Review

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Glycolipozyme membrane protein integrase (MPIase): recent data

Abstract: A novel factor for membrane protein integration, from the cytoplasmic membrane of *Escherichia coli*, named MPIase (membrane protein integrase), has recently been identified and characterized. MPIase was revealed to be essential for the membrane integration of a subset of membrane proteins, despite that such integration reactions have been, thus far, thought to occur spontaneously. The structure determination study revealed that MPIase is a novel glycolipid comprising a glycan chain with three *N*-acetylated amino sugars connected to diacylglycerol through a pyrophosphate linker. As MPIase catalyzes membrane protein integration, we propose that MPIase is a glycolipozyme on the basis of its enzyme-like function. The glycan chain exhibits a molecular chaperone-like function by directly interacting with substrate membrane proteins. Moreover, MPIase also affects the dimer structure of SecYEG, a translocon, thereby significantly stimulating preprotein translocation. The molecular mechanisms of MPIase functions will be outlined.

Keywords: glycolipozyme; membrane protein integration; MPIase; preprotein translocation; SecYEG.

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Introduction

All living cells express integral membrane proteins in every biomembrane. As integral membrane proteins possess transmembrane (TM) domains that are hydrophobic and water insoluble, membrane integration of such proteins proceeds coupled with translation to avoid aggregation. To achieve this cotranslational integration, many components function in a concerted manner. The integration reaction can be explained by the extended 'signal hypothesis' (1). SRP, a signal recognition particle, binds to a hydrophobic TM domain emerging from a ribosome and targets the SRP-nascent chain-ribosome complex to the SRP receptor (SR) on a membrane. The nascent chain is next transferred to a translocon (SecYEG in bacteria and the Sec61 complex in eukaryotic cells), followed by integration. YidC or TRAM interacts with integrating membrane proteins (Figure 1A and B). Beside the Sec-dependent pathway described above, it is known that a subset of membrane proteins of small size or a C-terminal TM is integrated independently of the SRP and Sec system because SRP only binds with a nascent chain (2–6) (Figure 1C). Recently, we clarified that a glycolipid named MPIase (membrane protein integrase) is involved in both Sec-dependent and -independent integration of membrane proteins (7–9). In this article, our recent studies on the identification and structure-function relation of MPIase will be outlined.

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Spontaneous insertion and diacylglycerol (DAG)

Membrane protein integration into inverted membrane vesicles (INV) can be easily analyzed by means of the protease protection assay (2). The membrane-integrated domain is protected by the membrane from externally added protease, giving a membrane-protected fragment (MPF) (Figure 2). In the case of MtlA (mannitol permease) with six TMs, an MPF of ~30 kDa is generated upon membrane integration (10, 11). The *in vitro* system involving INV prepared from mutant strains demonstrated that MtlA is targeted to a membrane by SRP and SR, followed by integration into the membrane at the SecYEG translocon; that is, it is Sec dependent (12, 13). Efficient integration into membranes was observed, even if the SecYEG

