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Nuclear Factor I Genes Regulate Neuronal Migration

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Abstract
Neuronal migration plays a central role in the formation of the brain, and deficits in this process can lead to aberrant brain function and subsequent disease. Neuronal migration is a complex process that involves the interaction of the neuron with the surrounding environmental milieu, and as such involves both cell-intrinsic and cell-extrinsic mechanisms. Studies performed in rodent models to investigate the formation of brain structures have provided key insights into how neuronal migration is coordinated during development. Within the cerebral cortex, glutamatergic neurons derived from the cortical ventricular zone migrate radially into the cortical plate, whereas interneurons derived within the ventrally located ganglionic eminences migrate tangentially into the cortex. Within the embryonic cerebellum, cerebellar granule neuron progenitors migrate from the rhombic lip over the surface of the cerebellar anlage, before differentiating and migrating radially into the internal granule layer of the cerebellum perinatally. In this review, we focus on one family of proteins, the nuclear factor I transcription factors, and review our understanding of how these molecules contribute to the formation of the hippocampus and the cerebellum via the regulation of neuronal migration.

Introduction

The migration of postmitotic neurons from proliferative ventricular zones to their final location, where they mature and form functional synaptic connections, is a key event during nervous system development. The importance of neuronal migration during development is highlighted by the range of human disorders that arise when this process does not occur correctly [1, 2]. These include lissencephaly, or 'smooth brain', where the aberrant migration of postmitotic pyramidal neurons within the cerebral cortex culminates in the failure of the brain to form normal sulci and gyri [3, 4], and subcortical band heterotopia, where neurons form inappropriate layers within the white matter of the cerebral cortex [5–7]. Both of these conditions are associated with cognitive deficits and mental retardation [1, 2].

Neuronal migration occurs via mechanisms that are conserved with respect to the regulation of other migratory cell populations [8]. When migration is first initiated, the neuron has to transit from a stationary mode to a mobile form, a process which entails significant changes to the cellular architecture. These include modifications to the actin and microtubule cytoskeleton that culminate in the acquisition of cellular polarity in the form of a leading process. Once mobile, the neuron has to navigate through the complex environment of the developing brain. This difficult process is performed with a high degree of fidelity through the use of guidance receptors expressed on the surface of the leading process, which en-

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able the migrating neuron to sense attractive and repulsive cues. How competing attractive and repulsive cues are integrated to achieve a directional response is still largely unknown, but extrinsic cues have been shown to elicit changes in intracellular second-messenger systems that ultimately manifest themselves as changes to actin and microtubule cytoskeletal dynamics [9]. Once at its final location, a neuron then needs to fully differentiate and to begin the process of maturation, which includes the extension of an axon, the elaboration of dendritic arbors and the formation of synaptic connections.

Recent research using model systems such as the developing rodent brain have identified many of the molecules that contribute to neurogenesis. In particular, studies into the development of the cerebral cortex and cerebellum have provided significant insights into the cellular and molecular mechanisms regulating neuronal migration. Here, we focus on one family of genes in particular, the nuclear factor I *(Nfi)* genes, and summarize our understanding of their contribution to neuronal migration via both cell-extrinsic and cell-intrinsic mechanisms within these regions of the developing brain.

Nfi Genes: Key Regulators of Nervous System Development

The *Nfi* genes have been shown to regulate a variety of facets of nervous system development [10–12]. The first report describing these transcription factors identified NFI as a host-derived factor needed for the initiation of adenovirus replication in vitro [13]. Subsequently, 4 different NFI family members were isolated in the vertebrate lineage, namely *Nfia*, *Nfib*, *Nfic* and *Nfix* [14, 15]. Like many transcription factors, the NFI proteins have a modular structure, consisting of a conserved N-terminal DNA-binding/dimerization domain and C-terminal transcriptional activation and/or repression domain [11]. NFI proteins bind as either hetero- or homodimers with high affinity to the dyad symmetric consensus sequence TTGGC(N5)GCCAA on duplex DNA [16]. They can also bind to consensus half sites (DNA sequence of TTGGC or GCCAA) with reduced affinity [17]. NFI proteins have been shown to either activate or repress gene expression, depending on the promoter and cellular context in which they are expressed [10, 18]. Homologs have been described in other vertebrates, from zebrafish [19] and *Xenopus* [20, 21] to humans [22]. Furthermore, a single *Nfi* gene is present in both the nematode *Caenorhabditis elegans* and *Drosophila* [23].

