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Lrmp/Jaw1 is Expressed in Sweet, Bitter, and Umami Receptor–Expressing Cells

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Abstract

Inositol 1,4,5-triphosphate–mediated calcium (IP_3-Ca^{2+}) signal cascade is an essential process in sweet, bitter, and umami taste signal transduction. Although the main components of this cascade have been identified, the candidate regulators of them in taste tissues are still unclear. In an effort to identify genes involved in taste signal transduction, we found that a gene encoding lymphoid-restricted membrane protein (Lrmp/Jaw1) was expressed in mouse taste tissues. Here we report that Lrmp/Jaw1 is specifically expressed in sweet, bitter, and umami taste receptor–expressing cells of mouse circumvallate, foliate, and fungiform papillae. In addition to this specific expression patterns, we found that Lrmp/Jaw1 is associated with type III IP_3 receptor (IP_3R3) via its coiled-coil domain in the COS7 heterologous expression system. These results raise the possibility that Lrmp/Jaw1 interacts with IP_3R3 in taste cells and suggest an important role for Lrmp/Jaw1 in the IP_3-Ca^{2+} signal cascade in sweet, bitter, and umami taste signal transduction.

Key words: co-immunoprecipitation, coiled-coil domain, immunohistochemistry, in situ hybridization, IP_3R3, taste

Introduction

Taste bud is a peripheral sensory system that receives chemical information on food and drink. In the mammalian tongue epithelium, taste cells occur in taste buds of 3 distinct types in taste papillae (circumvallate, CV; foliate, FL; and fungiform, FF) and respond to sweet, bitter, sour, salty, and umami taste substances.

In this decade, notable progress has been made in understanding the molecular mechanisms of taste signal transduction. Two families of G protein–coupled receptors, taste receptor family 1 and 2 (T1rs and T2rs), have been identified as sweet/umami and bitter taste receptors, respectively (Hoon et al. 1999; Adler et al. 2000; Matsunami et al. 2000; Kitagawa et al. 2001; Max et al. 2001; Sainz et al. 2001; Zhao et al. 2003; Mueller et al. 2005; Chandrashekar et al. 2006). The taste cells expressing T1rs and T2rs also express a number of signaling molecules such as G protein β subunit 3 ($G\beta_3$), G protein γ subunit 13 ($G\gamma_{13}$), phospholipase C β_2 ($PLC\beta_2$), type III inositol 1,4,5-triphosphate receptor (IP_3R3), and transient receptor potential cation channel, subfamily M, member 5 (Trpm5) (Huang et al. 1999; Clapp et al. 2001; Miyoshi et al. 2001; Pérez et al. 2002). It is known that Gβγ subunits activate $PLC\beta_2$ to produce IP_3, which induces calcium (Ca^{2+}) release through IP_3R3 located on the membrane of endoplasmic reticulum (ER). This IP_3-mediated calcium (IP_3-Ca^{2+}) signal cascade is an essential process in taste signal transduction because mice lacking $PLC\beta_2$ or IP_3R3 show a major deficiency in their ability to detect sweet, bitter, and umami taste substances (Zhang et al. 2003; Hisatsune et al. 2007).

The IP_3-Ca^{2+} signal cascade also plays important roles in other tissues in development, proliferation, and neural function processes (Kume et al. 1997; Takei et al. 1998; Itoh et al. 2001). Because IP_3Rs are known as targets of protein–protein interaction, the cascade is involved in complex regulation. For instance, IP_3R-binding protein released with IP_3 and IP_3

agonistically bind to IP_3Rs and determine the threshold of Ca^{2+} release in cerebellar neurons (Ando et al. 2003), and IP_3R-associated cyclic guanonsine monophosphate kinase substrate (IRAG) binds to IP_3Rs via its coiled-coil domain and regulates IP_3-mediated Ca^{2+} release in smooth muscle cells (Schlossmann et al. 2000; Geiselhöringer et al. 2004). In taste cells, calmyrin/CIB1 has been suggested to interact with T1r2 and modulates IP_3-evoked calcium release, recently (Hennigs et al. 2008). However, little is known about the candidate regulators of the IP_3-Ca^{2+} signal cascade.

