Mol Neurobiol
DOI 10.1007/s12035-008-8048-6

Nuclear Factor One Transcription Factors in CNS Development

Sharon Mason · Michael Piper · Richard M. Gronostajski · Linda J. Richards

Received: 27 August 2008 / Accepted: 12 November 2008

Abstract Transcription factors are key regulators of central nervous system (CNS) development and brain function. Research in this area has now uncovered a new key player–the nuclear factor one (NFI) gene family. It has been almost a decade since the phenotype of the null mouse mutant for the nuclear factor one A transcription factor was reported. *Nfia* null mice display a striking brain phenotype including agenesis of the corpus callosum and malformation of midline glial populations needed to guide axons of the corpus callosum across the midline of the developing brain. Besides NFIA, there are three other NFI family members in vertebrates: NFIB, NFIC, and NFIX. Since generation of the *Nfia* knockout (KO) mice, KO mice for all other family members have been generated, and defects in one or more organ systems have been identified for all four NFI family members (collectively referred to as NFI here). Like the *Nfia* KO mice, the *Nfib* and *Nfix* KO mice also display a brain phenotype, with the *Nfib* KO forebrain phenotype being remarkably similar to that of *Nfia*. Over the past few years, studies have highlighted NFI as a key payer in a variety of CNS processes including axonal outgrowth and guidance and glial and neuronal cell differentiation. Here, we discuss the importance and role of NFI in these processes in the context of several CNS systems including the neocortex, hippocampus, cerebellum, and spinal cord at both cellular and molecular levels.

Keywords Pax6 · Ngn · Emx · Cerebellum · Spinal cord · Cerebral cortex · Hippocampus · Cellular differentiation

S. Mason · M. Piper · L. J. Richards (✉)
Queensland Brain Institute, The University of Queensland,
Building #79,
St Lucia 4072, Australia
e-mail: richards@uq.edu.au

L. J. Richards
School of Biomedical Sciences, The University of Queensland,
Brisbane 4072, Australia

R. M. Gronostajski
Dept. of Biochemistry, Program in Neurosciences,
Developmental Genomics Group,
Center of Excellence in Bioinformatics and Life Sciences,
State University of New York at Buffalo,
Buffalo, NY, USA

Published online: 05 December 2008

Introduction

Nuclear factor one (NFI) was first described as a host-encoded protein required for the initiation of Adenovirus replication in vitro [1]. The NFI proteins are site-specific transcription factors [2] and bind as either hetero- or homodimers [3] to the dyad symmetric consensus sequence TTGGC(N5)GCCAA on double-stranded DNA with high affinity [4, 5]. NFI binding affinity can be modified by sequences adjacent to the consensus sequence and sequences within the degenerate 5-nucleotide spacer region [6, 7]. NFI can also bind to consensus half sites (TTGGC or GCCAA) at a lower affinity [8].

A single NFI gene is present in *Caenorhabditis elegans*, *Drosophila*, and the cephalochordate *Amphioxus* [9–11]. No NFI genes have been detected in single-celled organisms [9]. In the early 1990s, the Sippel laboratory identified four NFI family members, NFIA, NFIB, NFIC, and NFIX, in chicken [12, 13]. Homologs were later described in other vertebrates, from Zebrafish [11] and *Xenopus* [14, 15] to humans [16, 17]. The same four family members have consistently been described in all vertebrates [10], and no additional family members have been found to date.

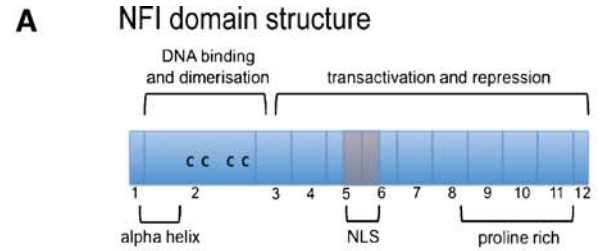
The NFI gene family controls the transcription of a variety of developmentally regulated genes in multiple organ systems. Here, we concentrate on the role of NFI in

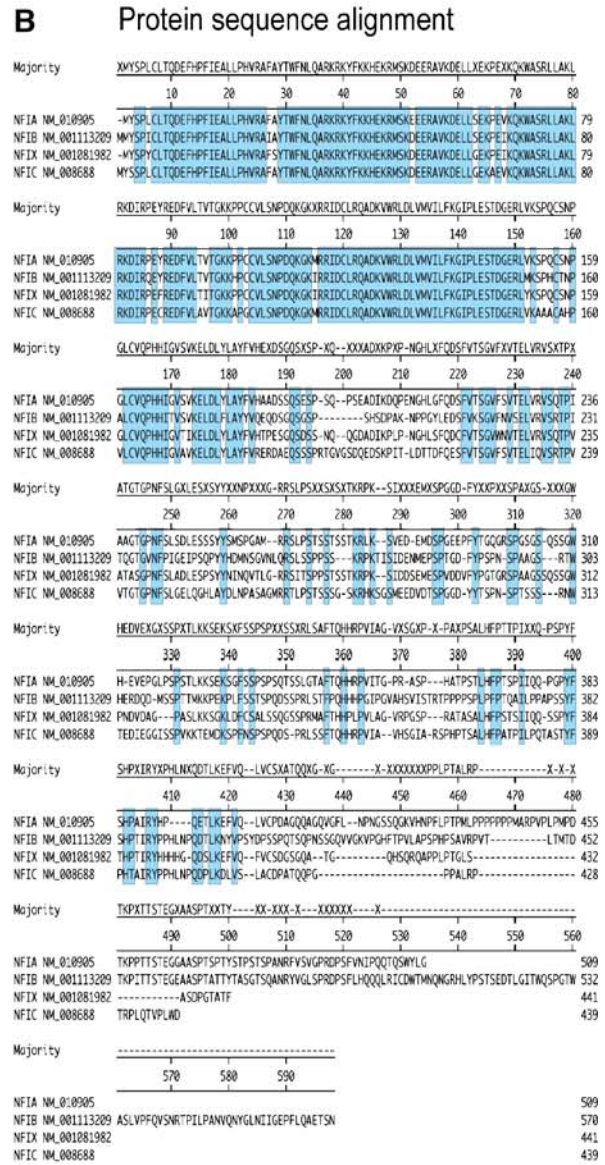
central nervous system development at both cellular and molecular levels.

Nuclear Factor One Transcription Factor Gene Structure (Including Splice Variants)

Through analysis of exon structure, it has been suggested the four vertebrate NFI genes have arisen from gene duplication of a *C. elegans*-like ancestral gene during chordate evolution [9]. The functional relevance of this is unknown, although clearly there is a correlation between NFI diversity and organism complexity.

Each of the four NFI transcription factors in vertebrates has an N-terminal DNA-binding and dimerization domain and a C-terminal transcription modulation domain involved in repression and activation of transcription [10, 18]. The ~200–220 amino acid N-terminal DNA binding domain is highly conserved between the four family members [9]. The N-terminal domain contains four conserved cysteine residues, three of which are essential for DNA binding [19]. The other cysteine residue is involved in redox control and is responsible for NFI proteins being sensitive to oxidative inactivation [19, 20]. The C-terminal activation/repression domain is proline rich, and sequence homology between the four NFI genes (Fig. 1) is not as highly conserved in this domain [16, 17, 21]. Figure 1 gives an overview of NFI domain structure and shows an alignment of the protein sequences of the different NFI family members in mouse and





C Protein sequence homologies

NFIA	+	+	-	+	-	-
NFIB	+	-	+	-	+	-
NFIX	-	+	+	-	-	+
NFIC	-	-	-	+	+	+
Percent Homology						
Full Length	61.9	73.2	60.9	66	65	61.2
N-terminus	87.4	89	85	85	80.7	80.8
C-terminus	45.5	60.4	39.2	52.1	50.5	42.9

Fig. 1 NFI gene structure and protein sequence alignment. **a** NFI domain structure in vertebrates. Exons are numbered *1–12*. Full-length *Nfib* transcripts contain 12 exons, while full length *Nfia*, *Nfix*, and *Nfic* transcripts contain 11 exons. The N-terminal region contains a DNA binding and dimerization domain (*bracketed and labeled DNA binding and dimerization*) and is largely encoded by exon 2. This domain contains four conserved cysteine residues (*labeled C*) which are required for DNA binding and redox control. There is also a basic alpha helical domain (*bracketed and labeled alpha helix*) at the start of exon 2. The C-terminal transactivation and repression domain (*labeled transactivation and repression*) is encoded from exon 3 onwards. The deduced nuclear localization signals are labeled *NLS* and are at the border of exon 5 and exon 6 (*coloured gray*). The proline-rich region of the activation/repression domain is bracketed. **b** Homology of the NFI family members in mouse. The predicted amino acid sequences of full length transcripts of the NFIA, NFIB, NFIX, and NFIC family members were aligned using Clustal W (MegAlign). The PubMed accession numbers of the messenger RNA transcripts for NFIA, NFIB, NFIX, and NFIC used in this alignment are NM_010905, NM_001113209, NM_001081982, and NM_008688, respectively. Conserved amino acids between all four family members are shaded blue. NFIB has an extended C-terminus of ~60 amino acids. **c** Percent homologies of all family members in mouse. Analysis was performed with the aid of Clustal W, with the N-terminus arbitrarily taken as the first 210 amino acids and the C-terminus taken as the remainder of the predicted polypeptide. NFIA and NFIX are the most homologous, with an overall homology of ~73%

a comparison of the homologies between them. Activation or repression of transcription by NFI depends on both the promoter type [21] and the cellular context [22].

