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RBFOX PROTEINS REGULATE ALTERNATIVE SPLICING OF NEURONAL SODIUM CHANNEL *SCN8A*

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Abstract

The *SCN8A* gene encodes the voltage-gated sodium channel Na_v1.6, a major channel in neurons of the CNS and PNS. *SCN8A* contains two alternative exons, 18N and 18A, that exhibit tissue specific splicing. In brain, the major *SCN8A* transcript contains exon 18A and encodes the full-length sodium channel. In other tissues, the major transcript contains exon 18N and encodes a truncated protein, due to the presence of an in-frame stop codon. Selection of exon 18A is therefore essential for generation of a functional channel protein, but the proteins involved in this selection have not been identified. Using a 2.6 kb *Scn8a* minigene containing exons 18N and 18A, we demonstrate that co-transfection with Fox-1 or Fox-2 initiates inclusion of exon 18A. This effect is dependent on the consensus Fox binding site located 28 bp downstream of exon 18A. We examined the alternative splicing of human *SCN8A* and found that the postnatal switch to exon 18A is completed later than 10 months of age. In purified cell populations, transcripts containing exon 18A predominate in neurons but are not present in oligodendrocytes or astrocytes. Transcripts containing exon 18N appear to be degraded by nonsense-mediated decay in HEK cells. Our data indicate that RBFOX proteins contribute to the cell-specific expression of Na_v1.6 channels in mature neurons.

Keywords

alternative splicing; Rbfox; neuronal specificity; Nav1.6

INTRODUCTION

The gene *SCN8A* encodes sodium channel Na_v1.6, one of the major voltage-gated sodium channels responsible for generation and propagation of action potentials in mammalian neurons. Na_v1.6 is concentrated at the axon initial segment and nodes of Ranvier, and is also present in dendrites and soma of most neurons in the central and peripheral nervous systems (Lorincz and Nusser, 2010; Schaller and Caldwell, 2000). Mutations of Na_v1.6 cause severe movement disorders in the mouse, including tremor, dystonia, ataxic gait, and premature lethality (Meisler et al., 2004). The critical role of Nav1.6 in the neuronal firing has been

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demonstrated by the abnormal firing patterns in neurons from mutant mice, including cerebellar Purkinje cells (Raman et al., 1997), dorsal root ganglia C-fibers (Black et al., 2002), spinal sensory neurons (Cummins et al., 2005), trigeminal neurons (Enomoto et al., 2007), subthallamic neurons (Do and Bean, 2004), retinal ganglion cells (Van Wart and Matthews, 2006), prefrontal cortical pyramidal neurons (Maurice et al., 2001), hippocampal CA1 neurons (Royeck et al., 2008), globus pallidus neurons (Mercer et al., 2007) and cerebellar granule cells (Levin et al., 2006). Reduced repetitive firing and reduced persistent current are common abnormalities. Haploinsufficiency of *SCN8A* has been associated with intellectual disability in a human pedigree (Trudeau et al., 2006), and rare coding variants were identified in patients with autism (Weiss et al., 2003).

Neuron-specific alternative splicing is an important component of regulated gene expression in the nervous system (Li et al., 2007). The alternative exons 18A and 18N of *SCN8A* are the product of exon duplication and encode domain 3 transmembrane segments 3 and 4 (Plummer et al., 1997) (Fig. 1A). Transcripts containing exon 18A predominate in brain and encode the active, full-length channel protein (Fig. 1B). Other tissues express two types of *SCN8A* transcripts that do not encode an active channel, transcripts containing exon 18N with an in-frame stop codon, and transcripts that skip exon 18 ($\Delta 18$) (Figure 1B). An invertebrate homolog of the truncated protein encoded by 18N transcripts was shown to be inactive in functional assays channel activity (Tan et al., 2002). The protein encoded by the $\Delta 18$ transcript has altered topology of transmembrane domains and is unlikely to fold into an active channel. Inclusion of exon 18A is thus essential for production of functional $\text{Na}_v1.6$.

SCN8A exon 18N is conserved in vertebrates from fish to mammals (Plummer et al., 1997; Tan et al., 2002). The related sodium channels *SCN1A*, *SCN2A*, *SCN3A*, and *SCN9A* contain different duplicated alternative exons with in-frame stop codons that also truncate the channel in domain 3 (Alessandri-Haber et al., 2002; Kerr et al., 2008; Oh and Waxman, 1998). Alternative splicing of these stop-codon-containing exons may reinforce the specificity conferred by transcriptional regulation, by preventing the expression of active channel in non-neuronal cells (Plummer et al., 1997).

