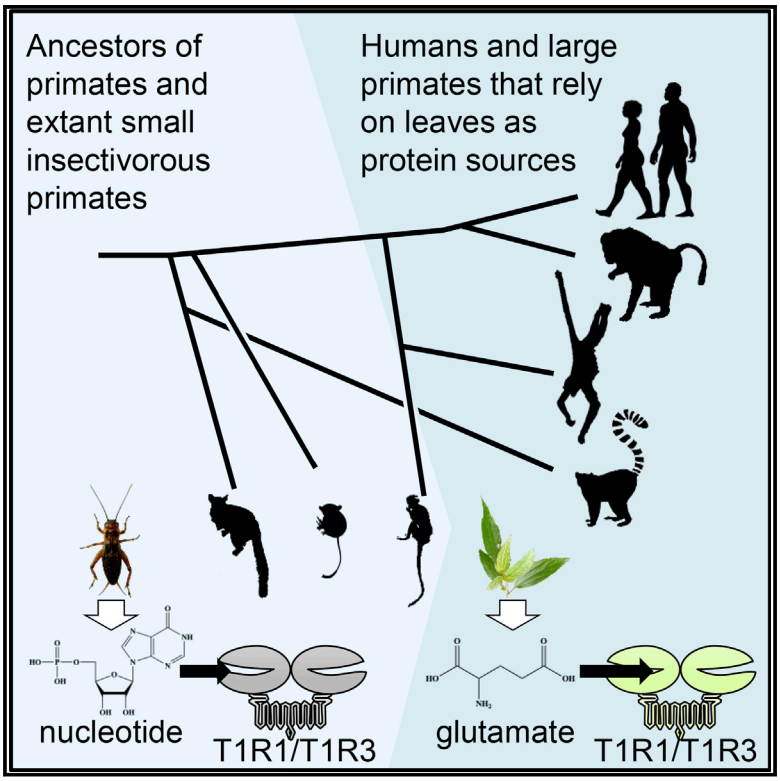
Report

Current Biology

Evolution of the primate glutamate taste sensor from a nucleotide sensor

Graphical abstract



Highlights

- The ancestral type of primate T1R1/T1R3 was sensitive to nucleotides of insects
- T1R1/T1R3 of large primates evolved to detect L-Glu found in folivorous diets
- L-Glu preference might overcome aversive tastes of leaves and promote consumption

Authors

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In brief

Toda et al. investigate the evolutionary plasticity of T1R1/T1R3 for detecting amino acids by using functional, behavioral, phylogenetic, and ecological approaches in diverse primate lineages. T1R1/T1R3 of large primates underwent adaptive evolution that improved their ability to detect L-glutamate, which is a major free amino acid in leafy diets.

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Report

Evolution of the primate glutamate taste sensor from a nucleotide sensor

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SUMMARY

Taste perception plays an essential role in food selection. Umami (savory) tastes are sensed by a taste receptor complex, T1R1/T1R3, that detects proteinogenic amino acids.[1] High sensitivity to L-glutamate (L-Glu) is a characteristic of human T1R1/T1R3, but the T1R1/T1R3 of other vertebrates does not consistently show this L-Glu response.[1,2] Here, we demonstrate that the L-Glu sensitivity of T1R1/T1R3 is a derived state that has evolved repeatedly in large primates that rely on leaves as protein sources, after their divergence from insectivorous ancestors. Receptor expression experiments show that common amino acid substitutions at ligand binding sites that render T1R1/T1R3 sensitive to L-Glu occur independently at least three times in primate evolution. Meanwhile T1R1/T1R3 senses 5′-ribonucleotides as opposed to L-Glu in several mammalian species, including insectivorous primates. Our chemical analysis reveal that L-Glu is one of the major free amino acids in primate diets and that insects, but not leaves, contain large amounts of free 5′-ribonucleotides. Altering the ligand-binding preference of T1R1/T1R3 from 5′-ribonucleotides to L-Glu might promote leaf consumption, overcoming bitter and aversive tastes. Altogether, our results provide insight into the foraging ecology of a diverse mammalian radiation and help reveal how evolution of sensory genes facilitates invasion of new ecological niches.

RESULTS AND DISCUSSION

In vertebrates, palatable tastants are detected by a class-C family of G protein-coupled receptors (GPCRs), T1Rs.[3] The T1R family contains only three receptors, T1R1, T1R2, and T1R3, encoded by *TAS1R1*, *TAS1R2*, and *TAS1R3* genes, respectively, although lineage-specific duplications of *TAS1R* genes have been observed in fish.[4] The T1R1/T1R3 heterodimer is the predominant receptor for the umami (savory) taste in humans,[5] whereas T1R2/T1R3 mediates sweet taste.[3] Retention of these receptors over evolutionary timescales is shaped by feeding ecology. For example, the giant panda, an obligate bamboo-eater, lost function of the *TAS1R1* gene[6] and many obligate carnivorous mammals have lost the *TAS1R2* gene, consistent with lack of sugars in their diet.[7] Birds, which evolved from a presumably carnivorous dinosaur ancestor, and have also lost *TAS1R2* gene,[8] although nectarivorous birds have subsequently acquired the ability to detect sugars by changing the function of their T1R1/T1R3.[8,9]

In humans, monosodium salts of acidic amino acids, especially monosodium L-glutamate (MSG), elicit the perception of umami taste.[10] Consistent with the strong umami taste perception in humans, the human T1R1/T1R3 is highly and specifically sensitive to L-glutamate (L-Glu).[1] In contrast, the mouse T1R1/T1R3 is broadly sensitive to various L-amino acids, but only weakly to acidic amino acids.[1] Members of the present authorship previously reported that this inter-species difference in the L-Glu sensitivity is primarily attributable to the properties of the

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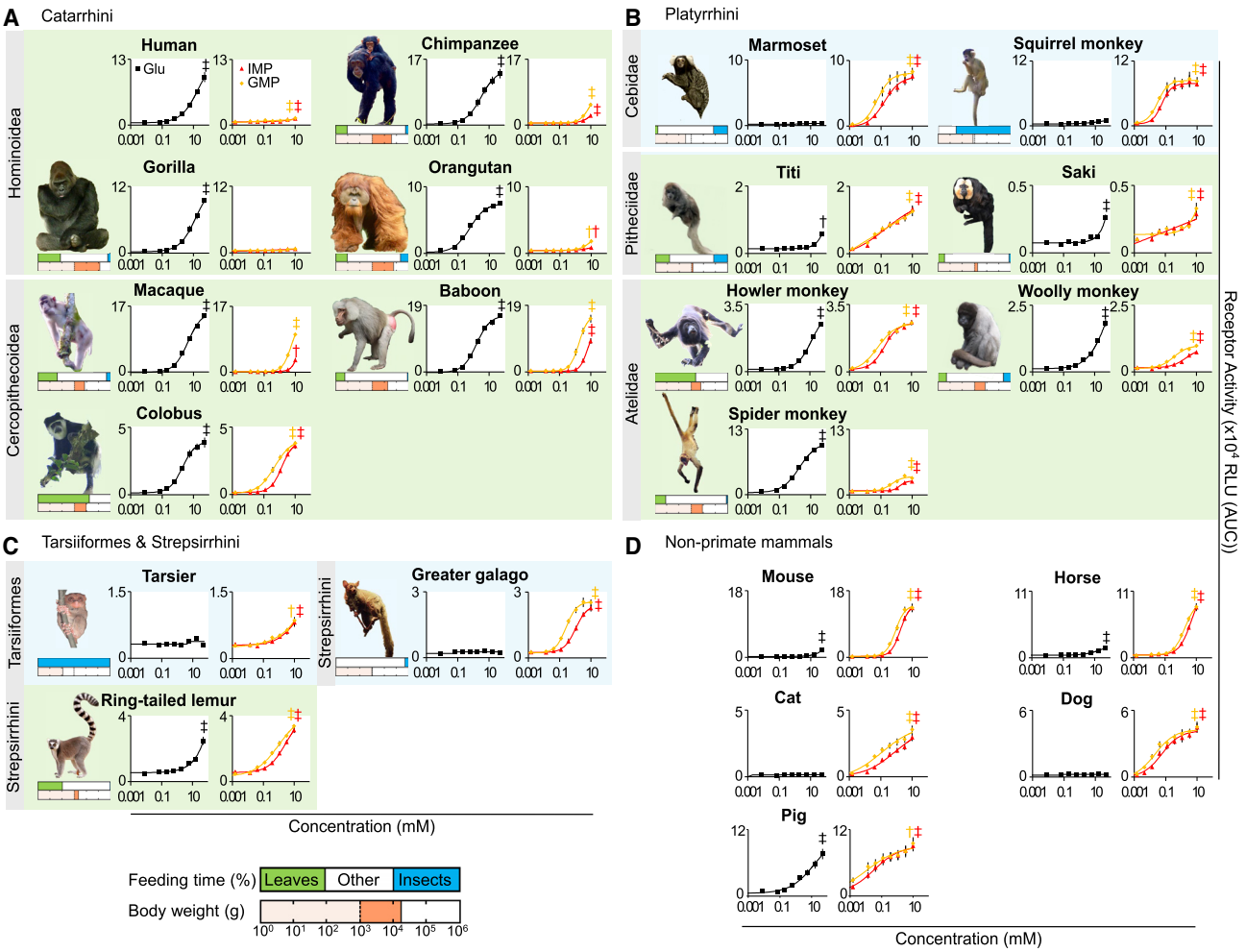

