ELSEVIER

FEMS Microbiology Letters 204 (2001) 215–221

FEMS MICROBIOLOGY Letters

www.fems-microbiology.org

MiniReview

Super-channel in bacteria: function and structure of the macromolecule import system mediated by a pit-dependent ABC transporter

Yumiko Mishima [a], Keiko Momma [a], Wataru Hashimoto [a], Bunzo Mikami [b], Kousaku Murata [a,*]

[a] *Department of Basic and Applied Molecular Biotechnology, Graduate School of Agriculture, Kyoto University, Uji, Kyoto 611-0011, Japan*
[b] *Department of Quality Design and Development, Graduate School of Agriculture, Kyoto University, Uji, Kyoto 611-0011, Japan*

Received 29 May 2001; received in revised form 6 August 2001; accepted 6 August 2001

First published online 19 September 2001

Abstract

In a soil isolate, *Sphingomonas* sp. A1, the transport of a macromolecule (alginate: 27 kDa) is mediated by a pit-dependent ATP-binding cassette (ABC) transporter. The transporter is different from other ABC transporters so far analyzed in that its function is dependent on a pit, a mouth-like organ formed on the cell surface only when cells are compelled to assimilate macromolecules, and in that it allows direct import of macromolecules into cells. The ABC transporter coupled with the pit, which functions as a funnel and/or concentrator of macromolecules to be imported, was designated the 'super-channel', and in this review, we discuss the three-dimensional structure and specific function of the 'super-channel' for macromolecule import found for the first time in a bacterium.

Keywords: Super-channel; Pit; ABC transporter; Macromolecule import; Three-dimensional structure; Alginate lyase

1. Introduction

During a study on the enzymatic depolymerization of biofilm alginate in cystic fibrosis [1], we found that when the cells of a bacterium, *Sphingomonas* sp. A1, were grown on alginate, a mouth-like pit formed on their surface and that high-molecular-mass alginate (27 kDa) was directly incorporated into the cells [2]. To the best of our knowledge, this is the first finding of a bacterium with a pit in the history of microbiology. Subsequent analysis of the system involved revealed the association of an ATP-binding cassette (ABC) transporter that allows the transport of macromolecules into the cytosol [3].

ABC transporters are indispensable not only for the supply of nutrients to cells, but also for their protection from hazardous compounds. They are also implicated in a variety of inherited human diseases such as cystic fibrosis [4], and in the multidrug resistance of cancer cells [5] and a wide range of microorganisms. The remarkable feature of ABC transporters is the extremely wide range of compounds that can be transported outward (export or secretion) or inward (import) using the energy provided by ATP hydrolysis, the latter of which is typical of many prokaryotic systems utilized for the uptake of small solutes [6].

The ABC transporters in bacteria have been shown to be able to import many compounds, but they are largely restricted to low-molecular-mass compounds, for example histidine, maltose, ribose, phosphate, sulfate, nickel, molybdenum, oligopeptides, fatty acids, and so on, and as far as we are aware, no bacterial ABC transporters for the import of high-molecular-mass compounds have been reported in the literature. Therefore, the pit-dependent macromolecule import system operating in *Sphingomonas* sp. A1 is worth studying not only to understand the dynamic structure–function relationship of the bacterial cell surface, but also to demonstrate novel molecular apparatuses latent on it.

Analysis of the pit in connection with the macromolecule (alginate) transport mediated by the ABC transporter indicated that the biopolymer is first concentrated in the pit and then translocated into the cytosol across the outer

* Corresponding author. Fax: +81 (774) 38-3767.
E-mail address: murata@food2.food.kyoto-u.ac.jp (K. Murata).

