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

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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

* Department of Anatomy, Fukushima Medical University School of Medicine, Fukushima 960-1295, Japan <E-mail: tmasu@fmu.ac.jp>
** Department of Anatomy, Graduate School of Medicine, Osaka City University, Osaka 545-8585, Japan

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′-acccgctgcctgcagtgctgccg-3′ and 5′-atgagtttgtgattg-ggactct-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-μm 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  ATGCCAGCCATCCTGGTGGCCTCCAAGATGAAGTCGGGACTGCCCAAACCCGTGCACAGC    60
      M  P  A  I  L  V  A  S  K  M  K  S  G  L  P  K  P  V  H  S

  61  GCCGCACCCATCCTGCACGTCCCGGCGGCCAGGGCGATCCCGCAGCCCTGCTACCTGAAG   120
      A  A  P  I  L  H  V  P  A  A  R  A  I  P  Q  P  C  Y  L  K

 121  TTCGGCAGCAGGGTGGAGGTGACGAAGCCGTCCTATTCCAGCCAGATCCCTCTCAAGTCC   180
      F  G  S  R  V  E  V  T  K  P  S  Y  S  S  Q  I  P  L  K  S

 181  CCCAGCGGGCAGGAGAGCGCCGGGGATGGACCGCCGCCGAGGAAGGGCAGCTCGGTGGAA   240
      P  S  G  Q  E  S  A  G  D  G  P  P  P  R  K  G  S  S  V  E

 241  AGCGCTTTTGACACTCAGATTTACACGGATTGGGCTAATCACTATCTGGCAAAATCTGGC   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  ATAATCCAGGTTGTAGCAAATGAAAAGATTGAAGACATCAATGGCTGCCCAAAAAACAGA   420
      I  I  Q  V  V  A  N  E  K  I  E  D  I  N  G  C  P  K  N  R

 421  TCTCAAATGATTGAAAACATAGATGCATGCCTGAGTTTCCTGGCTGCTAAAGGGATTAAC   480
      S  Q  M  I  E  N  I  D  A  C  L  S  F  L  A  A  K  G  I  N

 481  ATACAGGGGCTGTCAGCAGAAGAAATCCGGAATGGGAATCTAAAGGCCATCTTGGGCCTT   540
      I  Q  G  L  S  A  E  E  I  R  N  G  N  L  K  A  I  L  G  L

 541  TTCTTCAGTTTGTCTCGATACAAGCAACAGCAGCAGCAGCCACAGAAACAGCCACCACAG   600
      F  F  S  L  S  R  Y  K  Q  Q  Q  Q  Q  P  Q  K  Q  P  P  Q

 601  CACCATCTGTCCCAGCCAGCTCCCTCCGTGTCCCAGCACACAGGGCCTCCATCTCAGGGC   660
      H  H  L  S  Q  P  A  P  S  V  S  Q  H  T  G  P  P  S  Q  G

 661  CAGGCTGGGCCGCAGCAGCAGCAGCAAGTCCCAGCTTCGCTCCAAACTCAGTGCCAACAG   720
      Q  A  G  P  Q  Q  Q  Q  Q  V  P  A  S  L  Q  T  Q  C  Q  Q

 721  CCTCAGCAAGCTGCACAGCATCCATTCAAAGCACAACCAGAAATGCAGTCAAGGCTCCCA   780
      P  Q  Q  A  A  Q  H  P  F  K  A  Q  P  E  M  Q  S  R  L  P

 781  GGTCCGACCACGAGGGTGTCCACTGCAGGCAGTGAGACAAAAACTCGTGGTTCTATCAGT   840
      G  P  T  T  R  V  S  T  A  G  S  E  T  K  T  R  G  S  I  S

 841  ACCAACAATCGCCGCAGCCAGAGCTTTAACAATTATGACAAGTCCAAGCCTGGAGTTCTT   900
      T  N  N  R  R  S  Q  S  F  N  N  Y  D  K  S  K  P  G  V  L