Figure 1. The acquisition of the L-Glu sensor in primates using leaf-based protein

(A–D) T1R1/T1R3 of primates that rely on leaves as their main protein source significantly responded to L-Glu, whereas T1R1/T1R3 of various mammals, including insectivorous primates, displayed strong responses to 5′-ribonucleotides. Dose-dependent responses of T1R1/T1R3 from the species indicated to L-Glu and 5′-ribonucleotides (n = 6, mean ± SE; differences between highest and lowest concentration of each tastant, †$p < 0.01$, ‡$p < 0.001$, Welch's t test). Species primarily using leaves versus insects for protein are shaded green and blue, respectively. Further information of the diet characteristics of the examined primates are shown in Table S1. The body weights are according to previous study.[17]

See also Figure S1.

amino acid binding site, which exists at the large extracellular Venus flytrap domain (VFTD) of T1R1.[11] Receptor expression studies have revealed intriguing variation among primates. High L-Glu affinity was observed in baboon and macaque receptors, but not in the squirrel monkey T1R1, when co-expressed with mouse T1R3.[11] However, the extent of the relationship between the L-Glu sensitivity of T1R1/T1R3 and primate diets remains unknown. Paleontological studies infer that early primates were small and their diets were dominated by insects and fruit,[12,13] although diets of extant species are more variable and also include leaves, gum exudates, mosses, and others.[14] Among these diets, fruit are widely used as a source of carbohydrates, whereas main protein sources are typically either insects or leaves,[15] with larger-bodied primates (> 1 kg) more readily able to digest the latter.[12] Here, we test the evolutionary plasticity of T1R1/T1R3 as a sensor for detecting proteinogenic amino acids by using functional, behavioral, phylogenetic, and ecological approaches in diverse primate lineages, and assess its potential to contribute to the adaptive radiation of mammals.

Ligand shift in the primate T1R1/T1R3

We cloned T1Rs of seventeen primates and five non-primate mammals from various lineages (Table S1). The responses to amino acids were analyzed by using heterologous expression systems.[11] T1R1/T1R3 of all tested catarrhines (human, apes, and African and Asian monkeys), non-cebid platyrrhines (monkeys of the Americas) (titi, saki, howler monkey, woolly monkey, and spider monkey), and the ring-tailed lemur, a strepsirrhine, exhibited significant responses to L-Glu (Figure 1). In contrast, T1R1/T1R3 of the other four primates (marmoset, squirrel monkey, tarsier, and greater galago) exhibited no or only slight responses to L-Glu, up to the highest concentration tested

(50 mM) (Figure 1). Behavioral response data from the previous studies for platyrrhine monkeys show excellent agreement with our functional assay; spider monkeys prefer MSG at approximately the same concentration (2 mM) as the detection threshold in humans, whereas squirrel monkeys show no preference for MSG up to 300 mM solution.[16] All of the primates that exhibited significant L-Glu responses weigh over 1 kg and spend more time feeding on leaves than on insects, whereas the primates with the low L-Glu sensitivity were smaller-bodied primates and do not rely on leaves as main protein sources (Figure 1 and Table S1).

Given that amino acid responses in human and mouse T1R1/T1R3 are strongly potentiated by 5′-ribonucleotides,[1,18] such as inosine monophosphate (IMP) and guanosine 5'-monophosphate (GMP), we also examined the responses to 5′-ribonucleotides. Although a previous study reported that 5′-ribonucleotide alone elicit no response of mouse T1R1/T1R3[1] and 5′-ribonucleotides were considered only as a positive allosteric modulator,[18] T1R1/T1R3 of all tested primates except gorillas was significantly activated by the 5′-ribonucleotide alone (Figure 1). In the primates that rely on insects as protein sources (marmoset, squirrel monkey, tarsier, and greater galago) (Table S1), 5′-ribonucleotides were even more potent than any amino acids tested (Figure S1). The high 5′-ribonucleotide sensitivities of T1R1/T1R3 were also observed in the non-primate mammals (mouse, cat, dog, horse, and pig) (Figure 1). This is concordant with previous electrophysiological studies for canine taste nerves, concluding that 5′-ribonucleotides serve as agonists, whereas L-Glu acts only as a modulator for this taste receptor in dogs.[19,20] A previous ligand binding study using the isolated VFTD also showed that IMP could bind to cat T1R1 in the absence of L-amino acids.[21] Altogether, although 5′-ribonucleotides were considered only as positive allosteric modulators,[1,18] these results indicate that 5′-ribonucleotides do serve as main agonists of T1R1/T1R3 in various mammalian species. Meanwhile, T1R1/T1R3 of some primates, including humans, that gained the high L-Glu sensitivity was not or only slightly activated by 5′-ribonucleotides alone. On the other hand, the synergistic (or at least additive) effect between amino acids and 5′-ribonucleotides was retained among all tested primates (Figure 2).

Key mutations for the L-Glu detection

To understand the genetic underpinning of taste sensitivities and preferences, we investigated critical changes in primate T1R1/T1R3 that contributes to high sensitivity for L-Glu. We previously reported that loss-of-charge mutations at the amino acid residues 170 and 302 in human T1R1 conferred a high affinity for L-Glu because of the elimination of the electrostatic repulsion at the ligand binding pocket[11] (Figure 3B). Humans, macaques, and baboons, which exhibited the high L-Glu sensitivity, have uncharged residues (Ala or Gly) at both 170 and 302.[11] In contrast, squirrel monkeys and mice, which exhibited the low L-Glu sensitivity, have acidic residues in either or both of the two residues, respectively.[11] Among the new primate species we tested in this study, the outgroup of anthropoids (catarrhines and platyrrhines) broadly shared the acidic residues, E170 and D302, and the last common ancestor (LCA) of placental mammals as well as the primate LCA was inferred to have these acidic residues at 170 and 302 (Figure 3A). On the other hand, we discovered that all tested catarrhines as well as spider monkeys and ring-tailed lemurs, which gain protein primarily from leaves, have uncharged residues at both 170 and 302 (Figure 3A). Introducing acidic residues at 170 and 302 of the spider monkey and the ring-tailed lemur T1R1 resulted in a diminished response to L-Glu (Figures 3C and 3D), although these receptors still functional and responded to L-Ala and/or 5′-ribonucleotides (Figure S2), indicating that the loss of charge mutations at 170 and 302, and resulting L-Glu detection, has evolved repeatedly in primates.

