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Shared and Unique G Alpha Proteins in the Zebrafish Versus Mammalian Senses of Taste and Smell

Yuichiro Oka and Sigrun I. Korsching

Institut für Genetik, Universität zu Köln, Zülpicher Strasse 47a, D-50674 Köln, Germany

Correspondence to be sent to: Yuichiro Oka, Institut für Genetik, Universität zu Köln, Zülpicher Strasse 47a, 50674 Köln, Germany. e-mail: okay@uni-koeln.de

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Abstract

Chemosensory systems in vertebrates employ G protein–coupled receptors as sensors. In mammals, several families of olfactory and gustatory receptors as well as specific G alpha proteins coupling to them have been identified, for example, gustducin for taste. Orthologous receptor families have been characterized in fish, but the corresponding G alpha genes have not been well investigated so far. We have performed a comprehensive search of several lower vertebrate genomes to establish the G alpha protein family in these taxa and to identify those genes that may be involved in chemosensory signal transduction in fish. We report that *gustducin* is absent from the genomes of all teleost and amphibian species analyzed, presumably due to independent gene losses in these lineages. However, 2 other G alpha genes, *Gi1b* and *G14a*, are expressed in zebrafish taste buds and 4 G proteins, *Go1*, *Go2*, *Gi1b*, and *Golf2*, were detected in the olfactory epithelium. *Golf2*, *Gi1b*, and *G14a* are expressed already shortly after hatching, consistent with the physiological and behavioral responses of larvae to odorants and tastants. Our results show general similarity to the mammalian situation but also clear-cut differences and as such are essential for using the zebrafish model system to study chemosensory perception.

Key words: teleost, gustducin, evolution, phylogeny, lamprey

Introduction

Chemicals are essential stimuli for animals to find food and prey, avoid predators, and select mates or nesting positions in the environment. Vertebrates are equipped with 2 main sensory systems to detect environmental chemicals, the olfactory and gustatory senses. These systems employ several families of G protein–coupled receptors (GPCRs) as receptors, odorant receptors (ORs), vomeronasal receptors (V1Rs and V2Rs), trace amine–associated receptors (TAARs), and formyl peptide receptors (FPRs) in the olfactory system, and taste receptors (T1Rs and T2Rs) in the gustatory sense (Mombaerts 2004; Liberles and Buck 2006; Liberles et al. 2009; Riviere et al. 2009). The alpha subunits of heterotrimeric G proteins through which mammalian receptors transduce signals have been characterized: ORs and TAARs couple to Golf (Belluscio et al. 1998; Liberles and Buck 2006), V1Rs and all FPRs but one to Gi2 (Berghard and Buck 1996; Norlin et al. 2003; Liberles et al. 2009; Riviere et al. 2009), V2Rs and FPR-rs1 to Go (Berghard and Buck 1996; Riviere et al. 2009; Liberles et al. 2009), and taste receptors mainly to gustducin (also known as Gt3 or Ggust) (McLaughlin et al. 1992; Wong et al. 1996; Chandrashekar et al. 2000; Ueda et al. 2003). However, because *gustducin* knockout mice show only partial loss of taste sensations, other G alpha proteins also found in taste cells, Gi2 and G14 may contribute to signal transduction in mammals (Wong et al. 1996; Caicedo et al. 2003; He et al. 2004; Shindo et al. 2008; Tizzano et al. 2008).

In contrast, our knowledge about chemosensory signaling in fish species is very limited. G alpha subunits have not been described in fish chemosensory systems, excepting some immunohistochemical studies that used antibodies raised against mammalian G alpha proteins (Teleosts, Hansen et al. 2003, 2004; Hansen and Zielinski 2005; Zhang et al. 2006; Koide et al. 2009; Cartilaginous fish, Ferrando et al. 2009, 2010). Recently, the zebrafish G alpha gene family has been characterized (Oka et al. 2009). One major difference to the mammalian situation is the existence of a fifth class of G proteins, Gv, beyond the 4 classes (Gs, Gi, Gq, G12) shared with mammals. Interestingly, gustducin was not detected in the zebrafish genome in that study. Here,

we have extended those studies to characterize the G alpha gene family in 4 additional teleost fish species as well as in the genome of an amphibian and a jawless vertebrate.

We report that *gustducin* appears to have been lost early in the teleost lineage and independently in the amphibian lineage. Both local and whole-genome duplications have increased the size of the fish G protein repertoires above that typically seen in mammals. We identified the G alpha genes expressed in zebrafish chemosensory tissues and showed that some of their teleost-specific paralogs are not found in chemosensory tissues and may thus have acquired new functions. We detected expression of G alpha genes in zebrafish chemosensory tissues already shortly after hatching, consistent with the early onset of sensory and behavioral responses to odorants and tastants (Lindsay and Vogt 2004; Li et al. 2005). The present study provides evidence that chemosensory receptors in mammals and teleosts mostly have homologous signaling molecules but also show some molecular differences, possibly resulting from their adaptation to the respective environment.