Additionally, variation in the NFI gene transcripts is achieved by the formation of multiple splice variants for each gene. For each of the four NFI family members, the largest messenger RNA (mRNA) transcript contains 11–12 exons that can be alternatively spliced through multiple mechanisms, including alternative splicing of exons in the 3′ region, resulting in a modified repression/activation domain. Up to nine different splice variants have been described per gene [10, 15, 16, 23]. Individual splice variants of the NFI family members are highly conserved across species as different as birds and mammals and typically display over 90% homology [24, 25]. Different splice variants of the NFI family have been identified in brain, [21, 26] but their functional relevance remains to be determined.

One study has demonstrated that all four NFI proteins (in chicken) can effectively form homo- and heterodimers in vitro [3]. Different dimer combinations display similar or identical DNA-binding affinity, specificity, and stability [3]. This is not surprising, given the homology of the DNA binding and dimerization domains between the different NFI family members. In vitro studies have shown that the formation of NFI homodimers or heterodimers requires cotranslation of NFI proteins, and heterodimers cannot be formed from mixing preformed homodimers [3, 18, 27]. The occurrence and abundance of different heterodimers and homodimers within a cellular system or in vivo has not been studied, and the functional relevance of different dimer combinations is also unknown. Finally, whether the formation of homo- or heterodimers also plays a role in the switch between activation or repression of transcription remains to be determined.

Posttranslational modifications of NFI proteins through either glycosylation or phosphorylation have been reported. *N*-glycosolation of an NFIC isoform has been reported during early mouse mammary gland involution in vivo [28], and O-linked glycosylation of an NFIB isoform in the JEG3 (human placental choriocarcinoma cell line) has also been described in vitro [29]. The functional outcomes of glycosylation of NFI proteins remain to be determined. Phosphorylation of various NFI isoforms have been reported in a few studies in vitro. Cell division cycle 2 (cdc2) kinase, a cell cycle control gene, has been demonstrated to phosphorylate NFI using an in vitro phosphorylation assay [30]. Differential phosphorylation states of NFI have been reported in brain lipid binding protein (BLBP) positive glioblastoma cell lines compared to BLBP negative glioblastoma cell lines. This study demonstrated that NFI is essential for the activation of the BLBP promoter, and proposed BLBP positive cell lines may contain a phosphatase activity required for NFI to activate the BLBP promoter [31]. Another study demonstrated that, in mammary epithelial cells, the amount of an active NFIC isoform was controlled by tyrosine phosphorylation by Jak2 (Janus kinase 2) [32].

Overall, the potential mechanisms of NFI transcriptional regulation in vertebrates including the promoter type of interest, NFI dimerization (homo- or heterodimers), variations within NFI consensus binding, splice variants of all four NFI family members, and posttranslational modifications of NFI proteins suggests that NFI transcriptional control during development may be highly complex.

The Expression and Role of NFI in the Development of the Central Nervous System

Using in situ hybridizaton, Chaudhry et al. [21] demonstrated that *Nfia*, *Nfib*, and *Nfix* have both distinct and overlapping mRNA expression patterns in the developing and postnatal mouse brain. This finding suggested the NFI transcription factor gene family may have an important role in brain development.

Subsequently, it was shown that the knockout (KO) mice for the NFIA, NFIB, and NFIX family members displayed interesting brain phenotypes [33–37]. All phenotypes discussed here are present on a stable C57BL/6J background unless stated otherwise. The stable backcross allows the phenotypes of the KO mice of the different family members to be compared directly. Both the *Nfia* and *Nfib* mutants display agenesis of the corpus callosum (ACC) and enlarged lateral ventricles [34, 36, 37]. *Nfia* KO mice exhibit perinatal lethality, probably caused by defects in renal development [38] and the *Nfib* KO mice also die at birth due to lung hypoplasia [37]. The generation of conditional mutants will enable analysis of potential postnatal roles of *Nfi* in mouse brain.

Nfix KO mice have a normal lifespan only if they are fed on a soft food diet and may have defects in the digestive system [33]. Postnatal analysis (P0 to P69) of the *Nfix* KO mice (C57BL/6J background) has revealed that, unlike the *Nfia* and *Nfib* KO mice, the *Nfix* mutants do not display ACC [33]. However, like the *Nfia* and *Nfib* KO mice, there is enlargement of the lateral ventricles of the brain [33]. Whether there are any defects in the development of the corpus callosum in the *Nfix* KO mice throughout embryogenesis remains to be determined.

Consistent with the relatively low level of expression of *Nfic* in the developing telencephalon, [21] the *Nfic* KO mice do not appear to have aberrant brain development. Disruption of this gene in mouse causes abnormal molar root formation and severe incisor defects in postnatal

development [39] due to a disruption in odontoblast differentiation [40]. If the *Nfic* KO mice are fed soft food, they have a normal life span [39].

In the peripheral nervous system, *Nfia* and *Nfic* are embryonically expressed in the dorsal root ganglia and cranial ganglia [21]. Expression of NFI genes in the central nervous system (CNS) is widespread throughout the axis of the CNS. The following sections will detail the expression and function of NFI in areas of the CNS from the cerebral cortex to the spinal cord.

NFI cell type expression in the telencephalon Dissociated primary cell cultures from the neocortex have demonstrated NFIA and NFIB are present in both neurons and glia [41]. Interestingly, NFIA and NFIB are not expressed by GABAergic interneurons throughout the telencephalon [41]. However, NFIA and NFIB co-localize with NeuN (a neuronal nuclear marker) in the cortical plate, suggesting that they may be expressed in pyramidal neurons [41]. Supporting this, further analysis using retrograde tracing from the pyramidal decussation in early postnatal development (P2-P3) has revealed NFIA and NFIB are expressed in the majority of corticospinal projection neurons [41]. Retrograde tracing from the cortex revealed that the projecting neurons of the corpus callosum do not express NFIB, and only a small subpopulation of these neurons express NFIA [41]. This suggests that ACC in the *Nfia* and *Nfib* mutants may occur thorough non-cell-autonomous mechanisms, such as regulating the development of midline glial populations. Such populations are required for corpus callosum formation and highly express NFIA, NFIB, and NFIX during their development [33, 41]. The expression of NFIX in specific neuronal populations of the cortex is currently not known. The role of the NFI transcription factors is beginning to be delineated in a variety of systems within the CNS, including the proliferative zones, neocortex, midline of the developing telencephalon, hippocampus, cerebellum, pons, and spinal cord.

Expression and role of NFI in the development of proliferative zones NFIX can be detected as early as embryonic day 11 (E11) in the ventricular zone (VZ), a proliferative zone lining the lateral ventricles where precursor cells in the developing telencephalon are generated [33]. By E12, NFIA, NFIB and NFI-X are expressed in the roof plate [33, 41]. NFIA and NFIB are expressed in the VZ and subventricular zone (SVZ) by E15 and E16, respectively [41]. In postnatal development, NFIA, NFIB, and NFIX positive cells also line the ventricles, and in the adult, both NFIA and NFIB are expressed in the SVZ [33, 41]. Table 1 gives an overview of NFI expression throughout development.

Interestingly, in the *Nfia* KO, quantitative real-time polymerase chain reaction (qRT-PCR) from E18.5 whole brain tissue demonstrated that transcript levels of *Tbr2* are increased 1.9-fold compared to wild-type (WT) controls [37]. Tbr2 is a marker of intermediate progenitor cells (progenitors that reside in the SVZ and predominantly give rise to upper layer neurons), implying NFIA may play a role in the differentiation of intermediate progenitors into neurons. Further studies are needed to confirm this. Postnatal analysis of *Nfix* KO mice has shown defects in progenitor cell proliferation or migration [33]. In *Nfix* KO mice, there is an increase in Pax6-positive and doublecortin-positive cells at the surface of the lateral ventricles of the brain at all postnatal ages analyzed [33]. Pax6 is a marker of progenitor cells, and doublecortin is expressed in migrating neurons. Thus, there may be a population of aberrant progenitor cells that are present around the VZ of the *Nfix* KO mice. An alternative hypothesis is that the cells may represent rostral migratory neurons, which fail to migrate to the rostral migratory stream [33].

Expression and role of NFI in the development of the neocortex NFIX can be detected as early as E11 in the preplate (the preplate constitutes the first postmitotic cells of the cortical plate) [33]. By E13, NFIA and NFIB are highly expressed in the preplate [41]. At E15, NFIA, NFIB, and NFIX are expressed in the deep layers of the cortex, the subplate, and the marginal zone only and not throughout the cortical plate [33, 41]. At E17, NFIX is expressed throughout the cortical plate [33], whereas NFIA and NFIB expression is most prominent in the subplate, marginal zone, and deep layers of the cortex [41]. Importantly, during embryogenesis, NFIA and NFIB do not show a graded expression pattern through the rostrocaudal axis, indicating that these transcription factors may not be involved in patterning and arealization of the cortex [41]. The expression of NFIX through the rostrocaudal axis remains to be determined. Postnatally, the cortex demonstrates a dynamic pattern of NFIA, NFIB, and NFIX expression [33, 41]. Given the defects in cerebellar granule neurons (CGNs) in the *Nfi* mutant mice, [42] it is of interest to determine if the postmitotic preplate cells of the neocortex in the *Nfia*- and *Nfib*-deficient mice also demonstrate cell-autonomous defects in differentiation or migration within the cortical plate.