 901  TTTCCAGCACCCACAAGTTGCAATGAAAAAGAGCCTGCAGACAGTACAGCTTTCCAGCCC   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  CTCAGCGCTAAGCACACAGCAACGTCTTCCATGCTGTCTGTAAAGCAGCCCGTACCAGAG  1140
      L  S  A  K  H  T  A  T  S  S  M  L  S  V  K  Q  P  V  P  E

1141  CCTCCAAAGCCACCCTCAGAAGTTGTCAAAACAACTCCCAATGGCCAAAAATCTATGCTG  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  GAAAAGTTGAAACTCTTCAATAGCAAAGGAGGCTCCAAAGCAGGGGGGACAACGCTTGAG  1260
      E  K  L  K  L  F  N  S  K  G  G  S  K  A  G  G  T  T  L  E

1261  TGTTCAGCGTCTCGTGACAACAGTTGTGAAAAGCTAGAGACACTTCCCAGCTTTGAGGAG  1320
      C  S  A  S  R  D  N  S  C  E  K  L  E  T  L  P  S  F  E  E

1321  AGCGAAGAAATCGATGCCACAAACCAGAATGTGAGCAATCCAGGATCGATGTCCAGTAGC  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  AAAAGTTCTCCCAAGGGCAATGAGAAGGAGAAAGAGAAACAGAAGGAGAAAGAAAAGGAT  1500
      K  S  S  P  K  G  N  E  K  E  K  E  K  Q  K  E  K  E  K  D

1501  AAAAGTAAAGACACGGGGAAAAGAACATCTATCACCGAAAAGCTGGATGTAAAAGAGGAA  1560
      K  S  K  D  T  G  K  R  T  S  I  T  E  K  L  D  V  K  E  E

1561  TCAAAAGAAGAACAGACAGTGCTAGCAACAACAGAGATGCCAAAAAAGTCCTCAAAGATT  1620
      S  K  E  E  Q  T  V  L  A  T  T  E  M  P  K  K  S  S  K  I

1621  GCAAGCTTTATTCCGAAAGGAGGAAAGCTGAACAGTGCCAAGAAGGAGGCCTCAGCCCCT  1680
      A  S  F  I  P  K  G  G  K  L  N  S  A  K  K  E  A  S  A  P

1681  TTGCACAGTGGAATACCAAAACCAGGAATGAAAAACACCGCAGGGAAATCCTCAAGTGCC  1740
      L  H  S  G  I  P  K  P  G  M  K  N  T  A  G  K  S  S  S  A

1741  CCAGTTTCTACAAAAGAAAGCGAGAGGAGCCGCAGTGGGAAACCTGGCTCGGGACTCTCG  1800
      P  V  S  T  K  E  S  E  R  S  R  S  G  K  P  G  S  G  L  S

1801  CATCAGAAGTCTCAGCTAGACAGCAGGAATTCCAGTTCCTCTTCAAGCTTAGCCTCTTCC  1860
      H  Q  K  S  Q  L  D  S  R  N  S  S  S  S  S  S  L  A  S  S

1861  GAAGGAAAAGGCATCGGAGGCCTCAACAGCAGCAACAGCAGCCAGTCTGTCAGCGGGCCG  1920
      E  G  K  G  I  G  G  L  N  S  S  N  S  S  Q  S  V  S  G  P

1921  GCCACCACACACAGCACGGGAAGCAACACCGTCAGTGTTCAGCTACCTCAGCCCCAGCAG  1980
      A  T  T  H  S  T  G  S  N  T  V  S  V  Q  L  P  Q  P  Q  Q

1981  CAATATAGCCACCCGAATACAGCCACAGTAGCTCCGTTCATGTACAGATCACAGACAGAG  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  TATGTCAAAACTGGACAGCCTGGTCTCGAAGACCTCTCAGGAGAGGATCCAGAAACTCGG  2160
      Y  V  K  T  G  Q  P  G  L  E  D  L  S  G  E  D  P  E  T  R

