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The evolution of Olig genes and their roles in myelination

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One of the special attributes of vertebrates is their myelinated nervous system. By increasing the conduction velocity of axons, myelin allows for increased body size, rapid movement and a large and complex brain. In the central nervous system (CNS), oligodendrocytes (OLs) are the myelin-forming cells. The transcription factors OLIG1 and OLIG2, master regulators of OL development, presumably also played a seminal role during the evolution of the genetic programme leading to myelination in the CNS. From the available ontogenetic and phylogenetic data we attempt to reconstruct the evolutionary events that led to the emergence of the Olig gene family and speculate about the links between Olig genes, their specific cis*-regulatory elements and myelin evolution. In addition, we report a putative myelin basic protein (MBP) ancestor in the lancelet* Branchiostoma floridae*, which lacks compact myelin. The lancelet 'Mbp' gene lacks the OLIG1/2- and SOX10-binding sites that characterize vertebrate Mbp homologs, raising the possibility that insertion of* cis*-regulatory elements might have been involved in evolution of the myelinating programme.*

Keywords: Myelin, Olig genes, evolution and development (evo-devo), behaviour, myelin basic protein

INTRODUCTION

Which came first – central or peripheral myelin?

Myelin is the multi-layered glial sheath that surrounds axons in the vertebrate central and peripheral nervous systems (CNS and PNS). The invention of myelin was a defining moment in the history of vertebrates, because it enabled axons to propagate electrical impulses at unprecedented speed and, in so doing, marked the start of a dramatic new phase of evolution that led ultimately to the emergence of intelligence (Colman *et al.*, 1996; Richardson *et al.*, 1997; Schweigreiter *et al.*, 2006; Zalc *et al.*, 2008). The crucial innovation was the appearance of specialized myelinating cells – oligodendrocytes (OLs) in the CNS and Schwann cells in the PNS. Since development of these cells and their myelinating programmes are controlled by gene regulatory networks, exploring the molecular evolution of myelin-specific transcription factors and their binding sites on DNA is likely to provide insights into myelin evolution.

Myelin sheaths and myelin-specific proteins are present in all jawed vertebrates (gnathostomes), but absent from the jawless fish (agnathans: hagfish and lampreys) (Bullock *et al.*, 1984). Studies of cranial nerve dimensions in fossil fish give reason to think that placoderms, the earliest jawed fish, were the first vertebrates to possess myelin (around 400 million years ago, Mya) (Zalc *et al.*, 2008). These observations give rise to the idea that hinged jaws and myelinated nerves might have evolved in parallel, permitting the evolution of a predatory lifestyle. Jawbones and the Schwann cells that myelinate cranial nerves both develop from the cranial neural crest, providing a rationale for their co-evolution (Colman *et al.*, 1996; Zalc and Colman, 2000; Donoghue *et al.*, 2008; Zalc *et al.*, 2008).

An alternative or parallel idea is that myelin evolved for rapid locomotion and the ability to *escape* from predators. In favour of this is the observation that most OLs in the spinal cord develop from the same precursors as motor neurons (MNs; Sun *et al.*, 1998; Richardson *et al.*, 2000; Lu *et al.*, 2002; Park *et al.*, 2002; Zhou and Anderson, 2002; Takebayashi *et al.*, 2002a), suggesting that CNS myelin might first have evolved to ensheath motor circuits and accelerate escape reactions (Richardson *et al.*, 1997, 2000). Moreover, myelin-like glial sheaths that are found in some invertebrates (annelids and crustaceans) are specifically associated with axons that drive escape and/or startle responses (Roots, 1993; Davis *et al.*, 1999; Hartline and Colman, 2007). On the other hand, hagfish (which lack myelin) swim slowly and cannot accelerate to escape capture (see supplementary movie online). Fish swim using axial muscles that lie adjacent to the spinal cord, so motor axons extend only a short distance outside the CNS. Perhaps motor axons were initially ensheathed along their entire length by OLs, if there was no barrier to their leaving the neural tube via the ventral roots.

