RESEARCH COMMUNICATION

Ventral neural patterning by *Nkx* homeobox genes: *Nkx6.1* controls somatic motor neuron and ventral interneuron fates

Maike Sander,^{1,2,6} Sussan Paydar,^{3,6}
Johan Ericson,^{4,5} James Briscoe,⁴
Elizabeth Berber,³ Michael German,¹
Thomas M. Jessell,⁴ and John L.R. Rubenstein^{3,7}

¹Hormone Research Institute, Department of Medicine, University of California–San Francisco, San Francisco, California 94143 USA; ²Center for Molecular Neurobiology, Hamburg, Germany; ³Nina Ireland Laboratory of Developmental Neurobiology, University of California–San Francisco, San Francisco, California 94143 USA; ⁴Howard Hughes Medical Institute, Department of Biochemistry and Molecular Biophysics, Center for Neurobiology and Behavior, Columbia University, New York, New York 10032 USA; ⁵Department of Cell and Molecular Biology, Medical Nobel Institute, Karolinska Institute, S 17177 Stockholm, Sweden

There is growing evidence that sonic hedgehog (Shh) signaling regulates ventral neuronal fate in the vertebrate central nervous system through *Nkx*-class homeodomain proteins. We have examined the patterns of neurogenesis in mice carrying a targeted mutation in *Nkx6.1*. These mutants show a dorsal-to-ventral switch in the identity of progenitors and in the fate of postmitotic neurons. At many axial levels there is a complete block in the generation of V2 interneurons and motor neurons and a compensatory ventral expansion in the domain of generation of V1 neurons, demonstrating the essential functions of *Nkx6.1* in regional patterning and neuronal fate determination.

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During the development of the embryonic central nervous system (CNS) the mechanisms that specify regional identity and neuronal fate are intimately linked (Anderson et al. 1997; Lumsden and Krumlauf 1996; Rubenstein et al. 1998). In the ventral half of the CNS, for example, the secreted factor Sonic hedgehog (Shh) has a fundamental role in controlling both regional pattern and neuronal fate (Tanabe and Jessell 1996; Ericson et al. 1997a; Hammerschmidt et al. 1997). Shh appears to function as a gradient signal. In the spinal cord, five distinct classes of neurons can be generated in

vitro in response to two- to threefold changes in the concentration of Shh, and the position at which each neuronal class is generated in vivo is predicted by the concentration required for their induction in vivo (Ericson et al. 1997a,b; Briscoe et al. 2000). Thus, neurons generated in more ventral regions of the neural tube require progressively higher concentrations of Shh for their induction.

The genetic programs activated in neural progenitor cells in response to Shh signaling, however, remain incompletely defined. Emerging evidence suggests that homeobox genes function as critical intermediaries in the neural response to Shh signals (Lumsden and Krumlauf 1996; Tanabe and Jessell 1996; Ericson et al. 1997a,b; Hammerschmidt et al. 1997; Rubenstein et al. 1998). Several homeobox genes are expressed by ventral progenitor cells, and their expression is regulated by Shh. Gain-of-function studies on homeobox gene action in the chick neural tube have provided evidence that homeodomain proteins are critical for the interpretation of graded Shh signaling and that they function to delineate progenitor domains and control neuronal subtype identity (Briscoe et al. 2000). Consistent with these findings, the pattern of generation of neuronal subtypes in the basal telencephalon and in the ventral-most region of the spinal cord is perturbed in mice carrying mutations in certain Shh-regulated homeobox genes (Ericson et al. 1997b; Sussel et al. 1999; Pierani et al., unpubl.).

Members of the *Nkx* class of homeobox genes are expressed by progenitor cells along the entire rostro-caudal axis of the ventral neural tube, and their expression is dependent on Shh signaling (Rubenstein and Beachy 1998). Mutation in the *Nkx2.1* or *Nkx2.2* genes leads to defects in ventral neural patterning (Briscoe et al. 1999; Sussel et al. 1999), raising the possibility that *Nkx* genes play a key role in the control of ventral patterning in the ventral region of the CNS. Genetic studies to assess the role of *Nkx* genes have, however, focused on only the most ventral region of the neural tube. A recently identified *Nkx* gene, *Nkx6.1*, is expressed more widely by most progenitor cells within the ventral neural tube (Pabst et al. 1998; Qiu et al. 1998; Briscoe et al. 1999), suggesting that it may have a prominent role in ventral neural patterning. Here we show that in mouse embryos *Nkx6.1* is expressed by ventral progenitors that give rise to motor (MN), V2, and V3 neurons. Mice carrying a null mutation of *Nkx6.1* exhibit a ventral-to-dorsal switch in the identity of progenitor cells and a corresponding switch in the identity of the neuronal subtype that emerges from the ventral neural tube. The generation of MN and V2 neurons is markedly reduced, and there is a ventral expansion in the generation of a more dorsal V1 neuronal subtype. Together, these findings indicate that *Nkx6.1* has a critical role in the specification of MN and V2 neuron subtype identity and, more generally, that *Nkx* genes play a role in the interpretation of graded Shh signaling.