Materials and methods

Data mining

Recursive TBLASTN search using 16 mouse G alpha genes as query was performed to detect candidate G alpha genes in the Ensembl genome database (http://www.ensembl.org/index.html) for 5 teleosts (zebrafish *Danio rerio*, medaka *Oryzias latipes*, three-spined stickleback *Gasterosteus aculeatus*, fugu *Takifugu rubripes*, and green spotted puffer *Tetraodon nigroviridis*), western clawed frog *Xenopus tropicalis*, and in Pre!Ensembl database (http://pre.ensembl.org/index.html) for sea lamprey *Petromyzon marinus*. An expectation cutoff value of 10^{-10} was used as an exclusion criterion. All searches were performed in release 48 and confirmed in release 58. All exons of each candidate G alpha genes were detected with GeneWise (http://www.ebi.ac.uk/Tools/Wise2/) with occasional minor manual extensions for N-terminals. Resulting candidate genes were confirmed to be G alpha genes by constructing a phylogenetic tree with neighbor-joining algorithm (Clustal X 2.0.9). Due to the fragmented nature of the database, individual exons of lamprey G alpha genes were annotated based on the family-specific motifs in some cases. These genes are named following mammalian nomenclature. Postfixes (a, b, etc.) for paralogs were first given to zebrafish genes according to the homology to mammalian orthologs (e.g., zebrafish G11a is closer to mouse G11 than G11b is) and next given to genes in other fish species according to the homology to zebrafish orthologs (e.g., medaka G11a is closer to zebrafish G11a than medaka G11b is). This unified nomenclature was applied to all the orthologs of the previously known medaka gene, Gαia, renaming them as *Gi1b* (zebrafish Gαia gene, accession number NM_ 198805).

Phylogeny and synteny analyses

All G alpha protein sequences from 5 teleosts, human (*Homo sapiens*), mouse (*Mus musculus*), and 5 plants (*Arabidopsis thaliana*, *Oryza sativa*, *Glycine max*, *Solanum tuberosum*, and *Nicotiana tabacum*) were aligned with MAFFT (http://www.ebi.ac.uk/Tools/mafft/). Phylogenetic trees were constructed with neighbor-joining algorithm (Clustal X 2.0.9) with 1000 bootstrap or Bayesian inference (MrBayes 3.1.2) with number of generations = 10^6, sampling frequency = 100, number of chains = 4, and burn-in = 5000. The resulting trees are visualized with NJplot 2.1.

Syntenic regions surrounding *Gt* and *Gi* genes in human, mouse, 5 teleosts, and xenopus were examined in Ensembl genome viewer up to 6 genes in both 5′ and 3′ directions, and the gene arrangements were compared among species and loci.

Reverse transcription-polymerase chain reaction (RT-PCR) cDNA cloning of G alpha genes from zebrafish

Total RNA was extracted from barbels and lips, olfactory epithelium, eyes, gills, and brain of adult zebrafish (8–12 months, males) with RNeasy mini Kit (QIAGEN). cDNA was synthesized using BioScript reverse transcriptase (Bioline). cDNA fragments for each G alpha gene were amplified with specific primers for *Gs1* (forward-AAGACTGAGGACCAGCGAAA, reverse-GCTGGACAGGCTAACTGGAC), *Golf1* (forward-TGCTTCGCTGCAGAGTGTTA, reverse-GCATGTCCTGCTTGTTCAGA), *Golf2* (forward-GCTACACACTTCCCCCTGAA, reverse-CACACATTCATTCCCCTCCT), *Gt1* (forward-CTCCCTTGAAGAGTGCTTGG, reverse-GGATCAACCTCTCCAGGTCA), *Gt2* (forward-ACGGTCAAACTTCTGCTGCT, reverse-AGCAGAGTCGTTCAGCTGGT), *Go1* (forward-CCAGTCCAACGCTGTCTTTT, reverse-CGCTCCTTGTCTCCGTACTC), *Go2* (forward-TCTTCTGTGTGGCAATGAGC, reverse-AAAGTCACAGGGGGTGTCAG), *Gi1a* (forward-CACGATTCAGTCCATCATCG, reverse-TGTGTCTCCACAATGCCTGT), *Gi1b* (forward-GAGTAATCAAGCGGCTCTGG, reverse-TTCGGTTCATCTCCTCATCC), *Gi2a* (forward-ATCAGGGAAGAGCACCATTG, reverse-CTGCTGGGTGGGAATGTAGT), *Gi2b* (forward-GGGAAGAGCACCATCGTAAA, reverse-CTGCTGGGTTGGGATGTAAT), *Gi3* (forward-CCGCGATGGAGAAGAGTAAG, reverse-AGGCTGCAGAATCGTTGAGT), *Gz* (forward-ACATCCCAACTGTCGAGGAC, reverse-GATGTTGCTGGTGTCAGTGG), *Gq* (forward-AGAATCATCCATGGCTCAGG, reverse-GCTGGGTTGGAATGTAGGAA), *G11a* (forward-CGATCAGGTTCTGGTGGAAT, reverse-TGAAAGGCGAGTTGGAGTCT), *G11b* (forward-CCATGCAGGCAATGATCCGC, reverse-TAGTGGAGTCAGAGAGCTGG), *G14a* (forward-GTCCATCAGAACACGCTGAG, reverse-TGTCGCTCCATAAACTGCTG), *G14c* (forward-GTCCTTTGCCAAACTGGTGT, reverse-TGGATCCACTTCCGTCTTTC), *G15a* (forward-GTATCACGGCTCCAGA

