quent cores, which were analyzed at three to five contiguous intervals of 5 to 40 cm. The topmost interval gave modern Hg concentrations and fluxes, whereas the bottom interval provided preindustrial values (before 1850 for the Midwest); the middle sections were used to calculate whole-core ^{210}Pb burdens required for dating by the c.r.s. model. The data are in: T. A. Henning, thesis, University of Minnesota (1989); M. E. Brigham, thesis, University of Minnesota (1992); D. R. Engstrom, E. B. Swain, T. A. Henning, M. E. Brigham, P. L. Brezonik, in *Environmental Chemistry of Lakes and Reservoirs*, L. A. Baker, Ed. (American Chemical Society, Washington, DC, in press).
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Evidence from 18*S* Ribosomal RNA Sequences That Lampreys and Hagfishes Form a Natural Group

David W. Stock* and Gregory S. Whitt

Lampreys and hagfishes (cyclostomes) traditionally were considered to be a natural (monophyletic) group. Recently, the consensus of opinion, based largely on morphological analyses, has shifted to a view that lampreys are more closely related to jawed vertebrates (gnathostomes) than to hagfishes. Phylogenetic comparisons of 18*S* ribosomal RNA sequences from two hagfishes, two lampreys, a tunicate, a lancelet, and a number of gnathostomes support the monophyly of the cyclostomes. These data force a reassessment of several features of early vertebrate evolution.

Lampreys and hagfishes are the only living vertebrates without hinged jaws. Because the earliest vertebrates in the fossil record also lack jaws, the two living forms have been studied extensively at a number of levels of biological organization in the hope that they have retained features of the earliest vertebrates (*1*). Lampreys and hagfishes appear more primitive than gnathostomes (jawed vertebrates) in a number of features in addition to the absence of jaws, including the absence of paired fins, hard tissues, ribs, a thymus, lymphatic vessels, and genital ducts. These characteristics, along with similarities in the structure of the gills (*2*) and the tongue mechanism (*3*), have led to the traditional view that the two taxa form a natural (monophyletic) group, the Cyclostomata (*4*). However, in a number of respects, hagfishes appear even more primitive than lampreys (*1*). Hagfishes are isosmotic with their marine environment and lack radial muscles associated with the fins, extrinsic eye muscles, nervous regulation of the heart, and any trace of vertebral arches. Because of the absence in hagfishes of these and other characteristics present in lampreys and gnathostomes, a number of researchers have concluded that lampreys are more closely related to gnathostomes than either group is to hagfishes (*1, 5–7*). This phylogeny has also been proposed on the basis of studies of fossil jawless fishes (agnathans) (*8*) and probably represents the consensus of opinion today. A third phylogenetic possibility, that hagfishes are the closest relative of the gnathostomes, has not been proposed seriously. Determining the phylogenetic relationships of lampreys, hagfishes, and gnathostomes is of fundamental importance in testing hypotheses about the order and nature of evolutionary transformation during the earliest stages of vertebrate evolution. For example, different phylogenetic arrangements of these groups have different implications for whether the osmoregulatory strategy of hagfishes is primitive or due to secondary loss. Distinguishing between these alternatives may contribute to the resolution of the question of whether vertebrates originated in marine or freshwater environments (*1*).

A significant problem with many of the morphological and physiological characters that have been used in phylogenetic studies of cyclostome relationships is the difficulty of determining which character states are ancestral (and therefore uninformative with respect to phylogenetic relationships) and which are derived. Character polarity is usually inferred by reference to an assumed outgroup, but for many features (for example, gill structure), it is not possible to make meaningful comparisons with an outgroup because no invertebrate or protochordate group possesses similar structures (*2, 5*). A further problem is the possibility that some proposed primitive features of hagfishes, especially those related to the eye, may actually be the result of more recent degenerative evolution linked to their burrowing habits and life at ocean depths. Molecular sequence comparisons provide a way of overcoming many of the difficulties with outgroup comparison, if the assumption is made that the sequences being used can be aligned unambiguously with protochordate or invertebrate sequences.

The only extensive molecular sequence analyses of cyclostome-gnathostome relationships have used globins (*9*). These analyses have been interpreted as supporting cyclostome monophyly but suffer from a number of limitations. The globin gene family has undergone numerous gene duplications, some of which have been depicted as occurring around the time of the divergences among lampreys, gnathostomes, and hagfishes. Therefore, the phylogeny of the globin genes may not match the phylogeny of the organisms. In addition, globins are short molecules with a relatively rapid rate of evolution (*10*). A slowly evolving molecule is more appropriate for investigating the relationships of these taxa because some of the lineages may have diverged more than 500 million years ago (*11*). The small subunit ribosomal RNA (rRNA) has been used extensively for investigating diver-

Department of Ecology, Ethology, and Evolution, University of Illinois, Urbana, IL 61801.

