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Genetics of sweet taste preferences*

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Abstract: Inbred mouse strains display marked differences in avidity for sweet solutions due in part to genetic differences among strains. Using several techniques, we have located a number of regions throughout the genome that influence sweetener acceptance. One prominent locus regulating differences in sweetener preferences among mouse strains is the saccharin preference (*Sac*) locus on distal chromosome 4. Afferent responses of gustatory nerves to sweeteners also vary as a function of allelic differences in the *Sac* locus, suggesting that this gene may encode a sweet taste receptor. Using a positional cloning approach, we identified a gene (*Tas1r3*) encoding the third member of the T1R family of putative taste receptors, T1R3. Introgression by serial back-crossing of a chromosomal fragment containing the *Tas1r3* allele from the high sweetener-preferring strain onto the genetic background of the low sweetener-preferring strain rescued its low sweetener-preference phenotype. *Tas1r3* has two common haplotypes, one found in mouse strains with elevated sweetener preference and the other in strains relatively indifferent to sweeteners. This study, in conjunction with complimentary recent studies from other laboratories, provides compelling evidence that *Tas1r3* is equivalent to the *Sac* locus and that the T1R3 receptor (when co-expressed with taste receptor T1R2) responds to sweeteners. However, other sweetness receptors may remain to be identified.

INTRODUCTION

Sweet taste perception is initiated by the interaction of a sweetener with a G protein-coupled taste receptor on the apical ends of the taste receptor cell [1]. Many compounds that taste sweet to humans (sweeteners) are palatable to other species, including mice [2,3]. The mouse provides a particularly valuable tool for the molecular analysis of sweet taste. Inbred mouse strains display marked differences in the avidity for sweet solutions [4–6]. A significant portion of the differences in sweetener preferences among mouse strains is attributed to allelic variation of the saccharin preference (*Sac*) locus, on distal chromosome 4 [6–10]. The *Sac* genotype also influences responses of taste nerves to sweeteners [8,10]. This suggests that the *Sac* gene encodes a sweet taste receptor.

It was previously suggested that the T1R family of putative taste receptors may include a sweet receptor [11]. This receptor family is coded by three genes (*Tas1r1*, *Tas1r2*, and *Tas1r3*) located on the distal chromosome 4 [12–16], which makes each of them a candidate for the *Sac* locus. The genes encoding the T1R1 and T1R2 receptors have been excluded as candidates for *Sac* based on their more proximal chromosomal location [10,12,14]. However, we and others have found that the gene encoding

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the T1R3 receptor maps to a more distal part of chromosome 4 corresponding to the *Sac* interval and is indeed *Sac* [12–16].

In this summary of our work on the *Sac* locus, we outline how we came to conclude that *Tas1r3* codes for the sweet receptor T1R3. This report closely follows our published description of this work [16]. Our strategy has been to use high-resolution linkage analysis and physical mapping and sequencing to determine the precise limits of the critical *Sac* region. Following this, we identified genes within the *Sac* interval. The gene coding for T1R3 was considered the most likely candidate for the *Sac* locus. To verify the function of the T1R3 receptor, the low sweetener-preferring phenotype was rescued by introgressing the taster allele from a high sweetener-preferring mouse strain using serial back-crossing during selection of a congenic strain. As further evidence that T1R3 is a sweet receptor, we demonstrated that sequence variants of the *Tas1r3* gene are related to sweetener preference. These in vivo data provide compelling evidence that *Tas1r3* is equivalent to the *Sac* locus and that it encodes a taste receptor responding to sweeteners. As described in the Discussion section, this conclusion has recently been confirmed by other investigators using other methods.

METHODS

Detailed methods have been described [16]. Briefly, C57BL/6ByJ (B6) and 129P3/J (129) mice were purchased from the Jackson Laboratory. These were used for breeding of F_2 hybrids and congenic strains. Taste solution intake and preference studies were conducted on individually housed mice using 96-h two-bottle tests, with water as the second choice. Genotyping followed standard protocols [16]. A bacterial artificial chromosome (BAC) contig was constructed to narrow the genetic interval encompassing *Sac*, and a BAC spanning the critical region was sequenced as previously described [16].

