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Positional cloning of the mouse saccharin preference (*Sac*) locus

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Abstract

Differences in sweetener intake among inbred strains of mice are partially determined by allelic variation of the saccharin preference (*Sac*) locus. Genetic and physical mapping limited a critical genomic interval containing *Sac* to a 194-kb DNA fragment. Sequencing and annotation of this region identified a gene (*Tas1r3*) encoding the third member of the T1R family of putative taste receptors, T1R3. Introgression by serial backcrossing of the 194-kb chromosomal fragment containing the *Tas1r3* allele from the high-sweetener preferring C57BL/6ByJ strain onto the genetic background of the low-sweetener preferring 129P3/J strain rescued its low sweetener preference phenotype. Polymorphisms of *Tas1r3* that are likely to have functional significance were identified using analysis of genomic sequences and sweetener preference phenotypes of genealogically distant mouse strains. *Tas1r3* has two common haplotypes, consisting of six single nucleotide polymorphisms: one haplotype was found in mouse strains with elevated sweetener preference and the other in strains relatively indifferent to sweeteners. This study provides compelling evidence that *Tas1r3* is equivalent to the *Sac* locus and that the T1R3 receptor responds to sweeteners.

Keywords

sweet; taste receptor; genetics; positional cloning

Introduction

Sweet taste transduction is thought to be initiated by the interaction of a sweetener ligand with a G protein-coupled taste receptor on apical ends of the taste receptor cells, which evokes a sensation of pleasantness and a consummatory behavioral response (Lindemann, 1996). Many compounds that taste sweet to humans, or sweeteners, are palatable to various species of animals, including mice (Beauchamp and Mason, 1991; Bachmanov *et al*., in press). Inbred mouse strains display marked differences in the avidity for sweet solutions (Lush, 1989; Capeless and Whitney, 1995; Lush *et al*., 1995). Much of the differences in sweetener preferences among mouse strains are attributed to allelic variation of the

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saccharin preference (*Sac*) locus, on distal chromosome 4 (Phillips *et al.*, 1994; Lush *et al.*, 1995; Bachmanov *et al.*, 1997b; Blizard *et al.*, 1999; Li *et al.*, 2001b). In addition to sweetener preferences, the *Sac* genotype influences the afferent responses of gustatory nerves to sweeteners (Bachmanov *et al.*, 1997b; Li *et al.*, 2001b) suggesting that the *Sac* gene is involved in peripheral taste transduction and may encode a sweet taste receptor.

The T1R family of putative taste receptors consists of three genes expressed in taste receptor cells and located on the distal chromosome 4 (Hoon *et al.*, 1999; Kitagawa *et al.*, 2001; Max *et al.*, 2001; Montmayeur *et al.*, 2001; Sainz *et al.*, 2001), which makes them candidates for the *Sac* locus. The *Tas1r1* (alias *Gpr70*) gene encoding the T1R1 receptor and the *Tas1r2* (*Gpr71*, T1R2) gene have been excluded as candidates for *Sac* based on their more proximal chromosomal location (Kitagawa *et al.*, 2001; Li *et al.*, 2001b; Montmayeur *et al.*, 2001). However the *Tas1r3* gene encoding the T1R3 receptor has been mapped to a more distal part of chromosome 4 corresponding to the *Sac* interval (Kitagawa *et al.*, 2001; Max *et al.*, 2001; Montmayeur *et al.*, 2001; Sainz *et al.*, 2001).

In this study, using high-resolution linkage analysis followed by physical mapping and sequencing, we determined the exact limits of the critical *Sac* region. Next, we identified genes within the *Sac* interval and found that *Tas1r3* is the most likely candidate for the *Sac* locus. The low-sweetener preferring phenotype of 129P3/J strain was rescued by introgressing an allele of *Tas1r3* from a high-sweetener preferring C57BL/6ByJ strain using serial backcrossing during selection of a 129.B6-*Sac* congenic strain. Finally, sequence variants of *Tas1r3* that are likely to have functional significance were identified using analysis of *Tas1r3* sequences and sweetener preference phenotypes in genealogically distant mouse strains. 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.