Expression and role of NFI in the development of the telencephalic midline At the midline of the dorsal telencephalon, NFIA, NFIB, and NFIX are expressed in the cingulate cortex by E15. At this stage, NFIA is expressed in the midline glial populations (glial wedge and indusium griseum glia), and the subcallosal sling and NFIB is expressed in the glial wedge [33, 36, 41]. By E18, NFIA, NFIB, and NFIX are expressed in overlapping patterns in the midline glial populations and subcallosal sling [33, 41].

Table 1 Spatial and temporal expression of nuclear factor one in mouse central nervous system

	E11	E12	E13	E15	E17	E18	P0	P5	P6	P7	P14	Adult
Proliferative/Patterning zones												
Roofplate	–	A*/B*/X*	–	–	–	–	–	–	–	–	–	–
Ventricular zone	$A^{ND}/B^{ND}/X$*	A#/B#/X*	A#/B#/X^{ND}	A*/B#/X^{ND}	A*/B*/X^{ND}	A*/B*/X^{ND}	A*/B*/X^{ND}	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	A*/B*/X*	A*/B*/X*	$A^{ND}/B^{ND}/X^{ND}$
Subventricular zone	$A^{ND}/B^{ND}/X^{ND}$	A#/B#/X^{ND}	A#/B#/X^{ND}	A*/B#/X^{ND}	A*/B*/X^{ND}	A*/B*/X^{ND}	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	A*/B*/X^{ND}
Cortex												
Preplate	$A^{ND}/B^{ND}/X$*	$A^{ND}/B^{ND}/X$*	A*/B*/X*	–	–	–	–	–	–	–	–	–
Marginal zone	–	–	–	A*/B*/X*	A*/B*/X*	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$
Subplate	–	–	–	A*/B*/X*	A*/B*/X*	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$
Cortical plate												
II–III	–	–	–	A*/B*X#	A*/B*/X*	$A^{ND}/B^{ND}/X^{ND}$	A*/B#/X*	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	A*/B#/X*	A#/B#/X*	A#/B#/X#
IV	–	–	–	A*/B*/X*	A*B*/X*	$A^{ND}/B^{ND}/X^{ND}$	A*/B#/X*	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	A*/B#/X*	A#/B#/X#	A#/B#/X#
V	–	–	–	A*/B*/X*	A*B*/X*	$A^{ND}/B^{ND}/X^{ND}$	A*/B#/X*	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	A*/B*/X*	A#/B#/X#	A#/B#/X#
VI	–	–	–	A*/B*/X*	A*/B*/X*	$A^{ND}/B^{ND}/X^{ND}$	A*/B*/X*	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	A*/B*/X*	A*/B*/X#	A#/B#/X#
Piriform	–	–	$A^{ND}/B^{ND}/X$*	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	A*/B*/X^{ND}	A*/B#/X^{ND}
Midline of cerebral cortex												
Glial wedge	–	–	–	A*/B*/X#	A*/B*/X*	A*/B*/X*	A*/B*/X*	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	A*/B*/X^{ND}	A*/B*/X^{ND}	A#/B#/X^{ND}
Indusium griseum glia	–	–	–	A*/B#/X#	A*/B*/X*	A*/B*/X*	A*/B*/X*	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	A*/B*/X#	A*/B*/X#	A*/B*/X*
Subcallosal sling	–	–	–	A*/B#/X#	A*/B*/X#	A*/B*/X*	A*/B*/X*	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	A*/B*/X^{ND}	A*/B*/X^{ND}	–
Cingulate cortex	–	–	–	A*/B*/X*	A*/B*/X#	A*/B*/X*	$A^{ND}/B^{ND}/X$*	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X$*	$A^{ND}/B^{ND}/X$*	A#/B#/X^{ND}
Hippocampal system												
Hippocampal primordium	–	–	A*/B*/X*	A*/B*/X*	A*/B*/X*	$A^{ND}/B^{ND}/X^{ND}$	–	–	–	–	–	–
CA1	–	–	–	A*/B*/X*	A*/B*/X*	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	A*/B#/X*	$A^{ND}/B^{ND}/X^{ND}$	A*/B*/X*	A*/B*/X*	A#/B#/X*
CA2	–	–	–	A*/B*/X*	A*/B*/X*	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	A*/B#/X*	$A^{ND}/B^{ND}/X^{ND}$	A*/B*/X*	A*/B*/X*	A*/B*/X*
CA3	–	–	–	A*/B*/X*	A*/B*/X*	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	A*/B#/X*	$A^{ND}/B^{ND}/X^{ND}$	A*/B*/X*	$A^{ND}/B^{ND}/X$*	A*/B*/X*
Dentate gyrus	–	–	–	–	A*/B*/X*	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	A*/B*/X*	$A^{ND}/B^{ND}/X^{ND}$	A*/B*/X*	A*/B*/X*	A*/B*/X*
Stratum oriens	–	–	–	A*/B*/X*	A*/B*/X*	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	A#/B#/X#	$A^{ND}/B^{ND}/X^{ND}$	A#/B#/X#	A#/B#/X#	A#/B#/X#
Stratum pyramidale	–	–	–	A*/B*/X*	A*/B*/X*	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	A#/B#/X#	$A^{ND}/B^{ND}/X^{ND}$	A#/B#/X*	A#/B#/X*	A#/B#/X#
Stratum radiatum	–	–	–	A*/B*/X*	A#/B#/X#	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	A#/B#/X#	$A^{ND}/B^{ND}/X^{ND}$	A#/B#/X#	A#/B#/X#	A#/B#/X#
Cerebellum												
External germinal layer	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	A*/B*/X*	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$
Molecular layer	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	A*/B*/X*	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$
Internal granule layer	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	A*/B*/X*	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$
Spinal cord												
Ventricular zone	A*/B*/X^{ND}	A*/B*/X^{ND}	$A^{ND}/B^{ND}/X^{ND}$	A*/B*/X^{ND}	$A^{ND}/B^{ND}/X^{ND}$	A*/B*/X^{ND}	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$
Motor neurons	A*/B*/X^{ND}	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$
Astrocytes	–	–	–	–	$A^{ND}/B^{ND}/X^{ND}$	A*/B*/X^{ND}	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$	$A^{ND}/B^{ND}/X^{ND}$

Expression of NFIA, NFIB, and NFIX throughout the central nervous system in mouse development. A dashed line indicates that the structure/cell population is not present at that stage in development. NFIA, NFIB and NFIX are abbreviated to A, B, and X. References: [33, 41, 42, 49]

ND the expression has not been determined, *E* embryonic day, *P* postnatal day, *CA* cornu ammons

* NFI is expressed

NFI is not expressed

In postnatal development, the NFIA and NFIB proteins are still detected at the midline [41].

In *Nfia* and *Nfib* KO mice, the indusium griseum glia and the glial wedge, specific midline glial populations that sit dorsal and ventral to the corpus callosum, respectively, fail to form [36, 37]. In both mutants, these populations were analyzed with different glial markers (glial fibrillary acidic protein (GFAP) and/or brain lipid-binding protein) [36, 37]. In the *Nfia* KO mice, the long, radial like processes of the glial wedge and the indusium griseum glia fail to develop, and the overall number of processes formed is reduced [36]. In the *Nfib* KO mice, the glial wedge is greatly reduced, and there are no detectable indusium griseum glia [37]. These midline glial populations act to guide axons of the corpus callosum across the midline of the developing brain by secreting the diffusible, short-range guidance ligand, Slit2 [43]. Using in situ hybridization, it was shown that *Slit2* expression was reduced in the *Nfia* KO mice [36], implying a non-cell autonomous role of NFIA in ACC, through the failure of the malformed midline glial populations to secrete Slit2. Finally, midline zipper glia, which may play a role in midline fusion, are reduced or absent in the *Nfib* KO mice [37]. Further investigation is required to understand the role of the NFI genes and each family member in the development of these midline glial populations. Overall, the defects in the *Nfia* and *Nfib* mutants at the midline are similar, providing evidence that NFIA and NFIB may coordinate development of these glial populations. Analysis of specific genes using qRT-PCR at E18.5 demonstrated that *Nfib* KO mice have approximately a 2.2-fold increase in *Nfia* transcripts compared to WT controls [37]. This suggests a possible mechanism for compensation by NFIA in the absence of NFIB.

The subcallosal sling is another midline cell population that is mainly made up of neurons that form a sling-like structure just ventral to the corpus callosum [44, 45]. In *Nfia* KO mice, the subcallosal sling neurons migrate aberrantly into the septum and do not form the characteristic sling structure just ventral to the corpus callosum [36].

Expression and role of NFI in development of the hippocampus and dentate gyrus NFIA, NFIB, and NFIX are expressed at E13 when the hippocampal primordium is forming [33, 41]. At E15, distinct layers appear in the hippocampus, and NFIA and NFIX are expressed throughout, including in both the ventricular surface and the differentiating cell layers of the hippocampus, whereas NFIB is highest in the stratum radiatum and is expressed at lower levels in the ventricular zone [33, 41]. At E17, the dentate gyrus is detectable and NFIA, NFIB, and NFIX are highly expressed in this structure [33, 41]. At this stage, expression of NFI in the stratum radiatum has ceased; however, NFIA, NFIB, and NFIX are expressed in the stratum oriens, stratum pyramidale, and ventricular surfaces [33, 41]. Interestingly, NFIA, NFIB, and NFIX label the dentate gyrus throughout development, postnatally, and in the adult [33, 41]. The dentate gyrus is a key site for neurogenesis in the adult. Given the strong expression of NFIA, NFIB, and NFIX in the dentate gyrus, further investigations are required to determine if NFI has a role in stem cell proliferation or migration and neurogenesis in the adult.