In our study, we found that Lrmp/Jaw1, a gene encoding lymphoid-restricted membrane protein (Behrens et al. 1994; Hoon and Ryba 1997), was coexpressed with molecules in the IP_3-Ca^{2+} signal cascade in taste cells. Lrmp/Jaw1 was originally identified in lymphoid B and T cell lineages and characterized as an ER resident, type II membrane protein containing a coiled-coil domain (Behrens et al. 1994, 1996). In taste tissues, however, no information regarding the expression patterns and roles exists. In this paper, we describe the selective expression patterns in taste tissues and the novel molecular properties of Lrmp/Jaw1 and discuss its possible role as a candidate regulator of IP_3-Ca^{2+} signal cascade in sweet, bitter, and umami taste signal transduction.

Materials and methods

Construction of a subtracted full-length cDNA library

Eight- to 20-week-old male C57BL/6NCrj mice were treated in accordance with the basic guidelines of the Ministry of Agriculture, Forestry, and Fisheries for laboratory animal study. Mouse tongues were removed, dissected, and placed in Ringer's solution (150 mM NaCl, 4.7 mM KCl, 3.3 mM $CaCl_2$, 0.1 mM $MgCl_2$, 2 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, and 7.8 mM glucose). Ringer's solution containing 2.5 mg/mL collagenase type IV (Worthington Biochemical) and 2 mg/mL elastase (Worthington Biochemical) was injected beneath a CV papilla. After incubation for 15 min at room temperature, the tongue epithelium was peeled and dissected into epithelial fragments with/without taste buds. Total RNA from each tissue was extracted using TRIzol reagent (Invitrogen), and the poly A^+ RNA was isolated by oligo dT affinity chromatography (Invitrogen). cDNA libraries of both samples were prepared from the poly A^+ RNA using SuperScript Plasmid System for cDNA Synthesis and Plasmid Cloning Kit (Invitrogen). Taste bud-enriched, subtracted cDNA library was prepared from the cDNA libraries by normalization and subtraction procedure (Bonaldo et al. 1996).

DNA sequence analyses and annotation

The 5′-end sequence of each clone in the cDNA library was analyzed by multicapillary DNA analysis system, CEQ2000 (Beckman Coulter Inc.). The sequence data were analyzed using the DNASPace program (Hitachi Software) and the UniGene database.

cRNA probes for in situ hybridization

The probe for Lrmp/Jaw1 (1497–1778, GenBank NM_008511) was produced using the cDNA clones in pSPORT1 vector (Invitrogen). The probes for Trpm5 (1–4383, GenBank NM_020277) and $G\beta_3$ (998–1798, GenBank NM_013530) were produced using the cDNA clone in pGEM-T easy vector (Promega Co.). The probes for Mash1, T1r3, and gustducin were prepared as previously described (Kusakabe et al. 2002; Kim et al. 2003). The cRNA probe for single-colored in situ hybridization was prepared using digoxigenin (DIG) RNA Labeling Kit (Roche Diagnostics). For double-colored in situ hybridization, Fluorescein RNA Labeling Kit (Roche Diagnostics) was also used.

In situ hybridization

In situ hybridization experiments were performed as described previously (Kim et al. 2003). Tissues of CV, FL, and FF papillae from 8-week-old male mice were frozen in Optimal Cutting Temperature (OCT) compound (Sakura Finetech USA), sectioned into 5-μm slices, and attached to aminopropyl silane (APS)-coated glass slides. All hybridization reactions were performed at 65 °C in hybridization buffer (50% formamide, 5× standard saline-citrate solution (SSC), 5× Denhardt's solution, 500 μg/mL salmon testis DNA, 250 μg/mL tRNA, and 1 mM dithiothreitol), and the slides were subsequently washed with 0.2× SSC at 65 °C. For single-colored in situ hybridization, signals were detected using alkaline phosphate (AP)-conjugated anti-DIG antibody (Roche Diagnostics; 1:400) in combination with 5-bromo-4-chloro-indolyl-phosphate p-toluidine salt (BCIP) and nitroblue tetrazolium chloride (NBT) (Roche Diagnostics). For double-colored in situ hybridization, signals were detected using AP-conjugated anti-DIG antibody and peroxidase (POD)-conjugated anti-fluorescein antibody (Roche Diagnostics; 1:400) in combination with Streptavidin–Alexa 488 (Molecular Probes)–Tyramide-Biotin (PerkinElmer Life and Analytical Sciences) complex and HNPP/FastRed AP substrate (Roche Diagnostics), respectively. In situ hybridization with sense probes was performed as a negative control. No specific signal was found with any of the sense probes.

