Timing of the Functional Diversification of α- and β-Adrenoceptors in Fish and Other Vertebrates

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Adrenoceptors (ARs) are G protein–coupled receptors found throughout the vertebrates. Their pharmacology and preliminary phylogenetic analyses suggest that ARs are classified as α_1, α_2 (and their subtypes), and β_1, β_2, and β_3. However, the relationships among subtypes of this superfamily, as well as both the pattern and the timing of their diversification, are poorly understood. In addition, fish AR subtypes possess pharmacologies and tissue distributions that only partially overlap with those of their mammalian counterparts, in spite of their apparent orthologous relationships within subtypes. Here we analyze 136 sequences in a range of vertebrates, including fish, to resolve these issues. We show that diversification of ARs occurred during duplication events that occurred within distinct time periods. Each period maps to whole-genome duplication events, two in vertebrates and one in fish. We also show that ARs underwent multiple duplications within these broad windows and that fish ARs underwent extensive gene loss after duplications that promoted their functional divergence with respect to other vertebrates.

Key words: **adrenoceptors; phylogeny; vertebrates; G protein–coupled receptors; evolution; genome duplication**

Introduction

Adrenoceptors (ARs) are 7-transmembrane, domain, G protein–coupled receptors found throughout the vertebrates. Based upon pharmacology and molecular phylogenetic studies, ARs are classified as α_1, α_2 (and their subtypes), and β_1, β_2, and β_3. Despite independent studies focusing on individual subtypes,[1] very little is known regarding the phylogenetic relationships among these subtypes.

Previous functional studies reported that adrenergic agonists (AAs) binding to both β_2- and α_1-ARs initiate metabolic changes in the fish liver, while skeletal muscle β-ARs are regulated by AAs,[2] and α_1-AR expression modifies blood pressure.[1] A functional divergence for the salmonid β_3-AR was reported, although the evolutionary significance of this is not known.[3] Fish ARs possess a limited number of potential phosphorylation sites within their third intracellular loop and the intracellular tail compared with their mammalian orthologues, suggesting that the regulation of these receptors in fish may be distinct from that in mammals. However, the diversification pattern that promoted or permitted these functional differences between fish and tetrapods remains unclear. Here we address these issues by analyzing a large number of AR sequences from fish and tetrapods and we provide new insights into the pattern and timing of their divergence.

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Materials and Methods

Coding sequence homologues of trout ARs were extracted from GenBank using reciprocal best tBLASTx hits[4] (RBH) for select chordates. The corresponding amino acid sequences were extracted and aligned with the Muscle software.[5] Protein alignments were then back translated to nucleotide alignments with Pal2nal.[6] Accession numbers and alignments are available upon request.

The most appropriate nucleotide substitution model was selected with ModelTest.[7] The phylogeny was then estimated under a Bayesian framework with Bayesian evolutionary analysis by sampling trees (BEAST)[8] permitting the coestimation of topology and of divergence times without assuming a strict molecular clock.[9] Rates across lineages followed an uncorrelated lognormal (LN) prior distribution and divergence times followed a pure-birth process. The relaxed molecular clock was calibrated with 15 minimum age constraints[10] for the following divergences: dog/cow (α_{1A}: 0.045 billion years ago [Ga]), dog/human (α_{1A}, α_B: 0.050 Ga), human/bird (α_{2A}, α_B: 0.300 Ga), human/mouse (α_{1A}, α_{2A}, α_B: 0.097 Ga), human/opossum (α_{2A}, α_B: 0.170 Ga), rabbit/mouse (α_{1A}, α_{2A}: 0.040 Ga), and mouse/rat (α_{1A}, α_{2A}, α_B: 0.012 Ga). Each divergence followed a prior mean-0.01 exponential distribution with an offset corresponding to the minimum age. The age of the root followed a diffuse LN(0.0, 0.5) with an offset of 0.250 Ga. Four independent Markov chain Monte Carlo samplers were run to check convergence, each for 20 million steps sampling every 2000 steps. Convergence and appropriate burn-in periods were determined with Tracer (tree.bio.ed.ac.uk/software/tracer).