In the hippocampus, fimbrial glial processes and glial processes within the dentate gyrus are reduced in the *Nfia* KO mice [36]. The hippocampal commissure fails to form in the *Nfia* mutants [36]. The *Nfib* mutants also display severe disruptions in formation of the hippocampus [37, 46]. The supragranular glial bundle, derived from the ammonic neuroepithelium, normally forms a glial scaffold required for neuronal migration [47, 48]. However the supragranular glial bundle fails to mature from radial progenitors in the *Nfib* KO mice as shown by immunohistochemistry with the astrocyte-specific transporter GLAST (solute carrier family 1 (glial high affinity glutamate transporter) member 3) and tenascin-C, both markers for glial precursors, and GFAP, a marker for mature glia [46]. In WT mice, the supragranular glial bundle can be observed as a fascicle of GFAP-positive processes that attach to the basement membrane to form the hippocampal fissure; however, the hippocampal fissure is absent in the *Nfib* KO mice [46]. This study proposed that the hippocampal fissure is needed for the subsequent formation of the dentate gyrus.

In vitro, dissociated nestin-positive cells of *Nfib* KO hippocampus have an increased number of processes and more branches per process compared to WT cells [46]. No differences are observed in the number of TuJ1 (class III β-tubulin) positive cells between WT and KO cell cultures, indicating that the increased branching is not due to an increase in the number of neurons in the cell cultures from *Nfib* KO mice [46]. In vivo, nestin-positive fibers appear wavier in the *Nfib* KO mice [46]. Together, this suggests the formation of radial glial processes is disrupted. At E18, in the *Nfib* KO mice, more phospho-histone H3 positive cells are located in the VZ, and there are fewer mitotic cells in the dentate migratory stream [46]. These data suggest that the radial progenitors of the ammonic neuroepithelium fail to differentiate and remain in a progenitor state. Fimbrial glia, derived from the fimbrial glioepithelum, appear normal in the *Nfib* KO mice, [46] demonstrating that *Nfib* controls the differentiation of a specific subpopulation of glia derived from the ammonic neuroepithelium and that the maturation of this glial population is necessary for the development of the hippocampal fissure and dentate gyrus.

There are also cell migration defects in the *Nfib* KO hippocampus, with neurons stalling and not reaching their

appropriate locations in the hippocampus. The dentate granule cells in the *Nfib* KO mice fail to populate to the dentate anlage by E18 [46]. The number of dentate granule neurons in the WT and *Nfib* KO hippocampus were quantified via expression of the transcription factor Prospero-related homeobox 1 (Prox1), a marker of differentiated dentate granule cells, and no differences between WT and *Nfib* KO mice were evident, [46] indicating that these defects may be due to migration alone and not due to defects in neuronal proliferation or differentiation. In *Nfib* KO mice, pyramidal neurons of the hippocampus appear to migrate to the hippocampal formation (as determined with immunohistochemistry using the marker Tbr1 (T-box brain 1) at E14) [46]. The different subfields of the cornu ammons (CA) of the hippocampus are specified in the *Nfib* KO as analyzed with the CA1 field marker *SCIP* (*Pou3f1*) and the CA3 field marker *KA1* (*Grik4*) but these regions are smaller in the *Nfib* KO compared to WT mice [46]. Furthermore, lamination of the pyramidal neurons is delayed in the *Nfib* KO mouse by approximately 2 days and is only evident at E18 [46]. Calretinin-positive interneurons migrate to the hippocampal formation in the *Nfib* mutants but are unable to populate the presumptive dentate gyrus [46]. Thus, the defects in *Nfib* KO hippocampal formation, including malformation of the hippocampal fissure and dentate gyrus and aberrant neuronal migration, may be attributed to both the failure of the supragranular glial bundle to mature and to the altered morphology of radial progenitors that normally form the glial scaffold of the hippocampus [46].

Postnatal analysis of *Nfix* KO mice has revealed that the hippocampus is distorted with an enlarged CA1 field and a shorter dentate gyrus [33]. In posterior sections, the appearance of the CA3/CA4 subfields displays a slightly wavy appearance, possibly indicating a change in the cytoarchitecture of the pyramidal cell layer [33]. Given the postnatal defects in the hippocampus of the *Nfix* mutants, it would be of interest to study the embryonic phenotype of these mutants.

Expression and role of NFI in development of the cerebellum and brain stem Early postnatal (P6) expression of the NFI transcription factors in the cerebellum has been investigated [42]. NFIA, NFIB, and NFIX are first up-regulated in the cell nuclei of cerebellar granule neurons as they become postmitotic in the premigratory zone of the external germinal layer [42]. This expression persists as the granule neurons migrate radially through the molecular layer (ML) and subsequently undergo final maturation within the internal granule cell layer (IGL) of the cerebellum [42]. Consistent with this expression analysis, this study has shown that NFI has a role in the postmitotic development of CGNs [42]. Using an *Nfi* dominant repressor, this study found that NFI influences axon formation, dendritogenesis, and migratory behavior in the cerebellum [42].

Specifically, axonal outgrowth was impaired in CGN reaggregrates but not in dissociated CGN cultures, suggesting NFI regulates axon outgrowth only under conditions of homotypic cell contact [42]. In dissociated CGNs, the study demonstrated that NFI is needed for dendrite formation, (which is a late differentiation event occurring in the IGL). CGN cell migration defects were demonstrated in reaggregates and slice culture. The migration defect is cell intrinsic as transwell assays demonstrated that CGNs treated with the *Nfi* dominant repressor had impaired migration. Finally, analysis of postnatal *Nfia* KO mice on a C57BL/6J/Ntac background revealed that parallel fibers within the ML were either not extended or were misorientated, and dendrite formation was dramatically impaired. In the *Nfia* KO mice, although many CGNs reached the IGL, numerous CGNs were still evident in the ML, indicating there is a migration defect in the mutants. The *Nfib* KO mice were on a C57BL/6J/129S background and displayed perinatal lethality, so postnatal analysis could not be performed. Previously, cerebellar foliation defects were described in *Nfib* KO embryos [37]. In the recent study discussed above, it was also shown axon formation may be altered in the early cerebellum of the *Nfib* KO embryo due to a reduction in phospho-neurofilament staining [42].

Nfia, *Nfib*, and *Nfix* are expressed in the basilar pons during embryogenesis [21]. The basilar pons is a hindbrain structure that supplies a major mossy fiber input into the cerebellum. Loss of *Nfib* results in major defects in basilar pons formation that are not evident in the *Nfia* KO mice. At E18.5, the basilar pons is virtually absent in the *Nfib* mutants, with only a small cap of cells remaining [37].

Expression and role of NFI in spinal cord development NFIA and NFIB are highly expressed in the ventricular zone of the spinal cord at the onset of gliogenesis (E5–E6 in chick and E11.5 in mouse) [49]. Electroporation of *Nfia* or *Nfib* over-expression or knock-down constructs in the embryonic chick in vivo have revealed that NFIA and NFIB are needed for glial fate specification in the developing spinal cord [49]. Over-expression of either *Nfia* or *Nfib* leads to an increase in *GLAST* (a glial precursor marker) expression in the VZ from E4 to E5 in chick. From E6 to E7, over-expression of *Nfia* or *Nfib* also causes precocious migration of *GLAST*-positive cells from the VZ [49]. Consistent with these data, siRNA knock-down of *Nfia* causes a loss of glial progenitor markers in the VZ [49]. From E5, the expression of *GLAST*, Olig2 (oligodendrocyte transcription factor 2) and *FGFR3* (fibroblast growth factor receptor 3) is blocked and at E6, (at the onset of NFIB

expression in chick), the up-regulation of *Nfib* is prevented [49]. These data suggest that NFIA is needed for the expression of NFIB, and both NFIA and NFIB family members are needed to up-regulate glial specific markers at the onset of gliogenesis in the developing spinal cord.

Additionally, NFIA is needed to maintain the cells in a progenitor state during the period of neurogenesis to provide precursors for gliogenesis at a later time in embryogenesis by inducing the expression of Hes5 (hairy and enhancer of split 5) an effector in the Notch pathway [49]. This was demonstrated by electroporating siRNA knock-down constructs of *Nfia* at E5 in chick embryos which resulted in the loss of multiple progenitor markers in the VZ, including Pax6, Olig2, and Sox9 (SRY-box containing gene 9) [49]. *Nfia* knock-down with siRNA also leads to the down-regulation of *Hes5* [49]. Conversely, over-expression of *Hes5* and *Notch* can rescue the loss in progenitor cell markers (Sox9 and Olig2) caused by the loss of *Nfia* [49]. However, *Nfia* and *Nfib* are not sufficient to inhibit neurogenesis, in contrast to the Notch effectors, as over-expression of *Nfia* or *Nfib* from E4-E7 in chick in vivo did not alter the expression of Neurogenin2 or NeuN, markers for neuron fate specification and neurons, respectively [49].