[Key Words: *Nkx*; spinal cord; motor neurons; neuronal specification; interneurons]

⁶These authors contributed equally to this work.

⁷Corresponding author.

E-MAIL jlr@cgl.ucsf.edu; FAX (415) 502-7618.

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Results and Discussion

To define the role of *Nkx6.1* in neural development, we compared patterns of neurogenesis in the embryonic spinal cord and hindbrain of wild-type mice and mice lacking *Nkx6.1* (Sander et al. 1998). In wild-type embryos, neural expression of *Nkx6.1* is first detected at spinal cord and caudal hindbrain levels at about embryonic day 8.5 (E8.5; Qiu et al. 1998; data not shown), and by E9.5 the gene is expressed throughout the ventral third of the neural tube (Fig. 1A). The expression of *Nkx6.1* persists until at least E12.5 (Fig. 1B,C; data not shown). *Nkx6.1* expression was also detected in mesodermal cells flanking the ventral spinal cord (Fig. 1B,C). To define more precisely the domain of expression of *Nkx6.1*, we compared its expression with that of 10 homeobox genes—*Pax3*, *Pax7*, *Gsh1*, *Gsh2*, *Irx3*, *Pax6*, *Dbx1*, *Dbx2*, and *Nkx2.9*—that have been shown to define discrete progenitor cell domains along the dorsoventral axis of the ventral neural tube (Goulding et al. 1991; Valerius et al. 1995; Ericson et al. 1997a,b; Pierani et al. 1999; Briscoe et al. 2000).

This analysis revealed that the dorsal boundary of *Nkx6.1* expression is positioned ventral to the boundaries of four genes expressed in by dorsal progenitor cells: *Pax3*, *Pax7*, *Gsh1*, and *Gsh2* (Fig. 1I,N; data not shown). Within the ventral neural tube, the dorsal boundary of *Nkx6.1* expression is positioned ventral to the domain of *Dbx1* expression and close to the ventral boundary of *Dbx2* expression (Fig. 1G,H,P). The domain of *Pax6* expression extends ventrally into the domain of *Nkx6.1* expression (Fig. 1O), whereas the expression of *Nkx2.2* and *Nkx2.9* overlaps with the ventral-most domain of *Nkx6.1* expression (Fig. 1O,Q).

To address the function of *Nkx6.1* in neural development, we analyzed progenitor cell identity and the pattern of neuronal differentiation in *Nkx6.1* null mutant mice (Sander et al. 1998). We detected a striking change in the profile of expression of three homeobox genes, *Dbx2*, *Gsh1*, and *Gsh2*, in *Nkx6.1* mutants. The domains of expression of *Dbx2*, *Gsh1*, and *Gsh2* each expanded into the ventral neural tube (Fig. 1K–M; data not shown). At E10.5, *Dbx2* was expressed at high levels by progenitor cells adjacent to the floor plate, but at this stage ectopic *Dbx2* expression was detected only at low levels in regions of the neural tube that generate motor neurons (Fig. 1K). By E12.5, however, the ectopic ventral expression of *Dbx2* had become more uniform and now clearly included the region of motor neuron and V2 neuron generation (Fig. 1L). Similarly, in *Nkx6.1* mutants, both *Gsh1* and *Gsh2* were ectopically expressed in a ventral domain of the neural tube and also in adjacent paraxial mesodermal cells (Fig. 1M; data not shown).