The mammalian Fox gene family of RNA binding proteins encodes three related proteins, Rbfox1 (*A2BP1*), Rbfox2 (*RBM9*) and Rbfox3 (*HRNBP3/NeuN*) (Kim et al., 2009; Lieberman et al., 2001; McKee et al., 2005; Shibata et al., 2000). Rbfox1 (Fox-1) and Rbfox2 (Fox-2) are known to regulate neuronal exon splicing (Underwood et al., 2005). In this report, we demonstrate a role for the Fox proteins in the alternative splicing of *SCN8A*.

MATERIALS AND METHODS

DNA constructs

Fox-1 and Fox-2 cDNAs in the vector pcDNA3.1 were previously described (Underwood et al., 2005) and generously provided by Dr. Douglas Black, UCLA. To generate the *SCN8A* minigene, a 2.6 kb genomic DNA fragment containing exon 18N and exon 18A with 0.9 kb of upstream sequence and 1.2 kb of downstream sequence was amplified from genomic DNA from mouse strain 129X1/SvJ (The Jackson Laboratory, Bar Harbor, ME) and cloned in the vector pDUP4-1 (Modafferi and Black, 1997) (Addgene, Cambridge, MA). pDUP4-1 contains exons 1 and 2 of the beta-globin gene, which provide initiation and termination signals and can be used to distinguish the minigene transcripts from endogenous $\text{Na}_v1.6$ transcripts. *ApaI* and *BglII* sites were added using primers 1 and 2 (Table S1). The previously described Fox consensus site mutations TGCgTG and TGacgt (Tang et al., 2009) were introduced into the minigene Fox site 28 bp downstream of exon 18A by QuikChange Mutagenesis (Agilent) with primer sets 3–4 and 5–6 (Table S1) using conditions recommended by the supplier.

Cell culture

HEK293T cells were grown in DMEM:F12 media containing 1% Penicillin-Streptomycin supplemented with 10% FBS at 37°C in 5% CO₂. Transfections were performed using Eugene 6 (Roche) and Opti-MEM. HEK293T cells were grown in BD Falcon 6-well plates to 50% confluence and transfected with 2 ug Fox cDNA. Medium was renewed after 24 hours and cells were cultured for an additional 48 hours. For minigene assays, cells were transfected with 1 ug of minigene DNA and 1 ug of Fox cDNA, the medium was changed after 24 hours, and cells were incubated for an additional 24 hours.

RT-PCR

Cells were recovered by scraping into RLT media (Qiagen) with b-mercapthoethanol (0.3 ml/well) and lysed by centrifugation over Qiashtedder columns (Qiagen, Valencia, CA). RNA was prepared using the RNEasy kit (Qiagen). Samples were treated with DNase I prior to 1st strand synthesis. cDNA was synthesized from 5 ug RNA using the Superscript 1st strand cDNA kit (Invitrogen, Carlsbad, CA) with an oligo-dT primer for endogenous transcripts and random hexamer probes for minigene transcripts. Primers 11–16 (Table S1) were used in various combinations for RT-PCR. Amplification was initiated by incubation for 3 min at 94°C, followed by 30–40 cycles of 45 seconds at 94°C, 45 seconds at 60°C, and 45 seconds at 72°C, with a final extension for 10 minutes at 72°C. Products were separated on 2% agarose gels, visualized by ethidium bromide staining, and isolated from the gel for sequencing.

Samples of human brain cortex were obtained from the Harvard Brain Tissue Resource Center (#B4925, 9 years; #B3829, 22 years; #B4503, 56 years) and from Stratagene (#540157, fetal 18 weeks). RNA preparation with the Trizol reagent (Invitrogen) and 1st strand cDNA synthesis using Superscript (Invitrogen) were carried out as described (Drews et al., 2005). The quality of RNA preparations was demonstrated by the presence of intact 18S and 28S ribosomal RNA on agarose gels.

Purified neurons, astrocytes and oligodendrocytes

Cell populations were isolated at Stanford; these populations have been characterized in detail (Barres et al., 1992; Barres et al., 1988). Rat retinal ganglion cells (P6–7) and mature oligodendrocytes (P10–12) were purified by immunopanning as described (Dugas et al., 2006; Goldberg et al., 2002). Cells adherent to the final positive selection panning plates after extensive rinsing (T11D7 for RGCs, GC for oligodendrocytes) were scraped off in RLT media. RNA was prepared with the RNEasy kit (Qiagen).

