Division of Biochemistry
- Biofunctional Design-Chemistry -

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Visitors

Prof PROCHIANTZ, Alain Louis — École Normale Supérieure, France, 24 September 2008
Prof GRASLUND, Astrid — Stockholm University, Sweden, 14 October 2008

Scope of Research

The ultimate goal of our research is the regulation of cellular functions by designed peptides and proteins. Current research subjects include (1) development of novel intracellular delivery systems aiming at elucidation and control of cellular functions using designed membrane permeable peptide vectors, (2) elucidation of the DNA binding and recognition modes of C2H2-type zinc finger proteins and design of artificial transcription factors with various DNA binding specificities, and (3) design of stimulation-responsible artificial peptides and proteins.

Research Activities (Year 2008)

Presentations

"Cellular Dynamics of Cell Penetrating Peptides", Futaki S, 2nd International Symposium "Cellular Delivery of Therapeutic Macromolecules 2008", Cardiff, UK, 24 June 2008.

"Effective Macropinocytosis Induction and Membrane Penetration by FHV Peptide", Nakase I, Hirose H, Takeuchi T, Futaki S, Cell-Penetrating Peptides (CPP) Satellite Symposium of 30th European Peptide Symposium, Helsinki, Finland, 31 August 2008.

"Novel Intracellular Delivery System using pH-Dependent Fusiogenic Peptide", Nakase I, Kobayashi S, Kawabata N, Futaki S, 30th European Peptide Symposium, Helsinki, Finland, 5 September 2008.

"Arginine-Rich Peptides as a Vector of Intracellular Delivery" Futaki S, 3rd International Workshop on Approaches to Single-Cell Analysis, Zurich, Switzerland, 12 September 2008.

"Internalization of Arginine-Rich Peptides into Cells", Futaki S, The 6th China-Japan-Korea Foresight Joint Symposium on Gene Delivery and International Symposium on Biomaterials, Sanya, China, 26 November 2008.

Grants

Futaki S, Chemical Biology in Translocation of Membrane Permeable Peptides into Cells, Grant-in-Aid for Scientific Research (A), 1 April 2007–31 March 2010.

Futaki S, Developing Methodologies of Efficient Intracellular Delivery for Cell Imaging and High-Throughput Analysis, Grant-in-Aid for Scientific Research on Priority Areas, 1 April 2007–31 March 2009.

Imanishi M, Screening and Evaluation of Novel Clock-

Efficient Cellular Uptake of Flock House Virus Derived Arginine-Rich Peptide

Arginine-rich cell penetrating peptides (CPPs), including HIV-1 Tat (48-60) and oligoarginine peptides, have been applied for intracellular delivery of various molecules (e.g., bioactive proteins, peptides, nucleic acids). Macropinocytosis has been shown to be one of the major pathways in the CPP internalization, and we have already reported that interaction of CPPs with membrane-associated proteoglycans leads actin organization and the eventual induction of macropinocytosis.

From the study on cellular uptake efficiency of various DNA/RNA binding peptides rich in arginines, we have found that a peptide derived from flock house virus coat protein [FHV coat (35-49): RRRRNRTRRNRRRVR] internalized ~20 times more efficiently than Tat peptide into Chinese hamster ovary (CHO-K1) cells. The FHV peptide (1 μM) was able to induce similar extent of macropinocytosis to that of the Tat peptide (10 μM), and extent of cell surface adsorption of the FHV peptide was also considerably higher than that of the Tat peptide. Additionally, when the cells were treated with Alexa-labeled FHV peptide (10 μM) for 10 min, the peptide could translocate into cytosol and nucleus efficiently. On the other hand, only endosome-like signals were observed in the case of the Tat peptide by a confocal microscopy. These results suggest that effective macropinocytosis induction by the FHV peptide would lead its efficient internalization.

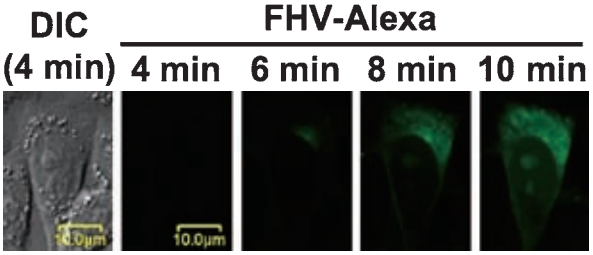

Figure 1. Confocal microscopic observation of CHO-K1 cells treated with Alexa-labeled FHV peptide (10 μM) at 37 °C. Times show incubation periods of the peptide on cells.

Rapid Transcriptional Activity *in Cellulo* and Slow DNA Binding *in Vitro* by Artificial Multi-Zinc Finger Protein

Artificial transcription factors targeting any desired genes are very attractive, but require specific DNA binding domains in order to address a single site for each gene promoter. By connecting various zinc fingers recognizing the corresponding 3- to 4-bp DNA, DNA binding domains for the desired and long sequences can be created. Though such a long sequence recognition is a marvelous property, we have found as the number of finger motifs increases, the equilibrium time with the target sequence is significantly longer as detected by *in vitro* EMSA experiments. In this study, we created 3- and 9-finger type artificial transcription factors, and compared the kinetics of the transcriptional activation *in vivo* as to whether or not a significant delay in the activation is observed for the 9-finger type. By using a ligand-inducing system, we demonstrated for the first time that finger multimerization does not affect the kinetics of the transcriptional activity; the 9-finger type artificial transcription factor activated the reporter gene as quickly as the 3-figner type. Our results suggest that the drawback of finger multimerization, i.e., the equilibrium time is prolonged depending on the number of finger motifs, can be surmounted in terms of its use for transcription factors *in vivo*. There is much interest in creating therapeutic molecules, and these findings suggest the significant potential of multi-zinc finger proteins as a tool for an artificial gene regulator.

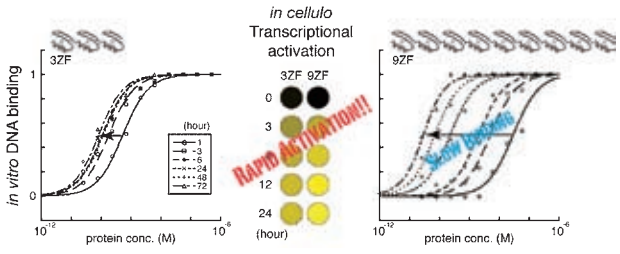

Figure 2. Rapid Transcriptional Activation by an Artificial Multi-Zinc Finger Protein.

Related Proteins Using Zinc-Finger Technology, PRESTO Program, Japan Science and Technology Agency, 1 October 2005–31 March 2009.

Nakase I, Development of New Cell-Targeting Peptides Having Functional Activities for Recognition of Various Proteoglycans on Cell Membrane, Grant-in-Aid for Young Scientist (B), 1 April 2007–31 March 2009.

Award

Imanishi, M, The Best Poster Prize, Chemistry in the New World of Bioengineering and Synthetic Biology (Royal Society of Chemistry), Oxford, UK, 24 September 2008.