CTAC, reverse-CTTTTCCTCCAGGATGTCCA), *G15b* (forward-ACAGTCGGCGCAGAGAGTAT, reverse-GGATGACGGAGGAATAAGC), *G15c* (forward-CTACAAAGACGGCGGACAGT, reverse-AGTAAACCACGGGTTGTGGA), *G15d* (forward-ATTCTCCGTGTCCAATCGTC, reverse-TCATAGCATCGTCAGCGTCT), *G12* (forward-AAAGCGCTGCTCGACTTCAG, reverse-ATGCCGCTATCATTCCAGAG), *G13a* (forward-AGAAATTGCACATCCCTTGG, reverse-TTTTGGCTGGGCAAGTAGTC), *G13b* (forward-CCGCAGTACCTGCCCTCAAT, reverse-GCTTTCGAAGCACTCAAACC), and *Gv1* (forward-GTGTGGCCGTTGTTTTGATG, reverse-CATGTCGTATCCACTCAGAG). Primers for controls are β-actin (forward-CCCCATTGAGCACGGTATT, reverse-AGCGGTTCCCATCTCCTG), phospholipase c (plc)-β2 (forward-TGGTGAAGCTGGACAAACTG, reverse-AAGCACAACCGGATACTTGG), and olfactory marker protein (omp, forward-CAAGGACACACAGTAGACGC, reverse-GGAACAGACTGACCAGAAGAG). All cDNA fragments amplified were cloned into pGEM-T vector (Promega).

In situ hybridization with adult tissue sections

Probe synthesis was done as described (Oka et al. 2009). Olfactory epithelia were dissected out, fixed in 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS, pH 7.5) at 4 °C for 2 h, and embedded in TissueTek (Sakura). Upper and lower jaws together with barbels were dissected out, fixed in 4% PFA in PBS containing 1 mM ethylenediaminetetraacetic acid at 4 °C overnight, and embedded in TissueTek. Sections of 8 μm and 10 μm were cut and collected onto Superfrost slide glasses (Thermo) for the olfactory epithelium and jaws, respectively. Pretreatment of sections, hybridization, high stringency washing, and signal detection were performed as described (Weth et al. 1996). Sections were observed and photographed with BZ-8100E (Keyence).

Whole mount in situ hybridization with 3 days post fertilization larvae

Whole mount in situ hybridization was performed as described (Thisse et al. 1993) using 3 days postfertilization (dpf) larvae, in which pigmentation was blocked by treatment with 0.045% phenylthiourea from late epiboly stage onward. Stained larvae were observed and photographed with Nikon SMU-Z binocular with Nikon Coolpix 950 attached.

Results

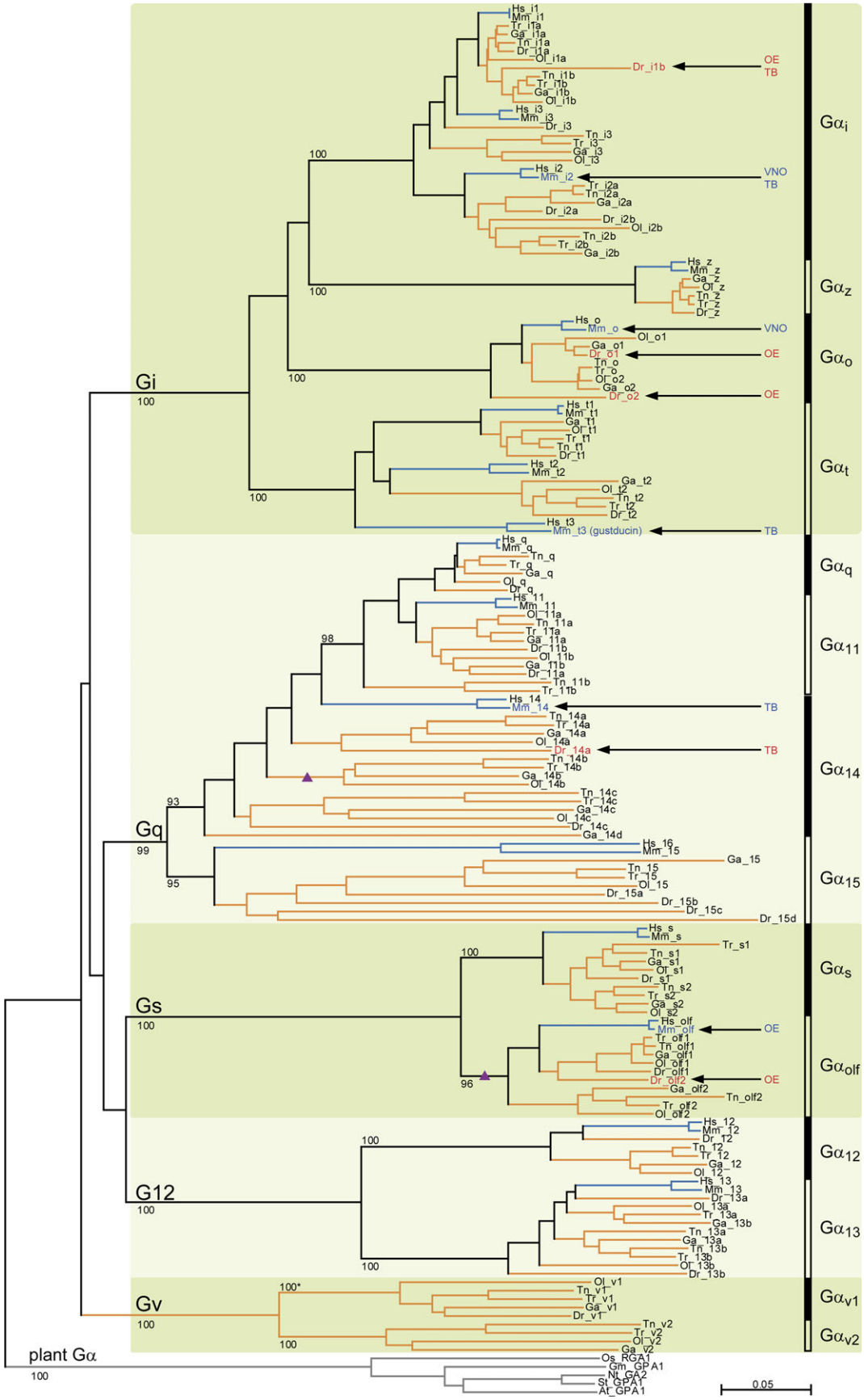
Gustducin gene is not present in any teleost and amphibian genomes analyzed.

