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TECHNOLOGY REPORT

A Cre Transgenic Line for Studying V2 Neuronal Lineages and Functions in the Spinal Cord

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Summary: During spinal neurogenesis, the p2 progenitor domain generates at least two subclasses of interneurons named V2a and V2b, which are components of the locomotor central pattern generator. The winged-helix/forkhead transcription factor Foxn4 is expressed in a subset of p2 progenitors and required for specifying V2b interneurons. Here, we report the generation of a Foxn4-Cre BAC transgenic mouse line that drives Cre recombinase expression mimicking endogenous *Foxn4* expression pattern in the developing spinal cord. We used this transgenic line to map neuronal lineages derived from Foxn4-expressing progenitors and found that they gave rise to all neurons of the V2a, V2b, and the newly identified V2c lineages. These data suggest that Foxn4 may be transiently expressed by all p2 progenitors and that the Foxn4-Cre line may serve as a useful genetic tool not only for lineage analysis but also for functional studies of genes and neurons involved in locomotion. genesis 48:667–672, 2010. © 2010 Wiley-Liss, Inc.

Key words: Foxn4; transcription factor; V2 interneuron; CPG

Spinal cord development begins with the formation of the neural tube, followed by the generation of distinct neuronal and glial cell types in the tube, and concludes with the establishment of functional sensory and motor circuits in the mature cord. The neuronal fates along the dorsoventral axis of the neural tube are controlled, in part, by signaling molecules secreted from the dorsal and ventral midlines in a concentration-dependent manner (Briscoe *et al.*, 1999, 2000; Ericson *et al.*, 1997a,b; Jessell, 2000; Tanabe and Jessell, 1996). For instance, the opposing activity gradients of Shh and BMPs/Wnts are shown to activate the expression of transcription factors (Lee *et al.*, 1998, 2000; Liem *et al.*, 1997, 2000; Mekki-Dauriac *et al.*, 2002; Patten and Placzek, 2002), which, in turn, define different progenitor domains from which distinct neuronal cell types arise (Briscoe *et al.*, 2000; Caspary and Anderson, 2003; Jessell, 2000; Lee and Jessell, 1999; Shirasaki and Pfaff, 2002).

In the ventral neural tube, the ventricular zone is organized into one motor neuron (MN) progenitor domain and four interneuron progenitor domains (p0–p3) that give rise to MNs and several classes of interneurons (V0–V3), respectively (Briscoe *et al.*, 2000; Burrill *et al.*, 1997; Ericson *et al.*, 1997a; Matise and Joyner, 1997; Pierani *et al.*, 1999). Each of the progenitor domains is defined by the combinatorial activities of a set of transcription factors, in particular, the homeoproteins and basic helix–loop–helix factors (Briscoe *et al.*, 2000; Jessell, 2000; Mizuguchi *et al.*, 2001; Muroyama *et al.*, 2005; Novitch *et al.*, 2001; Pierani *et al.*, 1999; Shirasaki and Pfaff, 2002; Zhou *et al.*, 2000, 2001). The p2 domain generates at least two molecularly distinct interneuron subclasses named V2a and V2b (Briscoe *et al.*, 2000; Karunaratne *et al.*, 2002; Li *et al.*, 2005; Muroyama *et al.*, 2005). V2a cells are mostly glutamatergic excitatory neurons defined by their expression of Chx10, whereas V2b cells are mostly inhibitory GABAergic/glycinergic neurons characterized by their expression of Gata2 and Gata3 (Al-Mosawie *et al.*, 2007; Briscoe *et al.*, 2000; Karunaratne *et al.*, 2002; Lundfald *et al.*, 2007). The binary V2a and V2b fates are selected by Dll-Notch signaling and consolidated by the activity of V2a- and V2b-specific LIM protein complexes involving Lhx3, LMO4, NLI, Scl, and Gata2 (Del Barrio *et al.*, 2007; Joshi *et al.*, 2009; Peng *et al.*, 2007). Although the V2b function is currently unknown, V2a neurons have been shown recently to control speed-dependent left–right locomotor coordination (Crone *et al.*, 2008, 2009).

Foxn4 is a winged-helix/forkhead transcription factor transiently expressed in a subset of progenitors during spinal neurogenesis and retinogenesis. It has been shown by gene targeting and gain-of-function analyses to play a crucial role in the generation of amacrine and hor-

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izontal cells during retinal development (Li *et al.*, 2004). In the spinal cord, Foxn4 is expressed by a subset of cells in the p2 progenitor domain (Li *et al.*, 2005). *Foxn4* inactivation causes a complete loss of V2b neurons, with a concomitant fate-switch to V2a neurons, whereas its misexpression promotes the V2b fate (Del Barrio *et al.*, 2007; Li *et al.*, 2005), thus demonstrating both the necessity and sufficiency for Foxn4 to promote V2b neuron differentiation. Foxn4 acts genetically upstream of the basic helix–loop–helix factors Mash1 and Scl, both of which are required for specifying the V2b fate (Li *et al.*, 2005; Muroyama *et al.*, 2005), as well as controls the expression of Dll4, a ligand for Notch signaling (Del Barrio *et al.*, 2007).

