Correspondence

Ancient colour vision: multiple opsin genes in the ancestral vertebrates

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Molecular investigation of the origin of colour vision has discovered five visual pigment (opsin) genes, all of which are expressed in an agnathan (jawless) fish, the lamprey *Geotria australis*. Lampreys are extant representatives of an ancient group of vertebrates whose origins are thought to date back to at least the early Cambrian, approximately 540 million years ago [1]. Phylogenetic analysis has identified the visual pigment opsin genes of *G. australis* as orthologues of the major classes of vertebrate opsin genes. Therefore, multiple opsin genes must have originated very early in vertebrate evolution, prior to the separation of the jawed and jawless vertebrate lineages, and thereby provided the genetic basis for colour vision in all vertebrate species.

The southern hemisphere lamprey *Geotria australis* (Figure 1A,B) possesses a predominantly cone-based visual system designed for photopic (bright light) vision [2,3]. Previous work identified multiple cone types suggesting that the potential for colour vision may have been present in the earliest members of this group. In order to trace the molecular evolution and origins of vertebrate colour vision, we have examined the genetic complement of visual pigment opsins in *G. australis*.

We used RT-PCR with a set of degenerate primers to amplify and clone partial cDNA sequences from each of the *G. australis* opsin genes. Overlapping cDNA sequences were isolated by 5′- and 3′-RACE-PCR and the complete coding sequence of each gene was reconstructed. The sequence of the *G. australis* opsin genes was confirmed by amplification of each cDNA as a single fragment using a proof reading DNA polymerase. The sequences of the PCR primers and sequence alignments of the five *G. australis* opsin genes are provided as supplementary material (Genbank accession numbers: AY366491–AY366495).

The five *G. australis* opsin cDNA sequences were compared with the major groups of opsin genes from a broad range of representative vertebrate species. A codon-matched nucleotide sequence alignment provided the basis for estimating the evolutionary distances between the gnathostome (jawed vertebrate) and *G. australis* opsins and the neighbour-joining method [4] was used to infer phylogenetic relationships. The resulting phylogenetic tree (Figure 1C) shows that the *G. australis LWS, SWS1* and *SWS2* opsin genes are orthologous to the *LWS, SWS1* and *SWS2* opsin genes of the jawed vertebrates, with the branching order within each gene group largely following the accepted order of appearance of the vertebrate species. The two remaining *G. australis* opsin genes, *RhA* and *RhB*, are most closely related to the gnathostome *Rh1* and *Rh2* genes. However, both *RhA* and *RhB* are equally distantly related to the gnathostome *Rh1* gene as they are to the *Rh2* gene. This suggests that the most recent common ancestor of the jawed and jawless vertebrates most likely possessed a single ancestral *Rh* opsin gene, as well as *LWS, SWS1* and *SWS2* opsin genes. After the separation of the agnathan and gnathostome groups, two independent gene duplication events occurred, one in the Agnatha to yield the *RhA* and *RhB* opsins, and the other in the jawed vertebrates to produce the *Rh1* (rod) and *Rh2* (green cone) opsin genes. Our analysis also shows that the previously isolated rhodopsin sequences from the northern hemisphere lamprey species *Petromyzon marinus* and *Lampetra japonica* are most closely related to the *G. australis RhA* opsin gene, rather than to the gnathostome *Rh1* gene, with which they have previously been grouped [5]. We therefore suggest that the northern hemisphere lamprey genes be renamed *P. marinus RhA* opsin and *L. japonica RhA* opsin.

Other investigators have speculated that the ancestors of all vertebrates must have possessed the five major types of opsin genes: *LWS, SWS1, SWS2, Rh1* and *Rh2* [5]. However, this study is the first to demonstrate unequivocally that the molecular events giving rise to four of the five classes of vertebrate opsin genes must have already taken place by the early Cambrian (approximately 540 mya [1]), prior to the evolution of the jawed vertebrates. Uniquely, our results suggest that the duplication of the ancestral *Rh* gene, to give rise to the true rod (*Rh1*) and the green cone (*Rh2*) opsins of the jawed vertebrates, occurred specifically within the gnathostomes and independently of the agnathan lineage. This finding provides insight into previous work, which identified rod-like photoreceptors in some jawless fishes. However, these rod-like cells also show some morphological [6,7] and physiological [8] characteristics of cone photoreceptors. As this study suggests that the true rod *Rh1* gene did not appear before the evolution of the jawed vertebrates, the agnathan lineage probably does not possess a true rod photoreceptor and retains cone-like features in all photoreceptors.

Therefore, the ancestral vertebrates possessed four major types of vertebrate cone opsins, each presumably sensitive to different parts of the spectrum. Although the selection pressure underlying the origins of colour vision is still unknown, the early vertebrates lived in a shallow water environment [9,10], where a wide light spectrum could be exploited by the evolution of multiple visual pigments. The presence of multiple cone opsins provides the potential for colour

