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Minireview
Protein Translocation across Biological Membrane

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Introduction

Protein translocation across biological membranes is a common phenomenon seen in both prokaryotes and eukaryotes. Genetic and biochemical studies on protein translocation have revealed that some steps of the process appear to be similar among these organisms. In bacteria, most secretory proteins and outer membrane proteins have N-terminal presequences (signal sequences) which consist of a few positively charged residues, followed by a stretch of about 7-15 largely hydrophobic residues, and these sequences are proteolytically cleaved before completion of translocation (1,2). In eukaryotes, several types of signal sequences with well established sorting functions have been identified (3), and two of them have been examined in detail. The signal sequences of proteins destined for membrane of endoplasmic reticulum resemble those cytoplasmic membrane of bacteria (2). On the other hand, the signal sequences of proteins targeting to mitochondria possess amphiphilic character with the following distinctive features (4). (i) The signal sequences usually form an amphiphilic α-helix with most hydrophobic residues on one side of the helix and hydrophilic residues on the other, (ii) the sequences bear a net positive charge, and acidic residues are rare ; (iii) the sequences are usually (but not always) accompanied by a cleavage site and processed by a matrix-localized protease (3).

Bacterial Signal Sequence Similar to Mitochondrial One

The bacterial phosphoenolpyruvate-dependent phosphotransferase system (PTS) is a unique and complex multifunctional enzyme system which is involved in sugar chemoreception, transport, and phosphorylation (5). The integral membrane proteins, called the Enzyme II of the PTS, have been sequenced (6). In contrast to most secretory or outer-membrane proteins of bacteria, these proteins have the N-terminal signal sequences with amphiphilic character (7,8). They resemble mitochondrial targeting sequences in their general features, except that they contain negatively charged amino acid residues in addition to positively charged ones. Although these signal sequences seem not to be cleaved after insertion into the membrane (internal signal sequences), evidence as described below suggests a role of these structures in envelope targeting and membrane insertion.

Physicochemical studies on the signal sequences of the nuclear-encoded mitochon-

drial proteins have revealed features consistent with a membrane targeting function. The mitochondrial signal peptides of cytochrome c oxidase subunit IV and of ornithine carbamyltransferase and some artificial mitochondrial targeting sequences exhibited amphiphilic α-helical or β-sheet structures when associated with detergent micelles or phospholipid vesicles (9-11). These and other mitochondrial signal peptides were capable of inserting efficiently into phospholipid monolayers and bilayers, and the insertion capacity of these peptides was shown to depend on both hydrophobic and charge interactions (12-14). Tamm et al (15) demonstrated that the 22-residue synthetic signal peptide of the glucitol permease inserted into phospholipid monolayers of various phospholipid compositions was induced to form a secondary structure (α-helix=65%). The more hydrophilic 15-residue signal peptide of the mannitol permease was also incorporated into monolayers. Experiments with protein fusions between mannitol permease and alkaline phosphatase revealed that the signal sequence actually functions as a targeting sequence into membrane (Fig.1, Y.Yamada, M.Yamada and M.H.Saier Jr, to be published). These results suggest that the signal sequences of PTS permease are capable of leading and inserting the proteins into bacterial membrane.

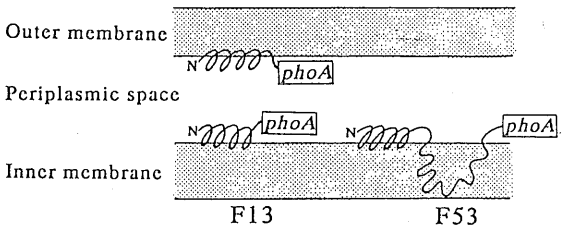

Fig. 1 Topological depiction of fused proteins between mannitol permease and alkaline phosphatase in *E. coli* membranes. The fused proteins were synthesized in the cytoplasm of *E. coli* and translocated. Final location of the proteins was depicted according to the experimental results of the membrane fractionation of the cells (Y.Yamada, M. Yamada and M.H. Saier, Jr, to be published). When the fused protein (F13) having 13-amino acid peptide of the N-terminal portion of mannitol permease and the mature form of alkaline phosphatase was synthesized, F13 was recovered in the outer membrane as well as the inner membrane fractions of *E. coli* cells. In contrast, the fused protein (F53) possessing N-terminal 53-amino acid peptide of mannitol permease was found exclusively in the inner membrane fraction. The discrepancy may be explained by the function of hydrophobic stretch from 24 to 44 amino acid residues as an anchor to the cytoplasmic membrane. Coiled lines and boxes represent the parts of mannitol permease and the mature form of alkaline phosphatase, respectively.

