Syd, a SecY-interacting Protein, Excludes SecA from the SecYE Complex with an Altered SecY24 Subunit*

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Syd is an Escherichia coli cytosolic protein that interacts with SecY. Overproduction of this protein causes a number of protein translocation-related phenotypes, including the strong toxicity against the secY24 mutant cells. Previously, this mutation was shown to impair the interaction between SecY and SecE, the two fundamental subunits of the membrane-embedded part of protein translocase. We have now studied in vitro the mechanisms of the Syd-directed inhibition of protein translocation. Pro-OmpA translocation into inverted membrane vesicles (IMVs) prepared from the secY24 mutant cells as well as the accompanied translocation ATPase activity of SecA were rapidly inhibited by purified Syd protein. In the course of protein translocation, high affinity binding of prepore-bearing SecA to the translocase on the IMV is followed by ATP-driven insertion of the 30-kDa SecA segment into the membrane. Our experiments using 125I-labeled SecA and the secY24 mutant IMV showed that Syd abolished both the high affinity SecA binding and the SecA insertion. Syd was even able to release the inserted form of SecA that had been stabilized by a nonhydrolyzable ATP analog. Syd affected markedly the proteolytic digestion pattern of the IMV-integrated SecY24 protein, suggesting that Syd exerts its inhibitory effect by interacting directly with the SecY24 protein. In accordance with this notion, a secY24 variant with a second site mutation (secY249) resisted the Syd action both in vivo and in vitro. Thus, Syd acts against the SecY24 form of translocase, in which SecY-SecE interaction has been compromised, to exclude the SecA motor protein from the SecYE channel complex.

Protein translocation across the E. coli plasma membrane is facilitated by multiple Sec factors (for reviews, see Refs. 1 and 2). A membrane-interacting ATPase, SecA, binds with high affinity to the membrane containing the functional SecY-SecE components (3). This is then followed by the ATP-dependent insertion of a SecA segment into the membrane (4). ATP hydrolysis-dependent release of the prepore and deinserter of SecA allow the next cycle of polypeptide movement (4). SecY, SecE, and SecG are the essential core components in the membrane, having 10, 3, and 2 transmembrane segments, respectively. They not only provide the high affinity binding site for SecA (5) but also productive SecA insertion (4, 5), and they are thought to provide a channel-like pathway for the movement of polypeptide chain. These membrane proteins form a trimeric complex (6–8) in which SecY interacts independently with SecE and SecG (9). Protoplastomes reconstituted only from SecY and SecE exhibit some basic translocation activities in conjunction with SecA (10). In addition, the inclusion of SecG markedly stimulates the translocation activities of the SecYE proteoliposomes (8, 11). In vivo, SecY requires SecE as the stabilizing partner (12, 13). Thus, uncomplexed forms of SecY are degraded rapidly by the FtsH proteolytic system (14). We proposed previously that a dominant negative variant of SecY, SecY<sup>d1</sup>, with a small internal deletion, sequesters SecE in an inactive complex (15). Intragenic suppressor mutations that abolish the dominant effect preferentially occur within or around cytoplasmic domain 2 (C2) of SecY (16). The secY24 amino acid substitution (Gly<sup>240</sup> → Asp) in C4 (17), which also suppresses secY<sup>d1</sup> intragenically, abolishes the SecY-SecE affinity co-isolation (9, 16). Thus, C4 is an essential cytoplasmic domain that is important for the interaction between SecY and SecE. The importance of the second cytoplasmic segment (C2) of SecE for SecY-SecE interaction was also shown (18).

The secY<sup>d1</sup> mutation allowed us to identify a new gene, syd, as a multicopy suppressor against this mutation (19). The syd gene product (Syd) is a hydrophilic protein of 181 amino acid residues, which is associated weakly with the membrane, presumably via its interaction with the SecY protein. When Syd is overproduced in SecY<sup>d1</sup>-expressing cells, it preferentially stabilizes the chromosomally encoded wild-type SecY protein, which is now given a greater opportunity to associate with SecE. This is the proposed suppression mechanism. Another striking phenotype associated with overproduction of Syd is its strong toxicity against the secY24 mutant cells. Presumably because of the defect in SecY-SecE interaction, the SecY24 mutant protein is degraded at 42 °C. This degradation is primarily responsible for the protein export defect (14). However, the Syd-mediated impairment of protein export and cell viability is not accompanied by degradation of the SecY protein (19). Thus, Syd seems to directly interfere with the functioning of the SecY24 mutant protein. These observations indicate that Syd provides a useful means to investigate into the nature of protein interactions involving SecY.

