeted to the ER membrane by interaction with the SRP receptor consisting of α-subunit (SRα) and β-subunit (SRβ) on the ER membrane. The SRP is released from the membrane-bound ribosome-nascent chain complex in a GTP-dependent manner (Connolly *et al.*, 1991) and then translocation arrest is relieved (Meyer *et al.*, 1982). The remaining ribosome-nascent chain complex associates with a complex of membrane proteins, Sec61p and TRAM which catalyzes membrane insertion and translocation of the nascent chain (Görlich *et al.*, 1992a; Görlich *et al.*, 1992b; Rapoport, 1992; Dobberstein, 1994). Thus, the SRP functions both as a cytosolic chaperone preventing premature folding of the secretory protein by coupling translation to translocation and as a 'pilot' to guide the protein to the SRP receptor complex in the membrane.

As mentioned above, it had been supported that the targeting system of the secretory proteins to membrane was mediated by quite different components in prokaryotes and eukaryotes. However, recently, genetic and biochemical evidence indicates that SRP-mediated targeting system may also occur in *E. coli* and *B. subtilis*. In *E. coli*, an SRP-like complex was identified which consists of one RNA molecule (4.5S RNA; 114 nucleotides) and one protein (fifty-four homologue; Ffh) that are homologues to the SRP 7S RNA and SRP54 of the eukaryotic SRP, respectively (Poritz *et al.*, 1990; Ribes *et al.*, 1990). Depletion of either 4.5S RNA or Ffh causes defects in the export of several secretory proteins (Ribes *et al.*, 1990; Phillips and Silhavy, 1992). The 4.5S RNA is primarily transcribed as a 130-nucleotide precursor, and its maturation requires the tRNA processing activity, RNase P (Bothwell et al., 1976). *E. coli* FtsY also exhibited a homology with SRα (Bernstein *et al.*, 1989; Römisch *et al.*, 1989). It was reported that FtsY was

in part located at the cytoplasmic membrane and that depletion and overexpression of FtsY affected both cell morphology and protein export (Luirink *et al.*, 1994). *In B. subtilis*, small cytoplasmic RNA (scRNA; 271 nucleotides) is a structural analogue to SRP 7S RNA (Struck *et al.*, 1988). SRP 7S RNA consists of four domains (domains I-IV) based on the predicted secondary structure. 4.5S RNA lacks structures resembling domains I and III, and more than half of domain II is missing. In contrast, the secondary structure of scRNA is strikingly similar to that of 7S RNA, although scRNA lacks domain III. Functional analysis of *B. subtilis* scRNA have been indicated that domains I and II are needed for the formation of heat-resistant spores (Nishiguchi *et al.*, 1994). The scRNA is also transcribed as a 354-nucleotide primary transcript and processing into 271-nucreotides by the action of RNaseIII and others (Oguro *et al.*, 1998). *B. subtilis* Ffh is also identified as a homologue of mammalian SRP54. In *B. subtilis*, the depletion of Ffh or scRNA in the cell led to defects in the translocation of the secretory proteins (Nakamura *et al.*, 1992; Honda *et al.*, 1993). The existence of homologues of two SRP components in *B. subtilis* raised the possibility that a SRP-like particle is involved in the pathway of protein secretion.

B. subtilis has a strong ability to secrete enzymes into extracellular. This microorganism forms the endospore when the nutrition in the medium is exhausted as described above. To clarify the mechanism of protein secretion especially to ascertain the presence of the SRP-SRP receptor mediated targeting system in *B. subtilis*, I cloned a *B. subtilis*, the *ftsY* (*srb*) gene encoding a homologue of the SRα and characterized the function of the gene product, FtsY. Furthermore, I showed that FtsY is required for normal spore formation during sporulation.

Fig. 1. Protein localization in the eukaryotic cell. Newly synthesized localizing proteins are transported into nucleus, mitochondorium, chloroplast, endoplasmic reticulum (ER) and so on in eukaryotic cells. Secretion proteins pass through the ER membranes are secreted to the extracellular through golgi apparatus and secretion granule.

