Biogenic Trace Amine–Associated Receptors (TAARs) Are Encoded in Avian Genomes: Evidence and Possible Implications

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Recent studies of mammals and fish indicate that most trace amine–associated receptors (TAARs) may be involved in the detection of volatile biogenic compounds. It has therefore been suggested that this new class of “olfactory” receptors could be highly relevant for social communication and individual recognition. To determine if TAAR orthologues are encoded in avian genomes, we initiated BLAST searches of the *Gallus gallus* genome and public avian expressed sequence tags databases and performed associated phylogenetic analyses of the TAAR homologues identified. Our results suggest that a minimum of 3 TAAR paralogues are encoded in the *G. gallus* genome and that these are putative orthologues of the human/mouse genes TAAR1, TAAR2, and TAAR5. It is noteworthy that TAAR5 is activated by compounds that have been found in avian feces. We tentatively suggest that avian TAARs may compensate for the lack of an avian equivalent of the mammalian vomeronasal system and therefore may be important mediators of socially important avian chemical cues.

Recently, a new gene family of G protein–coupled receptors (GPCRs), denoted trace amine–associated receptors (TAARs), have been identified in both mammalian and fish genomes (Borowsky et al. 2001; Gloriam et al. 2005). Because trace amines are both structurally similar to conventional amine neurotransmitters and are present in neural tissue, albeit in small amounts, TAARs have been investigated for functions in neurotransmission and neuro-modulation (Lindemann and Hoener 2005; Lewin 2006). Although some evidence for such functions has been reported, recent studies point to TAARs being mainly involved in olfaction (Liberles and Buck 2006). Most TAAR genes are selectively expressed in small subsets of olfactory sensory neurons within the olfactory epithelium. In contrast to the large, and structurally diverse, odorant receptor family, which mediates general olfaction, TAARs appear to be specialized for detecting biogenic volatile amines. It has therefore been suggested that this new class of chemosensory TAAR receptors is associated with the detection of social cues and thus may be highly relevant for the study of behaviors associated with individual recognition and/or mate choice (Liberles and Buck 2006). In light of growing evidence that birds do indeed have a well-developed sense of smell that may be of considerable behavioral significance, we sought to determine if avian genomes encode TAAR orthologues.

**Methods and Results**

To determine if TAAR orthologues are encoded in avian genomes, we initiated a search for TAAR homologues in both the chicken (*Gallus gallus*) genome and additional public avian expressed sequence tags (EST) databases. The human TAAR1 protein sequence (RefSeq accession number NP_612200) was used as the query string for TBLASTN searches of the National Center for Biotechnology Information (NCBI) *G. gallus* reference genome database (build 2.1). To distinguish genuine TAAR orthologues from homologous non-TAAR GPCRs (e.g., serotonin, dopamine, and adrenergic receptors), the cutoff $E$ value for significant matches was set, on the basis of initial searches, at $10^{-50}$. Using the NCBI default BLAST search settings TBLASTN searches returned 4 matches with $E$ values $<10^{-50}$ on a contig assigned to *G. gallus* chromosome 3 (accession number NW_001471670.1) with $E$ values of $10^{-132}$, $10^{-87}$, $10^{-77}$, and $10^{-72}$. However, the aligned sequence of the last match was substantially shorter (289 amino acids) than the first 3 matches (between 326 and 335 amino acids). There is no associated RefSeq gene annotation in the chicken genome, but sequence conservation with other vertebrate taxa appears to be high in the matched regions.

To obtain the full-length coding sequences of the putative *G. gallus* TAAR genes, the *G. gallus* genome assembly of May 2006 (University of California Santa Cruz...
genome browser) was searched for the longest open reading frames (ORFs) corresponding to each of the putative genes, using the software package BioEdit (Hall 1999). Three continuous ORFs with lengths of either 996 or 1026 bp were found. The fourth putative ORF was truncated by an assembly gap, however, the 894 bp of sequence found was 100% identical with 1 of the 3 other ORFs, suggesting a duplication error during genome assembly. The lengths of the 3 unique putative TAAR ORFs (Figure 1) conformed to the expectation of intronless ORFs encoding 316—384 amino acid proteins (Gloriaim et al. 2005). In addition, the predicted proteins share the TAAR-specific peptide fingerprint motif (Figure 1) proposed to distinguish TAARs from other GPCRs (Lindemann and Hoener 2005).