To determine whether sweetener preference data were related to variations in the sequence of the *Sac* candidate gene, *Tas1r3,* data were taken from previous studies for the following mouse strains: 129/Rr, 129/Sv, AKR/J, BALB/cA, BALB/cByJ, C3H/He, C57BL/6ByJ, C57BL/6Ty, C57L/Lac, CBA/Cam, DBA/2Ty, IS/Cam, SEA/GnJ, ST/bJ, SWR/J [4], and CAST/Ei (A. Bachmanov et al., unpublished data). When preferences were available for two substrains, they were averaged. Comparison of the sequences from six strains (three high-preferring: C57BL/6ByJ, SWR/J, CAST/Ei, and three low-preferring: 129P3/J, AKR/J, DBA/2J) identified a haplotype of six single nucleotide polymorphisms (SNPs) associated with sweetener preference. Next, the regions contributing to this haplotype were sequenced in additional mouse strains (BALB/cByJ, C3H/HeJ, C57L/J, CBA/J, IS/CamEi, SEA/GnJ, and ST/bJ).

RESULTS

Linkage mapping

The initial linkage analysis was conducted using an F_2 intercross between B6 mice with high sweetener acceptance and 129 mice with low sweetener acceptance. The F_2 mice were phenotyped using 96-h two-bottle tests with sucrose and saccharin, and genotyped with markers polymorphic between the B6 and 129 strains. Interval mapping narrowed the region containing *Sac* to ~5 cM (Figs. 1a and 1b). This region was further reduced to 0.7 cM during the marker-assisted selection of a 129.B6-*Sac* segregating partially congenic strain (Figs. 1b and 1c).

Physical mapping and identification of genes within the *Sac* interval

A contig (Fig. 1d) of BAC clones representing the *Sac*-containing region was constructed by screening a mouse BAC library. The sequence of a 194 Kb critical region for the *Sac* locus was obtained by sequencing a BAC clone and searching GenBank. Of the 12 predicted genes within the critical region,

