The Brl Domain in Sec63p Is Required for Assembly of Functional Endoplasmic Reticulum Translocons*

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Protein translocation into the endoplasmic reticulum occurs at pore-forming structures known as translocons. In yeast, two different targeting pathways converge at a translocation pore formed by the Sec61 complex. The signal recognition particle-dependent pathway targets nascent precursors co-translationally, whereas the Sec62p-dependent pathway targets polypeptides post-translationally. In addition to the Sec61 complex, both pathways also require Sec63p, an integral membrane protein of the Hsp40 family, and Kar2p, a soluble Hsp70 located in the ER lumen. Using a series of mutant alleles, we demonstrate that a conserved Brl (Brr2-like) domain in the COOH-terminal cytosolic region of Sec63p is essential for function both in vivo and in vitro. We further demonstrate that this domain is required for assembly of two oligomeric complexes of 350 and 380 kDa, respectively. The larger of these corresponds to the heptameric "SEC complex" required for post-translational translocation. However, the 350-kDa complex represents a newly defined hexameric SEC' complex comprising Sec61p, Sss1p, Sbh1p, Sec63p, Sec71p, and Sec72p. Our data indicate that the SEC' complex is required for co-translational protein translocation across the yeast ER membrane.

Proteins destined for the secretory pathway are initially translocated across the membrane of the endoplasmic reticulum (ER) 4 at pore-forming structures known as translocons. Two pathways have been described by which this translocation can occur. The first occurs co-translationally in a manner dependent upon the actions of both signal recognition particle (SRP) and its cognate membrane receptor (SR). In this pathway, nascent precursors are targeted to the ER membrane, wherein the ribosome engages with the translocon, enabling the growing polypeptide chain to be inserted directly into the translocon pore (for a review, see Ref. 1). In the second pathway, fully translated precursors are targeted to the translocon in a post-translational manner that requires cytosolic chaperones and the membranous receptor Sec62p (for a review, see Refs. 2 and 3). In this pathway, translation and translocation are uncoupled, so the luminal chaperones Kar2p (4), Sec62p (for a review, see Refs. 2 and 3), and Sec63p-dependent pathway targets polypeptides post-translationally. In addition to the Sec61 complex, both pathways also require Sec63p, an integral membrane protein of the Hsp40 family, and Kar2p, a soluble Hsp70 located in the ER lumen. Using a series of mutant alleles, we demonstrate that a conserved Brl (Brr2-like) domain in the COOH-terminal cytosolic region of Sec63p is essential for function both in vivo and in vitro. We further demonstrate that this domain is required for assembly of two oligomeric complexes of 350 and 380 kDa, respectively. The larger of these corresponds to the heptameric "SEC complex" required for post-translational translocation. However, the 350-kDa complex represents a newly defined hexameric SEC' complex comprising Sec61p, Sss1p, Sbh1p, Sec63p, Sec71p, and Sec72p. Our data indicate that the SEC' complex is required for co-translational protein translocation across the yeast ER membrane.

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4 The abbreviations used are: ER, endoplasmic reticulum; SRP, signal recognition particle; SR, SRP cognate membrane receptor; BN-PAGE, blue native PAGE; Bistris, 2-(bis(2-hydroxyethyl)amino)-2-(hydroxymethyl)propane-1,3-diol.
show that whereas the 52-residue acidic domain at the extreme COOH terminus is required for post-translational translocation, it is nonessential for the co-translational reaction. We further define a novel Brl domain and show that deletion of residues 550–611 within this region results in a complete defect in ER translocation by either pathway. We go on to use blue native gel analysis to examine Sec61p-containing protein complexes and to determine that the Brl domain of Sec63p is required for the assembly of both the 380-kDa SEC complex and a newly defined 350-kDa SEC complex. Functional analysis of various mutant strains has revealed that cells lacking the SEC and SEC’ complexes are incompetent for protein translocation into the yeast endoplasmic reticulum. Our results indicate that the SEC and SEC’ complexes correspond to functional translocons in the yeast ER.

**EXPERIMENTAL PROCEDURES**

**Materials**—DNA restriction and modification enzymes were purchased from Roche Applied Science. [14C]-alpha amino acid mixture was from PerkinElmer Life Sciences. Oligonucleotides were from MWG-Biotech AG (Table 1). Site-directed mutagenesis was performed using the QuikChange site-directed mutagenesis kit from Stratagene according to the manufacturer’s instructions. All other reagents were from Roche Applied Science, Sigma, or Melford Laboratories (Suffolk, UK) at analytical grade.