In contrast, T1R1/T1R3 of other platyrrhine monkeys except cebids exhibited the significant L-Glu responses (Figure 1) but had an acidic residue at 302 (Figure 3A). There are many studies proposing that the common ancestor of anthropoids had a larger body size and heavier reliance on non-insect foods.[17,22] Considering that a single mutation, spider monkey T1R1-A302D, exhibited the smaller but still significant L-Glu response (Figure 3C), the ancestral anthropoids had presumably gained the small L-Glu sensitivity through the mutation to alanine at 170, and subsequently each of the primary leaf-eaters might have independently evolved additional substitutions to increase their L-Glu sensitivity. In support of this hypothesis, the maximum-likelihood (ML) trees of *TAS1R1* genes using either complete coding sequences or intron sequences showed the monophyly of pitheciids, which exhibited significant L-Glu responses, and cebids, which had the low L-Glu sensitivity (Figure S3). These suggest that the L-Glu sensitivity correlated more strongly with folivory than the sequence similarity of *TAS1R1*.

Another parallel evolution of the loss of charge at 170 and 302 occurred in lemurs (Lemuriformes) (Figure 3A). Like anthropoids, lemurs are remarkably diverse, ranging from the small mouse lemur to the large megaladapid, with associated variation in diet.[14] Lemurs number some ~100 extant species of four families. We anticipate that similar diversities in the evolution in T1R have contributed to the lemuroid adaptive radiation inviting future genetic and functional analysis.

Key residues for nucleotide detection

We find that the acidic mutations at 170 and 302 in spider monkey T1R1 (A170E and A302D) confer significantly higher affinities for 5′-ribonucleotides (Figures 3E and S2; Table S2). These results indicate the possibility that the loss of charges at 170 and 302 contributed to the low sensitivities of 5′-ribonucleotide, which we observed in some large primates (Figure 1). To search for additional residues that affect 5′-ribonucleotide detection, we constructed protein chimeras between spider monkey T1R1 and squirrel monkey T1R1, which retained strong 5′-ribonucleotide responses (Figure S4). Only two squirrel-monkey-type mutations for 302 and 379 in spider monkey T1R1 (A302D and K379T) were needed to impart 5′-ribonucleotide sensitivities as high as those of squirrel monkey T1R1/T1R3 (Figures 3E and S4B), and vice versa (Figures 3F and S4C). Comparing human and mouse receptors, we previously proposed that the residue 379, which exists at the outer side of the ligand binding pocket (Figure 3B), modulates the receptor activity of T1R1/T1R3 and affects the sensitivities of various amino acids.[11] The mouse-type Gly residue at this position caused the higher receptor activity and rendered the human T1R1/T1R3 sensitive to various amino acids.[11] Our present results suggest that the residue 379 affects not only the amino acid sensitivities but also the

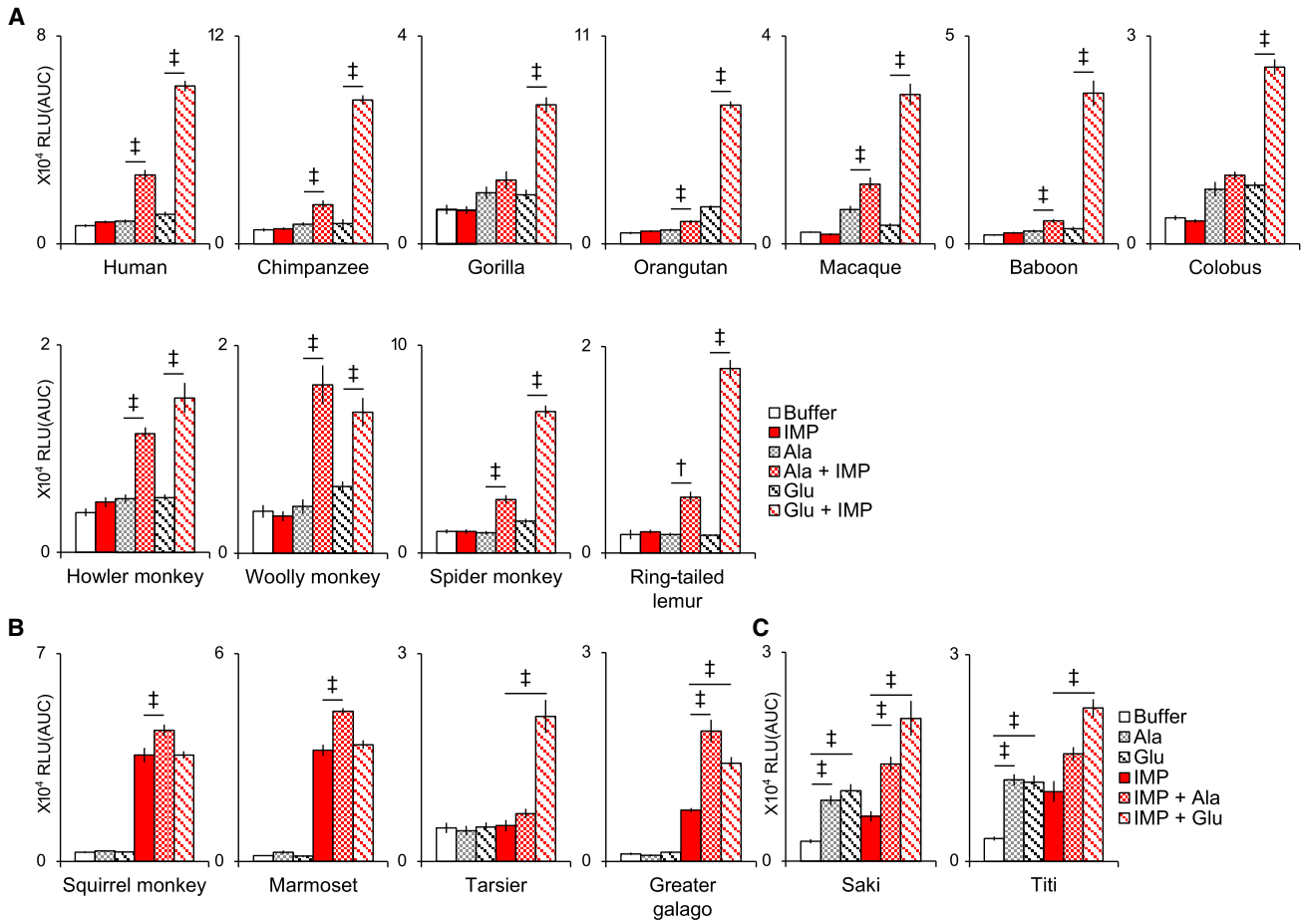

Figure 2. The synergistic effect between amino acids and a 5′-ribonucleotide in primate T1R1/T1R3

(A) Responses of T1R1/T1R3 of primary leaf-eaters to L-Ala or L-Glu in the absence and presence of IMP. The IMP concentration was chosen so that the cellular responses were not significantly increased by IMP alone.

(B and C) Responses of T1R1/T1R3 from insectivorous primates (B) and pitheciids (C) to IMP in the absence and presence of L-Ala or L-Glu. For insectivorous primates (B), the amino acid concentrations were chosen so that the cellular responses were not significantly increased by the amino acid alone. In case of pitheciids, we could not confirm the significant increase when we applied the low concentrations of the amino acids and IMP. To confirm T1R1/T1R3 of pitheciids could be activated by the mixture of the amino acid and IMP at least additively, the mixture of the amino acid and IMP at over the threshold concentrations to activate T1R1/T1R3 was employed for these monkeys (C).

n = 6, mean ± SE, two-sided t test after Benjamini-Hochberg adjustment for differences between with and without IMP (A) or between with and without amino acids (L-Ala or L-Glu) (B, C); $^{\dagger}p \leq 0.01$, $^{\ddagger}p \leq 0.001$. The concentrations (mM) of IMP/L-Ala/L-Glu used in the test were 1/15/0.5 (human), 0.1/5/0.1 (chimpanzee), 0.1/15/0.5 (gorilla), 0.5/1/0.1 (orangutan), 0.1/5/0.1 (macaque), 0.1/1/0.05 (baboon), 0.01/1/0.1 (colobus), 0.01/0.2/0.5 (howler monkey), 0.1/1/1 (woolly monkey), 0.1/1/0.1 (spider monkey), 0.1/1/0.5 (ring-tailed lemur), 0.005/0.01/2 (squirrel monkey), 0.1/0.01/2 (marmoset), 1/15/15 (tarsier), 1/5/5 (galago), 0.5/50/50 (saki), 0.05/10/50 (titi), respectively.