The ventral limit of *Pax6* expression was unaltered in

Figure 1. Selective changes in homeobox gene expression in ventral progenitor cells in *Nkx6.1* mutant embryos. Expression of *Nkx6.1* in transverse sections of the ventral neural tube at E9.5 (A), E10.5 (B), or E12.5 (C). Summary diagrams showing domains of homeobox gene expression in wild-type mouse embryos (D) and the change in pattern of expression of these genes in *Nkx6.1* mutants (E), based on analyses at E10.0–12.5. Expression of *Nkx6.1* (F,I), *Dbx2* (G,H,K,L), and *Gsh1* (I,M) in the caudal neural tube of wild-type (F–I) and *Nkx6.1* mutant (J–M) embryos. Horizontal lines, approximate position of dorsoventral boundary of the neural tube; vertical lines, expression levels of *Dbx2* and *Gsh1*. Expression of *Shh* (N,R), *Pax7* (N,R), *Nkx2.2* (O,S), *Pax6* (P,S), *Dbx1* (P,T), and *Nkx2.9* (Q,U) in wild-type (N–Q) or *Nkx6.1* mutant (R–U) embryos at spinal (N–P,R–T) and caudal hindbrain (Q,U) levels. Arrowheads, approximate position of the dorsal limit of *Nkx6.1* expression. Scale bar shown in J = 100 μ m (A–C), 50 μ m (F–M), or 60 μ m (N–U).

Nkx6.1 mutants, although the most ventrally located cells within this progenitor domain expressed a higher level of *Pax6* protein than those in wild-type embryos (Fig. 1O,S). We detected no change in the patterns of expression of *Pax3*, *Pax7*, *Dbx1*, *Irx3*, *Nkx2.2*, or *Nkx2.9* in *Nkx6.1* mutant embryos (Fig. 1R–U; data not shown).

Importantly, the level of Shh expression by floor plate cells was unaltered in *Nkx6.1* mutants (Fig. 1N,R). Thus, the loss of *Nkx6.1* function deregulates the patterns of expression of a selected subset of homeobox genes in ventral progenitor cells without an obvious effect on Shh levels (Fig. 1D,E). The role of Shh in excluding *Dbx2* from the most ventral region of the neural tube (Pierani et al. 1999) appears therefore to be mediated through the induction of *Nkx6.1* expression. Consistent with this view, ectopic expression of *Nkx6.1* represses *Dbx2* expression in chick neural tube (Briscoe et al. 2000). The detection of sites of ectopic *Gsh1/2* expression in the paraxial mesoderm as well as the ventral neural tube, both sites of *Nkx6.1* expression, suggests that *Nkx6.1* has a general role in restricting *Gsh1/2* expression. The signals that promote ventral *Gsh1/2* expression in *Nkx6.1* mutants remain unclear but could involve factors other than Shh that are secreted by the notochord (Hebrok et al. 1998).

The domain of expression of *Nkx6.1* within the ventral neural tube of wild-type embryos encompasses the progenitors of three main neuronal classes: V2, MN, and V3 interneurons (Goulding et al. 1991; Ericson et al. 1997a,b; Qiu et al. 1998; Briscoe et al. 1999, 2000; Pierani et al. 1999; Fig. 2A–D). We examined whether the generation of any of these neuronal classes is impaired in *Nkx6.1* mutants, focusing first on the generation of motor neurons. In *Nkx6.1* mutant embryos there was a marked reduction in the number of spinal motor neurons, as assessed by expression of the homeodomain proteins *Lhx3*, *Isl1/2*, and *HB9* (Arber et al. 1999; Tsuchida et al. 1994; Fig. 2E–L) and by expression of the gene encoding the transmitter synthetic enzyme choline acetyltransferase (data not shown). In addition, few if any axons were observed to emerge from the ventral spinal cord (data not shown). The incidence of motor neuron loss, however, varied along the rostrocaudal axis of the spinal cord. Few if any motor neurons were detected at caudal cervical and upper thoracic levels of *Nkx6.1* mutants analyzed at E11–E12.5 (Fig. 2M,N,Q,R), whereas motor neuron number was reduced only by 50%–75% at more caudal levels (Fig. 2O,P,S,T; data not shown). At all axial levels, the initial reduction in motor neuron number persisted at both E12.5 and p0 (Fig. 2M–T; data not shown), indicating that the loss of *Nkx6.1* activity does not simply delay motor neuron generation. Moreover, we detected no increase in the incidence of TUNEL⁺ cells in *Nkx6.1* mutants (data not shown), providing evidence that the depletion of motor neurons does not result solely from apoptotic death.