**SEC63 Allele Construction**—Truncation alleles of Sec63p were constructed by cassette integration into the diploid MET3-Sec63 strain BYY4 as previously described for the sec63-D28 strain BYY8 (11). Strains AJY1, containing a sec63-D52 allele, and AJY2, containing a sec63-D113 allele, were generated by transformation of BYY4 with a linear 3HA-His3MX6 cassette (30). Oligonucleotides for amplification of the cassette were SEC63D52 for plus 63d/R1 for AJY1 and SEC63D113 for plus 63d/R1 for AJY2. The plasmid-borne sec63-Δ550–611 allele was made by introducing BglIII sites into pKR2 (12) by site-directed mutagenesis using the oligonucleotides Sec63cid1a, Sec63cid1b, Sec63cid2a, and Sec63cid2b. pA7 was then made by the excision of the BglIII fragment, resulting in residues 550–611 of the SEC63 open reading frame being replaced by an arginine and a serine residue.

**Yeast Strains and Growth Conditions**—Yeast strains (Table 2) were grown on standard media as previously described (11). Repression of the MET3 promoter in minimal medium for total extract preparation and pulse labeling was achieved by the addition of l-methionine to early log phase cultures to a final concentration of 2 mM and growing for 7 h. For microscope preparation, MET3 repression was achieved by growth in YPD for 7 h.

**Radiolabeling and Immunoprecipitation**—10 A2800 equivalents of cells were pulse labeled for 5 min with the [14C]-alpha amino acid mixture, and immunoprecipitation of the Sec62p-dependent substrate CPY and SRP-dependent substrate DPAP B was carried out as previously described (11). Samples were analyzed by SDS-PAGE and phosphorimaging analysis.

**In Vitro Translocation Assays**—Microsome preparation and in vitro translocation assays were carried out as previously described (12). In brief, a-factor and D10c-a-factor mRNAs were transcribed from linearized template with Ribomax (Promega) T7 RNA polymerase and Ribopax SP6 RNA polymerase, respectively, according to the manufacturer’s instructions. Translations/translocations in the presence of [35S]-methionine were carried out in the presence of microsomes (50 A280/ml) added to a final concentration of 10%. Samples were analyzed by SDS-PAGE and phosphorimaging analysis, and quantification was carried out using AIDA (version 2.31).

**Blue Native PAGE Analysis**—BN-PAGE was adapted from the method previously described (31). Two A280 units of microsomes were harvested by centrifugation at 10,000 x g, resuspended in 100 μl of S-buffer (2% digitonin, 250 mM NaCl, 2 mM dithiothreitol, 20 mM Tris-HCl, pH 7.6, 5 mM MgOAc, 12% glycerol, 1% (v/v) protease inhibitor mixture (Sigma)) and then incubated on ice for 30 min. Unsolubilized material was removed by centrifugation at 10,000 x g, and the ribosomes were removed from the supernatant by centrifugation for 1 h at 400,000 x g. The supernatant was then diluted to 180 μl as S-buffer but minus NaCl and digitonin, followed by the addition of 20 μl of 10X sample buffer (5% Coomassie Brilliant Blue G250, 50 mM 6-aminoacrylic acid, 100 mM Bistris-HCl, pH 7.0). 0.8 A280 unit aliquots was loaded onto a 6–12% polyacrylamide gradient gel (buffered with 500 mM 6-aminoacrylic acid, 50 mM Tris-HCl, pH 7.0). The samples were run at 200 V for 18 h with Coomassie-containing cathode buffer (50 mM Tricine, pH 7.0, 15 mM Bistris-HCl, pH 7.0, 0.02% Coomassie Brilliant Blue G250) and then for a further 3 h at 500 V in Coomassie-lacking buffer (50 mM Tricine, pH 7.0, 15 mM Bistris-HCl, pH 7.0). Anode buffer (50 mM Bistris-HCl, pH 7.0) was constant throughout. The samples were then transferred to polyvinylidene difluoride membrane and analyzed by Western blotting. Complex sizes were determined using the high molecular weight calibration kit for native electrophoresis (Amer-
These results demonstrated the expression of the various deletion constructs and also confirmed that the growth conditions effectively depleted wild-type Sec63p following repression of the MET3 promoter.

