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Can Clues from Evolution Unlock the Molecular Development of the Cerebellum?

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Abstract The cerebellum sits at the rostral end of the vertebrate hindbrain and is responsible for sensory and motor integration. Owing to its relatively simple architecture, it is one of the most powerful model systems for studying brain evolution and development. Over the last decade, the combination of molecular fate mapping techniques in the mouse and experimental studies, both in vitro and in vivo, in mouse and chick have significantly advanced our understanding of cerebellar neurogenesis in space and time. In amniotes, the most numerous cell type in the cerebellum, and indeed the brain, is the cerebellar granule neurons, and these are born from a transient secondary proliferative zone, the external granule layer (EGL), where proliferation is driven by sonic hedgehog signalling and causes cerebellar foliation. Recent studies in zebrafish and sharks have shown that while the molecular mechanisms of neurogenesis appear conserved across vertebrates, the EGL as a site of shh-driven transit amplification is not, and is therefore implicated as a key amniote innovation that facilitated the evolution of the elaborate foliated cerebella found in birds and mammals. Ellucidating the molecular mechanisms underlying the origin of the EGL in evolution could have significant impacts on our understanding of the molecular details of cerebellar development.

Keywords Granule cell · Purkinje cell · Atonal · Sonic hedgehog · Zebrafish · Shark

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The cerebellum (Latin for 'little brain') develops from the dorsal half of rhombomere 1 in the hindbrain [1] and is involved in sensory integration and control of motor functions; mouse mutants with cerebellar phenotypes all have ataxic phenotypes and include *staggerer* [2], *reeler* [3] and *weaver* [4]. Knowledge of the molecular events underpinning cerebellar development has advanced dramatically over the last two decades, in large part because of a number of well-recognised features that make it an excellent model for understanding neural development. In addition to the prolonged developmental time period (extending to 3 weeks post-natal in mouse and 2 years in human), a relatively simple circuit architecture is reflected in a comparatively small number of cell types. In addition, in the light of three recent papers [5–7] investigating cerebellar development in more basal vertebrate lineages, we will argue that it also represents an excellent model for studying the evolution of the nervous system in vertebrates and that an "evo-devo" approach can shed considerable light on the molecular nature of development in this context.

The Geography of Precursor Pools

As with much of the central nervous system, progenitors in rhombomere 1 are located in the ventricular region of the neuroepithelium, and post-mitotic cells derived from asymmetric progenitor divisions migrate away from the

proliferative zone and instigate their terminal differentiation programme. In the amniote cerebellar anlagen, as in the spinal cord, dorsal cell fates are specified in response to extracellular signals, particularly TGF-βs [8, 9], derived from the roof plate [10–12]. At the ventricular surface of the cerebellar neuroepithelium, this leads to a fundamental division of progenitors into those in the ventricular zone (VZ) that express *Ptf1a* and give rise to GABAergic neuronal populations [13] and those situated in the most dorsal region of the neuroepithelium, the rhombic lip (RL), that express *Atoh1* and give rise to glutamatergic cells [14–17].

These two germinal zones give rise to a limited number of different cell types, including granule cells and unipolar brush cells (both RL derived), and Purkinje cells, stellate, basket, Golgi and Lugaro interneurons (all VZ derived). Each of these occupy a stereotyped position within the cortex of the amniote cerebellum [18]; the subpial molecular layer, which is composed largely of dendritic trees of Purkinje cells and parallel axonal fibres of granule cells sits atop a monolayer of GABAergic Purkinje cells, internal to the heavily populated internal granule layer (IGL) composed overwhelmingly of granule cell bodies (Fig. 1a), the most numerous cell type in the mammalian brain. The primary output neurons of the cerebellum, the deep cerebellar nuclei, comprise both glutmatergic and GABAergic populations, which lie within the white matter.