Antibodies

Rabbit polyclonal anti-Lrmp/Jaw1 antibody has been raised against peptides (TRKPSLSE, corresponding to amino acid residues 439–446 GenBank NP_032537) conjugated with keyhole limpet hemocyanin (KLH). Mouse monoclonal anti-IP_3R3 antibody (BD Biosciences) and rabbit polyclonal anti-FLAG antibody (Sigma) were purchased. Rhodamine-conjugated anti-rabbit immunoglobulin (IgG) and fluorescein isothiocyanate (FITC)–conjugated anti-mouse IgG polyclonal antibodies (Santa Cruz Biotechnologies Inc.) were used

as the secondary antibodies for immunohistochemistry and immunocytochemistry.

Heterologous expression

The coding region of Lrmp/Jaw1 and its deletion construct (GenBank NM_008511; Δ961–1389) were cloned into the pCMV-Tag 2A mammalian expression vector (Stratagene). The expression plasmid of rat IP_3R3 (Blondel et al. 1993) was kindly provided by Dr Graeme I. Bell. These plasmids were transfected into COS7 cells using LipofectAmine 2000 reagent (Invitrogen). After transfection, cells were cultured in Dulbecco's modified Eagle's medium medium containing 10% fetal bovine serum (Sigma) at 37 °C for 24–48 h.

Immunohistochemistry

Five-micron-thick fresh frozen sections from CV on glass slides were fixed in a 4% paraformaldehyde–phosphate-buffered saline (PBS) for 15 min at room temperature and washed 3 times in PBS. The sections were treated with blocking solution (PBS containing 5% nonimmune horse serum and 0.05% Triton X-100) for 1 h and then incubated overnight at 4 °C with anti-Lrmp/Jaw1 antibody (1:5000 in blocking solution) and anti-IP_3R3 antibody (1:200 in blocking solution). Then, rhodamine-conjugated anti-rabbit IgG and FITC-conjugated anti-mouse IgG polyclonal antibodies (each 1:500 in blocking solution) were applied for 30 min, and the slides were observed using a fluorescence microscope (Leica Microsystems). The images from each staining were merged using Photoshop (Adobe Systems Inc.).

Immunocytochemistry

Transfected COS7 cells cultured on chamber slides (Asahi Techno Glass) were fixed in cold methanol for 10 min at 4 °C. After incubation with anti-IP_3R3 antibody (1:200 in blocking solution) and rabbit polyclonal anti-FLAG antibody (1:200 in blocking solution), the secondary antibodies described above were applied for 30 min. The slides were observed by confocal fluorescence microscopy (Olympus Co.). The images from each staining were merged using Photoshop.

Immunoprecipitation

Immunoprecipitation was performed as described (Harlow and Lane 1988). At 48 h posttransfection, COS7 cells were harvested and lysed in lysis buffer (1% Nonidet P-40, 150 mM NaCl, 50 mM Tris–HCl [pH 7.5], 1 mM ethylenediaminetetraacetic acid, 1 mM phenylmethylsulfonyl fluoride, 10 IU/mL aprotinin, and 10 μg/mL leupeptin) with a micro-homogenizer. The homogenate was centrifuged at 15 000 × *g* for 10 min. The supernatant including soluble and microsomal fraction was incubated with 5 μL of Protein G-Sepharose fast flow beads (Sigma) for 2 h at 4 °C to eliminate nonspecific binding to the beads. The supernatant was then added to mouse monoclonal anti-FLAG M2 antibody affinity gel (Sigma) for 4 h at 4 °C. The affinity gel was washed 5 times with lysis buffer and boiled for 10 min with sodium dodecyl sulfate (SDS) sample buffer (10% glycerol, 6% 2-mercaptoethanol, 50 mM Tris–HCl [pH 6.8], and 2% SDS). The proteins prepared from the affinity gels were subjected to SDS–polyacrylamide gel electrophoresis with a 3–10% gradient gel (ATTO) and transferred onto polyvinylidene difluoride membranes by semidry blotting. After blocking with Block Ace (Dainippon Sumitomo Pharma Co.) for 1 h at room temperature, the membranes were incubated with the rabbit polyclonal anti-FLAG antibody or anti-IP_3R3 antibody (1:1000 in PBS containing 0.05% Triton X-100) for 16 h at 4 °C, followed by POD-conjugated anti-mouse or rabbit IgG (GE Healthcare UK Ltd). Immunoreactive bands were visualized with an enhanced chemiluminescence detection system (GE Healthcare UK Ltd).

