DEPARTMENT OF VIRAL ONCOLOGY
LABORATORY OF GENE ANALYSIS

I. First Group

The research projects carried out in this group are concerned with post-translational events in the expression of genetic information. Specifically, processes of protein translation, protein translocation across and integration into the membrane, membrane protein proteolysis and extracytoplasmic stress responses are investigated by combined molecular genetic, biochemical and structural approaches.

1) A pair of circularly permutated PDZ domains control RseP, the S2P family intramembrane protease of *E. coli*: K. INABA [1], M. SUZUKI [2], K.-I. MAEGAWA[1], S. AKIYAMA[3], K. ITO, and Y. AKIYAMA ([1] Medical Institute of Bioregulation. Kyushu University, [2] Institute for Protein Research, Osaka University, [3]RIKEN Harima Institute)

The σ^E pathway of extracytoplasmic stress responses in *E. coli* is activated through sequential cleavages of the anti-σ^E protein, RseA, by membrane proteases, DegS and RseP. Without the first cleavage by DegS, RseP is unable to cleave full-length RseA. We previously showed that a PDZ-like domain in the RseP periplasmic region is essential for this negative regulation of RseP. We now isolated additional deregulated RseP mutants. Many of the mutations affected a periplasmic region that is N-terminal to the previously defined PDZ domain. We expressed these regions and determined their crystal structures. Consistent with a recent prediction (1), our results indicate that RseP has tandem, circularly permutated PDZ domains (PDZ-N and PDZ-C). Strikingly, almost all the strong mutations have been mapped around the ligand-binding cleft region in PDZ-N (2). These results together with those of an *in vitro* reaction reproducing the two-step RseA cleavage suggest that the proteolytic function of RseP is controlled by ligand-binding to PDZ-N.

(1) Kinch, L. N., Ginalski, K., and Grishin, N. V. (2006) Protein Sci. 15, 84-93. (2) Inaba, K., Suzuki, M., Maegawa, K.-i,, Akiyama, S., Ito, K., and Akiyama, Y. (2008) J. Biol. Chem. 283, 35042-35052.

2) Involvement of RseP, the S2P family intramembrane protease of *E. coli*, in proteolytic degradation of secretary protein signal peptides: A. SAITO and Y. AKIYAMA

Signal peptides (SP) of secretory protein precursors are cleaved off by the action of signal peptidase. It is believed that they are subsequently degraded further by the action of signal peptide peptidase (SPP), an intramembrane proteases, in eukaryotic cells. Prokaryotic organisms including *E. coli* have no SPP homolog and little is known about the metabolism of their SPs. Our previous

results suggested that RseP, an *E. coli* S2P protease homolog, is involved in cleavage of SP of β-lactamase (Bla) *in vivo* (1). We now extended this finding and showed that the cleavage of Bla SP indeed depends on proteolytically active RseP. Purified RseP cleaved a synthetic peptide with the amino acid sequence of Bla SP, indicating that RseP directly catalyzes proteolysis of Bla SP. Mapping of the *in vivo* and *in vitro* cleavage sites showed that RseP introduces a cleavage(s) into the central hydrophobic region of Bla SP. We found further that signal peptides of 12 other secretory proteins received RseP-dependent cleavage *in vivo*, supporting the notion that RseP is a general enzyme responsible for SP proteolysis in *E. coli*. A combination of disruptions of RseP and FtsH, a membrane protease crucial for membrane protein degradation, resulted in synthetic growth defects even in the presence of suppressor mutations that rescue lethality caused by each single mutation. Thus, RseP appears to have a role in proteolytic quality control of membrane proteins in addition to the role in the stress response.

(1) Akiyama, Y., Kanehara, K, and Ito, K. *EMBO J.* 23, 4434-4442.

3) An attempt to identify cellular factors involved in "membrane stress response" in *E. coli*: T. HATTORI, H. MORI and Y. AKIYAMA

The cell envelope of *E. coli* is composed of the outer and inner membranes and the periplasmic space in-between. *E. coli* senses protein abnormalities in the periplasm and the outer membrane and induces expression of a set of proteins including cell surface proteases and chaperones, which is called "extracytoplasmic stress response (ESR)". We found previously that generation of misfolded inner membrane proteins caused by a class of dysfunction mutations in SecY, a central subunit of the translocon, led to activation of ESR (1). To gain insight into how a cell recognizes misfolded inner membrane proteins to induce ESR and how it copes with their toxic effects, we are trying to isolate multicopy suppressors against the secY351 mutant, a secY allele that specifically impairs membrane protein assembly/folding processes, using a cloned gene archive that covers all the predicted *E. coli* ORFs (ASKA library). We are screening for clones that alleviate ESR and the growth defects caused by this mutation, and obtained several candidates. We will continue further screening and characterization of multicopy suppressors.

(1) Shimohata, N., Nagamori, S., Akiyama, Y., Kaback, H.R., and Ito, K. (2007) *J. Cell Biol.* **176**, 307-317.