The division of progenitors within the mouse cerebellum into essentially two populations, the VZ and the RL, defined by their expression of *Ptf1a* and *Atoh1* respectively, has highlighted the conservation of specification mechanisms between the hindbrain and the spinal cord [19–22], and enabled the detailed molecular fate maps of the cerebellum mentioned above to be constructed [13, 15, 17]. Understanding how different neuronal populations are generated within these progenitor pools has now become a

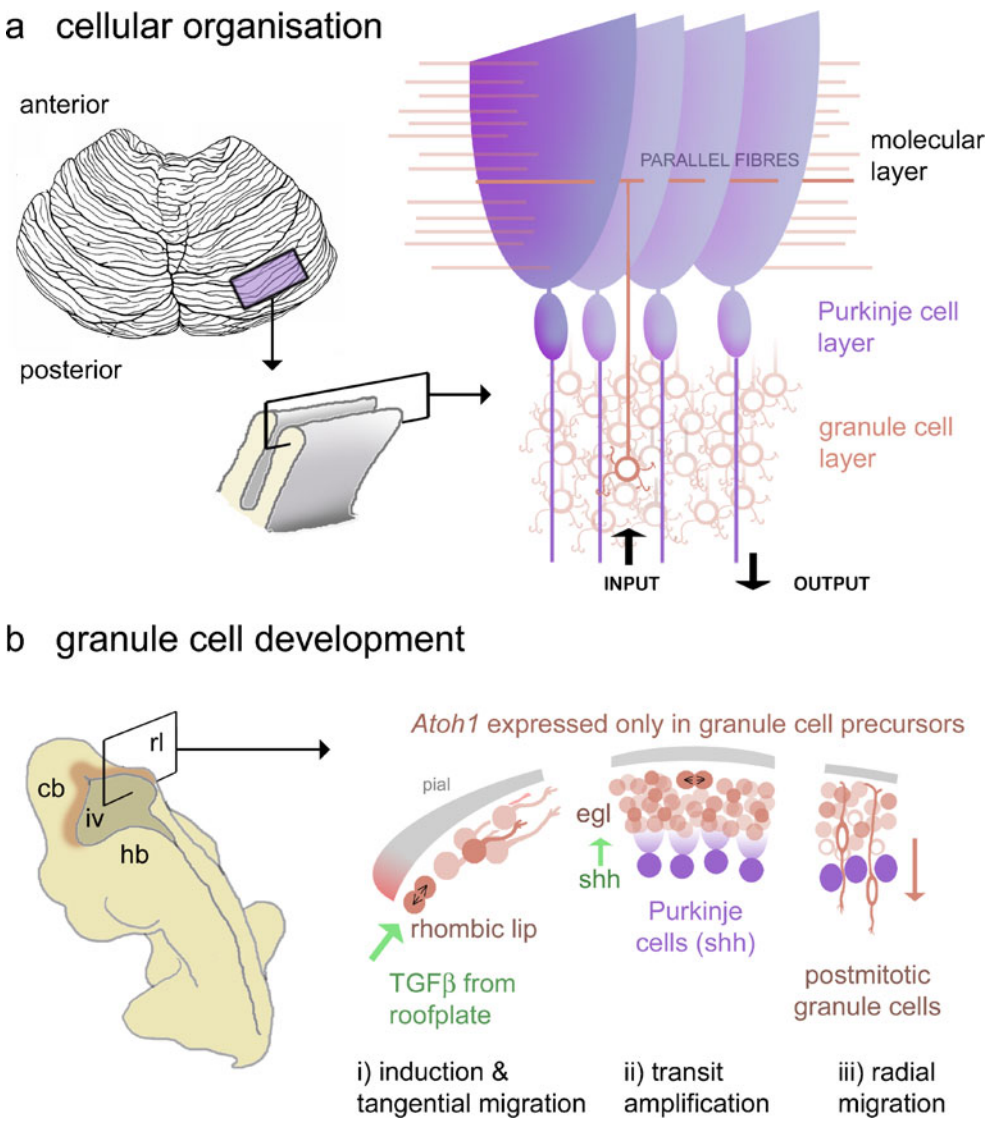

Fig. 1 Cerebellum architecture and amniote development. **a** Arrangement of Purkinje cells and granule cells in a layered cerebellum follows a stereotyped arrangement from fish to human. A subpial molecular layer is composed of Purkinje cell dendritic trees and granule cell axons and few cell bodies. A monolayer of Purkinje cell bodies separates the molecular layer from hugely populous internal granule layer. **b** This arrangement develops, in amniotes, via two phases of proliferation and migration. At the rhombic lip (*i*), TGF-β signalling from the roof plate induces *Atoh1* expression in neighbouring neural tube. *Atoh1* expression is maintained in GNPs, which migrate tangentially into a superficial, transient external granule layer. Here, they proliferate (*ii*) in a series of symmetrical divisions in response to shh signalling from underlying Purkinje cells. The vastly expanded post-mitotic derivatives of the EGL then migrate (*iii*) through the Purkinje cell layer, switching off *Atoh1* expression. Key: Granule cells (*empty brown*), granule cell precursors (*solid brown*), Purkinje cells (*purple*), diffusible proliferative signals (*green*)

major goal, and is one that has met with variable success to date.