Delineation of neuronal lineages is important for providing a framework to understand the molecular basis of neurogenesis. Our previous immunostaining and knock-in analyses have localized Foxn4 to a subset of p2 progenitors and identified a subset of V2a and V2b cells as the progeny of Foxn4-expressing progenitors (Del Barrio *et al.*, 2007; Li *et al.*, 2005), implicating the expression of Foxn4 only by a subset of p2 progenitors. However, because of the transient nature of Foxn4 expression, a possibility exists that Foxn4 may be transiently expressed by all p2 progenitors, which give rise to all V2a and V2b cells. Thus, the neural lineages of Foxn4-expressing progenitors have yet to be clearly described during spinal cord development. In this work, we generated a Foxn4-Cre BAC transgenic mouse line and used the Cre-loxP fate-mapping strategy to determine the cell lineages derived from Foxn4-expressing progenitors in the ventral spinal cord. Our results indicate that the Foxn4-Cre transgene can drive Cre recombinase expression mimicking that of the endogenous *Foxn4* in the developing spinal cord and, therefore, should be suitable for conditional deletion or ablation of genes and neurons in the V2 domain.

RESULTS AND DISCUSSION

To generate the Foxn4-Cre mice, a BAC containing the *Foxn4* locus was modified by recombineering in *E. coli* to insert the *Cre* coding region at the Foxn4 translation initiation site (Fig. 1a) (Copeland *et al.*, 2001; Gong *et al.*, 2002; Warming *et al.*, 2005; Yang *et al.*, 2006). This BAC transgenic construct contains ~125 kb 5′ flanking sequence, all exons and introns, and ~90 kb 3′ flanking sequence (Fig. 1a), thereby increasing the likelihood of sufficient regulatory information to recapitulate the endogenous *Foxn4* expression pattern as demonstrated for many other genes (Battiste *et al.*, 2007; Heintz, 2001; Yang *et al.*, 1997). We obtained three founder lines for this transgenic construct, and all of them displayed a similar Cre expression pattern in the developing ventral spinal cord (Fig. 1c–g).

To ask whether Foxn4-Cre can mimic the spatial expression pattern of the endogenous *Foxn4* gene (Fig. 1b), we carried out double-immunolabeling between Cre and markers for p2/V2 and adjacent domains. In the ventral spinal cord of E10.5 Foxn4-Cre transgenic embryos, Cre was seen in clusters of cells at the p2/V2 position, many of which co-expressed Foxn4, Chx10, or Gata2 (Fig. 1c–e). The presence of Cre in $Chx10^+$ and $Gata2^+$ cells is likely because of the perdurability of Cre because Foxn4 is normally expressed only in progenitors but not in V2a and V2b interneurons (Li *et al.*, 2005). This perdurability may also explain why Cre is present in more cells than Foxn4 (Fig. 1c). There was no expression of Cre in $En1^+$ V1 cells or in $Isl1/2^+$ MNs (Fig. 1f,g). Thus, the Foxn4-Cre transgene seems to be able to largely recapitulate the expression pattern of the endogenous *Foxn4* gene in the developing spinal cord. However, given the presence of Cre in more numerous cells than Foxn4, there is still a possibility that the BAC used may not contain all the regulatory elements necessary to completely mimic endogenous *Foxn4* expression.

To determine the neuronal offspring of Foxn4-expressing progenitors, we crossed the Foxn4-Cre line with the conditional reporter R26R-YFP mice, in which all cells would constitutively express yellow fluorescent protein (YFP) after a Cre-mediated recombination event to remove a transcription stop cassette (Fig. 1a) (Srinivas *et al.*, 2001). When spinal cord sections from E14.5 *Foxn4-Cre;R26R-YFP* embryos were double-labeled with an anti-green fluorescent protein (GFP) antibody and the nuclear marker 4′,6-diamidino-2-phenylindole, we found that Foxn4-expressing progenitors gave rise to many YFP^+ cells scattered within the ventral gray matter, with their axons projected into the ventrolateral funiculus (Fig. 2a,e). The ventral commissure seemed to be largely devoid of YFP^+ processes (Fig. 2a,e), consistent with the finding that V2 interneurons are mostly noncommissural neurons that project ipsilaterally (Al-Mosawie *et al.*, 2007; Lundfald *et al.*, 2007).