Results

Lrmp/Jaw1 was expressed in a subset of taste receptor cells

To identify genes related to taste signal transduction, we isolated and sequenced 600 clones of subtracted cDNA library derived from mouse CV papillae and tongue epithelium. According to sequence annotations, approximately 50 genes predicted to code membrane proteins or signal transduction–related proteins were selected for subsequent in situ hybridization analyses using mouse CV sections. As a result of this process, we found that Lrmp/Jaw1 was distinctly expressed in a specific subset of taste cells (Figure 1). To investigate the expression patterns in detail, we performed in situ hybridization using FL and FF in addition to CV papillae sections. Robust signals were observed in a subset of taste cells of all taste buds in each papilla but not in surrounding tongue epithelium (Figure 1).

Lrmp/Jaw1 was expressed in sweet/umami and bitter taste receptor cells

A taste bud consists of 50–100 taste cells of various functions and developmental stages (Beidler and Smallman 1965; Chandrashekar et al. 2006); thus, the expression patterns could predict the role of a gene in taste tissues. To understand the role of Lrmp/Jaw1, coexpression patterns with other

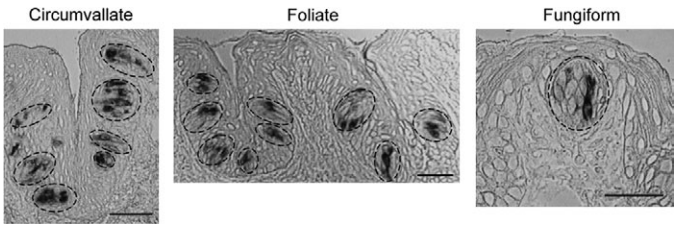

Figure 1 Cellular distribution for mRNA of Lrmp/Jaw1 gene in taste buds. Mouse CV, FL, and FF papillae slices (5 μm) were used for in situ hybridization. The broken lines indicate the outline of each taste bud. The expressions of the genes were visualized by NBT–BCIP. The scale bars indicate 50 μm.

taste-related genes were examined by double-colored in situ hybridization using CV papillae sections (Figure 2a). Trpm5 and $G\beta_3$ were used as a marker for both sweet/umami and bitter taste–responsive cells. Mash1, a basic helix-loop-helix–type transcription factor, was used as a marker for the taste cells that express neural cell adhesion molecule, which do not overlap Trpm5-expressing taste cells (Miura et al. 2005). A component of sweet and umami taste receptor, T1r3, and the taste bud–specific G protein, gustducin, were used as markers for sweet/umami and bitter taste–responsive cells, respectively. Double-colored in situ hybridization showed that Trpm5 was mostly coexpressed with Lrmp/Jaw1 (92.3–94.5%). A similar relationship of expression patterns was observed between Lrmp/Jaw1 and $G\beta_3$ (86.5–92.8%). In contrast, Mash1 was rarely coexpressed with Lrmp/Jaw1 (3.4–3.5%). T1r3 or gustducin-expressing cells were mostly included in a subset of Lrmp/Jaw1-expressing cells (Table 1). The coexpression pattern between Lrmp/Jaw1 and Trpm5 was also observed in FF papillae sections (Figure 2b). These results suggest that Lrmp/Jaw1 was selectively expressed in sweet/umami and bitter taste receptor cells.