Expression of Nfi *Proteins during Nervous System Development*

The expression patterns of the *Nfi* genes in the developing mouse brain were described via in situ hybridization over 20 years ago [24], revealing that *Nfia*, *Nfib* and *Nfix* are expressed in partially overlapping patterns within the developing nervous system. Within the neocortex, NFIA and NFIB are expressed in the ventricular zone, marginal zone and cortical plate during embryogenesis [25]. At the developing telencephalic midline, glial cells within the indusium griseum and the glial wedge also express NFIA and NFIB, as do the cells that form the subcallosal sling [26, 27]. Within the developing hippocampus, NFIA is expressed by multiple cellular populations, including ventricular zone progenitor cells, glia within the fimbria and postmitotic neurons within the ammonic neuroepithelium and dentate gyrus. The expression of NFIB within the embryonic hippocampus overlaps with expression of NFIA except for within progenitor cells within the fimbrial glioepithelium, which do not express *Nfib* during embryogenesis [28]. Finally, within the cerebellum, expression of NFIA and NFIB is upregulated within the nuclei of cerebellar granule neurons as they become postmitotic within the premigratory zone of the external granular layer. This expression persists throughout the migration of cerebellar granule neurons through the molecular layer to the internal granule cell layer [29].

The expression pattern of *Nfix* overlaps substantially with the expression of NFIA and NFIB [30]. Within the developing telencephalon, NFIX is expressed within the preplate and the ventricular zone from embryonic day 11 (E11). By E13, expression of NFIX is more widespread, encompassing the preplate, the septum, the piriform cortex and the ganglionic eminences. By E17, all of the layers within the cortical plate express NFIX. NFIX is also expressed at the ventricular surface and the differentiating cell layers of the developing hippocampal primordium at E13 [25, 30]. At E17, NFIX is highly expressed in the dentate gyrus and also expressed in the stratum oriens, stratum pyramidale and within the ventricular zone [25, 30]. Finally in the cerebellum, cerebellar granule neurons in the external granular layer display upregulation of *Nfix* expression, which persists as the granule neurons migrate into the internal granule layer of the cerebellum [29].

Investigation of the Function of Nfi *Genes during Development*

Investigation of the roles of the *Nfi* genes has predominantly been performed through the generation and analysis of individual gene knockout mice. *Nfia*$^{-/-}$ mice, gen-

erated through the deletion of the 3′ splice acceptor site and 219 bp of exon 2 [31], exhibit a range of neurological deficits. These include agenesis of the corpus callosum, enlarged lateral ventricles, cerebellar and hippocampal malformation, hydrocephalus, urinary tract defects and disrupted midline glia formations [27, 31, 32]. *Nfia* mice exhibit perinatal lethality due to renal deficits, precluding analysis of this gene in the postnatal development of the brain. Interestingly, however, recent studies have reported that mutations within the human *Nfia* gene are correlated with neurological disorders such as a thin, hypoplastic or absent corpus callosum, and hydrocephalus or ventriculomegaly, providing evidence for the evolutionarily conserved role for this gene in brain development [32, 33].

Nfib$^{-/-}$ mice, generated by targeting the 523 bp of the 3′ portion of exon 2 and the first 177 bp of the 5′ portion of intron 2 [34], die at birth, due to aberrant maturation of the lungs, but have been shown to have a variety of defects with regard to nervous system development [34]. For instance, mice lacking this transcription factor display enlarged lateral ventricles, dysgenesis of the corpus callosum and failure of glial maturation at the telencephalic midline [26], while also exhibiting cerebellar and hippocampal defects [28, 34]. Development of the pons within *Nfib* mutants is also abnormal, with development of neurons within the pontine nuclei occurring substantially later than in control mice [35].