5′-ribonucleotide sensitivities. Remarkably, the primate LCA as well as the LCA of placental mammals was presumed to have G379 (Figure 3A), and the K379G substitution in spider monkey T1R1 also resulted in the higher affinities for both L-Glu and 5′-ribonucleotides (Table S2). Furthermore, the triple mutations of spider monkey T1R1-A170E, A302D, and K379G exhibited higher affinities for the 5′-ribonucleotides than each of the single mutations for these three residues (Figures 3E and S2; Table S2). It appears that the introduction of Ala mutations at 170 and 302, which alone had a small effect on the 5′-ribonucleotide sensitivities, caused a dramatic reduction in 5′-ribonucleotide sensitivities when combined with other mutations, like G379K. Considering the positions of 170, 302, and 379 (Figure 3B), these residues seem not to bind a 5′-ribonucleotide directly but to induce the global conformational changes (e.g., steric and/or electric changes) of the binding pocket. These changes probably prevent a 5′-ribonucleotide from binding to its binding site solely unless an amino acid binds to the hinge region of the binding pocket. Thus a 5′-ribonucleotide functions only as a modulator for some primate receptors. Given that amino acids and 5′-ribonucleotides could activate T1R1/T1R3 synergistically (Figure 2), retaining the high agonist activities of 5′-ribonucleotides was perhaps less important than the acquisition of the high L-Glu sensitivity for the primates that switched their protein source to leaves. However, the small-sized insect-eating ancestors of cebids (marmoset and squirrel monkey)[17,22] appeared to regain the

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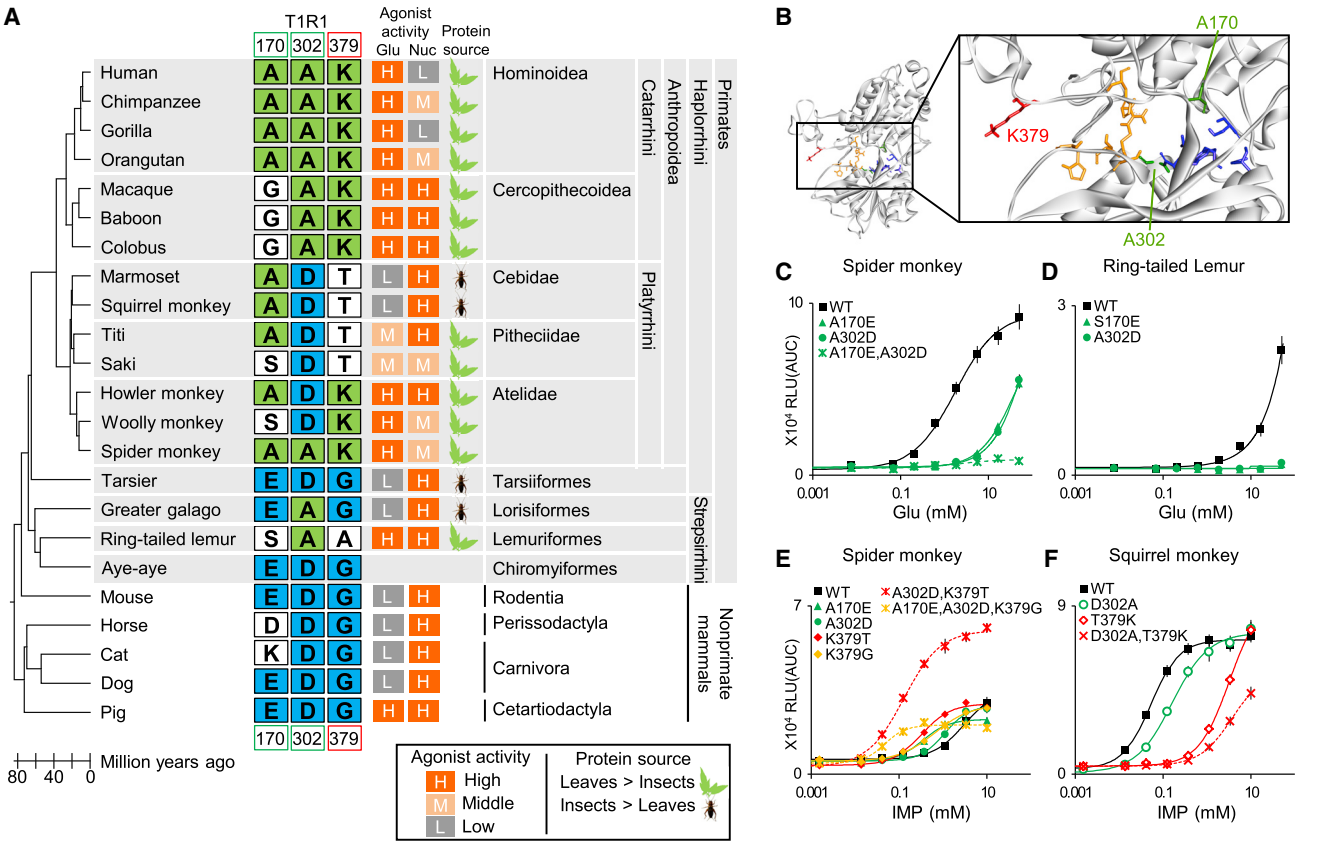

Figure 3. Independent evolution of the high L-Glu response in leaf-eating primates associated with convergent charge-altering residues

(A) A phylogenetic tree of 18 primates, together with 5 non-primate mammals, focusing on T1R1 (*TAS1R1*) evolution. Amino acids at the three T1R1 key sites are shown. The corresponding residues for the three sites are 171, 303, and 380 and 172, 304, and 381 for mouse and horse T1R1, respectively. The agonist activities of L-Glu and a 5′-ribonucleotide were classified as either high, middle, or low on the basis of the responses to each ligand versus the maximum cellular responses (see STAR Methods). The main protein source (leaves versus insects) of each primate is also shown. Further information of the diet characteristics are shown in Table S1. See also Figure S3.

(B) A homology model of the VFTD of human T1R1 shows the ligand binding site for L-amino acids (blue and green)[11,18] and for 5′-ribonucleotides (yellow).[18] The residue 379 is shown in red.

(C and D) Loss of charges at 170 and 302 of T1R1 contributed to the L-Glu detection. Dose-dependent responses of T1R1 mutants and T1R3 from spider monkey (C) and ring-tailed lemur (D) to L-Glu (n = 6, mean ± SE).

(E and F) Loss of charges at 170 and 302 also caused the reduction of the 5′-ribonucleotide sensitivities in cooperation with the mutation at 379. Dose-dependent responses of T1R1 mutants and T1R3 from spider monkey (E) and squirrel monkey (F) to IMP (n = 6, mean ± SE). Figures S2 and S4 and Table S2 provide additional information.

high agonist activities of 5′-ribonucleotides by other mutations, such as K379T (Figures 3E and 3F).