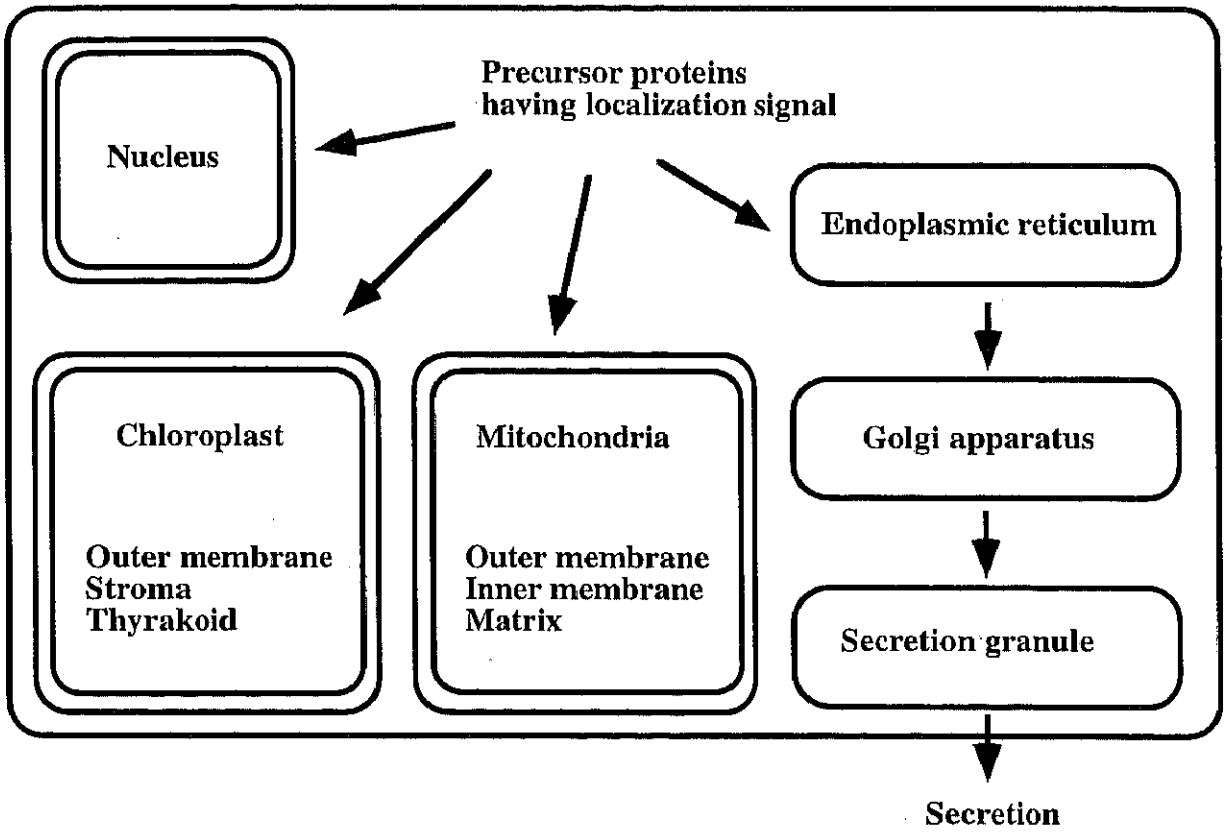

Precursor proteins
having localization signal
Nucleus
Endoplasmic reticulum
Chloroplast
Outer membrane
Stroma
Thyrakoid
Mitochondria
Outer membrane
Inner membrane
Matrix
Golgi apparatus
Secretion granule
Secretion

Fig. 2. Protein localization pathway in a gram-positive bacterium, *B. subtilis*. Newly synthesized localizing proteins of *B. subtilis* are divided into the extracellular space, the cytoplasmic membrane, the cytosol, and the forespore. The secretion proteins are directly secreted from the cytoplasm to extracellular space across the cytoplasmic membrane.

