1P109 チャネルロドプシンの構造変化におけるカチオンの効果
Structural changes of channelrhodopsin under various cation conditions
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Optogenetics has revolutionized neuroscience, where ion-transporting microbial rhodopsins are utilized as tools for light-induced neuronal excitation and suppression. Channelrhodopsin (ChR), a light-gated cation channel, is used to excite neurons by light. Recently, several chimeric proteins have been designed for better optogenetics application. Here we applied low-temperature spectroscopy to the chimeric ChR, ReaChR (Red-activatable ChR). We found accumulation of photointermediates at low temperatures by UV-vis spectroscopy. Light-induced difference FTIR spectra were measured between dark state and photointermediates in the presence of various cations. Structural changes of ReaChR and its cation effects will be discussed.

1P110 Effect of partial fluorination on bacteriorhodopsin reconstituted in dimerized Di-o-tetradecylphosphatidylcholine vesicle
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A comparative study of bacteriorhodopsin (bR) reconstituted in vesicles composed of a pseudocyclic Di-o-tetradecylphosphatidylcholine (PC-DTPC), which corresponds to a homologous dimeric molecule through a linkage of a single alkyl chain between two DTPC molecules, and of its partially fluorinated analog (PC-F4DTPC) has been performed by using several biophysical techniques. Upon partial fluorination of PC-DTPC, absorption maximum of reconstituted bR shifted from 575 nm to 565 nm and bR trimeric structure was highly stabilized against heat up to ~70°C. Laser flash photolysis experiments showed that after photo-excitation, bR in PC-F4DTPC recovers to the original ground state faster than bR in PC-DTPC. More details of fluorination effect on bR will be discussed.

1P111 Ca^2+-ATPaseの第2膜貫通ヘリックス（M2）とロングレンジの共役
Second Transmembrane Helix (M2) and Long-range Coupling in Ca^{2+}-ATPase
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The Actuator (A) domain of sarcoplasmic reticulum Ca^{2+}-ATPase undergoes large rotational movements that influence the distant transport sites through connections with transmembrane helices M1 and M2. Here we explore the importance of M2 and its junction with the A domain in coupling between Ca^{2+}-transport and ATP hydrolysis, E2P hydrolysis, and dephosphorylated enzyme transition by a series of mutations. Results are most clear with five-glycine insertions. The results pinpoint which parts of M2 control cytoplasm gating and which are critical for luminal gating at each stage, and suggest that proper gate function requires appropriate interactions, tension and/or rigidity in the M2 region at appropriate times for coupling with A-domain movements and catalysis.

1P112 アジ化物結合型チトクロム酸酵素の高分解能X線結晶構造解析によるアジ化物結合様式の解析
High-resolution crystal structural analysis reveals the two azide ions bind to Cytochrome c oxidase in different manner
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Cytochrome c oxidase (CcO) pumps protons coupled with O2 reduction. The azide-bound oxidized resting fast CO has been studied to probe the function of the O2 reduction site. The reported structure of azide-CcO (2.9 Å) shows that one azide ion bridges between CuB and Fea3 in the O2 reduction site. In this study, effect of azide concentration between 2 to 20 mM was examined on the X-ray structure of the O2 reduction site at high resolution (1.9 to 1.7 Å) and the azide binding to CcO was confirmed by absorption spectrum. These results reveal that previously determined electron density assigned as the azide ion is due to that of a peroxide ion. Namely, the peroxide in the O2 reduction site was replaced completely by azide-binding to both CuB and Fea3 at 20 mM azide.

1P113 NDQモチーフを持つpseudo geneの機能復元
Functional restoration of apseudo gene of rhodopsin with NDQ motif
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Pseudo gene is the gene which is not expressed in vivo. However, many pseudo genes have similar sequences to the functional genes. We focused on pseudo genes with the characteristic NDQ motif of the sodium pump rhodopsins. These rhodopsin genes encoded in the locus of pseudo gene must not be expressed because codon-frame shift occurs by the loss of base(s) and mutation(s). We fixed the frame shift of putative pseudo genes of rhodopsins with NDQ motif by introducing identical sequence as sodium pump rhodopsin KR2. As the result, we successfully expressed the rhodopsins. They showed sodium pump functions. However, their expression levels were low and pump activities were weaker than KR2. We discuss the molecular mechanism of the two proteins.

1P114 Role of a unique arginine residue on the assembly of the translocator domain in a trimeric autotransporter
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Haemophilus influenzae adhesin (Hia) belongs to a trimeric autotransporter family and consists of a passenger domain and a translocator domain. The crystal structure of Hia translocator domain (HiaT) has shown that HiaT forms a transmembrane β-barrel of 12 β-strands, four of which are provided from each subunit. This protein has a unique arginine residue at 1077. Arg1077 side chains from three subunits protrude toward the center of the β-barrel and are close to each other. To investigate role of this residue on the trimer assembly and stability, we replaced this arginine with the neutral amino acid, methionine, and the properties of the mutant were investigated. Although the mutation accelerated the reassembly, it seems to compromise the integrity of the trimer.