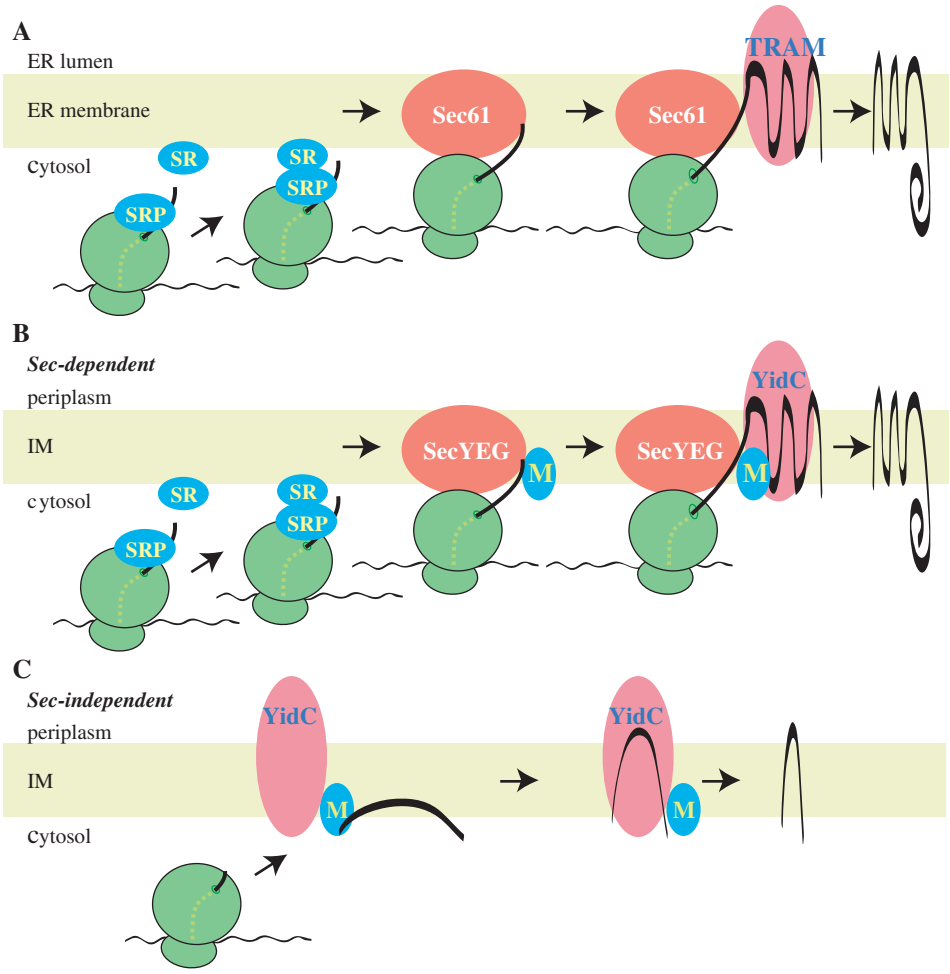

Figure 1 Mechanisms for membrane protein integration.
Protein integration into the ER membrane (A) or the cytoplasmic (inner) membrane of *E. coli* (B and C). The Sec-dependent (B) and -independent (C) pathways are shown. MPIase is shown as 'M'.

translocon was absent, when MtlA was *in vitro* synthesized with liposomes composed of *Escherichia coli* phospholipids (7). This observation is not compatible at all with the *in vitro* results obtained using INV prepared from mutant strains (12, 13); therefore, it must be an artifact owing to the artificial membrane system (7). Nonetheless, it should reflect membrane integration because it was stimulated by SRP/SR, and resistant against urea and high-salt extraction. Thus, MtlA was proven to be spontaneously integrated into liposomes (7), while such spontaneous integration is strictly blocked in INV (12, 13). These findings led us to the hypothesis that membrane components such as minor lipids and membrane proteins in INV inhibited the spontaneous integration. A lack of such a component in pure liposomes could be the reason why MtlA was disorderly integrated into membranes. A search for such membrane components revealed that DAG, a native membrane component of *E. coli*, prevents the spontaneous integration of MtlA (7). Inclusion of DAG in liposomes in several ratios as to phospholipids, corresponding to a physiological concentration in INV, completely blocked the spontaneous integration of MtlA (7). Moreover, DAG also blocked the intrinsic spontaneous integration of a subset of proteins that are known to be Sec independent. M13 procoat is a membrane protein composed of a TM domain preceded by a cleavable signal sequence (14). Pf3 coat carries a TM of N-out and C-in topology (15). These proteins contain a TM with the same topology. Membrane integration of these proteins has been extensively examined during the last 30 years (3). Mutant analysis revealed that insertion of M13 procoat and Pf3 coat proteins is independent of both Sec factors and the SRP system. In *in vitro* analysis, in which liposomes with protease preloaded inside were used, M13 procoat was digested as a result of membrane integration (16). Therefore, it has been widely believed that these proteins are spontaneously integrated