Cortical astroglia (P1–2) were purified by the shake-off method, as described by McCarthy and de Vellis (McCarthy and de Vellis, 1980). Cortical tissue was papain-digested and plated in medium that does not allow neurons to survive. After 4 days, non-adherent cells were removed by shaking and adherent cells were incubated another 2–4 days to allow the monolayer to refill. Medium was then replaced with fresh medium containing AraC (10 mM) to eliminate contaminating fibroblasts, and incubated for 48 hours. Astrocytes were trypsinized and plated onto 10 cm tissue culture dishes at 2×10^6 cells/plate. After 2 days, cells were removed by scraping for preparation of RNA.

RESULTS

Fox-1 and Fox-2 catalyze splicing of exon 18A from an *Scn8a* minigene

We constructed an *Scn8a* minigene containing a 2.6 kb genomic fragment with exon 18N, exon 18A, and adjacent introns, including the Fox binding site located 28 bp downstream of exon 18A (Fig. 2A). In the minigene vector, the *Scn8a* sequences are located between globin

exon 1, which provides the initial splice donor site for minigene transcripts, and globin exon 2, which provides the final splice acceptor site. HEK cells transfected with the minigene alone produced transcripts that were spliced from the beta-globin donor exon 1 in the construct to exon 18N, and then to globin exon 2, by passing exon 18A completely (Fig. 2B, lane 1). This pattern is typical for non-neural tissues and cells (Plummer et al., 1997). Co-transfection of the minigene with either Fox-1 cDNA or Fox-2 cDNA generated a novel product that contains exon 18A (Fig. 2B). This product results from splicing of the minigene transcript from exon 18N to 18A. This novel transcript is not seen *in vivo*, and may reflect the overexpression of minigene transcript relative to available splice factors, or the absence of a critical factor from HEK cells. However, the production of the 18N plus 18A transcript demonstrates the capability of the Fox proteins to initiate inclusion of exon 18A.

Splicing of minigene exon 18A is dependent on the downstream Fox binding site

A perfect copy of the Fox consensus site TGCATG is located 28 bp downstream of exon 18A (Fig. 1C). To determine whether this site is required for splicing of exon 18A by Fox-1 and Fox-2, we introduced a 1 bp mutation that was previously shown to impair Fox protein function (Tang et al., 2009) (Fig. 2C). Co-transfection of the mutated minigene with Fox-1 or Fox-2 resulted in greatly reduced inclusion of exon 18A (Fig. 2C). The data indicate that Fox binding to the consensus site is required for Fox-mediated inclusion of exon 18A.

Fox-1 alters splicing of endogenous SCN8A transcripts in HEK cells

To avoid the high level of minigene transcript expression and to determine whether direct splicing from exon 17 to exon 18A could be generated by the Fox proteins, we examined the splicing of endogenous *Scn8a* transcripts in HEK cells. In non-transfected HEK cells, the 18N and Δ 18 transcripts of *Scn8a* can be detected, but expression of exon 18A is missing, as expected in non-neural tissue (Fig. 3A). Transfection of HEK cells with the Fox-1 cDNA resulted in the appearance of transcripts that are directly spliced from exon 17 to exon 18A, using a reverse primer in exon 18A (Fig. 3C). Fox-1 is thus sufficient to initiate normal inclusion of exon 18A in a non-neuronal cell.

Effect of cycloheximide on transcripts containing exon 18N

The stop codon in exon 18N is located more than 55 bp upstream of the final exon junction, making transcripts containing exon 18N candidates for nonsense-mediated decay (Nicholson et al., 2010). Since cycloheximide inhibits the pioneer round of mRNA translation that is required for nonsense-mediated decay, it can be used as an indicator of transcript susceptibility to nonsense-mediated decay. In untreated HEK cells, as shown above, the abundance of transcripts containing exon 18N is low (Fig. 4A). However, treatment with cycloheximide for 5 hours resulted in a substantial increase in the abundance of exon 18N-containing transcripts (Fig. 4A). There was no increase of Δ 18 transcripts amplified by the same primers (Fig. 4A) or of the control transcripts ATP7A and FIG4 that lack internal in-frame stop codons (Fig. 4B). Although cycloheximide does not specifically affect the proteins involved in nonsense mediated decay, the data are consistent with the possibility that transcripts containing exon 18N are degraded *in vivo* by nonsense-mediated decay.