Methods

Animals

C57BL/6ByJ (B6) and 129P3/J (formerly 129/J, abbreviated here as 129) mice were purchased from The Jackson Laboratory. Details of breeding F_2 hybrids (Bachmanov *et al.*, 1997b) and of marker-assisted selection of a 129.B6-*Sac* congenic strain (Li *et al.*, 2001b) are described elsewhere.

Taste preference tests

Consumption of 120 mM sucrose and 17 mM saccharin was measured in individually caged mice using 96-hr two-bottle tests, with water as the second choice, described in detail elsewhere (Bachmanov *et al.*, 1997b; Li *et al.*, 2001b). Results are presented as sweetener solution intakes expressed per 30 g of body weight (BW; the approximate weight of an adult mouse) per day, or as a preference score (ratio of solution intake to total fluid intake, in percent). Indexes of sweetener consumption used for interval mapping were standardized within each gender and experimental group relative to the group mean and standard deviation.

Mouse genotyping and linkage analysis

Mouse genomic DNA was purified from tails by NaOH/Tris (Truett *et al.*, 2000) or phenol/ chloroform extraction. Simple sequence length polymorphism (SSLP) markers were tested using a standard protocol (Dietrich *et al.*, 1992), with minor modifications (Bachmanov *et al.*, 1997b; Li *et al.*, 2001b). All other markers were tested using a single-strand conformation polymorphism (SSCP) protocol (Orita *et al.*, 1989) or by sequencing the PCR products. Marker and primer details are available upon request. Polymorphic markers were

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mapped by genotyping F_2 and partially congenic mice. Chromosomal positions of non-polymorphic markers were confirmed using radiation hybrid mapping of the T31 mouse-hamster radiation hybrid panel (Research Genetics, Huntsville, AL) according to a standard protocol (http://www.jax.org/resources), and the data were submitted for analysis to the Mouse Genome Database. Markers that mapped in the *Sac* region were used to screen the RPCI-23 mouse bacterial artificial chromosome (BAC) library (Osoegawa *et al*., 2000).

Construction of a BAC contig

The RPCI-23 female (C57BL/6J) mouse BAC library (Osoegawa *et al*., 2000) was screened by hybridization (Church and Gilbert, 1984) with radioactively labeled probes (Feinberg and Vogelstein, 1983). The library was screened twice, first with a probe generated from the yeast artificial chromosome (YAC) clone 178B3 (http://carbon.wi.mit.edu:8000/cgi-bin/mouse/sts_info) and second with pooled probes of markers in the *Sac* region. The YAC 178B3 was chosen for screening because based on its STS content, it mapped in the *Sac* region and appeared to be non-chimeric. Positive clones identified by the initial screenings were re-arrayed and hybridized against individual probes. The secondary screening results were confirmed by PCR. BAC insert sizes were determined using pulsed–field gel electrophoresis after digestion with *NotI* (Lengeling *et al*., 1999). The BAC contig was assembled using SEGMAP, version 3.48 (Green and Green, 1991).

Analysis of nucleic and amino acid sequences

DNA from BAC 118E21 was isolated using an alkaline lysis protocol followed by CsCl density gradient centrifugation, subcloned and sequenced. Sequence data were assembled using Phred, Phrap and Consed packages of programs. After BAC 118E21 was sequenced, the sequence-tagged site (STS) content of this BAC and overlapping BACs was confirmed by aligning the STS and BAC end sequences with the 118E21 sequence (Sequencher, Gene Codes Corporation, Ann Arbor, MI). Repeat sequences were identified using RepeatMasker (Smit and Green, 2000), and GENSCAN (Burge and Karlin, 1998) was used to predict gene content and exon-intron organization. The predicted proteins were submitted to a TBLASTN searches against the *nr* and the *mouse EST* databases at NCBI or against Unigene.