0378-1097/01/$20.00
PII: S0378-1097(01)00388-3

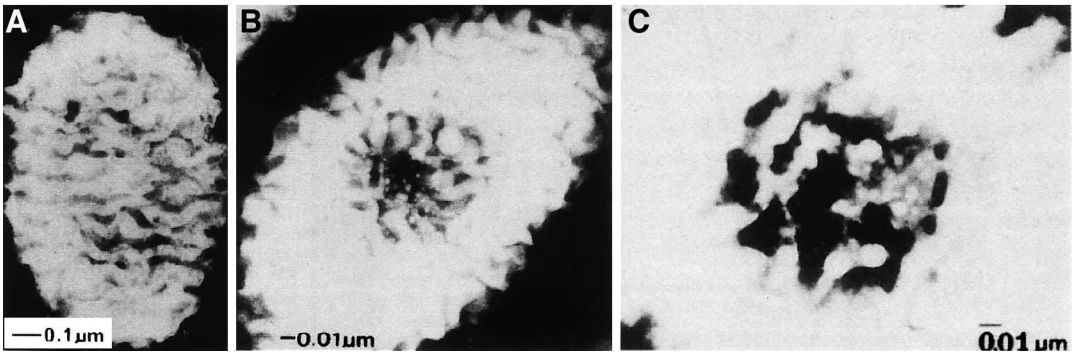


Fig. 1. Pit formation. Changes in the cell surface structure (pit formation) of *Sphingomonas* sp. A1 during growth in the presence of alginate. Culture time (h): A, 0; B, 14; C, 20. The small particles in the pits are alginate gel.

and cytoplasmic (inner) membranes, and the periplasm which lies between them. Direct macromolecule transfer from the cytosol across both membranes has also been observed only in the case of the export of a protein (hemolysin) in *Escherichia coli* [7].

Although the protein export system in *E. coli* is greatly different from the alginate import one of *Sphingomonas* sp. A1 in both the molecular components and molecular mechanisms involved, analysis of the cleverly equipped system for the direct transport of macromolecules may lead to a better understanding of the ABC transporter systems, and the results obtained will give rise to original ideas regarding the transport mechanisms for proteins, DNA, and other macromolecules.

In this review, we describe the nature of the transport pathway for alginate as a representative macromolecule, especially from the standpoint of the three-dimensional structures of the proteins that constitute the pathway.

2. Pit structure

Alginate is a linear polysaccharide produced by brown seaweed and certain pathogenic bacteria such as *Pseudomonas aeruginosa* [8]. This polymer is composed of β-D-mannuronate and C5 epimer α-L-guluronate, arranged in three different ways: poly-β-D-mannuronate, poly-α-L-guluronate, and heteropolymeric regions, in which there is random arrangement of monomers [9]. The cells of a Gram-negative bacterium, *Sphingomonas* sp. A1, isolated from a soil sample as a potent producer of alginate lyases are covered with many large plaits, and a mouth-like pit (0.02–0.1 μm) is formed on the cell surface, possibly through the rearrangement and/or reconstitution of the plaits, when the cells are grown on alginate as the sole source of carbon (Fig. 1) [2,10]. Thin sections of alginate-grown cells show an irregular site where the cell membrane sinks deeply into the cytosol [11]. The formation of the pit is reversible and is repressed when cells are grown on glucose or when alginate-grown cells are transferred to a medium without alginate [2]. Cell surface staining for mucopolysaccharides indicated the extensive accumulation of alginate in the pit [11]. These results apparently indicate that the pit functions as a funnel or concentrator for alginate to be transported into the cells.

3. Import system

A genomic fragment (15 kb) obtained through the complementation of an alginate uptake-deficient mutant of *Sphingomonas* sp. A1 contains 10 possible open reading frames (ORF) (Fig. 3). As discussed below, five of them (*algS*, *algM1*, *algM2*, *algQ1* and *algQ2*) constitute an operon, and are considered to encode components of the ABC transporter for alginate import [3]. About 1 kb upstream of *algS*, the alginate lyase gene, *aly*, is present in the opposite direction, and there are some ORFs encoding amino acid sequences homologous to those of *E. coli*: catalase (accession number p13029; cat), MDR protein (accession number p39843; mdr), and catabolite control protein (accession number p46828; ccp).

3.1. Periplasmic binding protein

Analysis of AlgQ1 (526 amino acids; 60 kDa) and AlgQ2 (516 amino acids; 60 kDa) by use of an antibody specific to AlgQ2 revealed that they are exclusively localized in the periplasm. The homology between AlgQ1 and AlgQ2 is appreciably high (74%), although the two proteins show much lower homology (22 and 23%, respectively) with maltose-binding protein (MalE) of *E. coli* [12]. Both AlgQ1 and AlgQ2 bind high-molecular-mass alginate (27 kDa) with high affinity ($K_d = 0.1–1$ μM) (unpublished results), which is comparable with those of other binding proteins [14], such as MalE and HisJ, the latter of which is a histidine-binding protein of *Salmonella typhimurium* [13].