Nfix knockout mice were also generated through the deletion of essential exon 2 [30]. In contrast to *Nfia* and *Nfib* knockout mice, *Nfix* knockout mice survive postnatally [30]. *Nfix*$^{-/-}$ mice do, however, exhibit a number of telencephalic defects. These include expansion of the cingulate cortex and the entire brain along its dorsoventral axis and the accumulation of cells within the subventricular zone of the postnatal brain. Aberrant development also occurs within the hippocampus in the absence of *Nfix*. Postnatal *Nfix* knockout mice display distorsions within the hippocampus, with an enlarged CA1 field and a shorter dentate gyrus [30]. The CA3/CA4 subfields also display morphological abnormalities [30], indicating a possible change in the cytoarchitecture of the pyramidal cell layer.

Downstream Targets of the NFI Transcription Factors

The *Nfi* genes encode transcription factors, and the wide range of nervous system abnormalities observed in the *Nfi* knockout mice described above have led to intensive research into the transcriptional targets of the NFI proteins. Putative targets of the NFI transcription factors have been primarily identified using candidate-based approaches in vitro. NFI binding sites have been characterized in the promoter regions of many neuronal and glial genes, including those of the astrocyte-specific glutamate/aspartate transporter (GLAST) [28], tenascin C [28], neurofilament M protein [36], myelin basic protein [37] and glial fibrillary acidic protein (GFAP) [38]. Support for these genes being direct targets for *Nfi*-mediated transcriptional activation has come from analysis of *Nfia* and *Nfib* knockout mice, which exhibit downregulated expression of GLAST, GFAP and tenascin C within the cortex and hippocampus [28, 39]. Furthermore, GFAP (a marker for astrocyte differentiation) has been shown to be a direct target of NFI in undifferentiated cortical progenitor cells prior to astrocyte differentiation using chromatin immunoprecipitation [40].

Within the developing nervous system, in vivo studies have also confirmed that *Nfia* is required to drive the expression of *Gfap*. An insight into one of the mechanisms underlying this process was recently provided by a study of the embryonic mouse cortex. Using cultured embryonic cortical progenitors, Namihara et al. [41] demonstrated that induction of the Notch signaling pathway resulted in the activation of NFIA expression. Furthermore, *Nfia* expression was correlated with the dissociation of the methyltransferase DNMT1 from the STAT3 binding site within the *Gfap* promoter, culminating in demethylation, and hence activation, of this promoter region [41]. As JAK/STAT signaling is required for gliogenesis [42], these findings place *Nfia* within the molecular cascade required to drive gliogenesis and further suggest that *Nfia* drives the transcription of glial-specific genes in part via the suppression of repressive DNA methylation.

The Notch signalling pathway is also a central component for the maintenance of progenitor cell identity. Recent research from our laboratory has demonstrated that *Nfia* promotes gliogenesis through the repression of the key Notch effector gene, *Hes1*. *Hes1* is a helix-loop-helix transcription factor that maintains neural progenitor cell identity through the inhibition of differentiation-specific gene expression [43]. *Nfia* knockout mice show significant upregulation of *Hes1* expression within the telencephalic ventricular zone during late embryogenesis. Furthermore, in silico promoter analysis, gel shift assays and chromatin immunoprecipitation demonstrated that the promoter of the *Hes1* gene contains a cluster of conserved NFI binding sites that are bound by NFIA both in vitro

and in vivo, and that *Nfia* can repress transcription under the control of the *Hes1* promoter in luciferase assays [39]. Taken together, these findings suggest that *Nfia* may drive progenitor cell differentiation both by repressing pathways involved in progenitor self-renewal and by the activation of differentiation gene programs that regulate neural development.

Nfi Genes and the Cell-Intrinsic Control of Neuronal Migration

As described above, the *Nfi* genes are expressed within the cerebellum by immature neurons within the premigratory zone, and by mature cerebellar granule cells within the internal granule cell layer. Targets for transcriptional control by the *Nfi* genes have also been identified in the developing cerebellum, and the phenotype of *Nfi* knockout mice is indicative of a central, cell-intrinsic role of these genes in the formation of this structure. Cerebellar granule neurons, one of the major neuronal subtypes found within the cerebellum, receive excitatory input from the mossy fibers originating within the pontine nuclei. Granule neurons send parallel fibers through the Purkinje layer into the molecular layer, where they branch and innervate the Purkinje cell dendritic arbors, forming thousands of excitatory synapses onto the intermediate and distal dendrites [44]. Developmentally, cerebellar granule cells undergo a stereotypical developmental program characterized by progressive stages of maturation. Granule progenitor cells first arise between E13 and E15 from a proliferative zone in the hindbrain known as the rhombic lip. They then migrate rostromedially along the surface of the cerebellar anlage, forming a layer of neuronal precursor cells known as the external granular layer. The cells within the external granular layer continue to proliferate during embryogenesis, producing a large pool of progenitors. Postnatally, these progenitors begin to differentiate, producing postmitotic granule cells, a process which occurs predominantly within the first 2 weeks in mice [45]. Newly differentiated granule cells cluster in the premigratory zone of the external granular layer, before migrating radially through the molecular layer and Purkinje cell layer to reach their final position within the internal granule layer. The process of granule cell migration is mediated by the radial processes of the Bergmann glia, which provide a scaffold for the migration of these neurons (fig. 1).