To determine the intron-exon boundaries of the *Tas1r3* gene, total RNA was extracted using TRIZol Reagent (Life Technologies Inc., Rockville, MD) from enzymatically separated mouse lingual epithelium (Ruiz *et al*., 1995; Spielman and Brand, 1995), which included fungiform, foliate and circumvallate taste papillae. The RNA was reverse transcribed (Superscript reverse transcriptase, Life Technologies). The cDNA samples were amplified using AmpliTaq DNA Polymerase with GeneAmp (Perkin Elmer Corporation, Branchburg, NJ) and intron-spanning primers selected to distinguish genomic and cDNA. Single bands of expected sizes were excised from the gel, purified and sequenced.

Mouse T1R3 protein sequence was predicted from cDNA obtained from tongue epithelium by RT-PCR, and it was analyzed using the computer programs HMMTOP (Tusnády and Simon, 1998) and TOPO (Johns and Speth, 1996) to determine the hydrophobicity and the transmembrane regions. Protein motif prediction was conducted using the Motif search service on GenomeNet (http://www.motif.genome.ad.jp).

Analysis of variation in sweetener preference and *Tas1r3* sequences among mouse strains

Sweetener preference 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 (Lush, 1989) and CAST/Ei (A. Bachmanov et al., unpublished data). When preferences were available for two substrains, they were averaged and shown as 129, BALB/c and C57BL/6.

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A 6.8 kb segment of genomic DNA, including ~2.6 kb upstream and ~1.2 kb downstream of *Tas1r3*, was sequenced in genealogically remote or unrelated (Beck *et al.*, 2000) mouse strains with high (C57BL/6ByJ, SWR/J and CAST/Ei) or low (129P3/J, AKR/J and DBA/2J) sweetener preferences. Comparison of the sequences from these six strains 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). The C57BL/6J strain sequence obtained from the sequencing of RPCI-23 BAC 118E21 was identical to the C57BL/6ByJ sequence. In some cases, the exact substrain tested for sweetener preference differed from the substrain sequenced, usually because the older substrains were not available for sequencing.

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-hr 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 (Figure 1*a,b*). This region was further reduced to 0.7 cM (flanked by *280G12-T7* and *D4Mon1* markers) during the marker-assisted selection of a 129.B6-*Sac* segregating partially congenic strain (Figure 1*b,c*).

Physical mapping

A contig of BAC clones representing the *Sac*-containing region was constructed by screening the RPCI-23 mouse BAC library (Figure 1*d*). A 246-kb BAC clone, 118E21, including most of the *Sac* interval, was sequenced. The remaining small proximal part of the *Sac* region was contained in a sequence from mouse genomic DNA (GenBank accession no. AF185591). The 0.7-cM Sac interval flanked by *280G12-T7* and *D4Mon1* had a physical size of 194 kb and contained twelve predicted genes (Figure 1*e*).

Identification of genes within the *Sac* interval

Of the twelve predicted genes, four were known (cyclin ania-6b, *Dvl, Ubc6p* and *Tas1r3*), two were similar to known human genes (*KIAA1716* and *SCNN1D*), and six were represented as cDNA clones with GenBank accession numbers NM_025338, AK004732, NM_024472, AK010425, AA435261 and NM_026125. Most of the genes identified within the *Sac*-containing interval are involved or potentially involved in cell division and differentiation [cyclin ania-6b and *Dvl* (MacLachlan *et al.*, 1995; Lee *et al.*, 1999)], maintenance of intracellular processes [*Ubc6p*, NM_024472, and genes similar to *KIAA1716* and *SCNN1D* (Waldmann *et al.*, 1995; Gilon *et al.*, 2000; Nagase *et al.*, 2000)], or collagen synthesis (NM_026125). The functions of the predicted genes represented by NM_025338, AK004732, AK010425 and AA435261 are unknown.

