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The role of Phox2B in chromaffin cell development

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Abstract

Phox2B, a homeodomain transcription factor closely related to Phox2A, is expressed in peripheral and central noradrenergic neurons. In neural crest (NC) derivatives Phox2B is restricted to sympathetic and parasympathetic ganglia, enteric neurons, and adrenal and extraadrenal chromaffin cells. Similar to MASH-1, Phox2B has been implicated in synchronizing pan-neuronal and catecholaminergic phenotype-specific aspects of neurogenesis. The role of Phox2B for the differentiation of the neuroendocrine NC derivatives, the adrenal medullary chromaffin cells, has not been explored. We have previously reported that in MASH-1-deficient mice most chromaffin cells are arrested at the early neuroblast stage and lack catecholaminergic differentiation. We show now that in Phox2B knockout/lacZ knockin mice the maturation of presumptive chromaffin cells is arrested at an even earlier stage of development. The cells lack the catecholaminergic marker enzyme TH and fail to form a centrally located medulla. In contrast to MASH-1 (−/−) mice they do not express dHand, Phox2A, c-ret, neurofilament, neuron-specific tubulin, and NCAM and appear ultrastructurally more immature. Many of these cells die by apoptosis. Despite the complete lack of differentiation, few lacZ-positive adrenal cells can still be found at E16.5. We conclude that Phox2B regulates very early events in the differentiation of adrenal chromaffin cells distinct to steps, which essentially require MASH-1.

Keywords: Sympathoadrenal cell lineage; Adrenal chromaffin cells; Phox2B; Phox2A; dHand; MASH-1

Introduction

Chromaffin cells and sympathetic neurons are both derivatives of the neural crest (NC) and have been hypothesized to share a common sympathoadrenal (SA) progenitor cell (Anderson, 1993; Anderson and Axel, 1986; Unsicker, 1993; Unsicker et al., 1978). SA progenitor cells first aggregate at the dorsal aorta, where they acquire a catecholaminergic neuronal fate. Subsequently, the cells migrate either in a dorsolateral direction to form sympathetic ganglia, or ventrally to colonize the adrenal gland, where they lose neuronal traits and differentiate into neuroendocrine chromaffin cells (Le Douarin and Kalcheim, 1999).

A variety of transcription factors have been identified that are involved in the generation of SA from NC cells. The homeodomain transcription factor Phox2B and the helix–loop–helix transcription factor MASH-1 are considered to play key roles in the early differentiation of SA cells (Guillemot et al., 1993; Pattyn et al., 1999; Stanke et al., 2004). In the chick embryo, initiation of CASH-1, the MASH-1 homolog, has been shown to precede expression of Phox2B (Ernsberger et al., 1995) after the cells have aggregated at the dorsal aorta. Phox2B and MASH-1 are both considered to promote neuronal and catecholaminergic differentiation in NC cells and to control the expression of other transcription factors, including Phox2A and dHand (Brunet and Pattyn, 2002; Hirsch et al., 1998; Lo et al., 1998).

Sympathetic neurons of MASH-1-deficient mice fail to undergo normal differentiation and subsequently die (Guillemot et al., 1993; Hirsch et al., 1998). Chromaffin cells have initially been reported to be largely unaffected by the MASH-1 mutation (Guillemot et al., 1993). In contrast, we have recently shown that catecholaminergic differentiation

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of the majority of adrenal chromaffin cells is impaired and that the cells are arrested at the early neuroblast level (Huber et al., 2002). Despite this deficit in differentiation about one-third of the SA cells survive in the adrenal gland of MASH-1-deficient mice at birth, in contrast to sympathetic neuronal progenitors which have almost completely disappeared by E12.5 (Hirsch et al., 1998).

The phenotype of Phox2B-deficient mice has been reported to be very similar to the phenotype of MASH-1-deficient mice regarding sympathetic ganglia (Pattyn et al., 1999). SA cells aggregate at the dorsal aorta but lack the catecholaminergic markers tyrosine hydroxylase (TH), dopamine β-hydroxylase (DBH), and Phox2A. However, at E13.5 there are no sympathetic ganglia in locations where they occur in heterozygous Phox2B mice. The development of the adrenal gland of Phox2B-deficient mice has not been analyzed.

