Expression of Alternatively Spliced Sodium Channel α-Subunit Genes

UNIQUE SPlicing PATTERNS ARE OBSERVED IN DORSAL ROOT GANGLIA

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Molecular medicine requires the precise definition of drug targets, and tools are now in place to provide genome-wide information on the expression and alternative splicing patterns of any known gene. DNA microarrays were used to monitor transcript levels of the nine well-characterized α-subunit sodium channel genes across a broad range of tissues from cynomolgus monkey, a non-human primate model. Alternative splicing of human transcripts for a subset of the genes that are expressed in dorsal root ganglia, SCN8A (Na\textsubscript{\textit{1.6}}), SCN9A (Na\textsubscript{\textit{1.7}}), and SCN11A (Na\textsubscript{\textit{1.9}}) was characterized in detail. Genomic sequence analysis among gene family paralogs and between cross-species orthologs suggested specific alternative splicing events within transcripts of these genes, all of which were experimentally confirmed in human tissues. Quantitative PCR revealed that certain alternative splice events are uniquely expressed in dorsal root ganglia. In addition to characterization of human transcripts, alternatively spliced sodium channel transcripts were monitored in a rat model for neuropathic pain. Consistent down-regulation of all transcripts was observed, as well as significant changes in the splicing patterns of SCN8A and SCN9A.

Alternative splicing of primary gene transcripts provides a mechanism to generate functionally distinct protein isoforms from a single gene. For the development of safe and efficacious therapeutic compounds, it is necessary to identify the repertoire of proteins that can arise from a gene targeted for therapeutic intervention and determine their tissue distribution within the body. The completion of several mammalian genome sequences, coupled with rich resources provided by extensive expressed sequence tag (EST) and cDNA sequencing, present opportunities for computational prediction of alternative splicing (1–4). The UCSC genome browser (genome.ucsc.edu), which displays overlapping tracks of mRNAs, ESTs, and comparative genomic conservation, can also facilitate the identification of potential alternative splice events. DNA microarrays that monitor exon-exon junctions directly across a broad range of transcripts provide an additional resource to detect alternative splicing on a genome-wide scale (5, 6). These combined computational and experimental approaches, coupled with traditional laboratory validation, provide a wealth of information about alternative splicing and tissue-specific expression, which are essential to define a drug target at the molecular level.

Sodium channels are multisubunit protein complexes that play a pivotal role in the propagation of action potentials along neurons (7, 8). The α-subunit genes encode the primary channel-forming pores within the cell membrane that allow ion-specific translocation. The α-subunit gene family contains nine paralogs (and one additional sodium channel-like gene, Na\textsubscript{\textit{V}}) that are highly conserved across vertebrate species (9). The channel protein structure includes four highly similar clusters of transmembrane helices that are connected by intracellular loops. A similar structure is found in calcium and potassium channels, indicating that this ion channel superfamily arose from a single primordial ion channel gene (8, 10). Voltage-gated sodium channels perform a broad spectrum of functions within vertebrate cells, as is evident from the large number of paralogous genes and their tissue-selective expression patterns. For a particular sodium channel gene, subtle differences in channel properties can be attributed to alternative splicing, post-translational modification, changes in the expression of ancillary β-subunits, and mutation (7, 8). Importantly, alternative splicing of transcripts derived from a common gene has been shown to generate biochemically and pharmacologically distinct sodium channel isoforms (11, 12).

We chose to focus our attention on sodium channels expressed in dorsal root ganglia (DRG), peripheral nervous system (PNS) structures found just outside the spinal column that play key roles in sensory transmission from the periphery to the brain. Channels expressed in DRG are known to play key roles in nociception (7, 8, 13). Modulation of DRG sodium channel activity may provide relief from neuropathic pain, a medical condition that is not well addressed by current medicinal therapies (13). Our goal was to catalog and quantify alternative splicing events that occur in SCN8A (encoding Nav1.6, PN4), SCN9A (encoding Nav 1.7, PN1), and SCN11A (encoding Nav 1.9, PN5). As an example, previous research had shown alternative splicing of SCN8A transcripts (14, 15). In rat DRG, alternative splicing extends the reading frame of exon 11, resulting in a channel that has altered kinetics of inactivation and reactivation relative to the non-extended isoform (12). Developmentally regulated alternative splicing of SCN8A coding exon 18 in mouse and human results in a transcript that encodes a truncated, nonfunctional channel that appears in

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBankTM/EBI Data Bank with accession number(s) AY682082, AY682081, AY682083, AY682085, AY682084, AY682086, and AY686824.