Transcripts containing exon 18A are present in neurons but not in two types of glia

It was previously reported that cultured hippocampal neurons express *Scn8a* transcripts containing exon 18A but epithelial cells from the inner ear do not (Mechaly et al., 2005). To extend this limited data, we examined astrocytes, oligodendrocytes and retinal ganglion neurons purified from whole rat brain between postnatal days P1 and P12. The characteristics of these purified cells have been described in detail (Barres et al., 1992; Barres et al., 1988). During the first two weeks of postnatal life, there is a major switch in

the proportion of *Scn8a* transcripts containing exon 18A in rat brain. At P1, there is a comparable abundance of transcripts with and without exon 18A, while at P12 there is a large excess of transcripts containing exon 18A (Fig. 5A). In cells isolated during this time interval, there was robust expression of exon 18A in the purified retinal ganglion neurons (Fig. 5A). In contrast, exon 18A could not be detected in RNA from purified astrocytes or oligodendrocytes (Fig. 5A). This data provides experimental support for the view that transcripts encoding the full length Na_v1.6 channel in brain are concentrated in neuronal cells as a consequence of neuron-specific splicing of exon 18A.

Developmental switch in splicing of human SCN8A

The expression of alternatively spliced transcripts of *SCN8A* in human brain has not previously been characterized. In human fibroblasts, transcripts containing exon 18N and the Δ 18 transcript can be amplified, similar to human HEK cells (Fig. 5B). In fetal brain at 18 weeks of gestation, transcripts containing exon 18A are present, at comparable levels to the other two transcripts (Fig. 5B). By 10 months postnatal, there is only small increase in the proportion of *SCN8A* transcripts containing exon 18A, relative to the two non-neuronal transcripts. The pattern in human brain at 10 months is similar to rodent brain in the early postnatal period up to weaning (Fig. 5A and (Plummer et al., 1997)). By 9 years of age, transcripts containing exon 18A predominate, and the other transcripts were not detected. This pattern persists in at 22 years and 56 years of age (Fig. 1D). The developmental switch to predominance of exon 18A is conserved in human brain, and appears to be delayed until later than 10 mos of age

DISCUSSION

Fox proteins and splicing of *SCN8A* exon 18A

The experiments described here provide evidence for a direct role of the neuronal splice factors Fox-1 and Fox-2 in generation of *SCN8A* transcripts that contain exon 18A and encode the active channel protein. These observations provide a satisfying answer to the mechanism of tissue specificity raised when this pair of duplicated, alternatively spliced exons was described 14 years ago (Plummer et al., 1997). The negative effect of mutating the Fox consensus binding site in the minigene construct strongly supports the role of the Fox proteins in splicing of exon 18A.

Recent studies using knock-out mice provide supporting evidence that the Fox proteins are involved in splicing of exon 18A *in vivo*. Mice with inactivation of RbFox1 were recently described, but splicing of exons 18A and 18N was not included in that study (Gehman et al., 2011). However, in mice that are homozygous for inactivation of Fox-2 and heterozygous for inactivation of Fox-1, the proportion of cerebellar *Scn8a* transcripts containing exon 18A is reduced from 80% to 40% (L. Gehman and D. Black, personal communication). The analysis of transfected cells described here, together with the observations in the knock-out mice, provide strong evidence that Fox-1 and Fox-2 contribute to the neuronal expression of full-length Na_v1.6 in mammalian brain.

This conclusion is consistent with previous evidence that the Fox proteins are expressed in neurons (Cahoy et al., 2008; Tang et al., 2009; Underwood et al., 2005). During development of mouse brain, both Fox proteins and *SCN8A* are first detected at embryonic day 12 (Plummer et al., 1997; Tang et al., 2009; Underwood et al., 2005), and in cultured P19 cells, differentiation towards a neuronal cell fate results in up-regulation of Fox-1 as well as initiation of splicing of *Scn8a* exon 18A (Hakim et al., 2010; Plummer et al., 1997). Fox proteins were previously shown to regulate splicing of another neuronal voltage-gated

ion channel gene, $Ca_v1.2$ (Tang et al., 2009), and 5 statistically significant changes in ion channel splicing were recently observed in Fox-1^{-/-} mouse brain (Gehman et al., 2011).

