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MOLECULAR CLONING AND EXPRESSION PATTERN OF THE SPLICING VARIANT OF CHICK NEURON NAVIGATOR 2

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Takayuki UENO*, Yuriko YAMADA* and Hiroyuki YAGINUMA*

Abstract

To identify novel genes differentially expressed in the dorsal spinal cord of mouse embryos, we used the Kazusa cDNA array system and laser capture microdissection. Through this process, we identified a cDNA encoding mouse *Neuron navigator 2* (*Nav2*) whose expression was found in the dorsolateral part of the spinal cord. To reveal the function of this gene in the spinal cord development, we isolated a cDNA encoding chick *Nav2* splicing variant (*Nav2s*). Sequence analyses revealed that chick *Nav2s* encodes a protein of 2393 amino acids. *In situ* hybridization analyses showed that chick *Nav2s* was detected in the spinal cord, the dermamyotome and in dorsal root ganglion neurons. These results suggest the possibility that *Nav2* may be involved in the early developmental process of the spinal cord or the navigation of axons beyond species.

Keywords: cDNA microarray, spinal cord, *in situ* hybridization, chick, mouse

Introduction

The *Caenorhabditis elegans unc-53* gene plays a crucial role in cell migration and outgrowth of axons (Hedgecock *et al.*, 1987; Hekimi and Kershaw, 1993; Stringham *et al.*, 2002). Three vertebrate homolog of *unc-53* (named *unc53H1*, *unc53H2* and *unc53H3*) were cloned based on the homology with *C. elegans*. *Unc53H2* is identical to *Neuron navigator 2* (*Nav2*) previously identified as the

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atRA-responsive gene (Maes *et al.*, 2002 ; Merrill *et al.*, 2002). Transcripts of the *Nav2* gene contain two putative actin-binding domains, two proline-rich sequences and a putative ATP/GTP nucleotide-binding site (AAA domain). A recent study using *Nav2* hypomorphic homozygous mutant mice suggests that *Nav2* is required for normal cranial nerve development (McNeill *et al.*, 2010). Furthermore, our previous study showed that *Nav2* is strongly expressed in the mouse embryonic spinal cord, suggesting the crucial role in the migration or outgrowth of neurons in the spinal cord (Masuda *et al.*, 2009). However, the function of the *Nav2* protein in the spinal cord development is not clear. For the first step to gain further insight into the molecular function of the *Nav2* protein in vertebrates, we cloned chick *Nav2* splicing variant (*Nav2s*) and investigated its expression pattern in the chick embryo.

Materials and Methods

cDNA cloning of a chick *Nav2* splicing variant

cDNA fragments of approximately 7.2 kbp were amplified from a chick cDNA library by the PCR using primers corresponding to the chick *Nav2* DNA sequence. The primers for the PCR are 5'-accgctgcctgcagtgtgccg-3' and 5'-atgagtttgattggactct-3'. The amplified DNA fragments were ligated to pCS+ vector and sequenced by using an ABI PRISM 3100 DNA sequencer (Life Technologies).

Animals

Chicken eggs were purchased from a local farm and incubated at 37.6°C until they reached the appropriate ages (stage 22 and 26 ; Hamburger and Hamilton, 1992).

In situ hybridization and immunohistochemistry

Transverse sections (25- μ m thick) of stage 22 and 26 chick embryos were cut on a cryostat and mounted on silane-coated slides. Hybridization and detection procedures were performed as described earlier (Masuda *et al.*, 2009).

Results and Discussion

We isolated 6 positive clones from a chick cDNA library, using 22- or 23-mer oligonucleotide probes originated from the registered chick *Nav2* gene sequence. Sequence analyses revealed that all independent clones shared identical nucleotide sequences that are different from the registered sequence. We named this chick *Nav2* splicing variant '*Nav2s*.' The nucleotide and amino acid sequences of chick *Nav2s* cDNA are shown in Figure 1. The chick *Nav2s* gene was 7,179 bp long which could encode a protein of 2393 amino acids.

