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## Fez family transcription factors: control of neurogenesis and cell fate in the developing mammalian nervous system

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### Abstract

*Fezf1* and *Fezf2* are highly conserved transcription factors that were first identified by their specific expression in the anterior neuroepithelium of *Xenopus* and zebrafish embryos. These proteins share an N-terminal domain with homology to the canonical engrailed repressor motif and a C-terminal DNA binding domain containing six C2H2 zinc-finger repeats. Over a decade of study indicates that the Fez proteins play critical roles during nervous system development in species as diverse as fruit flies and mice. Herein we discuss recent progress in understanding the functions of *Fezf1* and *Fezf2* in neurogenesis and cell fate specification during mammalian nervous system development. Going forward we believe that efforts should focus on understanding how expression of these factors is precisely regulated, and on identifying target DNA sequences and interacting partners. Such knowledge may reveal the mechanisms by which *Fezf1* and *Fezf2* accomplish both independent and redundant functions across diverse tissue and cell types.

### Introduction

Development of the mammalian nervous system encompasses multiple steps including differentiation of immature neurons and glia from stem cell precursors, migration of these immature cells to their final locations, extension of axons and dendrites, and establishment of mature neural circuits. This process is highly dependent upon transcriptional regulators that function to determine a cell's identity, position, and connections within the nervous system. In this review we discuss recent progresses in understanding the roles of the zinc-finger transcription factors *Fezf1* and *Fezf2* during neurogenesis and the specification of distinct neuronal fates. We provide an overview of their distinct and overlapping functions during development of the olfactory system and forebrain, and highlight some unresolved questions. Determining the functions of these transcription factors during neural development has important implications for understanding brain patterning, neurogenesis, and cell fate specification.

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## The Fez family of zinc-finger transcription factors is evolutionarily conserved

The Forebrain Embryonic Zinc Finger (Fez) family is a highly conserved family of transcription factors (Figure 1) that play roles in neurogenesis, developmental patterning, cell-fate specification and axon guidance [1]. The *Drosophila* genome contains a single Fez family homologue, *Earmuff* (*erm*), while in vertebrates there are two family members, *Fezf1* (also known as *Fez*) and *Fezf2* (also known as *Fezl*, and *Zfp312*) [2,3] (Figure 1B). These genes were first identified on the basis of their specific expression in the anterior neuroepithelium of *Xenopus* and zebrafish embryos [4–6]. Both evolutionarily and within the same species, the Fez proteins exhibit high levels of homology, particularly within their DNA binding and protein-protein interaction domains (Figure 1A).

Interestingly, within these lower organisms the Fez family appears to play conserved roles in the development and fate specification of multiple neuronal structures (Table 1). In the developing *Drosophila* larval brain, *erm* is expressed in intermediate neural progenitor cells and is required to prevent their de-differentiation back into type II neuroblasts [2,7]. This function of *erm* is likely dependent on physical association with the *Drosophila* homologue of the SWI/SNF chromatin-remodeling complex [8]. The genomes of both *Xenopus laevis* and zebrafish contain homologues of both *Fezf1* and *Fezf2* (Figure 1A–B). In *Xenopus* these genes are expressed in the forebrain during early development [4,9]. However, aside from a conserved role in diencephalon patterning [9], little has been reported about the functions of these genes during development of the frog nervous system. In contrast, more extensive work in zebrafish has demonstrated that these factors are expressed in the forebrain and function in diencephalon patterning and neuronal differentiation [5,10–12]. Additionally, *Fezf2* has been shown to play critical roles in the proper development of forebrain dopaminergic neurons [13–16].

Fez Family members all share an N terminal Engrailed homology 1 (Eh1) repressor domain that is known to interact with the Groucho/TLE family of transcriptional co-repressors [5]. In addition, they contain six C2H2 zinc-finger DNA binding motifs at the C terminal. Previous work indicates that FEZF1 and FEZF2 can function redundantly to repress the transcription of a common set of target genes [3,17]. This observation should become increasingly important as new knowledge is gained about the genetic and molecular pathways in which these factors function.

## *Fezf1* and *Fezf2* are expressed in unique and overlapping domains during development of the olfactory system and forebrain

In mice, expression of *Fezf1* is first detectable in the head fold on embryonic day 7.5 (E7.5) [18]. At E8.5 *Fezf1* and *Fezf2* exhibit similar and overlapping expression patterns in the developing forebrain, where *Fezf1* is expressed across a slightly larger domain than *Fezf2* [19]. Two days later at E10.5, *Fezf1* and *Fezf2* are expressed in similar and overlapping patterns in the forebrain and olfactory system (Figure 2A). *Fezf1* is expressed in neural progenitors throughout the forebrain and olfactory pit [17,19,20]. *Fezf2* is also broadly

expressed in progenitor cells of the forebrain, but in contrast to *Fezf1*, its expression within the olfactory pit is restricted to the progenitor cells that will give rise to the vomeronasal organ (VNO) [17,19,20]. This early restriction of *Fezf2* expression to the future VNO positions it as the earliest marker that distinguishes progenitor cells that will give rise to the VNO from those that will generate the main olfactory epithelium (MOE).