*Present address and to whom correspondence should be addressed: Hopkins Marine Station, Department of Biological Sciences, Stanford University, Pacific Grove, CA 93950.

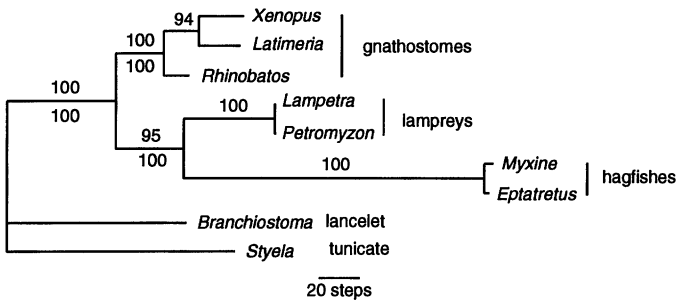

Fig. 1. Phylogenetic relationships of cyclostomes and gnathostomes. Sequences were aligned manually and regions of ambiguous alignment excluded from phylogenetic analyses (*22*). Regions used in the phylogenetic analyses correspond to the following positions in the human 18*S* rRNA sequence (*15*): 3 to 74, 79 to 125, 138 to 178, 185 to 191, 210 to 234, 287 to 303, 309 to 320, 327 to 705, 709 to 722, 725 to 731, 749 to 781, 798 to 834, 841 to 899, 902 to 1136, 1140 to 1147, 1151 to 1411, 1424 to 1430, 1438 to 1554, 1556 to 1751, and 1779 to 1830. Tree topology and branch lengths are from a maximum parsimony analysis with the computer program PAUP and a branch-and-bound search (*23*). The total tree length is 577 steps; branch lengths were optimized according to the Acctrans option, and the scale bar indicates the inferred number of nucleotide changes. Trees grouping hagfishes with gnathostomes or lampreys with gnathostomes required nine or ten extra steps, respectively. The degree of support for internal branches of the tree was further assessed by bootstrapping (*24*). Numbers above each branch indicate the percentage of most parsimonious trees in which it was found in 1000 bootstrap replications performed with PAUP (with the branch-and-bound search option). Numbers below branches refer to the results of a bootstrap analysis with maximum likelihood carried out with the SEQBOOT and DNAML programs from the PHYLIP package, version 3.4 (*25*). One hundred bootstrap replications were performed with the global search option, a random input order of taxa, empirically derived base frequencies, a single category of substitution rates, and a transition/transversion ratio of 2. The sequences of *Eptatretus, Lampetra,* and *Xenopus* were omitted from this analysis to reduce computational time.

gences of this age and much greater (*12, 13*). We therefore determined small subunit (18S) rRNA sequences from the hagfishes *Eptatretus stouti* and *Myxine glutinosa*, the lampreys *Petromyzon marinus* and *Lampetra aepyptera*, the chondrichthyan (cartilaginous) fish *Rhinobatos lentiginosus* (a ray), the urochordate (tunicate) *Styela plicata*, and the cephalochordate (lancelet or amphioxus) *Branchiostoma floridae* (*14*). The hagfishes examined represent the two extant families of this group, and the chondrichthian sequence, when considered with human (*15*), coelacanth (*16*), and frog (*17*) sequences, provides representatives of the two major divisions of gnathostomes. The sample of lampreys includes two divergent North American genera (*18*). The outgroups (*Styela* and *Branchiostoma*) represent the lineages commonly proposed to be the closest relatives of vertebrates (*19*).

A high degree of 18S rRNA sequence divergence of hagfishes relative to other vertebrates is reflected in the length of the branch leading to the hagfishes in the phylogenetic tree shown in Fig. 1. This long branch is found regardless of the tree topology onto which the changes are mapped, although its length is decreased in trees grouping lampreys with gnathostomes. In addition to the substitutions depicted in Fig. 1, hagfishes have insertions in several areas of the sequence that are conserved in length among all other vertebrates examined (*20*). In contrast, there is little sequence divergence between the two hagfishes or between the two lampreys in the regions that could be aligned among all taxa.