Later in spinal cord development, NFIA and NFIB also control astrocyte differentiation [49]. At E18.5 in the mouse spinal cord, NFIA and NFIB colocalize with the astrocyte markers GFAP and s100β [49]. By comparing *Nfia* or *Nfib* KO mice to WT, it was shown that GFAP-expressing cells in the spinal cord are greatly reduced at both E15.5 and E18.5 in the KO mice. Additionally, over-expression of *Nfia* or *Nfib* in the chick spinal cord in vivo accelerates GFAP expression by about 3 days [49]. Co-electroporation of *Olig2* with *Nfia* or *Nfib* constructs inhibits this expression [49]. Also, co-expression of *Olig2* with an excess amount of *Nfia* or *Nfib* rescued the loss of GFAP caused by the over-expression of *Olig2* alone, demonstrating that the antagonistic effect of Olig2 can be prevented by elevating the levels of NFIA or NFIB. This implies the relative levels of NFIA, NFIB, and Olig2 are important determinants of astrocytic fate [49]. Olig2 may inhibit NFIA through protein–protein interactions, as it was shown that Olig2 and NFIA co-immunoprecipitate in human embryonic kidney (HEK)293 cells [49]. Hence, paradoxically, Olig2 antagonises the NFIA and NFIB family members' ability to promote astrocyte differentiation, yet at the onset of gliogenesis, NFIA is needed for the expression of Olig2. This seminal study demonstrated that the NFI transcription factors control glial fate specification and at a later time in development, NFIA and NFIB control astrocytogenesis and are inhibited in cells of the oligodendrocyte lineage. Figure 2 gives an overview of the role of NFIA and NFIB in gliogenesis in the chick spinal cord.

Potential NFI Signaling Pathways

Up-stream NFI signaling pathways *Nfi* family members have been identified as significantly misregulated in a number of screens using mutant mice, including mice deficient in the patterning genes, *Pax6* (paired box gene 6) or *Emx2* (empty spiracles homolog 2). Microarray analysis of WT and homozygous *Pax6 Small-eye* (*Sey*/*Sey*) (Sey is the result of a point mutation in the *Pax6* gene) cortical tissue showed that *Nfia*, *Nfib*, and *Nfix* are down-regulated in the KO mice compared to WT at embryonic day 12. *Nfia* expression was validated using quantitative real-time PCR at E12 and E15. Finally, over-expression of *Pax6* in slice cultures increased *Nfia* mRNA levels compared to control tissue [50]. This suggests that Pax6 may up-regulate NFIA, NFIB, and NFIX during corticogenesis. Interestingly, Pax6 is needed for the proper differentiation of cortical radial glia [51]. Given the defects in the cortical midline glia and glial populations in the hippocampus of the *Nfia* and *Nfib* KO mice [36, 37], it is possible NFI may be down-stream of Pax6 in signaling pathways involved in the differentiation of radial glia. The transcriptional profiles of neurospheres dissected from the rostral periventricular region of E18.5 embryos in WT vs *Emx2* KO brains have also been analyzed using microarrays. In this study, *Nfia* was significantly up-regulated, [52] suggesting Emx2 or Emx2-controlled pathways may be up-stream of NFIA. Emx2 is needed to maintain cells in a progenitor state [53] as in *Emx2* KO mice, progenitors are quickly depleted [54]. Thus, in normal development, Emx2 may repress NFIA and inhibit the premature onset of differentiation of precursors in the developing telencephalon.

A subtractive hybridisation screen in *Neurogenin* (*Ngn*) *1* and *Ngn2* KO mice compared to WT revealed that, in *Ngn2* and *Ngn1;Ngn2* mutants, *Nfib* expression is reduced in the postmitotic preplate neurons of the neocortex at E13.5 [55]. The neurogenins are basic helix–loop–helix transcription factors that regulate neuronal specification in the developing cortex [56]. These data indicate that *Nfib* may be a down-stream effector of neurogenin in cells of the preplate.

Down-stream NFI signaling pathways Down-stream effectors of the NFI family members during development have not been widely investigated to date. A recent study has used microarrays to assess transcript levels in whole brains of WT and *Nfia* KO mice at E18 and postnatal day 16 (P16) [57]. All animals were F1 hybrids of C57BL/6J and 129S6. On this hybrid genetic background, 38.5% of offspring survive until P30, unlike *Nfia* and *Nfib* KO mice on a C57BL/6J background, which die at birth. Genes found to be significantly (at least 1.2-fold) misregulated in the microarray analysis were involved in a variety of cell functions and, of most importance to brain development, oligodendrocyte

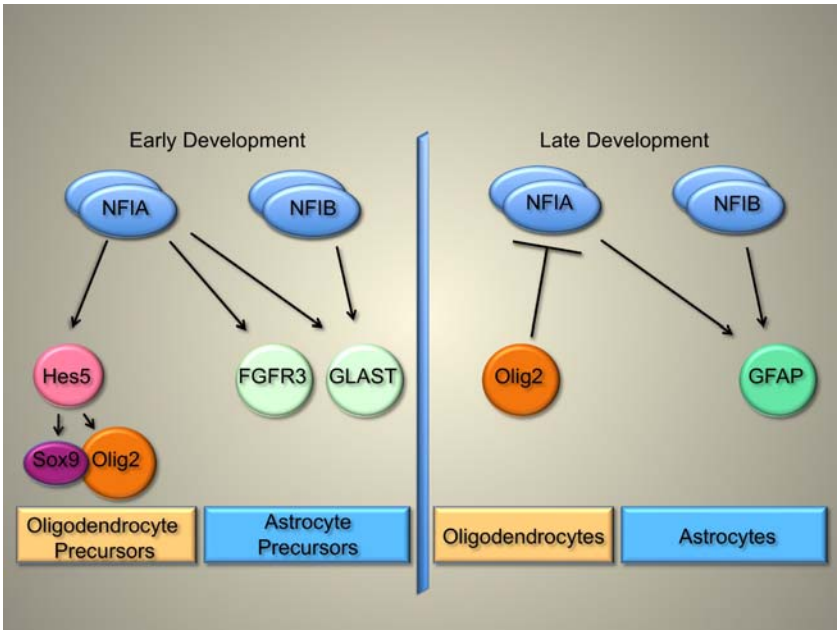


Fig. 2 Summary of the role of NFIA and NFIB in controlling gliogenesis in the chick spinal cord. The *left panel* gives an overview of the role of NFIA and NFIB in gliogenesis in chick between E5 and E7. At this stage, NFIA and NFIB are needed for the generation of glial precursors. The *right panel* demonstrates the role of NFIA and NFIB at a later time point in development; here, NFIA and NFIB are needed for the formation of astrocytes (as determined using the astrocyte marker GFAP). Paradoxically, in early development (*left panel*), NFIA controls expression of Olig2 through Hes5, yet later in development (*right panel*), Olig2 opposes the role of NFIA and NFIB in astrocytogenesis, possibly through protein–protein interactions. *E* embryonic day, *Olig2* oligodendrocyte transcription factor 2, *FGFR3* fibroblast growth factor receptor 3, *Sox9* SRY-box containing gene 9, *Hes5* hairy and enhancer of split 5, *GLAST* solute carrier family 1 (glial high affinity glutamate transporter) member 3, *GFAP* glial fibrillary acidic protein

precursor markers were up-regulated in the KO mice at P16, coinciding with a down-regulation of mature oligodendrocyte markers in the KO mice compared to WT mice. These data, together with the data in the spinal cord [49], suggest NFIA may control oligodendrocyte differentiation.

This study also performed *in silico* promoter analysis on all genes significantly misregulated in the microarray analysis. From the promoter analysis, ~70 out of ~360 misregulated genes were found to possess phylogenetically conserved *Nfi* binding sites up to 2 kilobases (kb) up-stream from the transcription start sites, suggesting that these genes may be direct targets of NFIA. These genes have a variety of functions, including cell adhesion, chromatin and cytoskeletal organization, oligodendrocyte differentiation, ion transport, and lipid metabolism. However, no genes were confirmed as direct targets using transcription factor binding assays.

Direct Targets of the NFI Family Members

Few direct targets of the NFI transcription factors are known at present. Binding sites for NFI were characterized in the promoter regions of neuronal and glial genes in the late 1980s and early 1990s, including the genes encoding neurofilament-M protein [58], myelin basic protein [59], and glial fibrillary acidic protein [60]. GFAP is a marker for astrocyte differentiation and has been demonstrated to be a direct target of NFI using chromatin immunoprecipitation (ChIP) [61]. Importantly, it was found that NFI occupies the *Gfap* promoter prior to the induction of astrocyte differentiation by comparing undifferentiated and differentiated primary cortical precursor cells from embryonic rat tissue [61]. This indicates NFI controls GFAP expression during astrocytogenesis, not only when astrocytes have terminally differentiated.

Electrophoretic-mobility-shift assays [26] have shown that NFI is the major DNA-binding protein interacting with the proximal region of the cerebellar-specific mouse $\alpha 6$ subunit of the γ-aminobutyric acid type A ($GABA_A$) receptor (GABRA6). GABRA6 is expressed in cerebellar granule neurons and is part of an intrinsic program directing their differentiation. In this study, luciferase assays using CGNs demonstrated that *Nfi* regulates *Gabra6* gene transcription. Finally, ChIP confirmed direct binding of NFI to the *Gabra6* promoter in CGNs in culture. More recent studies have shown that NFI binds the promoter of the cell adhesion molecules *N-Cadherin* and *Ephrin-B1* in CGNs again using ChIP analysis [42]. Interestingly, using reaggregate cultures, transwell migration assays and slice cultures in conjunction with inhibitors of *N-Cadherin* and *Ephrin-B1*, this study strongly implicated *Nfi*-mediated

expression of both Ephrin-B1 and N-cadherin to be responsible for proper axonal outgrown, cell migration, and dendrite formation in CGNs. Figure 3 provides an overview of the current literature on the up-stream and down-stream NFI signal transduction pathways in central nervous system development.