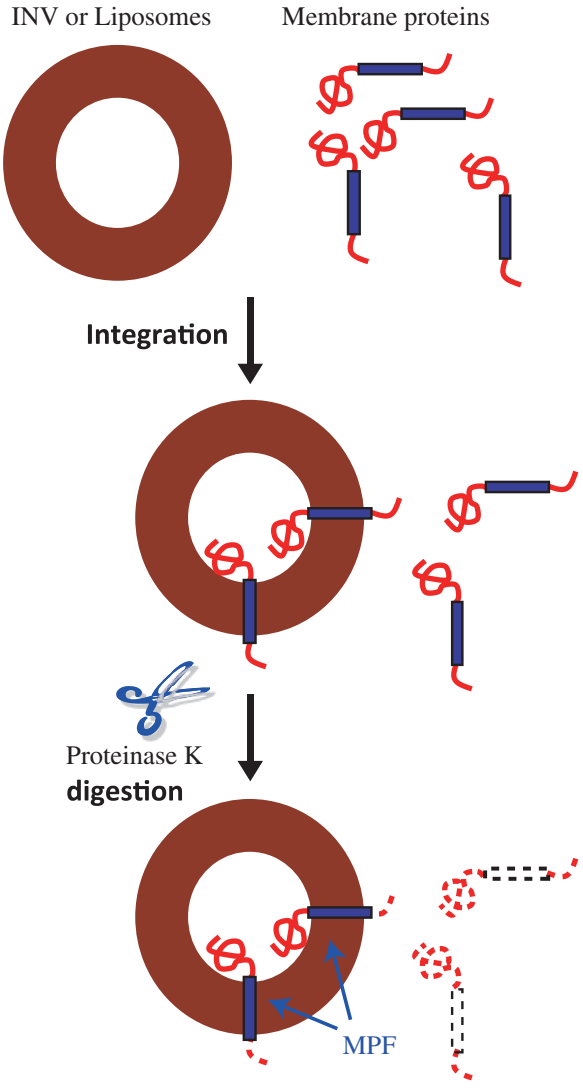

Figure 2 The *in vitro* assay system for protein integration into INV or liposomes.
Membrane proteins are *in vitro* synthesized in the presence of INV or liposomes to allow membrane integration. After the reaction, the membrane-integrated domain is protected against the externally added proteinase K, giving MPFs (indicated by blue arrows).

into membranes by means of the hydrophobic interaction between TMs and lipid bilayer leaflets. When liposomes containing DAG were used for M13 procoat and Pf3 coat integration, DAG also blocked the spontaneous integration of these proteins completely (7, 17). 3L-Pf3 coat is a mutant version of Pf3 coat in which three leucine residues are inserted in the middle of the TM domain (18). Although integration of M13 procoat and Pf3 coat proteins is stimulated by the membrane potential, 3L-Pf3 coat does not require the membrane potential (18). In this sense, insertion of this protein is the most spontaneous. Even if 3L-Pf3 coat was used for the integration assay, DAG clearly blocked its spontaneous integration. These results strongly suggested that the integration of proteins, which had thus far been thought be spontaneous, requires another unknown factor(s).

The blockage of spontaneous integration of MtlA by DAG enables negative control of the reconstitution of MtlA integration. However, proteoliposomes containing SecYEG and YidC in the presence of DAG were not active in MtlA integration, again strongly suggesting the presence of a new integration factor (7).

DAG is a physiological component of *E. coli* membranes (19–21). The existence of DAG has been shown; however, its precise function remains unclear. It is feasible that the *in vivo* role of DAG is to block deregulated spontaneous insertion (7, 17). Wild-type *E. coli* cells express DAG at ~1.5% as to phospholipids (17). A *dgkA* mutant, deficient in DAG kinase, expresses up to ~10% DAG (19, 20). However, overproduction of *dgkA* did not affect the DAG expression, suggesting the occurrence of tight regulation as to DAG expression (17). Several genes are known to be involved in DAG biosynthesis, i.e., *mdoB* (22, 23), *pgpB* (24–26), and *eptB* (27). The products of these genes produce DAG as committed reactions. Even with the triple knockout of these genes, DAG is still expressed as in the wild type (17). Even if *dgkA* was overproduced in the triple knockout background, the DAG level did not change (17). These results suggest an important role of DAG, such as blockage of spontaneous insertion. Depletion of DAG will be necessary to demonstrate the precise role of DAG in *E. coli*.

YidC

YidC has been identified as a factor that interacts with the SecYEG translocon (28). Although inclusion of YidC with SecYEG did not affect the efficiency of translocation of presecretory proteins, the gene knockout resulted in cell death, causing defects in membrane protein integration (29). YidC is homologous to mitochondrial Oxa1p and Alb3p in chloroplasts, both of which are involved in membrane protein biogenesis (30). It is known that integration of many membrane proteins, including not only M13 procoat and Pf3 coat, but also F0c, CyoA, etc., becomes defective upon YidC depletion (31, 32). In *in vitro* experiments, the integrating nascent chains of membrane proteins are efficiently cross-linked to YidC, confirming that YidC interacts and functions together with SecYEG (33, 34). A membrane chaperon function has also been suggested (35). Proteoliposomes containing YidC are reported to stimulate the integration of a subset of membrane proteins, indicating that YidC is a membrane protein insertase (36).