The crystal structures of AlgQ1 and AlgQ2 were determined (Fig. 3) (unpublished results). AlgQ2 has two lobes (N- and C-terminal lobes), each of which consists of α-helices and β-strands. The overall structure of AlgQ2 resembles that of other substrate-binding proteins including MalE [15]. As has been observed in the case of the

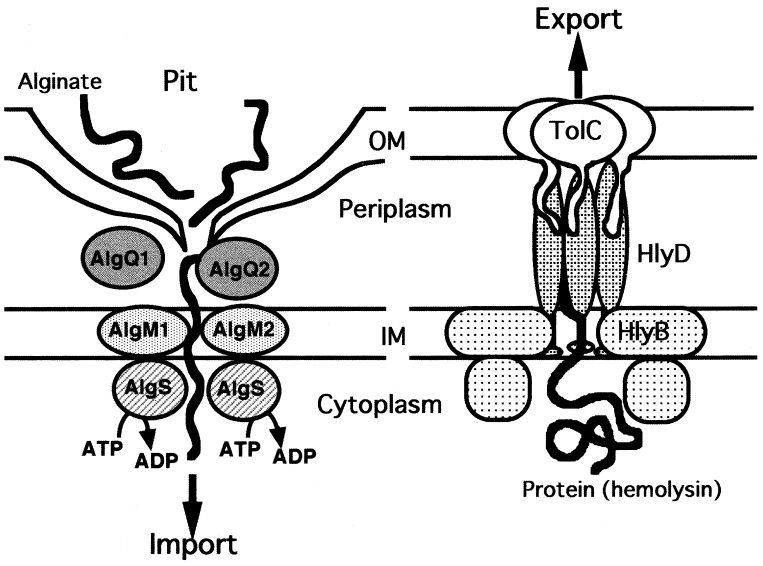


Fig. 2. Macromolecule import and export systems. Left: Polysaccharide (alginate) import system of *Sphingomonas* sp. A1. Right: Protein (hemolysin) export system of *E. coli*. The model is taken from [32]. The mechanisms of the two systems are discussed in detail in the text. IM: inner membrane; OM: outer membrane.