1 ATGCCAGCCATCCTGGTGGCCTCCAAGATGAAGTCGGGACTGCCAAACCCGTGCACAGC 60
 M P A I L V A S K M K S G L P K P V H S
 61 GCCGCACCCATCCTGCACGTCGCCGGCGCCAGGGCGATCCCGCAGCCCTGCTACCTGAAG 120
 A A P I L H V P A A R A I P Q P C Y L K
 121 TTCGGCAGCAGGGTGGAGGTGACGAAGCCGCTCCTATTCCAGCCAGATCCCTCTCAAGTCC 180
 F G S R V E V T K P S Y S S Q I P L K S
 181 CCCAGCGGGCAGGAGAGCGCCGGGGATGGACCGCCGCGAGGAAGGGCAGCTCGGTGGAA 240
 P S G Q E S A G D G P P P R K G S S V E
 241 AGCGCTTTTGACACTCAGATTTACACGGATTGGGCTAATCACTATCTGGCAAATCTGGC 300
S A F D T Q I Y T D W A N H Y L A K S G
 301 CACAAGAGATTGATAAAGGATCTGCAGCAAGATGTGACCGATGGAGTCTTACTAGCTGAA 360
H K R L I K D L Q Q D V T D G V L L A E
 361 ATAATCCAGTTGTAGCAAATGAAAAGATTGAAGACATCAATGGCTGCCAAAAAACAGA 420
I I Q V V A N E K I E D I N G C P K N R
 421 TCTCAAATGATTGAAAACATAGATGCATGCCTGAGTTTCTGGCTGCTAAAGGGATTAAC 480
S Q M I E N I D A C L S F L A A K G I N
 481 ATACAGGGGCTGCAGCAGAAGAAATCCGGAATGGGAATCTAAAGGCCATCTTGGGCCTT 540
I Q G L S A E E I R N G N L K A I L G L
 541 TTCTTCAGTTTGTCTCGATACAAGCAACAGCAGCAGCCACAGAAACAGCCACCCACAG 600
F F S L S R Y K Q Q Q Q Q P Q K Q P P Q
 601 CACCATCTGTCCAGCCAGCTCCCTCCGTGCCAGCACAGGGCCTCCATCTCAGGGC 660
 H H L S Q P A P S V S Q H T G P P S Q G
 661 CAGGCTGGGCCGAGCAGCAGCAGCAAGTCCAGCTTCGCTCCAACTCAGTGCCAACAG 720
 Q A G P Q Q Q Q Q V P A S L Q T Q C Q Q
 721 CCTCAGCAAGCTGCAGCAGCATCCATTCAAAGCACAAACAGAAATGCAAGTCAAGGCTCCCA 780
 P Q Q A A Q H P F K A Q P E M Q S R L P
 781 GGTCCGACCACGAGGGTGTCCACTGCAGGCAGTGAGACAAAACCTCGTGGTTCTATCAGT 840
 G P T T R V S T A G S E T K T R G S I S
 841 ACCAACAAATCGCCGAGCCAGAGCTTTAAACAATTATGACAAGTCCAAGCCTGGAGTTCTT 900
 T N N R R S Q S F N N Y D K S K P G V L
 901 TTTCCAGCACCCACAAGTTGCAATGAAAAGAGCCTGCAGACAGTACAGCTTTCCAGCCC 960
 F P A P T S C N E K E P A D S T A F Q P
 961 ACAGGAATGAATGAAAATGTATCATCTTCAGTTCAGAGCTGCAGCAGTACTACTAGTTGC 1020
 T G M N E N V S S S V Q S C S S T T S C
 1021 AATAACTCCTCGGCCATCCCTCAGCCCAGCTCTGCTATTAAGCCTTGGCGCAGCAAGTCC 1080
 N N S S A I P Q P S S A I K P W R S K S
 1081 CTCAGCGCTAAGCACACAGCAACGCTTCCATGCTGTCTGTAAGCAGCCCGTACCAGAG 1140
 L S A K H T A T S S M L S V K Q P V P E
 1141 CCTCCAAAGCCACCTCAGAAGTTGTCAAAACAACCTCCCAATGGCCAAAATCTATGCTG 1200
 P P K P P S E V V K T T P N G Q K S M L

Figure 1. Nucleotide and amino acid sequences of chick *Nav2s* cDNA. The coding region is numbered starting from the translation initiation codon. The calponin homology domain (80–189 aa) and the ATPases associated with a variety of cellular activities (AAA) domain (2053–2207 aa) are underlined.