Through our reversion studies of the secY24 mutant with respect to its susceptibility to Syd overproduction, we identified three new alleles of secY (20). While two of them affected residue 240 (the residue altered by secY24) of SecY, the third mutation, termed secY249 (for Ala<sup>249</sup> → Val) alleviated the Syd sensitivity of the secY24 alteration (when placed in cis configuration with secY24) without restoring the temperature resistance or the SecY-SecE interaction. Thus, the secY249 mutation...
may affect the interaction between SecY and Syd.

This work was focused on the ability of Syd to inhibit protein translocase when its SecY subunit is altered to the SecY24 mutant form. Our *in vitro* experiments show that Syd interferes with productive interaction between SecA and the membrane-integrated SecY complex.

**EXPERIMENTAL PROCEDURES**

*Escherichia coli* Strains and Plasmids—Plasmid pST67 contained the *syd* gene placed under the lac promoter and a translation-initiating SD sequence (5′-AAGGAG-3′) and was used as a *Syd*-overproducing plasmid for its construction, a polymerase chain reaction was programmed on pST30 (19) with primers 5′-GGGTTACCCCAAGGAAGACAAGCATGCGGATATTGAGCCG-3′ and 5′-GCCCTAGCGGCATCTGGTTCTCGCAAGTT-3′ to produce a 0.57-kilobase pair fragment, which was then treated with *Kpn*I and BamHI and cloned into pUC18. The resulting plasmid was confirmed for the *Syd*-coding region by sequencing. *E. coli* strain AD202 was a derivative of MC4100 (21) carrying ompT::kan (22). EM147 was a derivative of MC4100 carrying *syd:*cat and constructed essentially as described previously (19), except that it carried the chlorphenicol resistance determinant (cat) (instead of kan previously used) in the middle of the *syd* gene. Mutants carrying the *secY24 or secY24–249* mutation were described previously (20). These mutations were transferred into the genetic background of strain TW156 (66) by P1 transduction from *E. coli* strains and plasmids. For its construction, a polymerase chain reaction was performed using the synthetic peptide conjugated with keyhole limpet hemocyanin. Kaleidoscope prestained standards (purchased from Bio-Rad) were used as molecular weight markers in electrophoresis.

**RESULTS**

*Syd Inhibits Protein Translocation into IMVs with the Mutated SecY24 Subunit of Protein Translocase—Overproduction of Syd in the *secY24* mutant cells leads to the severest extent of inhibition of protein export known for *E. coli sec* mutants (19) and appeared to merit investigations *in vitro*. We prepared IMVs from *wild-type* and *secY24* mutant cells grown at 30 °C with the permissive temperature for the mutant. An *in vitro* translation product of *pro-*OmpA, labeled with [35S]methionine, was subjected to posttranslational translocation into IMV. When IMVs from *secY24* mutant cells were preincubated at 0 °C with increasing concentrations of Syd, translocation of *pro-*OmpA was progressively inhibited (Fig. 1). In contrast, *pro-*OmpA translocation into the wild-type IMV was not appreciably affected by Syd of up to 100 μg/ml (Fig. 1). Dose-dependent inhibition of translocation was also observed when a crude cytosol fraction from the *Syd*-overproducing cells was added to the reaction with the SecY24 IMV. Immunoblotting experiments showed that the concentrations of Syd protein required for a particular level of inhibition were similar between the purified sample and the crude cytosolic fraction, indicating that Syd had not been inactivated during the purification procedures (data not shown). Neither bovine serum albumin, up to 1 mg/ml, nor heat-treated Syd exerted any appreciable inhibitory effect (data not shown). Thus, this inhibition was due to the functional Syd protein and not to some nonspecific factors such as increased protein concentration.