Of these twelve genes within the 194-kb *Sac* interval, 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 (encoded by *Tas1r1*, alias *Gpr70*, 32%; Figure 2*a*), T1R2 (encoded by *Tas1r2*, alias *Gpr71*, 30%) and mGluR4 (encoded by *Gpcr1d*, 23%) (Hoon *et al.*, 1999; Chaudhari *et al.*, 2000). Sequence comparison of cDNA from mouse lingual epithelium and genomic DNA showed that *Tas1r3* contains 6 coding exons (Figure 2*b*). 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 (Figure 2*c*). This structure is typical of the G protein-

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coupled receptor family 3, which includes the metabotropic glutamate and extracellular calcium-sensing receptors. There is substantial evidence that a G protein-coupled mechanism is involved in sweet taste transduction (Lindemann, 1996), suggesting that *Tas1r3* is the most likely candidate for the *Sac* locus.

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 (see details in Methods). Two haplotypes consisting of six SNPs (Figure 2*b*) distinguished strains with high and low sweetener preferences (Figure 3). 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; Figure 2*a*), both within the predicted extracellular N-terminal domain of T1R3 (Figure 2*c*). Two other variants occurred in the upstream regions, the fifth polymorphism was intronic, and the sixth variant was a silent mutation (Ser) in exon 1.

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 (Kitagawa *et al.*, 2001; Max *et al.*, 2001; Montmayeur *et al.*, 2001; Sainz *et al.*, 2001). Based on the effects of the *Sac* genotype on peripheral sweet taste responsiveness (Bachmanov et al., 1997; Li et al., 2001b), and on the known mechanism of sweet taste transduction (Lindemann, 1996), *Tas1r3* is the most likely candidate for *Sac*.

If *Tas1r3* is identical to *Sac*, substitution of *Tas1r3* alleles must result in phenotypical changes attributed to the *Sac* locus. This can be tested through transgenic (‘phenotype rescue’ or ‘knock-out’) experiments. We obtained equivalent data using the 129.B6-*Sac* congenic mice. Introgression of the 194-kb chromosomal fragment containing the *Tas1r3* allele from the high-sweetener preferring B6 strain onto the genetic background of the 129 strain fully rescued its low sweetener preference phenotype: sweetener intake of the congenic mice was as high as that of mice from the donor B6 strain. This introgression was essentially equivalent to production of BAC-transgenic mice (Antoch *et al.*, 1997; Probst *et al.*, 1998) because size of the donor chromosomal fragment was comparable with the size of a typical BAC. These data demonstrate that substitution of *Tas1r3* alleles results in behavioral changes attributed to the *Sac* locus and therefore provide the strongest evidence to date that *Tas1r3* is identical to *Sac,* and that the T1R3 receptor responds to sweeteners.

Further evidence that *Tas1r3* is equivalent to *Sac* was obtained from correspondence of *Tas1r3* sequence variants to sweetener preference phenotypes in inbred mouse strains, which are largely attributed to allelic variation of the *Sac* locus. *Tas1r3* has multiple SNPs among the inbred strains we examined. We reasoned that variants that sort mouse strains into high- and low-sweetener preferring categories are more likely to be responsible for the *Sac* phenotype. To minimize the chances for sharing of chromosomal fragments due to identity-by-descent, we analyzed mouse strains with unrelated or distant genealogies. Using this approach, we identified a haplotype of six SNPs concordant with sweetener preferences. Two of these SNPs resulted in amino acid substitutions at positions 55 (Thr55Ala) and 60 (Ile60Thr) within the predicted extracellular N-terminus domain of T1R3. The same Thr55Ala and Iso60Thr polymorphisms have been identified by others (Kitagawa *et al.*, 2001; Max *et al.*, 2001; Montmayeur *et al.*, 2001; Sainz *et al.*, 2001), and they could affect ligand binding (Rana *et al.*, 2001) or receptor dimerization (Max *et al.*, 2001). The SNPs in

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non-coding upstream and intronic regions may affect expression of the gene (Phillips, 1999; Rana *et al.*, 2001).

What are the ligands of T1R3? The B6 and 129 strains differ in responses to several different classes of sweeteners, and to ethanol, which has a sweet taste component (Bachmanov *et al.*, 1996; Bachmanov *et al.*, in press). Our genetic analysis of the B6 × 129 F_2 hybrids demonstrated that the *Sac* genotype affects behavioral and neural responses to representatives of several different sweet-tasting chemicals (sugars, noncaloric sweeteners, sweet amino acids) and also ethanol consumption (Bachmanov *et al.*, 1997a; Tordoff *et al.*, 1998). The effects of *Sac* genotype on taste responses to a variety of compounds suggest that they evoke sweet taste sensation via a common receptor, T1R3.