Discovery of MPIase

It seems obvious that YidC functions as a protein insertase. Now, let us look back at 3L-Pf3 coat integration. Serek et al. (36) reported that YidC is a membrane protein insertase, but that 3L-Pf3 coat insertion occurs spontaneously irrespective of the presence and absence of YidC. In contrast, 3L-Pf3 coat insertion into liposomes was completely blocked by the presence of a physiological level of DAG (17). Moreover, in the presence of DAG, YidC-proteoliposomes were totally inactive in protein integration (7, 17, 37). Then, what is involved in the integration of these proteins if the spontaneous mechanism is not operative? Also, an attempt to reconstitute MtlA integration suggested that SecYEG, SRP, and YidC are not enough for protein integration (7). Therefore, we embarked on a search for the responsible factor in cytoplasmic membranes of *E. coli*.

A cholate extract of *E. coli* inner membranes does not contain detectable levels of Sec factors, YidC, or SRP, after it has been precipitated with acetone and then solubilized in a detergent solution (7). When this cholate extract was coreconstituted with SecYEG, MtlA integration was obviously observed in an SRP-dependent manner (7), suggesting that this extract contains the required integration factor. The cholate extract was also active in M13 procoat integration (7). The integration activity was further enhanced through several column chromatographies (7, 8). A factor-enriched fraction gave a single band corresponding to ~7 kDa on a urea-SDS gel (7, 8). Note that this band is detected at the position of ~12 kDa in the absence of urea (9). This fraction was active in both M13 procoat and MtlA integration (7, 17), suggesting that the same factor functions in both Sec-independent and -dependent integration. Proteinase K digestion of this fraction caused a slight shift of the band and inactivation (7). These results suggested that this factor is proteinaceous; however, it turned out that this was not the case. As described hereinafter, we reveal that the factor is a glycolipid (7, 9). This factor is characteristic in that it is soluble in trichloroacetic acid (TCA) in the presence of a detergent (7). Lipopolysaccharides (LPS), a major outer membrane component, exhibit similar TCA solubility and overlap with the factor on an SDS gel (8, 38). Moreover, mutant INV prepared from a deep rough mutant that expresses LPS with shorter saccharides (39) revealed reduced integration activity (7). From these data, we suspected that this integration factor is an LPS derivative at this stage (7). Later, it was found that this sample was slightly contaminated by LPS (8). TLC analysis involving a chromogenic reaction with anisaldehyde revealed that the factor gives a brown spot, while LPS gives a gray one (8). Further purification by liquid-liquid partition chromatography enabled separation of the factor from LPS. Thus, an LPS-free preparation was obtained. This highly pure factor exhibited high integration activity (8). Stoichiometric analysis indicated that the factor caused the integration of a higher number of 3L-Pf3 coat molecules if its molecular weight is assumed to be 7 kDa (8); that is, it catalyzed membrane integration like an enzyme. Thus, we named this factor MPIase after its function (8).

Structure and function of MPIase

Structure determination of MPIase

Amino acid analysis after HCl hydrolysis of the highly purified MPIase gave no amino acids but only two peaks corresponding to glucosamine and ammonia, suggesting that MPIase is non-proteinaceous and consists of unstable amino sugars (9). Composition analysis by gas chromatography-mass spectrometry (GC-MS) after methanolysis of MPIase also demonstrated the presence of glucosamine and fatty acids, indicating that MPIase is a glycolipid (9).

Matrix assisted laser desorption (MALDI)-time of flight (TOF)-MS of MPIase showing a characteristic repeating pattern with 608 or 650 mass intervals suggested that MPIase comprises multiple units of these sizes. The presence of many peaks at 42 mass unit intervals indicates various extents of acetylation in the molecule. MS/MS analyses of the material at m/z 608 showed that it consists of three components. One of the components was identified as *N*-acetyl-glucosamine (GlcNAc), and GlcNAc residues were non-stoichiometrically *O*-acetylated. The other building blocks were presumed to be *N*-acetyl-aminodeoxyhexose and *N*-acetyl-aminohexuronic acid, respectively, from their mass numbers (9).