Multiple aspects of cerebellar granule cell development and maturation are regulated by the NFI transcription factors [29]. *Nfia*$^{-/-}$ mice maintained on a mixed C57Bl/6J and 129S6 genetic background (where 38.5% of homozygous mice survive until postnatal day P30) display cerebellar defects, in particular a decrease in cerebellar size at P17, coupled with aberrant foliation and a delay in the postmitotic maturation of cerebellar granule neurons [29]. *Nfib* knockout mice have also been reported to exhibit foliation defects [34]. By the use of a dominant negative repressor construct (which represses *all Nfi* genes), Wang et al. [46] have shown that NFI proteins act to directly regulate the transcription of *Gabra6,* which encodes a protein intrinsic to the program of neuronal differentiation within granule neurons. More pertinently with regard to the migration of cerebellar granule neurons, the lentiviral delivery of the NFI dominant repressor construct has shown that *Nfi* genes are vital for neuronal migration to occur postnatally. In vitro, migration of postmitotic cerebellar granule neurons within reaggregate cultures was shown to be inhibited upon repression of NFI function. Additional evidence to support a role for *Nfi* genes in the cell-intrinsic control of cerebellar granule neuron migration came from modified Boyden assays, which demonstrated a significant reduction in neuronal migration in those cells transfected with the dominant negative NFI repressor. Finally, P17 *Nfia* knockout mice exhibit a significantly increased number of cerebellar granule neurons within the premigratory zone of the cerebellum, whereas these cells were not apparent in the wild-type control, indicative of a delay in the migration of these cells (fig. 1) [29].

An insight into exactly how *Nfi* genes regulate the migration of cerebellar granule neurons has come from the identification of genes that are directly regulated by NFI proteins during development of the cerebellum, namely N-cadherin and ephrin B1. Both of these cell adhesion molecules have previously been implicated in the migration of cerebellar granule neurons [47, 48]. Treatment of cultured cerebellar granule neurons with the NFI dominant repressor elicited a significant reduction in the mRNA expression for these genes, whereas the expression of other cell adhesion molecules, such as L1 and integrin β_1, was not significantly changed [29]. The expression of N-cadherin and ephrin B1 was also shown to be diminished in vivo, as expression of these proteins was markedly downregulated in the cerebellum of P17 *Nfia* knockout mice. Finally, using chromatin immunoprecipitation with a pan-NFI antibody, NFI proteins were shown to localize specifically to consensus binding sites within the promoters of N-cadherin and ephrin B1 [29]. Although transcriptional reporter assays were not per-