The question of whether PNS (Schwann cell) or CNS (OL) myelin is ancestral therefore boils down to what came first – vertebrate predation, or escape from predators? That sounds like a 'chicken and egg' situation but it is worth bearing in mind that formidable predators existed already before the advent of the vertebrate jaw – the top predators in Ordovician waters (~450 Mya) were arthropods (including Erypterids or 'sea scorpions', some of which came to exceed 2 metres in length; Braddy *et al.*, 2008). These probably preyed on bottom-dwelling ostracoderms – extinct armoured fish that are regarded as the (jawless) precursors of all present

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day fish (Forey and Janvier, 1994; Janvier, 1996). Thus, there could have been evolutionary pressure for primitive vertebrates to 'get moving' in order to escape predation long before the advent of the vertebrate jaw.

Regardless of where the primary selection came from, once the myelinating programme started to evolve in one cell type, all or part of the programme could have been activated in other cells, given appropriate cues. Therefore, evolution of CNS and PNS myelin would have gone largely hand in hand. In the CNS, OL precursors (OLPs) acquired the ability to migrate and myelinate widely and thereby myelin became ubiquitous throughout the CNS, with major advantages for all kinds of neural processes and tasks. Comparative studies of the gene regulatory networks that underlie CNS and PNS myelination might allow us to reconstruct the chain of molecular events that led to myelin in early vertebrates and might provide general insights into the evolutionary dialogue between genotype and phenotype. In the remainder of this article, we focus on the evolution and functions of the Olig genes, which play an important role in myelination of the CNS but not – as far as we know – in the PNS.

Olig genes – expression and function

Identification of the OL lineage (Olig) genes has been one of the more striking findings in glial biology in the past decade. Olig genes encode basic-helix-loop-helix (bHLH) transcription factors. Three members of the Olig family (Olig1–3) are known in all vertebrates except cartilaginous fish and chicken, and a fourth member Olig4 in teleost fish and amphibians. So far only Olig2/3 have been identified in cartilaginous fish (three species; see Fig. 1).

Expression of Olig1/2 is co-ordinately induced in the ventral neural tube of vertebrates by Sonic hedgehog (SHH) signalling from the floor plate. In mouse spinal cord, Olig1/2 are first expressed broadly in the ventral neuroepithelium starting at embryonic day 8.5 (E8.5), then soon afterwards narrows down to a single progenitor domain, pMN, which gives rise to MNs and OLPs (Lu *et al.*, 2000; Zhou *et al.*, 2000). MNs are generated first, between ~E9–E12 and OLPs after ~E12.5. Olig2 is expressed in pMN throughout MN development but is rapidly down-regulated in migrating post-mitotic MNs. By contrast, Olig1 expression is intermittent in pMN during MN development. After the pMN domain switches fate to produce OLPs, both Olig1 and Olig2 are continuously expressed in the whole OL lineage, from migratory OLP to myelinating OL, although the level of expression seems to decrease in differentiated OLs. In zebrafish, Olig2 is expressed in the common progenitors of MNs and OLPs, as in mammals (Park *et al.*, 2002), whereas Olig1 is expressed only in the OL lineage (Li *et al.*, 2007).

No MNs or OL lineage cells develop in spinal cords of Olig2 null mice, though at more anterior levels of the neuroaxis – especially forebrain and hindbrain – small groups of $SOX10^{+}$/$PDGFRA^{+}$ OLPs do form (Lu *et al.*, 2002). Since no OLPs or OLs form anywhere in the CNS of Olig1/Olig2 double-null mice, this implies that OLIG1 can partly compensate for loss of OLIG2 – but for the OL lineage only and only in certain parts of the CNS. There is still a lack of consensus over whether OLIG1 is obligatory for OL development. It was initially reported that OL development and maturation is normal in Olig1 null mice (Lu *et al.*, 2002) but a subsequent study (Xin *et al.*, 2005) reported a severe defect in OL maturation and myelination, leading to post-natal lethality. Xin *et al.* (2005) suggested that the lack of phenotype in the study of Lu *et al.* (2002) was due to compensatory over-production of OLIG2, caused by a *cis*-regulatory effect of the PGK-Neo cassette used to disrupt the Olig1 locus – the Olig1 null mice of Xin *et al.* (2005) did not contain PGK-Neo. This uncertainty needs to be resolved. In any case – and despite their lack of a developmental phenotype – the Olig1 null mice described by Lu *et al.* (2002) fail to remyelinate following acute experimental demyelination (Arnett *et al.*, 2004), so OLIG1 clearly can play a role in OL maturation in certain circumstances.