We performed a recursive search for G alpha genes in the genomes of 5 teleost fish. In total, 26, 27, 25, 25, and 25 genes were found for zebrafish, stickleback, medaka, tetraodon, and fugu, respectively. This is considerably larger than the mammalian family size of 16 functional genes (Birnbaumer 2007). Most of the increase in family size is due to the retention of many duplicated paralogs, originating in the whole-genome duplication early in teleost evolution (Hoegg et al. 2004). Additionally, several cases of local gene duplication were observed, most noticeably in *G15*, which is a single gene in mammals, but a family of 4 in zebrafish, due to 3 local duplications (Figure 1).

On the other hand, we observe occasional gene losses, always of paralogs, in individual teleost species, that is, late events occurring after divergence of the species analyzed here. However, there is one major exception which concerns the gene specialized for taste signaling in mammals, *gustducin*. Orthologs for this gene were not detected in any of the 5 teleost genomes analyzed in spite of the high genomic coverage in the databases (×6.5 to ×11). We searched for the *gustducin* ortholog also in the amphibian, reptilian, avian, and mammalian genome and expressed sequence tag databases. We found a *gustducin* ortholog in reptiles, avians, and mammals but not in amphibians (×7.7 coverage). These observations raise 2 possibilities: either the land-living vertebrates acquired the *gustducin* gene or the teleosts and the amphibians lost it independently during evolution.

To decide this question, we analyzed the genomic environment of all *Gt* genes. Several G alpha genes occur in evolutionary stable pairwise configuration, *Gq*/*G14*, *G11*/*G15*, and *Gi*/*Gt*. In the human and mouse genome, all 3 *Gt* genes are arranged in such a pairwise fashion, each together with a *Gi* gene (Wilkie et al. 1992). We find that these 3 gene pairs are conserved down to reptiles and 2 of them are found even in teleosts and amphibians. However, of the *Gi1*/*gustducin* gene pair, only the *Gi1* gene is present in all teleosts and amphibian species analyzed (Figure 2), always in a genomic location clearly syntenic with that of the mammalian *Gi1*/*gustducin* pair (data not shown). This synteny is consistent with the *gustducin* loss model but would be difficult to reconcile with the gene gain hypothesis.

To further examine the evolutionary history of *gustducin*, we established the G alpha protein repertoire in a more primitive fish species, the jawless lamprey, as well as in early chordates (lancelet and ascidians). A single *Gi* gene, but no *Gt* was found in early chordates (lancelet and ascidians, data not shown), that is, the local duplication to generate the first *Gi*/*Gt* gene pair may have occurred in the vertebrate lineage after divergence from the chordates. In lamprey, there are already 2 *Gt* genes present (transducins, Muradov et al. 2008) as well as 4 *Gi* genes, but no *gustducin* ortholog. Although no *Gi*/*Gt* gene pairs can be observed presumably due to the fragmented state of the genome assembly, all 4 *Gi* sequences found are orthologs of individual *Gi* genes in teleosts and tetrapods, and the same holds true for the 2 *Gt* genes (Muradov et al. 2008), consistent with the existence of 3 *Gi*/*Gt* gene pairs before separation of the jawless fish lineage. Genomic coverage of the lamprey database is not significantly lower (×5.9) than those for the other species



analyzed (×6.5 to ×11). Although some of the G alpha genes detected were thus only partial, it is rather unlikely that all 8 exons of the *gustducin* gene were missed in our search.

Furthermore, the position of the mammalian Gt3 (gustducin) in the phylogenetic tree is basal to the Gt1 and Gt2 branches (Figure 1), consistent with an early divergence of the *gustducin* gene but not with the recent gene gain model.