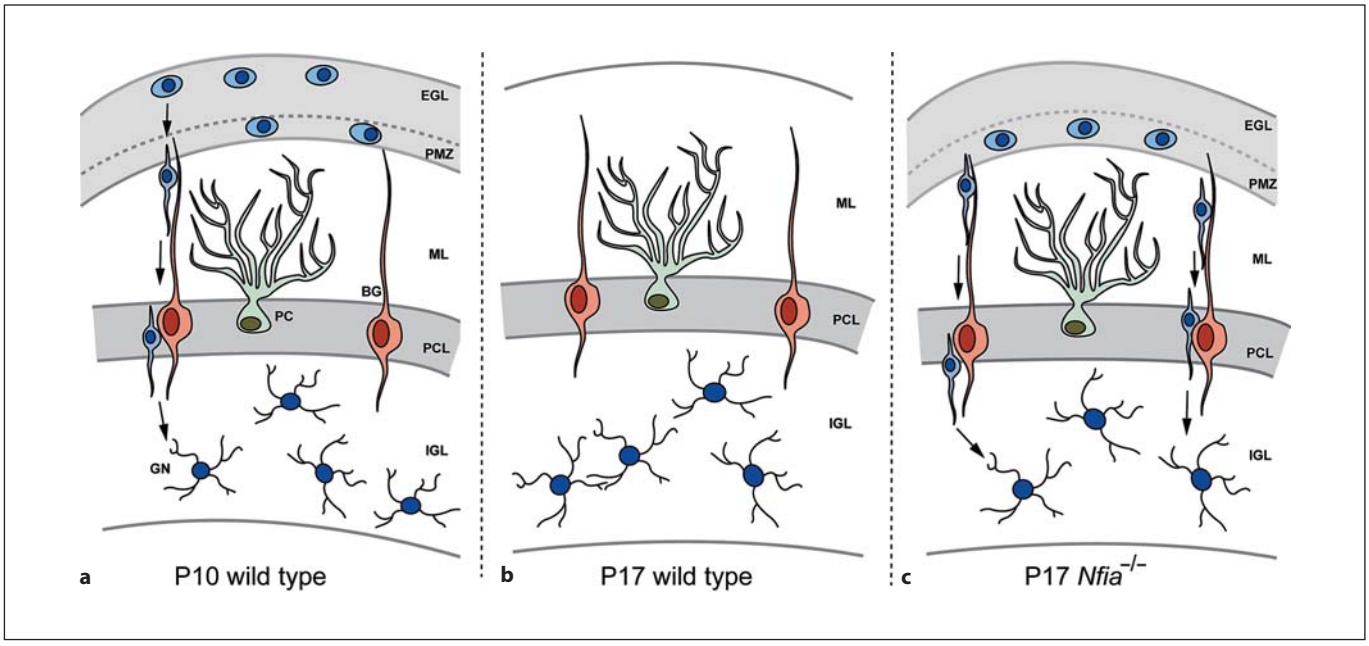

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Fig. 1. Summary of postnatal cerebellar development and of cerebellar defects observed within *Nfia* knockout mice. EGL = External granular layer; PMZ = premigratory zone; ML = molecular layer; PCL = Purkinje cell layer; IGL = internal granule layer; GN = granule neuron; PC = Purkinje cell; BG = Bergmann glia. **a** During postnatal development of the cerebellum, progenitor cells within the external granular layer differentiate into immature cerebellar granule neurons. These immature granule neurons pause within the premigratory zone before migrating through the molecular layer and Purkinje cell layer en route to the internal granule layer. Cerebellar granule cells migrate in close apposition to Bergmann glia. The schematic represents cerebellar development in wild-type mice at postnatal day 10. **b** By postnatal day 17, progenitor cells in the external granular layer of wild-type mice have differentiated, and this zone has subsequently disappeared. Furthermore, the formation of the cerebellum is essentially complete, with granule cell neurons having completed their migration into the internal granule layer. **c** In *Nfia* knockout mice, however, there are still immature neurons within the premigratory zone, as well as within the molecular layer, suggestive of delayed cerebellar development in the absence of this transcription factor [29].

formed to demonstrate NFI-regulated activity from these promoter regions, these findings nevertheless provide strong support for the notion that NFI proteins contribute to the migration of cerebellar granule neurons via activation of the expression of the cell adhesion molecules N-cadherin and ephrin B1.