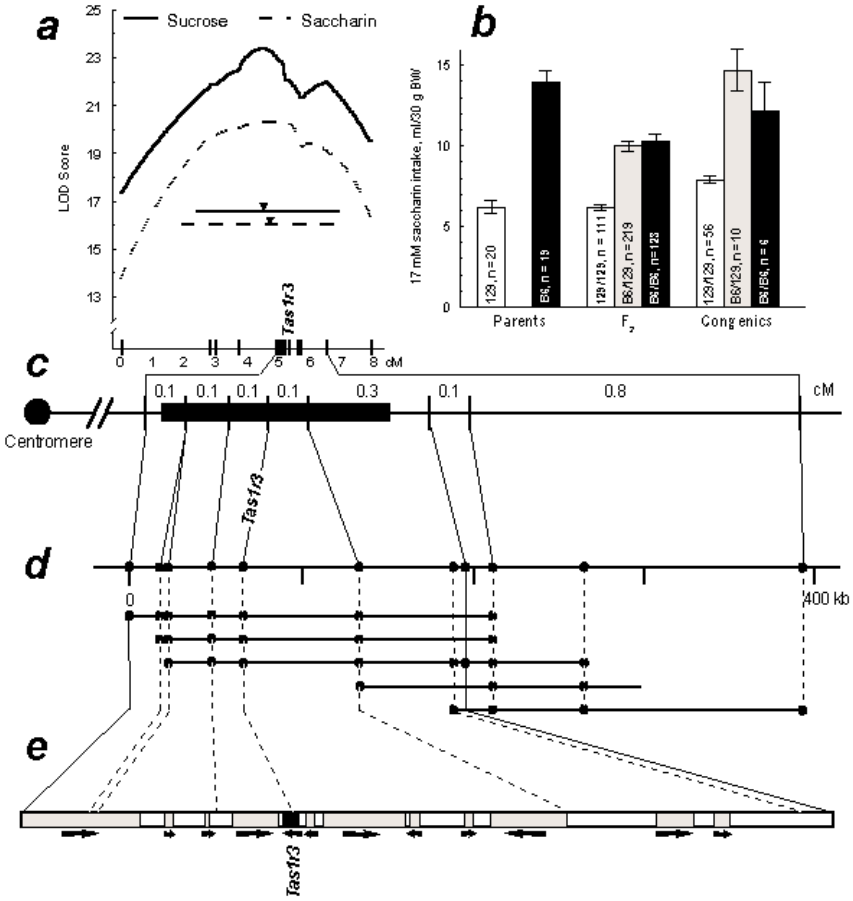

Fig. 1 Genetic and physical maps of the *Sac* region. (a) Interval mapping of sucrose and saccharin consumption to distal chromosome 4 using MAPMAKER software. Distances between markers were estimated based on data from the B6 × 129 F_2 intercross (n = 629). Curves trace the logarithm of the odds ratio (LOD) scores calculated using an unconstrained model (LOD threshold for significant linkage 4.3, 2 d.f.). The horizontal lines show the 2-LOD drop in confidence intervals for saccharin (dotted line, 5.3 cM) and sucrose (solid line, 4.5 cM); black triangles indicate the respective LOD score peaks (LOD 20.3 for saccharin and 23.3 for sucrose). This locus explained 18.6 % and 16.2 % of the total variance in saccharin and sucrose intakes, respectively. (b) Average daily 17 mM saccharin consumption by mice from parental 129 and B6 strains (left), F_2 hybrids (center), and segregating partially congenic 129.B6-*Sac* mice (right) in 96-h two-bottle tests with water (means ± SE). Genotypes of the F_2 and congenic mice for *Tas1r3* and their numbers are indicated on the bars. Each group had approximately equal numbers of males and females. Differences between parental strains and among the F_2 and congenic genotypes were significant ($p < 0.0001$). (c) Linkage map of the *Sac*-containing region. Distances between markers were obtained from the B6 × 129 F_2 intercross (see panel *a*). A black box depicts the donor fragment of the 129.B6-*Sac* partially congenic mice whose saccharin intakes are shown on panel *b*, right. (d) BAC contig and physical map of distal chromosome 4 in the *Sac* region. Dots indicate presence of markers within BACs detected by hybridization and confirmed by polymerase chain reaction (PCR) and, in some cases, by sequencing. (e) Genes within the *Sac*-containing interval. Filled areas indicate predicted genes. Arrows indicate the predicted direction of transcription.

four were known, two were similar to known human genes, and six were represented as cDNA clones. Most of the genes identified within the *Sac*-containing interval are involved or potentially involved in cell division and differentiation, maintenance of intracellular processes, or collagen synthesis. The func-

tions of four predicted genes are unknown. Of the 12 genes within the 194-kb *Sac* interval (Fig. 1e), only one, *Tas1r3* (taste receptor, type 1, member 3), was a G protein-coupled receptor. A predicted *Tas1r3* protein, T1R3, has moderate sequence homology to putative G protein-coupled taste receptors T1R1 and T1R2 and to glutamate taste receptor mGluR4. Sequence comparison of cDNA from mouse lingual epithelium and genomic DNA showed that *Tas1r3* contains 6 coding exons. It is translated into an 858-amino acid protein with a predicted secondary structure that includes seven transmembrane domains and a large hydrophilic extracellular *N*-terminus. This structure is typical of the G protein-coupled receptor family 3, which includes the metabotropic glutamate and extracellular calcium-sensing receptors.

Sequence variants of *Tas1r3*

As a candidate for *Sac*, *Tas1r3* should have sequence variants corresponding to phenotypical *Sac* alleles. To assess this correspondence, sequences of *Tas1r3* and surrounding genomic DNA were analyzed in mouse strains with known sweetener preferences. Two haplotypes consisting of six SNPs distinguished strains with high (81 ± 4 % preference score) and low (57 ± 1 % preference score) 1.6 mM saccharin preferences. Two of these SNPs resulted in amino acid substitutions of threonine (found in all high-preferring strains) for alanine (found in all low-preferring strains) at position 55 (Thr55Ala) and isoleucine for threonine at position 60 (Ile60Thr), both within the predicted extracellular *N*-terminal domain of T1R3.