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1 The abbreviations used are: DRG, dorsal root ganglia; PNS, peripheral nervous system; nt, nucleotides; RT, reverse transcriptase; CNS, central nervous system.
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**TABLE I**

| Primer name | 5’ to 3’ sequence | Purpose |
|-------------|--------------------|---------|
| SCN8.5F     | CAGAGCTGTTCTTCACGTAGGCTTATC | Amplify human SCN8A exon 5 to exon 13 |
| SCN8.13R    | CATTATCTCTGATCTCCTGAGCCGCCC | Amplify human SCN8A exon 5 to exon 13 |
| SCN9.4F     | CTGATGAGGAAAATCTGCTGATTAGCTT | Amplify human SCN9A exon 4 to exon 12 |
| SCN9.12F    | GGAGATAGTTTTCAAAGCTCGGAGAAC | Amplify human SCN9A exon 4 to exon 12 |
| SCN11.15F   | AGATGCCATCATTCTTCGGTAAAGA | Amplify human SCN11A exon 15 to exon 19 |
| SCN11.19R   | CAAATCCAGAGTACCCATTTGTA | Amplify human SCN11A exon 15 to exon 19 |

**TABLE II**

| DNA sequence | GenBank™ accession number |
|--------------|--------------------------|
| Human SCN8A - exon 6N to exon 12EXT | AY682082 |
| Human SCN8A - exon 6A to exon 12RS | AY682081 |
| Human SCN9A - exon 5N to exon 11EXT | AY682083 |
| Human SCN9A - exon 5A to exon 11RS | AY682084 |
| Human SCN9A - exon 5A to exon 11EXT | AY682086 |
| Human SCN11A - exon 16 skip | AY682224 |

**TABLE III**

| TaqMan® assay | 5’–3’ Forward primer sequence | 5’–3’ Reverse primer sequence | Purpose |
|---------------|-------------------------------|-----------------------------|---------|
| Human SCN8A exon 6N | ACAAGATTTTTGAAATACGGCGATATG | GTCAATGAGGAAATGTTGGAATAG | TaqMan® assay-exon 6N |
| Human SCN8A exon 6A | AGATGCCATCATTCTTCGGTAAAGA | Amplify human SCN8A exon 5 to exon 13 |
| Human SCN9A exon 5N | AAGAGATAGTTTTCAAAGCTCGGAGAAC | Amplify human SCN9A exon 4 to exon 12 |
| Human SCN9A exon 11wt | AAGATGCCATCATTCTTCGGTAAAGA | Amplify human SCN9A exon 4 to exon 12 |
| Human SCN9A exon 11ext | AAGATGCCATCATTCTTCGGTAAAGA | Amplify human SCN9A exon 4 to exon 12 |
| Rat SCN8A exon 5N | CAGAGGCTCTCCAGGACGACGG | Amplify human SCN9A exon 4 to exon 12 |
| Rat SCN8A exon 5A | CAGAGGCTCTCCAGGACGACGG | Amplify human SCN9A exon 4 to exon 12 |
| Rat SCN8A exon 11wt | CAGAGGCTCTCCAGGACGACGG | Amplify human SCN9A exon 4 to exon 12 |
| Rat SCN8A exon 11ext | CAGAGGCTCTCCAGGACGACGG | Amplify human SCN9A exon 4 to exon 12 |
| Rat SCN9A exon 5N | CAGAGGCTCTCCAGGACGACGG | Amplify human SCN9A exon 4 to exon 12 |
| Rat SCN9A exon 5A | CAGAGGCTCTCCAGGACGACGG | Amplify human SCN9A exon 4 to exon 12 |
| Rat SCN9A exon 11wt | CAGAGGCTCTCCAGGACGACGG | Amplify human SCN9A exon 4 to exon 12 |
| Rat SCN9A exon 11ext | CAGAGGCTCTCCAGGACGACGG | Amplify human SCN9A exon 4 to exon 12 |