When the SecY24 IMV was first incubated with excess Syd, sedimented, and subjected to the translocation assay, substantially levels of translocation of *pro-*OmpA were observed. Syd concentration in the second incubation mixture was about 15% of that before centrifugation. We suggest that Syd inhibits translocation while in an equilibrium of association and dissociation with IMV. In fact, preincubation was not necessary, since Syd added after the initiation of translocation reaction also prevented the subsequent increase of the translocated molecules (data not shown). None of the translational subunits was proteolyzed during the pretreatment with Syd or after the incubation at 37 °C with Syd, as confirmed by direct immunoblotting experiments (data not shown).

**Syd Rapidly Uncouples SecA Functions from Protein Translocation into the SecY24 IMV**—The ATP hydrolysis activity of SecA that is stimulated by a precursor protein and membrane vesicles with intact SecYEG complex is termed translocation ATPase (29). We found that Syd was a potent inhibitor of translocation ATPase when SecA was combined with the SecY24 IMV. Whereas Syd (100 μg/ml) inhibited this activity almost completely (Fig. 2, solid triangles), the same concentration of Syd only negligibly affected the activity in combination with the wild-type IMV (Fig. 2, solid circles). The inhibitory effect of Syd was specific for ATP hydrolysis that was coupled...
with translocation, such that neither the membrane ATPase activity (in the absence of preprotein) nor the intrinsic ATPase activity (without any other macromolecules) was affected significantly (data not shown). The addition of Syd into the reaction mixture in which SecA translocation ATPase was already activated by pro-OmpA and SecY24 IMV resulted in almost instantaneous inhibition of the ongoing reaction (Fig. 3).

We examined the SecA insertion reaction (4) using 125I-labeled SecA and urea-washed wild-type and SecY24 IMVs. In the absence of added Syd, both wild-type IMV and SecY24 IMV supported the pro-OmpA- and ATP-dependent formation of a membrane-protected 30-kDa fragment (Fig. 4, lane 4), although the efficiency was slightly lower for the SecY24 IMV. A nonhydrolyzable ATP analog, AMP-PNP, supported apparently pro-OmpA-independent insertion of SecA (Fig. 4, lane 6) as reported previously (5, 30). When Syd was included in these reactions, the SecY24 IMV did not support any detectable insertion of SecA, irrespective of whether ATP or AMP-PNP was used (Fig. 4, middle panel, lanes 1–3). In contrast, Syd inhibited SecA insertion into the wild-type IMV only slightly (Fig. 4, upper panel, lanes 1–3). These results indicate that Syd uncouples the SecA functions from the SecY24-mediated protein translocation reaction.

Syd Interferes with High Affinity Binding of SecA to the SecY24 IMV—To define the target reaction of the Syd inhibition, we carried out SecA binding assays. Various concentrations of SecA and IMV were incubated at 0 °C, and the membrane-bound SecA was isolated for the Scatchard analysis of the binding. SecA binding to the SecY24 IMV was found to be abolished almost completely by Syd. Thus, the high affinity phase of the binding was hardly observed in the presence of Syd (Fig. 5B, solid triangles). High affinity binding of SecA to the wild-type IMV was lowered by Syd to only a small extent (Fig. 5A, compare open and solid circles). These results suggest that the Syd action to the SecY24 IMV can essentially be ascribed to the elimination of the high affinity SecA binding.