1201 GAAAAGTTGAAACTCTTCAATAGCAAAGGAGGCTCCAAGCAGGGGGGACAACGCTTGAG 1260
 E K L K L F N S K G G S K A G G T T L E
 1261 TGTTCAGCGTCTCGTGACAACAGTTGTGAAAAGCTAGAGACACTCCAGCTTTGAGGAG 1320
 C S A S R D N S C E K L E T L P S F E E
 1321 AGCGAAGAAATCGATGCCACAACCAGAATGTGAGCAATCCAGGATCGATGTCCAGTAGC 1380
 S E E I D A T N Q N V S N P G S M S S S
 1381 CCCAAAATTGCACTCAAGGGAATCGCACAAAGGACTTTTAGCCGGGCACTGACTAATAAG 1440
 P K I A L K G I A Q R T F S R A L T N K
 1441 AAAAGTCTCCCAAGGGCAATGAGAAGGAGAAAGAAACAGAAGGAGAAGAAAAGGAT 1500
 K S S P K G N E K E K E K Q K E K E K D
 1501 AAAAGTAAAGACACGGGAAAAGAACATCTATCACCGAAAAGCTGGATGTAAAAGAGGAA 1560
 K S K D T G K R T S I T E K L D V K E E
 1561 TCAAAGAAGAACAGACAGTGTAGCAACAACAGAGATGCCAAAAAGTCTCAAGATT 1620
 S K E E Q T V L A T T E M P K K S S K I
 1621 GCAAGCTTTATCCGAAAGGAGAAAGCTGAACAGTGCCAAGAAGGAGGCCCTCAGCCCCT 1680
 A S F I P K G G K L N S A K K E A S A P
 1681 TTGCACAGTGGAAATACAAAACCAGGAATGAAAAACCCGAGGAAATCCTCAAGTGCC 1740
 L H S G I P K P G M K N T A G K S S A
 1741 CCAGTTTCTACAAAAGAAAGCAGAGAGGAGCCGAGTGGGAAACCTGGCTCGGGACTCTCG 1800
 P V S T K E S E R S R S G K P G S G L S
 1801 CATCAGAAGTCTCAGCTAGACAGCAGGAATCCAGTTCCTCTTCAAGCTTAGCCTCTTCC 1860
 H Q K S Q L D S R N S S S S S S L A S S
 1861 GAAGGAAAAGGCATCGGAGGCCTCAACAGCAGCAACAGCAGCCAGTCTGTACGCGGGCCG 1920
 E G K G I G G L N S S N S S Q S V S G P
 1921 GCCACCACACAGCAGCGGGAAGCAACCCGTCAGTGTTCAGCTACCTCAGCCCCAGCAG 1980
 A T T H S T G S N T V S V Q L P Q P Q
 1981 CAATATAGCCACCCGAATACAGCCACAGTAGCTCCGTTTATGTACAGATCACAGACAGAG 2040
 Q Y S H P N T A T V A P F M Y R S Q T E
 2041 AATGAAGGAAATGTAACAGCTGAGGCCAGCACGGGAGGGGTCAGCATGGATTCTACTCTC 2100
 N E G N V T A E A S T G G V S M D S T L
 2101 TATGTCAAACTGGACAGCCTGGTCTCGAAGACCTCTCAGGAGAGGATCCGAAAACCTCGG 2160
 Y V K T G Q P G L E D L S G E D P E T R
 2161 CGATTACGAACTGTGAAAACATTGCCGATCTTCGACAGAACTTGGAGGAAACAATGTCC 2220
 R L R T V K N I A D L R Q N L E E T M S
 2221 AGTTTGCAGGAAACCCAGGTCACAGCAGCTTGGAAACTACATTTGACACCAATGTG 2280
 S L R G T Q V T H S T L E T T F D T N V
 2281 ACCACCGAGATAAGGGGTCGCAGCATTCTCAGCTTGACAGGGGACCAACCCCTTTGTG 2340
 T T E I S G R S I L S L T G R P T P L S
 2341 TGGAGACTGGGGCAGTCCAGCCCCGCCTGCAGGAGGTGATGCTCCATCCATGGGAAAT 2400

Figure 1. (continued).

