1P091 膜たんぱく質の進化学手法「リボソームディスプレイ法」の構築と実践：リボソームと無細胞翻訳系による α -ヘモリンの *in vitro* 分子進化

Liposome Display: Directed evolution of membrane protein, α hemolysin, by using liposome and cell-free translation system

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In vitro methods have enabled the rapid and efficient evolution of proteins and successful generation of novel and highly functional proteins. However, the available methods deal only with globular proteins (e.g., antibodies and enzymes), and not membrane proteins. Here, we report the development of a method named liposome display that can evolve the properties of membrane proteins entirely *in vitro*. This method, which involves *in vitro* protein synthesis inside liposomes was applied to the pore-forming activity of α -hemolysin (AH), a membrane protein derived from *Staphylococcus aureus*. The obtained AH mutant possessed only two point mutations but exhibited a 30-fold increase in its pore-forming activity compared with the wild-type.

1P092 ニフトリクリプトクロム 1 のヘム結合モチーフ(HRM)の解析

Characterization of HRM in Chicken Cryptochrome1

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Molecular properties of a group of heme-binding proteins are regulated by binding of heme to the heme-regulatory motif (HRM) such as CP motif. Although the heme-binding is not shown, HRM is retained commonly in vertebrate cryptochromes (CRYs), which are known to play a pivotal role in the vertebrate circadian clockwork by closing a transcription-translation-based negative feedback loop as a transcriptional repressor. CRYs are also implied to function as a blue-light photoreceptor or light-driven magnetoreceptor. In order to examine the binding and explore the possible regulatory role of HRM in CRYs, we prepared a partial recombinant protein of chicken CRY1 and its point mutants to examine its heme-binding ability by UV-visible spectroscopy.

1P093 線虫 cytochrome b561 ファミリーの生理機能解析
Analyses on the physiological functions of the cytochrome *b*₅₆₁ protein family in *C.elegans*

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Cytochrome *b*₅₆₁ (b561) is a heme protein residing in neuroendocrine vesicles and regenerates intravesicular ascorbate (AsA) for the biosynthesis of neurotransmitters by transferring electrons from cytosolic AsA to intravesicular monodehydroascorbate radical. In higher animals, there are homologs belonging to the b561 family, but their physiological functions are not known in detail. In this study, we focused on Cecytlb-5 protein, one of seven b561 homologs in *C.elegans*, and found that it has two isoforms, which are different in the length of cytosolic loop. Heterologously expressed Cecytlb-5 proteins using *Pichia pastoris* system showed slightly different visible absorption spectra. To reveal the functions of them, we conducted *in situ* hybridization and RNAi on *C.elegans*.

1P094 線虫 cytochrome b561 ホモログ Cecytlb-1 の機能解析
Analyses on the novel function of Cecytlb-1, a cytochrome b561 homolog in *Caenorhabditis elegans*

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Cytochrome b561 is a membrane protein and has a role in regeneration of vesicular ascorbate (AsA) by a transmembrane electron transfer. Recently, b561 family proteins were found to distribute in eukaryotes. There are seven homologs in *C.elegans*. However, their physiological functions remain unclear. We attempted to clarify the function of Cecytlb-1, the closest homolog to neuroendocrine b561, and showed previously its distribution in muscle tissues. Analysis of the microsomal fraction obtained from *Pichia pastoris* containing the heterologously expressed Cecytlb-1 protein showed a peak at 561 nm in AsA-reduced spectrum. In the present study, we continued to clarify the nature by solubilization of Cecytlb-1 protein with n-octyl- β -D-glucoside.

1P095 Substrate access to slow substrate binding P450cam with mutation at the proposed gate for water egress/ingress from/to the active site

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P450cam, heme-bearing D-camphor (Cam) monooxygenase, contains in the catalytic center waters that are removed by Cam. We have proposed the water egresses via the gate formed by Asp297: Its mutation to Leu drastically slows Cam binding. Here we report x-ray structure of the Cam-free and -partially bound Leu297-mutant. The former exhibits open substrate channel; its top narrower than that of the WT. Analysis by Caver 3.0 program revealed the mutation renders no change in Cam access to heme. The latter retains open channel; Cam anchors to heme by hydrophobic interaction displacing liganded water. Crystallization with DTE closed the channel of the Cam-bound form, indicating normal channel. The present results suggest the mutation does not block the Cam access.

1P096 Heme serves as scaffold for substrate-driven active site structuring in cytochrome P450cam

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P450cam containing heme *b* in the catalytic center catalyzes absolute regio- and stereo-specific hydroxylation of D-camphor (Cam). Exclusive binding of properly oriented Cam to the Cam site achieves the specificity. Cam-free protein exhibiting open substrate channel (open form) changes upon interaction with Cam to the closed channel form (closed form), structuring the active site, although this process, essential to the catalysis, is unknown. Here we report x-ray structure of P450cam in a Cam bound open form, a possible encounter complex. Cam anchors to heme by hydrophobic interaction, displacing liganded water yielding a high-spin state. A loop near the Cam is disordered. The present results suggest heme serves as scaffold for Cam driven transition to the closed form.