As development proceeds, expression of these factors begins to demarcate distinct neuronal structures. At E15.5 *Fezf1* is broadly expressed in progenitor cells and neurons of the developing MOE, but the expression declines within the developing VNO once it segregates away from the MOE [17,20,21] (Figure 2B). Within the brain, *Fezf1* expression has subsided within the cerebral cortex, but it remains in the developing amygdala, ventral thalamus and hypothalamus [17–22] (Figure 2B). In contrast, *Fezf2* is expressed within the VNO, neocortical progenitor cells, and newly born deep-layer projection neurons [17,20,21,23]. By this stage, *Fezf2* expression begins to show a characteristic high expression level in layer five (L5) neurons, and lower levels of expression in L6 neurons and progenitors [23]. In addition, *Fezf2* is expressed within the thalamic eminence, prethalamus, developing amygdala and hypothalamus [18,19,22] (Figure 2B).

By birth, expression of these factors becomes restricted to domains that largely mimic their adult expression patterns (Figure 2C). Expression of *Fezf1* is confined to progenitor cells and sensory neurons of the MOE as well as neurons of the hypothalamus and amygdala [17,21,22]. In contrast, *Fezf2* is expressed at high levels within L5 neurons of the cerebral cortex and at lower levels within L6 neurons and progenitors [23–26]. Additionally, it is expressed within the supporting cells of the vomeronasal organ, and neurons within the hypothalamus and amygdala [17,22]. The dynamic and overlapping expression patterns of these genes underscore their essential functions during development of multiple structures within the nervous system. However, the cellular and molecular mechanisms that regulate the expression of these genes remain largely unknown.

## The Fez family is required for development of the main and accessory olfactory systems

During development of the murine olfactory system, *Fezf1* is expressed in olfactory sensory neurons (OSNs) and their precursors. Genetic loss-of-function studies indicate that it is required for the maturation of OSNs and their innervation of the olfactory bulb [17,20,21]. OSNs that lack *Fezf1* expressed decreased levels of mature OSN markers including olfactory receptors (ORs), G-protein subunit alpha, and olfactory marker protein (OMP) [17,20,21] (Figure 3A). Although the proneural gene *Mash1* exhibited slightly lower levels of expression in the *Fezf1* mutant MOE, neurogenesis appeared to be largely unaffected, as assayed by expression of NeuroD1, NeuroD6 and Ki67 [21]. In agreement with the failed maturation of *Fezf1*<sup>-/-</sup> OSNs, axons from these neurons failed to cross the cribriform plate and innervate the olfactory bulb [17,20,21] (Figure 3A). This defect was attributed to the inability of these axons to penetrate the meninges of the developing brain. Although the exact mechanisms remain to be explored, it seems likely that impaired protease function may at least in part underlie this defect [21].

*Fezf1* also plays a crucial role in the proper specification of OSN versus vomeronasal sensory neuron (VSN) identity [17]. Expression profiling of *Fezf1*<sup>-/-</sup> OSNs demonstrated that these neurons expressed genes normally enriched within VSNs. These included vomeronasal receptors class 1 and 2 (V1Rs, V2Rs), trace amine associated receptors (TAARs), and the VNO-specific ion channel *Trpc2* [17] (Figure 3A). The ectopic expression of genes normally transcribed at high levels within the VNOs suggests that *Fezf1* is a major cell fate determinant for specifying OSN identity. In addition, this raises the possibility that defects in OSN maturation and innervation of the OB may in part be due to the mixed cellular identity of the *Fezf1*<sup>-/-</sup> OSNs.

*Fezf2* is expressed at high levels specifically within the developing VNO and is excluded from OSNs and progenitor cells of the MOE [17]. Within the VNO, *Fezf2* is initially expressed in progenitor cells. However, by E16.5 its expression becomes restricted to sustentacular cells. In contrast to OSNs lacking *Fezf1*, *Fezf2*<sup>-/-</sup> VSNs exhibited decreased proliferation and underwent apoptotic cell death beginning around E12.5 (Figure 3B). These defects in cell proliferation, combined with increased cell death, ultimately resulted in the complete loss of VSNs and supporting cells by birth [17]. The early degeneration of the VNO in *Fezf2* mutant animals precluded the analysis of cellular identity. However it seems probable that - similarly to up-regulation of VSN genes in the *Fezf1*<sup>-/-</sup> OSNs - the sustentacular cells in the VNO of *Fezf2*<sup>-/-</sup> mice may exhibit a mixed cellular identity. Supporting this, *in utero* electroporation of *Fezf1* cDNA into upper-layer cortical neurons had the same effect on cortical neuron development as mis-impression of *Fezf2* [17], hence indicating that these transcription factors can regulate the expression of a common set of genes. This observation raises the question of whether FEZF1 and FEZF2 are functionally interchangeable during the development of the mammalian nervous system. Additionally, it is unclear why some cell types express both *Fezf1* and *Fezf2* while others express only one of these factors.