2161  CGATTACGAACTGTGAAAAACATTGCCGATCTTCGACAGAACTTGGAGGAAACAATGTCC  2220
      R  L  R  T  V  K  N  I  A  D  L  R  Q  N  L  E  E  T  M  S

2221  AGTTTGCGAGGAACCCAGGTCACTCACAGCACGTTGGAAACTACATTTGACACCAATGTG  2280
      S  L  R  G  T  Q  V  T  H  S  T  L  E  T  T  F  D  T  N  V

2281  ACCACCGAGATAAGCGGTCGCAGCATTCTCAGCTTGACAGGGCGACCAACCCCTTTGTCG  2340
      T  T  E  I  S  G  R  S  I  L  S  L  T  G  R  P  T  P  L  S

2341  TGGAGACTGGGGCAGTCCAGCCCCCGCCTGCAGGCAGGTGATGCTCCATCCATGGGAAAT  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 ATCTCAGACAAAGGAAGTGATGAAATAGATCTGGAAGGCATCACCATGGATGCCACCGGC 2580
     I  S  D  K  G  S  D  E  I  D  L  E  G  I  T  M  D  A  T  G

2581 TACATGAGTGATGGAGATGTGCTGGGCAAGAATATCAGGACTGACGATATCACCAGTGGG 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 ATGGCTGCAGTGCGAGAGACGATGCAGCGCAACACGTCCCTGGGACTCGGGGATGCTGAC 2760
     M  A  A  V  R  E  T  M  Q  R  N  T  S  L  G  L  G  D  A  D

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

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

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

2941 TTATGGTCCAGTGATGAAGTCAAGAAATCAGACGGAGGATCCGACAGTGGCATAAAAATG 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 ACTTCTGGTAGGAAGAACACTGTTATTTCGCAGACGGGTTCCTGGAGACGGGGCATGTCG 3120
     T  S  G  R  K  N  T  V  I  S  Q  T  G  S  W  R  R  G  M  S

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

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

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

3301 AAAAAGCTTCCCACAAGTAACTCTAGAACAACTGCTGCTAATGCTAATACATTCGGATTT 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 ACCAGTAGATCAGCTACTCTGGGAAAAATCCCAAAGTCATCCGGACTCATGGGTAGGACC 3480
      T  S  R  S  A  T  L  G  K  I  P  K  S  S  G  L  M  G  R  T

3481 ACTGGTCGGAAGACTAGTGTTGATGGCTCACAGAACCAGGATGATGGCTACTTAGCACTT 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 AGTGCCCGAACTAACCTTCAGTATCGTAGTTTACCCCGGCCCAGTAAATCAAGTAGCAGA 3600
     S  A  R  T  N  L  Q  Y  R  S  L  P  R  P  S  K  S  S  S  R

3601 AGTGGAGCTGGGAATAGATCTAGCACTAGTAGCATAGACTCCAACATAAGCAGCAAATCA 3660
     S  G  A  G  N  R  S  S  T  S  S  I  D  S  N  I  S  S  K  S

3661 GCTGGGTTGCCTGTCCCTAAAATGAGAGAGCCTGCCAAGGTAATTCTTGGAAGCTCTCTG 3720
     A  G  L  P  V  P  K  M  R  E  P  A  K  V  I  L  G  S  S  L

3721 CCAGGATTAGTCAATCAGACTGATAAAGAGAAAGGGATTTCGTCTGACAACGAAAGCGTG 3780
     P  G  L  V  N  Q  T  D  K  E  K  G  I  S  S  D  N  E  S  V

3781 GCCTCATGTAATTCTGTTAAAGTGAACCCTGCATCACAGACTGCTTCTAGTGGAGCTCAA 3840
     A  S  C  N  S  V  K  V  N  P  A  S  Q  T  A  S  S  G  A  Q