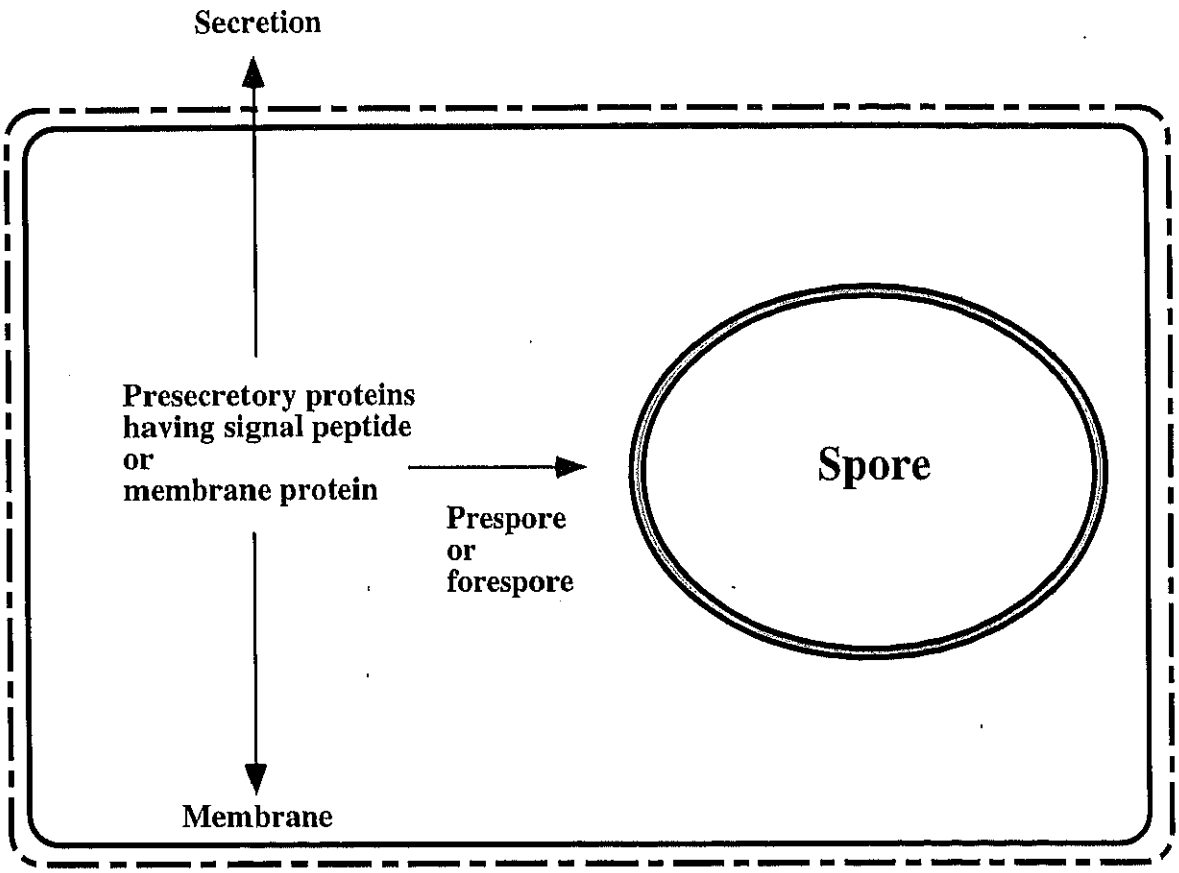

Secretion
Presecretory proteins
having signal peptide
or
membrane protein
Prespore
or
forespore
Spore
Membrane

Fig. 3. The morphological stages of sporulation in *B. subtilis*. The stages are designated by Roman numerals (0 to VII). The wavy lines are chromosomes. The sporangia are surrounded by a cytoplasmic membrane (thin line) and a cell wall (thick line). The developing spore (stage IV-VI) is encased in a layer of cortex (light stippling) and a coat layer (dark stippling). The four specific sporulation sigma factors are shown in the cells where they become active.