4) X-ray crystal structural analysis of membrane bound ATP-dependent protease FtsH: R. SUNO[1, 2], Y. AKIYAMA[2], S. IWATA[3] and M. YOSHIDA[1] ([1]the Chemical Resources Laboratory, Tokyo Institute of Technology, [2]Insitute for Virus Research, Kyoto University, [3]Department of Medicine, Kyoto University)

ATP-dependent proteases are involved in various cellular processes including cell division,

cell differentiation, signal transduction, and stress response. Among them, FtsH is unique, because it is the only protease that is anchored to the cytoplasmic membrane and the only essential protease for bacterial growth. Some closely related homologues of FtsH have been found in eukaryotic mitochondria and chloroplast. FtsH degrades not only misassembled subunits of membrane protein complexes for their quality control but also some short-lived cytosolic regulatory proteins for cellular regulation. FtsH comprises an N-terminal transmembrane segment and a C-terminal cytosolic region, which consists of AAA^+ (ATPases associated with diverse cellular activities) and protease domains. Previously, we successfully crystallized and determined a soluble region of FtsH (sFtsH) containing ADP from *Thermus thermophilus* at 3.9 Å resolution. In the hexameric structure, a substrate polypeptide can reach the active protease catalytic sites through a tunnel leading from AAA^+ domain of the adjacent subunit, but not from the central axial region. This raises a possibility of direct delivery of a polypeptide through this tunnel. Now, to understand the molecular mechanism of FtsH in detail, we examine the condition of crystallization of FtsH to solve the structure bound several kinds of ATP analogue to high resolution.

5) Biochemical analysis of the substrate-translocating mechanism of ATP-dependent Protease FtsH: R. SUNO[1, 2], Y. AKIYAMA[2] and M. YOSHIDA[1] ([1]the Chemical Resources Laboratory, Tokyo Institute of Technology, [2]Insitute for Virus Research, Kyoto University)

In the sFtsH structure, the synchronized open-close motions of subunits in FtsH would drive the translocation of a substrate polypeptide to the protease catalytic sites. In fact, we proposed the model that one subunit of FtsH bound a substrate and sent it into the other subunit. We constructed reconstitution method of sFtsH to confirm this model. Both of Two mutants, E419Q and F229A, have no protease activity, because E419Q was mutated at protease catalytic site, and F229A was mutated at substrate-binding site. But, by reconstitution of sFtsH complex from subunits of both E419Q and F229A, the ATP-dependent protease activity of this hybrid sFtsH was recovered. This result suggested that E419Q mutant bound a substrate and sent it into F229A mutant, and was consistent in out model. Furthermore, we will examine ATP hydrolysis cycle of FtsH using the same reconstitution method.

6) Conformational transition of sec machinery inferred from bacterial SecYE structures: T. TSUKAZAKI[1], H, MORI, S. FUKAI[2], R. ISHITANI[1], T. MORI[3], N. DOHMAE[4], A. PEREDERINA[5], Y. SUGITA[3], D. G. VASSYLYEV[5], K. ITO and O. NUREKI[1] ([1]Institute of Medical Science, University of Tokyo, [2]Life Science Division, Synchrotron Radiation Research Organization, University of Tokyo, [3]Advanced Science Institute, RIKEN, [4]Biomolecular Characterization Team, RIKEN, [5]Department of Biochemistry

and Molecular Genetics, University of Alabama)

Evolutionarily conserved membrane protein complex, the Sec translocon, functions as a protein-conducting channel to facilitate secretory protein export and membrane protein integration. We determined the first atomic (3.2 Å)-resolution crystal structure of the SecYE translocon from a SecA (bacterial translocation motor)-containing organism, *T. thermophilus*, in complex with an anti-SecY Fab fragment. The structure has revealed a significant conformational change from the previously determined archaeal SecYE structure (1), in that several SecY transmembrane helices are shifted to create a hydrophobic crevasse open to the cytoplasm. Disulfide mapping and molecular dynamics analyses indicate that this "pre-open" state of the bacterial SecYE complex was induced by the Fab fragment and SecA, which in common bind to SecY residues at the tip of the cytoplasmic domain. Our disulfide crosslinking experiments revealed that some of these SecY residues contact specific residues of SecA that are otherwise buried and unavailable in the isolated SecA molecule. Thus, SecA may also undergo a major conformational change upon binding to the translocon. These results suggest that the channel and the motor components of the Sec machinery undergo cooperative conformational changes upon their interaction, presumably as important steps for the entrance of the preprotein-SecA complex and enhancement of the ATPase activity of SecA. This study (2) and the simultaneously published work from the laboratory of Tom Rapoport (3, 4) represent major advances in our understanding of how secretory proteins are transported across the membrane through the interplay between the SecA ATPase and the SecYE translocon.