W R L G Q S S P R L Q A G D A P S M G N

2401 GGGTATCCTCCCAGAGGGAATGCCAGCCGCTTCATCAACACGGAATCGGGACGTTACATG 2460
G Y P P R G N A S R F I N T E S G R Y M

2461 TATTCAGCACCTTTGCGAAGACAGCTAGCATCTCGTGGCAGCAGTGTCTGCCATGTGGAC 2520
Y S A P L R R Q L A S R G S S V C H V D

2521 ATCTCAGACAAGGAAGTGTGAAATAGATCTGGAAGGCATCACCATGGATGCCACCGGC 2580
I S D K G S D E I D L E G I T M D A T G

2581 TACATGAGTGTGGAGATGTGCTGGGCAAGAATATCAGGACTGACGATATCACCAGTGGG 2640
Y M S D G D V L G K N I R T D D I T S G

2641 TATATGACTGATGGTGGCTTGGGCCTCTACACTCGAAGGCTAAACCGGCTGCCTGATGGC 2700
Y M T D G G L G L Y T R R L N R L P D G

2701 ATGGCTGCAGTGCAGAGACGATGCAGCGCAACAGTCCCTGGGACTCGGGGATGCTGAC 2760
M A A V R E T M Q R N T S L G L G D A D

2761 AGCTGGGATGACAGCAGCTCTGTGAGCAGTGGGATCAGTGACACCATAGATAATCTCAGC 2820
S W D D S S S V S S G I S D T I D N L S

2821 ACTGATGACATTAACACCAGCTCCTCTATCAGCTCTTATGCCAACACCTGCCTCCTCC 2880
T D D I N T S S S I S S Y A N T P A S S

2881 CGTAAAACTTAGATGCACAGACTGATGCAGAAAAGCATTCCAGGTCGAGGGGAATCC 2940
R K N L D A Q T D A E K H S Q V E R N S

2941 TTATGGTCCAGTGTGAAGTCAAGAAATCAGACGGAGGATCCGACAGTGGCATAAAAAATG 3000
L W S S D E V K K S D G G S D S G I K M

3001 GAGCCAGGATCTAAATGGAGGCGGAATCCCTCTGATGTGTCTGATGAATCTGATAAAAGC 3060
E P G S K W R R N P S D V S D E S D K S

3061 ACTTCTGGTAGGAAGAACAACACTGTTATTTCCGACAGCGGGTTCCTGGAGACGGGGCATGTCG 3120
T S G R K N T V I S Q T G S W R R G M S

3121 GCTCAGGTTGGCATTACCACCAAGGACTAAACCTTCAACCCTCGGGCACATTAAG 3180
A Q V G I T T P R T K P S T T S G T L K

3181 ACACCTGGAACAGGGAAAACACTGATGACGCCAAGGTATCAGAAAAGGGTAGACTATCTCCT 3240
T P G T G K T D D A K V S E K G R L S P

3241 AAGGCTGGACATGTTAAACGTTCCCATCAGATGCAGGACGCAGCAGTGGTGTGATGAATCC 3300
K A G H V K R S P S D A G R S S G D E S

3301 AAAAAGCTTCCCACAAGTAACTCTAGAACAACCTGCTGCTAATGCTAATACATTCGGATTT 3360
K K L P T S N S R T T A A N A N T F G F

3361 AAGAAACAGAGCGGGTCAGCCGTAGGCATGACTATAATTACTGCCAGTGGGGCAACTATC 3420
K K Q S G S A V G M T I I T A S G A T I

3421 ACCAGTAGATCAGCTACTCTGGGAAAATCCCAAAGTCATCCGGACTCATGGGTAGGACC 3480
T S R S A T L G K I P K S S G L M G R T

3481 ACTGGTCGGAAGACTAGTGTGATGGCTCACAGAACCAGGATGATGGCTACTTAGCACTT 3540
T G R K T S V D G S Q N Q D D G Y L A L

Figure 1. (continued).