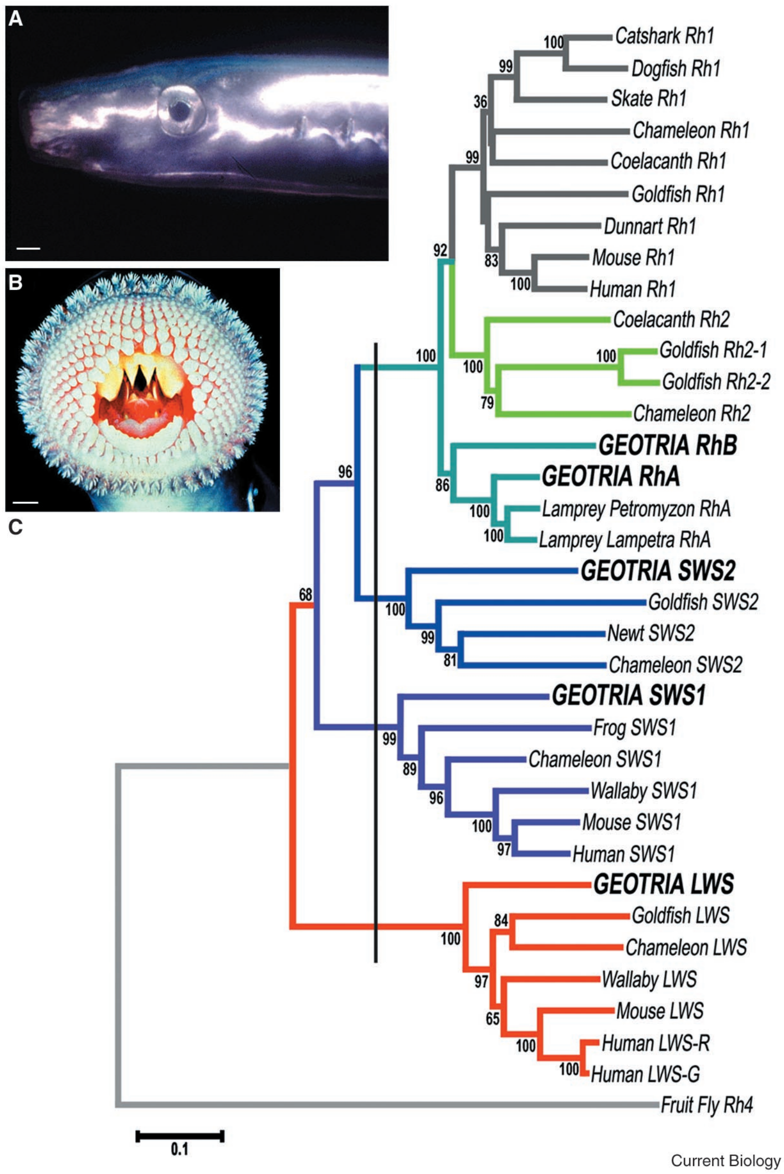

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Figure 1.
The head (A) and suctorial mouth (B) of the southern hemisphere lamprey *Geotria australis* (reproduced from [3] with permission from Cambridge University Press). Scale bars, 1mm (A), 10 mm (B). (C) Phylogenetic tree showing the relationships between the opsin genes of *G. australis,* the northern hemisphere lampreys *Lampetra japonica* and *Petromyzon marinus*, representative jawed vertebrates and an invertebrate outgroup. The black vertical line indicates the predicted genetic complement of opsins present in the most recent common ancestor of the jawed and jawless vertebrates, approximately 540 million years ago [1]. A codon-matched nucleotide sequence alignment was used to estimate evolutionary distances between sequences and the tree was generated using the neighbour joining method [4]. The number at each branch point reflects the robustness of that branch point (maximum 100). The scale bar is calibrated in nucleotide substitutions per site. Details of the sequences and methods are provided as Supplemental Data.

discrimination and thus a significant selective advantage for these early vertebrates. Based on our findings, we suggest that photopic (bright light, cone-based) colour vision originated first in the ancestral vertebrates, while full scotopic (dim light, rod-based) vision developed later, being dependent on the evolution of the *Rh1* opsin gene probably only within the jawed vertebrate lineage.

Supplemental Data

Supplemental data are available at http://www.current-biology.com/supplemental

References

1. Shu, D.G., Morris, S.C., Han, J., Zhang, Z.F., Yasui, K., Janvier, P., Chen, L., Zhang, X.L., Liu, J.N., Li, Y., et al. (2003). Head and backbone of the early Cambrian vertebrate *Haikouichthys.* Nature *421*, 526–529.
2. Collin, S.P., Potter, I.C., and Braekevelt, C.R. (1999). The ocular morphology of the southern hemisphere lamprey *Geotria australis* Gray, with special reference to optical specializations and the characterisation and phylogeny of photoreceptor types. Brain Behav. Evol. *54*, 96–111.
3. Collin, S.P., Hart, N.S., Shand, J., and Potter, I.C. (2003). Morphology and spectral absorption characteristics of retinal photoreceptors in the southern hemisphere lamprey (*Geotria australis*). Vis. Neurosci. *20*, 119–130.
4. Saitou, N., and Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. *4*, 406–425.
5. Yokoyama, S. (2000). Molecular evolution of vertebrate visual pigments. Prog. Retin. Eye Res. *19*, 385–419.
6. Dickson, D.H., and Graves, D.A. (1979). Fine structure of the lamprey photoreceptors and retinal pigment epithelium (*Petromyzon marinus* L.). Exp. Eye Res. *29*, 45–60.
7. Collin, S.P., and Potter, I.C. (2000). The ocular morphology of the southern hemisphere lamprey *Mordacia mordax* Richardson with special reference to a single class of photoreceptor and a retinal tapetum. Brain Behav. Evol. *55*, 120–138.
8. Govardovskii, V.I., and Lychakov, D.V. (1984). Visual cells and visual pigments of the lamprey, *Lampetra fluviatilis* L. J. Comp. Physiol. [A] *154*, 279–286.
9. Parker, A.R. (1998). Colour in Burgess Shale animals and the effect of light on evolution in the Cambrian. Proc. R. Soc. Lond. B Biol. Sci. *265*, 967–972.
10. Maximov, V.V. (2000). Environmental factors which may have led to the appearance of colour vision. Philos. Trans. R. Soc. Lond. B Biol. Sci. *355*, 1239–1242.

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