(1) Van den Berg, B., Clemons, W. M., Jr., Collinson, I., Modis, Y., Hartmann, E., Harrison, S. C., and Rapoport, T. A. (2004) *Nature* **427**, 36-44. (2) Tsukazaki, T., Mori, H,, Fukai, S., Ishitani, R., Dohmae, N., Perederina, A., Sugita, Y., Vassylyev, D. G., Ito, K. and Nureki, O. (2008) *Nature* **455**, 988-991 (3) Zimmer, J., Nam, Y. and Rapoport, T. A. (2008) *Nature* **455**, 936-943 (4) Erlandson, K. J., Miller, S. B. M., Nam, Y., Osborne, A. R., Zimmer, J. and Rapoport, T, A, (2008) *Nature* **455**, 984-987

7) Identification of SecG Nearest Neighbors Using *in vivo* Site-directed Cross-linking: N. TANAKA, G. KOBAYASHI, N. DOHMAE[1], K. ITO, Y. AKIYAMA, and H. MORI

([1]Biomolecular Characterization Team, RIKEN)

While SecG, an auxiliary and dynamic component of the bacterial translocon, is thought to stimulate protein translocation by assisting in the SecA reaction cycles, its molecular mechanism remains poorly understood. In order to study how SecG interacts with other cellular factors, including SecA, *in vivo*, we used the method of *in vivo* site-directed photo crosslinking developed by P. Schultz (1). After systematic examinations of the SecG sub-domains (two transmembrane (TM) segments, one cytoplasmic loop and one periplasmic tail), we found that the cytoplasmic region approaches SecA and SecY alternately (2). In addition, two cross-linked adducts of different SDS-PAGE mobilities have been observed when pBPA (p-benzophenylalanine, a photo reactive

amino acid derivative) was introduced into positions 52 and 72 near the membrane boundaries of the second TM segment of SecG. They did not cross-react with antibodies against any other Sec factors examined. To identify the partner proteins of the cross-linked products, we have been attempting to purify them by affinity chromatography using either SecG antibody-conjugated resin or metal chelating resin. In the latter case, we use His_6-tagged versions of the SecG (pBPA) derivatives. Purified samples will then be subjected to mass spectrometry analysis.

1) Chin, J. W. and Schultz, P. G. (2002) *ChemBioChem* 3, 1135–1137; 2) Kobayashi, G., Ito, K. and Mori, H. (2006) *Annual Report Inst. Virus Res.* 49, 60-61

8) Coupling between membrane insertion and release of elongation arrest of MifM: S. CHIBA and Y. AKIYAMA

Bacillus subtilis mifM encodes a single N-terminal transmembrane (TM) region and the C-terminal 'arrest motif' that interacts with the polypeptide exit tunnel of the ribosome to induce translational elongation arrest of *mifM* (1). Elongation arrest of *mifM* allows for efficient *yidC2* translation by unfolding a secondary structure of the *mifM-yidC2* mRNA. Because SpoIIIJ (YidC1)-dependent membrane insertion of MifM releases the elongation arrest, *yidC2* is expressed only when SpoIIIJ activity is lowered. Thus, SpoIIIJ and YidC2 may serve as the primary and a backup membrane protein insertases, respectively. Systematic mutant analyses revealed that insertion of more than 10 amino acid residues between TM and the arrest motif of MifM resulted in loss of SpoIIIJ-dependent release of elongation arrest. These and other results suggest that elongation arrest is released only within a limited time window during membrane insertion of MifM. Dynamism of membrane insertion rather than just membrane tethering of MifM seems to be crucial in the release of elongation arrest of *mifM*.

(1) Chiba, S. and Pogliano, K. in preparation.

9) Peculiarities in the Biosynthesis of SecM: K. ITO, S. CHIBA and Y. AKIYAMA

SecM is unique in that its translation is subject to elongation arrest within the ribosome, whereas the engagement of its N-terminal region in the Sec secretion reaction releases the ribosomal stall. We have refined *in vivo* experimental approaches to this protein and are now able to detect directly SecM peptidyl-tRNA, either in its steady states by immunoblotting or in its transient states by pulse-labeling/immunoprecipitation. We plan to explore various combinations of "arrest sequences" and "release cues" *in vivo* to understand the generality and specificity of this emerging class of regulatory mechanisms (see also Chiba and Akiyama, this volume). Meantime, it was found that newly synthesized SecM behaves differently according to the expression levels. While instability was noted previously for secreted SecM molecules that were also highly

overproduced, we now found that SecM expressed at a lower (more physiological) level cannot be detected unless its secretion is blocked, even if it carries a Met_6 sequence at the C-terminus, which was previously shown to stabilize the highly overproduced SecM. Thus, SecM may be co-translationally or co-translocationally degraded by some unknown mechanism.

