1P178 嗅上皮に見いだされたリガンド結合タンパク質の研究

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Cp-PLBP: A possible ligand-binding protein found in the olfactory epithelium of *Cynops pyrrhogaster*

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We constructed and investigated a cDNA library of the olfactory epithelium of *Cynops pyrrhogaster*, and found a group of clone at relatively high frequency. This clone encoded a part of an open reading frame composed of 436 amino acids, but the N-terminal part was lacked. The deduced amino acid sequence showed homology to rat RY2G5, which is expressed exclusively in the olfactory epithelium and represented a high homology to bactericidal/permeability-increasing protein of rabbit and lipopolysaccharide-binding protein of human. RY2G5 is, thus, expected to bind lipopolysaccharide. Therefore, we supposed that the isolated clone may encode a protein that binds molecules such as lipopolysaccharide, and tentatively named the clone Cp-PLBP (*Cynops pyrohogaster* Possible Ligand-Binding Protein). According to an *in situ* hybridization analysis of frozen sections of the nasal organ of the newt, Cp-PLBP was expressed in Bowman's gland. The Bowman's gland that express Cp-PLBP, however, distributed in the different pattern from that of Cp-OSP or Cp-Lip, which are the proteins belong to the lipocalin superfamily and found in the newt olfactory epithelium. The results suggest that the distribution of the "ligand binding" proteins is different depend on the part of the mucus layer in the newt nasal organ. To investigate whether Cp-PLBP has binding capacity of molecules or not, we constructed an expression vector of Cp-PLBP and expressed it in *E. coli*. Now we try to purify the expressed protein with a physiological conformation.

1P179 味刺激で誘発される活動電位に依存したマウス味細胞の ATP 放出

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ATP release from mouse taste cells with action potentials in response to a tastant

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Taste cells release neurotransmitters in response to a tastant for sending the signals to gustatory nerve fibers. Recent reports have highlighted the role of ATP as a key neurotransmitter from taste cells to gustatory nerve fibers. However, ATP release properties of taste cells and the mechanisms have not been elucidated. Here we tried to detect tastant-evoked ATP release from single taste cells with action potentials of mouse fungiform papillae. The action potentials were recorded with the electrode basolaterally attached to a single taste cell. The electrode solution was collected and applied for luciferase assay to determine the ATP just after an increase in the action potentials was observed in response to a tastant. To identify taste cells, we used transgenic mice expressing GFP in gustducin-positive (Type II) cells with sweet/bitter/umami taste receptors and no conventional synapses, or GAD67-positive (Type III) cells with putative sour taste receptors and synaptic vesicles. When Type II cells increased the firing rate in response to saccharin, ATP was detected in the electrode solution. The amount of ATP increased in a firing rate-dependent manner. When Type III cells responded to HCl, ATP was below the detection limit of the luciferase assay. The results suggest that the amount of ATP released from single taste cells differ with the response properties, or that taste cells responsive to HCl release another neurotransmitter. Supported by JSPS Grants-in-Aid 18077004, 18109013 (YN) and 19791367 (RY).

1P180 味細胞の細胞型による応答特性の差異

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Differences in response properties among cell types of taste cells

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Taste buds contain four types of cells, Type I ~III and basal cells. Among them, Type II cells express sweet, bitter and umami taste receptors and transduction components but conventional synapses have not been observed in these cells. On the other hand, Type III cells possess synapses but not these taste related molecules. Recently, taste cells expressing markers for Type III cells were shown to be co-expressed with putative sour taste receptors, suggesting some Type III cells may be involved in sour sensation. However, little is known about physiological responses of Type II cells and Type III cells. In this study, we investigated response properties of specified taste cells by means of transgenic mice which express green fluorescent protein (GFP) in gustducin-positive (Type II) and GAD67-positive (Type III) cells. By using a loose patch recording technique, we found spontaneous firing of action potentials in GFP expressing taste cells of both Gustducin-GFP and GAD67-GFP mice. Some of Gustducin-GFP cells responded to saccharin and other sweeteners, suggesting that a part of Type II cells may be sweet taste receptor cells. Some of GAD67-GFP cells showed specific responses to sour taste stimuli such as HCl and citric acid, suggesting that a part of Type III cells may be sour taste receptor cells. These results suggest that both type II and Type III cells may be taste receptor cells that are devoted to different taste qualities. Supported by JSPS Grants-in-Aid 18077004, 18109013 (YN) and 19791367 (RY).

1P181 コレラ菌のタウリン走性に関与する化学受容体ホモログの解析

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A chemoreceptor homolog mediates an attractant response to taurine in *Vibrio cholerae*

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In response to various stimuli, *Vibrio cholerae* controls the rotation of its single polar flagellum to migrate toward more favorable environments, an ability known as chemotaxis which is implicated in pathogenicity as well as survival in various environments. The *V. cholerae* genome is deduced to encode 45 chemoreceptor homologs, which we name methyl-accepting chemotaxis protein-like proteins (MLPs). However, it is largely unknown what kind of stimuli these putative chemoreceptors sense. We have therefore launched to explore the functions of 45 MLPs. We found that a classical biotype strain of *V. cholerae* shows taxis to bile. Among the bile components, taurine attracted the *V. cholerae* strain. Analyses of the putative amino acid chemoreceptors and mutant strains, in which the gene(s) encoding these receptors are deleted, revealed that Mlp37 mediates an attractant response to taurine. Mlp37 may elicit an attractant signal by binding directly to taurine or by binding to some other (periplasmic) protein in complex with taurine. Interestingly, marine Vibrio species (*V. alginolyticus and V. parahaemolyticus*) did not respond to taurine, whereas their genomes contain orthologs of the mlp37 gene. Comparative analyses on Mlp37 and its orthologous proteins are in progress to identify which feature of Mlp37 is responsible for taurine sensing.