### ***Fezf1* and *Fezf2* function redundantly during patterning of the diencephalon**

The developing diencephalon can be subdivided into prethalamus, thalamus, hypothalamus, and pretectum. No defect in diencephalic patterning was observed in either *Fezf1*<sup>-/-</sup> or *Fezf2*<sup>-/-</sup> mice. However, examination of *Fezf1*<sup>-/-</sup>; *Fezf2*<sup>-/-</sup> double mutant mice revealed that these factors play redundant roles in patterning of these structures [19]. In double mutant animals, the prethalamus was completely lost and this was accompanied by a rostral expansion of the pretectum and a marked reduction in thalamic size [19]. Analysis of regional markers such as *Gbx2* and *Lhx1* during early development demonstrated that the rostral diencephalon failed to be specified, thus allowing a rostral expansion of the caudal diencephalon. These defects were accompanied by a loss of the zona limitans intrathalamica (ZLI) [19], an important patterning center located between the boundary of the prethalamus and thalamus [27]. Both *Fezf1* and *Fezf2* are expressed rostral to the ZLI boundary, which is demarcated by the expression of the transcription factor *Irx1*. Indeed, misexpression of either *Fezf1* or *Fezf2* caudal to the ZLI was sufficient to repress formation of caudal diencephalon structures [19]. These results suggest that *Fezf1* and *Fezf2* function redundantly during early diencephalon patterning to repress a caudal diencephalon fate

within the rostral diencephalon; furthermore, they raise the possibility that these factors may play hitherto unrecognized roles in the patterning of other neural structures.

### ***Fezf1* and *Fezf2* play unique and redundant roles in forebrain neurogenesis**

*Fezf1* and *Fezf2* have both been implicated in controlling the neurogenic programs of neocortical progenitors during development of the cerebral cortex [3,28]. These factors were shown to bind the promoter of the basic helix-loop-helix transcription factor *Hes5* and repress its transcription during early cortical neurogenesis [3]. In *Fezf1*<sup>-/-</sup> *Fezf2*<sup>-/-</sup> double mutant mice, expression of *Hes5* was increased throughout the forebrain [3]. This led to decreased neurogenesis as assayed by expression of the proneural gene *Neurogenin2* and the postmitotic neuron marker *Tuj1* [3] (Figure 4A). Additionally, the TBR2-expressing basal progenitor cell population was reduced (Figure 4A). These neurogenic defects ultimately resulted in decreased numbers of projection neurons born during early corticogenesis (Figure 4A). Notably, defects in cortical neurogenesis were not observed in *Fezf1* or *Fezf2* single mutants, suggesting that these factors function redundantly in the regulation of *Hes5* transcription and that loss of a single Fez family member is not sufficient to impair early neurogenesis [3]. Recent work has extended these observations, and suggests that the histone H2B ubiquitylation factor *Bre1a* functions upstream of *Fezf1* and *Fezf2* in the control of *Hes5* expression [28]. Accordingly, perturbation of *Bre1a* expression in neural stem cells resulted in altered *Hes5* levels and defects in neurogenesis [28].

In contrast to *Fezf1*, which is only expressed in the cortex during its early stages of development, *Fezf2* is expressed in neocortical progenitors throughout cortical neurogenesis [23]. Analyses of *Fezf2*<sup>-/-</sup> mice indicate that it is required for the generation and fate specification of subcerebral projection neurons in layer 5 (L5) [24–26,29,30] (Figure 5A). However, since expression of *Fezf2* is also enriched in these post-mitotic neurons of L5, whether *Fezf2* is required in L5 neurons or in neocortical progenitors has remained unclear. Indeed, it was speculated that expression of *Fezf2* in early neocortical progenitors may direct these cells to generate deep-layer neurons, and thus *Fezf2*-expressing progenitors were predicted to be lineage-restricted to generate early-born, deep-layer neurons [31,32]. Recent *in vivo* lineage tracing experiments using the *Fezf2* locus indicate that this is not that case, and that -- at both the population and clonal level -- *Fezf2*-expressing progenitor cells sequentially generate deep- and upper-layer projection neurons and glia in accordance with their birthdates [33] (Figure 4B). This observation suggests that *Fezf2* likely has divergent functions within cortical progenitor cells and postmitotic neurons. Given that mis-expression of *Fezf2* in late cortical progenitor cells (see below) is sufficient to generate ectopic subcerebrally-projecting neurons [24,26,29], the above finding indicates that simple expression of *Fezf2* alone is not sufficient to promote a deep-layer neuron identity. To further our understanding of the mechanisms by which *Fezf2* controls projection neuron fates, it will be important to dissect its precise functions in progenitor cells versus postmitotic neurons.

## ***Fezf2* is required for the proper fate specification of multiple classes of cortical projection neurons**

The mammalian neocortex contains 6 layers of projection neurons. Neurons within the same layer tend to share similar morphology, axonal projection patterns, and gene expression profiles [34]. Based upon their axonal projection patterns, cortical projection neurons can be broadly divided into two classes: corticofugal projection neurons (CFuPNs) and cortico-cortical projection neurons. CFuPNs can be further divided into corticothalamic projection neurons (CThPNs) and subcerebral projection neurons (SCPNs). CThPNs send axons into the thalamus and their cell bodies predominantly reside within L6. In contrast, SCPN cell bodies are highly enriched in L5 and these neurons extend axons into subcerebral targets including the spinal cord, superior colliculus and brainstem. Cortico-cortical projection neurons send axons to other cortical areas, either within the ipsi- or contralateral cortical hemisphere (callosal projection neurons, CPNs). These neurons are present throughout layers 2–6, but are most concentrated within upper cortical layers (L2–3).