** MATERIALS AND METHODS **

**Sodium Channel Expression Compendium (Body Atlas)—**Gene expression profiling of cynomolgus monkey mRNA was performed on ink-jet synthesized oligonucleotide microarrays designed to monitor ~47,000 human transcripts. This two-array set (Hu50K) is an updated version of human microarrays described previously (17). Probes for these alternatively spliced isoforms were most pronounced in DRG. These data indicate that effective treatment of neuropathic pain via antagonism of sodium channels must account for multiple channel isoforms.
transformed the log ratios to ratios and normalized linearly by scaling the largest tissue ratio to 1.

RT-PCR and Quantitative Real Time PCR (TaqMan®)—Reverse transcription-polymerase chain reaction (RT-PCR) amplification from tissue-specific mRNA or total RNA was performed as described previously (5). The oligonucleotides used in this study (Table I) were obtained from Qiagen (Valencia, CA). Amplicons were subcloned into pCR2.1 using a TOPO-TA cloning kit (Invitrogen). Sequencing was performed by a commercial vendor (Lark Technologies Inc., Houston, TX). The sequences of all isoforms described in this study were deposited into GenBank™ (Table II).

TaqMan® is a registered trademark of Roche Applied Science. TaqMan® primer probe reagents were obtained through the Applied Biosystems Assays-by-Design custom assay service (Foster City, CA). Amplicons were subcloned into pCR2.1 using a TOPO-TA cloning kit (Invitrogen). Sequencing was performed by a commercial vendor (Lark Technologies Inc., Houston, TX). The sequences of all isoforms described in this study were deposited into GenBank™ (Table II).

TaqMan® is a registered trademark of Roche Applied Science. TaqMan® primer probe reagents were obtained through the Applied Biosystems Assays-by-Design custom assay service (Foster City, CA). The primer-probe sets used in this study are shown in Table III. Probe sequences were designed to straddle the unique splice junctions characteristic of each alternative splice form. TaqMan® assays were performed on an ABI 7900 real time PCR instrument in 10-μl assays that were run in triplicate in a 384-well format optical PCR plate. The assays were calibrated with isoform-specific RT-PCR clones using the standard curve method. Standard curves generated from plasmid clones were linear across at least six orders of magnitude, and all reported values derived for total tissue RNA fell within the range of these standard curves.

Total RNA from human tissue was obtained from Clontech. Total rat dorsal root ganglia RNA from control and treated animals from a spinal nerve ligation neuropathic pain model was obtained as a gift from Dr. Hao Wang and colleagues (Merck Research Labs, West Point, PA). All of the handling of the animals and testing was performed in accordance with the policies and recommendations of the International Association for the Study of Pain (19) and received approval from the Institutional Animal Care and Use Committee of MRL, West Point, PA. The experimental treatment of the animals was exactly as described in Ref. 20. RNA was converted to cDNA for TaqMan® measurements using a commercially available kit from Applied Biosystems. All assays were normalized on a tissue-to-tissue basis by adding a constant amount of

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Fig. 1. Sodium channel body atlas from cynomolgus macaque monkey. Microarray measurements were made of sodium channel α-subunit transcripts across multiple tissue-derived RNA samples. For each gene, fluorescence intensity values were measured for labeled cRNA hybridized to 3’-positioned probe sequences. The tissue sample that generated the highest intensity value was assigned a value of 1, and all remaining samples were normalized on a linear scale. The tissues were sorted by SCN8A expression level, first by PNS, then CNS, and then all remaining tissues. Error bars estimate 1 S.D. of average measurements and are described in more detail under “Materials and Methods.”