Loss-of-function studies in zebrafish suggest a conserved role of OLIG2 in MN and OL development (Park *et al.*, 2002). OLIG1 is not required for MN development, either in zebrafish or mice (Lu *et al.*, 2002; Li *et al.*, 2007). OLIG1 does not seem to be absolutely required for OL development in zebrafish either, although it can synergize with SOX10 to augment the expression of myelin basic protein (MBP) (Li *et al.*, 2007). Thus, in both mouse and zebrafish, the role of OLIG1 is ambiguous. In cartilaginous fish, OLIG1 seems to be missing (though the genome projects are incomplete so this conclusion should be regarded as preliminary). It is as though OLIG1, in those animals that have it, is at a transitional state of evolution where its functional separation from OLIG2 is still not complete.

Molecular properties of Olig genes

OLIG1/2 are members of the class A bHLH transcription factor superfamily (Atchley and Fitch, 1997). However, unlike other family members, OLIG1/2 are considered to be transcriptional repressors, not activators. Both OLIG1 and OLIG2 can form strong homodimers, as well as weak heterodimers with the ubiquitous bHLH proteins E12 and E47 (Zhou *et al.*, 2001; Gokhan *et al.*, 2005; Li *et al.*, 2007). Both OLIG1 and OLIG2 bind to a common 'E-Box' motif (CANNTG). There are two other members of the OLIG subfamily – OLIG3 and OLIG4 – that function differently from OLIG1/2 and have roles in the development of several kinds of interneurons and astrocytes (Takebayashi *et al.*, 2002b; Filippi *et al.*, 2005; Bronchain *et al.*, 2007; Liu *et al.*, 2008; Storm *et al.*, 2009).

OLIG1/2 are important at several sequential, interdependent stages of neural development – (1) neuroepithelial 'patterning', (2) MN and OL lineage specification, (3) OL differentiation and myelination. In the embryonic spinal cord and hindbrain, OLIG2 helps to pattern the ventral neuroepithelium by demarcating the pMN domain and distinguishing it from neighbouring domains. It does this by repressing competing transcription factors that specify alternative fates – for example, IRX3 to the dorsal side (p2 domain) and NKX2.2 on the ventral side (p3 domain) (Briscoe and Ericson, 2001). While marking out the pMN domain, OLIG2 prevents premature differentiation of pMN progenitors by repressing expression of HB9, a transcriptional regulator of MN differentiation, via E-box motifs associated with the Hb9 gene (Lee *et al.*, 2005). Complexes of OLIG2 with Neurogenin 2 (NGN2) and/or other transcription factors of the same or different classes subsequently regulate MN differentiation then fate-switching from MN to OLP production. In the OL lineage, OLIG1 regulates the transcription of major myelin-specific genes including Mbp, Plp and Mag (Xin *et al.*, 2005), either on its own or through interactions