Taken together, it is likely that an ancestral *gustducin* gene was present in the early stage of the vertebrate evolution but has been lost independently in lamprey, bony fishes, and amphibians.

Gi1b and G14a genes are expressed in the taste buds in the adult zebrafish

Although gustducin in mammals seems to play a major role in signal transduction from sweet, umami, and bitter taste sensation, it is not the only transducer for taste cells because mice without functional gustducin have a residual ability to respond to these stimuli (Wong et al. 1996; Caicedo et al. 2003; He et al. 2004). Furthermore, additional G alpha genes, *Gi2* and *G14*, have been detected in taste cells (Caicedo et al. 2003; Shindo et al. 2008; Tizzano et al. 2008). As we did not find a *gustducin* gene in the genomes of fish species, we next examined which of the 26 zebrafish G alpha genes are expressed in taste tissues. RT-PCR analysis detected the expression of 19 and 23 G alpha genes in the cDNA prepared from lips/barbels and gills, respectively (Figure 3A), where taste buds have been described (Hansen et al. 2002). Many of these genes are expressed rather ubiquitously in nervous tissue but some show enrichment in taste-related tissue, among them *Gi1b* and *G14a*.

In situ hybridization with cRNA probes for all genes detected by RT-PCR in adult chemosensory tissues demonstrated the expression of *Gi1b* and *G14a* genes in small clusters of cells, which show very similar morphology and distribution to taste buds, as identified by *PLC-β2* and *TrpM5* expression (Figure 3B and data not shown). Both *Gi1b* and *G14a* genes were detected in lips, barbels, and oral cavity, the areas known to contain taste buds in fish. However, and in contrast to mammalian taste buds, neither of the 2 *Gi2* paralogs was detected using in situ hybridization (Supplementary Figure 1). These results strongly suggest that *Gi1b* and *G14a* but not *Gi2* genes are expressed in zebrafish taste buds.

Gi1b, Golf2, Go1, and Go2 genes are expressed in the olfactory epithelium of the adult zebrafish

RT-PCR analysis detected the expression of 23 G alpha genes in the cDNA prepared from adult olfactory epithelium (Figure 3A). *Go1*, *Go2*, *Gi1b*, and *Golf2* are among those which showed enrichment in the olfactory epithelium. In situ hybridization for all genes detected by RT-PCR demonstrated the expression of these 4 genes in the adult olfactory epithelium (Figure 3A). *Golf2*, *Go1*, and *Go2* showed broad expression within the sensory region, with Golf2-expressing cells somewhat more concentrated in the basal layer than Go-expressing cells, whereas *Gi1b* was expressed in a sparse population of cells, most of which were located close to the apical surface of the epithelium (Figure 3C).

Of the paralog pair *Golf1*/*Golf2*, only the *Golf2* gene was detected in the olfactory epithelium (Figure 4B and Supplementary Figure 1). Golf1 could be detected in the brain by in situ hybridization (Supplementary Figure 1), but in the olfactory epithelium only by RT-PCR, presumably due to the efficient amplification of minor transcripts that would be below the detection limit of the in situ hybridization. Similarly, the *Gi1b* paralog *Gi1a* is below the detection limit of in situ hybridization in the olfactory epithelium (data not shown).

The chemosensory G alpha genes are expressed already in early larvae

Previous studies have shown that zebrafish larvae can respond to olfactory and gustatory stimuli already in rather early stages (Lindsay and Vogt 2004; Li et al. 2005). G alpha proteins serving as chemosensory signal transduction molecules would have to be expressed in such early stages, too. We therefore investigated by whole mount in situ hybridization with zebrafish larvae, which G alpha genes are expressed at 3 dpf. We report that *Gi1b* and *G14a* were expressed in small patches in the mouth region, presumably the taste buds (Figure 4A). Zebrafish larvae normally hatch around 2–3 dpf and start feeding around 5 dpf. Thus, our results are consistent with the hypothesis that *Gi1b* and *G14a* may serve as gustatory signal transduction molecules.

Gi1b was also observed in the olfactory pit, delineated by *OMP* gene expression (Figure 4B), where it was expressed in a sparse population of cells, consistent with the finding in the adult tissue. *Golf2* was likewise expressed in the olfactory pit (Figure 4B), but in a broad pattern reminiscent of

Figure 1 Phylogenetic tree of the teleost G alpha protein family. Phylogenetic tree (neighbor-joining algorithm) with all G alpha proteins in 5 teleosts, Orange (Dr, *Danio rerio*; Ol, *Oryzias latipes*; Ga, *Gasterosteus aculeatus*; Tr, *Takifugu rubripes*; and Tn, *Tetraodon nigroviridis*) and 2 mammals, Blue (Hs, *Homo sapiens* and Mm, *Mus musculus*). G alpha genes from 5 plants (At, *Arabidopsis thaliana*; Os, *Oryza sativa*; Gm, *Glycine max*; St, *Solanum tuberosum*; and Nt, *Nicotiana tabacum*) were used as an outgroup (Gray). Five G alpha classes and 14 families are alternately shaded and shown with alternating bars to the right, respectively. Branching to each class and to most families is supported by near-maximal to maximal bootstrap values (numbers in percent of 1000 trials). Asterisk indicates the branch that is moderately supported by Bayesian inference; triangles depict branches that change positions in Bayesian analysis. G alpha subunits found in chemosensory organs are colored, and corresponding organs are indicated as OE, olfactory epithelium; VNO, vomeronasal organ; and TB, taste buds. The scale bar shows amino acid substitution rate for the neighbor-joining tree.