3841 AGTACTCACCAGCAAGGAGCCAAGTACCCTGATGTGGCCTCTCCCACTTTGCGCAGACTT 3900
     S  T  H  Q  Q  G  A  K  Y  P  D  V  A  S  P  T  L  R  R  L

3901 TTTGGTGGAAAGCCTAGTAAACAAGTTCCCATCACAACAGCAGAAAATATGAAAAATTCA 3960
     F  G  G  K  P  S  K  Q  V  P  I  T  T  A  E  N  M  K  N  S

3961 GTAGTCATCTCCAATCCTCATGCTACTATGAACCAGCAGGGTAATCTTGATTCACCATCT 4020
     V  V  I  S  N  P  H  A  T  M  N  Q  Q  G  N  L  D  S  P  S

4021 GGCAGTGGTATACTAAGCAGTGGGGGCAGCAGTCCTCTCTATAGTAAAAACACAGATTTG 4080
     G  S  G  I  L  S  S  G  G  S  S  P  L  Y  S  K  N  T  D  L

4081 AACCAGTCTCCACTAGCTTCTAGTCCCAGTTCTGCACATTCAGCTCCTTCCAACAGTTTA 4140
     N  Q  S  P  L  A  S  S  P  S  S  A  H  S  A  P  S  N  S  L

4141 ACATGGGGCACCAACGCAAGTAGCTCTTCAGCTGTTAGCAAGGATGGCATTGGCTATCAG 4200
     T  W  G  T  N  A  S  S  S  S  A  V  S  K  D  G  I  G  Y  Q

4201 TCTGTCAGCAGTCTTCATACCAGCTGTGAATCCATTGATATCTCTCTGAGCAGTGGAGGT 4260
     S  V  S  S  L  H  T  S  C  E  S  I  D  I  S  L  S  S  G  G

4261 GGGCTGAGCCATAACTCCTCCGGTAGCTTGATTCCAGCCTCTAAAGATGATTCTCTGACT 4320
     G  L  S  H  N  S  S  G  S  L  I  P  A  S  K  D  D  S  L  T

4321 CCCTTTGTCCGAACCAACAGTGTTAAGACCACACTGTCTGAAAGGTATACTCCTTCCTCC 4380
     P  F  V  R  T  N  S  V  K  T  T  L  S  E  R  Y  T  P  S  S

4381 CAACTTCGTAGCCAGGAAGATGCAAAAGAATGGCTACGGTCACATTCAGCAGGAGGGCTC 4440
     Q  L  R  S  Q  E  D  A  K  E  W  L  R  S  H  S  A  G  G  L

4441 CAGGACACTGCTGGCAATTCTCCATTTTCATCAGGATCCAGCATAACATCACCTTCTGGA 4500
     Q  D  T  A  G  N  S  P  F  S  S  G  S  S  I  T  S  P  S  G

4501 ACTAGATTTAACTTCTCCCAGCTTGCAAGCCCAACCACTGCAGCCCAGATGAGCTTGTCA 4560
     T  R  F  N  F  S  Q  L  A  S  P  T  T  A  A  Q  M  S  L  S

4561 AATCCAACCATGCTGCGGACCCATAGCCTTTCCAATGCAGATGGCCCCTATGACCCCTAT 4620
     N  P  T  M  L  R  T  H  S  L  S  N  A  D  G  P  Y  D  P  Y

4621 AGTGACACACGCTTCAGGAACAGCTCCATGTCCTTGGACGAGAAGAGCAGAACAATGAGC 4680
     S  D  T  R  F  R  N  S  S  M  S  L  D  E  K  S  R  T  M  S

4681 CGATCTGGCTCGTTCCGTGATGGCTTTGAAGAAGTGCATGGTTCTTCTCTCTCTTTGGTA 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  CTACGAAGAGAGTTGGATGCATCCCAAGAGAAAGTATCAGCTCTGACAACTCAGCTGACT  4860
      L  R  R  E  L  D  A  S  Q  E  K  V  S  A  L  T  T  Q  L  T