3541 AGTGCCCGAACTAACCTTCAGTATCGTAGTTTACCCGGCCAGTAAATCAAGTAGCAGA 3600
 S A R T N L Q Y R S L P R P S K S S S R
 3601 AGTGGAGCTGGGAATAGATCTAGCACTAGTAGCATAGACTCCAACATAAGCAGCAAAATCA 3660
 S G A G N R S S T S S I D S N I S S K S
 3661 GCTGGGTGCGTGTCCCTAAAATGAGAGAGCCTGCCAAGGTAATCTTGAAGCTCTCTG 3720
 A G L P V P K M R E P A K V I L G S S L
 3721 CCAGGATTAGTCAATCAGACTGATAAAGAGAAAGGATTTTCGTCTGACAACGAAAGCGTG 3780
 P G L V N Q T D K E K G I S S D N E S V
 3781 GCCTCATGTAATCTGTAAAAGTGAACCTGCATCACAGACTGCTTCTAGTGGAGCTCAA 3840
 A S C N S V K V N P A S Q T A S S G A Q
 3841 AGTACTCACCAGCAAGGACCAAGTACCCTGATGTGGCCTCTCCACTTTGCGCAGACTT 3900
 S T H Q Q G A K Y P D V A S P T L R R L
 3901 TTTGGTGGAAAGCCTAGTAAACAAGTCCCATCACAACAGCAGAAAATATGAAAATTCA 3960
 F G G K P S K Q V P I T T A E N M K N S
 3961 GTAGTCATCTCCAACTCCTCATGCTACTATGAACCAGCAGGGTAATCTTGATTCACCATCT 4020
 V V I S N P H A T M N Q Q G N L D S P S
 4021 GGCAGTGGTATACTAAGCAGTGGGGCAGCAGTCTCTCTATAGTAAAAACAGATTTG 4080
 G S G I L S S G G S S P L Y S K N T D L
 4081 AACCACTCTCCACTAGCTTCTAGTCCAGTTCTGCACATTCAGCTCCTTCCAACAGTTTA 4140
 N Q S P L A S S P S S A H S A P S N S L
 4141 ACATGGGGCACCAACGCAAGTAGCTTTCAGCTGTTAGCAAGGATGGCATTGGCTATCAG 4200
 T W G T N A S S S S A V S K D G I G Y Q
 4201 TCTGTGAGCAGTCTTCATACCAGCTGTGAATCCATTGATATCTCTCTGAGCAGTGGAGGT 4260
 S V S S L H T S C E S I D I S L S S G G
 4261 GGGCTGAGCCATAACTCCTCCGGTAGCTTGATTCCAGCCTCTAAGATGATTCTGTGACT 4320
 G L S H N S S G S L I P A S K D D S L T
 4321 CCCTTTGTCGAACCAACAGTGTTAAGACCACACTGTCTGAAAGGTATACTCCTTCTCCTCC 4380
 P F V R T N S V K T T L S E R Y T P S S
 4381 CAACTTCGTAGCCAGGAAGATGCAAAAGAATGGCTACGGTCACATTGAGCAGGAGGGCTC 4440
 Q L R S Q E D A K E W L R S H S A G G L
 4441 CAGGACACTGTGGCAATCTCCATTTTCATCAGGATCCAGCATAACATCACCTTCTGGA 4500
 Q D T A G N S P F S S G S S I T S P S G
 4501 ACTAGATTTAACTTCTCCAGCTTGAAGCCCAACCACTGCAGCCCAGATGAGCTTGCA 4560
 T R F N F S Q L A S P T T A A Q M S L S
 4561 AATCCAACCATGCTCGGGACCCATAGCCTTTCCAATGCAGATGGCCCTATGACCCCTAT 4620
 N P T M L R T H S L S N A D G P Y D P Y
 4621 AGTGACACAGCTTCAGGAACAGCTCCATGTCCTTGGACGAGAAGAGCAGAAACAATGAGC 4680
 S D T R F R N S S M S L D E K S R T M S
 4681 CGATCTGGCTCGTCCCGTATGGCTTTGAAGAAGTGCATGGTCTTCTCTCTCTTTGGTA 4740
 R S G S F R D G F E E V H G S S L S L V

Figure 1. (continued).