Double-immunostaining of E14.5 spinal cord sections with antibodies against V2 markers revealed the expression of YFP in all $Chx10^+$ and $Gata2^+$ cells (Figs. 2a–h and 3a), indicating that all V2a and V2b interneurons arise from Foxn4-expressing progenitors. However, only about 37.2% and 25.5% of YFP^+ cells co-expressed Chx10 and Gata2, respectively (Figs. 2a–h and 3b), suggesting that many V2 interneurons may completely downregulate the expression of V2 markers by E14.5 and/or Foxn4 may be expressed in additional unidentified V2 interneurons or glial cells. In spinal cord sections of E12.5 *Foxn4-Cre;R26R-YFP* embryos, we investigated whether YFP^+ cells co-express Sox1, which is found in a small subset of interneurons that are derived from the p2 domain and designated as V2c (Panayi *et al.*, 2010). As shown in Fig. 2i–l and by quantification (Fig. 3a), all ventrolaterally located $Sox1^+$ cells were immunoreactive for GFP, demonstrating that Foxn4-expressing progenitors also give rise to all $Sox1^+$ V2c interneurons. Approximately 4% of all YFP^+ cells co-expressed Sox1 in the ventrolateral area (Fig. 3b), consistent with V2c cells forming a very small subclass of V2 interneurons (Panayi *et al.*, 2010). By contrast, MNs are not generated from