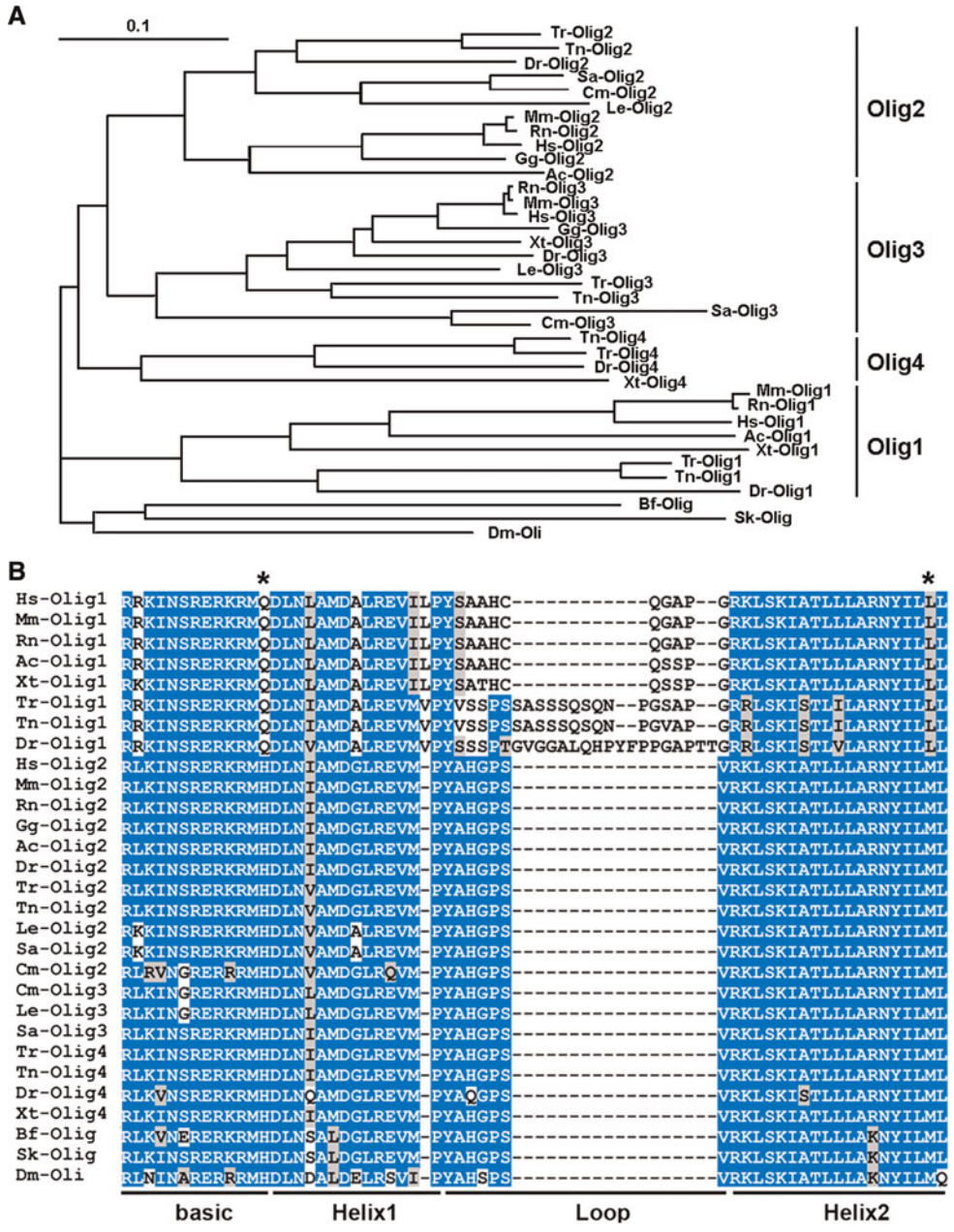

Fig. 1. Evolutionary relationships among OLIG protein family members. (A) OLIG protein sequences were downloaded from NCBI (http://www.ncbi.nlm.nih.gov), Ensembl (http://www.ensembl.org), JGI (http://genome.jgi-psf.org), MDIBL (http://www.mdibl.org/research/skategenome.shtml) and IMCB (http://esharkgenome.imcb.a-star.edu.sg). BioEdit/ClustalW was used to draw the rooted phylogenetic tree. Scale bar represents 10% protein sequence divergence. (B) Multiple alignment of bHLH domains of OLIG proteins. OLIG1 has a different loop region from that of other OLIGs (even including fly OLI). Key amino acid changes in helix regions are starred. Abbreviations: Hs: *Homo sapiens*; Mm: *Mus musculus* (house mouse); Rn: *Rattus norvegicus* (brown rat); Gg: *Gallus gallus* (chicken); Ac: *Anolis carolinensis* (Carolina anole); Xt: *Xenopus tropicalis* (clawed frog); Dr: *Danio rerio* (zebrafish); Tr: *Takifugu rubripes* (fugu fish); Tn: *Tetraodon nigroviridis* (puffer fish); Le: *Leucoraja erinacea* (little skate); Cm: *Callorhinchus milii* (elephant shark or chimera); Sa: *Squalus acanthias* (spiny dogfish); Bf: *Branchiostoma floridae* (Florida lancelet); Sk: *Saccoglossus kowalevskii* (acorn worm); Dm: *Drosophila melanogaster* (fruitfly).

with other OL transcription factors including SOX10 (Li *et al.*, 2007). OLIG1 changes its cellular localization from nucleus in OLPs to cytoplasm in myelinating OLs, a behaviour that is apparently not shared by OLIG2 (Arnett *et al.*, 2004).

In brief, OLIG2 is required for the early events of neuroepithelial patterning, progenitor fate specification and MN differentiation (and possibly also OL differentiation), whereas OLIG1 is involved only in late events including OL differentiation and adult re-myelination. It is not yet known whether OLIG2 is required for OL differentiation and/or remyelination.

Phylogeny of Olig genes

Several phylogenetic trees of Olig genes have been proposed (Lowe *et al.*, 2006; Li *et al.*, 2007; Bronchain *et al.*, 2007). With continuing progress in genomic sequencing projects, the number of online hits of Olig genes in public genomic databases is increasing rapidly. It is therefore appropriate to attempt a revised phylogeny (Fig. 1A). We chose all Olig-related gene sequences from three mammals (human, rat and mouse), one reptile (a lizard, the Carolina anole), one bird (chicken), one amphibian (clawed frog, *Xenopus*