More recently, another cell adhesion molecule, transient axonal glycoprotein 1 (Tag1), has been implicated downstream of *Nfi* genes during cerebellar development. *Nfib* knockout mice show a significant reduction in Tag1 expression in the developing cerebellum [49]. Chromatin immunoprecipitation performed with a pan-NFI antibody again demonstrated binding of NFI proteins to the Tag1 promoter, and luciferase experiments demonstrate that both *Nfia* and *Nfib* can activate reporter gene expression under the control of the Tag1 promoter [49]. Taken together, these findings suggest that the NFI family controls the expression of a suite of cell adhesion molecules that are required for the migration of cerebellar granule cells during the postnatal development of the cerebellum. However, many things remain unknown with regard to the role of the *Nfi* genes within the cerebellum. For example, the respective contribution of each of the *Nfi* genes expressed within the cerebellum remains unknown. The use of the dominant negative NFI repressor does not enable the identification of *which* NFI proteins are critical to control neuronal migration. Both *Nfia* and *Nfib* mutant mice exhibit cell-intrinsic defects in cerebellar neuronal migration, but their relative importance is unclear. Furthermore, NFIX is also expressed by cerebellar granule neurons [29], and the development of the cerebellum in the *Nfix* knockout mouse has yet to be investigated. Finally, the migration of cerebellar granule neurons occurs in close apposition with the fibers of the Bergmann glia. Given the importance of *Nfi* genes for gliogenesis, it will be important to study the development of Bergmann glia within *Nfi* knockout mice to determine whether the delayed migration of cerebellar granule neurons also has a cell-extrinsic component.

Nfi Genes and Cell-Extrinsic Regulation of Neuronal Migration

While these studies have demonstrated a role for the *Nfi* genes in coordinating the migration of cerebellar granule neurons in a cell-intrinsic manner, *Nfi* genes have also been shown to regulate neuronal migration via cell-extrinsic mechanisms during development, particularly within the embryonic hippocampus.

Morphological Development of the Hippocampus

Developmentally, the pyramidal neurons and dentate granule neurons that comprise the principal excitatory neurons of the hippocampus are derived from the caudomedial telencephalic ventricular zone (fig. 2). As with many neuronal cells, postmitotic hippocampal neurons migrate from their place of origin during development, a process that is guided by the processes of radial glia and of more mature, GFAP-expressing astrocytic cells [50]. The formation of the dentate gyrus provides one example of this process. In wild-type mice, the migration of dentate granule cells begins at around E14. These cells, derived from the primary dentate neuroepithelium (the area of ventricular zone adjacent to the fimbria), consist of both postmitotic dentate granule neurons and dividing dentate granule cell precursors. Migration from the ventricular zone into the future dentate gyrus occurs along the dentate migratory stream, a process that is not concluded until the end of the first week of postnatal life. Once they reach the dentate gyrus, the dentate granule cells assemble into the lower and upper blades of this structure (fig. 2) [50].

Importantly, mature, GFAP-expressing glia are thought to play a key role in the morphological formation of the dentate gyrus. There are two glial scaffolds within the nascent hippocampal formation, the supragranular glial bundle, which is derived from the ammonic neuroepithelium, and the fimbrial glial bundle, which is derived from the fimbrial glioepithelium. These glia form GFAP-positive fascicles that act as scaffolds for migrating dentate granule cells [50, 51]. The importance of these glial scaffolds in the cell-extrinsic regulation of neuronal migration within the hippocampus is highlighted by the phenotype of *Nfi* knockout mice. Glial development is markedly reduced in the hippocampus of both *Nfia* and *Nfib* knockout mice. In *Nfia* mutants, the development of both the supragranular glial bundle and the fimbrial glial bundle is reduced in comparison to that of wild-type controls [39]. The phenotype of *Nfib* mutants is subtly different, as, although the supragranular glial bundle fails to form, the fimbrial glial bundle appears relatively normal (fig. 2) [28].

Despite this difference, *Nfia* and *Nfib* knockout mice exhibit very similar hippocampal phenotypes, namely the morphological absence of the dentate gyrus [28, 39]. This is thought to be due to the failure of the mature glial bundles to differentiate from ventricular zone-derived progenitor cells. The consequence of this failure of glial differentiation is that dentate granule cells within the forming hippocampus of *Nfi* mutant mice fail to populate the presumptive dentate gyrus. Using the dentate granule cell marker prospero-related homebox 1 (Prox1), granule cells have been shown to migrate only to the farthest extent of the fimbrial glial bundle in both *Nfia* and *Nfib* knockout mice [28, 39]. This suggests that the scaffold provided by the supragranular and fimbrial glial bundles is necessary for the formation of the dentate gyrus, and illustrates the requirement of the *Nfi* genes for the cell-extrinsic control of neuronal migration within the hippocampus (fig. 2). One caveat to these studies is that the *Nfi* genes are also expressed within dentate granule cells themselves [25]. Thus, there is also the potential that the migration defects observed in these mice could also have cell-intrinsic components. Conditional ablation of the *Nfi* genes within the dentate granule cell lineage may provide one way to address the role of the *Nfi* genes in the cell-extrinsic versus cell-intrinsic control of dentate granule cell migration.

