Division of Biochemistry
– Biofunctional Design-Chemistry –

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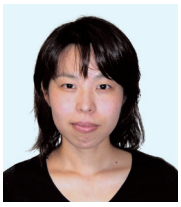

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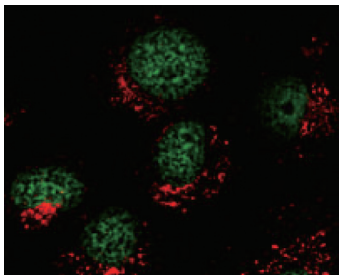
Prof DIBO, Gabor Institute of Chemistry, Eotvos Loránd University, Hungary, 12 December

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.

KEYWORDS

Membrane-Permeable Peptides
Intracellular Delivery
Peptide Design
Zinc Finger Protein



Selected Publications

Imanishi, M.; Nakamura, A.; Doi, M.; Futaki, S.; Okamura, H., Control of Circadian Phase by an Artificial Zinc Finger Transcription Regulator, *Angew. Chem. Int. Ed.*, **50,** 9396-9399 (2011).

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Azuma, Y.; Imanishi, M.; Yoshimura, T.; Kawabata, T.; Futaki, S., Cobalt(II)-Responsive DNA Binding of a GCN4-bZIP Protein Containing Cysteine Residues Functionalized with Iminodiacetic Acid, *Angew. Chem. Int. Ed.*, **48,** 6853-6856 (2009).

Inomata, K.; Ohno, A.; Tochio, H.; Isogai, S.; Tenno, T.; Nakase, I.; Takeuchi, T.; Futaki, S.; Ito, Y.; Hiroaki, H.; Shirakawa, M., High-Resolution Multi-Dimensional NMR Spectroscopy of Proteins in Human Cells, *Nature*, **458,** 106-110 (2009).

Nakase, I.; Hirose, H.; Tanaka, G.; Tadokoro, A.; Kobayashi, S.; Takeuchi, T.; Futaki, S., Cell Surface Accumulation of Flock House Virus-derived Peptide Leads to Efficient Internalization via Macropinocytosis, *Mol. Ther.*, **17,** 1868-1876 (2009).

In Vivo Tumor Accumulation of Arginine-rich Cell-penetrating Peptides and Anticancer Drug Delivery

In this study, we investigated the biodistribution of arginine-rich cell-penetrating peptides (CPPs) in tumor-xenografted nude mice using *in vivo* imaging after the intravenous injection of the fluorescently labeled CPPs. There have been several reports about the *in vivo* distribution of arginine-rich CPPs and their conjugates that often suggest their tendencies to accumulate in some organs. However, few reports have been published that studied the biodistribution of CPPs in tumor-xenografted mice.

Using typical arginine-rich CPPs, this study determined that there is a considerable difference among these peptides regarding their tendencies of tumor accumulation. Tat, Penetratin and octaarginine (R8) showed almost a similar degree of accumulation in the kidney, liver and lung, however, R8 showed a higher accumulation in the tumor xenografts. A comparison of the tendency for tumor accumulation of the oligoarginines composed of different numbers of argninines (R2, R8, R12, and R16) revealed that the R8 peptide showed the highest accumulation in a tumor. A further increase in tumor accumulation was observed by the D-substitution of the amino acids of R8 peptide (r8).

To exemplify the applicability of the r8 peptide for cancer therapy, the conjugate of doxorubicin with r8 was prepared. The r8-doxorubcin conjugate (4 mg doxorubcin/kg) effectively suppressed the tumor proliferation without a decrease in the mouse weight after intravenous injection of the conjugate. A higher dose of doxorubicin (6 mg/kg) was necessary to obtain the same extent of tumor growth suppression effect without conjugation to r8, but this was accompanied by a significant weight loss of the mice. These results suggested the potential of r8 as a prototype of tumor-targeting vectors.

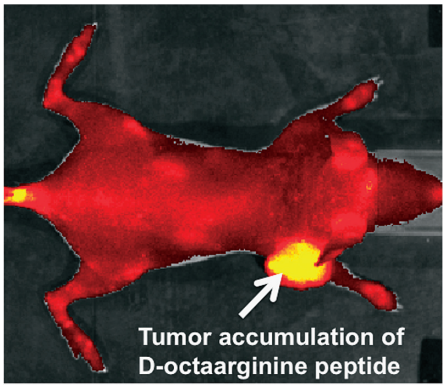

Figure 1. Tumor accumulation of fluorescently labeled r8 peptide intravenously administrated into tumor-bearing mouse.

Control of Circadian Phase by an Artificial Zinc Finger Transcription Regulator

Circadian time originates from clock genes interlocked in transcription/translation feedback loops. In spite of the increase in fundamental knowledge of the circadian molecular machinery in the past decade, practical applications have been so far lacking, and no attempts have been performed in the adjustment of circadian time via a direct action on the core-clock components. Recently, C2H2-type zinc-finger-based artificial DNA binding proteins have seen tremendous development, and can be used to specifically and efficiently manipulate the expression of a target gene.

Here we designed a C2H2-type zinc-finger-based artificial DNA binding protein, ZF(dGRE), that can specifically recognize the glucocorticoid responsive element (GRE) in the regulatory region of the *Period1* gene. A chromatin immunoprecipitation (ChIP) assay indicated that the DNA binding activity and sequence specificity of ZF(dGRE) are functionally preserved even when its DNA target site is chromosomally structured within the cells. To control the time-specificity of our zinc-finger construct, we added the ligand-controllable destabilizing domain and a transcriptional activation domain to ZF(dGRE). The ligand-inducible accumulation of this artificial transcription factor in the nucleus led to the consolidation of circadian rhythms and to circadian phase resetting (Figure 2). To our knowledge, this is the first report of an artificially designed protein that can externally control cellular clock at the genomic level. In addition, our results suggest that *mPeriod1* is a state variable in the generation of circadian rhythms. This artificial transcription factor, which directly controls the clock machinery, potentially opens the way towards novel and effective chronotherapy by tuning circadian time.

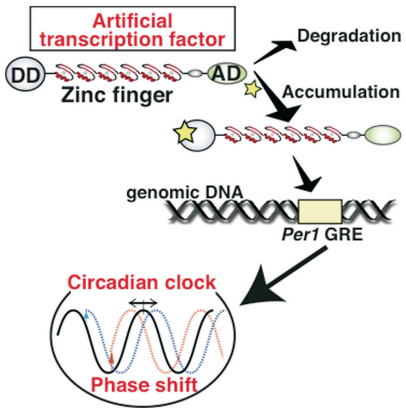

Figure 2. Induction of circadian phase shifts by an artificial transcription factor specifically targeting a glucocorticoid response element of the *mPeriod1* gene.