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Researchers Discover Human Gene that May Produce Sweet Taste Receptor

Two research groups led by Howard Hughes Medical Institute (HHMI) investigators have independently identified a human gene that encodes a likely receptor for sweet compounds. The researchers say finding the gene, which is expressed by the tongue's taste cells, opens an important research pathway that may help answer fundamental questions such as how the brain perceives sweet taste and why molecules with dramatically different chemical structures can taste sweet. Discovery of the candidate sweet taste receptor adds to a repertoire of recently discovered receptors thought to be involved in the perception of bitter and umami tastes.

Discovery of the candidate sweet receptor gene, called *T1r3*, was reported in articles published in the May 2001 issue of *Nature Neuroscience* by HHMI's Linda Buck and colleagues at Harvard Medical School; and in the May 2001 issue of *Nature Genetics* by HHMI's Robert F. Margolskee and colleagues at the Mount Sinai School of Medicine at New York University.

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— **Linda B. Buck**

Taste receptors are proteins that nestle in the surface of taste bud cells and bind to specific chemicals. When the appropriate chemical activates a taste receptor, it launches a cascade of molecular events that culminates in the brain's perception of taste information.

The starting point for both research groups was the *Sac* locus in the mouse genome a region of mouse chromosome 4 known to govern the preference for sweet tasting substances.

"The *Sac* locus in mice was known to be the most important determinant for differentiating 'taster' strains of mice that preferred sweetened solutions from 'non-taster' strains that didn't prefer a sweetened solution over plain water,"

said Margolskee. "But we didn't know whether the *Sac* locus was one gene or multiple genes, or a genetic control element."

Margolskee's group built on earlier studies by Alexander Bachmanov and Gary Beauchamp of the Monell Chemical Senses Center that had narrowed the location of the *Sac* locus to a small chromosome region near a known marker. Once Margolskee's team located the homologous marker in human DNA, "we put together a contiguous region of about a million base pairs of DNA around this marker using both finished human sequence and unfinished high throughput human sequences," said Margolskee. In fact, since the human genome sequence in that region had not yet been completed, the scientists had to fit the pieces together themselves, like a jigsaw puzzle, to create an organized search region.

Within this contiguous human genome sequence, the researchers discovered numerous genes. But only one coded for a protein that fit their working assumption that the *Sac* gene product should be a signal transduction component such as a G protein-coupled receptor (GPCR).

Buck and her colleagues used a different approach in searching for a GPCR near the *Sac* locus. "We first determined where the region corresponding to the mouse *Sac* locus was in humans and looked for genes encoding G protein-coupled receptors in that region," said Buck. "In the finished human genome database, we didn't find anything, but in the draft sequence, we found a piece of DNA that would fit in that region, and which had a gene encoding what appeared to be a GPCR." Studies by Buck and her colleagues revealed that the gene was related to similar previously identified genes, called *Tlr1* and *Tlr2* that had been found in taste cells, but whose taste-reception function was unknown. *Tlr1* and *Tlr2* had been previously identified by HHMI investigator Charles Zuker at the University of California, San Diego and Nicholas Ryba at the National Institutes of Health.

Both Buck's and Margolskee's groups named the potential sweet receptor gene product T1r3, for "taste receptor family 1, member 3."

"This receptor really shouted out to us as a strong candidate to be the *Sac* gene and to be a sweet receptor, because of its similarity to *Tlr1* and *Tlr2*," said Margolskee. Also, the *Tlr3* protein has a large extracellular loop that juts outside the cell membrane, as would be required for a receptor that had evolved to attach to large carbohydrate sugar molecules, said Margolskee. Importantly, studies of *Tlr3* expression by both groups confirmed that the gene was selectively expressed in the membranes of taste cells and not in other cells in the tongue or elsewhere in the body.

"This protein is physically in the taste cell where it ought to be if it is the *Sac* protein," said Margolskee. "And within the taste cells, it's in the apical membranes, where a sweet receptor should be."

Both teams of scientists had predicted that the *Tlr3* gene would show sequence differences between sweet-preferring taster mice and sweet-indifferent non-taster mice. And, indeed, they did find such differences

in gene sequence between the strains that argued for the gene's role in sweet tasting. In a molecular model of the structure of T1r3, Margolskee and colleagues identified a specific amino acid sequence in the non-taster T1r3 protein that is predicted to add a new carbohydrate group at a portion of the receptor likely to be critical for its function.

In a discovery that hinted at how very different molecules might trigger the sweet taste sensation, Buck and her colleagues found that the majority of mouse taste bud cells that expressed *T1r3* also expressed *T1r2*.

"We find the co-expression of these two genes extremely intriguing," said Buck. "It implies that individual taste cells are probably recognizing at least two tastes. They might be functioning independently as taste receptors; or the receptor proteins might interact to form various combinations that give the opportunity to recognize different sweeteners."

Buck and Margolskeewhose laboratories are now exploring the molecular function of the candidate sweet receptoremphasize that such work could have important clinical benefits.

"A large percentage of people in the United States and other western countries are overweight. And the artificial sweeteners now used in an attempt to control weight just don't do a very good job of mimicking the taste of the natural sweetener," said Margolskee. "Until now, developing those sweeteners has been a hit-or-miss proposition; but if we understood the sweet receptor and its binding mechanism, we could design a sweetener molecule that would fit perfectly and be a million times more potent than sugar yet have the same sweetness as a natural sugar sweetener."

"Also, the loss of the sense of taste is a major quality-of-life problem for the elderly, and one that can contribute to malnutrition," said Margolskee. "If we could enhance the activity of taste receptors, including those for sweet and amino acids, it might help patients with nutritional problems."

Buck emphasized that identifying the sweet receptor offers an important pathway for exploring how the brain processes taste information. "If you have genes that code for receptors that recognize particular tastes such as sweet versus bitter, you can use those genes as tools to actually visualize what's happening inside the brain. For example, is there a sweet spot in the brain, a bitter spot, or a sour spot?" Furthermore, researchers would be able to explore whether the olfactory and taste senseslong known to function in concertshare neural circuitry.