Conclusions and Future Directions

The *Nfi* genes have emerged as key molecular regulators of central nervous system development, controlling the development of structures including the spinal cord, cerebellum, pons, hippocampus and neocortex [11]. The roles of this gene family in these developmental processes have also been shown to be diverse, with *Nfi* genes implicated in processes including the generation of astrocyte and oligodendrocyte precursors within the spinal cord [42], driving glial-specific gene expression both in vitro and in vivo [27, 40] and the formation of cortical axon tracts [27, 34]. It is now increasingly evident that *Nfi* genes can also contribute to the process of neuronal migration through both cell-extrinsic and cell-intrinsic mechanisms, as evidenced by the defects in the formation of the cerebellum [29] and hippocampus [28, 39] in mice lacking these genes.

Whether the *Nfi* genes regulate neuronal migration in other cellular contexts is unknown. This is particularly

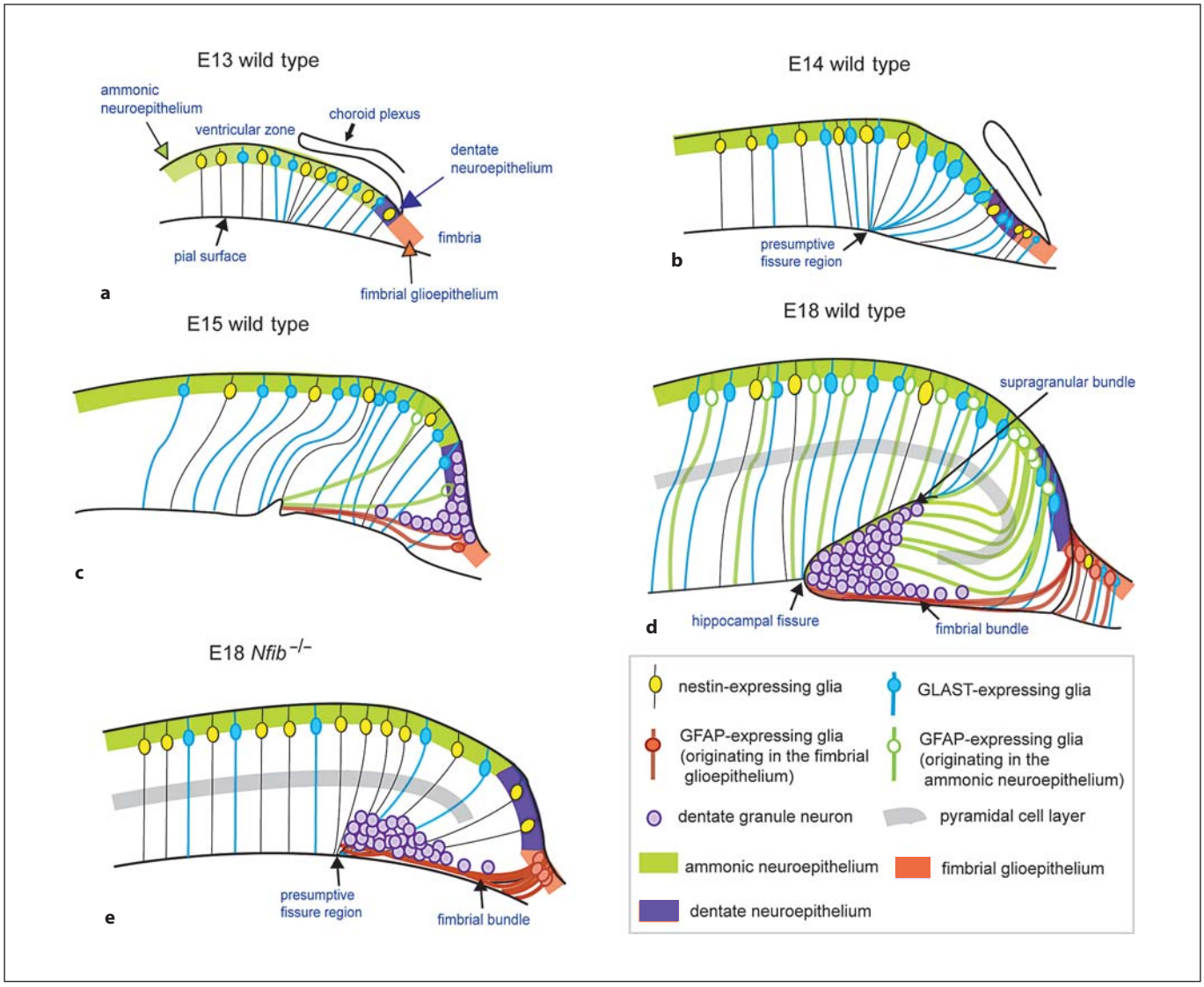

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Fig. 2. The formation of glial scaffolds facilitates neuronal migration within the developing hippocampus. **a** Within the developing hippocampal formation at E13, the ventricular zone comprises 3 distinct areas, the ammonic neuroepithelium, the dentate neuroepithelium and the fimbrial glioepithelium. Both nestin- and GLAST-expressing progenitor cells are found at this time. **b** By E14, the presumptive hippocampal fissure has begun to form at a morphological level. **c** By E15, GFAP-expressing glia arising from the ammonic neuroepithelium and fimbrial glioepithelium are seen. Moreover, dentate granule neurons differentiate from progenitors within the dentate neuroepithelium at this stage. **d** By E18, the hippocampal fissure has formed, and dentate granule neurons have migrated along the supragranular glial bundle and the fimbrial glial bundle to form the blades of the dentate gyrus. **e** In *Nfib*$^{-/-}$ mice, the supragranular glial bundle does not form from progenitor cells within the ammonic neuroepithelium. As such, dentate granule neurons only migrate along the fimbrial glial bundle. These results suggest that dentate granule cell migration, and thus correct morphogenesis of the dentate gyrus, requires both the supragranular glial bundle and the fimbrial glial bundle.