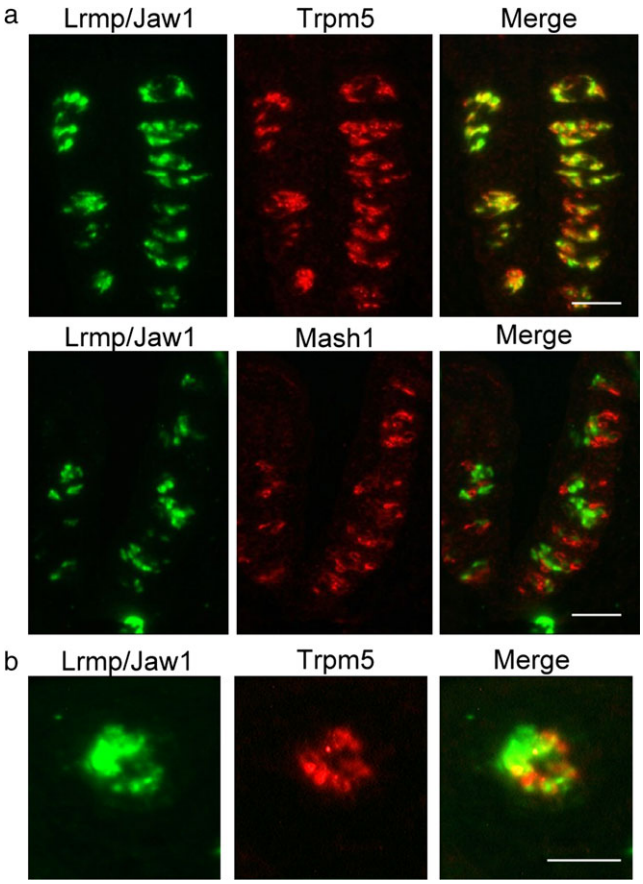

Figure 2 Coexpression patterns of Lrmp/Jaw1 and known taste-related genes. **(a)** Comparison of the mRNA expression pattern of Lrmp/Jaw1 (green) with Trpm5 (red, upper) and Mash1 (red, lower) in CV papillae by double-colored in situ hybridization. **(b)** Comparison of the mRNA expression pattern of Lrmp/Jaw1 (green) with Trpm5 (red) in FF papillae by double-colored in situ hybridization. After merging, colocalization is indicated in yellow. The coexpression ratios in CV papillae are summarized in Table 1. The scale bars indicate 50 μm.

Lrmp/Jaw1 was coexpressed with IP_3R3

It has been reported that Lrmp/Jaw1 has a well-conserved α-helical coiled-coil domain that consists of 140 amino acids in the middle third of the protein and an ER membrane anchor domain in the carboxyl-terminal of the protein (Behrens et al. 1994, 1996). The coiled-coil domain of Lrmp/Jaw1 shared 44% identity with the domain of IRAG, which associates with IP_3R1. Because IP_3R3 was coexpressed with Trpm5 in taste buds (Pérez et al. 2002), we predicted that Lrmp/Jaw1 may colocalize and interact with IP_3R3. To investigate the coexpression patterns of Lrmp/Jaw1 and IP_3R3 at the translational level, we performed double-colored immunohistochemistry using CV papillae sections. As expected, the results showed that almost all Lrmp/Jaw1-expressing taste cells also expressed IP_3R3 in CV papillae (Figure 3).

Lrmp/Jaw1 associated with IP_3R3 in COS7 heterologous expression system

To investigate the protein–protein interaction between Lrmp/Jaw1 and IP_3R3, we utilized the COS7 heterologous expression system and the FLAG epitope–tagged Lrmp/Jaw1 construct with/without a coiled-coil domain (FLAG-Lrmp/Jaw1 and FLAG-Lrmp/Jaw1Δ, Figure 4a). Immunocytochemistry showed that both constructs of Lrmp/Jaw1 were similarly colocalized with IP_3R3 on the ER membrane of transfected COS7 cells, whereas immunoprecipitation experiments showed that FLAG-Lrmp/Jaw1 and FLAG-Lrmp/Jaw1Δ had different molecular properties (Figure 4b,c). We found

Table 1 The coexpression ratio of Lrmp/Jaw1 and taste-related genes in CV papillae

	Trpm5	$G\beta_3$	Mash1	T1r3	Gustducin
(Lrmp/Jaw1)/gene[a]	92.3% (709/768)	86.5% (532/615)	3.5% (11/316)	98.3% (347/353)	85.7% (276/322)
Gene/(Lrmp/Jaw1)[b]	94.5% (709/750)	92.8% (532/573)	3.4% (11/328)	53.1% (347/653)	58.0% (276/476)

Numbers of counted cells were given in parentheses.
[a]The coexpression ratio of the cells expressing both Lrmp/Jaw1 and a taste-related gene per the cells expressing a taste-related gene.
[b]The coexpression ratio of the cells expressing both Lrmp/Jaw1 and a taste-related gene per the cells expressing Lrmp/Jaw1.

that IP_3R3 was co-immunoprecipitated with FLAG-Lrmp/Jaw1 but not with FLAG-Lrmp/Jaw1Δ in the lysate of transfected COS7 cells. Therefore, the deletion of the coiled-coil domain did not affect subcellular localization of Lrmp/Jaw1 and IP_3R3 but altered its interaction with IP_3R3. These results suggest that Lrmp/Jaw1 is associated with IP_3R3 via its coiled-coil domain in the COS7 heterologous expression system.