Significance of NFI in Human Neurological Conditions and Cancer

We previously reported expression of NFIA in the human fetal brain at 15 weeks gestation. NFIA is expressed in the neocortex, indusium griseum glia, and the subcallosal sling at this stage of development [62]. A recent study has demonstrated NFIA may be the cause of a human neurological disorder occurring in a small percentage of the population [38]. This study described five individuals with *Nfia* haploinsufficiency that presented with a CNS malformation syndrome consisting of a thin, hypoplastic, or absent corpus callosum and hydrocephalus or ventriculomegaly. The majority of the patients also exhibited Chiari type I malformation, tethered spinal cord, and urinary tract defects including vesicouretheral reflux.

The brain phenotype coincides with the *Nfia* KO mouse data that has been previously published. This study also investigated urinary tract defects in the *Nfia* mutants and discovered that, at reduced penetrance, *Nfia* is required in a dosage-sensitive manner for ureteral and renal development [38]. However, other genes besides *Nfia* were also disrupted or deleted and may contribute to the phenotype in these patients [38]. These genes varied widely between the patients, with *Nfia* being the only gene commonly disrupted in all five patients. In one individual, *C20orf32* was the only other gene disrupted besides *Nfia* [38]. Given the expression pattern of *C20orf32* [38], it is unlikely to be a contributing factor in the CNS malformation syndrome. In the two most extreme cases (involving half-siblings), deletions involved either a further 39 or 47 genes, in addition to *Nfia* [38]. Although these deleted genes involved different chromosomes and did not overlap between the two patients, it is possible these deletions may contribute to the CNS malformation syndrome in these patients. The morphology of the hippocampus in these patients is also of interest to study given the malformed glial populations in the hippocampus of the *Nfia* KO mice and complete absence of the hippocampal commissure in these mutants.

In addition to the possibility that NFIA is involved in a human congenital syndrome, NFI has been implicated in a range of other human diseases. The over-expression of *Nfib* through recombination events has been implicated in the myeloproliferative disorder, polycythemia vera, [63] possibly

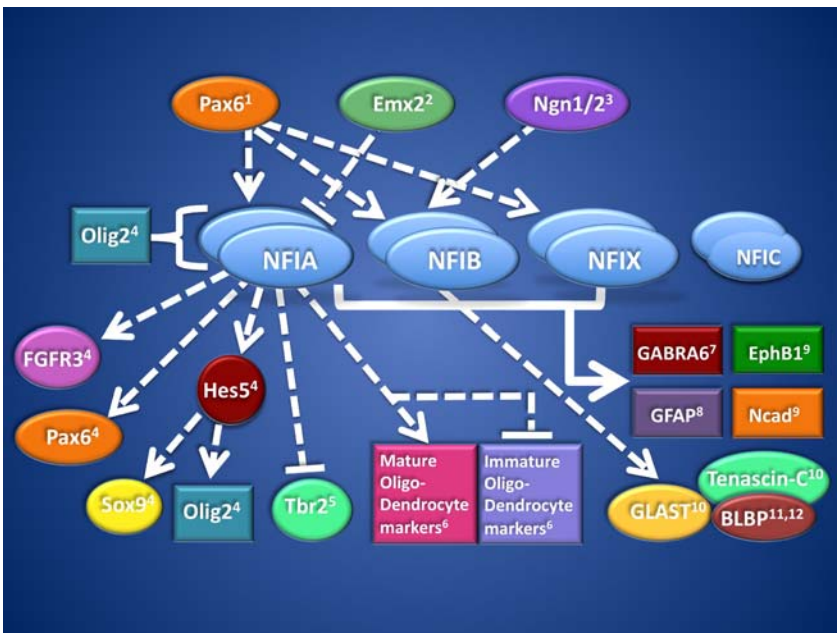


Fig. 3 NFI regulatory pathways in central nervous system development. *Brackets* indicate direct protein–protein interactions. *Dashed lines* represent targets that are currently not known to be direct. *Closed lines* represent NFI target genes that have shown to be direct through transcription factor binding assays. All molecules are linked to a numbered citation *1* [50], *2* [52], *3* [55], *4* [49], *5* [37], *6* [57], *7* [26], *8* [61], *9* [42], *10* [46], *11* [36], *12* [31, 36]. *Pax6* paired box gene 6, *Emx2* empty spiracles homolog 2, *Ngn2* neurogenin 1/2, *Olig2* oligodendrocyte transcription factor 2, *FGFR3* fibroblast growth factor receptor 3, *Sox9* SRY-box containing gene 9, *Hes5* hairy and enhancer of split 5, *Tbr2* T-box brain gene 2, *GLAST* solute carrier family 1 (glial high affinity glutamate transporter) member 3, *BLBP* brain lipid-binding protein, *GABRA6* gamma-aminobutyric acid (GABA) A receptor alpha 6, *EphB1* Ephrin-B1, *GFAP* glial fibrillary acidic protein, *Ncad* N-cadherin

through inducing resistance to transforming growth factor-beta (TGF-β1) which normally inhibits hematopoiesis [63]. *Nfib* has also been implicated in lipoma pathobiology, [64] again through recombination events. Through nonrandom amplification at 9p23-24, *Nfib* is over-expressed in squamous cell carcinoma of the esophagus [65] and triple negative breast cancer (a subdivision of the basal breast cancer group defined as estrogen receptor negative, progesterone receptor negative, and HER2 receptor negative, which results in a poor clinical outcome) [66]. *Nfib* has also been shown to be a translocation partner of high-mobility group (nonhistone chromosomal) protein isoform I-C in pleomorphic adenomas [67]. *Nfia* is up-regulated in neoplastic primary glioblastomas [68], and a role for NFIA in the pathogenesis of glioblastoma warrants further investigation. Overall, NFI genes play important roles in both development and cancer, and misexpression of NFI may be involved in the pathogenesis of a variety of human diseases.

Conclusion

The NFI gene family control a range of key processes in CNS development including axon guidance and outgrowth, glial and neuronal cell differentiation, and neuronal migration in one or more CNS systems. NFI is essential for proper axonal outgrowth and dendrite formation in cerebellar granule neurons in vitro, and there are clear defects in axonal projections and dendrite formation of cerebellar granule neurons in *Nfia* KO mice postnatally [42]. It also appears NFIA and NFIB control non-cell autonomous mechanisms of axon guidance at the midline of the developing brain through inducing the maturation of the midline glial populations, the glial wedge, and the indusium griseum glia, which are needed to guide callosal projection neurons [41]. In *Nfia* and *Nfib* KO mice, these glial populations fail to mature, and the corpus callosum fails to form [36, 37]. The role of NFI in glial differentiation is not restricted to the midline of the developing telencephalon.

NFIA and NFIB control gliogenesis in the embryonic chick spinal cord and, later in spinal cord development, are needed for astrocytogenesis [49]. In the postnatal mouse brain, a gene expression screen indicated that NFIA is needed for the development of oligodendrocytes in the brain [57]. Defects in glial development and differentiation are also present in the *Nfia* and *Nfib* KO hippocampus [36, 37]. Specifically, in the *Nfib* mutant, the supragranular glial bundle fails to form because radial glial progenitors do not differentiate [46]. As a result, the glial scaffold of the hippocampus does not form, and this may in turn cause malformation of the dentate gyrus and hippocamal fissure and failure of the dentate granule neurons to migrate to the dentate anlage [46].

NFI is needed for the cell intrinsic control of cerebellar granule neuron migration, as determined using an in vitro assay [42]. Migration of cerebellar granule neurons is also impaired in *Nfia* KO mice [42]. In *Nfia* KO mice, the subcallosal sling cells migrate aberrantly into the septum and fail to reach the midline [36]; whether this occurs through cell autonomous mechanisms remains to be determined.

Overall, the generation of null mutants for each of the NFI transcription factors, NFIA, NFIB, NFIC, and NFIX, has greatly accelerated our understanding of the role of the NFI family members in CNS development. Further detailed expression studies and phenotypic analyses in the *Nfi* KO mice at the cellular level are needed. Furthermore, additional molecular approaches such as whole transcriptome profiling and chromatin immunoprecipitation to delineate the down-stream targets of the NFI transcription factors, that may account for the phenotype of the *Nfi* KO mice, are also required. The generation of conditional mutants will also allow NFI to be deleted in a spatially and temporally dependent manner and will therefore aid in the study of specific brain regions and cell populations at both the cellular and molecular level. Furthermore, defining how the different NFI family members coordinate the development of specific brain regions and specific cell populations within these regions will also need to be addressed. It is currently not known if different NFI family members are expressed in the same cells. All antibodies to date have been made in rabbit, making this a difficult question to address; thus, monoclonal antibodies are needed. Double and triple crosses of the different NFI mutants may be used to address the interplay between the different family members and may give more insight into the role of individual NFI family members. All of these approaches may assist to further define the role of NFI in CNS development. Possible compensation and redundancy between the different NFI family members is important to address, given the similarities in the KO phenotype of the *Nfia* and *Nfib* family members. Finally, the role of individual splice variants is of interest because it is likely that NFI splice variants add to the complexity of NFI transcriptional control during development. The NFI genes have provided important insights into the development of the nervous system. A further 10 years of work will reveal their complex interactions with other developmentally expressed genes and will define their precise role in nervous system development and function.

