日本農芸化学会主催

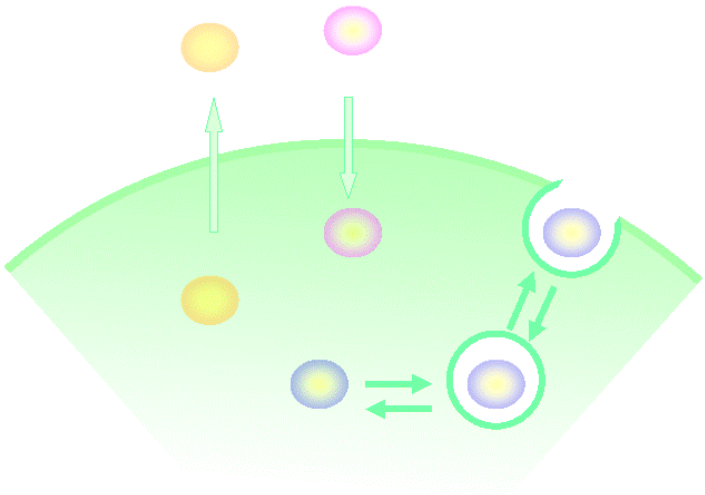
Japan Society for Bioscience, Biotechnology, and Agrochemistry

第34回　化学と生物シンポジウム

The 34th JSBBA Symposium on Chemistry and Biology

Unconventional Pathway of Protein Secretion and Internalization: Mystery across Cell Membranes

タンパク質の分泌と細胞内導入の非定型的経路
～　細胞膜透過のミステリー　～



2008 年 9 月 27 日（土）

名古屋大学野依記念学術交流館

September 27, 2008 (Saturday)

Noyori Conference Hall, Nagoya University

Japan

S p e a k e r s

Dr. Walter Nickel

Dr. Akitada Ichinose

Dr. Marco E. Bianchi

Dr. Yohei Hirai

Dr. Alain Prochiantz

Dr. Shiroh Futaki

Registration (12:30-13:00)

P r o g r a m

13:00 Opening remark: Sakayu Shimizu (Vice President of JSBBA)

Chair: Masatoshi Maki (Nagoya University, Japan)

13:05 Walter Nickel (Heidelberg University Biochemistry Center, Germany)
Overview - The mystery of unconventional protein secretion: How does fibroblast growth factor 2 get transported into the extracellular space?

13:45 Akitada Ichinose (Yamagata University School of Medicine, Japan)
Moving outside: Translocation of a 'cytosolic' transglutaminase, the A subunit of coagulation factor XIII.

14:20 Marco E. Bianchi (San Raffaele Scientific Institute, Milan, Italy)
Extracellular function of high mobility group box I protein (HMGB1) secreted from necrotic and non-necrotic cells.

Coffee Break (14:55-15:15)

Chair: Walter Nickel (Heidelberg University Biochemistry Center, Germany)

15:15 Yohei Hirai (Institute for Frontier Medical Science, Kyoto University, Japan)
Extracellular function of epimorphin/syntaxin2 as a morphogenic protein.

15:50 Alain Prochiantz (Ecole normale supérieure, Paris, France)
Secretion and internalization of homeoprotein transcription factors as direct signaling proteins.

16:25 Shiroh Futaki (Institute for Chemical Research, Kyoto University, Japan)
Internalization mechanism of cell penetrating peptides.

17:00 Closing remark: Akira Isogai (President of JSBBA)

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Free Discussion over Snacks and Drinks (17:10 ~ 18:40)

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THE MYSTERY OF UNCONVENTIONAL PROTEIN SECRETION: HOW DOES FIBROBLAST GROWTH FACTOR 2 GET TRANSPORTED INTO THE EXTRACELLULAR SPACE?

Walter Nickel

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A number of secretory proteins are released from cells by mechanisms that are distinct from the classical ER/Golgi-mediated secretory pathway. Recent studies revealed two critical requirements for the secretion of one such protein, fibroblast growth factor 2 (FGF-2). FGF-2 secretion is initiated by its recruitment to the cytoplasmic leaflet of plasma membranes mediated by the phosphoinositide $PI(4,5)P_2$. Based on mutational studies and RNAi silencing of PIP kinases, it was shown that this interaction is required for efficient FGF-2 secretion. A second interaction that is critical for FGF-2 transport into the extracellular space is mediated by cell surface heparan sulfate proteoglycans (HSPGs). This has been shown by FGF-2 variant forms that are both deficient for binding to heparan sulfates and for secretion. In a complementary approach cell lines that do not express functional HSPGs were demonstrated not to secrete the wild-type form of FGF-2. Additionally, based on affinity-purified plasma membrane vesicles with inverted topology, FGF-2 was shown to be secreted by direct translocation across the plasma membrane in an ATP- and membrane potential-independent manner. I propose that FGF-2 membrane translocation is a diffusion-controlled process that is initiated by FGF-2 binding to the phosphoinositide $PI(4,5)P_2$. Cell surface HSPGs are functioning as an extracellular molecular trap that drives directional transport of FGF-2 across the plasma membrane. As illustrated in Fig. 1, these findings resulted in a current working hypothesis on how FGF-2 physically traverses the plasma membrane.