Both the maximum parsimony and maximum likelihood analyses shown in Fig. 1 provide strong support for the hypothesis that the cyclostomes form a monophyletic group. In both cases, this result was found in at least 95% of the trees from the bootstrap analyses. To estimate further the robustness of this conclusion, we varied the gnathostome taxa included in the parsimony analyses, with different combinations of the chondrichthyan, frog, human, and coelacanth sequences, as well as sequences of most of the major groups of both cartilaginous and bony fishes (*20*). Bootstrap support for cyclostome monophyly ranged from 85 to 100%; the lower values generally were found in analyses including more rapidly evolving taxa.

Because of the controversy surrounding the sister group of the vertebrates (*19*), we also tried several combinations of outgroup taxa with both maximum likelihood and maximum parsimony. Using either the tunicate or the lancelet as the sole outgroup (and the frog and the coelacanth as gnathostome representatives) in maximum parsimony analyses had a larger effect on the results than varying the ingroup. Cyclostome monophyly was found in 64% of the bootstrap trees with the lancelet alone, whereas a relationship between lampreys and gnathostomes was found in 62% of the bootstrap trees with the tunicate alone. The choice of outgroup had less effect on the results of maximum likelihood analyses; cyclostome monophyly was supported in 97% of the trees when the lancelet was the outgroup and 81% with the tunicate as the outgroup. The decrease in support for cyclostome monophyly with maximum parsimony with either outgroup alone may be a result of the rapid rate of evolution in the hagfish lineage. Because there is a tendency for long branches to attract in parsimony analyses (*21*), one might expect that the hagfish branch would cluster with the outgroup; this would result in a sister-group relationship between lampreys and gnathostomes. This grouping would be due to convergence of the hagfish sequence with that of the outgroup. Such convergence would not necessarily involve the same set of positions in two distantly related outgroups, however, and this proposal may explain why there is less support for cyclostome paraphyly when both outgroups are included (that is, the two sets of positions showing convergence might tend to cancel the effects of each other). In support of the proposed effect of long branches in the analyses, the highest tendency for the hagfish sequence to cluster with the outgroup is found when the tunicate is the sole representative, and this sequence is more divergent from the vertebrate sequences than is that of the lancelet.

Although our analyses with conventional methods of tree-building support cyclostome monophyly, some caution is required in interpreting the results. In our analyses, we treated all characters as independent and having equal weight, two conditions that are almost certainly unrealistic. Additional parsimony analyses of our data with the technique of giving more weight to transversions than to transitions still support cyclostome monophyly. More sophisticated models of sequence evolution derived empirically from analyses of gnathostome sequences or from other groups may allow increased resolution with the 18S rRNA data. The likelihood of accelerated 18S rRNA evolution in the hagfishes is also a potential cause for concern. However, unequal rate effects might be expected to yield a paraphyletic Cyclostomata rather than the monophyletic Cyclostomata seen in most of our analyses.

Definitive phylogenetic resolution of cyclostome relationships may require the analysis of sequences from additional genes. Nevertheless, our results challenge the prevailing view of a paraphyletic Cyclostomata. If additional sequences yield similar results, then many of the supposed primitive features of hagfishes will have to be ascribed to secondary loss or to a parallel acquisition of features by lampreys and gnathostomes.

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Long-Term Survival of Xenogeneic Pancreatic Islet Grafts Induced by CTLA4Ig

Deborah J. Lenschow, Yijun Zeng, J. Richard Thistlethwaite, Anthony Montag, William Brady, Marylou G. Gibson, Peter S. Linsley, Jeffrey A. Bluestone*

Antigen-specific T cell activation depends on T cell receptor-ligand interaction and costimulatory signals generated when accessory molecules bind to their ligands, such as CD28 to the B7 (also called BB1) molecule. A soluble fusion protein of human CTLA-4 (a protein homologous to CD28) and the immunoglobulin (Ig) G1 Fc region (CTLA4Ig) binds to human and murine B7 with high avidity and blocks T cell activation in vitro. CTLA4Ig therapy blocked human pancreatic islet rejection in mice by directly affecting T cell recognition of B7$^+$ antigen-presenting cells. In addition, CTLA4Ig induced long-term, donor-specific tolerance, which may have applications to human organ transplantation.

At present, the major therapies to prevent the rejection of organ transplants rely on panimmunosuppressive drugs, such as cyclosporine A or monoclonal antibodies (MAbs) to CD3. These drugs must frequently be taken for the life of the individual, depress the immune system, and often result in increased infections and cancer. We attempted to develop a treatment that affected only the transplant antigen-specific T cells, thus inducing donor-specific tolerance. The binding of CD28 by its ligand, B7/BB1 (B7), during T cell receptor engagement is critical for proper T cell signal-