Results and Discussion

Phylogeny and Timing of Adrenoceptors Diversification

RBH searches resulted in 136 unique AR sequences for the α and β families. Three dopamine sequences were included for reference, as used previously.[1] The most appropriate nucleotide substitution model was GTR + Γ_5 + I (e.g., Ref. 11). The first two 2×10^6 steps of the Bayesian analyses were discarded as burn-in. Trees are rooted by using a relaxed molecular clock.

Our analysis recovered a deep basal divergence between α- and β-ARs with high confidence (posterior probability (PP) = 1.00; Fig. 1). All α- and β-AR types were confidently resolved (PP = 1.00) except for the α_1/α_2 split (PP = 0.54). While the divergence of the D1β/D1x dopamine receptor sequences is poorly resolved, species trees for all AR paralogues were correctly estimated.

Our phylogeny is consistent with the 2R hypothesis of two rounds of whole-genome duplication events in vertebrates (events "1R" and "2R"[12]) and a third whole-genome duplication in fish (event "3R" of the 3R hypothesis[13]). Yet, the history of ARs shows a more complicated pattern where multiple duplications took place within three main time periods. These periods are defined by the upper and lower limits of the 95% credibility intervals for duplication dates (stars, Fig. 1). These three periods are between 1.79–0.91 Ga (red star), 0.86–0.37 Ga (blue star), and 0.50–0.16 Ga (pink star). Recent (<0.01 Ga) segmental duplications are also identified (black star). Despite extensive and sensitive tBLASTx searches, our data do not show any evidence for the third round of duplication in β-ARs; this is likely a result of extensive gene loss following this fish-specific duplication event.

It is tempting to identify these three periods with the three rounds of whole-genome duplications: the first corresponds to the time just after urochordate divergence when event "1R" is predicted to have happened,[14,15] the second to the divergence of tetrapods (event "2R"[12]), and the third to the split of ray-finned fish (event "3R"[16]). Our results suggest that if the 3R hypothesis is correct, whole-genome duplications could produce a genomic context that is conducive to segmental duplications. This