T1R1/T1R3 agonists of primate diets

To examine the relationship between T1R1/T1R3 ligands and diets, we analyzed the occurrence of free L-amino acids and 5′-ribonucleotides in representative primate diets (insects, fruit, and leaves). In both the insects and the plant samples (fruit and leaves), L-Glu was one of the major free amino acids (Figure 4A) and its concentration was almost one order of magnitude higher than the sum of free 5′-ribonucleotides (Figure 4B and Table S3). We also found that all plant samples contained smaller amounts of 5′-ribonucleotides than did insects (Figure 4B). A previous study analyzing the extracts of the plants consumed by ring-tailed lemurs showed that L-Glu was one of the major free amino acids in their diets during their leaf-eating period (> 60% time spent feeding on leaves).[23] Another study, which analyzed free amino acid composition of leaves consumed by howler monkeys, also showed that L-Glu was ranked in the top five in the most commonly consumed leaves (> 10% in their diets in each season).[24] These data indicate that L-Glu could be useful for detecting the protein sources of primate diets and the acquisition of the high L-Glu sensitivity in T1R1/T1R3 contributes to feeding on plant-based diets, which scarcely contain 5′-ribonucleotides, through positive taste associations.

As found in marine arthropods,[25] the most common 5′-ribonucleotide of the insects was adenosine monophosphate (AMP) (Figure 4B). For the cell-based assay, we could not examine AMP responses because of unexpected non-specificity of cellular responses. Instead, to test whether insectivorous primates could show the preference of AMP, we developed two-bottle preference tests for squirrel monkeys (Figure 4C),

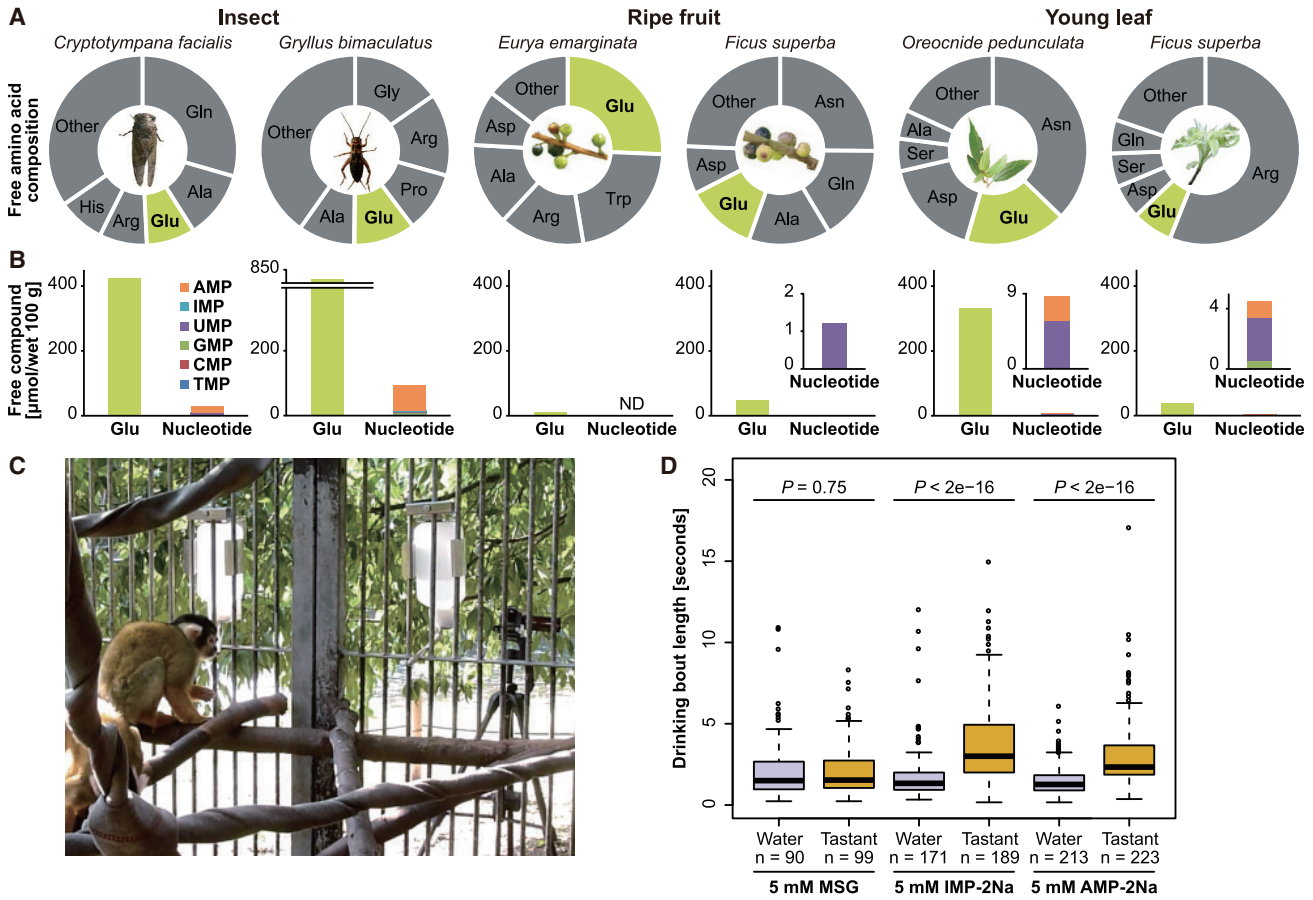

Figure 4. The ancestral nucleotide taste sensor for insect-based diets is not useful to detect folivorous diets

(A and B) Free L-Glu is abundant in all primate diets and free 5′-ribonucleotides are comparatively poor in folivorous diets. Ratio of five top-most abundant amino acids in the detected free amino acids in molarity (A). Contents of free L-Glu and 5′-ribonucleotides in the primate diets (B). See also Table S3.

(C and D) The insectivorous squirrel monkeys displayed behavioral responses to 5′-ribonucleotides. Captive squirrel monkeys (n = 5) were presented with solutions of test stimuli (5 mM MSG, IMP, or AMP) and water (C). The box-and-whisker plot of drinking bout lengths showed that squirrel monkeys prefer 5′-ribonucleotides (IMP and AMP) but not MSG (p values from GLMM) (D). See also Table S4 and Data S1.

which are insectivores and possess T1R1/T1R3 that was highly sensitive to 5′-ribonucleotides (Figures 1 and 3). As predicted, squirrel monkeys showed a strong preference for 5 mM AMP-2Na as well as for IMP-2Na over water ($p < 2e{-}16$; generalized linear mixed-effects model, GLMM), whereas MSG was not preferred to water at the same concentration ($p = 0.75$, GLMM) (Figure 4D). Altogether with the fact that other purine nucleotides, IMP and GMP, could activate T1R1/T1R3 (Figure 1), these suggest that AMP can serve as an agonist of T1R1/T1R3 and enables insectivorous animals to perceive and prefer tastes of insects.

L-Glu sensing for leaf-eating primates

It has been proposed that the primordial class-C GPCR had a high affinity for L-Glu.[26] Our data suggest that early mammals have evolved the T1R1/T1R3 function to detect 5′-ribonucleotides, which contributed to the detection of their insect-based diets. By retaining the synergistic activity between 5′-ribonucleotides and amino acids, T1R1/T1R3 subsequently regained the high L-Glu affinity through mutations at the amino acid binding site independently in several lineages. It is possible that sensitivity to L-Glu facilitated folivory by increasing the palatability of leaves that contain plenty of secondary metabolites,[27] which would be detected by bitter taste receptors (T2Rs).[28] Although bitter taste is believed to detect potentially toxic compounds, humans sometimes prefer bitter foods and beverages with the contribution of other preferable tastes and aroma. For example, L-Glu and L-theanine, which is an amino acid found primarily in green tea, have a considerable effect on tea flavor, and we previously revealed that L-theanine, as well as L-Glu, elicits an umami taste via T1R1/T1R3.[29] It is possible that changing taste receptor function enabled large primates to perceive and prefer the taste of leaves given that we can prefer the taste of green tea. The strong preference for the mixture of L-Glu and 5′-ribonucleotides has perhaps contributed to the detection of not only leaves but also other diets. For example, many primate species consume fermented fruit as fallback foods during periods of low food availability.[30] Because fermentation releases L-Glu and 5′-ribonucleotides, high L-Glu sensitivity and the coincidence detection of 5′-ribonucleotides could affect perception of the products of fermentation[31] and extend periods of fruit exploitation. Such a scenario has been

suggested to contribute to the emergence of the human propensity for fermented foods.[32] Intriguingly, coincidence detection of both L-Glu and 5′-ribonucleotides together is consistent with hypotheses of fermentation detection[31] and the role of fermented foods in shaping the evolution of taste sensitivity might be a profitable area for future research. Furthermore, T1R1/T1R3 expresses not only in taste tissues but also in variety of organs.[33,34] Although the function of T1R1/T1R3 in non-taste tissue remains unclear, the difference in the ligand selectivity between species probably also affect the physiological events other than taste perception.