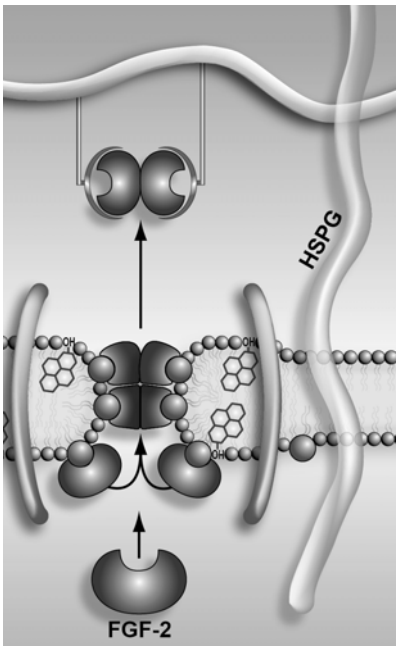

Fig. 1: A current model of the molecular mechanism of FGF-2 secretion.

Moving Outside: Translocation of a 'Cytosolic' Transglutaminase, the A Subunit of Coagulation Factor XIII

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Factor XIII (FXIII) is a plasma Transglutaminase circulating in blood as a heterotetramer consisting of two catalytic A subunits (FXIII-A) and two non-catalytic B subunits (FXIII-B). FXIII-A mainly exists in plasma and the cytoplasma of megakaryocytes/platelets, monocytes/macrophages, and osteoblasts/chondrocytes, etc. When we cloned the cDNA and gene for FXIII-A, we first noticed that there was no signal peptide for secretion; nor could we find any internal signal. The results of the expression of FXIII-A and/or FXIII-B in a heterologous mammalian cell system suggested that FXIII-A is not secreted through the conventional secretory pathway, but is released, at least in vitro, from the cells by cell damage or apoptosis. However, it has been demonstrated that FXIII-A is expressed on the cell surfaces of human monocytes and macrophages (Kradin et al, 1987; Conkling et al. 1989); moreover the cell surface expression of FXIII-A was augmented by macrophage activators. It was also reported that in cultured mouse osteoblasts and chondrocytes FXIII-A is produced as a small processed form (Al-Jallad et al, 2006; Nakano et al, 2007); ascorbic-acid treatment dramatically stimulated the externalization of FXIII-A to the cell surface and to the extracellular matrix. To explore the possible intracellular function(s) and mechanism of extracellular release, we investigated the intracellular localization of FXIII-A using a megakaryocytic cell line, MEG-01. In the endogenously synthesizing cells, three typical localizations of FXIII-A were observed: 1) a diffuse pattern in the cytoplasm; 2) a filamentous structure around the nucleus; and 3) a diffuse pattern in the nucleus. Intracellular FXIII-A partly co-localized and interacted with an intermediate filament protein, vimentin. Furthermore, FXIII-A was also found in the lipid raft fraction (raft) of the plasma membrane, the amount of which varied under culture conditions. FXIII-A had also been detected on the surface of bovine and human platelets. When human washed platelets were activated by thrombin, fibrinogen in non-raft fractions transformed into fibrin and translocated to the raft on the platelet surface. It is important to note that this fibrinogen/fibrin translocation was completely blocked by the addition of cystamine, a potent Transglutaminase inhibitor including activated FXIII (FXIIIa). Moreover, a fibrinogen mutant of Q398A&Q399A, where both the γ－γ cross-linking sites by FXIIIa were destroyed, did not move to the raft, suggesting that FXIII-A is essential for fibrin translocation to the surface. These findings may facilitate our understanding of extracellular release mechanisms and intracellular functions of FXIII-A in the cellular events of megakaryocytes/platelets.

Extracellular function of high mobility group box I protein (HMGB1) secreted from necrotic and non-necrotic cells.