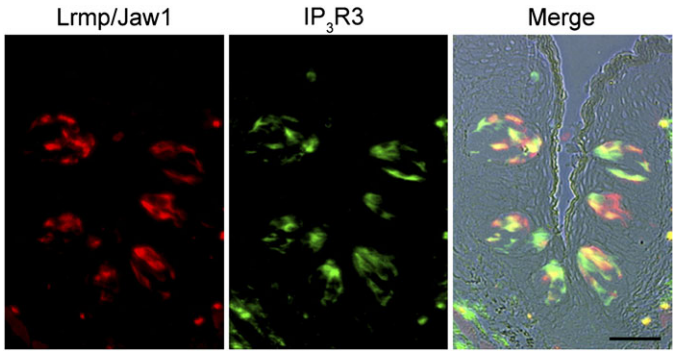

Figure 3 Coexpression patterns of Lrmp/Jaw1 and IP_3R3 in CV papillae. Comparison of the protein expression pattern of Lrmp/Jaw1 (red) and IP_3R3 (green) by double-colored immunohistochemistry. After merging, colocalization is indicated in yellow. The scale bars indicate 50 μm.

Discussion

In this paper, we demonstrated that Lrmp/Jaw1 was selectively expressed in sweet, umami, and bitter taste–responsive cells. Lrmp/Jaw1 was originally identified in lymphoid B and T cell lineages and was assumed to have a role in the development of lymphoid cells (Behrens et al. 1994, 1996). Hoon and Ryba (1997) also reported that Lrmp/Jaw1 was identified in rat CV cDNA library by analysis of partial sequences of clones. These reports, however, did not provide any indications as to the expression patterns and roles of Lrmp/Jaw1 in taste tissues; therefore, we found the expression of Lrmp/Jaw1 in taste cells for the first time. Coexpression of Lrmp/Jaw1 with the molecules related with IP3-Ca^{2+} signal cascade such as $G\beta_3$, Trpm5, and IP_3R3 suggests that Lrmp/Jaw1 is component of the taste mechanism in sweet/umami and bitter receptor cells.

Furthermore, we showed that Lrmp/Jaw1 may engage in direct protein–protein interaction with IP_3R3. A number of IP_3Rs-binding proteins are known to regulate the IP_3-Ca^{2+} signal cascade (Choe and Ehrlich 2006), and their differential distribution is thought to produce various signal transduction in various organs, although little is known about such regulators in taste cells. Our results raise the possibility that Lrmp/Jaw1 regulates the IP_3-Ca^{2+} signal cascade