In the cerebellar VZ, the order of cell specification is relatively poorly understood. Spatially distinct patterns of proneural gene expression are present in the VZ [23] and indeed these correlate to some degree with the expression of differentiation markers in post-mitotic cells derived from the VZ [10, 24]. However, whether these molecular distinctions reflect the different cell types that are known to be produced from the VZ is unknown, and whether there is any absolute temporal distinction between cell fates at the VZ must await (inducible) molecular fate maps of the subsets of *Ptf1a*-positive VZ cells.

In contrast, considerable progress has been made in recent years in defining the subdivisions within the RL. An interesting aspect of cerebellar neurogenesis at the RL in mammals is that distinct cell populations are born during specific temporal windows of specification [25]. The first cerebellar cells to be born from the RL, between E10.5 and E12.5 in mouse [17] are the deep cerebellar neurons that migrate tangentially over the pial surface of the cerebellar anlagen to the nuclear transitory zone, before gradually moving radially to their mature position in the most medial of the deep cerebellar nuclei (DCN) [26]. After E12.5, there is a temporal switch in cell fate and granule neuron progenitors (GNPs) are born and follow this same migratory stream, gradually populating the entire subpial surface of the cerebellum. After birth, there is a change in the responsiveness of GNPs to signals, both attractive and repulsive, from their surroundings [27–31] that instigates radial migration to their mature location in the IGL. The production of GNPs extends to the post-natal period and the radial migration of GNPs is not complete until the third week after birth in mouse [32].

The third cell population to derive from the murine RL, the unipolar brush cells (UBCs), are also born following the temporal switch, with a peak of production between E15.5 and E17.5, though they undertake a distinct migration path through the white matter toward their terminal location within the IGL [33]. Whether UBCs originate from a distinct subpopulation of the RL that does not produce granule neurons is an intriguing question, and a distinct possibility given novel evidence from the mouse that a molecular subdivision, *Lmx1a* expression, marks GNPs that will populate the most posterior part of the cerebellum [34]. A central remaining issue is the molecular nature of the temporal switch from DCN to GNP fate. Interestingly, while the early born cells, in common with all other RL-derived hindbrain populations, lose expression of *Atoh1* as they leave the RL, GNPs retain *Atoh1* expression, implying that the cell fate switch is intrinsic to RL progenitors rather than a result of extrinsic cues during migration. Additionally heterochronic, heterotopic transplantation experiments in the chick have shown that the change in cell fate is nevertheless dependent upon an extrinsic signal, which must therefore be present at the RL [35].

The GNPs derived from the RL are of crucial importance for understanding the morphogenesis of the cerebellum in both development and evolution, and in amniotes form a secondary proliferative progenitor pool, the external granule layer (EGL) above the molecular layer (Fig. 1b). This pool of progenitors is committed to a granule neuron fate and undergoes transit amplification in response to sonic hedgehog (shh) signalling from underlying Purkinje cells [36–39]. Shh expression is initiated directly downstream of retinoic acid orphan receptor-αexpression [40] in mature post-mitotic Purkinje cells at E17.5 [37]. Symmetrical transit amplification in the EGL continues until the third post-natal week [41], leading to the huge number of granule cells (80 billion in humans) in the mature mammalian cerebellum. The proliferative response is facilitated by the retention of *Atoh1* expression in GNPs [42, 43] and the level of shh signal transduction has been shown to be intimately linked to the extent of foliation of the mature cerebellum [44, 45]. Whilst decreased levels of signalling lead to the formation of a much less foliated cerebellum, the converse is also true, with increased shh signalling causing the mouse cerebellum to adopt an ectopic extra folium that is found naturally in the rat [44].