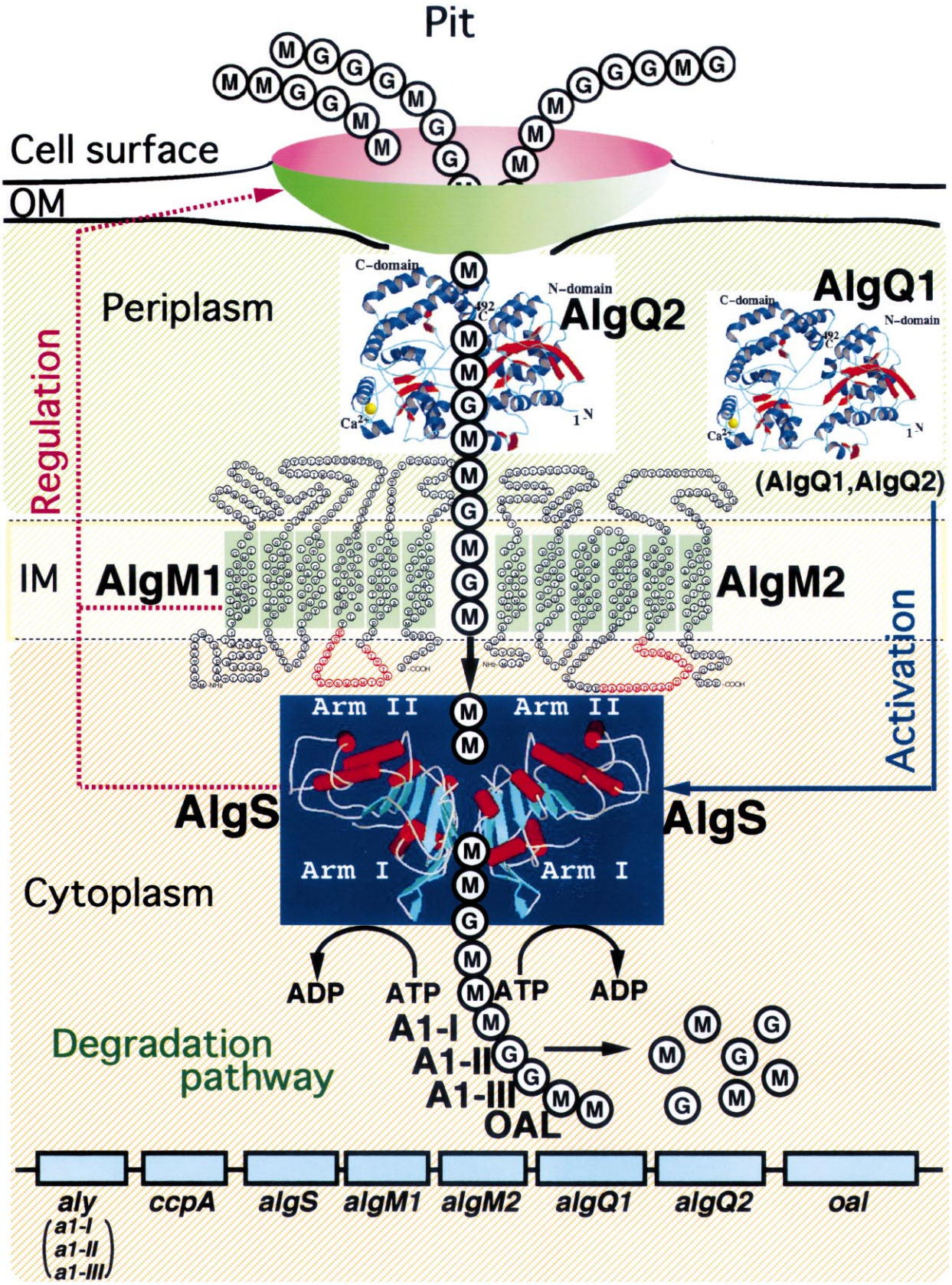
maltose/MalE complex [16], in the absence of alginate, the structures of binding proteins are in the open form so that they can bind a large ligand, alginate. Once the ligand is trapped in the cleft formed between the two lobes, the structures of the binding proteins are converted to the closed form and then they deliver the ligand to the permease of the ABC transporter. Phylogenetic analyses of MalE indicated that the N- and C-terminal lobes each associate with either MalF or MalG, both of which are membrane-bound permease domains of ABC transporters [17]. Similarly, one lobe of AlgQ1 or AlgQ2 interacts with AlgM1 and the other one with AlgM2. Several kinds of substrate-binding proteins have been characterized, but they are for proteins having affinity toward low-molecular-mass compounds. AlgQ1 and AlgQ2 are therefore the first macromolecule-binding proteins identified. Although the overall structure of AlgQ2 resembles that of other substrate-binding proteins, there are distinct differences between AlgQ2 and MalE of *E. coli* or HisJ of *S. typhimurium*, as follows. (i) Both AlgQ1 and AlgQ2 are 100 amino acids longer than MalE (396 amino acids), although the significance of the difference is obscure. (ii) Two copies of the alginate-binding protein (AlgQ1 or AlgQ2) are present. Typical bacterial traffic ATPases (transport systems for maltose or histidine) have one copy of the substrate-binding protein, MalE or HisJ, respectively. (iii) The two alginate-binding proteins contain calcium ions near their surface (Fig. 3). The function of this bivalent cation is not yet clear.

3.2. Membrane permease

AlgM1 (324 amino acids: 37 kDa) and AlgM2 (293 amino acids: 33 kDa) exhibit little similarity with UgpA (26%) and UgpE (24%) [3], respectively; UgpA and UgpE are both constitutive membrane-bound permease domains of the ABC transporter for the import of glycerol 3-phosphate in *E. coli* [18]. The hydropathy profiles indicate that both AlgM1 and AlgM2 contain six putative transmembrane helices. Most membrane-bound permease domains of bacterial ABC transporters have an EAA motif of approximately 20 amino acids (EAA...G.....I.LP) at a distance of about 100 amino acid residues from the C-terminus, which leads to the formation of a loop in the direction of the cytoplasmic fraction and to contact with the ATP-binding protein of the ABC transporter [19]. A sequence matching the consensus permease EAA motif (EAA...G.....I.LP) is present in both AlgM1 (**E**S**A**QV-D**G**ATRWQMITR**ITLP**) and AlgM2 (**EAA**RMD**G**AND-LQILWKVYI**P**) [3]. Therefore, AlgM1 and AlgM2 are thought to interact with ATP-binding proteins (AlgS), and to constitute the ABC transporter for alginate import as membrane-bound permeases.