II. Second Group

1) Analysis of molecular mechanism underlying keratin-associated protein 13-induced activation of canonical wnt signaling pathway: S. YANAGAWA

Low-density lipoprotein receptor-related protein 6 (LRP6) is a component of cell-surface receptors for Wnt proteins and Wnt is known to promote recruitment of Axin by LRP6 thereby inhibiting β-catenin's degradation. I found that Keratin associated protein (Krtap) 13, a cysteine-rich cytoplasmic protein which contains six tandem repeats of 10 amino acids with the CQ motif, binds to both the intracellular portion of LRP6 and a Wnt signaling effector, Dvls and that Krtap13 overexpression markedly stimulated TCF-dependent-reporter activities in both HEK293T cells and Drosophila S2R+ cells. Krtap13 is a cytoplasmic protein and Krtap13 never induced production of any Wnt proteins in 293T cells. Thus I concluded that Krtap13 activates Wnt signaling in the absence of Wnt, probably by mimicking some aspects of normal Wnt signal transduction. Krtap13-mediated activation of reporter activities were counteracted by Axin, suggesting that Krtap13 functions upstream of β-catenin. Actually, Krtap13 overexpression induced accumulation of β-catenin and its fly homolog Armadillo in 293T and S2R+ cells, respectively. Furthermore, RNAi experiments revealed that Dvls are required for Krtap13-induced reporter activation. The finding that Krtap13 binds to both LRP6 and Dvls and that Dvls are required for Krtap13-mediated activation of Wnt signaling suggested a possible molecular mechanism by which the overexpression of Krtap13 activates Wnt signaling.

Recently, Bilic et al. (Science 316, 1619-1622, 2007) reported that Wnt treatment induces plasma membrane–associated LRP6 aggregates (namely, LRP6 Signalosomes) that functions as a platform where Dvl and Axin co-polymerize. In view of these findings, Krtap13 appears to be a unique tool to analyze molecular mechanism of Wnt receptor activation.

2) Modulation of HPV-infected Cell Proliferation and Invasion by oncogenic Ras Protein: S. YOSHIDA, K. SASAKI, A. SATSUKA, N. KAJITANI, H. NAKAMURA and H. SAKAI

HPVs are small DNA viruses that require unscheduled S-phase entry in terminally differentiated epithelial keratinocytes for viral genome amplification. Two viral proteins, E6 and E7, are considered as main factors of HPV-induced tumorigenesis. E6 and E7 protein binds to and

degrades p53 and pRb respectively, inducing cell cycle progression and abolishes cell cycle arrest and epithelial differentiation. HPVs are associated with more than 90% of all human cervical carcinoma, but it is understood that HPV infection alone is not sufficient for cancer formation. Cervical carcinogenesis is considered as the multi-step process accompanied by host mutagenesis. Ras, that is a host proto-oncoprotein, is activated in approximately 30% human cancers, and also activated in progressive cervical cancer. Its activation is related to the cancer metastasis, resulting from the induction of matrix metalloproteases. The mutation is particularly detected in high-grade cervical lesion. We are investigating the effects of Ras activation on HPV-infected cells.

First, we introduced active-form of H-ras expression in primary keratinocytes expressing HPV oncoproteins, and analyzed the cell growth potential and the regulators for cell cycle checkpoints. We found that the single activation of H-ras in keratinocytes induces premature senescence, but E7-expressed keratinocytes could escape from the senescence. It was indicated that pRb pathway was important for ras-induced senescence in keratinocytes. Second, we analyzed the effect of ras activation on the HPV-infected epidermis using the (organotypic) raft culture system. We found that the E7 and H-ras expression conferred invasive potential on the epidermis into basal layer. This invasion resulted from up-regulations of MT1-MMP and MMP9 by H-ras and E7, respectively. This invasion was suppressed either by MEK or MMP specific inhibitors. It is suggested that HPV-infected cell could acquire metastasis by ras activation. E7 and constitutive active MEK1 expressed cells partly reproduced the invasion induced by both E7 and H-ras expressions, suggesting that the other activity of Ras contributed to the invasive potential.

3) Molecular mechanism of bystander cell death induced by HIV-1 infected cells: H. NAKAMURA, A. SATSUKA, N. KAJITANI, S. YOSHIDA, K. SASAKI and H. SAKAI

Human immunodeficiency virus type 1 (HIV-1) infection causes rapid CD4-positive T cells depletion that is one of the hallmarks of acquired immunodeficiency syndrome (AIDS). Although infection of HIV-1 directly causes the cell death, bystander apoptotic cell death is induced, resulting the massive loss of CD4-positive T cells in infected individuals. Thus, studying the biological mechanisms of bystander effect of infected cells is essential to understand AIDS pathogenesis.

In order to investigate the molecular basis of bystander cell death induced by HIV-1 infected cells, we are intending to infect NL43 mutants containing single or combined deletions of Nef, Vif, Vpr, and Vpu, to CD4-positive T cells, and analyze whether or not these viral factors are involved in depletion of CD4-positive T cells.

4) Identification of Novel Function of Human Papillomavirus E4: N. KAJITANI, A. SATSUKA, S. YOSHIDA, H. NAKAMURA, K. SASAKI and H. SAKAI

HPV infection begins in the basal cells of the epithelium, and as these cells divide, differentiate, and migrate toward the surface of the epithelium, the virus is able to complete its life cycle. The viral life cycle depends on the differentiation of the epithelium, but how the life cycle is controlled is not well understood. It is interesting that viral oncoproteins cause the increase of cellular proliferation and/or transformation, but terminally cellular differentiation of epithelium is required for the viral life cycle is completed.

