research. Because the labeling technique was still immature, however, conclusions regarding the significance of the different conformations are difficult to make. Here, we describe the preparation of fully active α -actin obtained from a baculovirus expression system for single FRET measurement. We developed α -actin recombinants, of which two domains of the tetramers have specific sites for fluorescent probes. This specific labeling technique offers to significantly expand the information acquired from actin studies.

1P202 セミインタクト細胞とそのリシール技術を用いたメンブレンダイナミクス研究
1C1240 Establishment of semi-intact cell system and application of its resealing technique: a study of membrane dynamics

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Semi-intact cells are cells, whose plasma membranes are permeabilized with detergent or toxins, and are called as “cell-type test tube”. We use streptolysin O (SLO) to permeabilize the cell. SLO-mediated pores are about 30 nm in diameter, which allows the entry of proteins, probes, and small chemical compounds, etc. into cells. By adding the cytosol, which is prepared from the cells under conditions different in cell cycle, differentiation stage, or disease state, we can change the intracellular environment and reconstitute the various physiological phenomena, keeping their experimental conditions synchronized, in semi-intact cells. So far we established the several assays for cell cycle-dependent changes of organelle morphology and membrane traffic by coupling semi-intact cell system with GFP visualization technique, and elucidated its regulatory mechanisms. Recently, it is found that the SLO-mediated pores can be resealed by calcium ion. We confirmed that the resealed cells restored the endocytosis and exocytosis driven by external stimulus, which are difficult to reconstitute in semi-intact cells due to the permeabilization of plasma membrane, and some cells gained the ability to proliferate. In addition, the resealing technique provided us the way to investigate the mechanisms of signal transduction pathways, or the one that takes time such as epigenetic alternation. In this paper, we will show our reconstitution systems of organelle dynamics and membrane traffic using semi-intact or resealed cells.

1P203 ラマンスペクトルで見る酵母と動物細胞のミトコンドリア
Raman spectroscopy of yeast and animal cell mitochondria

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Mitochondrion has a very important role for cell activity because of its ability to make energy in the form of ATP. Various methods for mitochondrial isolation have been established to study its mechanism. With the progress in genetic engineering, new methods to study mitochondria in living cell have also been developed. However, isolation methods may suffer from unknown contamination and genetic analysis is restricted only to specific known species. Raman spectra can be obtained from living cell as well as from isolated mitochondria and contain rich molecular information. In this report, yeast and animal mitochondria are studied using Raman spectroscopy, instead of conventional genetic methods or assays. Isolated and in vivo mitochondria are compared to study the effect of extraction/isolation. Centrifugal separation (which is the most widely used method of mitochondria separation) is used for isolating mitochondria from cells at each stage of the growth curves.

1P204 細菌べん毛蛋白質輸送における FliH ATPase と FliT シャペロンとの相互作用
Interaction between FliH ATPase and a substrate-specific chaperone FliT during bacterial flagellar protein export

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The bacterial flagellum, which is responsible for motility, is a biological nanomachine consisting of a rotary motor, a universal joint and a helical screw. For construction of the flagellum, most of the flagellar proteins are translocated into the central channel of the growing structure by the flagellar protein export apparatus. The proton motive force across the cytoplasmic membrane is utilized as an energy source for protein export. FliH ATPase forms a complex with its regulator FliH in the cytoplasm. The FliH-FliI complex delivers export substrates along with FliJ into the export gate made of integral membrane components of the export apparatus and facilitates the initial entry of the substrates into the narrow pore of the gate. FliT acts as a substrate-chaperone responsible for the filament-capping protein FliD. The FliT-FliD complex directly binds to FliI and FliJ. Our recent structural and functional analyses have revealed that the C-terminal segment of FliT controls its interaction with FliI and FliJ. However, it remains unknown

how it does. In this study, we analyzed the FliI-FliT interaction in more detail. We show that FliT binds to the extreme N-terminal region of FliI with high affinity and to the C-terminal domain with low affinity. We also show that the C-terminal segment of FliT inhibits the interaction with the extreme N-terminal region of FliI. Based on these results, we will discuss the regulatory mechanism of the FliI-FliT interaction during the export of FliD.

1P205 酢酸菌 *Gluconobacter oxydans* におけるべん毛運動の誘導
Induction of the flagellar motility in *Gluconobacter oxydans*, an acetic acid bacterium

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Gluconobacter oxydans, a member of acetic acid bacteria, catalyzes oxidative fermentation such as acetic acid from ethanol and L-sorbose from D-sorbitol. Through such the oxidative fermentation, *G. oxydans* can produce energy as the form of proton motive force. Thus, we hypothesize that energy metabolism is crucial in the oxidative fermentation. Flagellar motility is a major locomotive mean of bacteria driven by the proton motive force. The *G. oxydans* 621H strain has a set of the flagellar genes and swims presumably by its flagella. Here, we examined conditions where the flagellar motility is induced in *G. oxydans*. Swimming fractions of, and production of flagellins in, *G. oxydans* were dependent on concentrations of carbon and energy sources, such as D-glucose, glycerol, and D-sorbitol. Their high concentrations induced the flagellar motility. It was prominent in the late exponential growth phase. Even under low concentrations of carbon and energy sources, the flagellar motility was induced by ethanol, a source of energy convertible to acetic acid. Furthermore, acetic acid itself induced the flagellar motility. These results suggest that the flagellar motility is induced under the conditions with enough energy and that actions of their metabolites are some cues for the induction in *G. oxydans*.

1P206 ペニバチルススのべん毛基部にあるらせん構造体の構造解析
A new spiral structure at the proximal end of *Paenibacillus* flagella

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Paenibacillus strains are Gram-positive bacteria and found in a variety of environmental niches such as soil, foods, tree roots and insect larvae. *Paenibacillus alvei* AUG6 strain, used in this study, was isolated from the oral cavity of a healthy dog. Ordinary motile bacteria generally formed a swarm ring on semi-solid (0.5 %) agar plates and tight colonies on hard (1.5 %) agar plates. On the other hand, *P. alvei* AUG6 strain, on the hard agar plates, formed interspersed punctiform colonies which are collectively called the nebula morphotype. By electron microscopy, we found a specialized spiral structure associated with the hook portion of the flagella. In order to elucidate the structure and function of the spiral structures, we attempted to purify flagella that retain the spiral structures and observed them under the electron microscope. The spiral structures were attached with more than 70 % of the flagellar hooks. The average number of helical turns of the spiral was 5, and the pitch was 12.8±1.6 nm, and the helical diameter was 28.4±1.6 nm. The hook length was 63.6±6.1 nm. Thus, the spiral structure may cover the whole length of the hook (5 x 13 = 65). We also found in the preparation spiral structures that are much longer than those attached to the hook. The number of helical turns of those structures was multiples of five, suggesting that spiral structures dropped off from flagella were polymerized through their ends. We will be discussing the function of the spiral structure in swarming.

1P207 細菌べん毛特異的蛋白質輸送に関わる FliH-FliI 複合体の X 線結晶構造解析
X-ray crystallographic study of the FliH-FliI complex responsible for bacterial flagellar protein export

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The bacterial flagellum acts as a rotary nanomachine, which consists of three parts: the basal body, the hook and the filament. Most of the flagellar proteins are translocated into the central channel of the growing flagellum by the flagellar protein export apparatus. FliH is a cytoplasmic component of the export apparatus. FliH exists as a homodimer and forms a heterotrimeric complex with FliJ ATPase, whose entire structure is similar to the α and β subunits of F₀F₁-ATP synthase. The extreme