pertinent to the developing neocortex, where neuronal migration plays a central role in the formation of the laminar structure of this region within the adult brain. During development, newly born cortical neurons migrate radially from the ventricular zone into the emerging cortical plate. As each successive wave of neurons is born, they migrate past the previous cohort, such that in the mature brain the earliest born neurons are located in the deep layers, whereas the latest born neurons are found in the superficial layers [8]. This process requires the radial glial fibers to be

intact, as neurons use these as a scaffold for migration into the cortical plate. In mutations where the radial glia are morphologically abnormal, such as in mice lacking the *Pax6* gene, radial neuronal migration occurs abnormally [52]. Whether the *Nfi* mutant mice exhibit defective migration and lamination within the neocortex has yet to be ascertained, but given that the radial glial fibers within the hippocampus of *Nfib* mutant mice have been reported to be morphologically abnormal [28], cortical abnormalities with regard to neuronal migration remain a possibility.

Future studies are also needed to define exactly how the *Nfi* genes interact during development, and to what extent the suite of transcriptional targets downstream of NFIA, NFIB and NFIX overlap. The similarities in the phenotypes of the cerebellum and hippocampus of *Nfia* and *Nfib* knockout mice suggest that these transcription factors regulate the expression of similar genes, a finding supported by the fact that both regulate *Hes1* within the hippocampus [39] and *Tag1* within the cerebellum [49]. Comparative microarray-based experiments performed with knockout tissue from each of the *Nfi* mutants would provide an ideal platform to investigate the genetic hierarchies controlled by these transcription factors. Furthermore, identifying which, if any, proteins interact with NFI proteins to regulate target gene expression would provide additional insights into how these transcription factors control gene expression.

Looking forward, the development of conditional alleles for the *Nfi* genes would provide a powerful tool to investigate the role of these genes in development, postnatally and within the adult brain. This is particularly relevant for studies into neuronal migration, as differentiating between cell-intrinsic and cell-extrinsic effects of these genes is difficult given their broad patterns of expression. For example, conditionally ablating *Nfia* from dentate granule cells would enable the role of this gene in the developing hippocampus to be investigated more rigorously. A similar approach within the cerebellum using conditional expression of Cre recombinase in postmitotic cerebellar granule neurons would allow the evaluation of cell-intrinsic versus cell-extrinsic contributions towards neuronal migration.

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