Next we examined protein translocation in these mutant strains by pulse labeling and immunoprecipitation of the Sec62p-dependent substrate CPY and the SRP-dependent precursor DPAP B (Fig. 2). As previously reported (11), Sec63-Δ28p causes an accumulation of the precursor form of CPY but not of the precursor form of DPAP B (Fig. 2, lanes 4 and 5), indicating a functional defect that is specific to the post-translational translocation pathway. As might then be expected, both Sec63-Δ52p and Sec63-Δ113p were also defective in CPY translocation. However, whereas Sec63-Δ52p led to an accumulation of low levels of
pDPAP B, the Sec63-Δ113p mutant was found to be completely defective in DPAP B translocation (Fig. 2, lanes 6–9). Thus, whereas removal of the acidic domain has only a mild effect upon the translocation of SRP-dependent precursors, additional disruption of the Brl domain in Sec63-Δ113p abolished ER protein translocation by either pathway.

Construction of a Δ550–611 Deletion Allele—The Brl domain is clearly required for SRP-dependent translocation; however, the loss of the carboxyl-terminal acidic domain in the sec63-Δ113 truncation could account for the observed Sec62p-dependent translocation defect. In order to test whether the Brl domain might also be required for Sec62-dependent translocation, a deletion allele was constructed that lacked only residues 550–611 (Fig. 3A). Phenotypic analysis of cells expressing the sec63-Δ550–611 allele was again performed following in vivo repression of the MET3-regulated copy of SEC63. The deletion allele proved unable to support growth of BYY5 cells from strains described in \( \text{lane } 2 \) and underwent methionine repression of genomic SEC63 as described previously. Total cell extracts were made, and 0.5 \( A_{260} \) equivalents were resolved by 10% SDS-PAGE followed by Western blotting for Sec63p. Wild type Sec63p and the Δ550–611 gene product are indicated.

Sec63p Is Present in Two Distinct Translocon-associated Complexes—The Brl domain is predicted to be involved in protein-protein interactions, so we reasoned that its role might be to promote Sec63p binding to some component of the translocation machinery. In order to test this hypothesis, we first sought to characterize translocon-related protein complexes from solubilized yeast membranes using a BN-PAGE approach (33). Yeast microsomes were prepared from the wild type strain \( \text{W303} \) and solubilized in S-buffer containing 2% digitonin and resolved by 6–16% BN-PAGE. Western blotting analysis revealed four distinct complexes, with apparent molecular masses of 140, 280, 350, and 380 kDa, respectively, each of which contained all three components of the Sec61 complex, namely Sec61p, Sbh1p, and Sss1p (data not shown). Sec63p was found to be present in three distinct complexes, two of which co-migrated with the 350- and 380-kDa complexes detected with anti-Sec61 antibodies (Fig. 5A). Together, these data demonstrate that disruption of the Brl domain of Sec63p perturbs both SRP-dependent and Sec62p-dependent translocation.

In order to determine which if any of the four Sec61p-containing complexes corresponds to the post-translational SEC complex, we next probed with antibodies specific for Sec62p and Sec63p (Fig. 5B, lanes 2 and 3) as well as Sec71p and Sec72p (data not shown). Sec63p was found to be present in three distinct complexes, two of which co-migrated with the 350- and 380-kDa complexes detected with anti-Sec61 antibodies (Fig. 5B, lanes 1 and 2). Both Sec71p and Sec72p exhibited the same pattern of distribution as Sec63p, being present in both the 350- and 380-kDa complexes as well as an additional complex that migrates at ~200 kDa (Fig. 5B, lane 2). This 200-kDa complex most likely corresponds to the previously characterized "Sec63p complex" identified by Brodsky and Schekman (34). In the case of Sec62p, immunoblotting identified a single immunoreactive band at 380 kDa (Fig. 5B). These data