Extensive nuclear magnetic resonance (NMR) investigations of MPIase supported that MPIase is composed of trisaccharide repeating units and a lipid moiety. The presence of two phosphorus atoms was demonstrated on ^{31}P-NMR, which were assigned as pyrophosphate diester linkers between the reducing terminal of the glycan and the lipid part. The pyrophosphate linker was cleaved through mild hydrolysis with 48% aqueous HF to give a hydrophilic glycan fraction (HF-MPIase). Composition analyses by GC-MS of HF-MPIase gave three major peaks, one of which proved to be GlcNAc. The other peaks were assigned as 4-acetamido-4-deoxyfucose (Fuc4NAc) and 2-acetamido-2-deoxymannuronic acid (ManNAcA) through comparison

with some synthetic authentic sugars. The sequence and linkage mode of the trisaccharide were determined by two-dimensional NMR of HF-MPIase. Moreover, we synthesized several model disaccharides and compared their ^{13}C-NMR spectra with that of HF-MPIase. The number of repeating trisaccharide units was determined to be 9–11 by MALDI-TOF-MS after hydrolysis of MPIase with cold NaOH (NaOH-MPIase) (9).

Taking the aforementioned information into account, we determined the structure of MPIase to be as shown in Figure 3 (9).

Structure-activity relation

The glycan chain of MPIase is similar to that of enterobacterial common antigen (ECA), an outer membrane component (40–43), except for the length of the glycan. The glycan length of MPIase is homogeneous (n=9–11) (9), while that of ECA is longer and heterogeneous (n=18–55) (40, 41). Irrespective of the similarity in glycan structure, ECA was completely inactive in integration, indicating that the length of the glycan is critical (9). Another difference between MPIase and ECA is the linker that connects the glycan chain to DAG. Pyrophosphate is used in MPIase, whereas monophosphate is used in ECA (40, 41). This difference might reflect the subcellular localization of the two compounds. A soluble glycan chain (PP-MPIase) can be prepared by digesting MPIase with pyrophosphatase.

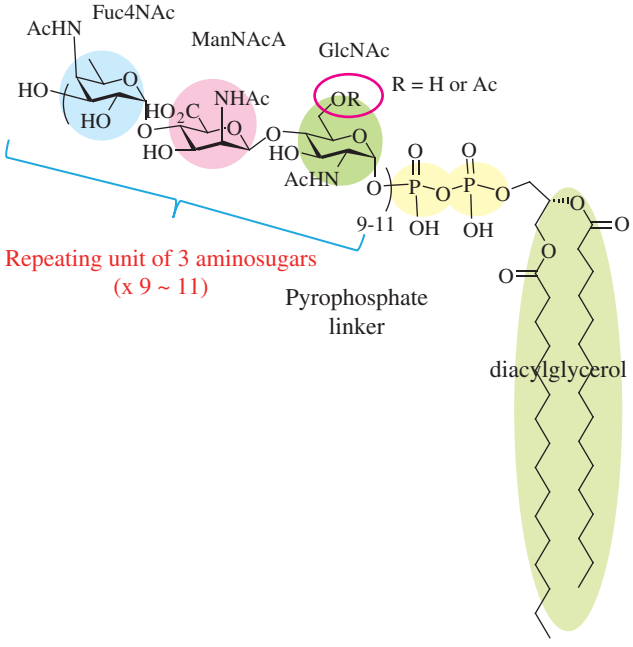

Figure 3 Structure of MPIase.

PP-MPIase was found to be more active than the intact MPIase (9). Therefore, the difference in linker structure is not important for the function. Mild alkaline-treated MPIase (NaOH-MPIase) has a similar structure to PP-MPIase except that NaOH-MPIase lacks *O*-acetyl residues. NaOH-MPIase was completely inactive, indicating that the *O*-acetyl residues are essential for the function (9). Proteinase K-inactivation of MPIase (7) can be explained if proteinase K would digest the *N*-acetyl residues with its low substrate specificity (44). These results indicate that the acetyl residues of MPIase are essential for the function. When MPIase directly binds to substrate membrane proteins, the hydrophobicity provided by the acetyl residues seems to be involved in this interaction. Gel filtration analysis also suggested that MPIase forms an oligomer (9). If MPIase is assumed to form an octamer, then one MPIase complex has 250–300 acetyl residues.