It is controversial as to whether single or multiple sweet taste receptors and transduction pathways exist (Bartoshuk, 1987; Bernhardt *et al.*, 1996; Lindemann, 1996; DuBois, 1997). Our data are consistent with the existence of a single sweet taste receptor. But it is also possible that the involvement of T1R3 in responses to diverse compounds is attributed to its dimerization with other members of the T1R family (Max *et al.*, 2001; Montmayeur *et al.*, 2001) or the existence of T1R3 splice variants (Max *et al.*, 2001; X. Li and D. Reed, unpublished data), which may create multiple forms of sweet taste receptors. However, the most abundant naturally-occurring sweeteners, sugars, are chemically similar and may not require multiple receptors for their recognition. This may explain why the family of putative sweet (T1R) receptors is much smaller than the family of putative bitter (T2R) receptors (Adler *et al.*, 2000).

Striking species differences in behavioral and physiological responses to sweeteners imply that the structure of sweet receptors vary among species (Beauchamp and Mason, 1991). A taste receptor for the sugar trehalose (Ishimoto *et al.*, 2000), identified in *Drosophila melanogaster*, has no homology with T1R3, suggesting the independent origin of insect and mammalian sugar taste receptors. Among mammals, cats, which are strict carnivores, apparently do not prefer carbohydrate sweeteners but do prefer certain amino acids humans describe as sweet (Beauchamp *et al.*, 1977). Even primate species differ in the ability to recognize certain high-potency sweeteners as sweet (Glaser *et al.*, 1995). *TAS1R3*, the human ortholog of mouse *Tas1r3*, exists in a region of conserved synteny, 1p36. Preliminary sequencing of *TAS1R3* detected common SNPs (D. Reed et al., unpublished data), which may be involved in genetic variation of the sensory and behavioral responses to sugars in humans (Reed *et al.*, 1997).

Finally, an understanding of sweet receptors may have practical applications. Most high-potency, low-calorie sweeteners were discovered by the accidental tasting of chemicals synthesized for other purposes (Walters, 1991). Models guided by the *Tas1r3* taste receptor structure should allow the design and development of new potent ligands (DuBois, 1997; Nofre, 2001).

To summarize, we have now defined the limits of the *Sac*-containing critical genomic interval, restricted to the 194-kb DNA fragment, and have identified twelve genes within this region. Although all these genes can be considered candidates for *Sac*, based on gene expression and predicted protein structures, *Tas1r3* is the most likely candidate. We further demonstrated that experimental manipulation of the genomic interval containing *Tas1r3* affects the taste responses to sweeteners attributed to the *Sac* locus, which provides novel *in vivo* evidence for the identity of *Tas1r3* and *Sac*. Finally, we conducted the first quantitative analysis of the relationship between the *Tas1r3* sequence variants and sweetener preference phenotypes in genealogically diverse mouse strains. In addition to *Tas1r3* missense polymorphisms reported by others, we have identified four other polymorphisms in

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noncoding regions that may affect *Tas1r3* function. Together, these data provide strong evidence that *Tas1r3* is identical to *Sac* and that it is associated with the taste quality of sweetness.