tropicalis), three teleosts (zebrafish, puffer fish and fugu fish), three cartilaginous fish (little skate, dogfish and elephant shark), one cephalochordate (lancelet) and a hemichordate (acorn worm). Consistent with previous reports, mammals, amphibians and teleosts all have Olig1/2/3, whereas Olig4 turns up only in teleosts and amphibians. According to a previous hypothesis (Bronchain *et al.*, 2007), three duplication events followed by gene loss led to the emergence of these four Olig homologs from the single ancestral Olig gene. According to this scenario, a local duplication initially gave rise to Olig1 and the ancestor of Olig2/3/4, then two subsequent genome-wide duplication events (Dehal and Boore, 2005) accompanied by large-scale gene loss produced the four present-day Olig genes. However, this interpretation is problematic. Firstly, Olig1 is not found in cartilaginous fish despite the presence of Olig2 and Olig3 (although we cannot absolutely rule out the possibility that Olig1 remains to be discovered, given that the datasets are still incomplete). Secondly, in all species that possess Olig1, it is co-located in a synteny block with Olig2, whereas no such synteny block can be found associated with Olig3 or Olig4 (Bronchain *et al.*, 2007). Thirdly, when we look at the bHLH domain of OLIGs, which acts as their functional centre for binding to DNA and other partners, OLIG1 stands apart in that it has a divergent loop region as well as several key amino acid changes compared to other OLIGs (even including very primitive fly OLI) (Fig. 1B). Considering these things together, we propose (Fig. 2A,B) that through two rounds of whole genomic duplication, followed by loss of one homolog, the single Olig ancestor gave rise to Olig2, Olig3 and Olig4. Then, possibly just before teleosts came into existence, a local duplication at the Olig2 locus generated a synteny block containing two Olig2 genes, one of which recombined with another distantly related bHLH family gene, subsequently undergoing further changes including domain insertion, rearrangement and/or reshuffling (Atchley and Fitch, 1997; Morgenstern and Atchley, 1999) to give rise to Olig1. As a result of positive selection, Olig1 ended up as the outlier of the present-day Olig gene family, being more distantly related to Olig2/3/4 than the latter are to each other (Fig. 1).