The key role of shh response of the EGL has important clinical consequences in humans. The inappropriate activation of shh signal transduction in GNPs is one of small number of likely causes of medulloblastoma [46], the most common paediatric brain malignancy [47]. This condition can be recapitulated in mice that lack a copy of the *Ptch1* gene [48], which results in the derepression of sonic hedgehog signalling. This experimentally induced medulloblastoma requires *Atoh1* expression in granule cells [49, 50]. As in normal development the transcriptional programme initiated in tumourigenesis is coordinated by Gli1, which together with Gli2 transduces sonic hedgehog signalling within the cerebellum [51].

The evolution (and pathogenesis) of cerebellar morphology in mammals is apparently therefore directly dependent upon the proliferative behaviour of the EGL, a finding that bears comparison with the subventricular zone (SVZ) of the mammalian neocortex [52]. In the rodent neocortex, radial glia in the ventricular zone gives rise to post-mitotic neurons and to intermediate progenitors that migrate to the SVZ and undergo a further symmetrical division, increasing neuronal cell number [53]. In humans, the outer SVZ is drastically expanded and is composed of both asymmetrically dividing stem cells, and progenitors that undergo transit amplification [54]. The implication is that alteration of the extent of transit amplification may

represent a powerful mechanism of developmental evolution in the brain.

Anatomical Diversity Across Vertebrates

The suggestion that different levels of shh signalling might be correlated with evolutionary changes in morphology amongst mammals [44, 45] takes on added significance from an evolutionary point of view in light of the extensive variation in cerebellar architecture found in different vertebrate groups. The presence of the cerebellum as a distinct brain structure is confined to gnathostomes [55], although the extent to which molecular architecture and function is conserved in jawless vertebrates and basal chordates are largely unexplored.

Amongst jawed vertebrates, the morphology of the cerebellum varies dramatically. In chondrichthyans, the cerebellum adopts a relatively elongated form that varies considerably in relative size and enormously in terms of foliation [56], and this variation has been correlated with behavioural strategies [56–58]. Additionally, lateral auricles or 'cerebellar-like' regions posterior or lateral to the cerebellar cortex receive inputs from the electroreceptive and lateral line systems [58]. In teleost fish, the form of the cerebellum exhibits huge variation largely as a result of the differing forms of the valvulus, a specialised region of the anterior cerebellum that is not found in non-teleost fish [55] and has been shown to be a source of adult stem cells [7, 59]. In tetrapods too, there is a considerable diversity of morphologies (Fig. 2a) ranging from the flat sheet found in amphibians to the elaborate, foliated mammalian and avian cerebellum. As in chondrichthyans [58], cerebellar morphology in tetrapods has been correlated with ecological and behavioural characteristics [60].

This range of extant morphologies raises the question of how such extreme diversity has evolved. Are the molecular mechanisms underlying neurogenesis conserved between different taxa, or are the precursor pools organised in a different way? Indeed, how conserved are the different cell types derived from these pools? With the complexity of the different migratory paths taken in amniotes by post-mitotic derivatives, and in the case of the EGL, proliferating neuroblasts, it is likely that alterations in cell migration paths have played a role in the evolution of form. It is also tempting to speculate that variations in the presence and extent (both spatial and temporal) of the EGL and transit amplification are also heavily involved in evolutionary alterations of the cerebellum, as has been implied in rodents [45]. The recent development of molecular lineage tracing technologies for cerebellar genes in the zebrafish [6, 7], coupled with comparative data from fish and shark [5] has now begun to address some of these questions.

A Diversity of Developmental Mechanisms

In chondrichthyans, the most basal lineage of extant jawed vertebrates, there is evidence for conservation with tetrapods of the molecular mechanisms of cell specification. In sharks, granule cells are organised in two midline eminences, and express Pax6 as they do in mammals [5, 61]. GNPs at the RL express *Atoh1* and mature Purkinje cells are GABAergic, possess the Zebrin II antigen, and express shh, implying that the two major components of the cerebellar circuit are conserved in at least some details of their molecular characteristics across gnathostomes. However, the spatial relationship between the two cell types is different. Whereas in amniotes, the cerebellar cortex has a stereotyped-layered organisation (Fig. 1a) throughout its extent, in the shark the mature granule cells occupy the medial eminences that are adjacent and ventral to the RL. Since the shark Purkinje cells occupy a subpial layer [5, 61], albeit not a monolayer, the implication is therefore that one of the underlying reasons for the difference in form is an alteration in granule cell migration patterns during evolution. In addition to tangential migration, the other defining feature of mammalian GNPs is their transit amplification in response to shh. However, the role of shh signalling in the shark cerebellum remains unclear. Expression of *Ptc2*, a readout of shh signal transduction, is confined to a region of the ventricular zone adjacent (and embryonically ventral of) the medial granule eminences, and has been hypothesised as a source of Purkinje cell progenitors [5]. What is clear though is that at present there is no evidence for *Atoh1*-mediated proliferation of GNPs in response to this signal; there appears to be no EGL in sharks.

