3P036 C2H2型ジンクフィンガータンパク質、Zic タンパク質の構造とGli 及びGlis タンパク質との系統学的関係の解析
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 A structural dynamics of Zic family proteins, C2H2-type zinc finger proteins, and their relationships with Gli/Glis family proteins
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Zinc finger (ZF) proteins constitute one of the biggest families of transcription factors. The *Zic* genes encode ZF proteins that mediate diverse events during the course of neuronal development. The *Zic*-ZF domains showed high homology to the corresponding domains of the Gli and Glis families, which also contain five C2H2-type ZF motifs and can function as activators or repressors of gene transcription regulating neuronal differentiation. For a better understanding of the molecular mechanism of the function of C2H2-type ZF proteins, we studied the relationships between the *Zic*-, *Glir*-, and *Glis*-ZFs in terms of protein conformation. Secondary structures were examined by means of circular dichroism spectroscopy (J-820, Jasco). The conformational behavior that depends on solution conditions was similar among all three families; but the secondary structures of these proteins differed slightly, and the α -helix contents of the *Zic*-ZFs were slightly smaller than those of the *Glir*- and *Glis*-ZFs. The addition of oligo DNA that included a GLI-binding sequence increased the α -helix content. Comparison of the *Zic*-, *Glir*-, and *Glis*-ZFs indicated that the α -helix content after the addition of oligo DNA correlated well with the affinity of each ZF for the oligo DNA (correlation coefficient, 0.85). These results suggest that the α -helix structure is important for DNA binding and profoundly related to functional and structural diversity among the three families.

3P038 ロドプシン細胞内第3ループと機能的に相同な、ファミリー2 G 蛋白質共役型受容体の細胞内ループの探索
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 The cytoplasmic loop of Family 2 G protein coupled receptor that is functionally homologous to the 3rd loop of rhodopsin
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G protein coupled receptors (GPCRs) are classified into several families by the sequence similarity. We previously reported that the third cytoplasmic loop (L3) of bovine rhodopsin (BvRh) has the similar function to L3 of family 1 GPCRs including muscarinic acetylcholine receptor and adrenergic receptor, and to the second cytoplasmic loop (L2) of metabotropic glutamate receptor in family 3. These insights were obtained by the loop-replacing method, in which we analyzed functional roles of cytoplasmic loops of various receptors by constructing the rhodopsin mutants having these cytoplasmic loops.

In this study, to investigate which of the cytoplasmic loops of family 2 GPCRs exhibits a role functionally homologous to L3 of BvRh, we applied the method to family 2 GPCRs. It has been reported that glucagon-like peptide-1 receptor (GLP-1R) can activate Gi/Go while secretin receptor cannot. We constructed the rhodopsin mutants for these receptors. Among the mutants, the expression levels of the mutants bearing L2 or L3 of the receptors were too low to analyze their G protein activation ability. However, the mutant bearing the first cytoplasmic loop (L1) of GLP-1R activated Gi/Go effectively, whereas the mutant bearing L1 of secretin receptor exhibited little Gi/Go activation. These results suggest that the chimeric receptors preserve G protein coupling selectivity, and that L1 of GLP-1R exhibits the role functionally homologous to L3 of BvRh in G protein activation, though they have little sequence similarity.

3P037 F1-ATPase の ϵ サブユニットのN末端ドメインはC末端ドメインへのATPの結合に影響する
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 N-terminal domain of F₁-ATPase ϵ subunit affects ATP binding to the C-terminal domain
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We have been trying to develop fluorescent ATP probes to visualize ATP within living cells. The ATP probes consist of cyan (CFP) and yellow (YFP) emitting fluorescent proteins, connected by ϵ subunit of *Bacillus* F₁-ATPase. The probes utilize change of fluorescence resonance energy transfer (FRET) efficiency between CFP and YFP upon binding of ATP to ϵ subunit. Probes with ϵ subunit from *Bacillus* sp. PS3 has about a thousand times higher affinity than that from *B. subtilis*. But their amino acid sequences are about 70% identical and amino acid residues involved in ATP binding are conserved. To identify amino acid residues important for determining affinity to ATP, we swapped an N-terminal domain between the two ϵ subunit. I measured fluorescent spectra of chimeric probes at various ATP concentrations. The YFP/CFP ratio showed that the probe with PS3 N-terminal domain (and *B. subtilis* C-terminal domain) has about twenty times higher affinity than the other. This result indicates that N-terminal domain of ϵ subunit influences an affinity to ATP, although the ATP binding site is located at C-terminal domain. We found that the residue at position 60 in N-terminal domain, which is not conserved between PS3 and *B. subtilis*, interacts with position 132 of C-terminal domain. We expected that the interaction between two domains via these residues will influence an affinity to ATP. Now, we are examining whether the affinity changes by substitution of the amino acid pair.

3P039 ラットS100 蛋白質中擬EFハンドの金属結合特性
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 Metal binding property of psudo EF hand in rat S100B protein
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S100 brain protein is one of the calcium-binding proteins in brain, and the characteristic features are especially associated with many diseases such as Alzheimer's disease, Down's syndrome, epilepsy, etc. S100B protein is a member of a group of low molecular weight acidic calcium-binding modulator proteins. They are composed of two distinct EF hands flanked by hydrophobic regions as either terminus and separated by a central hinge region. The C-terminal EF hand is usually referred to as the canonical calcium ion binding loop and encompasses 12 amino acids, whereas the N-terminal loop is formed of 14 amino acids called psudo EF hand and has a lower affinity of calcium ion. However, the interaction of psudo EF hand in S100 protein with metal ions have not addressed in detail. Here, we have designed a synthetic fragment encoding psudo EF hand in S100B protein from rat brain and have overexpressed it in *Escherichia coli*. Here, we will report on the metal binding properties of psudo EF hand domain in S100B protein.