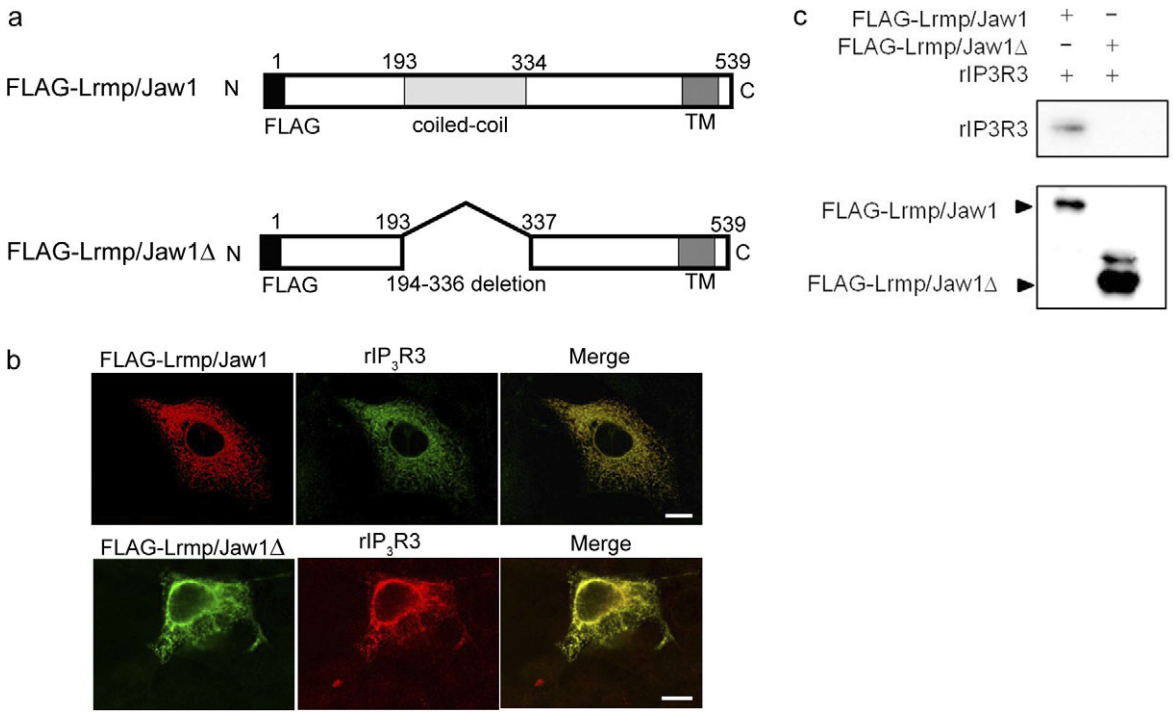

Figure 4 Association between Lrmp/Jaw1 and IP_3R3 in COS7 heterologous expression system. **(a)** Schematic diagrams of FLAG epitope–tagged Lrmp/Jaw1 constructs with/without a coiled-coil domain (FLAG-Lrmp/Jaw1 and FLAG-Lrmp/Jaw1Δ). TM, transmembrane domain, numbers indicate each amino acid residue. **(b)** Comparison of the subcellular localizations of IP_3R3 (green, upper panel; red, lower panel) and FLAG-Lrmp/Jaw1 (red, upper panel) or FLAG-Lrmp/Jaw1Δ (green, lower panel) by double-colored immunocytochemistry. After merging, colocalization is indicated in yellow. The scale bars indicate 10 μm. **(c)** Co-immunoprecipitated proteins with anti-FLAG antibody were detected by anti-IP_3R3 antibody (upper) or anti-FLAG antibody (lower). Arrows indicate the predicted molecular weight (65 kDa for FLAG-Lrmp/Jaw1, 50 kDa for FLAG-Lrmp/Jaw1Δ). The genes transfected in COS7 is indicated by symbols (+ or −).

not only in sweet, umami, and bitter taste–responsive cells but also in other cells such as related lymphoid systems.

The possibility remains that Lrmp/Jaw1 performs an alternative role in taste tissues. Because Lrmp/Jaw1 expression was abundant in mature B, pre-B, and pre-T cell lines, whereas it was low or undetectable in mature T cell lines, Behrens et al. (1994) predicted that Lrmp/Jaw1 could have a role in lymphoid development, such as developmentally regulated intracellular trafficking. It is considered that Lrmp/Jaw1 participates in the trafficking systems in taste cells. However, this is difficult to justify, at least for IP_3R3 trafficking, because deletion of the potential binding domain (coiled-coil domain of Lrmp/Jaw1) did not alter IP_3R3 expression on the ER membrane in the COS7 heterologous expression system. Further investigation using mice lacking Lrmp/Jaw1 would provide insights regarding the role of Lrmp/Jaw1–IP_3R3 interaction in taste tissue in vivo.

It has also been reported that several single nucleotide polymorphisms (SNPs) were detected in the Lrmp/Jaw1 sequence and were possibly associated with the type I diabetes phenotype and the pulmonary adenoma susceptibility phenotype in mice (Grimm et al. 2003; Wang et al. 2003). Thus, future investigation of the relationships between these SNPs and phenotypes may help us to understand the role of Lrmp/Jaw1 in taste tissue.

In summary, the present study showed that Lrmp/Jaw1 was selectively expressed in sweet, bitter, and umami taste–responsive cells. We also showed that Lrmp/Jaw1 may engage in direct protein–protein interaction with IP_3R3. These findings suggest that Lrmp/Jaw1 participates in taste signal transduction by regulating IP_3R3. Although further experiments are needed, we consider that Lrmp/Jaw1 has an important role in sweet, bitter, and umami taste signal transduction by regulating the IP_3-Ca^{2+} signal cascade.

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