3.3. ATP-binding protein

AlgS (363 amino acids: 40 kDa) contains two short, highly conserved consensus sequences, i.e. the Walker A (GXXGXGKST) and B (hhhhDEPT; h, hydrophobic amino acid) motifs that constitute a nucleotide-binding pocket. AlgS shows significant homology with ATP-binding domains (ATPase) of bacterial ABC transporters such as UgpC [18] (52%) and MalK [20] (48%) of *E. coli*, which are components of the ABC transporters for the import of glycerol 3-phosphate and maltose, respectively. Furthermore, a C motif of the SGG region (LSGG), which is thought to interact with bound Mg^{2+}-ATP [21], is present between 'Walker' motifs A and B of AlgS [3,22]. AlgS



expressed in *Sphingomonas* sp. A1 is localized in the membrane fraction and exhibits apparent constitutive ATPase activity in the form of a homodimer in the presence of divalent cations such as Mg^{2+}, Mn^{2+}, and Co^{2+} [3]. These results indicate that AlgS is the ATP-binding domain of an ABC transporter. In general, ABC transporters in prokaryotic cells are composed of two ATP-binding domain molecules interacting with two membrane-bound permeases. Therefore, the ABC transporter for alginate import in *Sphingomonas* sp. A1 probably consists of two molecules of AlgS and two permeases (AlgM1 and AlgM2).

The crystal constants are presented in [23], and X-ray crystallographic analysis is now being undertaken. A three-dimensional model of AlgS has been constructed by means of a run of the SWISS-Model program using HisP [24] (ATP-binding protein of the histidine transporter of *S. typhimurium*) as a template (Fig. 3) [25]. AlgS is L-shaped, consisting of two arms, I and II. Arms I and II are composed of β-strands and α-helices, respectively. Comparison of the structure of the AlgS model with that of the HisP/ATP complex [24] indicated that 'Walker' motifs A and B are located in arm I. The mechanism of binding of AlgS to ATP is assumed to be as follows. The sequence (GCGK) in 'Walker' motif A directly interacts with the β-phosphate of ATP and two amino acids (DE) in 'Walker' motif B associate with the γ-phosphate via a water molecule. Recently, several amino acid residues in the α-helices of arm II in MalK (ATP-binding protein of the maltose transporter of *E. coli*) have been reported to interact with the EAA loops of MalF and MalG, which are membrane-bound permeases of the transporter [26]. Therefore, arm II of AlgS is also thought to be responsible for the association with the EAA loops of AlgM1 and AlgM2.

4. Regulation of import system

The alginate import system 'super-channel' consists of a pit, binding proteins (AlgQ1 and AlgQ2), and an ABC transporter (permeases, AlgM1 and AlgM2; and an ATP-binding protein, AlgS), which might be linked to degradation enzyme processes (alginate lyases [27], A1-I, -II, and -III [28]; and oligoalginate lyase, OAL [29]) (Fig. 3). The import system and depolymerization process may be closely and simultaneously regulated in a unified manner for efficient incorporation of macromolecules, as easily seen from the clustering of the relevant genes in an operon (Fig. 3). It appears that some regulation mechanisms are operating in the ABC transporter for alginate import (Fig. 3). First, the disruption of *algM1* and/or *algS* results in the failure of cells to form a pit in the presence of alginate [3]. This indicates that the formation of a pit in the presence of alginate (Fig. 1) is controlled by the ABC transporter. Secondly, although AlgS is active in vitro even after separation from the transport complex, the ATPase activity of AlgS is substantially enhanced in the presence of both permeases (AlgM1 and AlgM2) and periplasmic binding proteins (AlgQ1 and AlgQ2) complexed with alginate or other macromolecules such as polygalacturonate, gellan, and xanthan (unpublished results). This observation is supported by the results of Nikaido et al. [30] and Morbach et al. [31]. They showed that purified HisP and MalK show constitutive ATPase activity in the absence of transport substrates, histidine and maltose, respectively, but their ATPase activities are enhanced in the presence of binding proteins and their substrates. However, the mechanism of the activation of AlgS (ATPase) in the presence of periplasmic binding proteins (AlgQ1 and AlgQ2) and permeases (AlgM1 and AlgM2) has not been elucidated yet. A fascinating finding was made by Ehrmann et al. [6] through analysis of a structural model of maltose-binding protein MalE of *E. coli*. They indicated that periplasmic binding proteins come into direct contact, across the cytoplasmic membrane, with ABC-ATPase in order to activate or trigger ATP hydrolysis. However, in the alginate transport system, activation of the ABC-ATPase by periplasmic binding proteins requires the participation of permeases, which thus indicates that the information regarding the structural change induced in the binding protein through the binding of alginate is transferred to the ABC-ATPase by way of mediation by permeases. Anyway, this is of great significance for characterization of the prokaryotic import system, since in the export system, no substrate-binding proteins are utilized and substrates are thought to come into direct contact with the membrane domains. Determination of the three-dimensional structures of permeases is essential for elucidating the intrinsic mechanism underlying the activation of ABC-ATPases.