The human and mouse orthologues of the 3 putative *G. gallus* TAAR genes were determined by BLASTP searches of the NCBI RefSeq database of human and mouse protein sequences. The significantly best “hits” for the putative *G. gallus* TAAR proteins were TAAR1 (human, NP_612200.1, *E* = 10^{-121} and mouse, NP_444435.1, *E* = 10^{-111}), TAAR2 (human, NP_001028252.1, *E* = 10^{-118} and mouse, NP_001007267.1, *E* = 10^{-124}), and TAAR5 (human, NP_003958.1, *E* = 10^{-119} and mouse, NP_001009574.1, *E* = 10^{-118}), with all 3 orthologues located on continuous regions of human chromosome 6 or mouse chromosome 10. These chromosomal assignments are consistent with previously reported synteny between large sections of chicken chromosome 3 with human chromosome 6 and mouse chromosome 10 (International chicken genome sequencing consortium 2004). In the chicken–human comparisons, the amino acid sequence identities of the putatively orthologous TAAR1, TAAR2, and TAAR5 proteins were 69%, 64%, and 70%, respectively, and the equivalent chicken–mouse comparisons were 63%, 64%, and 69%, respectively. These values of TAAR protein identities lie between values reported for analogous fish–mammal comparisons (35–50%) and mammal–mammal comparisons (80–98%) (Gloriam et al. 2005). TBLASTN searches of the total avian EST database at NCBI did not return any alignments with *E* values <10^{-37}, indicating that no homologues of the *G. gallus* TAAR sequences were present in the avian EST databases.

A phylogenetic analysis using the coding sequences of all known mouse, human, and *G. gallus* TAAR genes verified the homology relationships among the TAAR genes of these 3 vertebrates (Figure 2). The 3 *G. gallus* TAAR sequences are clearly located within the TAAR gene family and cluster within the TAAR1, TAAR2, and TAAR5 mammalian orthologues as expected.

**Discussion**

Our results suggest that a minimum of 3 TAAR paralogues are encoded in the *G. gallus* genome and that these are putative orthologues of the mammalian genes TAAR1, TAAR2, and TAAR5. Two of the 3 genes (TAAR1 and

Figure 1. ClustalW alignment of 3 *Gallus gallus* predicted TAAR protein sequences. A motif thought to define TAAR proteins is highlighted, whereas positions of identity in all sequences are indicated by asterisks.
TAAR2) were detected but are not explicitly described in any detail, in a report of the complete chicken GPCR family (Lagerstrom et al. 2006). Bearing in mind the current draft status of the G. gallus genome, our estimate of the number of chicken TAAR genes must be regarded as conservative and is at the lower end of the estimates described for other species: human, 6; mouse, 15; zebra fish, 57; and fugu, 8 (Gloriam et al. 2005; Liberles and Buck 2006). Evidence from mouse and fish indicates that most TAAR genes, with the exception of TAAR1, are expressed in the olfactory epithelium (Liberles and Buck 2006). The absence of TAAR homologous sequences from the avian EST databases is likely due to both the typically low levels of TAAR transcripts (Lindemann and Hoener 2005; Lewin 2006) and the paucity of cDNA libraries generated from avian olfactory epithelia. Assuming that chicken TAAR1, like its murine and piscine orthologues, is not expressed in the olfactory epithelium, we propose that the avian TAAR2 and TAAR5 orthologues are candidate avian receptors for the detection of volatile biogenic compounds. Clearly, investigations of TAAR2/5 gene expression patterns in avian olfactory epithelium and other tissues as well as functional studies are necessary to confirm this hypothesis. It is noteworthy that TAAR5 is activated by compounds—specifically trimethylamine, N-methylpiperidine, and isoamylamine—found in the urine of sexually mature male mice (Liberles and Buck 2006). Interestingly, in the context of this report, volatile trimethylamine has been found in feces of the black-bellied whistling duck (Dendrocygna autumnalis) (Robacker et al. 2000). We tentatively suggest that similar amino acid derivatives, although perhaps less structurally diverse than in mammals, in interaction with their cognate TAAR receptors may mediate avian social communication and/or individual recognition in some contexts. For example, it has been shown that the Antarctic prion (Pachyptila desolata) is able to recognize its breeding partner by individual specific odors (Bonadonna and Nevitt 2004). Furthermore, the significance of TAAR receptors in mediating social cues may be particularly pronounced in birds as they seem to lack both the vomeronasal organ and vomeronasal receptors thought to mediate social chemocommunication in mammals (International Chicken Genome Sequencing Consortium 2004).

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**References**


**Figure 2.** Maximum parsimony tree of all human, mouse, and predicted G. gallus TAAR-coding DNA sequences. The bootstrap percentages of 500 bootstrap replications are given at the nodes, and the following prefixes were used: h = human, m = mouse, and g = G. gallus in bold. The maximum parsimony analysis is based on the heuristic search algorithm close-neighbor interchange as implemented by MEGA (Tamura et al. 2007).

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