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Bitter Taste Receptors Identified

Howard Hughes Medical Institute (HHMI) researchers at the University of California, San Diego and their colleagues at the National Institute of Dental and Craniofacial Research (NIDCR) have identified a new family of genes that encode proteins that function as bitter taste receptors.

"Bitterness clearly evolved with the sole purpose of warning you against the ingestion of toxic substances."

— Charles S. Zuker

The research, which is reported in two articles in the March 17, 2000, issue of the journal *Cell*, provides important insight into the organization of the taste system.

"We've been trying for the past four years to understand how the taste system works, focusing primarily on sweet and bitter signaling," says Charles Zuker, an HHMI investigator at the University of California, San Diego. Now, the researchers have conducted a series of experiments that they say demonstrates conclusively that a single family of genes indeed contains human and rodent taste receptors.

"We now have the means to really start to investigate how taste works, not just in the tongue, but also in the brain," says Nicholas Ryba of NIDCR.

The research group includes Zuker and colleagues Ken Mueller, Jayaram Chandrashekar and Wei Guo of the University of California, San Diego; Elliot Adler, Mark Hoon and Ryba of the NIDCR; and Luxin Feng of Aurora Biosciences in La Jolla, Calif.

In 1999, a team led by Zuker and Ryba reported the discovery of two genes, *TIR₁* and *TIR₂*, which had most of the characteristics expected of taste receptor genes. The genes resembled other known sensory receptor genes and were expressed in the appropriate places inside taste receptor cells on the tongue and palate. But Zuker and Ryba hypothesized that two receptors

seemed far too few to handle the huge number of chemicals that produce sweet and bitter substances. What's more, *T1R₁* and *T1R₂* generally were not found in the same places as gustducin, a coupling protein critical in sending the bitter signal from taste buds to the brain.

"That meant that we were missing the family of taste receptors that coupled with gustducin," say study co-authors Mueller and Adler, "so we set out to identify those receptors." In their latest studies, the researchers focused on a specific interval of DNA on one human chromosome that was known to be associated with the ability to taste the bitter compound PROP (6-n-propylthiouracil). They identified a likely looking receptor sequence in that stretch of DNA, and showed that it belonged to a family of some 80 genes, which they dubbed *T2Rs*. Like *T1R₁* and *T1R₂*, the *T2R* genes were selectively expressed in taste receptor cells, but there was even better news.

"If you look at the expression of this new family, you find that every cell that expresses one of these receptors is a gustducin-expressing cell," says Zuker.

Next, the researchers screened libraries of mouse genes in a search for the mouse versions of the new gene family. Mice are useful in studying taste because strains have been bred with the inborn ability to taste or not taste certain bitter substances. Studies of these mice have pinpointed a cluster of gene positions on mouse chromosome six that are associated with the tasting of a number of bitter substances. When Zuker's and Ryba's group mapped the mouse versions of their new gene family to mouse chromosomes, "Bingo, a whole set of them sat right on top of that bitter cluster!" says Zuker.

Everything so far hinted that *T2Rs* were bitter taste receptors, but the researchers still did not have definitive proof. "To get that, we needed to show that when we put in a bitter compound, the compound binds to the receptor, and that triggers activity in the receptor cells," Zuker explains. That's difficult to do in a living system, so the researchers engineered laboratory-cultured cells to "report" activity when properly triggered.

"We were able to show that three of the receptors - two mouse and one human - specifically signaled in response to bitter taste," says Chandrashekar. One of the mouse receptors responded to cyclohexamide, a bitter compound for which there are mouse "taster" and "nontaster" strains. "It turns out that the receptor gene from the nontasters differed from that in the tasters," representing two alternate forms, or alleles, of the gene, says Zuker. When the researchers compared engineered cells containing the nontaster allele to those containing the taster allele, "we saw a corresponding shift in their sensitivity to cyclohexamide."

The new work helps explain, on a molecular level, the "logic" behind the taste system and how it differs from the olfactory system. The olfactory system

system is designed to recognize a wide range of odors and to discriminate one odor from another an essential ability if one is to avoid such inappropriate responses as mistaking a mate for a snack. The organization of the olfactory system reflects this need, with each olfactory neuron expressing only one of the 1,000 or so olfactory receptor genes.

Taste is a different matter, especially where bitter compounds are concerned. Virtually every naturally occurring toxin tastes bitter, "so bitterness clearly evolved with the sole purpose of warning you against the ingestion of toxic substances," says Zuker. The important thing is to recognize and reject anything bitter, not to get hung up on distinctions among different compounds. Indeed, experimental evidence indicates that humans are unable to discriminate one bitter substance from another.

"This imposes an interesting contrast with the olfactory system, and we now have found the logic behind it," says Hoon. Every cell that expresses genes in the *T2R* family expresses nearly all the genes in that family. "So rather than having one receptor per cell, like olfaction, you have many. This dramatically increases the repertoire of bitter things you can taste, but since the receptors are all in the same cell and the cell simply fires when activated, you do not discriminate."

Zuker is satisfied that the *T2R* family of genes represents at least a subset of bitter taste receptors, but there's more work to be done: tracing pathways from receptor cells to the brain, generating "knockout" mice that lack *T2Rs* and studying their taste deficits, and searching for more gustducin-linked receptors.