### **Specification of subcerebral projection neurons**

Recent studies indicate that *Fezf2* is an essential cell-fate determining gene for SCPNs. *Fezf2* is expressed in radial glia cells (RGCs) in the ventricular zone and deep-layer neurons of the neocortex, exhibiting a high expression level in L5 and lower expression in L6 [23,35,36] (Figure 5A). In *Fezf2* mutant mice, deep-layer projection neurons were not properly specified; SCPNs in L5 failed to send axons to subcerebral targets including the pons and spinal cord, and instead switched their fate to become CThPNs or CPNs [24–26,29,30,37]. Electrophysiological recording from individual L5 neurons in *Fezf2* mutant mice demonstrated that mutant neurons changed their electrophysiology properties from that of a non-adapting cell type (subcerebral neuron identity) to an adapting cell type (callosal neuron identity) [29]. Further, expression of the transcription factor SATB2, which marks callosal projection neurons, was significantly increased in deep cortical layers [29] (Figure 5B).

In addition to the subcerebral-to-callosal identity switch, some L5 neurons in *Fezf2* mutant mice changed their identity to become CThPNs. Expression of TBR1, a transcription factor that is specifically expressed in CThPNs, and is essential for their identity, was expanded into L5 [24,30]. A combination of retrograde tracing and birthdating experiments demonstrated that neurons born during the peak-time of L5 neurogenesis projected axons into the thalamus instead of their normal subcerebral targets. Collectively, these results indicate that *Fezf2* is a crucial cell-fate determinant for subcerebral projection neurons, and suggest that subcerebral neuron fate is achieved through the repression of alternate callosal and corticothalamic fates (Figure 5C).



## Genetic cross-repression specifies distinct neuronal fates in the developing cortex

Additional studies aimed at identifying cell-fate determining genes for other cortical projection neuron subtypes provide further support for the model that cortical projection neuron identity is achieved through the repression of alternate neuronal fates. SATB2, a DNA binding protein involved in chromatin remodeling, is expressed at high levels in callosal projection neurons, and was reported to be essential for the specification and execution of a callosal neuron fate [38,39]. In *Satb2*<sup>-/-</sup> mice, the mutant neurons did not send axons across the corpus callosum, and instead projected axons to subcortical targets [38,39]. Moreover, expression of the subcerebral neuron marker CTIP2 was significantly increased in upper cortical layers, suggesting a partial callosal to subcerebral neuron identity switch [38,39].

Similarly, analysis of *Tbr1*<sup>-/-</sup> mice demonstrated that it is required for the specification of corticothalamic neuron identity [30,40–42]. In *Tbr1*<sup>-/-</sup> mice, corticothalamic projections were absent and L6 neurons switched their identity to become SCPNs; they expressed SCPN markers and projected axons into subcerebral targets [30,40,41]. Among the observed gene expression changes was an increase in *Fezf2* expression, and chromatin-immunoprecipitation experiments demonstrated that TBR1 binds directly to a conserved region in the 3' end of the *Fezf2* gene [30,40]. This interaction may function to repress high-levels of *Fezf2* expression and a subcerebral neuron identity within L6 neurons. In support of this, misexpression of TBR1 in L5 neurons prevented these neurons from sending axons into the brainstem [30,40]. These results demonstrate that TBR1 is a major cell-fate determinant for CThPNs, and that it achieves this, at least in part, through repression of subcerebral neuron identity.

A common theme emerging from studies of *Fezf2*<sup>-/-</sup>, *Satb2*<sup>-/-</sup> and *Tbr1*<sup>-/-</sup> mice is that cortical projection neuron fate is achieved through the inhibition of alternate fates [43]. Indeed, analysis of *Fezf2*<sup>-/-</sup>; *Tbr1*<sup>-/-</sup> mice demonstrated that some of the defects observed in *Fezf2*<sup>-/-</sup> animals -- including development of corticospinal projections -- was partly restored [30]. In the future, it will be important to identify the downstream pathways of FEZF2, TBR1 and SATB2, and to determine whether, in addition to repressing alternate neuronal identities, these genes also activate the pathways required for subcerebral, corticothalamic and callosal neuron differentiation, respectively.

### ***Fezf2* is sufficient to reprogram cortical projection neurons**

Recent studies suggest that mis-expression of *Fezf2* is sufficient to generate ectopic SCPNs. Initial experiments utilized *in utero* electroporation to introduce high-levels of *Fezf2*, driven from an extrachromosomal plasmid, into late-stage neocortical RGCs [24,26,29]. At this developmental time point, these RGCs normally generate upper-layer CPNs. However, projection neurons derived from electroporated RGCs extended axons to subcortical targets including the thalamus, pons and spinal cord [24,26,29]. In some cases, the induction of genes normally associated with deep-layer SCPNs was also reported, including TBR1 and CTIP2 [24,26]. Interestingly, electroporated neurons migrated to their normal positions in

upper cortical layers, indicating that laminar position can be functionally dissociated from axonal and gene expression patterns [26,29].

## Reprogramming of ventral forebrain progenitors

Building upon these observations, more recent work focused on the ability of *Fezf2* to reprogram ventral forebrain progenitors into CFuPNs [44]. Rouaux and Arlotta (2010) reported that mis-expression of *Fezf2* in striatal progenitors by *in utero* electroporation was sufficient to induce the expression of genes enriched in deep-layer cortical projection neurons. These included SOX5, TBR1, BHLHB5 and FOG2 (also known as ZFPM2). The induction of CFuPN markers correlated with changes in cellular morphology from a stellate-like morphology (medium-spiny neuron) to a pyramidal-like morphology (cortical projection neuron). In addition, electroporated cells were reported to extend axons to subcerebral targets including the thalamus, cerebral peduncle and substantia nigra. The authors also reported that progenitors that had been electroporated with a *Fezf2*-expressing plasmid maintained expression of the ventral progenitor cell markers *Mash1* and *GSH2*, and did not turn on expression of dorsal progenitor markers such as *PAX6* or *TBR2*. This suggests that the reprogramming does not transition through a dorsal-forebrain like progenitor cell stage. However, whether the reported reprogramming was initiated at the progenitor cell or postmitotic stage remains unclear.