Sympathetic neurons and chromaffin cells are not only profoundly different in their phenotypes but also differ with respect to a number of molecular requirements for their development, including MASH-1 and c-Ret signaling (Allmendinger et al., 2003; Huber et al., 2002; cf. Unsicker, 1993). It has even been argued that SA progenitors giving rise to sympathetic neurons and chromaffin cells may already be distinct prior to reaching their final destinations (Ernsberger et al., 2005). The present study was undertaken to reveal whether sympathetic neurons and chromaffin cells also differ with respect to their requirements for Phox2B. We show here that, in contrast to sympathetic ganglia, the adrenal gland harbors SA progenitors in Phox2B-deficient mice at least until E16.5. However, these cells lack TH, Phox2A, dHand, and NCAM and by ultrastructural criteria appear even more immature than chromaffin progenitor cells in MASH-1-deficient mice.

Materials and methods

Experimental animals

Wild-type and phox2B$^{LacZ/LacZ}$ mice were obtained from intercrosses of Phox2B$^{+/LacZ}$ mice (Pattyn et al., 1999). The embryos were staged considering midday of the day of the vaginal plug as embryonic day 0.5. To rescue the embryos beyond midgestation drinking water of pregnant females was supplemented with 100 μg/ml of L-phenylephrine, 100 μg/ml of isoproterenol, and 2 mg/ml of ascorbic acid, from E8.5 onwards (Pattyn et al., 2000). Genotyping was carried out by PCR-analysis as described previously (Pattyn et al., 1999).

Histology

Pregnant mice were killed by CO_2 asphyxiation. Embryos were removed and washed with phosphate buffer (PB, pH 7.4) followed by fixation in PB containing 4% paraformaldehyde (PFA) for at least 12 h. Tissues were then rinsed three times with PB and placed in PB containing 30% sucrose for cryoprotection. After overnight immersion in sucrose the tissue was coated with OCT™ compound (Tissue Tek), frozen on dry ice, and stored at −70°C until further processing. Embryos were cut into serial 12-μm sections, mounted on Superfrost™ slides, and air-dried for 30 min, before performing in situ hybridization or X-Gal staining.

In situ hybridization

Non-radioactive in situ hybridization on cryosections and preparation of digoxigenin-labeled probes for mouse TH (Zhou et al., 1995), mouse MASH-1 (Casarosa et al., 1999), mouse dHand (Srivastava et al., 1997; courtesy of E. N. Olson), mouse Phox2A (Valarché et al., 1993), mouse neurofilament 68 (Huber et al., 2002), mouse NCAM, mouse SF-1, and LacZ were carried out using a modification of the protocol of D. Henrique (IRFDBU, Oxford, UK) as previously described (Ernsberger et al., 1997). Mouse NCAM (bp: 230–924) and mouse SF-1 (bp: 735–1336) were cloned by RT-PCR using a pGEM-T vector system (Promega) following the manufacturer's instructions. The plasmids were linearized with *Sal*I (NCAM) or *Not*I (SF-1) and transcribed with T7 (antisense). Appropriate sense controls have been carried out. Bp 839–1955 of the *Escherichia coli* LacZ gene were subcloned into pBluescriptII SK using *Cla*I and *Sac*I restriction sites. The plasmid was linearized with *Cla*I (antisense) and *Sac*I (sense control) and transcribed with T3 (antisense) and T7 (sense control).

X-gal staining

Cryosections were washed twice for 10 min with detergent solution [phosphate-buffered saline (PBS, pH7.4), 2 mM $MgCl_2$, 0.02% NP40, and 0.01% NA-deoxycholate], and then incubated for at least 12 h with staining solution containing phosphate-buffered saline (PBS, pH 7.4), 20 mM Tris buffer (pH 7.5), 2 mM $MgCl_2$, 5 mM $K_3Fe(CN)_6$, 5 mM $K_4Fe(CN)_6$, 0.02% NP40, 0.01% NA-deoxycholate, and 0.75 mg/ml X-gal (AppliChem). The reaction was stopped by washing the slides three times for 5 min in 5 mM EDTA/PBS (pH 7.4). TUNEL was performed subsequently.

TdT dUTP nick end labeling (TUNEL) analysis

For the detection of apoptotic LacZ-positive cells TUNEL was performed using an ApopTag peroxidase in situ apoptosis detection kit (Chemicon) following the manufacturer's instructions.