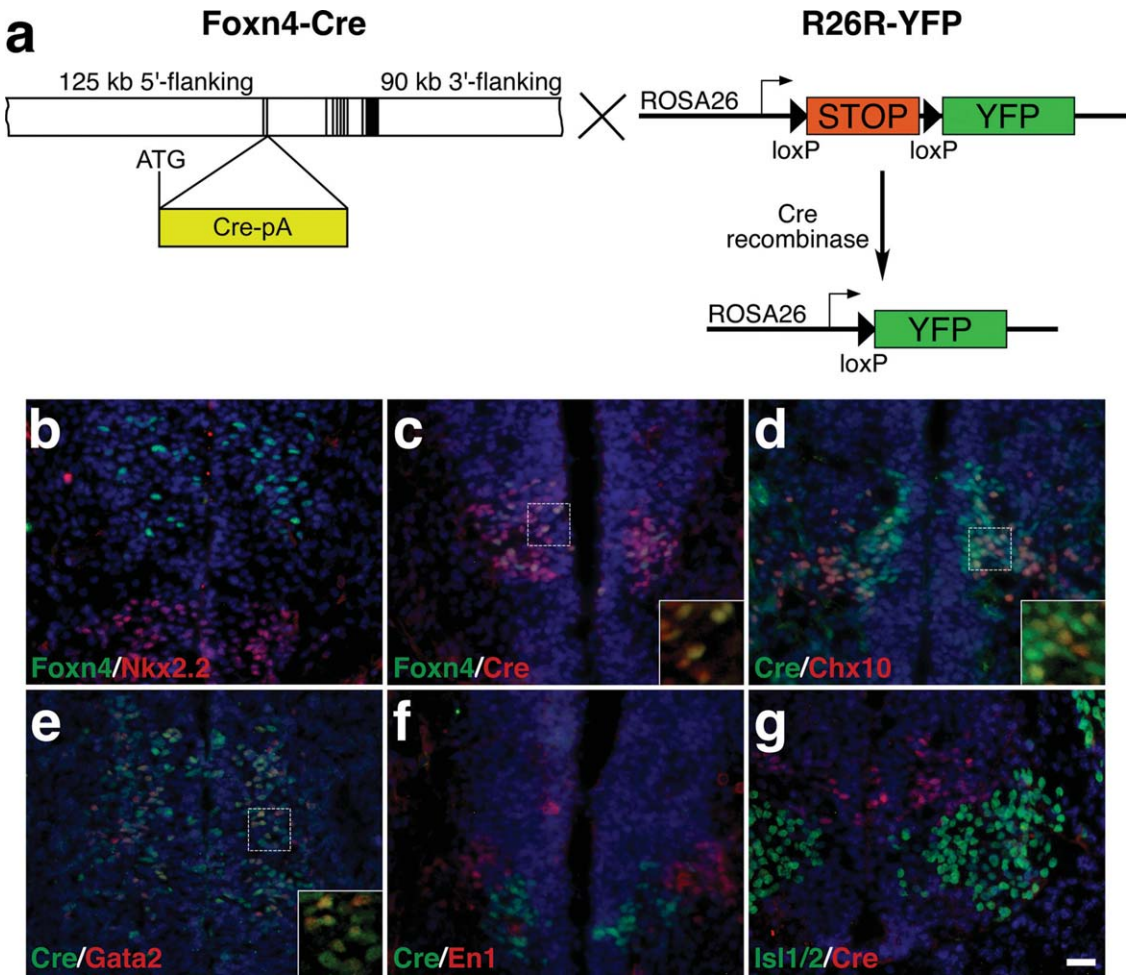

FIG. 1. Generation of Foxn4-Cre transgenic mice that express Cre recombinase in the p2/V2 domain of the spinal cord. (**a**) The Cre-loxP system for conditional activation of reporter YFP expression using Foxn4-Cre and R26R-YFP mice. To generate the Foxn4-Cre BAC, a BAC containing the *Foxn4* locus was modified by recombineering to insert the *Cre* coding region (Cre-pA) at the Foxn4 translation initiation site (ATG). The exons are indicated by vertical black bars, and the estimated lengths of 5′ and 3′ flanking sequences are also indicated. The transgenic line was crossed with R26R-YFP mice to activate YFP expression in the spinal cord. (**b–g**) Spinal cord sections from E10.5 wild-type (b) and Foxn4-Cre (c–g) embryos were labeled by double-immunofluorescence using the indicated antibodies and weakly counterstained with nuclear 4′,6-diamidino-2-phenylindole. There is colocalization between Cre and Foxn4, Chx10 and Gata2 in cells of the p2/V2 domain but no colocalization between Cre and En1 or Isl1/2. Insets in (c–e) show corresponding outlined regions at a higher magnification. Scale bar equals 25 μm (b–g).

the p2 progenitor domain, and, correspondingly, we did not observe YFP^+ cells that co-expressed the MN marker Isl1/2 (Fig. 2m–p).

Foxn4 is seen only in a limited number of p2 progenitors at a given stage during spinal cord development (Fig. 1b,c) (Li *et al.*, 2005). Moreover, the progeny marked by β-gal expressed from the knock-in *lacZ* reporter in $Foxn4^{lacZ/+}$ spinal cords encompasses only a proportion of V2a and V2b interneurons (Del Barrio *et al.*, 2007; Li *et al.*, 2005). These observations raise questions about whether all p2 progenitors transiently express Foxn4 and whether Foxn4-expressing progenitors give rise to all V2 interneurons or only a subset. Our lineage analyses suggest that all V2a, V2b, and V2c interneurons arise from p2 progenitors that express Foxn4 at some point in life. Consistent with this, there is a complete absence of V2b and V2c neurons in the spinal cord of *Foxn4* null mutant mice (Li *et al.*, 2005; Panayi *et al.*, 2010). Thus, despite the apparent expression of Foxn4 only in a subset of p2 progenitors (Li *et al.*, 2005), a broader than anticipated complement of V2 interneurons arise from Foxn4-expressing progenitors. Similarly, a recent fate-mapping study has revealed that Ascl1-expressing progenitors in the spinal cord generate oligodendrocytes more extensively than previously appreciated (Battiste *et al.*, 2007).

V2 neurons constitute part of the locomotor central pattern generator (CPG) network in the spinal cord, which includes also V0, V1, and V3 interneurons and MNs (Goulding, 2009). The CPG is an intrinsic neural