DISCUSSION

Using a positional cloning approach, we narrowed the *Sac*-containing region to a 194-kb interval. One gene within this interval, *Tas1r3*, encodes a G protein-coupled receptor (T1R3) that is expressed in taste receptor cells [12–16]. Based on the effects of the *Sac* genotype on peripheral sweet taste responsiveness [8,10], and on the known mechanism of sweet taste transduction [1], *Tas1r3* is the most likely candidate for *Sac*.

The study of this locus and its identification as a sweet receptor was significantly advanced by three very recent papers [17–19]. The first paper by Nelson et al. [17] showed that a *Tas1r3*-containing transgene from a taster strain rescued the non-taster phenotype. They also provided new evidence on localization of T1R3 in taste tissue. The authors then used a heterologous expression system to demonstrate that TlR3, co-expressed with TlR2 (T1R3+2), responds physiologically to some sweet compounds. In their second paper, Nelson et al. [18] showed that certain amino acids reported by humans to be sweet also activated the mouse T1R3+2 combination, whereas others did not (e.g., L-proline). Additionally, they found that the T1R3+1 combination acts as a broadly tuned L-amino acid sensor that synergizes with ribonucleotides. As these authors indicate, these receptor combinations cannot explain how mice distinguish and discriminate sweeteners, particularly since the L-amino acids can be sweet, bitter, or umami in character. Even more recently, Li et al. [19] used a modified assay system to demonstrate a broader responsiveness to sweeteners in the rat T1R3+2 combination, and sensitivity of the human T1R3+2 combination to sweeteners to which mice and rats are unresponsive. In sum, these papers convincingly demonstrate that T1R3 (in combination with T1R2) functions as a sweet taste receptor.

However, allelic variation in the members of the T1R family of receptors cannot account for all variations in behavioral and physiological responses to sweeteners in mice. This may be due to the existence of as yet unknown additional sweet receptors and/or other regulatory factors that impact upon the phenotype, which is an intake of sweet solutions in the long-term tests. Briefly, the reasons for this conclusion are as follows:

1. More than 50 % of the genetic variance in sweetener intake in the B6x129 F_2 cross used for identification of T1R3 [8] cannot be explained by polymorphisms in the gene coding for this taste receptor.
2. Unpublished data from our laboratories on acceptance of the sweet amino acid glycine by 28 strains of mice indicate that known variations in the sequence of *Tas1r3* cannot account for the pattern of strain differences. Glycine weakly activates the T1R3+2 receptor combination under some circumstances [18,19], but it is possible that a separate glycine receptor exists.
3. We have found (e.g., [16]) that some inbred mouse strains with identical *Tas1r3* haplotypes exhibit marked differences in acceptance of the non-nutritive sweetener saccharin. Polymorphisms of additional genes must underlie this behavioral difference.
4. We have found [20] that strain and individual differences among rats' responses to sweeteners are not related to genotypic variation of the rat *Tas1r3* ortholog. Although we cannot eliminate other T1Rs as being involved, the mapping work of other investigators [21] is not consistent with the conclusion that these genes are involved.
5. Regional tongue distributions of sweet sensitivities in mice determined electrophysiologically are not consistent with the histological distributions of the T1R3+2 combination of receptors [22,23]. Additionally, single fiber analyses detect fibers that are more or less responsive to different sweeteners. For example, some fibers respond well to sucrose, fructose, and saccharin, but not to glucose and maltose. Finally, mouse sweetness inhibitors selectively inhibit some sweeteners [24–27]. Taken together, these physiological and anatomical studies are consistent with the existence of multiple sweet receptor types.
6. On a theoretical basis, additional sweet receptors/sweet receptor families may exist in the genome as suggested from invertebrate research that has found highly divergent receptor types [18,28,29].

In summary, this research program illustrates the value of in vivo studies using inbred mouse strains to help identify genes important in sweet taste perception. A similar strategy will be used in future studies aimed at identifying novel genes that may specify additional receptors or important proteins involved in sweet taste transduction and processing. This effort is aided immeasurably by the recent progress in specifying the human and mouse genomes. When the critical region for a genetic locus is narrowed to a small number of genes, it is now relatively easy to survey this region and search for candidates using available databases from the human and mouse genome projects. With these tools, we look forward to rapid progress to further our understanding of the peripheral and central processing of sweet taste information.

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