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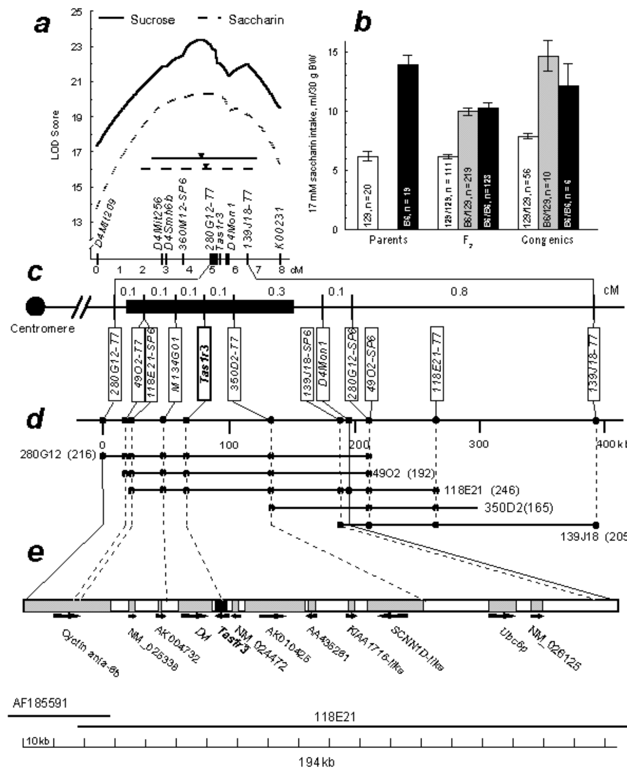

Fig. 1.

Genetic and physical maps of the *Sac* region

a, Interval mapping of sucrose and saccharin consumption to distal chromosome 4 using MAPMAKER software (Lander *et al.*, 1987). Distances between markers were estimated based on data from the B6 × 129 F_2 intercross (n = 629). Curves trace the LOD scores calculated using an unconstrained model [LOD threshold for significant linkage 4.3, 2 d.f. (Lander and Kruglyak, 1995)]. The horizontal lines show the 2-LOD drop 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 variance in saccharin and sucrose intakes respectively. Analyses of LOD scores under dominant and additive models (not shown) demonstrated that the B6 allele is dominant over the 129 allele. Analysis of preference scores showed similar results (not shown).

b, Average daily 17 mM saccharin consumption by mice from parental 129 and B6 strains (left), F_2 hybrids (center), and N_6, N_7, N_4F_4 and N_3F_5 segregating partially congenic 129.B6-*Sac* mice (right) in 96-hr 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 ($F > 39.5$, $p < 0.000001$, ANOVA). Females consumed more saccharin than males ($F > 26.5$, $p < 0.000005$), and the differences among genotypes were more pronounced in females than in males (interaction gender × strain or genotype, $F > 6.4$, $p < 0.02$). However, the main effect of genotype was the same for females and males: F_2 and congenic B6 homozygotes and heterozygotes for *Tas1r3* did not differ from each other, and had higher saccharin intakes than did 129 homozygotes ($p < 0.000001$, planned comparisons). Intakes of 120 mM sucrose were 14.2 ± 0.6 ml/30 g BW for the F_2 mice homozygous for B6 allele of *Tas1r3* (n = 170), 13.8 ± 0.5 ml/30 g BW for the F_2 heterozygotes (n = 299) and 7.4 ± 0.4 ml/30 g BW for the F_2 mice

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homozygous for 129 allele of *Sac* (n = 152); results of statistical analyses were similar to those for saccharin. Haplotype of the donor fragment in the *Sac*-congenic mice is depicted in panel *c*.

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. Location and size of the donor fragment were determined based on the presence of B6 alleles of polymorphic markers in mice from the N_4, N_6, N_7, N_4F_4 and N_3F_5 generations. The donor fragment ends proximally between *280G12-T7* and *49O2-T7*, and distally between *350D2-T7* and *D4Mon1*.

d, BAC contig and physical map of distal chromosome 4 in the *Sac* region. BAC sizes (kb) are shown in parentheses. Dots indicate presence of markers within BACs detected by hybridization and confirmed by PCR and, in some cases, by sequencing. *139J18-SP6* and *D4Mon1* were found within the 118E21 sequence, but were not used for BAC screening (*D4Mon1* was used for genotyping F_2 and congenic mice). In this region, linkage distance of 1 cM corresponds to ~0.25 Mb of physical distance instead of typical 2 Mb, and therefore the frequency of recombinations is ~8 times higher than the average throughout the genome.

e, Genes within the *Sac*-containing interval flanked by *280G12-T7* and *D4Mon1*. The full sequence of this region is assembled from BAC 118E21 and from *Mus musculus* cyclin ania-6b gene (GenBank accession no. AF185591). Filled areas indicate predicted genes. Arrows indicate the predicted direction of transcription.