**FIGURE 3. Deletion of residues 550–611 of the cytosolic domain of Sec63p.** A, an in-frame excision of the region of the SEC63 open reading frame corresponding to residues 550–611 was created as described under "Experimental Procedures." B, the haploid strain BYY5 (pMET3-SEC63) transformed with pRS316 (vector control), pJKR2 (wild type SEC63), or pAPJ (Δ550–611) was plated onto selective media either lacking or supplemented with 2 mw methionine and incubated for 3 days. C, cells from strains described in B underwent methionine repression of genomic SEC63 as described previously. Total cell extracts were made, and 0.5 \( A_{260} \) equivalents were resolved by 10% SDS-PAGE followed by Western blotting for Sec63p. Wild type Sec63p and the Δ550–611 gene product are indicated.
are entirely consistent with the 380-kDa species being equivalent to the heptameric SEC complex first identified in cross-linking studies (35). The data further suggest that the 350-kDa species corresponds to a hexameric subcomplex, which we term SEC/H11032, comprising Sec61p, Sss1p, Sbh1p, Sec63p, Sec71p, and Sec72p but not Sec62p. In order to confirm this, we next examined Sec61-containing complexes in membranes depleted of either Sec62p or Sec63p. Membranes were prepared from methionine-treated cultures of either W303/H9251 (wild type), BYY5 (pMET3-SEC63), or MWY50 (pMET3-SEC62) and then resolved by either 6–12% BN-PAGE (Fig. 6, A and B, upper panels) or 10% SDS-PAGE (Fig. 6, A and B, lower panels) before Western blotting for Sec61p, Sec63p, or Sec62p. In membranes depleted of Sec62p, we found that the 380-kDa complex could no longer be detected with antibodies to either Sec61p or Sec63p. These results confirm that all three proteins are components of a single 380-kDa complex corresponding to the SEC complex. None of the other complexes were absent following Sec62p depletion (Fig. 6A), which confirms our finding that they do not contain Sec62p (see Fig. 5B) but further indicates that the appearance of

FIGURE 4. The Δ550–611 allele is defective in SRP-dependent and Sec62p-dependent translocation both in vivo and in vitro. A, BYY5 cells containing pJKR2 (SEC63; lanes 1–3), pRS316 (vector control; lanes 4 and 5), or pAJ7 (Δ550–611; lanes 6 and 7) were treated with methionine, as described previously. Cells were pulse-labeled, and translocation substrates were immunoprecipitated as described previously. 5 A260 units of yeast microsomes from W303/H9251 were prepared for BN-PAGE and analyzed by autoradiography. The different forms of CPY and DPAP B are indicated as previously described. B, upper panel, in vitro translation/translocation reactions were carried out using RNA encoding gpoF (lanes 1–3) or DΔαF (lanes 4–6), and microsomes were prepared following repression of genomic SEC63. Reactions were resolved by 12.5% SDS-PAGE. Microsomes are from BY55 + pRS316 (vector control; lanes 1 and 4), pJKR2 (SEC63; lanes 2 and 5), or pAJ7 (Δ550–611; lanes 3 and 6). Lower panel, 0.5 A260 equivalents of the microsomes described were resolved by 10% SDS-PAGE and Western blotted with anti-Sec63p and anti-Sec61p antibodies. Sec61p, Sec63p, and Sec63-Δ550–611p are indicated. C, AIDA software (version 2.31) was used to quantify the percentage translocation of each precursor from five data sets.
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these complexes is independent of any activity of Sec62p. Intriguingly, the signal at 350 kDa is enhanced upon depletion of Sec62p, which would be consistent with our hypothesis that this species represents a complex related to SEC but lacking the Sec62p component. This was confirmed upon depletion of Sec63p, where we found that both the 350- and 380-kDa Sec61-containing complexes were now absent but that the 140 and 280-kDa species were unaffected (Fig. 6B). These results demonstrate that Sec63p is required for the assembly of Sec61p into both the SEC and SEC′ complexes.

The Brl Domain in Sec63p Is Required for Complex Formation—To determine whether the Brl domain is required for the assembly of the Sec63p-containing SEC and SEC′ complexes, we next analyzed protein complexes from membranes prepared from BYY5 cells carrying either pAJ7 (ΔSSO–611), pRS316 (vector control), or pJKR2 (wild type SEC63). As expected, membranes derived from BYY5 + pJKR2 exhibited the wild type pattern of translocon complexes (Fig. 7A, upper panel, lane 2). However, in those derived from both BYY5 + pAJ7 (ΔSSO–611), both the SEC and SEC′ complexes are absent, whereas the 140- and 280-kDa Sec61p complexes remain unaffected (upper panel, lanes 1 and 3). SDS-PAGE and Western analysis of the same microsomes confirmed repression of wild type SEC63 and expression of ΔSSO–611 (lower panels). The absence of both the SEC and SEC′ complexes in ΔSSO–611-containing microsomes correlates with complete defects in both translocation pathways.

