

**EVOLUTION OF GLUTAMINE SYNTHETASE IN VERTEBRATES:
MULTIPLE GLUTAMINE SYNTHETASE GENES EXPRESSED IN
RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)**

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Glutamine synthetase (GSase; L-glutamate:ammonia ligase (ADP forming); E.C. 6.3.1.2) catalyses the ATP-dependent formation of glutamine from ammonia and glutamate. In fish, GSase is a multifunctional enzyme. GSase is a critical enzyme in detoxification of the highly mobile and toxic ammonia (for review, see Ip et al. 2001). The enzyme is also key to the ‘fish type’ ornithine urea cycle, with glutamine as the N-donor substrate for the initial step catalysed by carbamoyl phosphate synthetase III (CPSase III) (for review, see Anderson 2001). The regulation of urea synthesis, at least in the facultatively ureogenic marine toadfish (*Opsanus beta*), is upstream of the urea cycle and presently attention is focused on GSase (e.g. Walsh et al. 1999). The objective of this study was to isolate and characterize the GSase gene(s) in the rainbow trout, *Oncorhynchus mykiss*.

Our data show direct evidence for four GSase genes in rainbow trout. Using GSase specific primers, the polymerase chain reaction (PCR) was found to amplify four expressed GSase sequences from a rainbow trout cDNA library constructed from mRNA isolated from the combined gill and kidney tissues of 12 trout. In a separate experiment, three of the four sequences isolated above were amplified via RT-PCR from a total RNA sample pooled from the isolates of six rainbow trout alevin. For two of these sequences, we characterized the

full length coding regions (*Onmy-GS01*, *GS02*), and for two others, we described partial sequences, lacking only the 5' end of the coding sequence (*Onmy-GS03*, *GS04*).

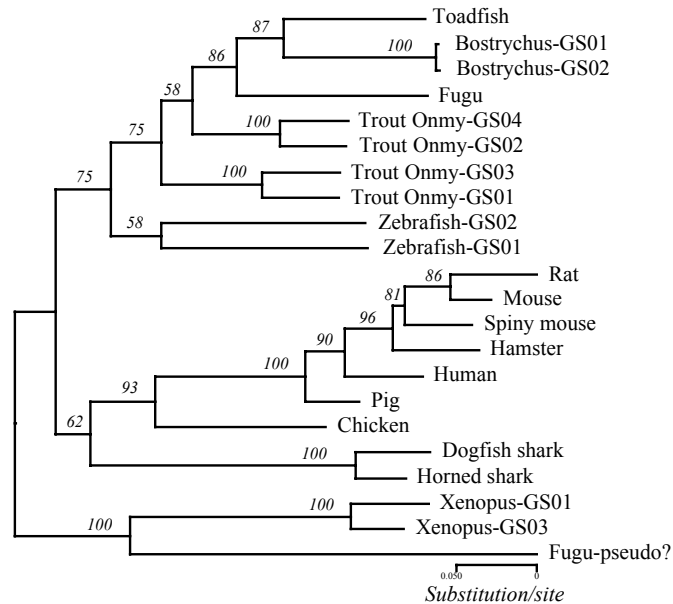


Fig. 1. Maximum likelihood radial phenogram based on a DNA alignment of the coding sequence of glutamine synthetase genes in vertebrates. Bootstrap values above 50% are placed at the appropriate nodes.

A maximum likelihood radial phenogram, based on the alignment of CDS nucleotides, was constructed (Fig. 1). This phylogeny shows strong bootstrap support (>70%) for four monophyletic clades, one containing all mammalian and avian genes, a second containing all teleostean fish genes, a third containing *Xenopus* and a single *Fugu* (possibly pseudogene) sequence and a fourth containing the shark sequences. The relationship among these four clades is not as well supported (62%). Among the bony fish genes, the present phylogeny suggests that there are two rainbow trout lineages, an *Onmy-GS01/3* and an *Onmy-GS02/04* clade. These clades are highly divergent and do not form a monophyletic grouping suggesting that these two lineages arose prior to

speciation. We speculate that two functional GSase lineages were present in the common ancestor of all salmonids prior to the tetraploidization event that preceded the rise to the modern salmonids. We also report the existence of two distinct GSase genes in zebrafish. The relationship of the zebrafish genes is not well resolved in the phylogeny, but their duplication appears to be independent of the duplication of the two trout lineages.