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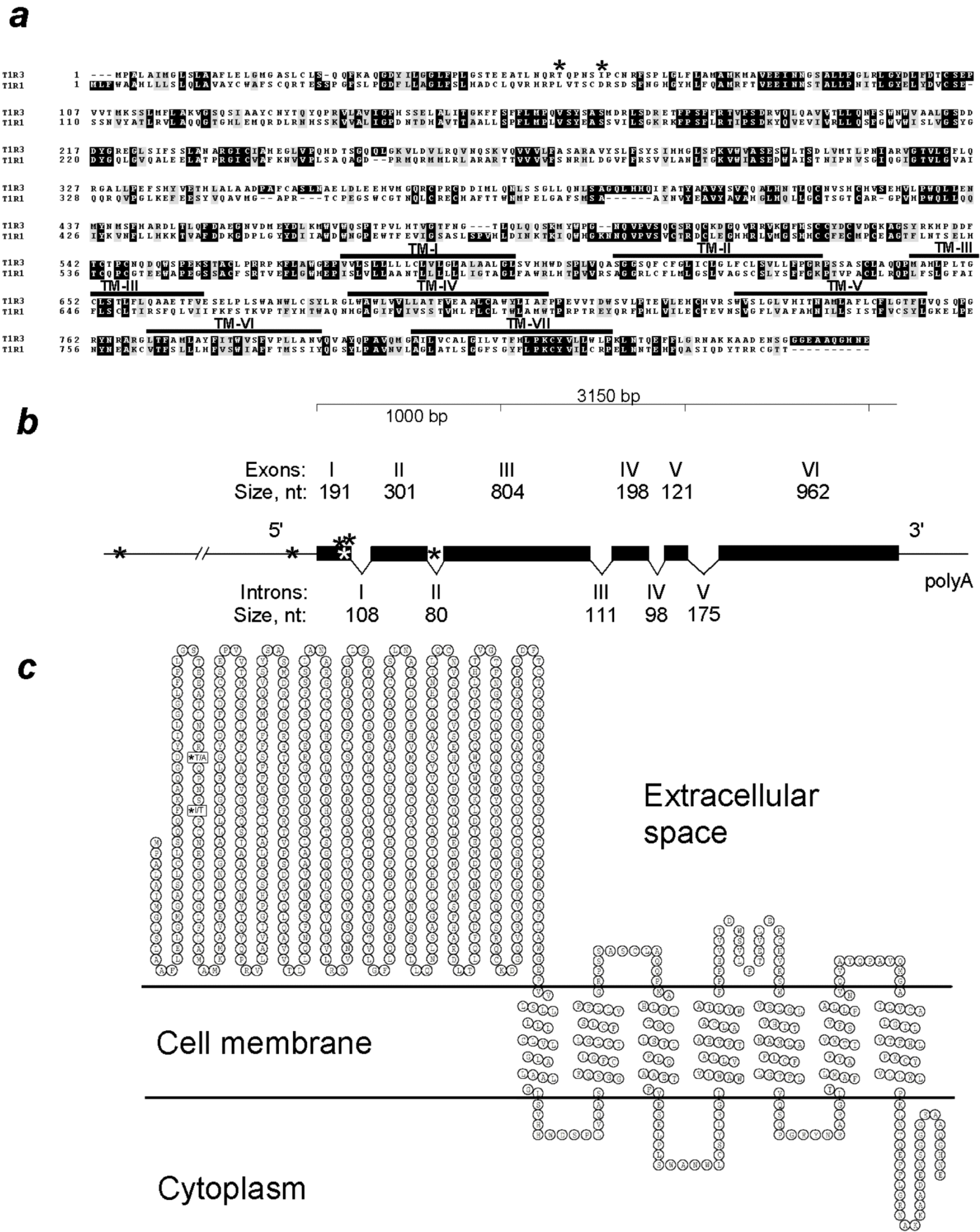

Fig. 2.