4861  GCGAATGCCCACCTTGTGGCAGCATTTGAGCAGAGTCTGGGGAACATGACGATCAGACTG  4920
      A  N  A  H  L  V  A  A  F  E  Q  S  L  G  N  M  T  I  R  L

4921  CAGAGCCTCACCATGACAGCTGAACAAAAGGACTCTGAACTGAATGAGCTAAGGAAGACT  4980
      Q  S  L  T  M  T  A  E  Q  K  D  S  E  L  N  E  L  R  K  T

4981  ATTGAACTACTGAAGAAGCAAAATGCTGCTGCCCAGGCTGCCATTAATGGAGTCATCAAC  5040
      I  E  L  L  K  K  Q  N  A  A  A  Q  A  A  I  N  G  V  I  N

5041  ACACCTGAGCTCAACTGCAAAGGAACTGGAGCTGCTCAACCCACAGACTTGCGGATCCGA  5100
      T  P  E  L  N  C  K  G  T  G  A  A  Q  P  T  D  L  R  I  R

5101  AGACAGCACTCTTCGGATAGCGTCTCCAGCATTAACAGTGCTACCAGCCACTCTAGCGTG  5160
      R  Q  H  S  S  D  S  V  S  S  I  N  S  A  T  S  H  S  S  V

5161  GGAAGCAACATAGAGAGTGATTCAAAGAAAAAGAAGAGGAAGAACTGGGTCAATGAGTTA  5220
      G  S  N  I  E  S  D  S  K  K  K  K  R  K  N  W  V  N  E  L

5221  CGCAGCTCCTTCAAGCAAGCTTTTGGTAAAAAGAAGTCTCCCAAGTCAGCATCTTCTCAT  5280
      R  S  S  F  K  Q  A  F  G  K  K  K  S  P  K  S  A  S  S  H

5281  TCGGATATTGAGGAGATGACAGATTCTTCATTACCTTCATCACCAAAGCTACCACACCAT  5340
      S  D  I  E  E  M  T  D  S  S  L  P  S  S  P  K  L  P  H  H

5341  AACTCTACCGTTTCTACACCATTGCTGAGAGCTTCTCATTCCAATTCTCTTATTTCTGAA  5400
      N  S  T  V  S  T  P  L  L  R  A  S  H  S  N  S  L  I  S  E

5401  TGCACAGACAGTGAAGCTGAAACAGTCATGCAGTTACGCAATGAACTAAGAGACAAGGAG  5460
      C  T  D  S  E  A  E  T  V  M  Q  L  R  N  E  L  R  D  K  E

5461  ATGAAGTTGACTGACATTCGTCTAGAAGCCCTTAGCTCTGCTCATCAGCTTGACCAGCTT  5520
      M  K  L  T  D  I  R  L  E  A  L  S  S  A  H  Q  L  D  Q  L

5521  CGGGAGGCAATGAACAGAATGCAGAGTGAAATTGAGAAGTTAAAAGCAGAAAATGATCGA  5580
      R  E  A  M  N  R  M  Q  S  E  I  E  K  L  K  A  E  N  D  R

5581  CTGAAGTCTGAAAACCACAGCAGCTGTAGCAGGGCTCAGTCTCAGGCTTCCATTTCATCC  5640
      L  K  S  E  N  H  S  S  C  S  R  A  Q  S  Q  A  S  I  S  S

5641  TCTCCAAGACATTCAGTGGGTCTCTCTCAACACAGTTTGAACCTCACAGAGTCAACTAGT  5700
      S  P  R  H  S  V  G  L  S  Q  H  S  L  N  L  T  E  S  T  S

5701  CTCGACATGCTGTTAGATGACACTGGTGATGGCTCTGCCCGGAAGGAAGGAGGCAGACAT  5760
      L  D  M  L  L  D  D  T  G  D  G  S  A  R  K  E  G  G  R  H