We then examined whether Syd interferes with the SecA-SecY interaction passively, for instance by steric hindrance, or if it acts more actively, for instance to change the subunit arrangement of the SecY-SecE complex. For this purpose, we studied the effect of Syd on a state of SecA that had already been inserted and stabilized as such by AMP-PNP. 125I-Labeled SecA was first allowed to insert into wild-type or SecY24 IMV in the presence of AMP-PNP and then subjected to the second incubation with either Syd or buffer alone. The second incubation in the presence of Syd resulted in the disappearance of the 30-kDa fragment inserted into the SecY24 IMV (Fig. 6, lower panel, lane 1), whereas SecA inserted into the wild-type IMV persisted (Fig. 6, upper panel, lane 1). Incubation without Syd did not cause any change in the intensity of the inserted SecA,

Syd and SecY-SecA Interaction

FIG. 1. In vitro effects of Syd on translocation of pro-OmpA. A, in vitro synthesized and 35S-labeled pro-OmpA was subjected to post-translational translocation reaction at 37 °C for 40 min into IMVs prepared from strains EM169 (secY+), EM168 (secY24), and EM167 (secY24–249) as indicated. IMVs had been premixed with the indicated concentration of purified Syd (Syd conc.) at 0 °C before the initiation of the assays. After proteinase K digestion and SDS-PAGE, 35S-labeled pOmpA was visualized by autoradiography. p and m represent the precursor and mature forms, respectively. B, the result in A is graphically depicted after quantification by a Fuji BAS 2000 phosphor imager. Open circles, wild type; solid triangles, secY24; open squares, secY24–249. Translocation efficiency into the wild type IMV in the absence of Syd was about 80%.

FIG. 2. Effects of Syd on SecA translocation ATPase activity. SecA translocation ATPase activities were assayed using urea-washed IMVs from the wild type (EM169; circles) or the secY24 (EM168; triangles) cells, in the presence (solid symbols) of Syd (100 μg/ml) or in its absence (open symbols).

FIG. 3. Effects of Syd on ongoing reactions of SecA translocation ATPase. Two reactions of SecA translocation ATPase using IMVs from the secY24 mutant (EM168) were initiated at 37 °C. After 5 min, Syd (100 μg/ml) was added to one reaction (solid triangles), whereas the other reaction received the same volume of the buffer (open triangles).
Syd and Sec-Y-SecA Interaction

FIG. 4. Effects of Syd on SecA insertion into IMVs. 

125I-Labeled SecA (4 μg) and urea-treated IMVs (60 μg of protein) from secY+ (EM154; upper panel), secY24 (EM153; middle panel), and secY24–249 (EM167; lower panel) cells were preincubated at 0 °C in a total volume of 230 μl, and IMV-SecA complex was isolated by centrifugation, resuspended, and divided into six portions, each of which was then subjected to a SecA insertion reaction at 37 °C for 15 min in a total volume of 50 μl containing the 125I-SecA-bound IMV (100 μg/ml), pro-3His-OmpA (66 μg/ml; lanes 1 and 4), either ATP (2 mM; lanes 1, 2, 4, and 5) or AMP-PNP (2 mM; lanes 3 and 6), and Syd (100 μg/ml; lanes 1–3). The samples were treated with trypsin (1000 μg/ml at 0 °C for 15 min), and the 30-kDa fragment was visualized by SDS-PAGE and autoradiography.

FIG. 5. Effects of Syd on SecA high affinity binding to IMVs. SecA (1–200 nM, in which 125I-SecA occupied 1 nM) was mixed with urea-washed IMVs (100 μg/ml) from secY+ (EM154; A) or secY24 (EM153; B) cells, in a 50-μl reaction. Syd (100 μg/ml) was also included for the reactions shown by solid symbols. Samples were incubated at 0 °C for 30 min and centrifuged to pellet down the IMV-SecA complex. Radioactivities of the pellet and the supernatant were determined by an LKB γ-ray counter, and the results are shown as Scatchard plots. The apparent Kd values for high affinity binding of SecA were 14.9 and 25.6 nM for the secY+ IMV in the absence and presence of Syd, respectively. The value for the secY24 IMV was 15.4 nM in the absence of Syd, while the high affinity binding was not clearly observed in the presence of Syd.