Internal Signal Sequence of Mitochondrial Protein

Adenylate kinase is known to contribute to homeostasis of the cellular adenine nucleotide composition (16). Three isozymes (AK1,AK2 and AK3) found in vertebrates differ from each other in cellular localizations and tissue distributions (17-22). The isozymes, thus, appear to be useful for analysis of the mechanism of protein translocation. Gene structures of AK1 in chicken and human were established (23-25). cDNA structure of bovine AK2 was determined (26) and it was demonstrated that no presequence is present in the protein to direct the import into mitochondria (26,27). cDNA of AK3 localized in the mitochondrial matrix was isolated and characterized (28). The AK3 cDNA encodes a 227-residue protein, whose predicted amino acid sequence agrees with the sequence previously determined with the purified protein, suggesting that AK3 has no N-terminal presequence (Fig.2). The experiment of in vitro protein synthesis confirmed this prediction (28).

Presequences of most mitochondrial proteins can potentially form positively charged amphiphilic α-helices (3,10). Amphiphilic feature of the peptide region which can be analyzed by helical wheel projection is characterized as a distribution pattern of the positively charged residues on one side of the

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  1  GGC GAC TAG GCC GCC CCC TGC TCA CCG CCC AGC CAG CCG GCC GGC ACG   48
             stop

 49  GGC GGC GAA GTG AGT GAG CGC GCA GTT AAG AGC CGA GTC AGG CCG AAG   96

                                                    Met Gly      2
 97  GAA GCT CCA CTG TCC GCG CGG GCC CAG CCT CTT TCT GCA GCT ATG GGG  144

  3  Ala Ser Ala Arg Leu Leu Arg Ala Ala Ile Met Gly Ala Pro Gly Ser  18
145  GCG TCT GCG CGG CTA CTG CGC GCA GCG ATC ATG GGG GCG CCG GGC TCT  192

 19  Gly Lys Gly Thr Val Ser Ser Arg Ile Thr Lys His Phe Glu Leu Lys  34
193  GGC AAA GGC ACC GTG TCC TCG CGC ATC ACC AAA CAC TTC GAG CTG AAG  240
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Fig. 2 The nucleotide sequence around N-terminal portion of AK3 cDNA. Predicted amino acids are shown above the nucleotide sequence. The N-terminal end of the amino acid sequence was determined from the following three lines of evidence. First, a stop codon (box) exists at the upstream position which is in frame of the AK3-coding sequence. Between the stop codon and the first Met codon, no ATG is found in the same frame. Second, the translation initiation signal is found around the first Met which is homologous to Kozak's consensus sequence (29, double underline). Third, when the AK3 mRNA was synthesized in vitro by the aid of the SP6 promoter and subjected to in vitro protein synthesis in rabbit reticulocyte lysates, the size of AK3 was coincident with that of AK3 purified from bovine heart mitochondria (28), indicating that AK3 has no N-terminal presequence.