## Postmitotic reprogramming of cortical projection neurons

The reprogramming ability of *Fezf2* was further explored in two studies published at the beginning of 2013, which investigated the effects of mis-expressing *Fezf2* in newly born postmitotic neurons [45,46]. De la Rosa et al. (2013) used a variant of *in utero* electroporation (iontoporation) to deliver *Fezf2*-expressing plasmids into postmitotic neurons of the L4 barrel cortex. These cells are the principal recipients of projections from the thalamus; they exhibit a characteristic spiny morphology and extend axonal projections within the cortex. When *Fezf2* was mis-expressed postmitotically in L4 spiny neurons, the authors reported a change in cellular morphology to a pyramidal-like morphology, a redirection of axons to subcortical targets and a change in electrophysiological properties that were reminiscent of L5 neurons. In addition, the electroporated neurons were reported to shut off the expression of L4 markers including *Ror $\beta$* , *SATB2* and *CUX1*, and turn on the expression of the L5 marker *ER81*. Notably, the induction of the more broadly expressed deep-layer markers *CTIP2* and *SOX5* was not reported.

Using a similar *in utero* electroporation based strategy, Rouaux and Arlotta (2013) demonstrated that during a short temporal window following exit from cell cycle, newly-born cortical neurons destined for layers 2–4 could be coaxed into extending axons subcortically to the thalamus, cerebral peduncle and spinal cord. Additionally, the authors reported the induction of genes normally enriched in deep layers including *ER81*, *SOX5*, *CRYM*, *CRIM1*, *TBR1* and *FEZF2* itself. The molecular changes associated with the reprogramming lasted through at least the first postnatal month. Both of these studies reported that reprogramming of postmitotic neurons is still possible several weeks after birth; however, the efficiency dropped dramatically within the first 3 postnatal days. This



suggests that the ability of newly-born neurons to respond to ectopic *Fezf2* decreases rapidly following their exit from the cell cycle.

Although the ability to redirect axons to subcortical regions remains consistent across all of the studies described above, the molecular changes associated with the reported fate-switch are variable. In one study only changes in axonal targeting were reported [29]. However, additional reports indicate that the expression of genes normally associated with CSMNs may be induced -- though the extent of this induction remains unclear [24,44–46]. Notably, the induction of broadly expressed deep-layer markers such as CTIP2 is variable between different studies [45,46]. In future, it will be crucial to repeat these experiments using less invasive methods for mis-expression of *Fezf2* such as inducible transgenic strategies. Combining stably expressed transgenes with the tamoxifen inducible Cre-Lox system would allow precise spatial and temporal control of *Fezf2* expression within defined cell types. Finally, identifying additional genes that function with *Fezf2* in controlling projection neuron fate specification should prove beneficial in understanding the full potential of *Fezf2* to reprogram the fate of projection neurons within the developing forebrain.

## Developmental regulation

*Fezf1* was first identified through a screen in *Xenopus* embryos for genes that were induced in response to overexpression of Noggin [4]. Similarly, *Fezf2* was isolated by screening for genes that were up-regulated in zebrafish in response to overexpression of the Wnt inhibitor *Dkk1* [5,6]. Less is known about the control of these genes by secreted factors in mammals. However, recent work using differentiated human embryonic stem cells demonstrated that application of exogenous Wnt3a to the culture medium resulted in decreased *Fezf2* expression while application of DKK1 increased *Fezf2* expression [47]. This indicates that at least some of the same regulatory mechanisms that control transcription of these factors are conserved throughout evolution.

What are the regulatory mechanisms that spatially and temporally control *Fezf2* transcription during development of the neocortex? Previous work indicates that multiple transcription factors directly bind to the *Fezf2* locus to regulate its expression. SOX5, an Sry box-containing transcription factor enriched in L6 CThPNs, was shown to bind a highly-conserved noncoding element downstream of *Fezf2*, enhancer 434 (also known as E4) [36,48]. Binding of SOX5 to this enhancer was sufficient to repress transcription of a luciferase reporter, and *Sox5*<sup>-/-</sup> mice exhibit increased levels of *Fezf2* expression within L6 of the cortex [48]. More recent work has identified two additional SOX family members, SOX4 and SOX11 that bind to enhancer 434 and, in contrast to SOX5, promote *Fezf2* transcription [36]. Deletion of enhancer 434 resulted in a dramatic reduction of *Fezf2* expression in the cortex and a loss of the corticospinal tract, phenocopying *Fezf2*<sup>-/-</sup> mice. Similarly, *Sox4*<sup>-/-</sup>; *Sox11*<sup>-/-</sup> double mutant mice displayed a loss of the corticospinal tract and reduced expression of deep-layer markers [36]. The temporal and molecular mechanisms by which these SOX family members function at enhancer 434 remain unknown; however a competition based model in which SOX5 and SOX4/SOX11 vie for occupancy of enhancer 434 to regulate *Fezf2* transcription seems probable.