Naturally, this is only one of several possible models (including the model of Bronchain *et al.*, 2007) and additional genomic data might suggest further revisions. We anticipate that the identification of Olig genes in the Agnathans will be particularly informative.

Olig genes and myelin evolution

An active debate in evolutionary biology is about what types of mutations make the dominant contribution to phenotypic diversity – mutations in non-coding *cis*-regulatory elements or in protein-coding regions of genes – for example, those encoding transcription factors (Lynch and Wagner, 2008; Wagner and Lynch, 2008). Numerous research findings suggest that in spite of significant sequence divergence, transcription factors from distantly related organisms can carry out equivalent or analogous functions in their respective hosts and can even substitute for one another in a transgenic context. A prime example of this is Pax6, a master control gene for eye development. Ectopic expression of either *eyeless* (fly counterpart of mammalian Pax6) or mouse Pax6 in *Drosophila* embryos can induce a compound eye structure

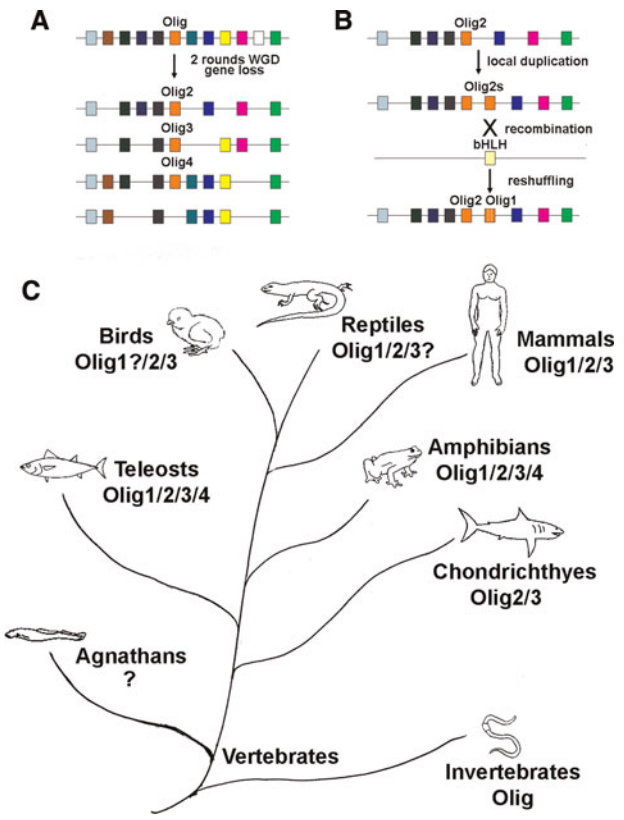

Fig. 2. Speculative scheme of Olig gene evolution. (A) After two rounds of whole genome duplication during early vertebrate evolution, accompanied by gene loss, the Olig gene ancestor gave rise to Olig2, Olig3 and Olig4. (B) A local duplication at the Olig2 locus generated a synteny block containing two Olig2 genes and one of these subsequently underwent recombination with another distantly related bHLH family gene resulting in Olig1. (C) An evolutionary timeline of Olig genes. It is not known whether Olig genes exist in Agnathans. Olig1 appears to be missing from chicken, although more bird species need to be examined to discover whether this is a general feature of birds or a gap in the (still incomplete) chicken genome. A lizard (*Anolis carolinensis*) possesses Olig1 as well as Olig2 but so far Olig3 has not appeared in the *Anolis* genomic database, which is still incomplete.