5. Macromolecule import and export systems

As far as we are aware, Gram-negative bacteria have two ABC transporter-dependent pathways for moving

←

Fig. 3. Pit-dependent macromolecule import system 'super-channel' in *Sphingomonas* sp. A1. The mechanism underlying the transport of alginate, which is used as a representative macromolecule to be imported, and the degradation pathway for alginate are described in the text. –►: Activation of AlgS by binding proteins AlgQ1 and AlgQ2 (ribbon model showing the position of Ca^{2+}). ...►: Regulation of pit formation by the ABC transporter (AlgM1/AlgM2:AlgS-AlgS). G, guluronate; M, mannuronate; *aly*, genes for alginate lyases (A1-I, A1-II, and A1-III); *ccpA*, catabolite control protein gene; *algS*, *algM1*, and *algM2*, ABC transporter genes for alginate import; *algQ1* and *algQ2*, genes for alginate-binding proteins; *oal*, oligoalginate lyase gene.

macromolecules across the cell envelope. One is the HlyB/HlyD/TolC pathway for the export of toxic proteins (e.g. α-hemolysin in *E. coli*) [32]. The other one is the import pathway for alginate presented here. It is of interest to note that in both pathways, macromolecules must cross the cytoplasmic (inner) and outer membranes, and the periplasm between them. However, as illustrated in Fig. 2, the two systems are essentially different in both the transportation orientation and the molecular machineries that constitute the system. In the export system (HlyB/HlyD/TolC) for the toxins, the pathway requires only three proteins and does not generate periplasmic intermediates [33,34]. The three export components are traffic ATPase and an accessory protein, which together form an energized substrate-specific complex (HlyB/HlyD) in the inner membrane, and a third protein, TolC. Other than *E. coli* hemolysin, the system can export many large proteins such as cyclolysin (170 kDa) of *Bordetella pertussis*, and proteases (60–80 kDa) of *Serratia marcescens* and *P. aeruginosa* (60–80 kDa) [35–37]. X-ray crystallography of the TolC protein indicated that a TolC homotrimer forms a hollow tapered cylinder comprising two domains: the tunnel domain (α-helical barrel) and the channel domain (β-barrel) anchoring the outer membrane to form a porin-like structure. The most striking feature of TolC is the long periplasmic tunnel. A protein (e.g. α-hemolysin) that has passed through the inner membrane through the action of permeases (HlyD) and ABC proteins (HlyB) is transferred to the tunnel-like TolC architecture and then released into the external medium through the porin-like gate of TolC.

Except for the essential components (i.e. the energized complex between the permease and ABC protein) in the inner membranes, the import system for macromolecule alginate of *Sphingomonas* sp. A1 is apparently different from the *E. coli* HlyB/HlyD/TolC export system. The hemolysin export system in *E. coli* generates no periplasmic intermediates and TolC has an essential function in the transport of macromolecules from the periplasm to the external medium across the outer membrane. However, the alginate import system of *Sphingomonas* sp. A1 has no TolC-like proteins, and the pit formed on the cell surface and macromolecule-binding proteins (AlgQ1 and AlgQ2) present in the periplasm allow the movement of alginate across the outer membrane, although the interaction between the pit and binding proteins is not yet clear, nor the detailed structure of the pit facing the periplasmic space.

Therefore, the mechanism by which the pit is formed through the reconstitution and/or rearrangement of plait molecules and how the pit enables the movement of alginate across the outer membrane are core problems to be solved. Perhaps cellular enzymes with the ability to degrade or modify the peptidoglycan layer are involved in these events, especially in the creation of the 'mesh' that allows the passage of macromolecules.