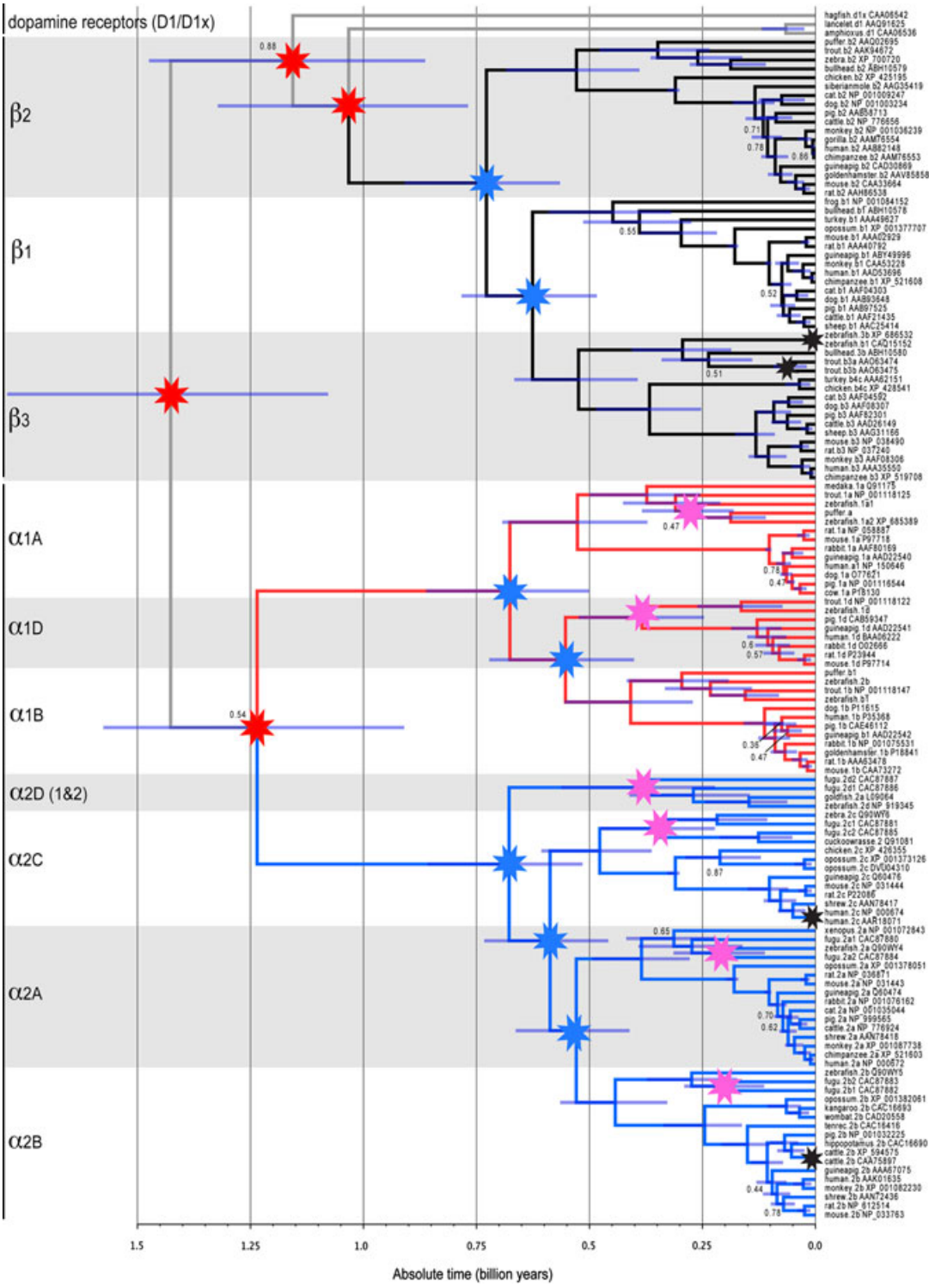

Figure 1. Chronogram of the 136 adrenoceptor sequences. Time is in billon years. Bars, 95% credibility intervals; numbers, clade posterior probability (when < 0.90); stars, duplication events [red: 1.79–0.91 billion years ago (Ga); blue: 0.86–0.37 Ga; pink: 0.50–0.16 Ga; black: < 0.01 Ga]. (In color in *Annals* online.)

hypothesis is consistent with the pattern observed, e.g., in plants[17] or in carp.[18]

Functional Implications

While fish and mammalian AR subtypes appear to be encoded by orthologous genes (Fig. 1), extensive gene loss following the fish-specific duplication might blur orthology and create cases of "hidden paralogy."[19] In this case, we expect to find evidence for relaxed selective pressures in fish sequences. We tested this prediction by computing maximum likelihood estimates of nonsynonymous (dN) to synonymous (dS) rate ratios, a measure of selection, between pairs of sequences.[20] The dN/dS ratios between a fish sequence and a nonfish sequence was significantly higher than for fish/fish and nonfish/nonfish comparisons (Kruskal Wallis: $H = 31.90$, df $= 2$, $P = 1.2 \times 10^{-7}$). Relaxed selective pressures imply faster rates of evolution that are potentially associated with functional divergence. Therefore, we propose that functional divergence between fish and other vertebrates[1] can be interpreted as hidden paralogy, where the third round of fish-specific whole-genome duplication was followed by extensive (for β-ARs) or differential (for α-ARs) gene loss events. Hidden paralogy is also likely to explain the functional difference between fish and tetrapod members of other superfamilies, such as corticosteroid receptors[21] and cytokines.[22]

Some additional questions remain. For instance, the α_2 type underwent four duplications during "2R," leading to five subtypes (Fig. 1, including the α_{2D} 1 and 2 types), whereas other subtypes, such as α_1, only underwent two duplications during the same period of time: is this differential pattern adaptive or is it the byproduct of specific chromosomal locations of gene duplicates or of other nonadaptive events? More detailed studies are required to fully understand the pattern of diversification of ARs.

Acknowledgments

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Conflicts of Interest

The authors declare no conflicts of interest.

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