5761  GTCAAAATAGTTGTCAGTTTTCAGGATGAAATGAAATGGAAGGAGGATTCAAGGCCGCGT  5820
      V  K  I  V  V  S  F  Q  D  E  M  K  W  K  E  D  S  R  P  R

5821  ACCTTCCTCATAGGTTGCATTGGAGTGAGCGGGAAGACCAAATGGGATGTTCTGGATGGT  5880
      T  F  L  I  G  C  I  G  V  S  G  K  T  K  W  D  V  L  D  G

5881  GTTGTTAGACGGCTGTTTAAGGAGTACATCATTCACGTGGATCCAGTGAGTCAGCTGGGG  5940
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Figure 1. (*continued*).

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

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

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

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

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

6181  GGCCCCAGTGGCACTGGTAAAACATACCTAGCAAACCGGCTCTCTGAGTATATGGTCCTG  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  GCTGTAGATATGCCTCTTGTAATTATTTTGGACAACTTGCATCATGTTAGCTCCCTAGGA  6420
      A  V  D  M  P  L  V  I  I  L  D  N  L  H  H  V  S  S  L  G

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

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

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

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

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

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

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

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

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

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

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

Figure 1. (*continued*).

```
7081  GCAGCCAACTACTCAAGTCCCCAGAGTTACGACAGTGACTCTAACAGCAACAGCCATCAC   7140
      A  A  N  Y  S  S  P  Q  S  Y  D  S  D  S  N  S  N  S  H  H

7141  GATGACATACTCGATTCATCTCTGGAATCAACGTTGTGA   7179
      D  D  I  L  D  S  S  L  E  S  T  L  *
```

Figure 1. (*continued*).