The expression of E4 occurs in the upper layers of the epithelium, coordinating with the onset of viral genome amplification and the expression of viral late genes. It is known that E4 disrupts the keratin networks. It is also known that E4 induces G_2/M cell cycle arrest. But it is not known well about the details of E4. To investigate novel functions of E4, we performed yeast two-hybrid assays and got several candidate proteins as which interacts with E4.We carry on the analysis about the interactions between the each candidates and E4 in vitro or in vivo. In the future, we will ascertain the function of E4 and its involvement in viral life cycle.

5) A novel human papillomavirus type18 replicon and its application in screening the anti-viral effects of cytokines: A. SATSUKA, N. KAJITANI, S. YOSHIDA, H. NAKAMURA, K. SASAKI and H. SAKAI

Human papillomaviruses (HPVs) infect to the basal cells of the multi-layered epithelial organ. The infection induces benign tumors, such as warts and condylomas, which occasionally progress into malignant tumors. Although the analysis of HPV life cycle is essential to elucidate the mechanism of the virus-induced tumorigenesis, it has been hampered because of the lack of suitable assay system for analyzing the virus lifecycle *in vitro*. In this report, we developed a new system for the analysis of the HPV lifecycle. The new system consists of a novel HPV replicon and an organotypic “raft” culture, by which the HPV DNA is maintained stably in normal human keratinocytes through long period and the vegetative replication of HPV is reproduced. It will promote the biochemical and genetic studies on the HPV lifecycle and the tumorigenesis.

We confirmed the usefulness by evaluating the anti-virus effect of cytokines. To examine the usefulness of the system, the anti-virus effects of three cytokines(IFNβ, TGFβ, TNFα) were examined with it, and the results suggested that the IFNβ treatment was effective to anti-HPV therapy in the early stage of the HPV-infected lesions, while the TNFα treatment or the induction of inflammatory response might have detrimental effect on the lesions. This system will be adapted to the screening for the other anti-HPV compounds. It also allows manipulating the genetic elements of both host cells and virus, thus it becomes accessible to analyze the regulatory mechanisms of the virus lifecycle and the virus-induced tumorigenesis.

LIST OF PUBLICATIONS
Department of Viral Oncology
Laboratory of Gene Analysis

I. First Group

Koide, K., Ito. K., and Akiyama, Y. Substrate recognition and binding by RseP, an *Escherichia coli* intramembrane protease. J. Biol. Chem. 283, 9562-9570, 2008.

Muto, H., and Ito, K. Peptidyl-prolyl-tRNA at the ribosomal P site reacts poorly with puromycin. Biochem. Biophys. Res. Commun., 366, 1043-1047, 2008.

Tsukazaki, T.[a)], Mori, H. [a)], Fukai, S., Ishitani, R., Mori, T., Dohmae, N., Perederina, A., Sugita, Y., Vassylyev, D. G., Ito, K.[b)] and Nureki O. [b)] Conformational transition of Sec machinery inferred from bacterial SecYE structures. Nature 455, 988-991, 2008.

[a] These authors contributed equally to this work. [b] Corresponding authors

Inaba, K., Suzuki, M., Maegawa, K., Akiyama, S., Ito, K., and Akiyama, Y. A pair of circularly permutated PDZ domains control RseP, the S2P family intramembrane protease of *E. coli*. J. Biol. Chem. 283, 35042-35052, 2008.

Inaba, K. and Ito, K. Structure and mechanisms of the DsbB-DsbA disulfide generation machine. Biochim. Biophys. Acta. 1783, 520-529, 2008.

Ito, K. and Inaba, K. The disulfide bond formation (Dsb) system. Curr. Opinion Struct. Biol., 18, 450-45, 2008.

Ito, K.: Structure of bacterial SecY translocon and its interaction with SecA. Beckwith Reunion Meeting "Microbial Genetics and Genomics" 16-19, May, Cassis, France, 2008

Ito, K.: Quality control pathways used to monitor membrane protein abnormalities and roles of translocon in the correct biogenesis of membrane proteins. Symposium "Insertion, Folding, Assembly and Quality Control", 108th ASM General Meeting, June 5, Boston, U.S.A., 2008

Mori, H.: How does SecA ATPase interact with translocon and drive protein translocation? G-COE in Chemistry " Frontier of Organelle Dynamics and Protein Functions " March 11-13, Nagoya, Japan, 2008

Suno, R., Tsuchiya, D., Niwa, H., Yoshida, M., and Morikawa, K.: X-ray crystallographic study on membrane-bound metalloprotease FtsH. The 15th East Asia Joint Conference on Biomedical Research, July 20-23, Seoul, Korea, 2008