The sequence similarity of *Onmy-GS01* to *Onmy-GS03* and *Onmy-GS02* to *Onmy-GS04* could either represent allelic diversity or alternatively reflect a relatively recent duplication event. To investigate these hypotheses, we conducted a sequence comparison of intron 4 variation amplified from a single fish. Four unique intron sequences, 146, 256, 120 and 113 bp corresponding to *Onmy-GS01 – GS04*, respectively, were found. Reflective of the maximum likelihood phylogeny, a dot plot analysis shows a slightly higher level of similarity between the *Onmy-GS01/GS03* genes sequences and also between the *Onmy-GS02/GS04* sequences. As expected, this similarity is most noticeable around the splice boundaries. For most of the remaining intron sequence no significant similarity exists, arguing against an allelic relationship between the sequence pairs. The analysis of the 3' UTR sequences agrees with the intron analysis. We conclude that the similarity within these gene sequence pairs reflects a recent gene duplication event. This predicts at least four GSase loci in trout. Consistent with this interpretation, a Southern blot of a single individual hybridised with a probe made up of both the *Onmy-GS01* and *Onmy-GS02* genes revealed five to seven bands in *Hin* fl and *Alu* I digests, respectively.

The genetic distance estimates between the GSase gene pairs, *Onmy-GS01/03* and *Onmy-GS02/04* (0.0948 and 0.0825 substitutions/site, respectively), are greater than those observed between *Mus* and *Rattus* (0.0798, Fig. 1). Assuming a molecular clock for bony fish GSase genes, the *Onmy-GS01/03* and *Onmy-GS02/04* values are, therefore, consistent with the duplication of these loci during the ancestral tetraploidization event estimated to have occurred 25 - 100 mya (Allendorf and Thorgaard 1984).

From total RNA extracted from ten adult trout tissues the relative expression of *Onmy-GS01* and *Onmy-GS02* was studied using semi-quantitative PCR (Fig 2). This analysis shows 1) *Onmy-GS02* is expressed at higher levels relative to *Onmy-GS01* in most adult tissues, 2) the highest level of expression is in the brain, and 3) the largest difference between expression of the two genes is found in the intestine. Clearly, rainbow trout possess multiple GSase loci with differing levels of tissue expression, implying manifold potential routes of

regulation. Our data also indicate that caution should be taken when interpreting mRNA expression data of a single gene, unless multiple genes have been ruled out.

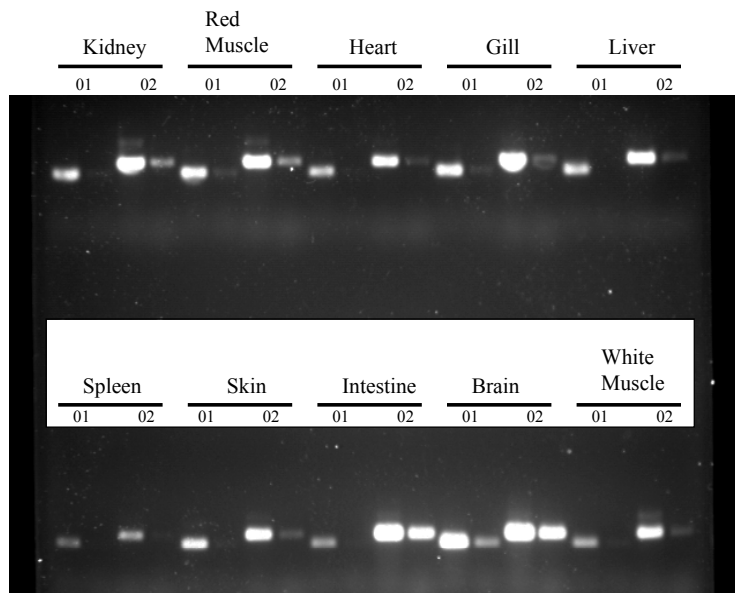


Fig. 2. Semi-quantitative PCR of glutamine synthetase genes *Onmy-GS01* and *Onmy-GS02* in adult tissue. An equal amount of each amplification was run on a 1.5% agarose gel. The reactions are organized according to tissue sample, in groups of four. The order of samples is *Onmy-GS01* (01), 30 cycles and 25 cycles total, and *Onmy-GS02* (02), 30 cycles and 25 cycles total.

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Acknowledgements

The authors wish to thank Dr. Steve F. Perry, University of Ottawa for his generous donation of the trout cDNA library, Phyllis Essex-Fraser for her help with isolating RNA in trout tissues and Monica Elliott for RT-PCR assays. Funding for this project was provided by a Premier's Research Excellence Award to P.A.W. and NSERC grants to P.A.W. and T.P.M.