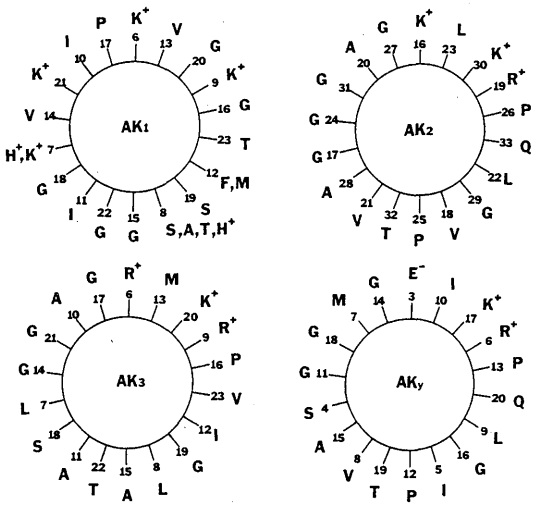

Fig. 3 Helical wheels of N-terminal portions of various adenylate kinases. Helical wheels (30) were drawn with 18-amino acid residues near the N-termini of AK1 (23), AK2 (26), AK3 (28) and yeast adenylate kinase (AKy,31). In the helical wheel of AK1, those of chicken, porcine, calf, rabbit and human were plotted together (23). Numbers are the positions of amino acid residues in each sequence.

wheel and the hydrophobic residues on the other side (30). The features of helical wheel plots at the N-terminal portions of AK2 and AK3 are quite similar to those of presequences of mitochondrial proteins (Fig.3). Since AK2 and AK3 are localized in the mitochondrial intermembrane space and matrix, respectively, those portions may function as targeting signals to the destination (internal signal sequence). In contrast, such an amphiphilic helical wheel can not be depicted in AK1 molecules (23) which are present in the cytosol. The helical wheel of yeast adenylate kinase (AKy,31) which is present in the cytosol resembles those of AK2 and AK3, except the negative charge of Glu-3, which may inhibite the interaction of the N-terminal segment with mitochondrial membranes, resulting in the adenylate kinase in the cytosol.

Export of Mitochondrial Protein in *Escherichia coli*

When the AK3 cDNA was incorporated into the expression vector and expressed in *E. coli*, the AK3 protein was synthesized and recovered in the periplasmic fraction (28). The protein secreted to the periplasm was purified to homogeneity and the N-terminal sequence was determined. The sequence was identical to that predicted from the DNA sequence. Thus, the AK3 protein without the presequence was secreted through the bacterial cytoplasmic membrane as it is imported through the mitochondrial membrane into the matrix.

To examine the function of the N-terminal portion of AK3 on protein translocation, a fused protein between AK3 and β-lactamase was constructed and analyzed (unpublished results). When the protein of the N-terminal

34-amino acid peptide of AK3 fused with β-lactamase which was lacking its own presequence was expressed in *E. coli*, the fused protein was recovered in the periplasm. From these results, it is suggested that the N-terminal portion of AK3 has a function similar to that of presequences of bacterial secretory proteins.

Conclusions

From the finding that PTS permeases of *E. coli* possess the signal sequences structurally and functionally analogous to those of mitochondrial proteins, evolutionary relationship between them could be speculated. The PTS system is considered to be established as the first major carbohydrate catabolic enzyme system that appeared on earth (32). It is postulated that the amphiphilic signal sequences of PTS permeases may propagate in mitochondrial proteins when symbiotic bacteria flourished within the host eukaryote as endosymbiouts.

For this decade, tremendous number of studies on protein translocation have been performed with various biological membranes of such as bacteria, endoplasmic reticulum, mitochondria, chloroplasts and peroxisomes (1-4,10,33). Protein translocation requires ATP, transmembrane electrochemical potential and protein factors. ATP is essential for translocation of proteins in all membrane systems. Although an electrochemical potential has proven to be used for bacterial and mitochondrial membranes, it probably helps to achieve optimal translocation rates. Indeed, AK3 is able to pass through both the bacterial inner membrane and the mitochondrial membrane even though the two membranes are charged with the opposite electrochemical potential.

Fig.4 illustrates the model of protein translocation. Most secretory proteins have N-terminal presequences which lead them to interact with membrane and finally are proteolytically cleaved. Membrane-bound proteins are also led to the membrane by presequences but stay in the membrane with hydrophobic domain(s) within the proteins. These translocations may be irreversible because the presequences are removed before completion of translocation. On the other hand, proteins possessing an internal signal sequence such as PTS permease, AK2 and AK3 seem to pass through or bind to membranes in a manner similar to those of proteins bearing presequence, except that the signal sequence is not removed. Since the internal signal sequence may be indispensable for the enzymatic function after translocation, the signal could be structurally protected from the attack of signal peptidases.