Evolution of the L-Glu sensor in primary leaf-eating primates might also have occurred in concert or synergistically with other genes such as urate oxidase for nucleotide metabolism[35] and chitinase for insect digestion.[15] Among non-primate mammals that we tested in this study, the pig T1R1/T1R3 showed the high L-Glu responses despite both acidic residues at 170 and 302 (D170 and D302) (Figure 1). Given that piglets are known to display strong preference for MSG,[36] this suggests that mammals outside primates also have a T1R1/T1R3 complex that has evolved to detect L-Glu and is adapted to their diets by distinct molecular mechanisms. We provide strong, multi-faceted evidence that the ancestors of extant primates, including humans, converted their T1R1/T1R3 from a 5′-ribonucleotide sensor into an L-Glu sensor in response to an insectivory-to-folivory dietary transition. This sheds new light on the feeding ecology of a radiational order of mammals and improves understanding of genetic underpinnings facilitating the widespread evolutionary process of niche differentiation.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2021.08.002.

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AUTHOR CONTRIBUTIONS

Y.T. designed the work, performed experiments (DNA work, cellular assay, and behavioral assay), analyzed data, and wrote the paper; T.H. designed the work, collected samples, performed experiments (DNA work, chemical analysis, and behavioral assay), analyzed data, and wrote the paper; Y.K. and A.I. collected samples and analyzed data; T.N., M.H., and R.A. analyzed data; A.D.M. collected samples and wrote the paper; Y.I. and T.M. designed the work and wrote the paper; H.I. and S.K. designed the work, collected samples, and wrote the paper.

DECLARATION OF INTERESTS

The authors declare no competing interest.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
western chimpanzee (*Pan troglodytes verus*)	Primate Research Institute, Kyoto University, Japan	Ayumu; GAIN ID 0608
western gorilla (*Gorilla gorilla*)	Higashiyama Zoo, Japan	Oki; GAIN ID 0005
Sumartan orangutan (*Pongo abelii*)	Higashiyama Zoo, Japan	Baran; GAIN ID 0008
southern pig-tailed macaque (*Macaca nemestrina*)	Sumatra, Indonesia	N/A
Abyssinian colobus (*Colobus guereza*)	Japan Monkey Centre, Japan	Fanta; Pr6353
common marmoset (*Callithrix jacchus*)	Primate Research Institute, Kyoto University, Japan	N/A
Bolivian gray titi monkey (*Plecturocebus donacophilus*)	Primate Research Institute, Kyoto University, Japan	N/A
white-faced saki (*Pithecia pithecia*)	Japan Monkey Centre, Japan	ID: 6038; Pr5605
mantled howler monkey (*Alouatta palliate*)	Kids Saving the Rainforest wildlife rehabilitation society, Costa Rica	N/A
Geoffroy's woolly monkey (*Lagothrix cana*)	Japan Monkey Centre, Japan	Miko; Pr6202
Geoffroy's spider monkey (*Ateles geoffroyi*)	Primate Research Institute, Kyoto University, Japan	N/A
Philippine tarsier (*Carlito syrichta*)	Ueno Zoo, Japan	N/A
greater galago (*Otolemur crassicaudatus*)	Primate Research Institute, Kyoto University, Japan	N/A
ring-tailed lemur (*Lemur catta*)	Japan Monkey Centre, Japan	Yarare; Pr5862
pig (*Sus scrofa*)	Zyagen, CA, USA	Cat#GP-160M
horse (*Equus caballus*)	Zyagen, CA, USA	Cat#GE-170
cat (*Felis catus*)	Zyagen, CA, USA	Cat#GC-130M
dog (*Canis lupus*)	Zyagen, CA, USA	Cat#GD-150M
Cryptotympana facialis	Inuyama, Aichi, Japan	N/A
Gryllus bimaculatus	Mikkabi Cricket, Japan	N/A
Eurya emarginata	Yakushima Island, Japan	N/A
Ficus superba	Yakushima Island, Japan	N/A
Oreocnide pedunculata	Yakushima Island, Japan	N/A
Chemicals, peptides, and recombinant proteins		
coelenterazine	Promega	Cat#S2001
Lipofectamine 2000 Transfection Reagent	Thermo Fisher Scientific	Cat#11668019
bovine serum albumin	Sigma Aldrich	Cat#A8022
Critical commercial assays		
QIAGEN Plasmid Mini Kits	QIAGEN	Cat#12125
Assay Plate, 96 well, Black with Clear Flat Bottom, CellBIND Surface	Corning	Cat#3340
FlexStation pipette tips Black, 96 for FlexStation Systems	Molecular Devices, LLC.	Cat#9000-0911
Deposited data		
chimpanzee T1R1 complete coding sequence	This study	DDBJ: LC616521
chimpanzee T1R3 complete coding sequence	This study	DDBJ: LC616526
gorilla T1R1 complete coding sequence	This study	DDBJ: LC616528
gorilla T1R3 complete coding sequence	This study	DDBJ: LC616530
orangutan T1R1 complete coding sequence	This study	DDBJ: LC616533

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
orangutan T1R3 complete coding sequence	This study	DDBJ: LC616536
macaque T1R1 complete coding sequence	This study	DDBJ: LC616537
macaque T1R3 complete coding sequence	This study	DDBJ: LC616540
baboon T1R1 complete coding sequence	Toda et al.[11]	DDBJ: LC616545
baboon T1R3 complete coding sequence	This study	DDBJ: LC616548
colobus T1R1 complete coding sequence	This study	DDBJ: LC616550
colobus T1R3 complete coding sequence	This study	DDBJ: LC616552
marmoset T1R1 complete coding sequence	This study	DDBJ: LC616555
marmoset T1R3 complete coding sequence	This study	DDBJ: LC616556
squirrel monkey T1R1 complete coding sequence	Toda et al.[11]	DDBJ: LC616557
squirrel monkey T1R3 complete coding sequence	This study	DDBJ: LC616558
titi monkey T1R1 complete coding sequence	This study	DDBJ: LC616560
titi monkey T1R3 complete coding sequence	This study	DDBJ: LC616561
white-faced saki T1R1 complete coding sequence	This study	DDBJ: LC616562
white-faced saki T1R3 complete coding sequence	This study	DDBJ: LC616563
howler monkey T1R1 complete coding sequence	This study	DDBJ: LC616565
howler monkey T1R3 complete coding sequence	This study	DDBJ: LC616566
woolly monkey T1R1 complete coding sequence	This study	DDBJ: LC616567
woolly monkey T1R3 complete coding sequence	This study	DDBJ: LC616570
spider monkey T1R1 complete coding sequence	This study	DDBJ: LC616571
spider monkey T1R3 complete coding sequence	This study	DDBJ: LC616572
tarsier T1R1 complete coding sequence	This study	DDBJ: LC616575
tarsier T1R3 complete coding sequence	This study	DDBJ: LC616576
galago T1R1 complete coding sequence	This study	DDBJ: LC616577
galago T1R3 complete coding sequence	This study	DDBJ: LC616581
ring-tailed lemur T1R1 complete coding sequence	This study	DDBJ: LC616582
ring-tailed lemur T1R3 complete coding sequence	This study	DDBJ: LC616583
pig T1R1 complete coding sequence	This study	DDBJ: LC616588
pig T1R3 complete coding sequence	This study	DDBJ: LC616591
horse T1R1 complete coding sequence	This study	DDBJ: LC616595
horse T1R3 complete coding sequence	This study	DDBJ: LC616598
cat T1R1 complete coding sequence	This study	DDBJ: LC616601
cat T1R3 complete coding sequence	This study	DDBJ: LC616602
dog T1R1 complete coding sequence	This study	DDBJ: LC616603
dog T1R3 complete coding sequence	This study	DDBJ: LC616604
human T1R1 complete coding sequence	NCBI	NM_138697.3
human T1R3 complete coding sequence	NCBI	NM_152228.1
mouse T1R1 complete coding sequence	NCBI	NM_031867.2
mouse T1R3 complete coding sequence	NCBI	NM_031872.2
Experimental models: Cell lines		
HEK293T	The Matsunami Laboratory, Duke University, USA	N/A
Experimental models: Organisms/strains		
Bolivian squirrel monkeys (*Saimiri boliviensis*)	Japan Monkey Centre, Japan	Haney; Bump; Hao; Harley; Hammer
Software and algorithms		
Clampfit v10.4.0.36	Molecular Devices, LLC.	https://www.moleculardevices.com/
SoftMax Pro software v5.4	Molecular Devices, LLC.	https://www.moleculardevices.com/
Discovery Studio Visualizer 2016 v16.1	Dassault Systemes	https://discover.3ds.com/discovery-studio-visualizer-download