Acknowledgments We thank Dr Guy Barry and Ms Erica Little for comments on the manuscript. This work was funded by a National Health and Medical Research Council (NHMRC) project grant (LJR) and a Clive and Vera Ramaciotti grant (MP). LJR is supported by a NHMRC Senior Research Fellowship, SM is supported by a

University of Queensland F.G Meade PhD Scholarship, and MP is supported by a NHMRC Howard Florey Centenary Fellowship.

References

1. Nagata K, Guggenheimer RA, Enomoto T, Lichy JH, Hurwitz J (1982) Adenovirus DNA replication in vitro: identification of a host factor that stimulates synthesis of the preterminal protein-dCMP complex. Proc Natl Acad Sci U S A 79(21):6438–6442
2. Nagata K, Guggenheimer RA, Hurwitz J (1983) Specific binding of a cellular DNA replication protein to the origin of replication of adenovirus DNA. Proc Natl Acad Sci U S A 80(20):6177–6181
3. Kruse U, Sippel AE (1994) Transcription factor nuclear factor I proteins form stable homo- and heterodimers. FEBS Lett 348 (1):46–50
4. Gronostajski RM, Adhya S, Nagata K, Guggenheimer RA, Hurwitz J (1985) Site-specific DNA binding of nuclear factor I: analyses of cellular binding sites. Mol Cell Biol 5(5):964–971
5. Leegwater PA, van Driel W, van der Vliet PC (1985) Recognition site of nuclear factor I, a sequence-specific DNA-binding protein from HeLa cells that stimulates adenovirus DNA replication. Embo J. 4(6):1515–1521
6. Gronostajski RM (1986) Analysis of nuclear factor I binding to DNA using degenerate oligonucleotides. Nucleic Acids Res 14 (22):9117–9132
7. Gronostajski RM (1987) Site-specific DNA binding of nuclear factor I: effect of the spacer region. Nucleic Acids Res 15(14):5545–5559
8. Meisterernst M, Gander I, Rogge L, Winnacker EL (1988) A quantitative analysis of nuclear factor I/DNA interactions. Nucleic Acids Res 16(10):4419–4435
9. Fletcher CF, Jenkins NA, Copeland NG, Chaudhry AZ, Gronostajski RM (1999) Exon structure of the nuclear factor I DNA-binding domain from *C. elegans* to mammals. Mamm Genome 10 (4):390–396
10. Gronostajski RM (2000) Roles of the NFI/CTF gene family in transcription and development. Gene 249(1–2):31–45
11. Strausberg RL, Feingold EA, Grouse LH, Derge JG, Klausner RD, Collins FS, Wagner L, Shenmen CM, Schuler GD, Altschul SF, Zeeberg B, Buetow KH, Schaefer CF, Bhat NK, Hopkins RF, Jordan H, Moore T, Max SI, Wang J, Hsieh F, Diatchenko L, Marusina K, Farmer AA, Rubin GM, Hong L, Stapleton M, Soares MB, Bonaldo MF, Casavant TL, Scheetz TE, Brownstein MJ, Usdin TB, Toshiyuki S, Carninci P, Prange C, Raha SS, Loquellano NA, Peters GJ, Abramson RD, Mullahy SJ, Bosak SA, McEwan PJ, McKernan KJ, Malek JA, Gunaratne PH, Richards S, Worley KC, Hale S, Garcia AM, Gay LJ, Hulyk SW, Villalon DK, Muzny DM, Sodergren EJ, Lu X, Gibbs RA, Fahey J, Helton E, Ketteman M, Madan A, Rodrigues S, Sanchez A, Whiting M, Young AC, Shevchenko Y, Bouffard GG, Blakesley RW, Touchman JW, Green ED, Dickson MC, Rodriguez AC, Grimwood J, Schmutz J, Myers RM, Butterfield YS, Krzywinski MI, Skalska U, Smailus DE, Schnerch A, Schein JE, Jones SJ, Marra MA (2002) Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences. Proc Natl Acad Sci U S A 99(26):16899–16903
12. Kruse U, Qian F, Sippel AE (1991) Identification of a fourth nuclear factor I gene in chicken by cDNA cloning: NFI-X. Nucleic Acids Res 19(23):6641
13. Rupp RA, Kruse U, Multhaup G, Gobel U, Beyreuther K, Sippel AE (1990) Chicken NFI/TGGCA proteins are encoded by at least three independent genes: NFI-A, NFI-B and NFI-C with homologues in mammalian genomes. Nucleic Acids Res 18(9):2607–2616
14. Puzianowska-Kuznicka M, Shi YB (1996) Nuclear factor I as a potential regulator during postembryonic organ development. J Biol Chem 271(11):6273–6282
15. Roulet E, Armentero MT, Krey G, Corthesy B, Dreyer C, Mermod N, Wahli W (1995) Regulation of the DNA-binding and transcriptional activities of Xenopus laevis NFI-X by a novel C-terminal domain. Mol Cell Biol 15(10):5552–5562
16. Apt D, Liu Y, Bernard HU (1994) Cloning and functional analysis of spliced isoforms of human nuclear factor I-X: interference with transcriptional activation by NFI/CTF in a cell-type specific manner. Nucleic Acids Res. 22(19):3825–3833
17. Kulkarni S, Gronostajski RM (1996) Altered expression of the developmentally regulated NFI gene family during phorbol ester-induced differentiation of human leukemic cells. Cell Growth Differ 7(4):501–510
18. Mermod N, O'Neill EA, Kelly TJ, Tjian R (1989) The proline-rich transcriptional activator of CTF/NF-I is distinct from the replication and DNA binding domain. Cell 58(4):741–753
19. Bandyopadhyay S, Gronostajski RM (1994) Identification of a conserved oxidation-sensitive cysteine residue in the NFI family of DNA-binding proteins. J Biol Chem 269(47):29949–29955
20. Bandyopadhyay S, Starke DW, Mieyal JJ, Gronostajski RM (1998) Thioltransferase (glutaredoxin) reactivates the DNA-binding activity of oxidation-inactivated nuclear factor I. J Biol Chem 273(1):392–397
21. Chaudhry AZ, Lyons GE, Gronostajski RM (1997) Expression patterns of the four nuclear factor I genes during mouse embryogenesis indicate a potential role in development. Dev Dyn 208(3):313–325
22. Chaudhry AZ, Vitullo AD, Gronostajski RM (1999) Nuclear factor I-mediated repression of the mouse mammary tumor virus promoter is abrogated by the coactivators p300/CBP and SRC-1. J Biol Chem 274(11):7072–7081
23. Santoro C, Mermod N, Andrews PC, Tjian R (1988) A family of human CCAAT-box-binding proteins active in transcription and DNA replication: cloning and expression of multiple cDNAs. Nature. 334(6179):218–224
24. Grunder A, Qian F, Ebel TT, Mincheva A, Lichter P, Kruse U, Sippel AE (2003) Genomic organization, splice products and mouse chromosomal localization of genes for transcription factor Nuclear Factor One. Gene 304:171–181
25. Kruse U, Sippel AE (1994) The genes for transcription factor nuclear factor I give rise to corresponding splice variants between vertebrate species. J Mol Biol 238(5):860–865
26. Wang W, Stock RE, Gronostajski RM, Wong YW, Schachner M, Kilpatrick DL (2004) A role for nuclear factor I in the intrinsic control of cerebellar granule neuron gene expression. J Biol Chem 279(51):53491–53497
27. Meisterernst M, Rogge L, Foeckler R, Karaghiosoff M, Winnacker EL (1989) Structural and functional organization of a porcine gene coding for nuclear factor I. Biochemistry 28(20): 8191–8200
28. Kane R, Murtagh J, Finlay D, Marti A, Jaggi R, Blatchford D, Wilde C, Martin F (2002) Transcription factor NFIC undergoes N-glycosylation during early mammary gland involution. J Biol Chem 277(29):25893–25903
29. Mukhopadhyay SS, Rosen JM (2007) The C-terminal domain of the nuclear factor I-B2 isoform is glycosylated and transactivates the WAP gene in the JEG-3 cells. Biochem Biophys Res Commun 358(3):770–776
30. Kawamura H, Nagata K, Masamune Y, Nakanishi Y (1993) Phosphorylation of NF-I in vitro by cdc2 kinase. Biochem Biophys Res Commun 192(3):1424–1431
31. Bisgrove DA, Monckton EA, Packer M, Godbout R (2000) Regulation of brain fatty acid-binding protein expression by differential phosphorylation of nuclear factor I in malignant glioma cell lines. J Biol Chem 275(39):30668–30676
32. Nilsson J, Bjursell G, Kannius-Janson M (2006) Nuclear Jak2 and transcription factor NF1-C2: a novel mechanism of prolactin