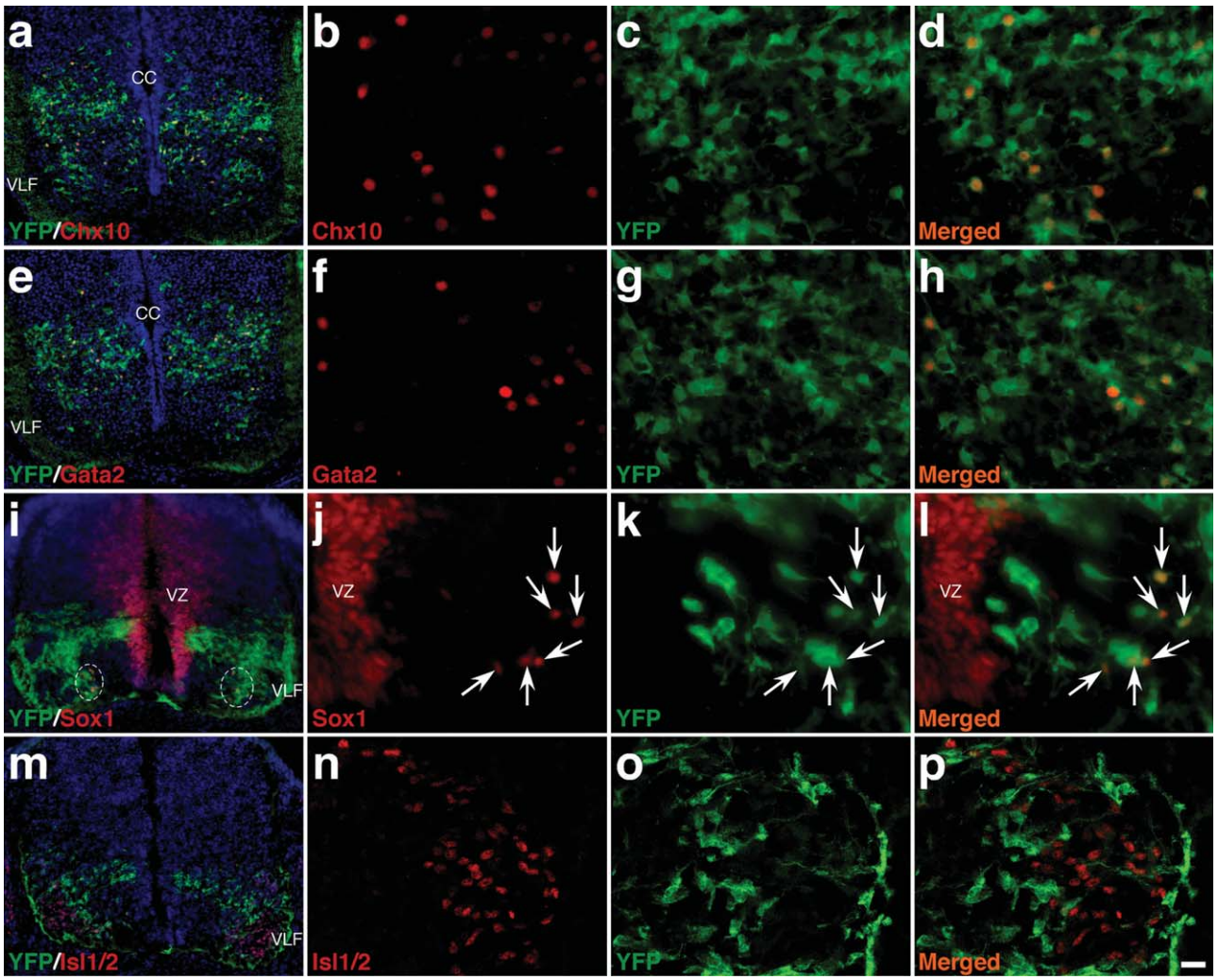

FIG. 2. Foxn4-expressing cells give rise to all interneurons of the three known V2 lineages. (**a–p**) Spinal cord sections from E14.5 (a–h) and E12.5 (i–p) *Foxn4-Cre;R26R-YFP* embryos were stained by double-immunofluorescence using the indicated antibodies. Sections in a, e, i, and m were also weakly counterstained with 4′,6-diamidino-2-phenylindole. Note the colocalization between YFP and Chx10 in V2a neurons (a–d), between YFP and Gata2 in V2b neurons (e–h), and between YFP and Sox1 in V2c neurons (indicated by arrows) (i–l). YFP and Isl1/2 are not colocalized in motor neurons (m–p). The dashed ovals in (i) outline the regions where Sox1-immunoreactive V2c cells are located. CC, central canal; VLF, ventrolateral funiculus; VZ, ventricular zone. Scale bar equals 50 (a, e, i, and m), 12.5 (b–d, f–h, and j–l), and 12 μm (n–p).

network capable of generating rhythmic patterns of motor activity without sensory inputs. At present, the developmental, cellular, and physiological events leading to locomotor rhythm still remain poorly defined. Gene-targeting experiments have shown that V0 neurons are essential for proper coordination of left–right locomotor activity (Lanuza *et al.*, 2004). Genetic ablation of V1 cells demonstrates a key role for these interneurons in the generation of fast locomotor outputs (Gosgnach *et al.*, 2006). V2a cells are mostly glutamatergic excitatory interneurons (Al-Mosawie *et al.*, 2007; Lundfald *et al.*, 2007). Selective ablation of V2a interneurons by diphtheria toxin results in disrupted left–right coordination at high speeds and increased variability in frequency and amplitude of locomotor rhythm (Crone *et al.*, 2008, 2009). This subclass of interneurons is, therefore, required for maintaining left–right alternation at high speeds of locomotion as well as for stabilizing the locomotor rhythm. V2b cells, on the other hand, are mostly GABAergic/glycinergic inhibitory interneurons (Al-Mosawie *et al.*, 2007; Lundfald *et al.*, 2007). It is currently unknown how the V2b and V2c subclasses of V2 interneurons participate in the CPG network. In this regard, our Foxn4-Cre transgenic line should provide a useful genetic tool not only for lineage analysis but also for domain- and neuron-specific gene manipulation to study functions of genes and neurons in the V2 domain.