Structure of the *Tas1r3* gene.

a, Protein alignment of the novel T1R3 and the previously described T1R1 [*Tas1r1 or Gpr70*; GenBank accession no. AF301161 (Li *et al.*, 2001b)] mouse genes. Identical amino acids are shaded in black; conservative amino acid substitutions are shaded in gray. The protein sequences of T1R3 and T1R1 were deduced from cDNA sequences of the B6 strain. * denotes missense polymorphisms in T1R3. Roman numerals with solid black bars underneath indicate the transmembrane domains.

b, Structure of the *Tas1r3* gene based on comparison between genomic DNA and cDNA sequences from mouse tongue epithelium. The six coding exons are shown as black boxes. Exon and intron sizes are given in nucleotide base pairs (sizes of exons I and VI are partial, excluding untranslated regions). Asterisks indicate SNPs defining haplotypes of high-sweetener preferring strains (B6-like) and low-sweetener preferring strains (129-like; see Fig 3). The B6-like/129-like haplotypes are as follows (the haplotype nucleotides are numbered with the A in the ATG start codon as nucleotide 1): T/A at nt −2383, A/G at nt −183, A/G at nt 135 (exon I, silent at amino acid position 45), A/G at nt 163 (exon I, missense T55A), T/C at nt 179 (exon I, missense I60T), and T/C at nt 651, (intron II). ***c***, Conformation of the predicted T1R3 protein. The missense mutations (Thr55Ala and Ile60Thr) are denoted with *.

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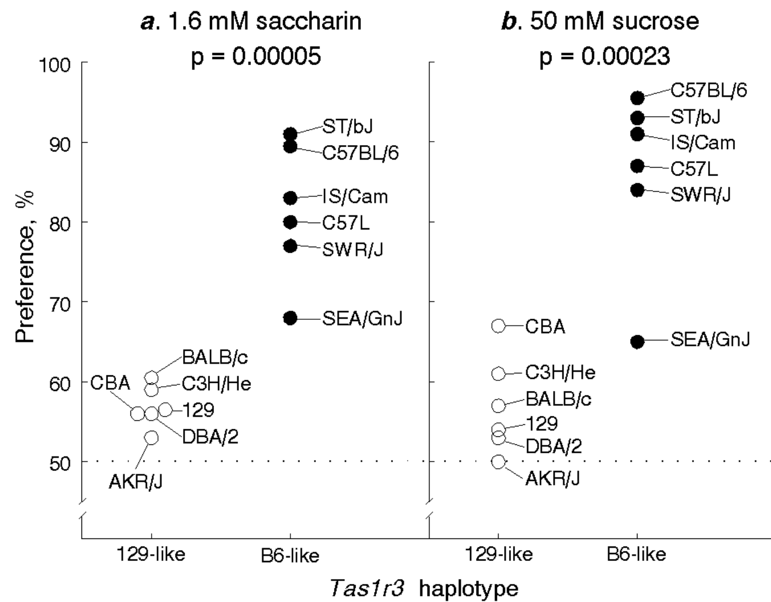

Fig. 3.

Relationship between *Tas1r3* haplotype and sweetener preference.

Saccharin (***a***) and sucrose (***b***) preferences by mice from inbred strains with two different haplotypes of the *Tas1r3* gene (see Figure 2*b*). The strains with the B6-like haplotype of *Tas1r3* strongly preferred saccharin (81 ± 4%) and sucrose (86 ± 5%), whereas strains with the 129-like haplotype were indifferent to these solutions (57 ± 1% and 57 ± 3% respectively, $p_s < 0.0003$, t-tests). The SEA/GnJ strain had lower sweetener preference compared with other strains with the B6-like haplotype. A mutation within the bone morphogenetic 5 protein in the SEA/GnJ strain (Kingsley *et al.*, 1992) may cause disturbance in calcium metabolism and reduce sweetener preference (Tordoff and Rabusa, 1998). The complete strain name is shown if identical substrains were used for genotyping and phenotyping; the strain name is truncated if the substrain genotyped differed from the substrain phenotyped.