to 7). Since *C.ele1* is the closest relative to the neural b561, we started analyses from *C.ele1*. The visible absorption spectra of the C.ele1 protein obtained from *P. pastoris* expression system showed characteristic properties as a member of the b561 family and was found to be reducible with AsA. However, immunohistochemical staining using a specific antibody indicated that C.ele1 protein was not expressed in neurons but in muscles. Furthermore, we found that knock-down of *C.ele1* gene did not show any effects on neural functions. These results indicated that *C.ele1* may have important functions in muscles, for the synthesis of collagens by utilizing AsA and, therefore, is a new type in the b561 family. To determine the physiological functions of other members of the b561 family, we are investigating other homologs of *C.elegans*.

3P115 ウシ心筋チトクロム c 酸化酵素のプロトンポンプ経路
Proton pumping pathway of bovine heart cytochrome c oxidase

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Cytochrome c oxidase catalyzes O₂ reduction to water coupled to proton pumping across membrane. X-ray structures of the bovine and bacterial enzymes suggest three proton transfer pathways (D-, K-, and H-pathways), each homologous between the two enzymes. Mutation results of bacterial D-pathway can be categorized into two groups. Glu242Gln and Asp91Asn (bovine numbering) mutations abolish O₂ reduction activity, while Asn98Asp and Asn163Asp (bovine numbering) abolish proton pumping without impairing O₂ reduction activity. These results are taken to support a proposal that D-pathway conveys both water-forming and pumping protons. The amino acid residues essential to D-pathway are conserved between the two enzymes, suggesting functional conservation. D-pathway proton pumping contradicts to our H-pathway proton pumping for the bovine enzyme. Analysis of bovine Glu242Gln and Asp91Asn mutations is now underway, whereas Asn98Asp and Asn163Asp mutations were found not to change the enzymatic activities, which is unexpected considering structural similarity of D-pathway between the two enzymes. Here, to examine any back mutation that restores the function of the mutant bovine/human hybrid enzyme expressed in HeLa cells, we determined DNA sequences of core subunit, subunits I-III. Bovine subunit I gene from chromosomal DNA and human subunits II and III from mitochondrial DNA of HeLa cells did not show any mutation other than those introduced. The present results suggest that bovine D-pathway does not function as a proton pump.

3P116 細胞表面ムチン CD43 は細胞接着阻害を介して微絨毛形成を促進する
Cell surface sialomucin, CD43, induces Microvillous formation via inhibition of cell adhesion.

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I found expression of CD43 molecule induced cell rounding and formation of microvilli in HEK293T cells. CD43 is a cell surface sialomucin rich in various leukocytes, and an established inhibitor for cell adhesion. Indeed, expression of CD43 inhibited integrin-mediated and cell to cell adhesion in HEK293T cells.

Then, how is formation of microvilli induced by CD43? Microvilli generated were cell surface protrusions of 0.1~0.2 micrometer in width, 0.5~5 micrometer in length. These protrusions have CD43, cellular membrane, polymerized actin, and C-terminal phosphorylated Ezrin/Radixin/Moesin (ERM). Since activated ERM have been considered linkers between cell membrane and actin cytoskeleton, mechanisms to recruit ERM to membrane were investigated. As the result, the extracellular domain of CD43, but not the intracellular domain, was responsible for microvillous formation by CD43 despite interaction of ERM with CD43's intracellular domain. Then, how does the extracellular domain induce phosphorylation of ERM was investigated. Since CD43's extracellular domain inhibits cell adhesion, HEK293T cells were trypsinized, inhibited cell adhesion by culturing on BSA-coated dish, and analyzed by anti-phospho-ERM immunoblotting. As the result, detachment from substrata and inhibition of cell adhesion induced phosphorylation of ERM, while integrin-mediated cell adhesion significantly decreased it. These observations strongly suggest CD43 induced phosphorylation of ERM and microvillous formation by inhibition of cell adhesion.

3P117 ウシ心筋 NADH-ユビキノン酸化還元酵素の完全性が二次元結晶の質に及ぼす影響
The integrity of the bovine heart NADH-ubiquinone oxidoreductase responsible for the quality of the 2D crystals.

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NADH-ubiquinone oxidoreductase (Complex I) locates at the entrance of the mitochondrial and bacterial respiratory chains. The enzyme transfers electrons from NADH to ubiquinone, coupled to proton pumping. The mitochondrial enzyme consists of 45 different subunits and is one of the largest known membrane protein complexes with molecular mass of about 1,000kDa. Both the mitochondrial and bacterial enzymes have a characteristic L-shaped structure, with the hydrophobic domain embedded in the membrane and the hydrophilic domain protruding into the mitochondrial matrix or the bacterial cytoplasm. Although the crystal structure of the hydrophilic domain and the whole complex of a bacterial Complex I have been determined at 3.1Å and 4.5Å resolution respectively, the atomic structure of the whole Complex I is not yet known.

We have established the method for purification of Complex I from bovine heart which maintained the sensitivity for specific inhibitors like Piericidin A (PA). Having the sensitivity for PA indicates the integrity of purified enzyme. We have surveyed the 2D crystallization conditions to provide 2D sheet crystals. The enzyme incorporated into the membrane without any crystalline array lost the sensitivity for PA. Only the enzyme in the membrane with crystalline array was maintained the high sensitivity for PA, indicating that the integrity of enzyme is indispensable for obtaining the 2D crystal and the Complex I in 2D crystals is active and intact.

3P118 界面活性剤がウシ心筋複合体 I の 2 次元結晶へ及ぼす影響
The effects of detergents for quality of 2D crystal of bovine heart NADH-ubiquinone oxidoreductase.

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NADH-ubiquinone oxidoreductase (complex I) is a main entry point of mitochondrial and bacterial respiratory chains. Bovine heart complex I is one of the largest known membrane protein complexes with molecular mass of about 1,000 kDa, containing at least 45 different subunits. The enzyme transfers electrons from NADH to ubiquinone, coupled to proton pumping. Recently, whole structure of complex I from *T. thermophilus* was obtained at relatively low resolution (Rouslan G. et al. Nature.).

To understand mechanism of this enzyme, the atomic structure has to be determined.

Using the enzyme preparations purified from bovine heart mitochondria by our improved method, extensive 2D crystallization trials have been conducted by mixing purified enzyme and lipids in the presence of a variety of detergents. The selection of lipid species for 2D crystals was conducted. The ordered crystals were obtained by using the bovine heart phosphatidylcholine (PC). Detergents also play a crucial role, they solubilize lipids which are to be added to a crystallization mixture effectively and they have to be removed from the mixture for making the reconstituted membrane. In this study, the 21 species of detergents for solubilizing PC were examined. Among these detergents, bile acid salts type detergents provided well ordered crystalline array. The best crystals were obtained with cholate. The crystalline sample had sensitivity to specific inhibitor PA, which indicates that complex I in 2D crystal consist of intact enzyme.

3P119 アシル鎖長の異なる 3 種類の脂質膜リポソームに再構成されたバクテリオロドプシンの分子集合状態の変化が引き起こす分子構造・構造安定性への影響
Effect of molecular assembly upon structure and structural stability of bacteriorhodopsin reconstituted into phospholipid liposomes

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Bacteriorhodopsin (bR), which forms 2D-crystalline assembly by nature, is a light-driven proton pump of Halobacteria. BR undergoes irreversible photobleaching, namely the protein denaturation by continuous irradiation of visible light at various conditions in which bR crystalline assembly is melt.