SecY24 Alteration of Subunit Arrangements of Translocase and Further Evidence for the Syd Interaction with SecY—The secY24 alteration in SecY weakens the interaction between SecY and SecE such that the SecY24-SecE complex comes apart after solubilization with a nonionic detergent (9, 26). We examined trypsin digestion patterns of SecY and SecE in the membrane-integrated state. Wild-type and SecY24 IMV were treated with increasing concentrations of trypsin, and SecY and SecE were detected by immunoblotting using antisera against the N-terminal sequence of SecY and the central cytoplasmic region of SecE, respectively. Treatment of the wild-type IMV with low concentrations of trypsin produced an N-terminal fragment of about 21 kDa (Fig. 7, lanes 17 and 18). Its apparent molecular size suggests that the site of this cleavage was within cytoplasmic region 4 (C4). The SecY24 IMV gave different trypsin digestion patterns in two respects. First, the overall trypsin sensitivity as assessed by the disappearance of the intact SecY molecules was significantly higher for the SecY24 IMV than the wild-type IMV (Fig. 7, compare lanes 6–10 with lanes 16–20). Second, the 21-kDa N-terminal fragment produced from the SecY24 IMV (Fig. 6, lane 7) was further shortened to a slightly faster migrating product when trypsin concentrations were increased (Fig. 7, lanes 8 and 9).

Although the SecY24 amino acid alteration (Gly240 to Asp) is also in C4 domain of SecY, it is unrelated to any amino acid residues directly relevant to the trypsin-mediated hydrolysis of peptide bonds. Thus, this sequence alteration may have caused a change in local conformation of SecY or in subunit arrangements of the SecYEG complex.

We found a striking difference in the trypsin susceptibility of SecE between the wild-type IMV and the SecY24 IMV. SecE in the former IMV resisted trypsin of the highest concentration examined (Fig. 7, lower panel, lanes 16–20), although some decrease in the intensity of SecE, in parallel with that of the intact SecY, was observed at higher trypsin concentrations...
Syd and SecY-SecA Interaction

FIG. 7. Effects of the secY24 mutation and Syd on trypsin digestion patterns of IMV-integrated SecY and SecE. Urea-treated IMVs prepared from the secY' (EM154; lanes 11–20) and secY24 (EM153; lanes 1–10) cells were treated with increasing concentrations of trypsin in the presence of Syd (100 μg/ml; lanes 1–5 and 11–15) or in its absence (lanes 6–10 and 16–20), at 0 °C for 20 min. The trypsin concentrations used were as follows: 0 (lanes 1, 6, 11, and 16), 1 (lanes 2, 7, 12, and 17), 10 (lanes 3, 8, 13, and 18), 100 (lanes 4, 9, 14, and 19), and 1000 (lanes 5, 10, 15, and 20) μg/ml. Note, however, that trypsin in the stock solution used in this particular experiment had been partially inactivated due to repeated freezings and thaws, and essentially identical results were obtained in confirmatory experiments using a freshly prepared trypsin solution of concentrations about 1/3 those used in this experiment. Samples were separated by SDS-PAGE and immunologically stained with antisera against the N-terminal part of SecY (upper panel) and antisera against the central part of SecE (lower panel). The solid arrowheads indicate the positions of the full-length SecY (upper panel) and SecE (lower panel), respectively, and the open arrowheads indicate the positions of N-terminal fragments of SecY.

A Second Site Mutation, secY249, Cancels the Inhibitory Effects of Syd—Previously, we isolated Syd-resistant variants from the secY24 mutant (20). The mutations converged into three alleles in addition to the true reversion. Two of the alleles change residue 240 affected by the secY24 mutation, restoring the SecY-SecE interaction (20). The other allele, secY249 (for an Ala249 to Val change), is an intragenic suppressor mutation that suppresses only the inhibitory effect of Syd, without any effect on the temperature-sensitive protein export defect of the secY24 mutation (20). IMVs prepared from the secY24–249 double mutant supported pro-OmpA translocation with an efficiency similar to that of the secY24 IMV. However, it was only slightly inhibited by Syd (Fig. 1). The double mutant IMV also supported the translocation ATPase activity of SecA even in the presence of Syd (data not shown). Insertion of the 30-kDa fragment into the double mutant IMV was not inhibited by Syd (Fig. 4, lower panel, lane 1). The SecY24–249 IMV gave trypsinic digestion patterns of SecY and SecE that were essentially identical to those of the SecY24 IMV. However, they were unaffected by the presence of excess Syd (data not shown). Thus, the effects of the secY249 mutation on the phenotypes of the secY24 mutation have been reproduced in vitro. These results suggest that the Syd effect is brought about through specific interaction between SecY and Syd, in which the residue 249 of SecY plays an essential role.