(Halder *et al.*, 1995). Such examples imply that *cis*-regulatory elements get the upper hand. However, there is also evidence that favours the opposing view that modification of transcription factors themselves plays the major role. The molecular functions of transcription factors did evolve through time and the introduced changes were not only in their conserved DNA binding domains but also in other parts of the molecule that carry out distinct molecular functions. For example, expressing mouse Nkx2.5, a critical gene in mammalian heart development, ectopically in *Drosophila* embryos fails to rescue the defective heart phenotype resulting from a loss-function mutation in the fly Nkx2.5 homolog (Park *et al.*, 1998; Ranganayakulu *et al.*, 1998). In all, it seems more reasonable to believe that *cis*-regulatory elements and transcription factors evolved in parallel to contribute to biodiversity (Lynch and Wagner, 2008).

To our knowledge, there has been only one study of Olig ancestors in an invertebrate – a hemichordate – where only one Olig gene was found (Lowe *et al.*, 2004). In that study, Olig expression was first detected in a cluster of dorsal cells of the prosome base, right after gastrulation. Later on, its expression expanded to the entire proboscis ectoderm and some cells along the dorsal midline. Similarly, in vertebrates,

Olig2/3/4 are all first detected at gastrulation. Later on, at the neurulation stage, Olig2 expression is restricted to the precursors of MNs and OLPs in the ventral neural tube, Olig3 expression can be seen in the dorsal part of the neural tube and Olig4 is also expressed in dorsal neuroepithelium, which gives rise to several kinds of interneurons as well as astrocytes (Zhou *et al.*, 2000; Lu *et al.*, 2000; Park *et al.*, 2002; Takebayashi *et al.*, 2002b; Bronchain *et al.*, 2007).

Piecing together the evidence, we can construct a plausible (but highly speculative!) version of events in the lead-up to CNS myelination. As a result of two rounds of genome duplication during early vertebrate evolution, a single ancestral Olig gene gave rise to Olig2, Olig3 and Olig4. These subsequently evolved independently and ultimately specified distinct neuronal identities, one of which (under Olig2 control) was MN identity. The ancestral Olig gene might have already been involved in neural specification – possibly even MN specification – prior to genome duplication. As OLIG2 function diverged further, MNs underwent reprogramming of their gene expression profiles by acquiring or losing OLIG2-binding *cis*-regulatory elements to enable other pre-existing or newly evolved genes to be expressed under OLIG2 control, leading to primitive glial cells ('motor glia') that resembled today's OLPs. Initially these motor glia were not highly migratory but interacted with and perhaps ensheathed neurons, including MNs, that lay close to their site of origin in pMN. Later, a myelination programme evolved, under the control of myelin determinants like SOX10 (Wegner and Stolt, 2005). After OLIG1 arrived on the scene in teleost fish, it gained the ability to bind directly to SOX10 (Li *et al.*, 2007), enhancing the myelination programme and adapting it to the needs of the CNS.

The lack of an Olig1 homolog in birds, which have CNS- and PNS-specific myelin like other vertebrates, is seemingly at variance with the above interpretation. Perhaps it is premature to conclude on the basis of one example (chicken) that all avian species lack Olig1. Nevertheless, if birds as a group do turn out to have lost Olig1 then this would imply that birds might have found an alternative way of regulating their CNS myelination programme.

Other refinements have occurred in the CNS. Some time between teleosts and tetrapods, OLPs gained expression of PDGFRA (Ana Mora, Nigel Pringle and W.D.R., unpublished results) and, in so doing, became highly proliferative and motile, able to disseminate widely from their point of origin in the ventral neural tube and to myelinate many new types of neuron. This naturally had great benefits for the evolution of higher brain functions and cognition. As the brain, particularly the forebrain, expanded in size from bird to rodent to primate, OLIG2 was activated in progressively more dorsal parts of the neuroepithelium, providing even greater capacity for generating OL lineage cells (Kessaris *et al.*, 2006; Richardson *et al.*, 2006).

A distant MBP ancestor in lancelet?

The major structural genes of the myelin sheath probably did not evolve specifically for myelin but were recruited from the pre-existing pool of proteins because they had appropriate physicochemical properties (Zimmer, 2000). This recycling of pre-evolved proteins might have involved insertion of a myelin-specific *cis*-regulatory element(s) into the vicinity of the 'captured' genes. For example, Mbp is expressed in the immune system and in some neurons in mammals (Landry *et al.*, 1998; Feng, 2007), so presumably was not selected solely for its role in myelin.