In the zebrafish, representatives in this context of the actinopterygian ('ray-finned fish') branch of bony vertebrates (the other branch being our own, the sarcopterygians or 'lobe-finned fish'), elegant molecular fate mapping data using BAC transgenic fish lines has shown that GNPs are likewise marked by *Atoh1* expression, with Purkinje cells originating from a *Ptf1a*-expressing VZ [6]. The primary division of precursor pools into an *Atoh1*-positive population producing glutamatergic neurons and a *Ptf1a*-positive population giving rise to GABAergic neurons thus appears to have a long phylogenetic history. Also in common with amniotes, *Atoh1* expression in the fish RL is correlated with TGF-β expression in the roof plate. Downstream of *Atoh1*, *NeuroD* is expressed in post-mitotic granule cells [6, 62]. Even more so than has been demonstrated in the shark therefore, the molecular cascades underpinning Purkinje and granule cell development appear conserved in bony fish. In addition to the molecular events underlying specification and differentiation, the tangential migratory path of GNPs appears conserved between teleosts and

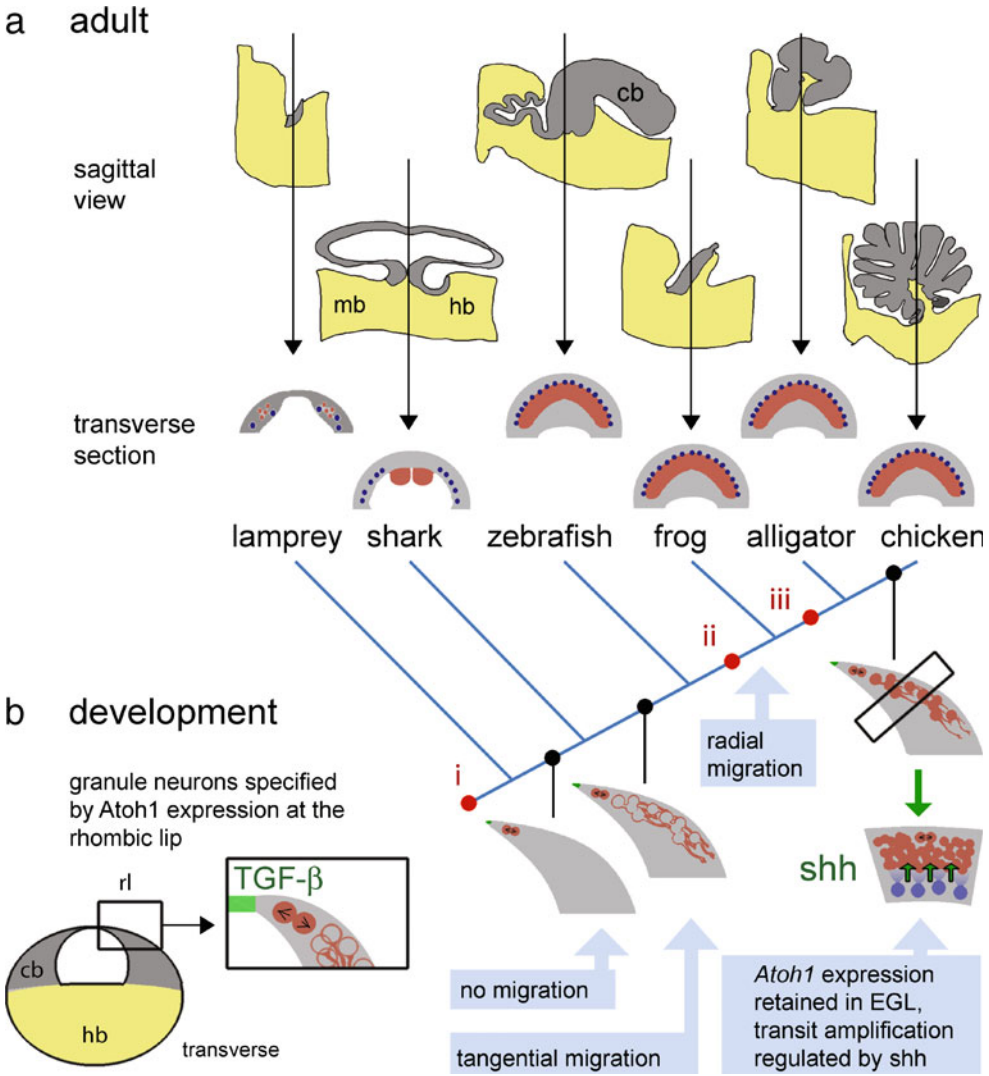

Fig. 2 Vertebrate cerebellar evolution. Shark, zebrafish and amniote developmental mechanisms are shown in the context of broader vertebrate phylogeny. **a** Sagittal views of adult cerebella after [55] from different vertebrates (*top row*) with matching schematic transverse sections (*below*) showing the relative spatial distribution of Puekinje cells (*purple*) and granule cells (*brown*). **b** Evolution of the cerebellum in terms of granule cell development. In the last common ancestor of jawed vertebrates, granule cells were simply specified at the rhombic lip by TGF-β signalling from adjacent roof plate and deposited next to their site of origin. In the osteicythian lineage, tangential migration evolved in post-mitotic granule neurons, leading to the common mature tissue architecture found in all extant bony fishes (including tetrapods) in tangential section, where a monolayer of Purkinje cells lies above a populous internal granule layer. In birds and mammals, tangential migration is intitiated in committed precursors of granule cell neurons (rather than definitive granule cells) leading to the formation of the transient, proliferative external granule layer. Cell division in this superficial layer of transit amplifying progenitors, is promoted by sonic hedgehog, which is secreted from underlying Purkinje cells. At their final, symmetrical division, post-mitotic granuel neurons migrate radially to form the internal granule layer. Important gaps in our knowledge remain about the (*i*) nature of the prototype vertebrate cerebellum, (*ii*) the processes that produce a distinct but non-proliferative EGL in frogs [66], and (*iii*) Shh-mediated transit amplification within the amniote clade. Colour coding as in Fig. 1