The persistence of some spinal motor neu-

rons in *Nkx6.1* mutants raised the possibility that the generation of particular subclasses of motor neurons is selectively impaired. To address this issue, we monitored the expression of markers of distinct subtypes of motor neurons at both spinal and hindbrain levels of *Nkx6.1* mutant embryos. At spinal levels, the extent of the reduction in the generation of motor neurons that populate the median (MMC) and lateral (LMC) motor columns was similar in *Nkx6.1* mutants as assessed by

Figure 2. Disruption of motor neuron differentiation in *Nkx6.1* mutant embryos. The relationship between the domain of *Nkx6.1* expression (A–C, green) by ventral progenitors and the position of generation of motor neurons and V2 interneurons (A–D, red) in the ventral spinal cord of E10.5 wild-type embryos. (A) *Isl1/2* motor neurons; (B) *HB9* motor neurons; (C) *Lhx3* (*Lim3*) expression (red) by motor neurons, V2 interneurons, and their progenitors is confined to the *Nkx6.1* progenitor domain. (D) *Chx10* (green) V2 interneurons coexpress *Lhx3* (red). Expression of *Isl1/2* (E,I), *HB9* (F,J), *Lhx3* (G,K), and *Phox2a/b* (H,L) in the ventral spinal cord (E,F,G) and caudal hindbrain (H) of E10.5 wild-type (E–H) and *Nkx6.1* mutant (I–L) embryos. Pattern of expression of *Isl1/2* and *Lhx3* at cervical (M,N,Q,R) and thoracic (O,P,S,T) levels of E12.5 wild-type (M–P) and *Nkx6.1* mutant (Q–T) embryos. Arrows, position of *Isl1* dorsal D2 interneurons. Scale bar shown in I = 60 μ m (A–D), 80 μ m (E–L), or 120 μ m (M–T).

the number of motor neurons that coexpressed *Isl1/2* and *Lhx3* (defining MMC neurons; Fig. 3A,B) and by the expression of *Raldh2* (defining LMC neurons; Sockanathan and Jessell 1998; Arber et al. 1999; Fig. 3C,D). In addition, the generation of autonomic visceral motor neurons was reduced to an extent similar to that of somatic motor neurons at thoracic levels of the spinal cord of E12.5 embryos (data not shown). Thus, the loss of *Nkx6.1* activity depletes the major subclasses of spinal motor neurons to a similar extent.

At hindbrain levels, *Nkx6.1* is expressed by the progenitors of both somatic and visceral motor neurons (Fig. 3E,F; data not shown). We therefore examined whether the loss of *Nkx6.1* might selectively affect subsets of cranial motor neurons. We detected a virtually complete loss in the generation of hypoglossal and abducens somatic motor neurons in *Nkx6.1* mutants, as assessed by the absence of dorsally generated HB9⁺ motor neurons (Fig. 3G,H; data not shown; Arber et al. 1999; Briscoe et al. 1999). In contrast, there was no change in the initial generation of any of the cranial visceral motor neuron populations, assessed by coexpression of *Isl1* and *Phox2a* (Briscoe et al. 1999; Pattyn et al. 1997) within ventrally generated motor neurons (Fig. 3I,J; data not shown). Moreover, at rostral cervical levels, the generation of spinal accessory motor neurons (Ericson et al. 1997a,b) was also preserved in *Nkx6.1* mutants (data not shown). Thus, in the hindbrain the loss of *Nkx6.1* activity selectively eliminates the generation of somatic motor neurons, while leaving visceral motor neurons intact. Cranial visceral motor neurons, unlike spinal visceral motor neurons, derive from progenitors that express the related

Nkx genes *Nkx2.2* and *Nkx2.9* (Briscoe et al. 1999). The preservation of cranial visceral motor neurons in *Nkx6.1* mutant embryos may therefore reflect the dominant activities of *Nkx2.2* and *Nkx2.9* within these progenitor cells.

We next examined whether the generation of ventral interneurons is affected by the loss of *Nkx6.1* activity. V2 and V3 interneurons are defined, respectively, by expression of *Chx10* and *Sim1* (Arber et al. 1999; Briscoe et al. 1999; Fig. 4A,G). A severe loss of *Chx10* V2 neurons was detected in *Nkx6.1* mutants at spinal cord levels (Fig. 4B), although at hindbrain levels of *Nkx6.1*, mutants ~50% of V2 neurons persisted (data not shown). In contrast, there was no change in the generation of *Sim1* V3 interneurons at any axial level of *Nkx6.1* mutants (Fig. 4H). Thus, the elimination of *Nkx6.1* activity affects the generation of only one of the two major classes of ventral interneurons that derive from the *Nkx6.1* progenitor cell domain.