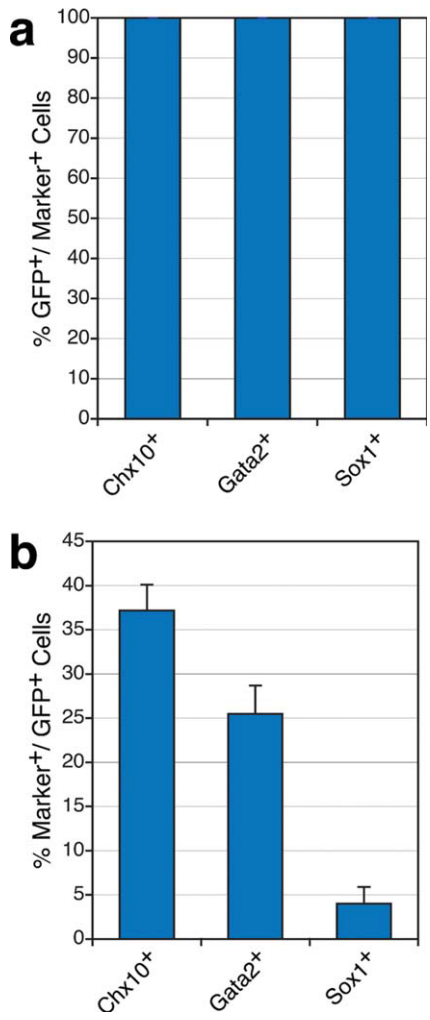

FIG. 3. Percentages of V2 marker-positive cells that are immunoreactive for GFP (**a**), and GFP-positive cells that are immunoreactive for V2 cell markers (**b**) in *Foxn4-Cre;R26R-YFP* spinal cords. Each histogram in (b) represents the mean ± SD for three different spinal cords. Note that $Chx10^+$ and $Gata2^+$ cells were counted at E14.5, but counts of $Sox1^+$ cells were obtained at E12.5 when these cells could be optimally labeled. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

MATERIALS AND METHODS

Generation of Transgenic Mice by BAC Recombineering

A *Foxn4* BAC (Clone-ID: RP23-154G3) was purchased from Invitrogen (Carlsbad, CA). The Foxn4-Cre BAC transgenic construct was prepared primarily following the online BAC recombineering protocols (http://web.ncifcrf.gov/research/brb/recombineeringInformation.aspx) (Copeland *et al.*, 2001). Two adapters located in the exon with the translation start site were used for homologous recombination, which resulted in the immediate control of *Cre* gene under the endogenous *Foxn4* promoter without any extra sequence. The two adapters are from two pairs of oligos: 5′ end: 5′ CCGCTCGA GATTGTCCTGCAATTGTGACCCTTTTGG 3′ and 5′ CGC AAGCTTATTTCCTTAGGGATGAAAAAAAAAAG 3′ and 3′ end: 5′ CGGACTAGTATAGAAAGTGGCATTTGGTCCA GAATG 3′ and 5′ TCCCCGCGGGTATTCCTGTGGAGAG CAGTGGTGG 3′. Three Foxn4-Cre transgenic founder lines were produced at the transgenic/knockout core facility of the Cancer Institute of New Jersey using F1 (C57BL/6xCBA) with the finished construct. All three lines exhibited a similar reporter expression pattern in the spinal cord, and the final analysis was mainly based on one line. The conditional reporter R26R-YFP mice were purchased from the Jackson Laboratory (Srinivas *et al.*, 2001).

Immunofluorescence

Staged mouse embryos were collected and fixed with 4% paraformaldehyde in phosphate-buffered saline and processed for cryosections. Immunofluorescent staining of cryosections was then carried out as previously described (Li *et al.*, 2004). The antibodies used in the immunostaining analysis are anti-GFP (for YFP) (goat, 1:1,000; Abcam, Cambridge, MA), anti-GFP (for YFP) (rabbit, 1:400; MBL International, Woburn, MA), anti-Foxn4 (rabbit, 1:50) (Li *et al.*, 2004), anti-Cre (mouse, 1:1,000; Covance, Princeton, NJ), anti-Cre (rabbit, 1:10,000; EMD Chemicals, Gibbstown, NJ), anti-Chx10 (sheep, 1:1,600; Exalpha Biologicals, Shirley, MA), anti-Gata2 (rabbit, 1:200, Santa Cruz Biotechnology, Santa Cruz, CA), anti-Gata2 (guinea pig, 1:2,000) (Peng *et al.*, 2007), anti-En1 (mouse, 1:25; Developmental Studies Hybridoma Bank [DSHB]), anti-Nkx2.2 (mouse, 1:50; DSHB), anti-Isl1 (mouse, 1:50; DSHB, Iowa City, Iowa), and anti-Sox1 (guinea pig, 1:500) (Panayi *et al.*, 2010).

Quantification

To quantify YFP^+ cells and YFP^+ cells colocalized with cell type-specific markers in the spinal cord, three comparable samples of each type were counted for positively labeled cells on serial semisections located at the thoracic region. The results were analyzed statistically.

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LITERATURE CITED

Al-Mosawie A, Wilson JM, Brownstone RM. 2007. Heterogeneity of V2-derived interneurons in the adult mouse spinal cord. Eur J Neurosci 26:3003–3015.