amniotes. In the zebrafish, granule cells born at the RL do not accumulate at the midline as in sharks, but rather migrate tangentially away from the RL as in amniotes [5–7, 63–65]. However, there appears to be no clear distinction between the tangential and radial phases of migration as there is in amphibians [66] and amniotes [27].

Despite the overall conserved pattern of cell specification mechanisms, a number of significant developmental differences are apparent between the zebrafish and amniote cerebellum. These differences shape the different adult morphologies and serve to illustrate the potential of the cerebellum as a model system for evolutionary developmental studies of central nervous system development. Whilst in the fish, there is a temporal distinction between the early derivatives of the RL that migrate out of the cerebellar territory and the later ones that form cerebellar

neuronal populations [65], there is no temporal switch between glutamatergic *cerebellar* cell fates (DCN vs. GNPs) as has been demonstrated in mammals (see above). Zebrafish eurydendroid cells are the output projections neurons of the cerebellum and, like amniote DCNs, are born from both the RL and the VZ. As yet, it is unclear whether they represent ontological homologues of the DCN or merely functional equivalents. What is clear though is that both granule neurons and eurydendroid cells are born from the RL during the same developmental time period [6]. The evolution of the temporal switch in cerebellar cell fates (from DCN to GNP) at the RL appears thus to be confined to amniotes, though its significance remains unexplored.

As outlined above, one of the most prominent features of the amniote cerebellum is the transit amplification of GNPs in the external granule layer in response to shh, and it is this that is likely to be a major causative factor driving the evolution of cerebellar morphology. In the zebrafish, whilst there are proliferating, *Atoh1*-positive cells in the molecular layer of the juvenile and adult cerebellum [6], there is no external granule layer of *Atoh1* positive progenitors that extends over the whole pial surface and no underlying shh signalling to drive its transit amplification [5, 7]. A proliferative external granule layer, therefore, is so far confined to the amniotes and their elaborate, heavily foliated cerebella.