Evx1⁺, Pax2⁺ V1 interneurons derive from progenitor cells located dorsal to the *Nkx6.1* progenitor domain (Fig. 4B) within a domain that expresses *Dbx2* but not *Dbx1* (Burrill et al. 1997; Matise and Joyner 1997; Pierani et al. 1999). Because *Dbx2* expression undergoes a marked ventral expansion in *Nkx6.1* mutants, we examined whether there might be a corresponding expansion in the domain of generation of V1 neurons. In *Nkx6.1* mutants, the region that normally gives rise to V2 neurons and motor neurons now also generated V1 neurons, as assessed by the ventral shift in expression of the *En1* and *Pax2* homeodomain proteins (Fig. 4B,C,E,F). Consistent with this, there was a two- to threefold increase in

the total number of V1 neurons generated in *Nkx6.1* mutants (Fig. 4C,D). In contrast, the domain of generation of Evx1/2 V0 neurons, which derive from the *Dbx1* progenitor domain (Pierani et al. 1999), was unchanged in *Nkx6.1* mutants (Fig. 4I,J). Thus, the ventral expansion in *Dbx2* expression is accompanied by a selective switch in interneuronal fates from V2 neurons to V1 neurons. In addition, we observed that some neurons within the ventral spinal cord of *Nkx6.1* mutants coexpressed the V1 marker *En1* and the V2 marker *Lhx3* (Fig. 4K,L). The coexpression of these markers is rarely if ever observed in single neurons in wild-type embryos (Ericson et al. 1996). Thus, within individual neurons in *Nkx6.1* mutants, the ectopic program of V1 neurogenesis appears to be initiated in parallel with a residual, albeit transient, program of V2 neuron generation. This result complements observations in *Hb9* mutant mice, in which the programs of V2 neuron and motor neuron generation coincide transiently

Figure 3. Motor neuron subtype differentiation in *Nkx6.1* mutant mice. Depletion of both median (MMC) and lateral (LMC) motor column neurons in *Nkx6.1* mutant mice. Expression of *Isl1/2* (red) and *Lhx3* (green) in E12.5 wild-type (A,C) and *Nkx6.1* mutant (B,D) mice spinal cord at forelimb levels. (E–J) Motor neuron generation at caudal hindbrain level. (E,F) *Nkx6.1* expression in progenitor cells and visceral motor neurons in the caudal hindbrain (rhombomere [r] 7/8) of E10.5–E11 wild-type (E) *Nkx6.1* mutant (F) mice. HB9 expression in hypoglossal motor neurons in E10.5–E11 wild-type (G) and *Nkx6.1* mutant (H) mice. Coexpression of *Isl1* (green) and *Phox2a/b* (red) in wild-type (I) or *Nkx6.1* mutant (J) mice. (h) hypoglossal motor neurons; (v) visceral vagal motor neurons. Scale bar shown in C = 50 μ m (A–D) or 70 μ m (E–J).

Figure 4. A switch in ventral interneuron fates in *Nkx6.1* mutant mice. *Chx10* expression in V2 neurons at rostral cervical levels of E10.5 wild-type (A) and *Nkx6.1* mutant (B) embryos. *En1* expression by V1 neurons at rostral cervical levels of wild-type (C) and *Nkx6.1* mutant (D) embryos. *Pax2* expression in a set of interneurons that includes V1 neurons (Burrill et al. 1997) at caudal hindbrain levels of wild-type (E) and *Nkx6.1* mutant (F) embryos. (G,H) *Sim1* expression by V3 neurons in the cervical spinal cord of wild-type (G) and *Nkx6.1* mutant (H) embryos. *Evx1* expression by V0 neurons at caudal hindbrain levels of wild-type (I) and *Nkx6.1* mutant (J) embryos. *En1* (red) and *Lhx3* (green) expression by separate cell populations in the ventral spinal cord of E11 wild-type (K) and *Nkx6.1* mutant (L) embryos. Scale bar shown in B = 60 μ m (A–D), 75 μ m (E,F), 70 μ m (G,H), or 35 μ m (K,L).

within individual neurons (Arber et al. 1999; Thaler et al. 1999).