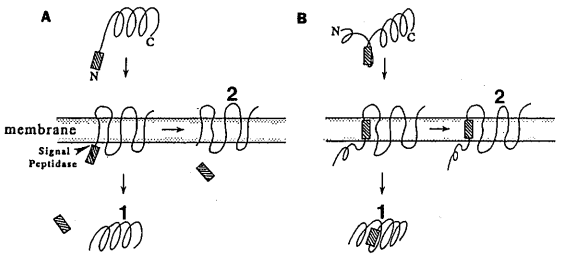

Fig. 4 Possible mechanisms of membrane translocation of proteins with presequence (A) and internal signal sequence (B) are illustated. Examples are : (A-1) secretory form ; β-lactamase (34), insulin (35) and albumin (36), (A-2) membrane-bound form ; M13 coat protein (37), insulin receptor (38) and immunoglobulin μ-chain (39),(B-1) secretory form ; colicin E1 as a toxin (40), 3-oxoacyl-CoA thiolase (41), AK2 and AK3, (B-2) membrane-bound form ; colicin E1 as a channel (42), band III (anion transporter, 43) and PTS permeases. In these protein translocations, ATP is required as a driving force. Protein factors specific to each membrane system might direct translocation. Translocating proteins are shown as coiled lines with shadowed boxes representing signal sequences. N and C mean N-and C-termini of proteins, respectively.

