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Effect of arginine and its analogs for the Na⁺-driven flagellar motors of alkaliphilic *Bacillus*.

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Some species of alkaliphilic *Bacillus* have Na⁺-driven flagellar motors that convert electrochemical potential energy into mechanical work of rotation. Specific inhibitors are useful for investigating energy transduction systems coupled with ion transport. Amiloride is a potent inhibitor of the Na⁺ channel in epitheliocyte that inhibits Na⁺-driven flagellar motors by competing with Na⁺ at the Na⁺-interaction site of the motors. The functional element of amiloride is protonated guanidino moiety, which is slightly larger than monohydrated Na⁺. We reported that arginine, an amino acid with guanidino moiety, inhibited Na⁺-driven flagellar motors of alkaliphilic *Bacillus* by competing with Na⁺. This result indicated that arginine bound to the Na⁺ interaction site of the motors. There did not appear to be any difference between the effects of L-arginine and D-arginine, so the structure around asymmetric carbon atoms of arginine was not involved in binding to the Na⁺ interaction site of the motors. This implies that the Na⁺ interaction site is located near the entrance, preventing the molecule group around the asymmetric carbon atom of arginine from reaching the area surrounding the Na⁺ interaction site due to the length of the alkyl chain of arginine. We now try to synthesis of derivatives of arginine to determine whether or not the motor will distinguish optical isomers when the alkyl chain of arginine is shortened.

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Loose coupling between chemical reaction and mechanical work in kinesin

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Kinesin is an ATP-driven molecular motor that converts chemical energy into a unidirectional motion, taking forward and backward 8-nm steps along a microtubule. The tight/loose dispute, which argues the stoichiometry between the chemical reaction and mechanical work production by kinesin, is a classical problem that many studies have targeted to solve, however it remains still unclear. Here we challenged this problem by probing ATP dependence of kinesin forward and backward stepping rate. The result showed that both of forward and backward steps are triggered by ATP binding, which strongly suggests a loose coupling between chemical reaction and mechanical work in kinesin. In addition, an external force against the movement of kinesin further strengthened this uncoupling without changing a Michaelis-Menten constant of ATP binding. These results can be explained by a rectified Brownian motion mechanism that enables varied mechanical events, but not a simple power-stroke mechanism that coerces a mechanical reaction rigidly coupled to chemical reaction. The molecular motor might economize energy consumption by using energy of thermal diffusion to make a mechanical motion.

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Gene regulation for a specific inhibition of the typeIII secretion of *Salmonella* virulence factors by monoterpenes of plant essential oils

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Plant essential oils are known to have insecticidal, anti-microbial, anti-fungal, and bactericidal activities, suppressing their growth. Monoterpenes are major components in the volatile oils. We have found that some monoterpenes (citral, linalool, and α -terpineol) specifically inhibited the secretion of virulence factors (Sip proteins) from *Salmonella* typhimurium at the concentration of less than 0.02% (0.2 μ /ml), under which neither growth nor motility was affected. Electron microscopy showed that the number of the TTSS apparatus was considerably reduced. Northern blotting showed that the *sipA* and *invJ* genes were greatly downregulated by the monoterpene treatments, indicating the suppression on the TTSS gene expression. Further analysis using RT-PCR showed that a global regulator gene *hilA* was also downregulated by the treatment. The *hilA* gene is regulated directly by upstream genes (*hilC*, *hilD*, *rtxA*) and some other environment sensor genes (*phoP*, *phoB*, *ompR*, *sirA*, *hilE*, *sirA*, *csrA*, *csrAB*, *lon*, *hupB*, *fis*). We have examined the influence of monoterpenes on the expression level of these genes and discuss possible pathways of the signal transduction triggered by monoterpenes.

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Coupling of Mechano- and ATP Signaling in Mammary Glands Revealed by Imaging of ATP Released from the Tissue

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ATP is now recognized as a ubiquitous extracellular messenger. ATP is often released by mechanical stress of the cells and tissues, and works as a mechano-sensing machinery or functional modulator. In mammary glands, milk filled alveoli contract by oxytocin from posterior pituitary to squeeze out milk. So, they suffer large mechanical stress in physiological conditions. Using primary cultured cells, we found that mammary epithelial cells are highly sensitive to mechanical stimuli such as touch and stretch, and release ATP. The ATP activates P2Y1 in myoepithelial cells and P2Y2 in secretory epithelial cells in surroundings, that forms propagating calcium waves. The contraction of myoepithelial cells by oxytocin was also found to work as a mechanical stress. These findings suggested that myo- and secretory epithelial cells interact mutually via mechanically released ATP. To confirm this, we have developed a real-time ATP imaging system under microscope, using Luciferin-Luciferase bioluminescence and a luminescence-imaging camera. The system also enables simultaneous Nomarski imaging with infrared. We have applied this method to a cultured tissue of mammary glands that maintains the alveolus structure. In this mammary tissue sample, touch induces the ATP-release and the contraction of alveoli. Stretch induces ATP-release dose-dependently. Application of oxytocin also generates ATP-release. These findings clearly show that mechano- and ATP signaling are coupled in mammary glands to enhance or regulate milk secretion.