This effect might be due to a general perturbation in the cytosolic domain of Sec63p rather than any specific role for the Brl domain in SEC′ assembly. In order to test this, we next examined translocon complexes present in Sec63Δ28p membranes. Two major Sec61-containing complexes were identified, the smaller corresponding to the 140-kDa complex and the larger migrating slightly faster than SEC′ (Fig. 7B). The larger complex also contained Sec63p (Fig. 7B) plus all other known components of the SEC′ complex (data not shown). Complex migration in BN-PAGE is known to be affected by charged domains, so the faster migration of SEC′ in this case may be due solely to the absence of the highly charged COOH-terminal region of Sec63p. A similar increase in migration can also be seen for the Sec63pΔ28p-Sec71p-Sec72p complex (Fig. 7B). Of course, we cannot exclude the possibility that the faster migration of these complexes might be due to the loss of some unknown component.

Sec63Δ28p membranes are specifically defective in post-translational translocation. Consistent with this, we found no detectable SEC complex, indicating that the COOH-terminal 28 residues of Sec63p are in fact essential for recruitment of Sec62p. Our results further demonstrate that the Sec63p Brl domain is required for formation of higher order protein translocation complexes identified in the yeast ER membrane.

The J-domain of Sec63p Is Required for Function of the SEC and SEC′ Complexes—The absence of the SEC and SEC′ complexes in the mutants analyzed thus far might be an artifact of the concomitant defect in protein translocation in these cells. In this scenario, complex formation would be dependent upon ongoing co-translational translocation and not vice versa. In order to examine this possibility, we screened existing mutants to identify any that might remain proficient in complex assembly despite a block in translocation. We have previously reported a derivative of Sec63p in which the majority of the luminal J-domain is deleted (sec63ΔJ), which results in the abolition of both translocation pathways both in vivo and in vitro (12). BN-PAGE analysis of membranes prepared from BYY5 cells containing pAJ8 (sec63ΔJ) revealed four Sec61-containing complexes that were indistinguishable in gel mobility from those observed in control cells (Fig. 7C). These results indicate that formation of the SEC and SEC′ complexes does not require ongoing protein translocation. Moreover, we can further conclude that the role of the Sec63p J-domain in protein translocation must only occur downstream of complex assembly.

DISCUSSION

Despite a number of recent structural studies of specific translocon components, a clear view has yet to emerge regarding the nature of the functional translocon. The highest resolution model to date of the archaeabacterial SecY complex has led to the suggestion that the protein-conducting channel might be formed by a single SecY trimer (23). However, numerous other studies have demonstrated that the homologous euarchea and eukaryotic translocon complexes form much larger oligomeric structures. EM models show eukaryotic complexes with an average outer diameter of 95 Å and a size of between 250 and 280 kDa (24). This led to the suggestion that the translocon might comprise three or four copies of the Sec61 complex, an observation supported by cryo-EM studies of the ribosome-associated yeast translocon (25, 27). EM models of the bacterial translocon have shown a dimeric SecYEG
complex associated with the ribosome (36, 37). The variation in complex size identified in these various studies means that the true oligomeric nature of the core-translocon complex in vivo is still unclear. In addition, the biophysical modeling methods are based solely upon the trimeric Sec61 complex or its homologues, and therefore the various associated factors that interact with Sec61 complexes during protein translocation are not accounted for.

Blue Native PAGE has been used to visualize both the mammalian Sec61- complex and bacterial SecYEG (29, 31). In this study, we have identified four distinct Sec61-containing complexes derived from yeast ER membranes. The two smaller complexes, 140 and 280 kDa, are strikingly similar to the 140-kDa complex identified in mammalian ER (34) and to the 130- and 270-kDa bacterial complexes identified as oligomers of the conserved SecYEG complex (29). The molecular masses of Sec61p (53 kDa), Ss1p (8.9 kDa), and Sb1p (8.7 kDa) would predict a molecular mass for the heterotrimer of 70.6 kDa. Thus, the observed gel mobilities of 140 and 280 kDa are remarkably consistent with those predicted for a dimer and tetramer of the Sec61 complex. Of course, we cannot exclude the possibility that some other factor(s) might be present in one or more of the complexes detected in our analysis.