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References

1) Randall, L.L.,Hardy, S.J.S. and Thom, J.R. : Export of protein. A biochemical view. *Annu. Rev. Microbiol.,* **41** : 507-541,1987.
2) von Heijne, G. : Signal sequences. The limits of variation. *J. Mol. Biol.,* **184** : 99-105,1985.
3) von Heijne, G. : Transcending the impenetrable. How proteins come to terms with membranes. *Biochim. Biophys. Acta,* **947** : 307-333,1988.
4) Roise, D.and Schatz, G. : Mitochondrial presequences. *J. Biol. Chem.,* **263** : 4509-4511, 1988.
5) Saier, M.H., Jr. : *Mechanisms and regulation of carbohydrate transport in bacteria,* Academic Press, Orlando, FL,1985.
6) Saier,M.H., Jr., Yamada,M., Erni,B., Suda, K., Lengeler,J., Ebner,R., Argos,P., Rak. B., Schnetz, K., Lee, C.A., Stewart, G.C., Breidt, F., Jr., Waygood, E.B.,Peri, K.G. and Doolittle, R.F. : Sugar permeases of the bacterial phosphoenolpyruvate-dependent phosphotransferase system. Sequence comparisons. *FASEB J.,* **2** : 199-208,1988.
7) Yamada,M. and Saier,M.H.,Jr. : Glucitol-specific enzymes of the phosphotransferase system in *Escherichia coli*. Nucleotide sequence of the *gut* operon. *J. Biol.Chem.,* **262** : 5455-5463.
8) Saier,M.H., Jr., Yamada, M.,Suda, K.,Erni, B.,Rak,B., Ebner,R., Lengeler,J., Stewart,G. C., Waygood,E.B. and Rapoport, G. : Bacterial proteins with N-terminal sequences resembling mitochondrial targeting sequences of eukaryotes. *Biochimie.,* **70** : 1743-1748.1988.
9) Roise,D., Horvath, S.J., Tomich,J.M., Richards,J.H. and Schatz,G. : A chemically synthesized pre-sequence of an imported mitochondrial protein can form an amphiphilic helix and perturb natural and atrificial phospholipid bilayers. *EMBO J.,* **5** : 1327-1334, 1986.
10) Epand,R.M.,Hui,S.-W.,Argan, C.,Gillespie,L. L. and Shore,G.C. : Structural analysis and amphiphilic properties of a chemically synthesized mitochondrial signal peptide. *J. Biol. Chem.,* **261** : 10017-10020,1986.
11) Roise,D.,Theiler,F.,Horvath,S.J.,Tomich,J. M.,Richards,J.H.,Allison,D.S. and Schatz, G. : Amphiphilicity is essential for mitochondrial presequence function. *EMBO J.,* **7** : 649-653,1988.
12) Tamm,L.K. : Incorporation of a synthetic mitochondrial signal peptide into charged and uncharged phospholipid monolayers. *Biochemistry,* **25** : 7470-7476,1986.
13) Sherjank,I.S.,Shore,G.C. and Silvius, J.R. : The interaction of a synthetic mitochondrial signal peptide with lipid membranes is independent of transbilayer potential. *EMBO J.,* **6** : 3117-3123,1987.
14) Myers,M.,Mayorga,O.L.,Emtage,J. and Freire,E. : Thermodynamic characterization of interactions between ornithine transcarbamylase leader peptide and phospholipid bilayer membranes. *Biochemistry,* **26** : 4309-4315,1987.
15) Tamm,L.K.,Tomich,J.M. and Saier, M.H., Jr. : Membrane incorporation and induction of secondary structure of synthetic peptides corresponding to the N-terminal signal sequences of the glucitol and mannitol permeases of *Escherichia coli. J. Biol. Chem.,* **264** : 2587-2592,1989.
16) Atkinson,D.E. : *In Cellular energy metabolism and its regulation,* Academic Press, Orlando,FL,1977,p.85-107.
17) Noda,L. : *In* P.D.Boyer(ed.), *The enzymes,* vol.8, Academic Press,Orlando,FL,1973,p.279-305.
18) Watanabe,K.,Itakura,T. and Kubo,S. : Distribution of adenylate kinase isozymes in porcine tissues and their subcellular localization. *J. Biochem.,* **85** : 799-805,1979.
19) Khoo,J.C. and Russell,P.J. : Isozymes of adenylate kinase in human tissue. *Biochimi. Biophys. Acta,* **268** : 98-101,1972.
20) Tomasselli,A.G. and Noda,L.H. : Mitochondrial GTP-AMP phosphotransferase.2. Kinetic and equilibrium dialysis studies. *Eur. J. Biochem.,* **93** : 263-270,1979.
21) Heldt,H.W. and Schwalbach,K. : The participation of GTP-AMP-P transferase in substrate level phosphate transfer of rat liver mitochondria. *Eur. J. Biochem.,* **1** : 199-206, 1967.
22) Tomasselli,A.G.,Frank,R. and Schiltz,E. : The complete primary structure of GTP : AMP phosphotransferase from beef heart mitochondria. *FEBS Lett.,* **202** : 303-308, 1986.
23) Kishi,F.,Maruyama,M.,Tanizawa,Y. and Nakazawa,A. : Isolation and characterization of cDNA for chichen muscle adenylate kinase. *J. Biol. Chem.,* **261** : 2942-2945,1986.
24) Suminami,Y.,Kishi,F.,Torigoe,T. and Nakazawa,A. : Structure and complete nucleotide

sequence of the gene encoding chichen cytosolic adenylate kinase. *J. Biochem.* **103** : 611-617,1988.

25) Matsuura,S.,Igarashi,M.,Tanizawa,Y., Yamada,M.,Kishi,F.,Kajii,T.,Fujii,H.,Miwa, S.,Sakurai,M. and Nakazawa,A. : Human adenylate kinase deficiency associated with hemolytic anemia.A single base substitution affecting solubility and catalytic activity of the cytosolic adenylate kinase. *J. Biol. Chem.,* **264** : 10148-10155,1989.

26) Kishi,F., Tanizawa,Y. and Nakazawa,A. : Isolation and characterization of two types of cDNA for mitochondrial adenylate kinase and their expression in *Escherichia coli*. *J. Biol. Chem.,* **262** : 11785-11789,1987.

27) Watanabe,K. and Kubo,S. : Mitochondrial adenylate kinase from chicken liver.Purification,characterization and its cell-free synthesis. *Eur. J. Biochem.,* **123** : 587-592,1982.

28) Yamada,M., Shahjahan,M., Tanabe,T., Kishi,F. and Nakazawa,A. : Cloning and characterization of cDNA for mitochondrial GTP : AMP phosphotransferase of bovine liver. *J. Biol. Chem.,* **264** : 19192-19199, 1989.