signaling in mammary epithelial cells. Mol Cell Biol 26 (15):5663–5674
33. Campbell CE, Piper M, Plachez C, Yeh YT, Baizer JS, Osinski JM, Litwack ED, Richards LJ, Gronostajski RM (2008) The transcription factor Nfix is essential for normal brain development. BMC Dev Biol 8:52
34. das Neves L, Duchala CS, Tolentino-Silva F, Haxhiu MA, Colmenares C, Macklin WB, Campbell CE, Butz KG, Gronostajski RM (1999) Disruption of the murine nuclear factor I-A gene (Nfia) results in perinatal lethality, hydrocephalus, and agenesis of the corpus callosum. Proc Natl Acad Sci U S A 96(21):11946–11951
35. Driller K, Pagenstecher A, Uhl M, Omran H, Berlis A, Grunder A, Sippel AE (2007) Nuclear factor I X deficiency causes brain malformation and severe skeletal defects. Mol Cell Biol 27 (10):3855–3867
36. Shu T, Butz KG, Plachez C, Gronostajski RM, Richards LJ (2003) Abnormal development of forebrain midline glia and commissural projections in Nfia knock-out mice. J Neurosci 23(1):203–212
37. Steele-Perkins G, Plachez C, Butz KG, Yang G, Bachurski CJ, Kinsman SL, Litwack ED, Richards LJ, Gronostajski RM (2005) The transcription factor gene Nfib is essential for both lung maturation and brain development. Mol Cell Biol 25(2):685–698
38. Lu W, Quintero-Rivera F, Fan Y, Alkuraya FS, Donovan DJ, Xi Q, Turbe-Doan A, Li QG, Campbell CG, Shanske AL, Sherr EH, Ahmad A, Peters R, Rilliet B, Parvex P, Bassuk AG, Harris DJ, Ferguson H, Kelly C, Walsh CA, Gronostajski RM, Devriendt K, Higgins A, Ligon AH, Quade BJ, Morton CC, Gusella JF, Maas RL (2007) NFIA haploinsufficiency is associated with a CNS malformation syndrome and urinary tract defects. PLoS Genet 3 (5):e80
39. Steele-Perkins G, Butz KG, Lyons GE, Zeichner-David M, Kim HJ, Cho MI, Gronostajski RM (2003) Essential role for NFI-C/CTF transcription-replication factor in tooth root development. Mol Cell Biol 23(3):1075–1084
40. Park JC, Herr Y, Kim HJ, Gronostajski RM, Cho MI (2007) Nfic gene disruption inhibits differentiation of odontoblasts responsible for root formation and results in formation of short and abnormal roots in mice. J Periodontol 78(9):1795–1802
41. Plachez C, Lindwall C, Sunn N, Piper M, Moldrich RX, Campbell CE, Osinski JM, Gronostajski R, Richards LJ (2008) Nuclear factor I gene expression in the developing forebrain. J Comp Neurol 508(3):385–401
42. Wang W, Mullikin-Kilpatrick D, Crandall JE, Gronostajski RM, Litwack ED, Kilpatrick DL (2007) Nuclear factor I coordinates multiple phases of cerebellar granule cell development via regulation of cell adhesion molecules. J Neurosci 27(23):6115–61127
43. Shu T, Richards LJ (2001) Cortical axon guidance by the glial wedge during the development of the corpus callosum. J Neurosci 21(8):2749–2758
44. Shu T, Li Y, Keller A, Richards LJ (2003) The glial sling is a migratory population of developing neurons. Development 130 (13):2929–2937
45. Silver J, Lorenz SE, Wahlsten D, Coughlin J (1982) Axonal guidance during development of the great cerebral commissures: descriptive and experimental studies, in vivo, on the role of preformed glial pathways. J Comp Neurol 210(1):10–29
46. Barry G, Piper M, Lindwall C, Moldrich RX, Mason S, Little E, Sarkar A, Tole S, Gronostajski R, Richards LJ (2008) Specific glial populations regulate hippocampal morphogenesis. J Neurosci 28(47):12328–12340
47. Rickmann M, Amaral DG, Cowan WM (1987) Organization of radial glial cells during the development of the rat dentate gyrus. J Comp Neurol 264(4):449–479
48. Sievers J, Hartmann D, Pehlemann FW, Berry M (1992) Development of astroglial cells in the proliferative matrices, the granule cell layer, and the hippocampal fissure of the hamster dentate gyrus. J Comp Neurol 320(1):1–32
49. Deneen B, Ho R, Lukaszewicz A, Hochstim CJ, Gronostajski RM, Anderson DJ (2006) The transcription factor NFIA controls the onset of gliogenesis in the developing spinal cord. Neuron 52 (6):953–968
50. Holm PC, Mader MT, Haubst N, Wizenmann A, Sigvardsson M, Gotz M (2007) Loss- and gain-of-function analyses reveal targets of Pax6 in the developing mouse telencephalon. Mol Cell Neurosci 34(1):99–119
51. Gotz M, Stoykova A, Gruss P (1998) Pax6 controls radial glia differentiation in the cerebral cortex. Neuron 21(5):1031–1044
52. Gangemi RM, Daga A, Muzio L, Marubbi D, Cocozza S, Perera M, Verardo S, Bordo D, Griffero F, Capra MC, Mallamaci A, Corte G (2006) Effects of Emx2 inactivation on the gene expression profile of neural precursors. Eur J Neurosci 23(2):325–334
53. Heins N, Cremisi F, Malatesta P, Gangemi RM, Corte G, Price J, Goudreau G, Gruss P, Gotz M (2001) Emx2 promotes symmetric cell divisions and a multipotential fate in precursors from the cerebral cortex. Mol Cell Neurosci 18(5):485–502
54. Pellegrini M, Mansouri A, Simeone A, Boncinelli E, Gruss P (1996) Dentate gyrus formation requires Emx2. Development 122 (12):3893–3898
55. Mattar P, Britz O, Johannes C, Nieto M, Ma L, Rebeyka A, Klenin N, Polleux F, Guillemot F, Schuurmans C (2004) A screen for downstream effectors of Neurogenin2 in the embryonic neocortex. Dev Biol 273(2):373–389
56. Schuurmans C, Armant O, Nieto M, Stenman JM, Britz O, Klenin N, Brown C, Langevin LM, Seibt J, Tang H, Cunningham JM, Dyck R, Walsh C, Campbell K, Polleux F, Guillemot F (2004) Sequential phases of cortical specification involve Neurogenin-dependent and -independent pathways. Embo J 23(14):2892–2902
57. Wong YW, Schulze C, Streichert T, Gronostajski RM, Schachner M, Tilling T (2007) Gene expression analysis of nuclear factor I-A deficient mice indicates delayed brain maturation. Genome Biol. 8 (5):R72
58. Elder GA, Liang Z, Snyder SE, Lazzarini RA (1992) Multiple nuclear factors interact with the promoter of the human neurofilament M gene. Brain Res Mol Brain Res 15(1–2):99–107
59. Tamura T, Miura M, Ikenaka K, Mikoshiba K (1988) Analysis of transcription control elements of the mouse myelin basic protein gene in HeLa cell extracts: demonstration of a strong NFI-binding motif in the upstream region. Nucleic Acids Res 16(24):11441–11459
60. Miura M, Tamura T, Mikoshiba K (1990) Cell-specific expression of the mouse glial fibrillary acidic protein gene: identification of the cis- and trans-acting promoter elements for astrocyte-specific expression. J Neurochem. 55(4):1180–1188
61. Cebolla B, Vallejo M (2006) Nuclear factor-I regulates glial fibrillary acidic protein gene expression in astrocytes differentiated from cortical precursor cells. J Neurochem 97(4):1057–1070
62. Ren T, Anderson A, Shen WB, Huang H, Plachez C, Zhang J, Mori S, Kinsman SL, Richards LJ (2006) Imaging, anatomical, and molecular analysis of callosal formation in the developing human fetal brain. Anat Rec A Discov Mol Cell Evol Biol 288 (2):191–204
63. Kralovics R, Guan Y, Prchal JT (2002) Acquired uniparental disomy of chromosome 9p is a frequent stem cell defect in polycythemia vera. Exp Hematol 30(3):229–236
64. Italiano A, Ebran N, Attias R, Chevallier A, Monticelli I, Mainguene C, Benchimol D, Pedeutour F (2008) NFIB rearrangement in superficial, retroperitoneal, and colonic lipomas with aberrations involving chromosome band 9p22. Genes Chromosomes Cancer 47(11):971–977
65. Yang ZQ, Imoto I, Pimkhaokham A, Shimada Y, Sasaki K, Oka M, Inazawa J (2001) A novel amplicon at 9p23–24 in squamous

cell carcinoma of the esophagus that lies proximal to GASC1 and harbors NFIB. Jpn J Cancer Res 92(4):423–428
66. Han W, Jung EM, Cho J, Lee JW, Hwang KT, Yang SJ, Kang JJ, Bae JY, Jeon YK, Park IA, Nicolau M, Jeffrey SS, Noh DY (2008) DNA copy number alterations and expression of relevant genes in triple-negative breast cancer. Genes Chromosomes Cancer 47(6):490–499
67. Geurts JM, Schoenmakers EF, Roijer E, Astrom AK, Stenman G, van de Ven WJ (1998) Identification of NFIB as recurrent translocation partner gene of HMGIC in pleomorphic adenomas. Oncogene 16(7):865–872
68. Scrideli CA, Carlotti CG Jr, Okamoto OK, Andrade VS, Cortez MA, Motta FJ, Lucio-Eterovic AK, Neder L, Rosemberg S, Oba-Shinjo SM, Marie SK, Tone LG (2008) Gene expression profile analysis of primary glioblastomas and non-neoplastic brain tissue: identification of potential target genes by oligonucleotide microarray and real-time quantitative PCR. J Neurooncol 88(3):281–291