Fox proteins and Scn8a in non-neural tissues

In addition to neuronal expression, Fox proteins are expressed in ovary and heart (Underwood et al, 2005). We recently reported the presence of transcripts containing exon 18A in isolated cardiac ventricular myocytes (Noujaim et al, 2011), and $Na_v1.6$ protein has been detected by immunostaining in transverse tubules of ventricular myocytes (Du et al., 2007; Lopez-Santiago et al., 2007; Maier et al., 2004). Fox proteins have also been detected in oligodendrocyte precursors, but not in mature oligodendrocytes (Cahoy et al., 2008). Similarly, sodium currents have been observed in oligodendrocyte precursors but not in mature oligodendrocytes (Barres et al., 1990; De Biase et al., 2010). Sodium currents have also been detected in other types of glia (Karadottir et al, 2008; Steinhauser et al, 2002). These observations suggest that Fox proteins may play a role in expression of full length $Nav1.6$ in non-neuronal cells.

Biological function of alternative exon 18N with the in-frame stop codon

Exons containing an in-frame stop codon have been referred to as “poison cassettes” (Lareau et al., 2007) that induce nonsense-mediated decay and down-regulate gene expression (Nicholson et al., 2010). Based on DNA sequence alone, inclusion of exon 18N appears to be favored over exon 18A by higher G-C content of adjacent introns and the more favorable nucleotide at the -6 position of the splice acceptor site. Although the level of *SCN8A* transcripts in non-neuronal cells is low, even a small amount of sodium channel protein in these cells could be deleterious to the maintenance of cellular membrane potential (Williams, 1970). Thus the requirement for a neuronal splice factor to generate active channel protein provides added protection against negative effects of low level expression of channel protein in non-neuronal cells (Fig. 6).

The cockroach invertebrate sodium channel *para* contains three alternatively spliced copies of the exon that corresponds to $Na_v1.6$ exon 18. The copy designated G3 contains an in-frame stop-codon and incorporation of this exon results in a truncated protein that is nearly identical to the 18N protein (Tan et al., 2002). The available genomic sequence for sodium channels from other invertebrates and vertebrates is not of sufficient quality to determine whether *para* exon G3 and vertebrate exon 18N are derived from a common ancestor or are of independent origin. The truncated cockroach sodium channel protein failed to generate sodium currents in the *Xenopus* oocyte system (Tan et al., 2002). This is consistent with biophysical studies of site-directed channel mutations in domain 3 that inactivate channel activity (Stuhmer et al., 1989). Truncating mutations of *SCN1A* in patients with Dravet Syndrome that are in domain 3 are clinically as severe as stop codons near the N-terminus of the protein, supporting the view that truncation in domain 3 inactivates the channel (Meisler and Kearney, 2005). These observations indicate that the truncated $Na_v1.6$ protein encoded by exon 18N is unlikely to retain channel activity. Consistent with our evidence for nonsense-mediated decay of transcripts containing exon 18N, we have been unable to detect the truncated protein on Western blots of brain homogenates or purified membrane fractions (unpublished observations).

Relationship to alternative exons 5A and 5N

SCN8A contains another pair of duplicated, developmentally regulated alternative exons, 5N and 5A, that share a common evolutionary origin with exon 18N and exon 18A (Plummer et al., 1997; Plummer and Meisler, 1999). The structural relationship between the two pairs of exons predicts that the exon duplication event occurred in a two-domain channel prior to generation of the modern four-domain channel. There is a consensus Fox binding site

located 359 bp downstream of exon 5A which could enhance inclusion of this exon (Kuroyanagi, 2009), and splicing of exon 5A is reduced in Fox-1^{-/-} mice (Gehman et al., 2011). Exons 5A and 5N confer different biophysical properties in the paralogous channel *SCN5A*, and the splicing switch from exon 5N to exon 5A may be important during neuronal development (Onkal et al., 2008). Additional heterogeneity of *SCN8A* brain transcripts results from use of four alternative 5-noncoding exons and two alternative polyadenylation sites (Drews et al., 2005).