In addition, we found two larger Sec61p-containing complexes migrating at 350 and 380 kDa, respectively. The largest of these corresponds to the previously defined heptameric SEC complex known to be required for post-translational translocation (7). The 350-kDa species is a newly defined SEC’ complex containing all of the components of the SEC complex apart from Sec62p.

In cells depleted of Sec62p, only the SEC complex was lost, with a concomitant block in post-translational translocation. However, upon repression of SEC63, both the SEC and SEC’ complexes were absent, and both translocation pathways were defective. Significantly, the 140- and 280-kDa Sec61-containing complexes remained abundant after Sec63p depletion, from which we must conclude that neither of these smaller complexes is sufficient for translocation. Thus, whereas the SEC complex is required for Sec62p-dependent translocation, SEC’ is required for SRP-dependent translocation. The pools of 140/280-kDa Sec61 complex and Sec63p-Sec71-Sec72 complex observed in wild type membranes might derive merely from unstable SEC’ or might correspond to bona fide intermediates of a translocon reaction cycle.

It has previously been reported that mammalian Sec61 complex plus SR was sufficient for co-translational translocation into reconstituted proteoliposomes (38). However, Sec63p is essential for co-translational translocation into intact yeast membranes in a manner that, importantly, is independent of the post-translational pathway (11, 12). This apparent anomaly might be explained were Sec63p to play a regulatory role, say in translocon gating, that is essential in intact membranes but not in artificial proteoliposomes. However, given the subsequent discovery that mammalian Sec63 co-purifies with Sec61 complex (17), it would be interesting to determine whether some catalytic level of Sec63 might be present in the proteoliposome system.

The presence of Sec71p and Sec72p in the co-translational SEC’ complex is interesting, given that neither is essential for this process. However, both of these components were first identified in a genetic screen...
for mutants defective in the integration of an SRP-dependent membrane protein (39). From this we must conclude that mutations in both SEC71 and SEC72 confer phenotypically significant defects in this pathway. Our data clearly demonstrate that the higher order SEC and SEC’ complexes provide the context in which Sec63p functions during protein translocation. Interestingly, it has recently been shown that residues within the acidic domain of Sec63p are phosphorylated and that this contributes to Sec62p binding. It has been suggested that this might provide a mechanism to regulate Sec62p binding and may therefore influence the relative levels of SEC and SEC’ complexes present within the ER membrane (40).

It seems likely that the translocation channel would be tightly regulated in order to maintain the permeability barrier of the ER membrane. Indeed, a number of studies on the mammalian translocation machinery have demonstrated that the Kar2p homologue BiP is required to gate the luminal face of the translocon (13, 14). More recently it has been demonstrated that BiP-mediated gating requires a membranous J-component that interacts with BiP at two points during the gating cycle (15). First, ADP-bound BiP requires a J-domain to be present to enable binding to the luminal face of the translocon. Subsequent to binding, a J-mediated stimulation of nucleotide exchange and hydrolysis recycles BiP from the translocon, leaving the channel open. The SEC/SEC’ complexes would provide a context in which the J-domain of Sec63p could recruit Kar2p to the luminal face of the active translocon in order to regulate the opening and closing of the channel. Similar complexes may prove to exist in mammalian cells; indeed, homologues of both Sec63p and Sec62p have been identified and found in association with purified Sec61a (16, 17), but no specific function has yet been ascribed to these proteins in the mammalian cells. There is also a second membrane-associated J-protein in the mammalian ER, Mtp1, that has been shown to interact with both the ribosome and BiP (41), raising the possibility that there might be some functional redundancy in the mammalian system.

Our results clearly demonstrate that the cytosolic domain of Sec63p plays two distinct roles in translocon assembly. The Brl domain is required for the formation of the SEC’ complex, and the acidic domain is then required for recruitment of Sec62p to form the larger SEC complex. The Brl domain is so-called due to its homology to two regions of the yeast U5 200-kDa RNA helicase Brr2p (bad response to refrigeration), a component of the yeast spliceosome (20, 21). The two “Brl regions” in Brr2p have been shown to interact with a number of different spliceosomal components during the dynamic structural rearrangements that occur within the spliceosome during the course of a splicing reaction (22). Our evidence is consistent with the Brl domain within Sec63p acting as a protein-binding domain during complex assembly. It is then interesting to speculate that, as in the spliceosome, it might also provide a mechanism for structural remodeling events within the translocon during this complex reaction process. It will be interesting to determine which factors interact with the Brl domain and whether these interactions change during the course of a translocation reaction.

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