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2 Essentials of Real Time PCR, www.appliedbiosystems.com/support/tutorials/pdf/essentials_of_real_time_pcr.pdf.
**RESULTS**

**Body Atlas Expression Patterns of Sodium Channels**—DNA microarrays afford the opportunity for genome-wide monitoring of transcription within any RNA sample. When RNA samples from diverse tissues throughout the body are hybridized, it becomes possible to assemble a transcriptional compendium or Body Atlas (5, 21, 22). This resource is essentially a semiquantitative, whole-genome Northern blot for all transcripts with corresponding probes on the array. The Body Atlas of the nine voltage-gated sodium channel paralogs across a broad range of tissues was obtained for cynomolgus macaque monkey transcripts that could be mapped to both human and chimpanzee genomic sequences, seven were perfect matches in both species. Finally, although sequence differences between human and cynomolgus monkey transcripts may affect individual probe intensities, they are unlikely to influence intensity ratios (between sample and pool), which are used here. Examination of the Body Atlas data reveals that SCN4A (Nav1.4) and SCN5A (Nav1.5) exhibit strikingly selective expression in striated muscle and heart, respectively. SCN3A (Nav1.3) appears to be transcribed in numerous tissues. Channels SCN1A (Nav1.1), SCN2A (Nav1.2) and SCN8A (Nav1.6) appear to be abundantly expressed in both PNS and CNS tissues. In contrast, SCN9A (Nav1.7), SCN10A (Nav1.8) and SCN11A (Nav1.9) expression is strikingly selective to DRG, with only minor expression levels detected elsewhere in the body.

**Detection of Alternative Splicing in SCN8A, SCN9A, and SCN11A**—Within the vertebrate sodium channel paralog family, parsimonious clustering by protein sequence indicates that SCN8A and SCN9A occupy one branch of the sodium channel family tree that also includes SCN1A, SCN2A, and SCN3A (8, 9). As mentioned, within SCN8A, two alternative splicing events with the potential to produce functional sodium channels have been described. The first involves the potential use of mutually exclusive, alternative exon 6 (coding exon 5) sequences that encode parts of transmembrane segments S3 and S4 within domain I (Fig. 2 and Refs. 14 and 15). The 92 nucleotide (nt) alternative exons, which are found in human, mouse, and rat genomic sequence and known to be used in other sodium channel family members, code for nearly identical amino acid sequences that differ at only two positions. The second, described in mouse and rat, involves the use of alternative 5′splice donor sites in exon 12 (coding exon 11), which

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**FIG. 2.** Alternative splicing of SCN8A, SCN9A, and SCN11A transcripts. A, neonatal (N) and adult (A) alternative splices involve the use of mutually exclusive alternative exons. The RefSeq (RS; human SCN8A [NM_014191.1], human SCN9A [NM_002977.1]), and extended (EXT) coding exon 11 sequences are generated by the use of alternative splice donor sites. The SCN11A variant was generated by exon skipping (3). B, sodium channel transcripts are represented as a line, with the encoded pore-forming transmembrane domains shown as numbered rectangles. SCN8A and SCN9A are represented by the top gene structure. Both gene transcripts undergo analogous splicing events. SCN11A is shown as the bottom transcript with the position of the exon-skipping event denoted. C, nucleotide sequences of alternative exons, exon extensions, and the skipped SCN11A exon.
encodes a portion of the cytoplasmic loop between domains I and II (Fig. 2 and Ref. 12). The resulting channels differ by 11 amino acid residues, and these isoforms exhibit distinct electrophysiological properties (Table I and Ref. 12). Given conservation of the respective genomic sequences, we hypothesized that these alternative splice events might be expressed in human tissues. Gene-specific amplification primers for SCN8A were used to generate RT-PCR products from human DRG, and sequencing confirmed usage of both mutually exclusive exon 6 cassettes and alternative splice donor sites in exon 12 (Fig. 2).