29) Kozak,M. : Compilation and analysis of sequences upstream from the translational start site in eukaryotic mRNAs. *Nucleic Acids Res.,* **12** : 857-872,1984.

30) Eisenberg,D., Schwarz,E., Komaromy,M. and Wall,R. : Analysis of membrane and surface protein sequences with the hydrophobic moment plot. *J. Mol. Biol.,* **179** : 125-142, 1984.

31) Tomasselli,A.G., Mast,E., Janes,W. and Schiltz,E. : The complete amino acid sequence of adenylate kinase from baker's yeast. *Eur. J. Biochem.,* **155** : 111-119,1986.

32) Saier,M.H., Jr. and Yamada,M. : Evolutionary considerations concerning the bacterial phosphoenolpyruvate : sugar phosphotransferase system. *In* J. Reizer and A. Peterkofsky(eds.), *Suger transport and metabolism in gram-positive bacteria.* Ellis-Harwood Ltd., Chichester, England, p.196-214,1987.

33) Eilers,M. and Schatz,G. : Protein unfolding and the energetics of protein translocation across biological membranes. *Cell,* **52** : 481-483,1988.

34) Satcliffe,J.G. : Nucleotide sequence of the ampicillin resistance gene of *Escherichia coli* plasmid pBR322. *Pro. Natl. Acad. Sci. USA,* **75** : 3737-3741,1978.

35) Villa-Komaroff,L., Efstradiatis,A., Broome, S., Lomedico,P., Tizard,R., Naber,S.P., Chick,W.L. and Gilbert,W. : A bacterial clone synthesizing proinsulin. *Proc. Natl. Acad. Sci. USA,* **75** : 3727-3731,1978.

36) Strauss,A.W., Bennett,C.D., Donohue,A.M., Rodkey,J.A. and Alberts,A.W. : Rat liver pre-proalbumin. Complete amino acid sequence of the pre-piece. Analysis of the direct translation product of albumin messenger RNA. *J. Biol. Chem.,* **252** : 6846-6855,1977.

37) Sugimoto,K., Sugisaki,H., Okamoto,T. and Takanami,M. : Studies on bacteriophage fd DNA.IV.The sequence of messenger RNA for the major coat protein gene. *J. Mol. Biol.,* **111** : 487-507,1977.

38) Ebina,Y., Ellis,L., Jarnagin,K., Edery,M., Graf,L., Clauser,E., Ou,J.-H., Masiarz,F., Kan,Y.W., Goldfine,I.D., Roth,R.A. and Rutter,W.J. : The human insulin receptor cDNA. The structural basis for hormon-activated transmembrane signalling. *Cell,* **40** : 747-758, 1985.

39) Rogers,J., Early,P., Carter,C., Calame,K., Bond,M., Hood,L. and Wall,R. : Two mRNAs with different 3′ ends encode membrane-bound and secreted forms of immunoglobulin μ chain. *Cell,* **20** : 303-312. 1980.

40) Yamada,M. and Nakazawa,A. : Factors necessary for the export process of colicin E1 across cytoplasmic membrane of *Eschericia coli*. *Eur. J. Biochem.,* **140** : 249-255,1984.

41) Amaya,Y., Arakawa,H., Taniguchi,M., Ebina,Y., Yokota,S. and Mori,M. : A non-cleavable signal for mitochondrial import of 3-oxoacyl-CoA thiolase. *J. Biol. Chem.,* **263** : 14463-14470,1988.

42) Shirabe,K., Cohen,F.S., Shaohua,X., Peterson,A.A., Shiver,J.W., Nakazawa,A. and Cramer,W.A. : Decrease of anion selectivity caused by mutation of Thr^{501} and Gly^{502} to Glu in the hydrophobic domain of the colicin E1 channel. *J. Biol. Chem.,* **264** : 1951-1957. 1989.

43) Wickner,W. and Lodish,H.F. : Multiple mechanisms of protein insertion into and across membranes. *Science,* **230** : 400-407, 1985.