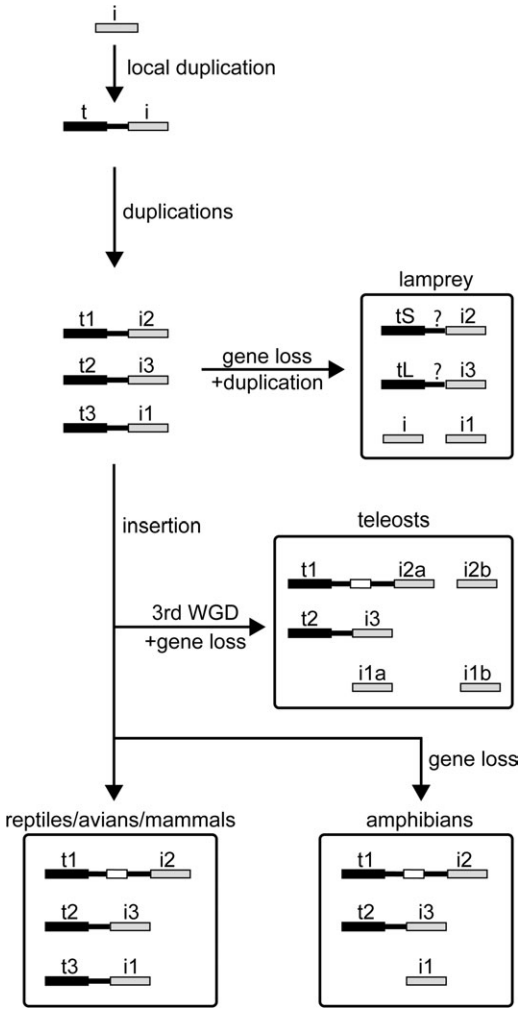


Figure 2 A schematic model of the evolution of Gi/Gt gene pairs in the vertebrate lineage. Gt and Gi genes are shown as filled or shaded boxes, respectively, and intergenic regions are drawn as lines. An ancestral Gi gene duplicated locally to make a Gi/Gt gene pair. The subsequent duplications lead to the presence of 3 Gi/Gt pairs, among them the Gi1/Gt3 (gustducin) pair. In lamprey (a jawless fish), 2 clear Gt orthologs (GtS and GtL) and 3 Gi genes (plus fragments from a fourth Gi gene) were identified (Muradov et al. 2008 and this study). Pairings of Gt and Gi genes cannot be observed in lamprey due to the current low degree of assembly and are therefore indicated by question marks. Before separation of teleosts and tetrapods, a gene inserted within the Gi2/Gt1 pair (open box) and additional insertions occurred in teleosts (data not shown). The teleost-specific third whole-genome duplication (WGD) and following gene losses resulted in 2 Gt genes and 5 Gi genes. Gi2a is lost in medaka (not shown). In amphibians, the Gt3 (gustducin) gene was lost independently.

the expression in the adult tissue. *Go1* and *Go2* were not detected, possibly due to a low level of expression at this stage.

Taken together, these data are consistent with the hypothesis that in zebrafish, Gi1b and G14a mediate the gustatory signals and that olfactory signals are transduced through Golf2, Gi1b, and Go proteins.

Discussion

In the present study, we have delineated the teleost G alpha protein family and found it to be consistently larger than the mammalian families due to the partial retention of duplicate genes, which may have been generated in a whole-genome duplication early in the teleost lineage (Hoegg et al. 2004). Occasional birth and death events of G alpha genes in individual species did not change the family size much among teleosts. However, the gene employed in mammalian taste signal transduction, *gustducin*, is absent from all teleost genomes studied. The lone observation of immunohistochemical staining for gustducin in catfish (Zhang et al. 2006) may conceivably be caused by cross-reactivity of the antibody because the homology between gustducin and other Gt proteins is rather high, nearly 80%.

We have shown that this absence is most likely due to a loss of the ancestral *gustducin* gene early in teleost evolution. In fact, *gustducin* appears to have been lost independently in amphibians as well. Such G protein losses in individual lineages are not unexpected as they are very common for the fifth class of G proteins, Gv (Oka et al. 2009). In the alternate explanation of a gene gain in tetrapods, *gustducin* would have to be generated as local duplication of a *Gi* gene. This is very unlikely, however, because *gustducin* is much closer related to the other *Gt* genes than to any of the *Gi* genes (Figure 1). Alternatively, a new *Gi/Gt* gene pair might have formed in the ancestor of land-living vertebrates by a translocation of a distant *Gt* gene into the *Gi1* surrounding. However, this appears to be an extremely unlikely event. Taken together, these observations favor the scenario in which the *gustducin* gene has been lost twice, one loss in the ancestor of teleosts and another independent one in the ancestor of amphibians.