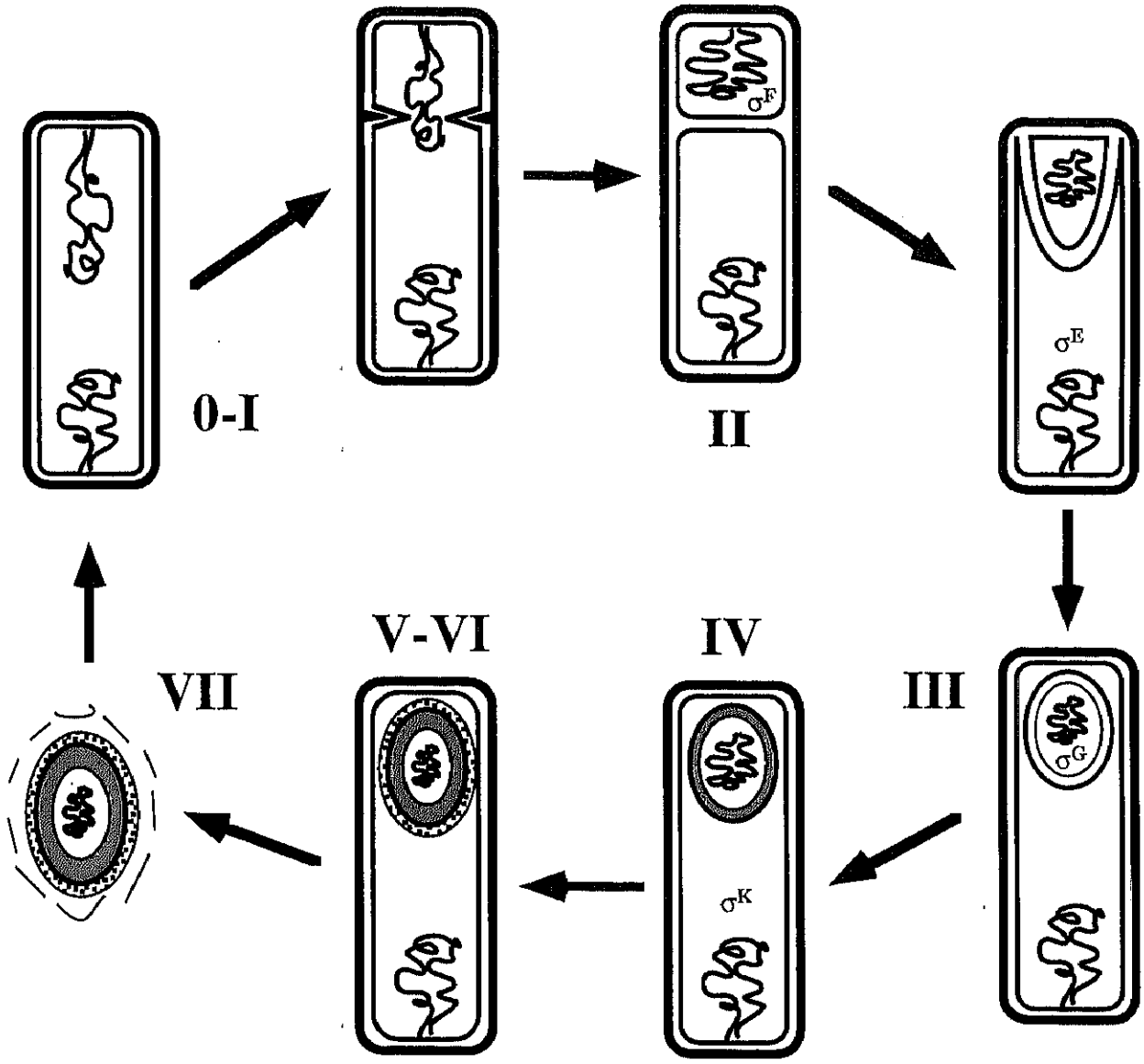

0-I
II
III
IV
V-VI
VII
σF
σE
σG
σK

Fig. 4. Crisscross regulation of cell-specific sigma factors. The central shaded area represents the intercompartment boundary between the forespore and the mother cell, which is initially, created through the combined action of the transcription factors σ^A, σ^H, and Spo0A. Sporulation is governed by two parallel pathway of intracellular gene control operating at the level of the transcription (thin arrows) of the genes for σ^G and σ^K and a crisscross pathway operating at the level of the activity (thick arrows) of four factors.