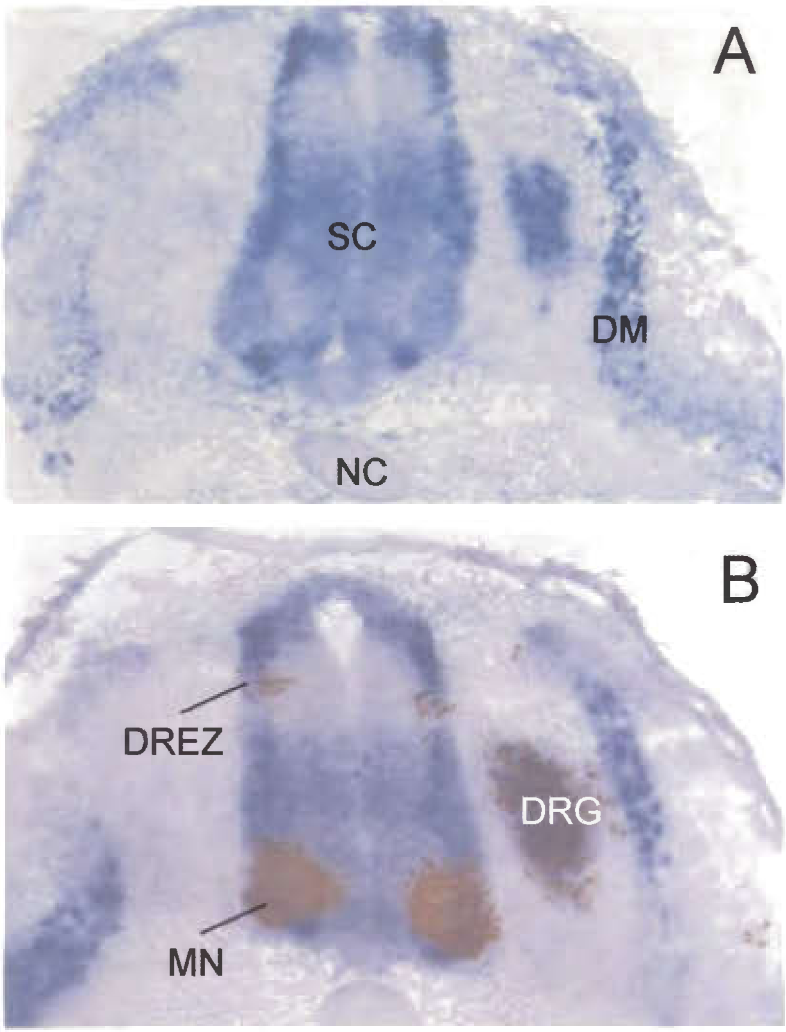

Figure 2. Expression of the *Nav2s* gene in the chick embryo at stage 22. (A) Transverse sections of stage 22 chick embryos were hybridized with the chick *Nav2s* probe. The chick *Nav2s* signal was shown in blue. (B) Staining with the anti-Islet-1/2 antibody (brown) was done to show DRG neurons and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

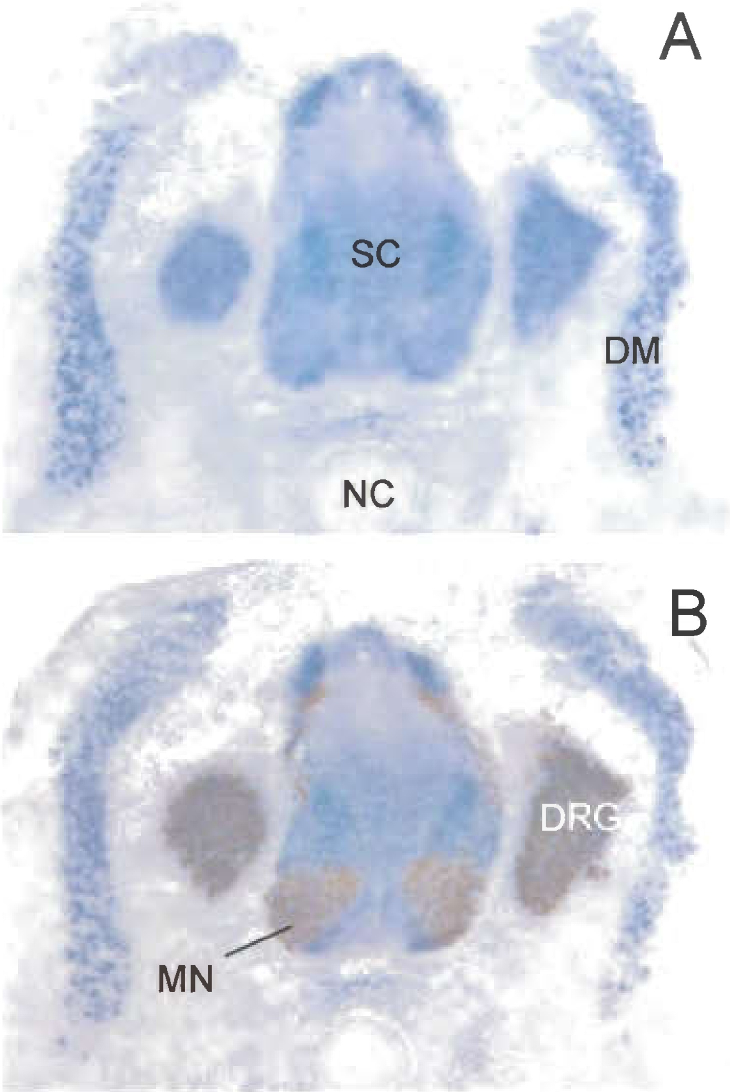

Figure 3. Expression of the *Nav2s* gene in the chick embryo at stage 26. (A) Transverse sections of stage 26 chick embryos were hybridized with the chick *Nav2s* probe. The chick *Nav2s* signal was shown in blue. (B) Staining with the anti-Islet-1/2 antibody (brown) was done to show DRG neurons and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