6. Features of macromolecule import

The alginate import pathway 'super-channel' in *Sphingomonas* sp. A1 is illustrated in Fig. 3, together with the depolymerization processes for the polymer imported. The bacterium forms a pit through rearrangement and/or reconstitution of plait molecules on the cell surface and concentrates the polymer in the pit, although the mechanism underlying targeting of alginate to the pit is not yet clear. The periplasmic binding proteins may be responsible for this process, and are expected to be localized just under the pit. The concentrated alginate is trapped by AlgQ1 and AlgQ2 present in the periplasm and then delivered to the ABC transporter. The ABC transporter directly incorporates alginate into cells through the action of permeases (AlgM1 and AlgM2) by use of energy generated through the hydrolysis of ATP by AlgS. As described above, the ABC transporter regulates the formation of the pit, and AlgS is activated through contact with AlgQ1 and/or AlgQ2 through an unknown mechanism. The pit-dependent macromolecule import system is universal, at least among the Sphingomonad bacteria, and we have confirmed that cells of *Sphingomonas mali*, when grown on alginate, form a pit on their surface, and incorporate alginate through the pit (unpublished results).

The pit-dependent macromolecule import system found in *Sphingomonas* sp. A1 is characteristic compared with the import systems so far found in other bacteria. First, this system requires no complicated apparatuses for the secretion of extracellular depolymerization enzymes or for the import of depolymerization products. Secondly, this system promises highly efficient utilization of biopolymers. This is often impossible, especially when a biopolymer is depolymerized outside cells by extracellular enzymes, since some of the depolymerized products are dispersed through diffusion. Furthermore, the pit-dependent macromolecule import system bears a striking resemblance to endo- and phagocytoses in that these systems accompany drastic changes in the structure of the cell surface and/or cell membrane. The bacterium *Sphingomonas* sp. A1 contains glycosphingolipids in place of lipopolysaccharides, a major component of the membranes of Gram-negative bacteria. Glycosphingolipids are a ubiquitous component of cell membranes of eukaryotes, and, in that respect, the membrane structure of *Sphingomonas* sp. A1 is essentially the same as that of eukaryotes. Therefore, phylogenetic analysis of the pit-dependent system would be useful for elucidating the common features as well as the evolutional origins of endo- and phagocytoses.

The crystal structures of bacterial ATP-binding proteins HisP [23] and MalK [38] and many substrate-binding proteins have been clarified. With determination of the three-dimensional structures of membrane domain units (permeases), the entire structures and overall functions of ABC transporters will be solved at one time.

Acknowledgements

This work was supported in part by Grants-in-Aid for Scientific Research (Nos. 10556017, 10145229, 09876026, 12019237, 11132237, 11460039, and 10556017 to K.M.; and 08760083, 09760085, and 11760062 to W.H.) from the Ministry of Education, Science, Sports and Culture of Japan, and a Grant-in-Aid for Scientific Research (DC1 03619 to Y.M.) from the Japan Society for the Promotion of Science (JSPS).