Taken together, our findings reveal an essential role for the *Nkx6.1* homeobox gene in the specification of regional pattern and neuronal fate in the ventral half of the mammalian CNS. Within the broad ventral domain within which *Nkx6.1* is expressed (Fig. 5A), its activity is required to promote MN and V2 interneuron generation and to restrict the generation of V1 interneurons (Fig. 5B). We favor the idea that the loss of MN and V2 neurons is a direct consequence of the loss of *Nkx6.1* activ-

ity, as the depletion of these two neuronal subtypes is evident at stages when only low levels of *Dbx2* are expressed ectopically in most regions of the ventral neural tube. Nonetheless, we can not exclude that low levels of ectopic ventral *Dbx2* expression could contribute to the block in motor neuron generation. Consistent with this view, the ectopic expression of *Nkx6.1* is able to induce both motor neurons and V2 neurons in chick neural tube (Briscoe et al. 2000). V3 interneurons and cranial visceral motor neurons derive from a set of *Nkx6.1* progenitors that also express *Nkx2.2* and *Nkx2.9* (Briscoe et al. 1999; Fig. 5A). The generation of these two neuronal subtypes is unaffected by the loss of *Nkx6.1* activity, suggesting that the actions of *Nkx2.2* and *Nkx2.9* dominate over that of *Nkx6.1* within these progenitors. The persistence of some spinal motor neurons and V2 neurons in *Nkx6.1* mutants could reflect the existence of a functional homologue within the caudal neural tube.

The role of *Nkx6.1* revealed in these studies, taken together with previous findings, suggests a model in which the spatially restricted expression of *Nkx* genes within the ventral neural tube (Fig. 5) has a pivotal role in defining the identity of ventral cell types induced in response to graded Shh signaling. Strikingly, in *Drosophila*, the *Nkx* gene *NK2* has been shown to have an equivalent role in specifying neuronal fates in the ventral nerve cord (Chu et al. 1998; McDonald et al. 1998). Moreover, the ability of *Nkx6.1* to function as a repressor of the dorsally expressed *Gsh1/2* homeobox genes parallels the ability of *Drosophila* *NK2* to repress

Figure 5. Changes in progenitor domain identity and neuronal fate in the spinal cord of *Nkx6.1* mutant embryos. (A) In wild-type mouse embryos, cells in the *Nkx6.1* progenitor domain give rise to three classes of ventral neurons: V2, motor (MN), and V3 neurons. V3 neurons derive from cells in the ventral most region of *Nkx6.1* expression that also express *Nkx2.2* and *Nkx2.9*. V1 neurons derive from progenitor cells that express *Dbx2* but not *Nkx6.1*. (B) In *Nkx6.1* mutant embryos the domain of *Dbx2* expression by progenitor cells expands ventrally and by embryonic day 12 (E12) occupies the entire dorsoventral extent of the ventral neural tube, excluding the floor plate. Checked blue indicates the gradual onset of ventral *Dbx2* expression. This ventral shift in *Dbx2* expression is associated with a marked decrease in the generation of V2 and MN neurons and a ventral expansion in the domain of generation of V1 neurons. A virtually complete loss of MN and V2 neurons is observed at cervical levels of the spinal cord. The generation of V3 neurons (and cranial visceral motor neurons at hindbrain levels) is unaffected by the loss of *Nkx6.1* or by the ectopic expression of *Dbx2*.

Nkx6.1 is required for ventral CNS specification

Ind, a *Gsh1/2*-like homeobox gene [Weiss et al. 1998]. Thus, the evolutionary origin of regional pattern along the dorsoventral axis of the central nervous system may predate the divergence of invertebrate and vertebrate organisms.

Materials and methods*Generation of Nkx6.1 null mutation*

A null mutation in *Nkx6.1* was generated by gene targeting in 129-strain ES cells by excising an 800-bp *NotI* fragment containing part of exon1 and replacing it by a PGK-neo cassette [M. Sander and M. German, unpubl.]. Mutants were born at Mendelian frequency and died soon after birth; they exhibited movements only upon tactile stimulation.

Immunocytochemistry and in situ hybridization

Localization of mRNA was performed by in situ hybridization following the method of Schaefer-Wiemers and Gerfin-Moser (1993). The *Dbx2* riboprobe comprised the 5' *EcoRI* fragment of the mouse cDNA [Pierani et al. 1999]. Probes for other cDNAs were cited in the text and used as described therein. Protein expression was localized by indirect fluorescence immunocytochemistry or peroxidase immunohistochemistry [Briscoe et al. 1999; Ericson et al. 1997b]. *Nkx6.1* was detected with a rabbit antiserum [Briscoe et al. 1999]. Antisera against Shh, Pax7, Isl1/2, HB9, Lhx3, Chx10, Phox2a/b, En1, and Pax2 have been described [Briscoe et al. 1999; Ericson et al. 1997b]. Fluorescence detection was carried out using an MRC 1024 Confocal Microscope (BioRad).

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Maike Sander, Sussan Paydar, Johan Ericson, et al.

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