More recent work using transgenic reporter mice has demonstrated that, when isolated from its endogenous locus, enhancer 434 drove strong reporter activity in cortical progenitor cells [35]. Additionally, this study uncovered, a minimal promoter element consisting of 2.7 kilobases (kb) upstream of the *Fezf2* start codon that was sufficient to drive reporter gene expression within both progenitor cells and postmitotic neurons of the cerebral cortex, throughout development. The authors also investigated the activity of a third non-coding element upstream of *Fezf2*, enhancer 1316. In contrast to enhancer 434, when assayed in isolation, enhancer 1316 was strictly active in postmitotic neurons. Collectively, this study concluded that the combined actions of enhancer 434, enhancer 1316, and the 2.7 kb promoter may function cooperatively to coordinate *Fezf2* expressions across multiple spatial and temporal domains [35]. However, none of the isolated promoter and enhancer elements drove reporter gene expression in a pattern identical to that of endogenous *Fezf2*. This suggests that these elements may represent only a fraction of the overall *cis*-regulatory program controlling *Fezf2* expression, similar to observations made in *Drosophila* through the study of pair-rule gene expression [49,50].

Additional work is needed to fully dissect the mechanisms that control *Fezf2* expression. For instance, how the activity of enhancers 434 and 1316 are integrated within additional regulatory programs during cortical development, such as binding of TBR1 to the 3' UTR of *Fezf2* is unclear. Multiple transcription factors that play important roles in cortical development have been shown to bind around the *Fezf2* locus [30,35,40,48]. However, the temporal order in which these factors occupy their binding sites as well as how they interact with one another is unknown. The recent identification of *Fezf2*-expressing radial glial cells as the progenitors for both deep- and upper-layer neurons and glia indicates that *Fezf2* expression levels in progenitor cells and different types of cortical projection neurons are precisely controlled and thus highlights the need to finely dissect the regulatory mechanisms that control its expression. As additional *cis*-regulatory elements and *trans*-acting factors are uncovered it will become increasingly important to understand the extent to which they independently, and in corporation, regulate *Fezf2* transcription.

## Conclusions and Outlook

*Fezf1* and *Fezf2* are conserved zinc-finger transcription factors that play important roles during development of the forebrain and olfactory system. In the generation of both tissues, they appear to have distinct as well as redundant functions. How, and why, they function redundantly in some cases but not others remains unclear. However, it is possible that these factors may cooperate on essential tasks such as early patterning and neurogenesis in the forebrain, and as development proceeds they increasingly perform unique functions.

Going forward, we believe that pressing issues are to identify the cofactors that function with *Fezf1* and *Fezf2*, to uncover their direct binding targets and to understand how transcription of these factors is spatially and temporally controlled. Given the high level of homology between *Fezf1* and *Fezf2* it seems likely that distinct protein-protein interactions may help to govern their activities in a tissue specific manner. Combining this knowledge with the identification of the regulatory elements and transcriptional regulators that control

*Fezf1* and *Fezf2* transcription should clarify the mechanisms by which these factors control neurogenesis and neuronal fate specification across multiple cell and tissue types.

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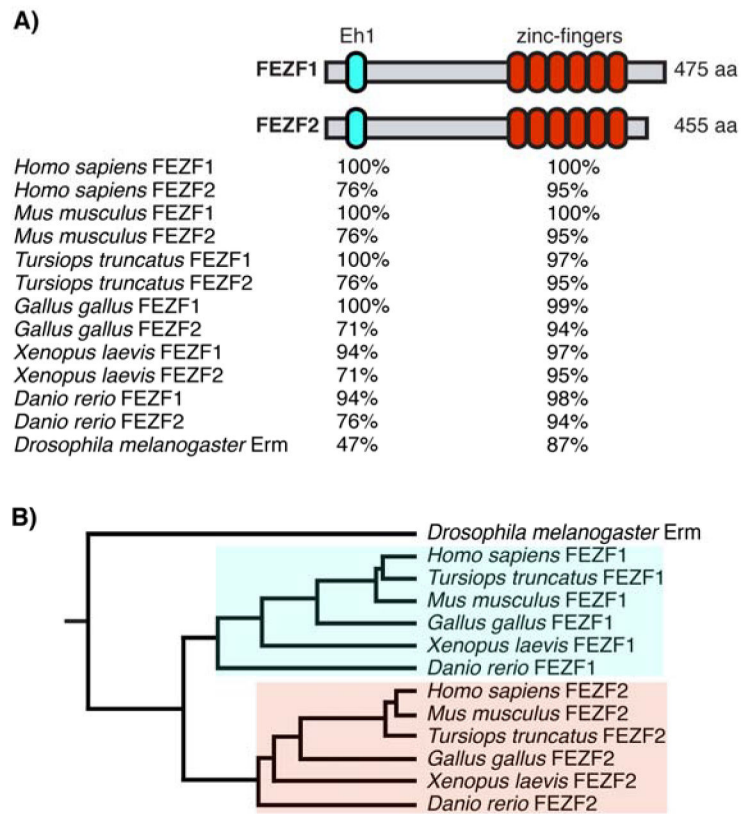
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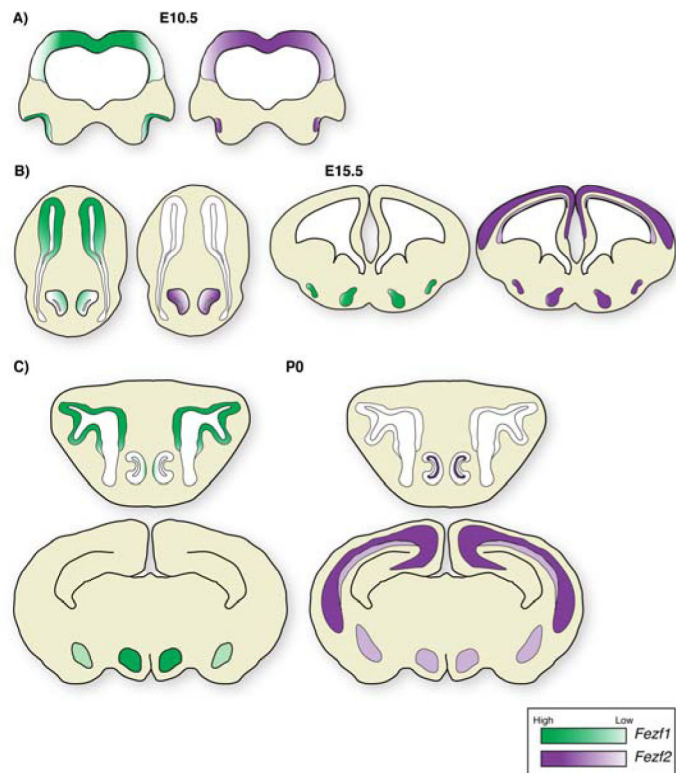
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**Figure 1.**