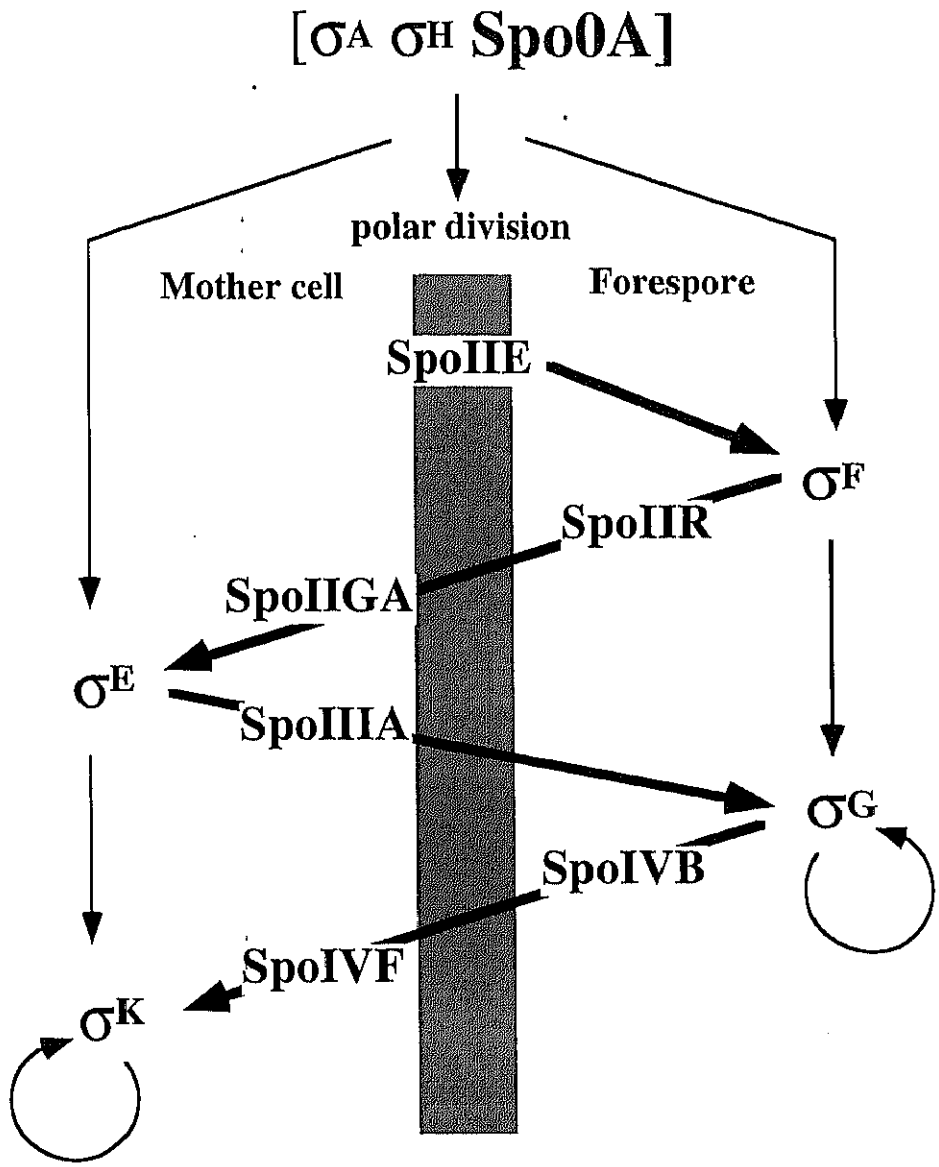

[σA σH Spo0A]
polar division
Mother cell
Forespore
SpoIIE
σF
SpoIIR
SpoIIGA
σE
SpoIIIA
σG
SpoIVB
SpoIVF
σK

Fig. 5. The schematic model for protein secretion pathway in *E. coli*. The secretory proteins translated on ribosomes in cytoplasm are recognized by chaperons like SecB. Then SecA recognizes and binds to the signal peptide. When SecA interacts with the cytoplasmic membrane and the signal peptide, the ATPase activity of SecA is activated. Using the energy produced by ATP hydrolysis, the translocation of the secretory protein is commenced and progressed pass through the translocation channel consists of SecY, SecE and SecG complex. The signal peptide is cleaved by signal peptidase after the translocation and the precursor protein turns into the mature protein.

