Figures and figure supplements

Non-classical amine recognition evolved in a large clade of olfactory receptors

Qian Li et al
Figure 1. Diamine recognition sites in the cadaverine receptor TAAR13c. (A) Structural modeling of zebrafish TAAR13c bound to cadaverine (yellow) reveals two aspartates (D112<sup>3.32</sup> and D202<sup>5.42</sup>) with carboxylates (red) predicted to form salt bridges to ligand amino groups (blue). (B) Cartoon of zebrafish TAAR13c topology depicts the location of D112<sup>3.32</sup> and D202<sup>5.42</sup>. (C) Charge-neutralizing mutation of D112<sup>3.32</sup> and D202<sup>5.42</sup> abrogates cadaverine responsiveness of zebrafish TAAR13c expressed in HEK-293 cells (mean ± sem, n = 3, **p<0.01 based on two-tailed unpaired Student's t-test). (D) D202A<sup>5.42</sup> mutation transforms TAAR13c from a diamine receptor into a monoamine receptor (mean ± sem, n = 3).

DOI: http://dx.doi.org/10.7554/eLife.10441.003
Figure 1—figure supplement 1. Functional analysis and structural modeling of TAAR13c mutants. (A) Structural modeling of TAAR13c bound to cadaverine (yellow) reveals residues within 5 Å of the amino group pointing towards transmembrane α-helix 5. (B) Charge-neutralizing mutation of Asp$^{3.32}$ eliminates cadaverine responsiveness in TAAR13c. HEK-293 cells were co-transfected with Cre-Seap and the D112A$^{3.32}$ mutant of TAAR13c, incubated with cadaverine or 3-methoxytyramine at various concentrations, and assayed for phosphatase activity (mean ± sem, n = 3). (C) Structural modeling of wild type TAAR13c bound to cadaverine (yellow) and the TAAR13c D202A$^{5.42}$ mutant bound to 3-methoxytyramine (yellow).

DOI: http://dx.doi.org/10.7554/eLife.10441.004
Figure 2. Asp\textsuperscript{5.42} is highly conserved in clade III TAARs. (A) Cartoon depiction indicates the location of positions 3.32 and 5.42 in GPCRs. (B) Bioinformatic analysis reveals the number of receptors with anions at positions 3.32 (X) or 5.42 (Y) across various biogenic receptor subfamilies in mouse, as well as TAARs in mouse, rat, humans, and zebrafish. Two mouse, rat, and human TAARs have Asp\textsuperscript{5.43} instead of Asp\textsuperscript{5.42}, and these are included in columns marked Y = Asp (*). (C) Phylogenetic analysis of the TAAR family in zebrafish (solid lines), mice (dashed lines), rats (dashed lines), and humans (dashed lines); scale bar = 0.5 substitutions per site. TAARs containing only Asp\textsuperscript{3.32} (blue), only Asp\textsuperscript{5.42} (red), or both Asp\textsuperscript{3.32} and Asp\textsuperscript{5.42} (green) are depicted. Mammalian TAARs with Asp\textsuperscript{3.32} and Asp\textsuperscript{5.43} instead of Asp\textsuperscript{5.42} are green. Glu\textsuperscript{3.32} containing rodent TAARs and the one zebrafish TAAR, TAAR19f, that lacks both Asp\textsuperscript{3.32} and Asp\textsuperscript{5.42} are depicted in black.

DOI: http://dx.doi.org/10.7554/eLife.10441.005
Figure 2—figure supplement 1. The phylogenetic tree of tetrapod and fish TAARs. (A) As in Figure 2, bioinformatic analysis reveals the number of receptors with anions at positions 3.32 (X) or 5.42 (Y). (B) Phylogenetic analysis of the TAAR family in fish (solid lines) and tetrapods (dashed lines); scale bar = 0.8 substitutions per site. TAARs containing only Asp$^{3,32}$ (blue), only Asp$^{5,42}$ (red), both Asp$^{3,32}$ and Asp$^{5,42}$ (green), and neither (black) are depicted.

DOI: http://dx.doi.org/10.7554/eLife.10441.006
Figure 2—figure supplement 2. Phylogenetic analysis of TAARs from different fish species. Using the phylogenetic tree in Figure 2—figure supplement 1B as a template, TAARs are highlighted from individual fish species indicated (solid, color), as well as from zebrafish and tetrapods (dashed, grey); scale bar = 0.8 substitutions per site. TAARs containing only Asp\(^{3.32}\) (blue), only Asp\(^{5.42}\) (red), both Asp\(^{3.32}\) and Asp\(^{5.42}\) (green), or neither (black) are depicted.

DOI: http://dx.doi.org/10.7554/eLife.10441.007
Figure 3. Identifying the first ligands for several zebrafish TAARs. HEK-293 cells were cotransfected with Cre-Seap and plasmids encoding zebrafish TAARs, incubated with test chemicals (100 μM), and assayed for phosphatase activity using a fluorescent substrate (mean ± sem, n = 3). Zebrafish TAAR10a and TAAR12h are clade I TAARs containing Asp$^{3.32}$ but not Asp$^{5.42}$ (blue), zebrafish TAAR13a, TAAR13c, TAAR13d, and TAAR13e, and TAAR14d contain both Asp$^{5.42}$ and Asp$^{3.32}$ (green), and zebrafish TAAR16c is a clade III TAAR containing Asp$^{5.42}$ but not Asp$^{3.32}$ (red).