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Schrödinger Suite v2019-1	Schrödinger, LLC	https://www.schrodinger.com/
MEGAX v10.0.4	Kumar et al.[37]	https://www.megasoftware.net
MAFFT v6.857b	Katoh et al.[38]	https://mafft.cbrc.jp/alignment/software/
R v > 3.0	N/A	https://www.r-project.org/
lme4 v1.1-10 (R package)	N/A	https://cran.r-project.org/web/packages/lme4/

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Takumi Misaka (amisaka@mail.ecc.u-tokyo.ac.jp).

Materials availability

This study did not generate new unique reagents.

Data and code availability

The accession number for the nucleotide sequences of mammalian *TAS1R1* and *TAS1R3* alleles determined and used in this study are available under the accession numbers DDBJ / GenBank / EMBL: LC616521 - LC616604. No codes were generated by this research.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Information about the animals

Our study used 5 group-reared Bolivian squirrel monkeys (*Saimiri boliviensis*) housed in large indoor-outdoor enclosures at the Japan Monkey Centre (Aichi, Japan). The group consisted of one adult female (named "Haney") and four adult males ("Bump," "Hao," "Harley," and "Hammer"). These males were all sons or grandsons of the female. The individuals were healthy and part of public zoological exhibitions at the Japan Monkey Centre. They were always provided a varied diet scattered in the enclosures and three-dimensional structures for environmental enrichment. They could also visually, vocally, directly interact with another group via a fence for social enrichment.

Information about the cultured cells

HEK293T cells from the Matsunami laboratory were maintained in high glucose concentration (4.5 g/L) Dulbecco's modified Eagle's medium (DMEM, Sigma Aldrich) supplemented with 10% fetal bovine serum (Invitrogen) at 37°C under 5% CO_2.

METHOD DETAILS

Ethics

The use of genetic materials of Japan Monkey Centre was approved by the Research Ethics Committee of the Japan Monkey Centre (#2017-018) and performed in accordance with the Ethical Guidelines for Research at the Japan Monkey Centre (1 April 2016). The use of genetic materials of Primate Research Institute, Kyoto University was approved by the Cooperative Research Program of Primate Research Institute, Kyoto University (#2015-D-20). The Yakushima Forest Ecosystem Conservation Center and Kagoshima Prefectural Government for their permission to conduct research in Yakushima. The behavioral assay in Japan Monkey Centre was approved as a collaborative research project with the Japan Monkey Centre (#2014018) and performed in accordance with the Guidelines for the Care and Use of Nonhuman Primates of Primates Research Institute, Kyoto University (Version 3, issued in 2010). The study was also performed as a collaborative research project with the Ueno Zoological Gardens (No. 31-445).

Cloning primate TAS1R1 and TAS1R3 sequences

Genomic DNA from genetic samples (liver, muscle, blood, cultured cells, and feces) of primates and non-primate mammals were extracted using conventional methods. PCR and Sanger sequencing for whole or partial coding sequences of their *TAS1R1* and *TAS1R3* genes were performed using specific primers designed based on the annotation from whole genome assemblies (based on the criteria in the previous study by members of the present authorship[37]) of their or phylogenetically related species. The coding and intron sequences in *Alouatta palliata* and *Callicebus donacophilus* were determined by capture sequencing of Illumina technology using RNA probes designed based on the annotation of whole genome assemblies of the platyrrhine monkeys (unpublished data). DNA fragments of the estimated coding sequences of *TAS1R1* and *TAS1R3* in these two species were artificially synthesized

by Integrated DNA Technologies, Inc for functional assay. Mammalian *TAS1R1* and *TAS1R3* nucleotide sequences were also retrieved from public databases: the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/); the UCSC Genome Browser (https://genome.ucsc.edu/); the Ensembl Genome Browser (https://www.ensembl.org/). The PCR products of each exon were assembled into one full-length sequence using overlapping PCR and were subcloned into the pEAK10 expression vector (Edge Biosystems, Gaithersburg, MD) at the AscI-NotI site. Chimeric and point mutant receptors were also prepared by PCR using overlapping primers and were subcloned into the pEAK10 expression vector. The Kozak consensus sequences were introduced upstream of the start codon for efficient translation.

T1R functional assay

Responses of T1R1/T1R3 were measured by using heterologous expression systems, as previously described.[11] HEK293T cells were transiently co-transfected with expression vectors for T1R1, T1R3, rat G15i2, and mt-apoclytin-II[11] using Lipofectamine 2000 regent (Invitrogen). After 48 h, the transfected cells were trypsinized, seeded in 96-well black-walled CellBIND surface plates (Corning), and cultured overnight at 37°C in 5% CO_2. After overnight culture, the medium was removed and replaced with coelenterazine loading buffer (10 μM coelenterazine, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 130 mM NaCl, 10 mM glucose, 5 mM KCl, 2 mM $CaCl_2$, and 1.2 mM $MgCl_2$, 0.1% BSA, pH adjusted to 7.4 using NaOH) for 4 h at 27°C in the dark. After 20 s of baseline reading, an aliquot of the assay buffer supplemented with 2X ligand was added, and the light emission was recorded using a FlexStation 3 microplate reader (Molecular Devices) for an additional 90 s. The response from each well was calculated based on the area under the curve (AUC) and expressed as RLU (relative light units). To examine the EC_{50} values, plots of the amplitudes versus concentrations were fitted to Hill equation by using Clampfit ver. 10.4.0.36 (Molecular Devices). Statistically significant increases were determined using Welch's two-sided paired t test; the Benjamini-Hochberg adjustment was used to correct for multiple comparisons (q = 0.01). The agonist activities of L-Glu and 5′-ribonucleotides were determined by calculating the ratio of the L-Glu or GMP response to the maximum cellular response observed among 19 tastants (17 amino acids and 2 5′-ribonucleotides) as the following formula by using the results of Figure S1, and classified as low (< 25%), middle (≥25%, < 50%), or high (≥50%). ("the L-Glu or GMP response"-"the buffer response") / ("the maximum cellular response" - "the buffer response"). In case of non-primate mammals, the responses of the highest and lowest concentration of either L-Glu or GMP on Figure 1 were applied to calculate and classify the agonist activities.