Inspection of genomic sequence of human, mouse and rat SCN9A suggested it has a gene structure similar to SCN8A. Specifically, the genome sequences from all three species encode potentially mutually exclusive exon 5 sequences, and evidence for alternative splicing of the rabbit paralog was deduced from cDNA sequences (24). Similarly, conservation of exon 11 alternative splice donor sites was found in human, mouse and rat genomic sequences, and evidence for alternative splicing was suggested by comparison of human, rat, and rabbit cDNA sequences (12). Confirmation that both of alternative splice events occur in human SCN9A was obtained by sequencing of RT-PCR products amplified from human DRG (Fig. 2).

Cross-species comparative studies using a combined paralog/ortholog approach also revealed a novel, alternatively spliced isoform of SCN11A. Sodium channel genes SCN5A, SCN10A, and SCN11A share a similar gene structure (25). Murine SCN5A is alternatively spliced in heart tissues, with one transcript that is missing the RefSeq (NM_021544.1) exon 17 (26). The same splicing event is observed in rat transcripts of SCN5A (GenBank™ AF356337). This 159-nt exon codes for 53 amino acids situated in the cytoplasmic loop region between domains II and III. Electrophysiology measurements suggest that the Na1.5 channels encoded by these isoforms are functionally similar (26). The human SCN11A gene encodes an analogous 114 nt, 38 amino acid exon in the same cytoplasmic loop-encoding region of the transcript. Moreover, this loop region has only 48% protein sequence identity between human and mouse, whereas the overall channel identity is 72%. Therefore, by analogy to murine SCN5A, this splice variant of SCN11A may encode a functional sodium channel. RT-PCR across this region of human SCN11A using DRG total RNA gave predominantly the expected exon 16 amplicon, however a clone containing a smaller amplicon was isolated, and sequencing revealed it encoded a splice variant lacking exon 16.

Quantitation of Alternatively Spliced Isoforms—Real-time PCR, wherein exon-specific primers flank a splice junction-specific, fluorescently labeled probe (commonly referred to as a TaqMan® assay) was used to quantify alternative splicing events. Using custom assays, we monitored the ratios of all of the alternative splicing events shown in Fig. 2 across two PNS (DRG and spinal cord) and two CNS (fetal and adult whole brain) tissue RNA samples (Fig. 3).

Several interesting conclusions can be drawn from these data. First, the tissue-specific differences in the overall expression levels of each channel transcript closely mirror the Body Atlas array measurements shown in Fig. 1. Second, almost all alternative splicing events are well represented, especially in DRG. This is particularly true of SCN9A, where we observed roughly equal abundance of both exon 5 and exon 11 alternative splice variants. The expression levels and splicing patterns observed in spinal cord were distinct from DRG, especially for SCN9A. Third, despite the relatively high abundance of certain splicing isoforms (e.g. SCN8A transcripts that include exon 6A), many of these alternatively spliced transcripts were not found in GenBank™. Finally, we were unable to reliably detect the SCN11A Δexon 16 splice variant, suggesting it is expressed at very low levels in human DRG (data not shown).

Alternative Splicing Events Appear to Be Unlinked—In DRG, SCN8A, and SCN9A transcripts undergo frequent alternative splicing at two sites (exon 6N versus 6A and exon 12RS versus 12ES). The data show that alternative splicing at these sites is independent, as evidenced by the uncorrelated expression levels of these two splice forms. Furthermore, this suggests that the splicing machinery is not acting as a single unit to promote the alternative splice event, but rather that each site is independently regulated. The implications of this finding for the regulation of sodium channel expression in the peripheral nervous system are discussed in the following sections.
exon 12EXT for SCN8A; exon 5N versus exon 5A and exon 11RS versus exon 11EXT for SCN9A). It is possible that these alternative splicing events are linked, e.g. SCN9A transcripts with exon 5N generally possess exon 11EXT. Conversely, alternative splicing at one site may not influence splicing at a distal site, in which case there should be a stochastic distribution of splicing-generated isoforms. To test this, we amplified cDNA prepared from DRG with PCR primers that span both alternative splice sites for SCN8A and SCN9A (Fig. 4). Individual amplicon clones were then screened at both alternative splice positions to determine the splicing pattern of each clone. We found all possible combinations of splice events, and the distribution of splicing events within the overall set of clones suggested that alternative splicing events were regulated independently with respect to one another. The implication of this finding is that DRG is populated with at least four distinct alternatively spliced isoforms of both SCN8A and SCN9A.