Tsukazaki, T., Mori, H., Fukai, S., Ishitani, R., Mori, T., Dohmae, N., Perederina, A., Sugita, Y., Vassylyev, D. G., Ito, K. and Nureki O.: Crystal structure of *T. thermophilus* Sec translocon with an Fab fragment implies a translocation-initiating state of SecY. シンポジウム「タンパク質機能発現システムーシャペロンからトランスロケーターまで」第３１回日本分子

生物学会年会、第８１回日本生化学会大会　合同大会　神戸　2008 年 12 月 9-12 日

森　博幸：　SecA ATPase はどのように translocon と相互作用し、タンパク質膜透過を駆動しているのか？　大阪大学蛋白質研究所セミナー「蛋白質の膜透過と膜挿入の分子メカニズム-その核心に迫る」　吹田　2008 年 1 月 24-25 日

武藤洋樹、森博幸、秋山芳展、伊藤維昭：　SecM の分泌は低温感受性の性質をもつ．大阪大学蛋白質研究所セミナー　「蛋白質の膜透過と膜挿入の分子メカニズム-その核心に迫る」　吹田　2008 年 1 月 24-25 日

森田 貴之、古澤 宏幸、塚崎 智也、森 博幸、伊藤 維昭、岡畑 恵雄：　水晶発振子上での Sec 系膜タンパク質を導入した平面脂質二分子膜の構築. 日本化学会第 88 春季年会 東京　　2008 年 3 月 26-30 日

森　博幸：タンパク質膜透過装置の構造と駆動メカニズム．　遺伝研研究会「細胞周期制御をめぐる単細胞システム分子生物学」 三島　2008 年 3 月 27-28 日

秋山芳展：　大腸菌 Rip プロテアーゼの機能と制御．ワークショップ「直接性、不可逆性を特徴とする新しいシグナル伝達のメカニズム」第 31 回日本神経科学大会 東京 2008 年 7 月 9-11 日

千葉志信：蛋白質の膜組込みをモニターする機構の解明．第 5 回 21 世紀大腸菌研究会 藤枝　2008 年 7 月 28-29 日

斎藤　啓、秋山芳展：大腸菌 S2P プロテアーゼ RseP の膜タンパク質品質管理における新たな役割．　第 5 回 21 世紀大腸菌研究会　藤枝　2008 年 7 月 28-29 日

斎藤　啓、秋山芳展：大腸菌 S2P プロテアーゼ RseP の膜タンパク質品質管理における新たな役割.タンパク社会若手ワークショップ　東京　2008 年 9 月 25 - 27 日

寿野良二：　AAA プロテアーゼ FtsH の構造と機能. 細胞機能科学セミナー　北海道　2008 年 9 月 19 日

斎藤　啓、秋山芳展：大腸菌 S2P プロテアーゼ RseP の膜タンパク質品質管理における新たな役割. 第 4 回学生フェスティバル　京都　2008 年 10 月 30 日

Tsukazaki, T., Mori, H., Fukai, S., Ishitani, R., Perederina, A., Vassylyev, D. G., Ito, K. and Nureki, O.：細菌型 Sec トランスロコン複合体の結晶構造解析　シンポジウム「膜を介した物質輸送・シグナル変換の構造基盤」第 46 回日本生物物理学会　福岡　2008 年 12 月 3-5 日

古沢宏幸、森田貴之、三友秀之、塚崎智也、森博幸、伊藤維昭、岡畑恵雄：　水晶発振子上での平面脂質二分子膜の構築と Sec 系膜タンパク質の固定化．　第 31 回日本分子生物学会年会・第 81 回日本生化学会大会合同大会　神戸　2008 年 12 月 9-12 日

中崎　洋介、寿野　良二、吉田　賢右、渡辺　洋平. 好熱菌 ClpP 結合型 ClpB 変異体:　第 81 回日本生化学会大会・第 31 回日本分子生物学会年会合同大会　神戸　2008 年 12 月 9-12 日

II. Second Group

Yoshida, S., Kajitani, N., Satsuka, A., Nakamura, H. and Sakai, H. Ras modifies proliferation and invasiveness of the cells expressing HPV oncoproteins. J. Virol. 82: 8820-8827, 2008.

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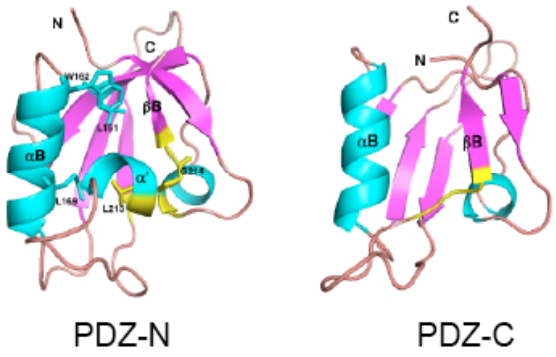
I. First Group（秋山、森）