In mouse, gustducin is a major transducing molecule and its loss leads to significant, but not complete, impairment of sensation to sweet, umami, and bitter taste modalities (Wong et al. 1996; Caicedo et al. 2003; He et al. 2004). The residual partial responses are thought to be mediated through Gi2 or G14 and are sufficient for the animals to survive and reproduce (Wong et al. 1996). It is conceivable that gustducin would serve to enhance the sensitivity or to modulate the signaling in the terrestrial vertebrates. A nonessential role would be consistent with its loss from the teleost fish and amphibian species.

Of the remaining taste-associated G alpha genes, only one, *G14a*, is the direct ortholog of the mammalian equivalent *G14*, the other, *Gi1b*, is from the same subfamily, but not the ortholog of the mammalian *Gi2* gene. The same observation was made for the smell-associated G alpha genes. Whereas *Golf2* is the direct ortholog of the mammalian *Golf*, *Gi1b* is not orthologous to the mammalian *Gi2*, as mentioned above. Nevertheless, both Gi1b and Gi2 are associated with members of the same olfactory receptor family, the *ora/v1r* family of putative pheromone receptor genes (Berghard and

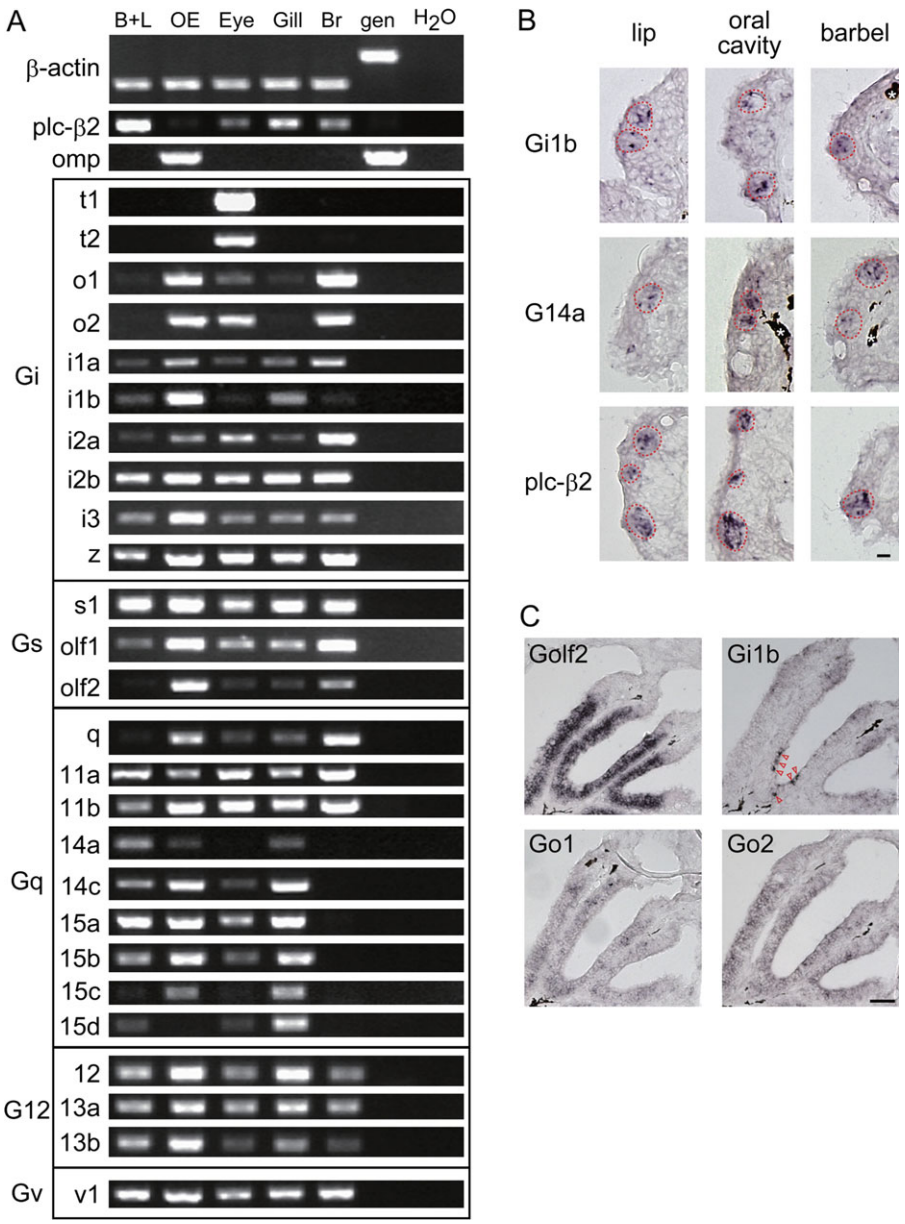


Figure 3 Expression of G alpha genes in adult zebrafish chemosensory tissues. **(A)** Expression of all 26 G alpha genes in sensory tissues and the brain of adult zebrafish was analyzed with RT-PCR using gene-specific primer pairs. The primers for β-actin were used to confirm equivalent cDNA inputs, and the ones for plc-β2 and omp were to confirm proper tissue preparations. The 5 classes of G alpha subunits are shown to the left, boxed. Tissues are indicated on top. B+L, barbels and lips; OE, olfactory epithelium; Br, brain; gen, genomic DNA. **(B)** Expression of Gi1b (top) and G14a (middle) in taste buds of adult zebrafish was examined with in situ hybridization. Staining was detected in lip, oral cavity, and barbels as small clusters of cells, the taste buds (encircled), identified by plc-β2 expression (bottom). Scale bar, 10 μm. **(C)** Expression of Golf2, Gi1b, Go1, and Go2 in the olfactory epithelium was examined with in situ hybridization. Staining for Golf2, Go1, and Go2 was broadly distributed within the sensory region, whereas Gi1b was detected in a sparse cell population (triangles). Scale bar, 50 μm.