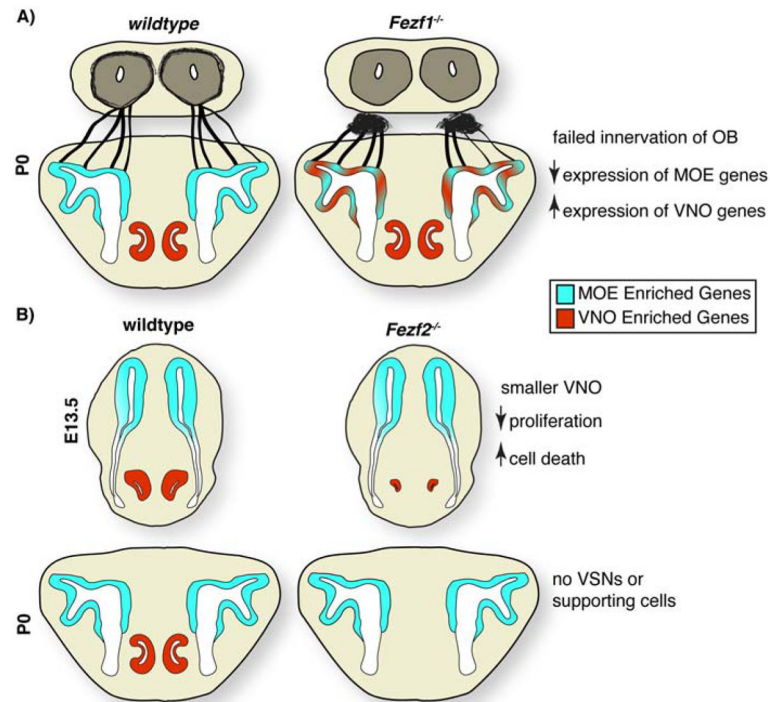
The Fez family of transcription factors. A: Schematic of FEZF1 and FEZF2 proteins highlighting the strong evolutionary conservation of the Engrailed homology 1 (Eh1) and zinc-finger DNA binding domains. Conservation is reported as percent homology to *Homo sapiens* FEZF1. B: Phylogenetic tree of FEZ family transcription factors. A single duplication event appears to have created the two FEZ proteins from a common ancestor.