Mechanism of Sec-independent integration as a membrane protein integrase

A soluble MPIase derivative, PP-MPIase, forms a soluble complex with a Sec-independent substrate of membrane proteins, indicating that MPIase possesses a molecular chaperone-like function dedicated to membrane proteins (9). The integration activity was observed even if liposomes were posttranslationally added to the PP-MPIase/membrane protein complex, indicating that formation of the complex is an intermediate step of the integration (9). Therefore, as a first step of integration, the MPIase complex receives a newborn membrane protein from a ribosome by means of its chaperone-like function. On the membrane surface, MPIase may open a putative gate into the lipid bilayer with a structure change of each subunit of MPIase, or may induce a conformational change of the protein. Through hydrophobic relay from MPIase to lipids, the substrate would be integrated into a membrane, resulting in its release from the MPIase. The MPIase free of a substrate protein gets ready for the next cycle of integration. Thus, MPIase catalyzes membrane protein integration like an enzyme, like a membrane protein integrase (Figure 4) (9). Indeed, even when a higher amount of membrane protein was synthesized than that of MPIase, MPIase drove the protein integration reaction beyond its stoichiometric amount (8). As PP-MPIase lacks the lipid anchor, it can receive a membrane protein more efficiently in a soluble milieu, explaining the higher activity of PP-MPIase (9). However, the membrane anchor of MPIase is essential for the Sec-dependent reaction (see below).

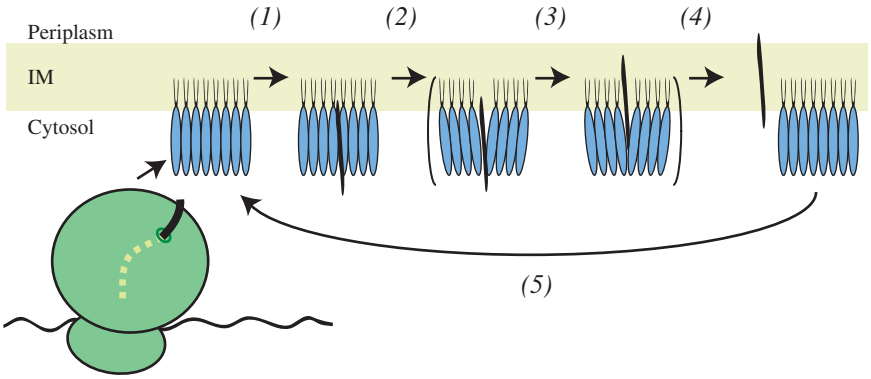

Figure 4 Working model for Sec-independent integration.
Newly synthesized membrane proteins are received by the glycan chains of MPIase (1). Possible structural changes of MPIase allow integration into the lipid bilayer (2–4). Upon release of a membrane protein, MPIase is recruited for the next cycle to ensure the catalytic reaction (5).

Mechanism of the Sec-dependent reaction

Identification and characterization of MPIase began with the reconstitution of MtlA integration, a Sec-dependent reaction, although a detailed analysis of the structure-function relation has been performed for a Sec-independent reaction, as summarized above. In the early analysis, we succeeded in inducing MtlA integration in an SRP-dependent manner when both SecYEG and MPIase were present (7). Therefore, it is highly likely that MPIase is involved in MtlA integration. However, it has been a matter of debate about whether MtlA integration requires MPIase, as it was reported that SecYEG or YidC is sufficient for MtlA integration (45). Nevertheless, the involvement of MPIase in preprotein translocation has been clearly demonstrated (7, 46). When MPIase was coreconstituted with SecYEG, the translocation activity of pOmpA was significantly (>10-fold) stimulated (46). One of the possible reasons for this stimulation was that MPIase affects the dimer orientation of SecYEG (46). The crystal structure of SecYEG revealed that SecYEG forms a dimer in a membrane with SecE being an interface, the so-called 'back-to-back' structure (47, 48). In this dimer, SecG is mapped at both distal sites. This dimer structure was confirmed by cross-linking experiments involving a mutant SecYEG in which a Cys residue had been introduced at a specified position (49). When SecYEG with Cys in TM 3 of SecE [SecYE(106C) G] was reconstituted into proteoliposomes, followed by oxidation, the SecE dimer was efficiently formed. In the case of SecYEG with Cys in TM 2 of SecG [SecYEG(60C)] (50), no SecG dimer was formed (46). However, when each SecYEG with a Cys residue was reconstituted together with MPIase, the SecG dimer but not the SecE dimer was efficiently formed, indicating that MPIase transformed the back-to-back dimer into a side-by-side dimer (Figure 5) (46). It has been reported that the SecG subunit of SecYEG, which significantly stimulates preprotein translocation (51–53), undergoes a topology inversion cycle coupled with preprotein translocation (54–57). It has also been demonstrated that this remarkable structure change of SecG is essential for the SecG function (54, 56). However, a controversial result has been reported, i.e., that a SecYEG mutant, in which SecY and SecG had been covalently cross linked, is fully functional (58). The difference in these observations originates from the experimental conditions. In the former study, INV prepared from wild-type cells were used, while in the latter, INV containing an overproduced