Sodium Channel Expression and Alternative Splicing in a Rat Neuropathic Pain Model—Nerve damage in the periphery can result in chronic neuropathic pain. Effective treatments for this condition may result from a more complete understanding of the biological changes that accompany nerve injury. We monitored isoform-specific changes in expression levels that occur in response to spinal nerve ligation in rat, a commonly used model for neuropathic pain (27). Total RNA from ipsilateral DRG of control-treated and nerve-damaged animals was harvested during the period in which maximal allodynia was observed in the injured animals (2 weeks post-injury). In control animals, the splicing pattern of rat SCN8A appeared similar to human SCN8A (Fig. 5A). In contrast, alternative splicing of rat SCN9A transcripts in control DRG was quantitatively different from human (Fig. 5B). In rat, 80% of the SCN9A transcripts include the exon 11 extension variant, whereas in human, this variant appeared to make up 45% of the overall DRG SCN9A transcript. The significance of this species-specific difference is unclear.

As reported previously, down-regulation of the SCN8A, SCN9A, and SCN11A sodium channel transcripts was observed in ipsilateral DRG in response to neuropathic injury (20, 28–30). SCN8A expression is reduced to 17% of control (Fig. 5A), SCN9A to 27% (Fig. 5B), and SCN11A to 2.5% (data not shown). We also observed a significant change in the expression pattern of alternatively spliced isoforms. In Fig. 5C, we show the retention of alternative splice forms, which is simply the post-injury value divided by the control value for each individual splicing event. For SCN8A, selective retention of exon 6N was observed. For SCN9A, the exon 11RS-containing transcripts were enriched in relative abundance in response to treatment.
Alternative splicing provides a mechanism to generate functionally diverse protein isoforms from a single genetic locus. As shown here, the SCN8A transcript undergoes both development-specific and tissue-specific splicing in humans. In development, exon 6N is highly expressed in fetal brain tissue, and splicing shifts to almost exclusive use of exon 6A in the adult brain. The extension of SCN8A exon 12 is observed almost exclusively in human DRG, with minor relative expression in spinal cord. The functional significance of these alternative splicing events is not yet clear. Interestingly, SCN9A shares similar gene architecture with SCN8A, with a duplicated exon 5 and alternative splice donor sites in exon 11. However, we find that the expression patterns of this transcript are entirely distinct. The SCN9A exon 5N is preferentially expressed in the PNS and CNS of adult tissues and significant usage of exon 5A was found only in DRG. Hence similar gene structure does not imply conserved patterns of splicing regulation.

The duplication of the exons encoding part of transmembrane helix S3 and all of helix S4 in domain I (exon 6 in SCN8A and exon 5 in SCN9A) is only partially conserved across the voltage-gated sodium channel gene family (Fig. 6). By examining 1) the degree of sequence conservation between human, mouse, and rat (genome.ucsc.edu), 2) human genomic sequence, 3) expressed mRNA and ESTs, and 4) published reports in the literature, this duplication appears in SCN2A (31), SCN3A (32), SCN5A (GenBank™ mRNAs), SCN8A (15), and SCN9A (Belcher et al., 1995 and this report). In human, SCN1A also appears to have a duplicated fifth coding exon, however the syntenic region in mouse and rat is interrupted by a single-base, frameshift mutation. It was not possible to find evidence of this exon duplication in the SCN4A, SCN10, or SCN11A genes. The functional significance of the duplicated exon is unclear. Characterization of alternatively spliced, exon 6N or exon 6A rat SCN2A channels failed to reveal detectable differences (33). On the other hand, conservation of this feature across family members and across species, coupled with clear examples of development-specific and/or tissue-specific regulation suggest these alternative exons play an important role that has yet to be identified.