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Tissue damage occurs often in the life of mammals and is usually repaired. Dying cells are swiftly phagocytosed, but before disappearing, they alert surrounding cells to activate homeostatic programs. They release signals that recruit inflammatory cells to the site of injury, promote cell migration and cell division to replace dead cells, and activate the immune system in anticipation of microbial invasion. Many of these events involve high-mobility group box 1 protein (HMGB1), a nuclear protein that is released passively when necrotic cells lose the integrity of their membranes. HMGB1 behaves as a trigger of inflammation, attracting inflammatory cells, and of tissue repair, recruiting stem cells and promoting their proliferation. Moreover, HMGB1 activates dendritic cells (DCs) and promotes their functional maturation and their response to lymph node chemokines. Activated leukocytes actively secrete HMGB1 in the microenvironment. Thus, HMGB1 acts in an autocrine/paracrine fashion and sustains long-term repair and defense programs. DCs secrete HMGB1 several hours after contact with the first maturation stimulus; HMGB1 secretion is critical for their ability to reach the lymph nodes, to sustain the proliferation of antigen-specific T cells, to prevent their activation-dependent apoptosis, and to promote their polarization towards a T-helper 1 phenotype. These immune responses will also be directed against self-antigens that DCs process at the time of injury and can lead to autoimmunity.

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Extracellular function of epimorphin/syntaxin2 as a morphogenic protein

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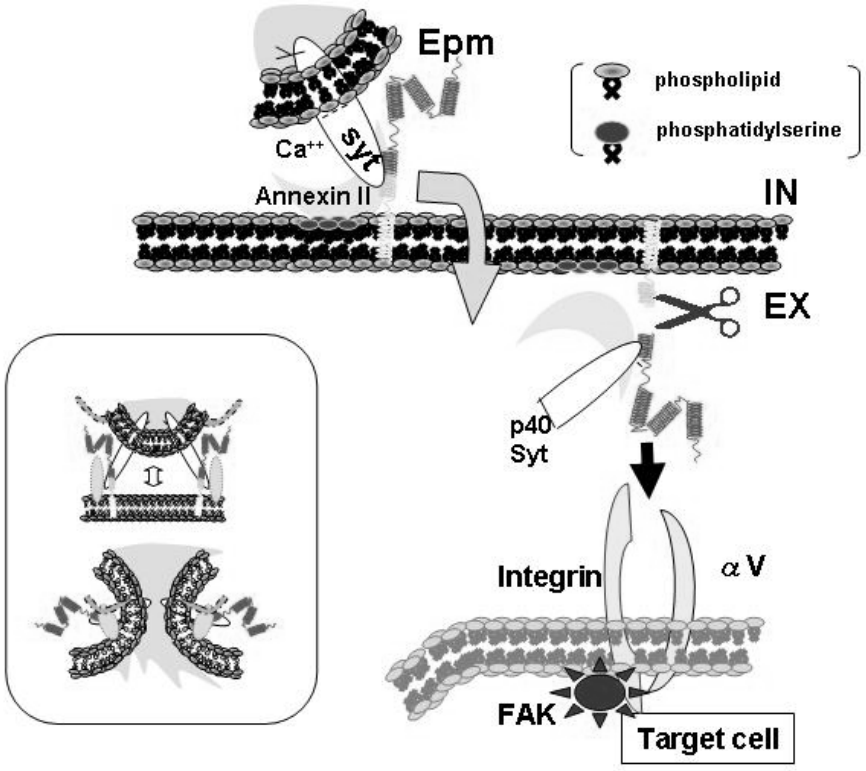
Elucidation of the processes involved in organogenesis requires a detailed understanding of complex spatio-temporal actions of morphoregulatory molecules. The morphogen epimorphin has been shown to function extracellularly in a wide variety of organ developmental processes, although this molecule lacks signal peptide and the major population exists in the cytosol as the vesicle fusion mediator syntaxin2. Here, I show the molecular mechanism underlying epimorphin's temporal extracellular localization, secretion and the following morphoregulatory action for the target epithelial cells. The SNARE domain of epimorphin, which is essential for the cytoplasmic vesicular fusion appeared to play a key role also in the membrane translocation of this molecule. The SNARE domain of epimorphin associates to phosphatidylserine-bound annexin II and synaptotagmin, and the flipping of phosphatidylserine caused by physiological stimuli leads to extracellular projection of the protein complex, which bears the similarity to the non-classical secretion mechanism of another leaderless protein FGF1. Epimorphin is then cleaved at Glu245-His246 and the C-terminally truncated form is released toward αv-integrin receptors on the target cells to activate focal adhesion kinase for their morphogenesis. These findings provide key information about the action of epimorphin in the extracellular milieu and shed light on the sophisticated regulatory mechanism in tissue morphogenesis by a protein of double life.