本年は 3 月に小出佳代さん、武藤洋樹さん、前河早希さんが学位（博士）を取得して研究室を去りました。一方、4 月に田中夏子さん、服部徳哉さんが生命科学研究科 M1 として、伊藤維昭博士、寿野良二博士がポスドクとして、さらに 5 月に千葉志信博士がポスドクとして新たに参加しました。

膜内切断プロテアーゼ RseP の機能制御

大腸菌では、外膜や内膜、ペリプラズムといった細胞表層に異常なタンパク質が蓄積するなどの「ストレス」に曝されると、その情報が内膜を越えて細胞質に伝達され、ストレスに対抗する働きを持つ細胞表層で働くプロテアーゼや分シャペロン等の発現が誘導されます。これは「表層ストレス応答」と呼ばれています。表層ストレス応答におけるシグナル伝達には複数の経路が存在しますが、そのうちの一つ、σ^E 経路では、発現誘導される遺伝子の転写に関わる転写因子σ^Eは通常膜結合型の anti-σ^E 因子 RseA の細胞質領域に強く結合して不活性な状態に保たれています。表層ストレスにより、まず、ペリプラズム側に活性部位を持つ膜プロテアーゼ DegS が直接活性化され、RseA のペリプラズム領域を切断します。これが引き金となって、引き続き、膜内切断プロテアーゼ RseP が RseA の膜貫通領域を切断することで、σ^E は膜から遊離し、最終的に活性化して標的遺伝子の転写に働きます。RseP は通常、DegS による切断を受けた「RseA 分解中間体」のみを切断し、完全長の RseA は切断できません。この RseP の機能抑制は DegS を介した表層ストレス応答の発動制御に重要な役割を果たしています。私達は、この RseP の機能制御に RseP のペリプラズム領域に存在する PDZ ドメインが関わることをこれまでに見出していました。今年度はさらに解析を進め、従来一つと考えられていた PDZ ドメインが、実際には 2 つ（PDZ-N と PDZ-C：図参照）存在すること、RseP の PDZ ドメインは他の多くの PDZ ドメインと異なり、circular permutation した 1 次配列を持つことを示し、また、PDZ ドメインへのリガンドの結合が RseP の機能制御に関わることを示唆しました。これらの結果は、RseP PDZ ドメインへのリガンドの結合を介した、新たなストレス応答メカニズムが存在する可能性を示唆しています。（秋山、伊藤）



PDZ-N　　PDZ-C

細菌型トランスロコン SecYE の構造解析から明らかになったタンパク質膜透過装置の構造変化

分泌タンパク質や膜タンパク質は、合成の場である細胞質から膜を越えた輸送過程を経て、機能する場所に配置されます。このため、あらゆる生物の膜には共通性の高いタンパク質透過チャネル（トランスロコン）が存在しています（当グループはトランスロコンの中心を成すSecYを25年前に発見しました)。バクテリアでは、トランスロコンであるSecYE複合体と駆動モーターであるSecA ATPaseの共同作用によりタンパク質の膜透過が起こります。トランスロコンの結晶構造として、従来はアーキアのものが唯一知られていましたが、その構造は静止状態のものでした。本研究では、東大・医科研、濡木理教授、塚崎智也助教らと協力して、高度好熱菌SecYEの立体構造をX線結晶構造解析によって決定しました（図１左)。SecYに対するモノクローナル抗体断片との複合体として解かれたSecYEの構造には、静止型構造にはない凹み（矢印）が細胞質領域に観察され、この構造をプレオープン構造と命名しました。凹みは、αヘリックスを収納できる程度の大きさで、保存された疎水性アミノ酸残基からなり、機能的に重要であることが示唆されました。プレオープン構造はSecAの結合によって静止構造から誘起されることが明らかとなりました。更に、トランスロコンへの結合によってSecA分子にも協調的な構造変化が起こることがわかり、ATP加水分解活性の昂進に到ることが示唆されました。これらの結果に基づき、膜透過初期過程においてSecAの結合によるプレオープン型への構造変化がトランスロコンに起こり、協調して活性化されたSecAからSecYの凹みに基質タンパク質が受け渡されるとの膜透過開始機構を提唱しました（図２)。

本研究並びに、我々の論文と同時に報告されたTom Rapoportのグループの研究は、「SecAは、トランスロコンとの相互作用を介して、どのように分泌タンパク質を膜透過させているのか？」という根本的な問題の解明に向けての重要な進展といえます。（森、伊藤）