Battiste J, Helms AW, Kim EJ, Savage TK, Lagace DC, Mandyam CD, Eisch AJ, Miyoshi G, Johnson JE. 2007. Ascl1 defines sequentially generated lineage-restricted neuronal and oligodendrocyte precursor cells in the spinal cord. Development 134:285–293.

Briscoe J, Pierani A, Jessell TM, Ericson J. 2000. A homeodomain protein code specifies progenitor cell identity and neuronal fate in the ventral neural tube. Cell 101:435–445.

Briscoe J, Sussel L, Serup P, Hartigan-O'Connor D, Jessell TM, Rubenstein JL, Ericson J. 1999. Homeobox gene *Nkx2.2* and specification of neuronal identity by graded sonic hedgehog signalling. Nature 398:622–627.

Burrill JD, Moran L, Goulding MD, Saueressig H. 1997. PAX2 is expressed in multiple spinal cord interneurons, including a population of $EN1^+$ interneurons that require PAX6 for their development. Development 124:4493–4503.

Caspary T, Anderson KV. 2003. Patterning cell types in the dorsal spinal cord: What the mouse mutants say. Nat Rev Neurosci 4:289–297.

Copeland NG, Jenkins NA, Court DL. 2001. Recombineering: A powerful new tool for mouse functional genomics. Nat Rev Genet 2:769–779.

Crone SA, Quinlan KA, Zagoraiou L, Droho S, Restrepo CE, Lundfald L, Endo T, Setlak J, Jessell TM, Kiehn O, Sharma K. 2008. Genetic ablation of V2a ipsilateral interneurons disrupts left-right locomotor coordination in mammalian spinal cord. Neuron 60:70–83.

Crone SA, Zhong G, Harris-Warrick R, Sharma K. 2009. In mice lacking V2a interneurons, gait depends on speed of locomotion. J Neurosci 29:7098–7109.

Del Barrio MG, Taveira-Marques R, Muroyama Y, Yuk DI, Li S, Wines-Samuelson M, Shen J, Smith HK, Xiang M, Rowitch D, Richardson WD. 2007. A regulatory network involving Foxn4. Mash1 and delta-like 4/Notch1 generates V2a and V2b spinal interneurons from a common progenitor pool. Development 134:3427–3436.

Ericson J, Briscoe J, Rashbass P, van Heyningen V, Jessell TM. 1997a. Graded sonic hedgehog signaling and the specification of cell fate in the ventral neural tube. Cold Spring Harb Symp Quant Biol 62:451–466.

Ericson J, Rashbass P, Schedl A, Brenner-Morton S, Kawakami A, van Heyningen V, Jessell TM, Briscoe J. 1997b. Pax6 controls progenitor cell identity and neuronal fate in response to graded Shh signaling. Cell 90:169–180.

Gong S, Yang XW, Li C, Heintz N. 2002. Highly efficient modification of bacterial artificial chromosomes (BACs) using novel shuttle vectors containing the R6Kgamma origin of replication. Genome Res 12:1992–1998.

Gosgnach S, Lanuza GM, Butt SJ, Saueressig H, Zhang Y, Velasquez T, Riethmacher D, Callaway EM, Kiehn O, Goulding M. 2006. V1 spinal neurons regulate the speed of vertebrate locomotor outputs. Nature 440:215–219.

Goulding M. 2009. Circuits controlling vertebrate locomotion: Moving in a new direction. Nat Rev Neurosci 10:507–518.

Heintz N. 2001. BAC to the future: The use of BAC transgenic mice for neuroscience research. Nat Rev Neurosci 2:861–870.

Jessell TM. 2000. Neuronal specification in the spinal cord: Inductive signals and transcriptional codes. Nat Rev Genet 1:20–29.

Joshi K, Lee S, Lee B, Lee JW, Lee SK. 2009. LMO4 controls the balance between excitatory and inhibitory spinal V2 interneurons. Neuron 61:839–851.

Karunaratne A, Hargrave M, Poh A, Yamada T. 2002. GATA proteins identify a novel ventral interneuron subclass in the developing chick spinal cord. Dev Biol 249:30–43.

Lanuza GM, Gosgnach S, Pierani A, Jessell TM, Goulding M. 2004. Genetic identification of spinal interneurons that coordinate left-right locomotor activity necessary for walking movements. Neuron 42:375–386.

Lee KJ, Dietrich P, Jessell TM. 2000. Genetic ablation reveals that the roof plate is essential for dorsal interneuron specification. Nature 403:734–740.

Lee KJ, Jessell TM. 1999. The specification of dorsal cell fates in the vertebrate central nervous system. Annu Rev Neurosci 22:261–294.

Lee KJ, Mendelsohn M, Jessell TM. 1998. Neuronal patterning by BMPs: A requirement for GDF7 in the generation of a discrete class of commissural interneurons in the mouse spinal cord. Genes Dev 12:3394–3407.