References

[1] Ramphal, R. and Pier, G.B. (1985) Infect. Immun. 47, 1–4.
[2] Hisano, T., Yonemoto, Y., Yamashita, T., Fukuda, Y., Kimura, A. and Murata, K. (1995) J. Ferment. Bioeng. 79, 538–544.
[3] Momma, K., Okamoto, M., Mishima, Y., Mori, S., Hashimoto, W. and Murata, K. (2000) J. Bacteriol. 182, 3998–4004.
[4] Ames, G.F.-L. and Lecar, H. (1992) FASEB J. 6, 2660–2666.
[5] Hyde, S.C., Emsley, P., Hartshorn, M.J., Mimmack, M.M., Gileadi, U., Pearce, S.R., Gallagher, M.P., Gill, D.R., Hubbard, R.E. and Higgins, C.F. (1990) Nature 346, 362–365.
[6] Ehrmann, M., Ehrle, R., Hofmann, E., Boos, W. and Schlosser, A. (1998) Mol. Microbiol. 29, 685–694.
[7] Andersen, C., Hughes, C. and Koronakis, V. (2000) EMBO Rep. 1, 313–318.
[8] Chakrabarty, A.M. (1991) Appl. Phycol. 8, 583–590.
[9] Preiss, J. and Ashwell, G. (1962) J. Biol. Chem. 237, 309–316.
[10] Murata, K., Inose, T., Hisano, T., Abe, S., Yonemoto, Y., Yamashita, T., Takagi, M., Sakaguchi, K., Kimura, A. and Imanaka, T. (1993) J. Ferment. Bioeng. 76, 427–437.
[11] Hisano, T., Kimura, N., Hashimoto, W. and Murata, K. (1996) Biochem. Biophys. Res. Commun. 220, 979–982.
[12] Duplay, P., Bedouelle, H., Fowler, A., Zabin, I., Saurin, W. and Hofnung, M. (1984) J. Biol. Chem. 259, 10606–10613.
[13] Doige, C.A. and Ames, G.F.-L. (1993) Annu. Rev. Microbiol. 47, 291–319.
[14] Kellerman, O. and Szmelcman, S. (1974) Eur. J. Biochem. 47, 139–149.
[15] Spurlino, J.C., Lu, G.-Y. and Quiocho, F.A. (1991) J. Biol. Chem. 266, 5202–5219.
[16] Sharff, A.I., Rodseth, L.E., Spurlino, J.C. and Quiocho, F.A. (1992) Biochemistry 31, 10657–10663.
[17] Hor, L.I. and Shuman, H.A. (1993) J. Mol. Biol. 233, 659–670.
[18] Overduin, P., Boos, W. and Tommassen, J. (1988) Mol. Microbiol. 2, 767–775.
[19] Saurin, W. and Dassa, E. (1994) Protein Sci. 3, 325–344.
[20] Gilson, E., Nikaido, H. and Hofnung, M. (1982) Nucleic Acids Res. 10, 7449–7458.
[21] Jones, P.M. and George, A.M. (1999) FEMS Microbiol. Lett. 179, 187–202.
[22] Walker, J.E., Saraste, M., Runswick, M.J. and Gay, N.J. (1982) EMBO J. 1, 945–951.
[23] Mishima, Y., Momma, K., Hashimoto, W., Mikami, B. and Murata, K. (2001) Acta Crystallogr. Sect. D 57, 884–885.
[24] Hung, L.W., Wang, I.X., Nikaido, K., Liu, P.Q., Ames, G.F. and Kim, S.H. (1998) Nature 396, 703–707.
[25] Peitsch, M.C. (1996) Biochem. Soc. Trans. 24, 274–279.
[26] Hunke, S., Mourez, M., Jehanno, M., Dassa, E. and Schneider, E. (2000) J. Biol. Chem. 275, 15526–15534.
[27] Wong, T.Y., Preston, L.A. and Schiller, N.L. (2000) Annu. Rev. Microbiol. 54, 289–340.
[28] Yonemoto, Y., Murata, K., Kimura, A., Yamaguchi, H. and Okayama, K. (1991) J. Ferment. Bioeng. 72, 152–157.
[29] Hashimoto, W., Miyake, O., Momma, K., Kawai, S. and Murata, K. (2000) J. Bacteriol. 182, 4572–4577.
[30] Nikaido, K., Liu, P.-Q. and Ames, G.F.-L. (1997) J. Biol. Chem. 272, 27745–27752.
[31] Morbach, S., Tebbe, S. and Schneider, E. (1993) J. Biol. Chem. 268, 18617–18621.
[32] Tanabalu, T., Koronakis, E., Hughes, C. and Koronakis, V. (1998) EMBO J. 17, 6487–6496.
[33] Koronakis, V., Koronakis, E. and Hughes, C. (1989) EMBO J. 8, 595–605.
[34] Koronakis, V., Hughes, C. and Koronakis, E. (1991) EMBO J. 10, 3263–3272.
[35] Glaser, P., Sakamoto, H., Bellalou, J., Ullmann, A. and Danchin, A. (1988) EMBO J. 7, 3997–4004.
[36] Koronakis, V. and Hughes, C. (1993) Semin. Cell Biol. 4, 7–15.
[37] Paulsen, I.T., Park, J.H., Choi, P.S. and Saier Jr., M.H. (1997) FEMS Microbiol. Lett. 156, 1–8.
[38] Diederichs, K., Diez, J., Greller, G., Muller, C., Breed, J., Schnell, C., Vonrhein, C., Boos, W. and Welte, W. (2000) EMBO J. 19, 5951–5961.