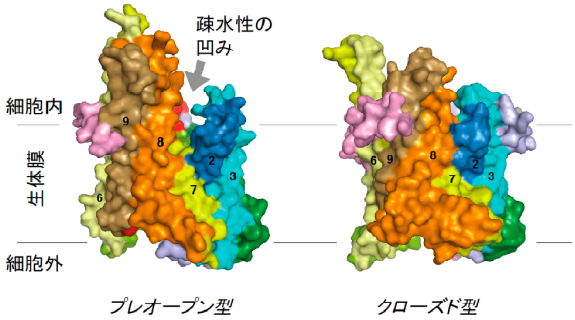


図１　SecYE トランスロコンの構造

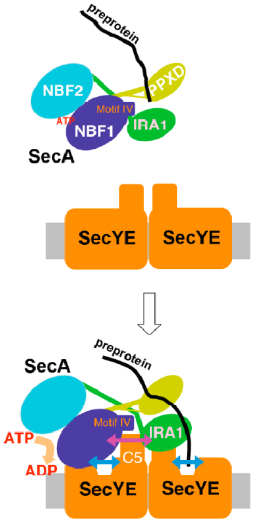


図２　SecA-SecY 相互作用

II. Second Group（酒井、柳川）

現在のスタッフは昨年度と変わらず酒井・柳川の二人で，大学院生としては生命科学研究科に所属する梶谷直子（D2），佐塚文乃（D2），そして医学研究科に吉田智志（D4），中村博保（D4），合わせて 4 名が在籍しています。それぞれが独立の研究課題に取り組んでおり、その詳細は英文の方を参照してください。吉田君は来年度から研究員として米国ウィスコンシン大学に留学します。その他の学生も将来の方向性を模索中です。技術補佐員としては新たに加藤木君（保健学科）と高木さん（法学部）が加わっています。

（１）ヒトパピローマウイルス（HPV）感染による悪性腫瘍形成機構の解明

HPV 感染症は代表的な STD（Sexually Transmitted Disease：性感染症）であり、広く蔓延していることが知られています。また近年ではその感染が若年層に広がっていることが問題となっています。HPV 感染は発がんと関連することが知られていて、特に子宮頚癌では，ほとんどの発症例で HPV の感染が確認されており，HPV 感染が子宮頚癌発症の主要なリスクファクターであると考えられています。HPV 感染によるがん化を防ぐためには，HPV の感染・複製を抑制することが効果的であると考えられます。しかし HPV の複製・遺伝子発現の制御は，上皮細胞の分化に強く依存していて，通常の組織培養では HPV の生活環を再現できないので，これまでその制御機構はほとんど分かっていませんでした。私たちは既に皮膚モデル培養系を用いて，HPV の複製を組織培養下で再現しています。この系をさらに発展させるべく，独自の HPV レプリコンを構築し，効率よく HPV の複製・遺伝子発現機構を解析する手法を検討しています。また一方で、機能のよく分かっていないウイルス制御遺伝子，特に E4 と E5 に注目し，それらの生物活性の同定を行っています。これらの遺伝子機能から、ウイルス複製の調節機構を探り、抗ウイルス剤開発の標的を見出したいと考えています。

（２）HIV-1 の病原性発現機構の解析

私たちの研究室では HIV の制御遺伝子の生物活性に興味を持ち研究を行っているが、現在は HIV-1 による細胞死誘導機構の解明も進めている。特に，感染細胞周辺の CD4 陽性細胞が apoptosis 誘導によって細胞死する“bystander cell death”と呼ばれる細胞障害機構に関して，そのウイルス側の要因を明らかにすると同時に，そこに関与する宿主側因子の同定を目指した研究を行っている。

（３）新規 LRP6 結合蛋白 Krtap13 を用いた Wnt 受容体活性化の分子機構の解析

分泌蛋白 Wnt による Canonical Wnt シグナル伝達経路では、Low density lipoprotein receptor related protein 6 (LRP6) と Frizzled がからなる複合体が、Wnt 受容体です。通常、β-catenin は、Axin を中核とした蛋白複合体の中でリン酸化を受け、Ub/プロテオソーム系にて分解されていますが、Wnt 刺激を受けると、Axin は LRP6 に結合して、そのβ-catenin 分解促進能を失い、その結果著しいβ-catenin

蛋白質の蓄積が生じます。β-catenin の一部は、核内で、転写因子 TCF の Co-activator となって Wnt の標的遺伝子の発現を促進します。柳川は、新規 LRP6 結合蛋白として、Keratin-associated protein 13 (Krtap13)を同定しました。Krtap13 は、197 アミノ酸からなり、他の Krtap と類似した Cys-Gln に富むリピート構造を持っていますが、その本来の機能は不明です。

TCF 依存性のレポーターアッセイを行うと、ヒト 293T、ハエ S2R+のいずれの細胞においても、Krtap13 の強制発現は、強く Wnt/Wingless 経路を活性化しました。従って Krtap13 は、その分子単独の強制発現により、著しい Wnt 経路の活性化をもたらす新規分子でした。さらに Krtap13 の効果は、Axin 蛋白質の共発現によって拮抗される事から、Krtap13 の作用点はβ-catenin の上流である事り、実際に、Krtap13 の発現は、293T 細胞, ハエ S2R+細胞において、それぞれβ-catenin、Armadillo 蛋白質の蓄積を誘導しました。また Krtap13 は Wnt 経路の重要な細胞質因子 Dvl とも結合しており、Krtap13 の作用には、この Dvl が、必要でした。Krtap13 は、Wnt 経路活性化の分子機構を解析する為の、ユニークなプローブとなるものと考えています。（柳川）