The Evolution of Cerebellar Development

The advances in understanding of the molecular underpinnings of cerebellar development through comparative descriptive studies in sharks, and through sophisticated molecular fate mapping techniques in zebrafish, now allow us to develop a cellular and molecular model of cerebellar developmental evolution (Fig. 2b). The overwhelming implication of recent studies is that the cerebellum evolved via a series of developmental innovations in cellular behaviour and signalling, rather than via the addition of novel cell types. The mechanisms of neurogenesis evolved very early and subsequent evolution has involved an elaboration of the numbers of cells and presumably the computational capacity of the cerebellum; and this has largely been achieved by the extension of the proliferative capacity of GNPs in space and time.

One of the obvious remaining questions is whether any recognisable cerebellar-like structures exist in more basal animals (i in Fig. 2b). Presuming that cerebellar circuitry evolved before the cerebellum as a distinct brain structure, then the question of the origin of the specification mechanisms that define the major cerebellar components —the granule cells, Purkinje cells and the output (deep cerebellar/eurydendroid) neurons—is a major one. Early vertebrate evolution was accompanied by the adoption of active predation driven by sophisticated sensory capabilities and swimming behaviour, coupled with extensive developmental innovations in the head region [67]. It is tempting to speculate therefore that the evolution of the vertebrate cerebellar circuit also accompanied this transition, and was in fact one of its pre-requisites. If so, how were the specification mechanisms of cerebellar cell types shaped by the whole genome duplications that were also coincident with early vertebrate evolution [68]? Also, did cerebellar circuits evolve de novo, or as an elaboration of pre-existing; however, rudimentary (perhaps just a few Purkinje-like and granule-like cells), circuitry that can still be found in extant outgroups, such as cephalochordates, hemichordates, or even protostomes? Cephalochordates, long thought to be the invertebrate group that is the closest related to the vertebrates [69], resemble the likely ancestor of the vertebrates, in that they are filter feeding fish-like animals with a central nervous system and a number of other vertebrate features, but a very small brain and unpaired sense organs [70]. Outside of the chordates, the basic ground pattern of the vertebrate CNS is conserved across deuterostomes (animals whose blastopore, following gastrulation, forms their anus) [71, 72], and indeed even in a representative, *Platynereis* [73, 74], of the other great clade of bilaterally symmetrical animals, the protostomes (whose blastopore forms their mouth). Theoretically at least, the need to process sensory and motor information would have been greatly increased with the evolution of bilateral symmetry, suggesting that the antecedents of the vertebrate cerebellar circuit may have a very long history indeed.

In terms of the evolution of morphology, a number of gaps in the vertebrate phylogeny remain unexplored. In particular, the extent to which the zebrafish and shark cerebella have taxon-specific developmental architectures is unclear at present. While the potential of the valvulus for generating elaborate morphologies in teleost fish is highlighted by its role as a source of adult stem cells [7], no analogous structure has yet been described in chondrichthyans, and yet some members of this clade possess hugely foliated cerebella [56]. Is this foliation the product of developmental proliferation of GNPs, and if so what is their spatial organisation? Molecular developmental investigations of these species would prove intensely interesting, and are essential in order to fully understand the ancestral gnathostome condition. Similarly, how does the organisation of precursor pools and proliferation in basal actinopterygians such as the paddlefish, and the lamprey (as the jawless outgroup) compare to that in chondrichthyans and teleost fish? Given the central role of the EGL and transit amplification in the evolution of the amniote condition, it will be of central importance to elucidate when this

evolved. In particular, the uniformly unspectacular form of amphibian cerebella [55] suggest that it will prove a fruitful comparative subject of research in illustrating the mechanisms that form the amniote cerebellum. Pioneering work from the 1970s shows that the frog displays a distinct, but non-proliferative, EGL [66]. By contrast, the degree of foliation in sauropsid reptiles varies significantly, ranging from the tortoise (which resembles an amphibian in having no cerebellar folia) through to the chicken with its highly folded cerebellum produced from a transit-amplifying EGL (Fig. 2). The birds and their close relatives therefore represent a series of variations ranging from the simple to the complex. Does this represent a convergent (with mammals) evolution of a Shh-dependent, proliferative EGL in gradual (still extant) reptilian steps, through to bird (iii in Fig. 2b)? Or did a proliferative EGL evolve in a common amniote ancestor (ii in Fig. 2b) with transit amplification having been secondarily *reduced* (to the level of an amphibian cerebellum) in multiple reptilian lineages? The answer to this and to how the amniote condition evolved from that of their amphibian ancestors will illustrate not only how cerebellar morphology evolves, but also will have significant implications for a mechanistic understanding of its development.