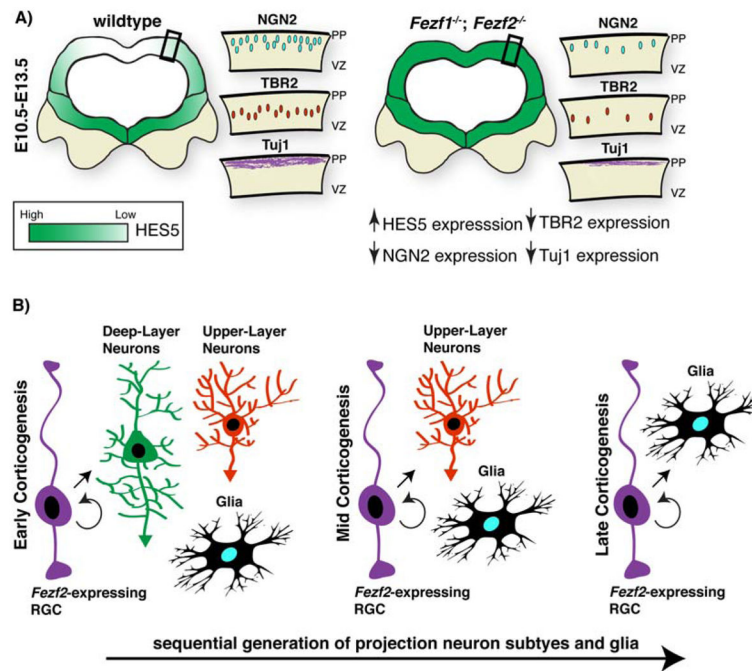
**Figure 2.**

*Fezf1* and *Fezf2* expression during embryogenesis. A: At E10.5 *Fezf1* is expressed in progenitor cells in the forebrain and olfactory pit. *Fezf2* is also expressed in forebrain progenitor cells; however within the olfactory pit its expression is restricted to the future VNO. B: By late gestation (E15.5), *Fezf1* expression remains within the olfactory epithelium, but is absent from the cerebral cortex, and is decreasing within the VNO. In addition, it is expressed within the developing amygdala and hypothalamus. In contrast, *Fezf2* expression remains high within the developing VNO and cerebral cortex. Within the cerebral cortex, *Fezf2* is expressed at high levels in deep-layer postmitotic neurons and at a lower level in progenitor cells. *Fezf2* is also expressed in the hypothalamus. C: At birth, expression of *Fezf1* and *Fezf2* largely mimics that at E15.5. Within the VNO, expression of *Fezf2* has become restricted to the sustentacular cell layer. Within the cerebral cortex it maintains a high expression level in L5 SCPNs and lower levels in L6 CThPNs. Green represents *Fezf1* expression, while purple represents *Fezf2* expression.

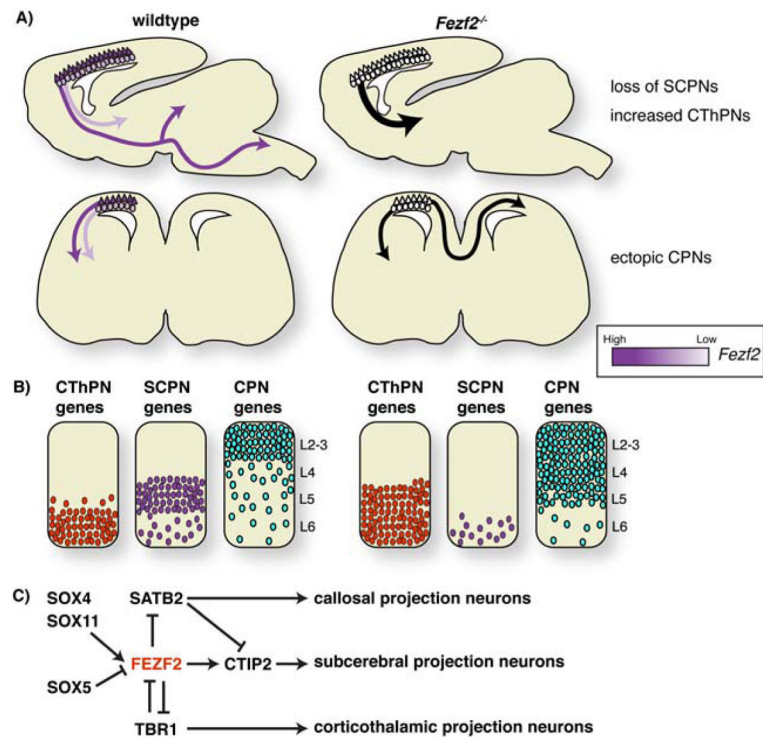


**Figure 3.**

Functions of *Fezf1* and *Fezf2* during olfactory system development. A: In *Fezf1<sup>-/-</sup>* mice, olfactory sensory neurons express decreased levels of MOE enriched genes and instead up-regulate VNO enriched genes. This is accompanied by the failure of OSN axons to cross the cribriform plate and innervate the olfactory bulb. B: The VNO initially segregates away from the developing olfactory pit in *Fezf2<sup>-/-</sup>* mice; however, it is substantially smaller. Because of decreased proliferation and increased apoptosis, VNO sensory neurons and sustentacular cells are completely absent at birth.



**Figure 4.** *Fezf1* and *Fezf2* functions during forebrain neurogenesis. **A:** Loss of both *Fezf1* and *Fezf2* during early forebrain neurogenesis results in an increase in HES5 expression. This leads to decreased neurogenesis and intermediate progenitor generation and decreases in the number of deep-layer projection neurons at birth. **B:** *Fezf2* is expressed in multipotent RGCs that sequentially generate all major cortical projection neuron subtypes and glia.

**Figure 5.**

Control of neocortical projection neuron fate by *Fezf2*. A: In wildtype mice *Fezf2* is expressed at high levels in L5 SCPNs that send axons to the midbrain, hindbrain and spinal cord and at lower levels in CThPNs that project to the thalamus. *Fezf2*<sup>-/-</sup> mice fail to generate subcerebral projections, and instead increased projections to the thalamus are observed. In the absence of *Fezf2*, L5 neurons extend axons across the corpus callosum, similar to CPNs. B: Gene expression changes after loss of *Fezf2* suggest that it represses alternate CPN and CThPN fates. C: A model of the genetic interactions involving *Fezf2* during generation of distinct cortical projection neuron subtypes.

**Table 1**

Expression and functions of the Fez family in non-mammalian species.

Organism	Gene Name(s)	Expression	Function(s)	References
<i>D. melanogaster</i>	<i>erm</i> ( <i>dfezf</i> )	intermediate neural progenitors (INPs)	maintenance of INP differentiation	2, 7–8
<i>X. laevis</i>	<i>fezf1</i> ( <i>fez</i> ) <i>fezf2</i> ( <i>fez-like</i> )	forebrain	diencephalon patterning	4,9
<i>D. rerio</i>	<i>fezf1</i> ( <i>fez</i> ) <i>fezf2</i> ( <i>fez-like, too-few</i> )	forebrain	diencephalon patterning, neuronal differentiation, dopaminergic neuron specification	5, 10–16

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