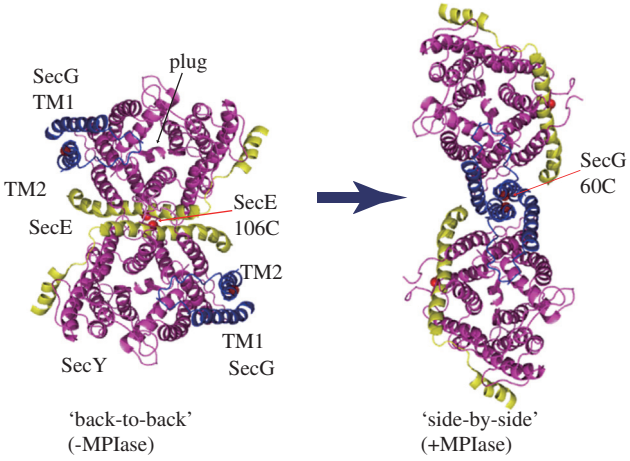

Figure 5 MPIase modulates the dimer orientation of SecYEG.
The structure of the SecYEG protomer (48) was modeled into back-to-back (left) and side-by-side (right) dimers. SecY, purple; SecE, yellow; SecG, blue. The positions of SecE 106C and SecG 60C are shown by red circles. The positions of TM 1 and 2 of SecG, and the plug domain in SecY (64) are also shown.

amount of SecYEG were used. INV containing overproduced SecYEG enabled the embedding of more proteins than in the case of the wild type (59–61); however, the specific activity of SecYEG was significantly diminished in the SecYEG-overproduced INV (46). As the expression level of MPIase is not affected by SecYEG overproduction, MPIase is relatively lacking in SecYEG-overproducing membranes, giving an artificial back-to-back SecYEG structure (46). Indeed, topology inversion of SecG efficiently occurs when both SecYEG and MPIase are present in proteoliposomes, demonstrating that MPIase is essential for SecG inversion (46). In summary, SecYEG on interaction with MPIase gives a side-by-side structure in which SecG inversion becomes possible, which results in much higher preprotein translocation activity.

MPIase as a glycolipozyme

As stated in the previous chapter, MPIase has two distinct functions; one involves its protein integrase-like activity in the Sec-independent integration, and the other is its involvement in the Sec-dependent reaction. While some steps during the integration reaction are speculative, the molecular mechanisms for the catalytic cycle in the Sec-independent integration have been elucidated (Figure 4) (9). Considering that MPIase catalyzes membrane protein integration, it is plausible that MPIase functions like an enzyme that can be called a 'glycolipozyme' (9). In the Sec-dependent reaction, it has been demonstrated that MPIase is crucial for the activation of SecYEG through a change in structure, and acts as a protein translocase/integrase (46). In this case, MPIase functions as if it is a coenzyme or an effector of SecYEG. Nevertheless, it is still possible that MPIase functions as an integrase for the Sec-dependent integration. Overall, we conclude that MPIase plays an important role in protein integration/translocation through its enzyme-like function. Researching MPIase will not only clarify the detailed mechanisms underlying protein integration/translocation observed in all living things, but also shed new light on glycolipid biology.