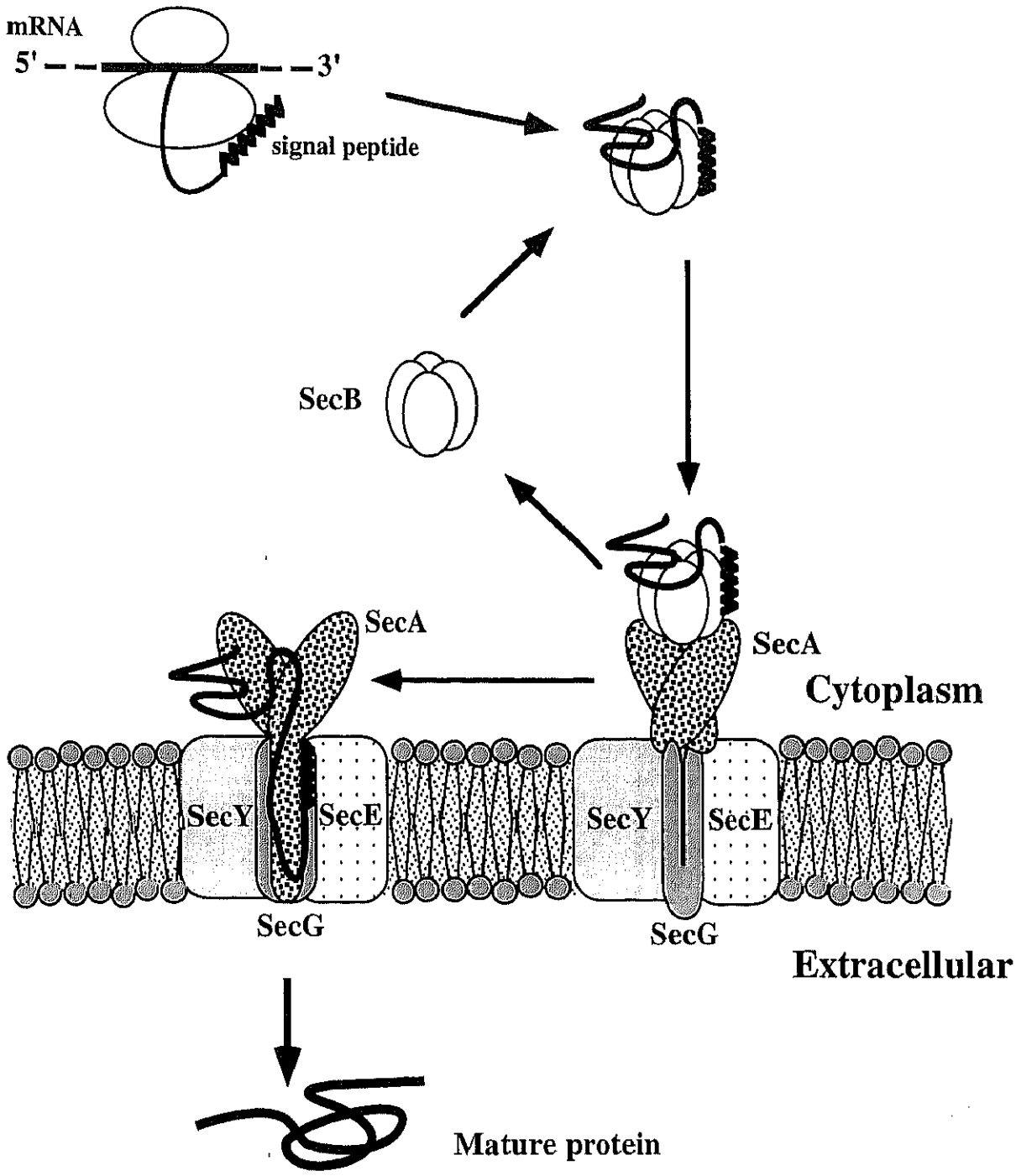
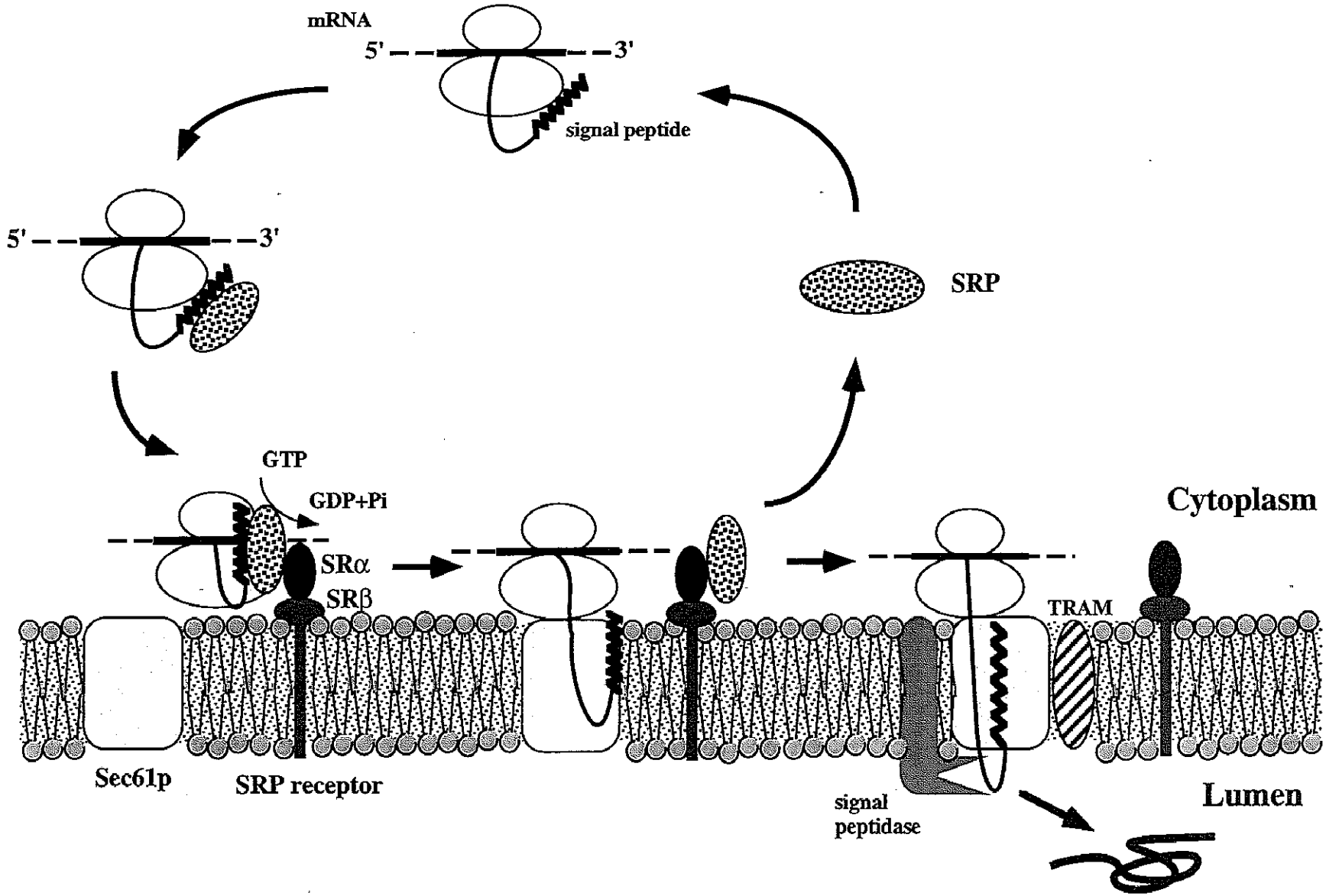

mRNA
5'
3'
signal peptide
SecB
SecA
SecA
Cytoplasm
SecY
SecE
SecY
SecE
SecG
SecG
Extracellular
Mature protein

Fig. 6. The schematic model for protein targeting pathway to the endplasmic reticulum in mammalian cell. The nascent secretory protein is recognized and arrested its translation by the signal recognition particle (SRP). When the SRP binds to the SRP receptor on the endoplasmic reticulum (ER) membrane, the secretory protein is released from the SRP and resumed its translation, and then translocated to the lumen of ER through the translocation channel. The signal peptide is cleaved by signal peptidase after the translocation and the precursor protein turns into the mature protein.


mRNA
5'
3'
signal peptide
SRP
GTP
GDP+Pi
SRα
SRβ
Cytoplasm
TRAM
Sec61p
SRP receptor
signal
peptidase
Lumen