Conclusions and Implications

The technical advances in molecular fate mapping in the mammalian cerebellum in the last 5 years have drastically improved our understanding of the molecular development of the hindbrain and cerebellum, but at the same time have posed a plethora of important questions regarding one of the best understood systems in developmental neurobiology. Coupled with the recent developments highlighting the molecular nature of neurogenesis in the zebrafish and the comparative data from sharks, the cerebellum now represents an excellent model for studying the evolution of development in the vertebrate central nervous system.

Aside from the outstanding comparative questions in regard to cerebellum evolution referred to above, many of the remaining questions arising from the work discussed in this review concern not only cerebellar evolution, but its molecular development in mammals too. A good example is the view of signalling interactions between granule and Purkinje cells during development. Given that the transduction of shh signalling in the EGL is dependent upon *Atoh1* expression in granule neuron precursors, how did the evolution of shh and *Atoh1* expression evolve? If they were not coincident, then what other functions do they serve? In terms of shh signalling, recent work has suggested that immature Purkinje cell and developing choroid plexus cell populations respond to and are dependent upon shh protein derived from mature choroid plexus and secreted into the cerebrospinal fluid of the fourth ventricle [75–77], highlighting that the function of shh signalling in the fourth ventricle is certainly not confined to transit amplification. Could Purkinje cell-derived shh augment these other, perhaps more ancestral, functions, and if so, are there further populations within the cerebellum that depend upon shh signalling, either for proliferation, specification, or some aspect of neuronal behaviour?

In relation to the course of granule neuron development, perhaps the most obvious question that arises from comparison of the different vertebrates discussed above, and which is of central importance for understanding the development of both the cerebellum and the CNS generally, is what molecular prerequisites are required for transit amplification. The ability to undergo transit amplification in GNPs is, as mentioned, dependent upon *Atoh1* expression [42, 43]. With comparative data suggesting that this ability (to express *Atoh1* away from the source of inductive signals) is a recent (so far amniote specific) evolutionary innovation, the implication is that a comparative approach represents a good strategy to elucidate the molecular basis of gene regulation in this context. It frames the central questions of whether a separate, and evolutionarily recent, regulatory mechanism controls *Atoh1* expression away from the source of TGF-βsignals at the roof plate, and of what the nature of this mechanism might be. The spatiotemporal specificity of *Atoh1* expression in the mouse has been shown to be imparted by an enhancer located 3′ of the coding sequence [21, 78]. This enhancer is capable of driving expression in both the RL and the EGL [78, 79]; the implication being that the output of this enhancer is different in different tissues. Additionally, it has been shown to be conserved across amniotes and capable of driving neural tube expression in both mouse and chick [80]. How this enhancer has evolved, and what other elements direct the expression of *Atoh1* in RL derivatives is therefore of considerable interest in explaining the evolution of *Atoh1* regulation and function. Interestingly, the expression of *Atoh1* in progenitors that are migrating away from the germinative rhombic lip as granule neuron progenitors is unique amongst all of the *Atoh1*-derived neuronal populations in the hindbrain; all other population switch off *Atoh1* expression as soon as they leave the RL. This suggests that an understanding of the evolution of gene regulation in GNPs may have significant implications for understanding the control of cell fate differences in the RL between R1 and the rest of the hindbrain.

The last few years have yielded dramatic advances in understanding cerebellar development. Whatever the extent to which evolutionary and molecular approaches to cerebellar development will interact in future, this recent progress has been facilitated partly because of the new

perspective that comparative studies between different model, and non-model, organisms provide. These studies have also begun to apply to the brain one of the central themes of evolutionary biology: that the elaboration of pre-existing characters drives phenotypic evolution. In the brain, this translates into a prediction that important evolutionary innovations in vertebrate history will be a result not of the de novo appearance of new cellular lineages, circuits and functions, but rather of elaboration of pre-existing lineages, in part through transit amplification. The combination of sophisticated genetic data and a comparative context has thus proved a powerful one. It has much left to contribute.

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