4741 TCCAGTACATCATCTATTTATTCAACACCTGAAGAGAAGTGCCAATCAGAGATTCGCAAG 4800
 S S T S S I Y S T P E E K C Q S E I R K
 4801 CTACGAGAGAGTTGGATGCATCCCAAGAGAAGTATCAGCTCTGACAACCTCAGCTGACT 4860
 L R R E L D A S Q E K V S A L T T Q L T
 4861 GCGAATGCCACCTTGTGGCAGCATTGAGCAGAGTCTGGGGAACATGACGATCAGACTG 4920
 A N A H L V A A F E Q S L G N M T I R L
 4921 CAGAGCCTCACCATGACAGCTGAACAAAAGGACTCTGAAGTGAATGAGCTAAGGAAGACT 4980
 Q S L T M T A E Q K D S E L N E L R K T
 4981 ATTGAACTACTGAAGAAGCAAAATGCTGCTGCCAGGCTGCCATTAATGGAGTCATCAAC 5040
 I E L L K K Q N A A A Q A A I N G V I N
 5041 ACACCTGAGCTCAACTGCAAAGGAAGTGGAGCTGCTCAACCCACAGACTTGGGATCCGA 5100
 T P E L N C K G T G A A Q P T D L R I R
 5101 AGACAGCACTCTTCGGATAGCGTCTCCAGCATTAAACAGTGTACCAGCCACTCTAGCGTG 5160
 R Q H S S D S V S S I N S A T S H S S V
 5161 GGAAGCAACATAGAGAGTGATTCAAAGAAAAGAGGAAGAAGTGGTCAATGAGTTA 5220
 G S N I E S D S K K K K R K N W V N E L
 5221 CGCAGCTCCTTCAAGCAAGCTTTTGGTAAAAAGAAGTCTCCAAGTCAGCATCTTCTCAT 5280
 R S S F K Q A F G K K K S P K S A S S H
 5281 TCGGATATTGAGGAGATGACAGATTCTTATTACCTTCATCACCAGCTACCACACCAT 5340
 S D I E E M T D S S L P S S P K L P H H
 5341 AACTCTACCGTITTTACACCAATTGCTGAGAGCTTCTCATTCCAATTCTTATTTTGAA 5400
 N S T V S T P L L R A S H S N S L I S E
 5401 TGACAGACAGTGAAGCTGAAACAGTCATGCAGTACGCAATGAAGTAAAGAGACAAGGAG 5460
 C T D S E A E T V M Q L R N E L R D K E
 5461 ATGAAGTTGACTGACATTCGTCTAGAAGCCCTTAGCTGCTCATCAGCTTGACCAGCTT 5520
 M K L T D I R L E A L S S A H Q L D Q L
 5521 CGGGAGGCAATGAACAGAATGCAGAGTAAAATTGAGAAGTAAAAGCAGAAAATGATCGA 5580
 R E A M N R M Q S E I E K L K A E N D R
 5581 CTGAAGTCTGAAAACACAGCAGCTGTAGCAGGGCTCAGTCTCAGGCTTCCATTTTCATCC 5640
 L K S E N H S S C S R A Q S Q A S I S S
 5641 TCTCCAAGACATTCAGTGGGTCTCTCAACACAGTTTGAACCTCACAGAGTCAACTAGT 5700
 S P R H S V G L S Q H S L N L T E S T S
 5701 CTCGACATGCTGTTAGATGACACTGGTGATGGCTCTGCCGGAAGGAAGGAGGCAGACAT 5760
 L D M L L D D T G D G S A R K E G G R H
 5761 GTCAAAATAGTTGTCAGTTTTTCAGGATGAAATGAAATGGAAGGAGGATTCAAGGCCCGGT 5820
 V K I V V S F Q D E M K W K E D S R P R
 5821 ACCTTCTCATAGTTGCATTGGAGTGAGCGGGAAGACCAATGGGATGTTCTGGATGGT 5880
 T F L I G C I G V S G K T K W D V L D G
 5881 GTTGTAGACGGCTGTTAAGGAGTACATCATTACCGTGGATCCAGTGAGTCACTGAGCTGGG 5940

Figure 1. (continued).