Non-coding regulatory elements and disease

Trans-acting genetic variation in the splice factor *SCNMI* and cis-acting variation in its binding site in exon 3 of *Scn8a* both modify disease severity in *Scn8a^{medJ}* mice (Buchner et al., 2003; Howell et al., 2007). Similarly, mutations in the Fox protein coding sequence or in its binding sites in *SCN8A* could influence the level of expression of full-length Na_v1.6. Haploinsufficiency of Na_v1.6 results in anxiety-related behavior and impaired sleep in the mouse (McKinney et al., 2008; Papale et al., 2010), and cognitive impairment in human patients (Trudeau et al., 2006). Cognitive impairment is also seen in patients with Fox-1 mutations (Bhalla et al., 2004; Martin et al., 2007), and copy number variants of Fox-1 have been associated with autism (Sebat et al., 2007). The overlap in phenotypes suggests that impaired splicing of *SCN8A* could contribute to the neuropsychiatric effects of Fox-1 mutations. The functional relationship between *SCN8A* and Fox proteins described here will contribute to understanding genetic interactions between these loci and their role in human disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Alternative splicing of *SCN8A*. (A) Structure of the full length, four domain, 1980 amino acid residue Nav1.6 channel protein. The transmembrane segments encoded by exon 18 are shown in black. (B) Genomic structure of exons 18A and 18N. *SCN8A* contains 26 protein coding exons; exons 17 to 19 are expanded. Exon 18N contains an in-frame stop codon. (C) A consensus Fox binding site is located downstream of exon 18A in *SCN8A* genes from human (H), mouse (M) and fugu (F).

Fig. 2. Splicing of an *Scn8a* minigene. (A) A. Structure of the 2.6 kb minigene containing exon 18N, exon 18A, and the Fox binding site TGCATG (asterisk). The CMV promoter and β-globin exons 1 and 2 are indicated; intron sizes in kb; exons not to scale. (B) RT-PCR products amplified from RNA isolated after transfection of HEK cells with the minigene alone (none) or co-transfection with Fox-1 or Fox-2. (C) RT-PCR products from HEK cells transfected with a minigene carrying a 1 bp mutation in the Fox binding site (from TGCATG to TGCgTG). w, wildtype minigene; m1, mutant minigene. RT-PCR primers 15 and 16 in β-globin exons 1 and 2, respectively (Table S1). Predicted lengths in bp: 8N +A=352, 18A=284, 18N=229, Δ18=161. The identity of all RT-PCR products in this and other figures was confirmed by sequencing.

Fig. 3.

Splicing of endogenous *SCN8A* transcripts in HEK cells. (A) RT-PCR products amplified from RNA from untransfected HEK cells with a forward primer in exon 17 and reverse primer in exon 19. PCR product lengths: 18N=216 bp; $\Delta 18$ =146 bp. The product containing 18A (269 bp) is not present. (B) Transcripts containing exon 18A alone can be amplified only after transfection of the Fox-1 cDNA. forward primer in exon 17, reverse primer in exon 18A. M, markers: 100-bp ladder.

Fig. 4.

Treatment with cycloheximide increases the abundance of *SCN8A* transcripts containing exon 18N. (A) HEK cells were incubated with cycloheximide (CHX, 500 ug/ml) for 5 hrs. RT-PCR was carried out with primers 11–12 (Table S1). Product sizes: 18N=216 bp, Δ 18=146 bp. (B) RT-PCR products from transcripts of *ATP7A* and *FIG4* that do not contain in-frame stop codons. Control, no CHX.

Fig. 5. *SCN8A* expression in purified neurons and human brain. (A) RT-PCR of RNA from rat brain at ages P1, P12 and adult demonstrates the postnatal switch to transcripts containing exon 18A. Purified retinal ganglia neurons (RGC) (P6–7) express predominantly exon 18A. Purified astrocytes (P1) and oligodendrocytes (P12) do not contain transcripts with exon 18A. M, molecular weight markers in bp. (B) Time course of developmental switch to exon 18A in human brain. The pattern is delayed in comparison with rodent brain; the human pattern at 10 months postnatal resembles rodent brain at postnatal day 1 (P1). fibr, human fibroblast control; E18w, 18 weeks of gestation. Forward primer in exon 17, reverse primer in exon 19; predicted product sizes, 18A=286, 18N=231, Δ18=163; identity of RT-PCR products was confirmed by sequencing. M, molecular weight markers in bp

Fig. 6. Model for restriction of active Na_v1.6 channels to neurons. Splice factors Fox-1 and Fox-2 in neurons generate transcripts containing exon 18A that encode the active sodium channel. In the absence of Fox-1 and Fox-2 in non-neuronal cells, transcripts containing exon 18N are produced and degraded by nonsense-mediated decay. Translation of remaining 18N transcripts generates a truncated protein lacking channel activity (Plummer et al., 1997). The much greater total abundance of Na_v1.6 transcripts in neurons compared with other cells is also a consequence of regulation at the level of transcription.