Although it has been reported that Mbp does not exist in a Urochordate (sea squirt, *Ciona*) (Gould *et al.*, 2005), we discovered a putative Mbp ancestor in a Cephalochordate (lancelet, *Branchiostoma floridae*) by tBLASTing (default Scoring Matrix, BLOSUM62) the *Branchiostoma* genomic database (http://genome.jgi-psf.org/cgi-bin/runAlignment?db=Brafl1&advanced=1, assembly v2) with *Xenopus* Golli-MBP (J37 isoform) protein as a query sequence (GenBank AB371427). By relaxing the expectation cut-off to 1 and using the default 'word size' 3, we found a single matched genomic fragment that encodes a 107-amino acid polypeptide with ~26% identity and ~40% 'similarity' to frog Golli-MBP ('expectation-value' 0.54). We used this genomic fragment to retrieve the corresponding lancelet cDNA (XP_002227648) from GenBank and confirmed its similarity to *Xenopus* Golli-MBP using ClustalW (see supplementary figure online).

Like vertebrate Golli-Mbp genes the lancelet gene has 11 exons, strengthening the idea of an ancestral relationship. It encodes a 737-amino acid protein; the MBP-like region referred to above lies close to the C-terminus, while the N-terminal region resembles part of another protein, Par-3 (partitioning defective 3) (data not shown). However, there is no similarity between lancelet and vertebrate Mbp in exon 5 where most of the core promoter elements for Mbp transcription lie (Campagnoni *et al.*, 1993). In particular, the lancelet gene lacks the conserved binding sites for OLIG1 and SOX10 that cooperate to drive Mbp expression in vertebrates (Li *et al.*, 2007) (Fig. 3). This is consistent with the idea that insertion of transcription factor binding sites might have been part of the evolutionary process that established the myelin genetic programme. However, considering the very distant relationship between MBP and its putative ancestor in lancelet, this conclusion must be regarded as tentative. Further studies of more highly conserved myelin genes such as Cnp or Mag would be required to put this conclusion on

Fig. 3. Multiple alignment of a vertebrate specific *cis*-regulatory element in exon 5 of Golli-Mbp genes. Fragments of exon 5 from human, mouse, rat, chick, zebrafish (Li *et al.*, 2007) and shark (Fors *et al.*, 1993) were aligned using BioEdit/ClustalW software. A conserved *cis*-regulatory element including an E-Box and an HMG (high mobility group) binding domain is underlined. The lancelet Golli-Mbp-like gene does not have this bipartite *cis*-regulatory element.

a firm footing, but so far no evolutionarily conserved OLIG or SOX10 binding sites have been mapped in genes other than Mbp.

CONCLUSION

It is likely that parallel evolution of Olig genes and their *cis*-regulatory elements in target genes were important for the emergence of myelination programmes in early vertebrates. Many questions are waiting to be answered. Which Olig genes are present in Agnathans, where are they expressed and what are their functions in these animals? Do they, for example, have ensheathing glia in their CNS that resemble OLPs? What are the direct gene targets of OLIG transcriptional regulation and how did their target set expand during evolution, to give rise first to a novel cell type (OLPs) and then the primitive myelination programme? Most pressing is the need to clarify the distinct functions of OLIG1 and OLIG2 in OL development and myelination, because our understanding of evolutionary events is predicated on developmental mechanisms. Also, we need to know more about the similarities and differences between CNS and PNS myelination programmes. Is there really no role in the PNS for Olig genes – e.g. Olig3 or Olig4, about which rather little is known so far? The rapid increase in the number and range of vertebrate and proto-vertebrate genomes that are coming on line is bound to help us address some of these questions – and is providing a fantastic resource for evolutionary biology in general.

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Statement of interest

None.

Supplementary material

The supplementary material referred to in this article can be found online at http://journals.cambridge.org/ngb.

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