Buck 1996; Norlin et al. 2003 and Oka Y and Korsching SI, unpublished observation). Interestingly, in both smell and taste, the same replacement, from *Gi1b* to *Gi2*, has occurred between the teleost and mammalian evolution. A recent study with transgenic medaka fish demonstrated that expression of the dominant negative form of rat Gi2 in taste cells can disturb the feeding response to attractive and repulsive tastants (Aihara et al. 2008). It remains to be seen in what sense Gi1b may be better suited for signaling of aquatic taste and smell stimuli compared with Gi2. The *Gi1b*-expressing cell population appears to be crypt neurons, a third type of olfactory receptor neurons found in fish but not in mammals. A detailed discussion of the nature of these neurons will be presented elsewhere.

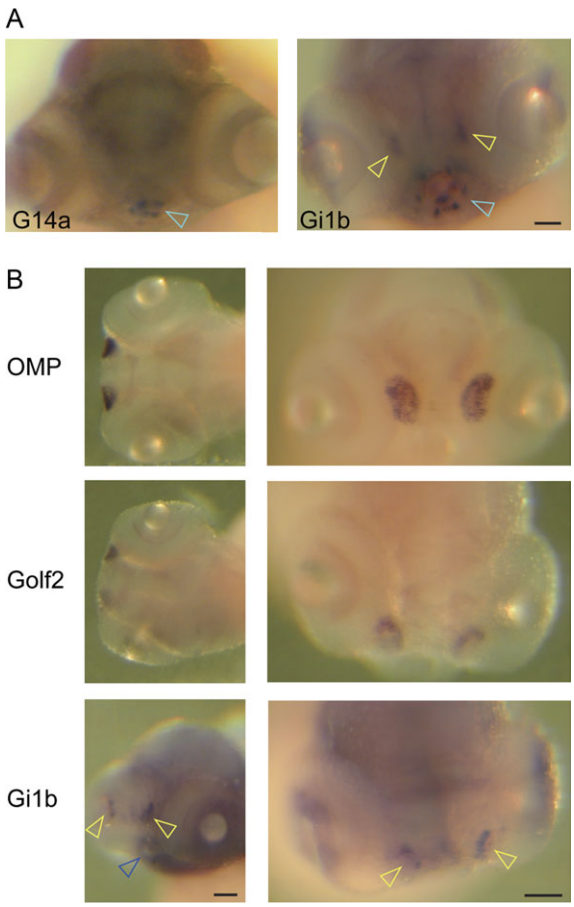


Figure 4 Chemosensory G alpha genes are expressed at an early larval stage. **(A)** Expression of G14a (left) and Gi1b (right) in 3 dpf zebrafish larvae was examined with whole mount in situ hybridization. Specific signals were detected in small patches in the mouth (blue arrowheads), which are larval taste buds. Gi1b was also detected in the olfactory pits (yellow arrowheads). Frontal views. Scale bar, 50 μm. **(B)** Expression of Golf2 (middle) and Gi1b (bottom) in 3 dpf larvae was analyzed with whole mount in situ hybridization. Specific signals were detected in the olfactory pits, which are visualized by omp expression (top). Left panels are dorsal (omp and Golf2) and fronto-lateral (Gi1b) views, and right panels are frontal views. Arrowheads are as in **(A)**. Scale bars, 50 μm.

It is worth pointing out that of those duplicate genes retained after the teleost-specific whole-genome duplication (Hoegg et al. 2004), only some remain expressed in the chemosensory organs, most notably the paralog pair *Go1* and *Go2*. Both genes are expressed in the olfactory epithelium, presumably in microvillous neurons expressing the *V2R*-like *OlfC* genes, exactly as the single mammalian *Go* gene, which is coexpressed with *V2R* genes. The somewhat more basal location of *Golf2*-expressing cells fits to an exclusive expression in ciliated receptor neurons expected from immunohistological study (Koide et al. 2009). For this paralog pair as well as for *Gi1a*/*Gi1b*, only one member is expressed in the chemosensory organs and the other member thus may have acquired new functions in other tissues.

In summary, we have established the G alpha gene repertoire used in zebrafish taste and smell organs and have demonstrated the expression of these genes both in larval and adult stages. We have shown that despite its generally high degree of conservation, the vertebrate G alpha gene family exhibits considerable flexibility and adaptation during evolution, from gene birth and death to re-specification and acquisition of new functions.

Supplementary material

Supplementary material can be found at http://www.chemse.oxfordjournals.org/.

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