DOI: http://dx.doi.org/10.7554/eLife.10441.008
Figure 3—figure supplement 1. Functional expression of TAAR10b, TAAR12i, and TAAR16e in Hana3A cells. Hana3A cells were co-transfected with Cre-Seap and TAAR-encoding plasmids, incubated with ligands, and assayed for phosphatase activity (mean ± sem, n = 6).

DOI: http://dx.doi.org/10.7554/eLife.10441.009
Figure 3—figure supplement 2. Structure-function studies of zebrafish clade I TAARs: TAAR10a and TAAR12h. HEK-293 cells were co-transfected with Cre-Seap and TAAR-encoding plasmids, incubated with ligands, and assayed for phosphatase activity.

DOI: http://dx.doi.org/10.7554/eLife.10441.010
Figure 4. Structure-activity studies of zebrafish TAARs. (A) Zebrafish TAAR13d displays highest affinity for putrescine among tested ligands, and reduced affinity for longer or shorter diamines. (B) Zebrafish TAAR16c recognizes several structurally related amines but not oxygen-containing analogs (1 mM). F: N-methylpiperidine; G: piperidine; H: N-methylpyrrolidine; I: pyrrolidine; J: tetrahydropyran; K: tetrahydrofuran. (C) Dose-dependent responses of TAAR16c for N-methylpiperidine and tetrahydropyran. (D) Zebrafish TAAR16f recognizes isoamylamine (L) but not isoamyl alcohol (M) at 1 mM. (E) Dose-dependent responses of TAAR16f for isoamylamine and isoamyl alcohol (mean ± sem, n = 3).

DOI: http://dx.doi.org/10.7554/eLife.10441.011
Figure 4—figure supplement 1. Several TAARs detect amines but not amino acids. HEK-293 cells were co-transfected with Cre-Seap and TAAR-encoding plasmids, incubated with ligands, and assayed for phosphatase activity (mean ± sem, n = 3).

DOI: http://dx.doi.org/10.7554/eLife.10441.012
Figure 4—figure supplement 2. Structure-function studies of the zebrafish clade III TAAR, TAAR16c. HEK-293 cells were co-transfected with Cre-Seap and plasmid encoding TAAR16c, incubated with ligands, and assayed for phosphatase activity (mean ± sem, n = 3–6).

DOI: http://dx.doi.org/10.7554/eLife.10441.013
Figure 5. Dose-dependent responses of zebrafish TAARs and charge-neutralizing TAAR mutants. (A) The identities of amino acids 3.32 and 5.42 in nine ‘de-orphaned’ zebrafish TAARs, as well as preferred ligands and corresponding EC$_{50}$s, are depicted. (B) Dose-dependent activation of TAARs and TAAR mutants by ligands indicated (mean ± sem, n = 3). Responses are shown in cells transfected with Cre-Seap alone (black) or together with wild type TAARs (red), D$_{3.32}$A mutant TAARs (blue), and D$_{5.42}$A mutant TAARs (green). D$_{3.32}$ is lacking in clade III TAARs (TAAR16c, TAAR16f) and D$_{5.42}$ is lacking in clade I TAARs (TAAR10a, TAAR12h), so the corresponding D$_{3.32}$A and D$_{5.42}$A mutants could not be generated.

DOI: http://dx.doi.org/10.7554/eLife.10441.014
Figure 6. Modeling the structure and evolution of non-classical amine recognition. (A) Structural modeling of zebrafish TAAR10a and TAAR16c bound to serotonin and N-methylpiperidine (yellow) respectively. (B) A model for the birth of a large clade of olfactory receptors with non-classical amine recognition. We propose that clade III TAARs evolved a non-classical mode of amine recognition in two steps. First, an ancestral TAAR gained the ability to recognize diamines by acquiring Asp$^{5.42}$. Subsequently, a diamine-detecting TAAR lost the canonical amine recognition site, Asp$^{3.32}$, leading to non-classical amine recognition through a transmembrane α-helix V salt bridge. Extensive gene duplication and mutation expanded and diversified clade III TAARs, leading to a large clade of olfactory receptors with non-canonical amine-detection properties.

DOI: http://dx.doi.org/10.7554/eLife.10441.015
Figure 6—figure supplement 1. Re-engineering the amine contact site of HTR6. HEK-293 cells were co-transfected with Cre-Seap and plasmids encoding HTR6 or an HTR6 double mutant (D^{3.32}A; A^{5.42}D), incubated with ligands (0, 10 or 100 μM), and assayed for phosphatase activity (mean ± sem, n = 3, *p < .05, **p < .01 based on one-way ANOVA analysis and Dunnett’s test to compare controls and ligand-activated responses).

DOI: http://dx.doi.org/10.7554/eLife.10441.016