DISCUSSION

Syd, originally identified as a multicopy suppressor of the secY–d1 mutation (19), directly interacts with SecY. Overproduction of Syd stabilizes the wild type SecY protein when it alone is overproduced or when it fails to associate with SecE because of the presence of the excess amounts of SecY–d1, the dominant negative SecY variant. The association of Syd with the plasma membrane is saturable such that SecY overproduction increases the membrane-bound state of Syd. We demonstrated in this study that Syd markedly altered the trypsin digestion pattern of SecY24 in IMV, lending further support to the notion that Syd interacts with SecY.

Evidence indicates that the secY24 mutation impairs SecY-SecE interaction. The temperature sensitivity of this mutant is partially suppressible by overproduction of SecE, and the mutation can act as an intragenic suppressor against the SecE-sequestering effect of the secY–d1 mutation (16, 20). After solubilization of the membrane with a nonionic detergent, a complex between SecY24 and SecE cannot be isolated (9, 16). The temperature-sensitive protein export defect of the secY24 mutant is due to the FtsH-mediated degradation of the SecY24 protein at 42 °C (14, 16). These observations suggest that the impaired SecY-SecE interaction is the primary cause of the secY24 defect. In further support of this notion, we found that SecE in the SecY24 IMV was more accessible to trypsin digestion than that in the wild-type IMV. The temperature sensitivity of the secY24 mutant could be due to temperature-dependent exacerbation of the interaction defect, temperature-dependent activation of the proteolytic system, or both.

When Syd is overproduced in the secY24 mutant, protein export is rapidly blocked followed by the loss of viability even at 30 °C (19). The severity of this block makes it the most useful

E. Matsuo, T. Taura, Y. Akiyama, and K. Ito, unpublished observations.
system to accumulate chemical amounts of a precursor protein with uncleaved signal sequence within the cell (24). The rapidity and the lack of accompanied SecY proteolysis suggest that this inhibition is brought about by direct interference by Syd with the translocase functions.

We reproduced the inhibitory effect of Syd in the in vitro translocation reaction of pro-OmpA. The concentration of Syd required for 50% inhibition of translocation was 5–10 μg/ml (or 250–500 nM) in a reaction containing IMV of 100 μg/ml proteins. Assuming that a cell contains 500 molecules of SecY (31) and that 10% of total proteins are located in the inner membrane, the above concentration of IMV corresponds to 250 ng/ml (or 5 nM) of SecY. Thus, numbers of the Syd molecules required for efficient inhibition far exceed those of SecY molecules. We have shown that Syd inhibits the high affinity binding of SecA to the IMV containing the SecY24 form of the translocase subunit. This could be due to a steric hindrance by the SecY-bound Syd with respect to SecA binding. There may be a competition between Syd and SecA for the interaction with SecYEG translocase. Indeed, increased concentrations of SecA acted to partially overcome the inhibition caused by a low concentration (10 μg/ml) of Syd. Although quantitative discussion requires knowledge about the Kd value of Syd-IMV binding, our IMV re-isolation experiments suggested that the dissociation rate of Syd is considerably high. Thus, real time binding/dissociation measurements will be required to determine the Kd value.

While the simple steric hindrance discussed above is possible, we believe it is more likely that Syd causes more drastic structural changes to the SecY complex. Syd was found to inhibit the SecA insertion reactions. We previously noted that the SecY205 mutant form of SecY (32) did not allow the pre-protein- and ATP-dependent mode of SecA insertion, while it still allowed SecA insertion in the presence of AMP-PNP (5). Our present results show, in contrast, that Syd abolishes both of these modes of SecA insertion reactions. Thus, it is conceivable that the SecY205 defect lies in a specific process of the SecA insertion reaction cascade, whereas Syd impairs the system more generally or inhibits a very early event. Indeed, Syd inhibited the high affinity binding between SecA and the SecY24 IMV. Furthermore, it acted to dissociate the inserted SecA segment that had been stabilized by AMP-PNP. In these cases, the subunit arrangement of the SecY complex may have been altered, leading to the elimination of both the high affinity SecA binding site and the structural element (or channel) required for maintaining the “inserted” state of SecA.