V V R R L F K E Y I I H V D P V S Q L G

5941 CTGAATT CAGACAGTGTTCGGTTACAGCATTGGAGAGATCAAACGCACAATAGTGCC 6000
L N S D S V L G Y S I G E I K R T N S A

6001 GAGACACCTGAGCTGTTCGCCTGGCTATCTGGTTGGAGAAAACAATACTATTTTCAGTT 6060
E T P E L L P C G Y L V G E N N T I S V

6061 ACCATCAAAGGTATCTGTGAAAACAGCTTGGACTGCCTGGTGTGTTGAATCACTGATCCCA 6120
T I K G I C E N S L D C L V F E S L I P

6121 AAGCCCACTACTGCAGCGCTACATCTCTCTCTGATGGAACACCGCGGATTATCTTGCT 6180
K P I L Q R Y I S L L M E H R R I I L S

6181 GGCCCCAGTGGCACTGGTAAAACATACCTAGCAAACCGGCTCTCTGAGTATATGGTCTG 6240
G P S G T G K T Y L A N R L S E Y M V L

6241 CGGGAGGGCAGGGAGCTGGCTGACGGAATTATTGCAACCTTCAACGTGGACCATAAGTCC 6300
R E G R E L A D G I I A T F N V D H K S

6301 AGTAAGGAACTTCGCCAATACCTGTCCAACCTAGCAGACCAATGTAATAGTGAAAATAAT 6360
S K E L R Q Y L S N L A D Q C N S E N N

6361 GCTGTAGATAGCCTCTTGAATATTTTGGACAACCTGCATCATGTTAGCTCCCTAGGA 6420
A V D M P L V I I L D N L H H V S S L G

6421 GAGATCTTCAATGGACTTCTAAATGCAAGTACCACAAATGTCGTATATTATTGGCACA 6480
E I F N G L L N C K Y H K C P Y I I G T

6481 ATGAACCAAGCCACTCCTCAACACCAATCTTCAACTTACCATAATTTAGATGGGTG 6540
M N Q A T S S T P N L Q L H H N F R W V

6541 CTATGTGTAACCACTGAGCCAGTCAAGGGCTTTCTTGCCGTTTCTGAGAAGAAAA 6600
L C A N H T E P V K G F L G R F L R R K

6601 CTGATTGAAAACAGAGATCAGTGGCAGAATGAGAAATGCAGAGCTGGTAAAATATTGAT 6660
L I E T E I S G R M R N A E L V K I I D

6661 TGGATTCAAAGGTCTGGCAACATCTGAACAAGTTCTTGGAGGCTCATAGCTCCTCTGAT 6720
W I P K V W Q H L N K F L E A H S S S D

6721 GTTACTATTGGTCCACGGCTCTCTCTCTTGTCCAATAGATGTAGATGGTTCAAGAGTT 6780
V T I G P R L F L S G P I D V D G S R V

6781 TGGTTTACTGACTGTGGAACACTCCATCATCCATACCTTCTGGAGGCAGTTAGAGAA 6840
W F T D L W N Y S I I P Y L L E A V R E

6841 GGGCTTACAGCTGTATGGGAGGAGCTCCCTGGGAGGATCCTGCCAAATGGGTAAATGGAC 6900
G L Q L Y G R R A P W E D P A K W V M D

6901 ACATACCCATGGGCAGCCACCCCGCAGCACCATGAGTGGCCTCCTCTGCTACAGTGCGG 6960
T Y P W A A T P Q H H E W P P L L Q L R

6961 CCTGAGGATGTGGGTTTGTGGCTACTCCTTGTACGGGAAGGCTCAACCAGCAAAACA 7020
P E D V G F D G Y S L S R E G S T S K Q

7021 GTTCCAGTGAGTACGCTGAAGGAGATCCACTGATGAACATGCTAATGAGACTGAAGAA 7080
V P V S D A E G D P L M N M L M R L Q E

Figure 1. (continued).