Phylogeny

Multiple alignments of nucleotide and amino-acid sequences were performed using MUSCLE[38] (implemented in MEGAX v10.0.4[39]) and MAFFT v6.857b.[40] Finding the best substitution pattern based on the lowest BIC (Bayesian information criterion) scores,[41] tree reconstruction and inference of ancestral sequences were conducted using MEGAX v10.0.4.[39] First, we constructed a *TAS1R1* gene tree using complete coding sequences, where the 6 exons were concatenated, in a total of 51 representative species of mammalian-wide taxonomy, including 29 primate species. The maximum likelihood (ML) tree (Figure S3) showed that the topology reflected the species tree of mammals as a whole,[42–44] except the three families of platyrrhine monkeys (pitheciids, atelids, and cebids). The tree showed the monophyly of pitheciid and cebid species and located atelids outside the monophyly. The consensus phylogenetic relationship of these three families remains unclear, but previous studies disposed this topology and alternatively accepted the topologies that make pitheciids or cebids the sister group of the other grouped families.[45]

To test whether this incongruence between species and *TAS1R1* gene tree topologies in platyrrhine monkeys is reproducible, the ML tree of *TAS1R1* intron sequences, where the 5 introns were concatenated after performing multiple alignment in each intron using G-INS-i in MAFFT (v6.857b),[40] was constructed in six species of the three platyrrhine monkey families (Figure S3A). The tree confirmed grouping pitheciids and cebids together with the high bootstrap value (81%) like the exon-based tree, indicating that *TAS1R1* in platyrrhine monkeys is under incomplete lineage sorting, that is, the early ancestor of each platyrrhine monkey family shared ancestral polymorphisms of *TAS1R1*.

Divergence time

To depict the phylogenetic tree, the divergence time of each taxon was based on the previous phylogenetic studies.[42–44,46] To calculate the divergence time between the atelid group and the two other platyrrhine monkey groups in the *TAS1R1* gene, RelTime-ML[39] was performed using the *TAS1R1* intron-based tree with calibration of 20.0-23.7 Mya as the divergence time of the last common ancestor of extant platyrrhine monkeys[43] (Figures 3A and S3B).

Literature survey of primate diet

Dietary patterns (i.e., the time spent for feeding or foraging each diet item in the wild) of the primate species analyzed for the functional assay were surveyed from literature of long-term field studies (Table S1). First, the list of dietary patterns was summed up from a review[47] based on published papers. For species whose dietary patterns were not reported there, other published papers were surveyed. Since there were few diet studies in *Macaca nemestrina*, *Papio hamadryas*, *Lagothrix cana*, and *Plecturocebus donacophilus*, the dietary patterns in species of the same genus were surveyed. There was no dietary report specific to *Carlito syrichta*, but any feeding ecology studies have not found evidence of substantial consumption of diet other than invertebrate prey in tarsiers. Therefore, the dietary pattern of *Carlito syrichta* was regarded as 100% time spent for insect eating.

Time averages of multiple studies in the same species (or genus) were calculated. Diet item categories were recategorized as "fruit" (including seeds), "leaves" (discriminated from terrestrial plant items such as grasses and herbs), "insects" (including any animal matter such as invertebrates and small vertebrates), "gum," "THV (terrestrial herbaceous vegetation)," and "MISC" (miscellaneous dietary items, which consequently make up < 15% of the diet, e.g., flowers, fungi, and soil).

T1R1/T1R3 agonists in primate diet

We collected representative dietary items consumed by non-human primates to analyze the composition of free amino acids and 5′-ribonucleotides. We sampled three diet types, i.e., insects, fruit, and leaves. Both ripe and unripe fruit and mature and immature leaves were analyzed. This is because primates generally prefer ripe fruit and young leaves to unripe ones and mature ones, respectively, due to their nutritive composition and low toxicity.[48] As key prey items in the diet of insectivorous primates, species from the families Hemiptera and Orthoptera were selected for chemical analysis.[49] Three wild cicadas (*Cryptotympana facialis*, Hemiptera) (11.2408 g wet weight in total) were directly collected in Inuyama, Aichi, Japan. 50 farmed crickets (*Gryllus bimaculatus,* Orthoptera) (17.3757 g wet weight in total) were purchased commercially. They were immediately frozen by liquid nitrogen and stored in a deep freezer (−80°C).

Natural plant samples from the diet of wild macaques (*Macaca fuscata yakui)* (> 15 g wet weight in total in each sample were collected in Yakushima Island (30°N, 130°E).[50] Sample collection was conducted outside of areas registered as a World Heritage Site due to environmental resource use restrictions. Unripe and ripe fruits of *Eurya emarginata* (Pentaphylacaceae) and *Ficus superba* (Moraceae) were collected on November 2015 and May 2016, respectively. Young and mature leaves of *F. superba* and *Oreocnide pedunculata* (Urticaceae) were collected on October 2015 and April 2016, respectively. These plant samples were transported to an on-site field station freezer (−30°C) in Yakushima as soon as possible, followed by 1-day shipment using a cold chain (−15°C) to the laboratory, and stored in a deep freezer (−80°C).

Frozen insect and plant samples were freeze-dried. Weights of dried samples were measured for the calculation of moisture. Quantification of 20 free proteinogenic amino acids and 6 free nucleoside monophosphate contained in these samples were outsourced to Japan Food Research Laboratories. Dried samples were massively homogenized with 5% perchloric acid and subject to the LA8080 High-Speed Amino Acid Analyzer (Hitachi High-Technologies Corporation) and high-performance liquid chromatograph using LC-20AD or LC-10ADvp (Shimadzu Corporation) for the quantification.

Two-bottle preference test

The two-bottle preference test was conducted in the usual condition for the group, i.e., without feeding/drinking limitation and without separation of group members. During behavioral trials, monkeys were presented simultaneously with two filled bottles (500 mL), one containing test stimuli and second containing water. Test stimuli included 5 mM MSG, IMP-2Na, or AMP-2Na. All the five members freely drink from the bottles placed in the outside enclosure (Figure 4C) as the following daily schedule: (*a*) a test starts at 10:00 am; (*b*) location of the two bottles is exchanged at 10:30 am; (*c*) bottles are removed at 11:00 am (break); (*d*) the new test starts at 13:00 pm with the solution and location same to the phase (*b*); (*e*) location of the two bottles is exchanged at 13:30 pm; (*f*) the bottles are removed at 14:00 pm (finish). Tests for each solution were conducted for 6 days, where the location of the solution was the right side in the phase (*a*) in three days and the left side in the three other days. All tests were directly observed by a habituated researcher and recorded by a 29.97-fps video. From the video records, supplemented with direct observation recodes, the number of video frames that spanned in each drinking bout was counted in each individual (Data S1A – F).

Homology modeling

The homology model of human T1R1 was prepared using the Schrödinger Suite 2019-1 (Schrödinger, LLC) under the OPLS3e force field. The active state of full-length structure of metabotropic glutamate receptor 5 (mGluR5) (PDB ID: 6N51) was used as a template.[51] The venus flytrap domain of the homology model was visualized using Discovery Studio Visualizer (Dassault Systemes) software.

QUANTIFICATION AND STATISTICAL ANALYSIS

For the T1R functional assay, quantification was represented by the mean value ± standard error for each response of receptors. Statistically significant increases were determined using Welch's two-sided paired t test; the Benjamini-Hochberg adjustment was used to correct for multiple comparisons (q = 0.01).

For the two-bottle preference test, statistical analysis was performed using the R package lme4 (Linear Mixed-Effects Models using 'Eigen' and S4) v1.1-10 (http://lme4.r-forge.r-project.org/). The result fit a generalized linear mixed-effects model (GLMM) with the random effects of the individual and session and Gamma distribution (family = Gamma(link = "log")) in a following formula: bout-length ~solution + bottle-position + (1 | individual) + (1 | session) (Data S1G–S1M).