Previous studies suggested that the high affinity binding of SecA requires both SecY and SecE (3, 33, 34). Thus, it seems to be a logical explanation that the presence of a high concentration of Syd acts to dissociate the SecY-SecE complex that is already compromised by the SecY24 alteration. An interesting possibility here is that Syd may bind preferentially to an uncomplexed state of SecY. This is consistent with the known ability of Syd to stabilize overproduced SecY (19). Although the physiological role of Syd is elusive (19), some speculations may be useful. For instance, Syd may act as a reservoir for unassembled forms of SecY, thus preventing its deleterious effects on the cell (14). Alternatively, it may serve as a regulatory factor that negatively controls the translocase function by interfering with either SecY-SecE or SecY-SecA binding. At any rate, interaction between Syd and SecY seems specific in that it is disrupted by the secY249 alteration of residue 249 of SecY. Syd will be useful to probe the architecture of protein translocation from the functional point of view.

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REFERENCES
1. Wickner, W., and Leonardi, M. R. (1996) J. Biol. Chem. 271, 29514–29516
2. Ito, K. (1996) Genes Cells 1, 337–346
3. Hartl, F.-U., Lecker, S., Schiebel, E., Hendrick, J. P., and Wickner, W. (1996) Cell 84, 269–279
4. Economou, A., and Wickner, W. (1994) Cell 78, 835–843
5. Matsumoto, G., Yoshihisa, T., and Ito, K. (1997) EMBO J. 16, 6384–6393
6. Brundage, L., Hendrick, J. P., Schiebel, E., Driessen, A. J. M., and Wickner, W. (1990) Cell 62, 649–657
7. Joly, J. C., Leonardi, M. R., and Wickner, W. T. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 4703–4707
8. Brundage, L., Fimmel, C. J., Mizushima, S., and Wickner, W. (1992) J. Biol. Chem. 267, 4166–4170
9. Homma, T., Yoshihisa, T., and Ito, K. (1997) FEBS Lett. 408, 11–15
10. Akiyama, J., Matsuyama, S., Tokuda, H., and Mizushima, S. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 6545–6549
11. Nishiyama, K., Mizushima, S., and Tokuda, H. (1993) EMBO J. 12, 3499–3415
12. Matsuyama, S., Akiyama, J., and Mizushima, S. (1990) FEBS Lett. 269, 96–100
13. Taura, T., Baba, T., Akiyama, Y., and Ito, K. (1993) J. Bacteriol. 175, 7771–7775
14. Kihara, A., Akiyama, Y., and Ito, K. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 4532–4536
15. Shiba, K., Ito, K., Yura, T., and Cerretti, D. P. (1984) EMBO J. 3, 631–635
16. Polischuk-Roder, M., Murphy, C., and Beckwith, J. (1996) J. Biol. Chem. 271, 19098–19104
17. Shiba, K., Ito, K., Yura, T., and Cerretti, D. P. (1984) EMBO J. 3, 631–635
18. Polischuk-Roder, M., Murphy, C., and Beckwith, J. (1996) J. Biol. Chem. 271, 19098–19104
19. Shiba, K., Ito, K., Yura, T., and Cerretti, D. P. (1984) EMBO J. 3, 631–635
20. Polischuk-Roder, M., Murphy, C., and Beckwith, J. (1996) J. Biol. Chem. 271, 19098–19104
21. Casadaban, M. J. (1976) J. Mol. Biol. 104, 541–555
22. Akiyama, Y., and Ito, K. (1999) Biochem. Biophys. Res. Commun. 267, 416–421
23. Akiyama, Y., and Ito, K. (1995) J. Biol. Chem. 270, 5519–5526
24. Matsuyama, S., Fujita, Y., Sagara, K., and Mizushima, S. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 1171–1181