Secretion and internalization of homeoprotein transcription factors as direct signaling proteins

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Homeogenes encode homeoprotein transcription factors with important developmental and adult functions. Indeed, they are key players within genetic networks that lay out the body plan and regulate morphological and physiological phenomena at the cellular and multi-cellular levels. In the early 1990s, we observed that homeoproteins are internalized by live cells and gain direct access their cytoplasm and nucleus. This finding has led to the identification of a small peptidic domain capable of translocating across biological membranes, thus opening the field of transduction peptides. These initial studies have allowed us and others to establish that homeoproteins share activities that extend beyond transcription to include translation regulation and intercellular transfer. Beyond the mechanistic and fascinating problem of how to get in (and out), it is likely that this novel signaling pathway serves important physiological functions. Based on recent experiments we propose that homeoprotein transfer may be at the origin of homeogenetic extension, boundary formation in developing organisms, and signaling both during development and in the adult. In the context of the nervous system, models will be presented that illustrate how homeoprotein transfer participates in the development of the eye anlagen, the establishment of retino-tectal maps and the regulation of the critical period for binocular vision.

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Internalization mechanism of cell penetrating peptides

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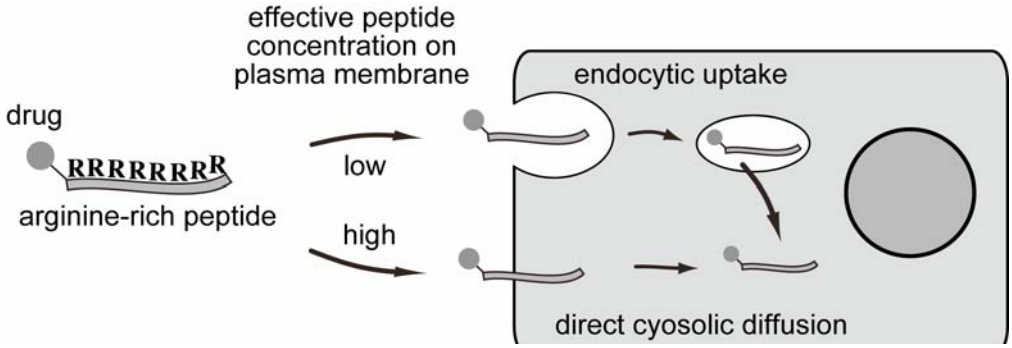
Arginine-rich peptides, including octaarginine (R8) and HIV-1 Tat peptides, belong to one of the major classes of cell-permeable peptides which deliver various proteins and macromolecules to cells. Involvement of the endocytic pathways has recently been demonstrated in the cellular uptake of these peptides. We have shown that macropinocytosis is one of the major pathways for cellular uptake and that organization of the F-actin together with activation of the Rac protein accompanies this process. Significant suppression of macropinocytic uptake and F-actin organization was observed in proteoglycan-deficient CHO cells. These results suggest that efficient internalization of the Tat and other arginine-rich peptides may be induced via interaction with membrane-associated proteoglycans by (i) concentration onto cell surface followed by (ii) stimulated uptake by macropinocytosis stimulated by the interaction of peptides with proteoglycans [1,2].

On the other hand, we have examined the methods of internalization of oligoarginines having different numbers of arginines (Rn, n=8, 12, 16). At relatively low concentrations, microscopic observation of the R12 and R16 peptides predominantly yielded endosome-like puctate signals, while upon raising the peptide concentration, the fractions labeling the cytosol and the amount of the internalized peptides increased dramatically [3]. Interestingly, this cytosolic peptide influx entry does not occur throughout the cell but initiates at defined locations. Detailed real-time observation of the R12 influx into cells performed in this study showed that the peptide influx accompanies the formation of unique ‘particle-like’ structures on the plasma membranes.



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Access map to Noyori Conference Hall

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JR名古屋駅・名鉄新名古屋駅・近鉄名古屋駅からの場合　…　地下鉄東山線藤が丘行きに乗車し、本山駅で地下鉄名城線右回りに乗り換え、名古屋大学駅下車。所要時間約30分（乗換を含む）

JR金山駅・名鉄金山駅からの場合　…　地下鉄名城線左回りに乗車し、名古屋大学駅下車。所要時間約25分

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