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7081 GCAGCCAAGTACTCAAGTCCCCAGAGTTACGACAGTGACTCTAACAGCAACAGCCATCAC 7140
      A A N Y S S P Q S Y D S D S N S N S H H
7141 GATGACATACTCGATTTCATCTCTGGAATCAACGTTGTGA 7179
      D D I L D S S L E S T L *

```

Figure 1. (continued).

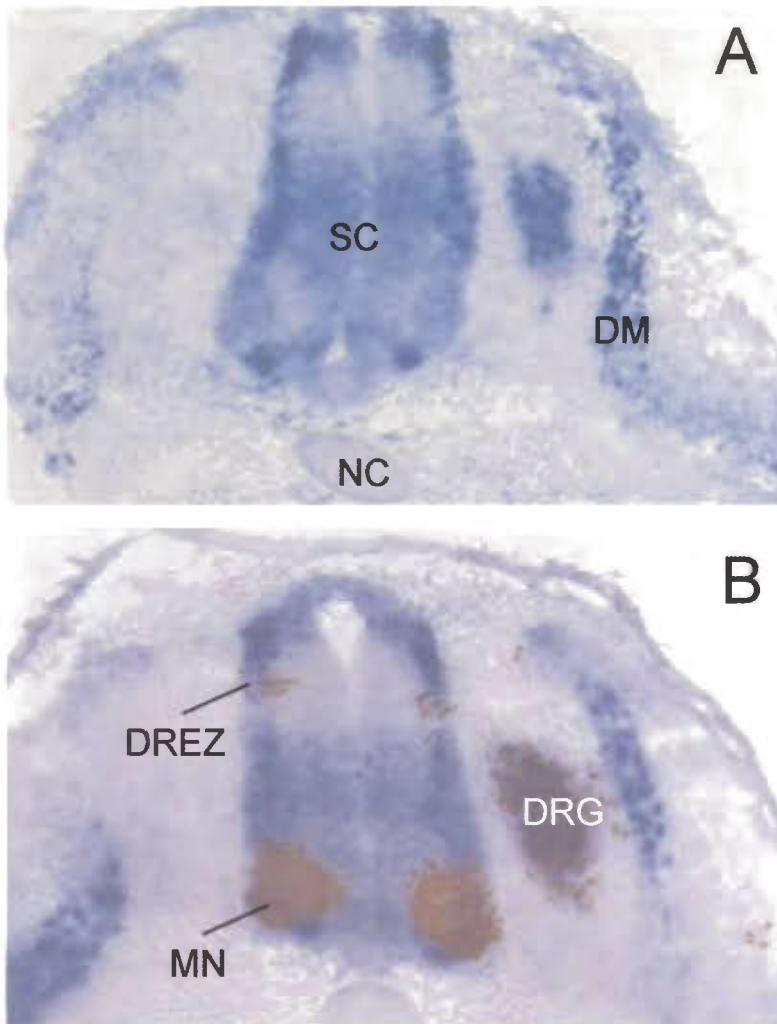


Figure 2. Expression of the *Nav2s* gene in the chick embryo at stage 22. (A) Transverse sections of stage 22 chick embryos were hybridized with the chick *Nav2s* probe. The chick *Nav2s* signal was shown in blue. (B) Staining with the anti-Islet-1/2 antibody (brown) was done to show DRG neurons and motor neurons (MN). DM, dermamyotome; DREZ, dorsal root entry zone; NC, notochord; SC, spinal cord.

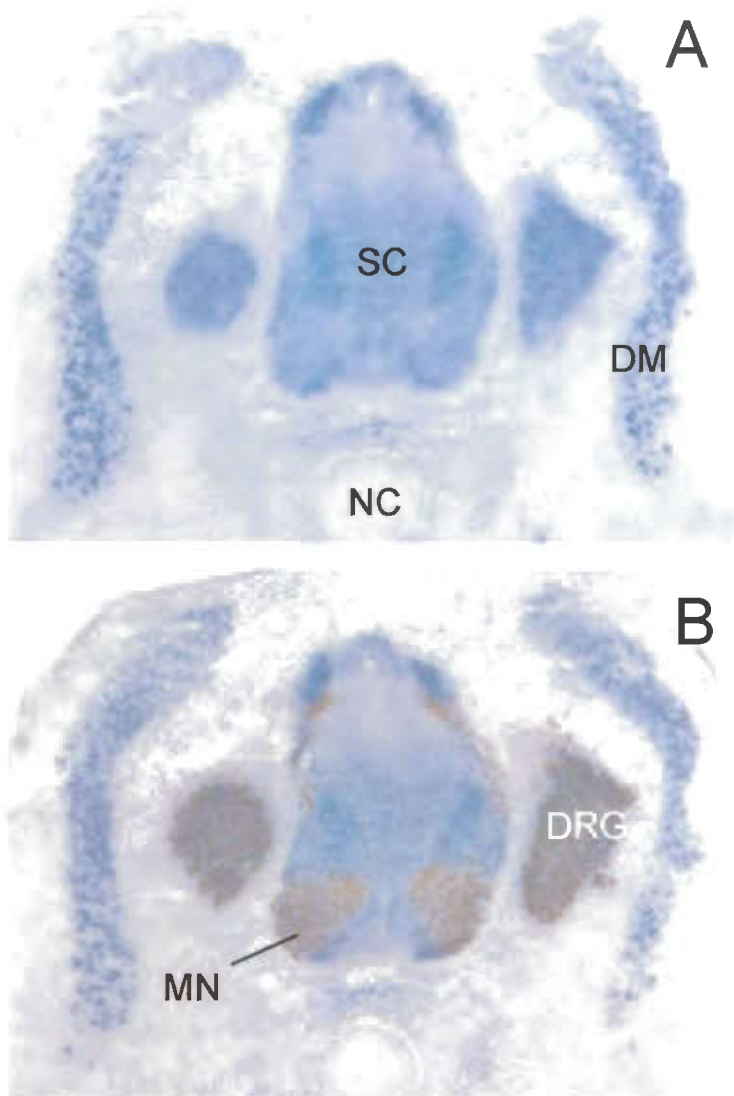


Figure 3. Expression of the *Nav2s* gene in the chick embryo at stage 26. (A) Transverse sections of stage 26 chick embryos were hybridized with the chick *Nav2s* probe. The chick *Nav2s* signal was shown in blue. (B) Staining with the anti-Islet-1/2 antibody (brown) was done to show DRG neurons and motor neurons (MN). DM, dermamyotome; NC, notochord; SC, spinal cord.

Next, we investigated the expression of chick *Nav2s* at the thoracic level of chick embryos (Fig. 2). At stage 22, the mRNA of the *Nav2s* gene was strongly expressed in the dorsolateral edges of the spinal cord (Fig. 2). Its expression was also detected in dorsal root ganglion (DRG) neurons and in the dermamyotome, and

slight expression was also noted in the dorsal root entry zone and in motor neurons (Fig. 2). At stage 26, the *Nav2s* gene was detected in the whole spinal cord and DRG neurons (Fig. 3). The dermamyotome continued to express *Nav2s* (Fig. 3).

Transcripts of Neuron navigator family genes are microtubule-associated protein (Martínez-López *et al.*, 2005). Together with our results that *Nav2s* is expressed in commissural neurons in the early spinal cord, it is highly possible that *Nav2s* could regulate the migration or axon guidance of commissural neurons in the spinal cord. In addition, we suppose that sensory deficits observed in *Nav2* mutant mice (Peeters *et al.*, 2004) may be a secondary effect from the disorganization of the spinal cord neurons.

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