The alternative splice donor sites in coding exon 11 that give rise to an 11 amino acid extension in the cytoplasmic loop between domains I and II are less prevalent in the human sodium channel gene family (Fig. 6). In addition to SCN8A and SCN9A, there is clear evidence from cross-species conservation for this alternative splice event in SCN1A, and alternative splicing of this exon in SCN1A has been observed in rat (12, 34). The GenBank™ collection of human mRNAs and ESTs indicate that SCN1A and SCN3A also undergo alternative splicing that deletes amino acids from this intracellular domain (Fig. 6). Characterization of the channels encoded by SCN8A alternatively spliced transcripts has demonstrated that the difference of 11 amino acids in the domain I-domain II cytoplasmic loop influences the inactivation and reactivation properties of the channel (12). It is intriguing that the extension isoforms are most highly expressed in PNS and CNS of adult tissues and significant usage of exon 5A was found only in DRG. Hence similar gene structure does not imply conserved patterns of splicing regulation.

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Alternative splicing of the heart-specific SCN5A transcript in mouse generates variability in the cytoplasmic loop that connects domains II and III (26). While there is no obvious functional consequence of this 53 amino acid deletion that results from the loss of coding exon 17, this variant is highly expressed in mouse heart and conserved between mouse and rat (GenBank™ accession AF353637). Alternative splicing has not been observed in human SCN5A transcripts. Here we report a comparable exon-drop isoform of SCN11A isolated from
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DRG, although this isoform appears to be rare. Further studies will determine if this transcript encodes a functional sodium channel.

Dorsal root ganglia are clearly unique with respect to sodium channel expression and alternative splicing. Specific sodium channel genes are expressed in DRG and almost nowhere else in the body. Moreover, alternative splicing within these transcripts generates a potentially diverse set of sodium channel isoforms. Similar DRG-selective alternative splicing of calcium channel CACNA1B transcripts has been reported recently (16). DRG is composed of heterogeneous cell types that were shown to differ in their expression patterns of CACNA1B transcripts. Similar observations were made with SCN9A transcripts in rat DRG (35). In this study, we have treated DRG as a homogenous tissue and detected a diverse spectrum of alternative splicing. It will be of interest to determine whether unique transcripts are constrained to specific cell subtypes, and more importantly, which sodium channel isoforms contribute most to neuropathic pain. Such channels would be preferred targets of a future class of channel-specific antagonists. Our investigation of a rat neuropathic pain model revealed selective enrichment of SCN8A mRNAs encoding exon 5N and SCN9A transcripts that included exon 11RS, suggesting these isoforms may selectively contribute to neuropathic pain.

Examination of DRG-specific, voltage-gated sodium channels highlight an important theme with respect to basic biology and pharmaceutical compound development. We found a surprising diversity of alternative splice forms in the highest expressing tissue, DRG, which is also a region of therapeutic focus with respect to neuropathic pain. Our search was by no pressing tissue, DRG, which is also a region of therapeutic interest. Our search was by no means exhaustive. We did not examine SCN10A transcripts for alternative splicing and are not certain we have identified all of the highly expressed splicing events that occur in SCN8A, SCN9A, or SCN11A. While our current knowledge of the human transcriptome is a powerful resource, we believe that much remains to be discovered about alternative splicing and that a thorough knowledge of these post-transcriptional events will be critical to the development of more effective and specific therapies in the treatment of disease and the maintenance of health.

Acknowledgments—We thank our colleagues Martin Kohler and Gregory Kaczorowski for enthusiastic support of this project, Hao Wang and colleagues for supplying RNA samples from the rat neuropathic pain model, Chris Roberts and Steve Milligan for coordination of the expression experiments, Shun Harada and Viera Kasparcova for production of the array data that supported these efforts.

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J. Biol. Chem. 2004, 279:46234-46241.
doi: 10.1074/jbc.M406387200 originally published online August 9, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M406387200

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