Li S, Misra K, Matise MP, Xiang M. 2005. Foxn4 acts synergistically with Mash1 to specify subtype identity of V2 interneurons in the spinal cord. Proc Natl Acad Sci U S A 102:10688–10693.

Li S, Mo Z, Yang X, Price SM, Shen MM, Xiang M. 2004. Foxn4 controls the genesis of amacrine and horizontal cells by retinal progenitors. Neuron 43:795–807.

Liem KF Jr, Jessell TM, Briscoe J. 2000. Regulation of the neural patterning activity of sonic hedgehog by secreted BMP inhibitors expressed by notochord and somites. Development 127:4855–4866.

Liem KF Jr, Tremml G, Jessell TM. 1997. A role for the roof plate and its resident TGFbeta-related proteins in neuronal patterning in the dorsal spinal cord. Cell 91:127–138.

Lundfald L, Restrepo CE, Butt SJ, Peng CY, Droho S, Endo T, Zeilhofer HU, Sharma K, Kiehn O. 2007. Phenotype of V2-derived interneurons and their relationship to the axon guidance molecule EphA4 in the developing mouse spinal cord. Eur J Neurosci 26:2989–3002.

Matise MP, Joyner AL. 1997. Expression patterns of developmental control genes in normal and Engrailed-1 mutant mouse spinal cord reveal early diversity in developing interneurons. J Neurosci 17:7805–7816.

Mekki-Dauriac S, Agius E, Kan P, Cochard P. 2002. Bone morphogenetic proteins negatively control oligodendrocyte precursor specification in the chick spinal cord. Development 129:5117–5130.

Mizuguchi R, Sugimori M, Takebayashi H, Kosako H, Nagao M, Yoshida S, Nabeshima Y, Shimamura K, Nakafuku M. 2001. Combinatorial roles of olig2 and neurogenin2 in the coordinated induction of pan-neuronal and subtype-specific properties of motor neurons. Neuron 31:757–771.

Muroyama Y, Fujiwara Y, Orkin SH, Rowitch DH. 2005. Specification of astrocytes by bHLH protein SCL in a restricted region of the neural tube. Nature 438:360–363.

Novitch BG, Chen AI, Jessell TM. 2001. Coordinate regulation of motor neuron subtype identity and pan-neuronal properties by the bHLH repressor Olig2. Neuron 31:773–789.

Panayi H, Orford M, Panayiotou E, Genethliou N, Mean R, Lapathitis G, Li S, Xiang M, Kessaris N, Richardson WD, Malas S. 2010. SOX1 is required for the specification of a novel p2-derived interneuron subtype in the mouse ventral spinal cord. J Neurosci. (in press).

Patten I, Placzek M. 2002. Opponent activities of Shh and BMP signaling during floor plate induction in vivo. Curr Biol 12:47–52.

Peng CY, Yajima H, Burns CE, Zon LI, Sisodia SS, Pfaff SL, Sharma K. 2007. Notch and MAML signaling drives Scl-dependent interneuron diversity in the spinal cord. Neuron 53:813–827.

Pierani A, Brenner-Morton S, Chiang C, Jessell TM. 1999. A sonic hedgehog-independent, retinoid-activated pathway of neurogenesis in the ventral spinal cord. Cell 97:903–915.

Shirasaki R, Pfaff SL. 2002. Transcriptional codes and the control of neuronal identity. Annu Rev Neurosci 25:251–281.

Srinivas S, Watanabe T, Lin CS, William CM, Tanabe Y, Jessell TM, Costantini F. 2001. Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. BMC Dev Biol 1:4.

Tanabe Y, Jessell TM. 1996. Diversity and pattern in the developing spinal cord. Science 274:1115–1123.

Warming S, Costantino N, Court DL, Jenkins NA, Copeland NG. 2005. Simple and highly efficient BAC recombineering using galK selection. Nucleic Acids Res 33:e36.

Yang XW, Model P, Heintz N. 1997. Homologous recombination based modification in *Escherichia coli* and germline transmission in transgenic mice of a bacterial artificial chromosome. Nat Biotechnol 15:859–865.

Yang Z, Jiang H, Chachainasakul T, Gong S, Yang XW, Heintz N, Lin S. 2006. Modified bacterial artificial chromosomes for zebrafish transgenesis. Methods 39:183–188.

Zhou Q, Choi G, Anderson DJ. 2001. The bHLH transcription factor Olig2 promotes oligodendrocyte differentiation in collaboration with Nkx2.2. Neuron 31:791–807.

Zhou Q, Wang S, Anderson DJ. 2000. Identification of a novel family of oligodendrocyte lineage-specific basic helix-loop-helix transcription factors. Neuron 25:331–343.