Future perspectives

Analysis of the MPIase function is about to begin. Therefore, many things remain to be clarified, for example, how MPIase is biosynthesized, whether MPIase is involved in *in vivo* reactions, and what is the relation between MPIase and YidC.

As no biosynthetic genes for MPIase have been identified thus far, it is presently impossible to deplete MPIase of membranes *in vivo* to examine whether MPIase functions as a protein integrase *in vivo* and *in vitro*. The glycan structure of MPIase is similar to that of ECA; however, all the ECA-deficient mutants thus far examined expressed MPIase like wild-type cells (9). Therefore, while genes for ECA biosynthesis might be involved in MPIase biosynthesis, a separate set of genes for MPIase biosynthesis should be operative.

YidC has been extensively analyzed both *in vivo* and *in vitro*. Moreover, the recently reported crystal structure provides detailed mechanisms for membrane integration (62). However, YidC-proteoliposomes carrying a sufficient amount of DAG exhibit marginal integration activity for Sec-independent substrates (7, 17, 37). As it is clear that YidC is involved in membrane protein biogenesis, it is likely that MPIase and YidC function in a concerted manner. Nonetheless, as an *in vitro* integration assay relies on that a part of membrane-integrated proteins confers proteinase resistance, even partially and incompletely integrated proteins may be evaluated as being integrated. Therefore, integration may be halfway through upon the MPIase function, and then YidC may receive a substrate from MPIase, followed by completion of integration. These possibilities will be verified in the near future.

About Sec-dependent integration such as MtlA integration, a consensus view of it has not yet been formed. In our view, both SecYEG and MPIase are necessary (7); however, in others' view, SecYEG or YidC is sufficient for MtlA integration (45). Future analysis is necessary in which spontaneous integration of MtlA is completely excluded because several detergents such as dodecylmaltoside form an insoluble complex with DAG (7, 63), which results in the depletion of DAG from liposomes that causes unexpected spontaneous integration. If MPIase is required for MtlA integration, there is another question. As MPIase affects the dimer structure of SecYEG, one could ask whether only the side-by-side dimer is active in MtlA integration or whether MPIase functions as an integrase even in the Sec-dependent integration. Subsequently, what is the role of YidC in MtlA integration, as it has been demonstrated that the nascent chain of MtlA is efficiently cross-linked with YidC (34)?

For preprotein translocation, it is believed that a preprotein is threaded through a pore in SecY, in which the opening is regulated by a plug domain (Figure 5) (64). Unlike translocation into endoplasmic reticulum (ER) membranes and in archaea where preprotein translocation proceeds cotranslationally, it is known that even a

partially folded domain can be translocated posttranslationally through the SecYEG translocon in *E. coli* (65, 66). In this case, the pore size of SecYEG should become larger. Therefore, it is possible that the SecYEG dimer forms a larger pore with a dynamic structure change. As MPIase affects the dimer structure of SecYEG (46), MPIase may be involved in this structure change.

The molecular mechanisms suggest that every kingdom of life uses a similar system for protein integration into membranes (Figure 1A and B). Bacteria to higher plants and animals all have the SRP system and a highly homologous translocon (SecYEG and Sec61). YidC homologues can be found in mitochondria and chloroplasts (30). TRAM in the ER membrane may have a similar function to YidC in that nascent membrane proteins can be efficiently cross-linked (67). Thus, the Sec-dependent integration into the bacterial cytoplasmic membrane and the ER membrane of eukaryotic cells is rather similar (Figure 1A and B). In this sense, it is possible that MPIase homologues may be widely conserved in all living cells; eukaryotic cells including mitochondria and chloroplasts may possess a compound that functions like a membrane protein integrase similar to MPIase, even if their chemical structures may greatly differ. Future discovery of such compounds is awaited.

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List of abbreviations

DAG	diacylglycerol
ECA	enterobacterial common antigen
INV	inverted membrane vesicles
LPS	lipopolysaccharides
MPF	membrane-protected fragment
MPIase	membrane protein integrase
PP-MPIase	pyrophosphatase-treated MPIase
SR	SRP receptor
SRP	signal recognition particle
TCA	trichloroacetic acid
TM	transmembrane

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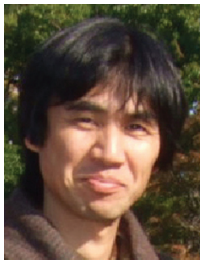
Bionotes



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