Electron microscopy

For electron microscopy adrenal glands from E14.5 wild-type and Phox2B-deficient mice were fixed by immersion in a mixture of glutaraldehyde (1.5%) and paraformaldehyde

(1.5%) in phosphate buffer at pH 7.3 for 48 h and rinsed several times with cacodylate buffer (0.1 M). Organs were then postfixed in 1% OsO_4/1.5% potassium hexacyanoferrate, rinsed in 0.1 M cacodylate buffer and 0.2 M sodium maleate buffer (pH 6.0), and block-stained with 1% uranyl acetate. Following dehydration through increasing concentrations of ethanol, the tissue was Epon embedded. Ultrathin sections (50 nm) were examined with a Zeiss EM10.

Results

We first investigated whether chromaffin cells of Phox2B-deficient mice invade the adrenal gland using LacZ expression as a marker for SA cells. We found that SA cells have colonized the adrenal anlagen at E13.5 (Fig. 1B). At this age, adrenal cortical cells and chromaffin cells are still intermingled in wild-type (not shown) and heterozygous mice (Fig. 1A). No LacZ-expressing cells could be detected at the site of the sympathetic suprarenal ganglion. (Fig. 1B; cf. arrow in Fig. 1A). While LacZ-positive cells gradually aggregate between E13.5 and E16.5 forming a centrally located adrenal medulla in heterozygous Phox2B mice (Figs. 1C, E, and G), LacZ-expressing cells in Phox2B-deficient mice disintegrate become more scarce and by E16.5 are sparsely scattered preferentially in the periphery of the adrenal gland (Figs. 1D, F, and H). As shown in Fig. 2, the percentage of TUNEL-positive LacZ-positive cells was strongly enhanced in Phox2B-deficient embryos at E15.5 as compared to heterozygous littermates, suggesting that many LacZ-positive cells apoptose between E15 and E16 in Phox2B-deficient mice. Despite the lack of a compact adrenal medulla, the size of the adrenal cortex as revealed by in situ hybridization for SF-1, a transcription factor of steroidogenic cells (Luo et al., 1994), appeared unaltered in $Phox2B^{LacZ/LacZ}$ mice (Fig. 3).

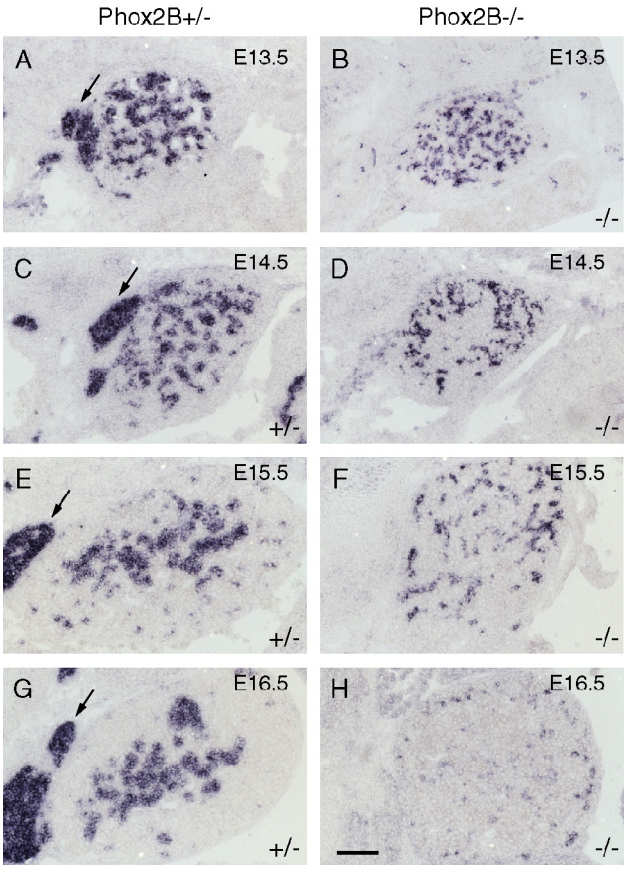

Fig. 1. LacZ in situ hybridization reveals SA cells in the adrenal glands of $Phox2B^{+/LacZ}$ (A, C, E, and G) and $Phox2B^{LacZ/LacZ}$ (B, D, F, and H) mice at different embryonic ages. Note that the formation of a centrally located adrenal medulla found in heterozygous animals (E and G) is impaired in the knockout (F and H). The suprarenal ganglion (arrows in A, C, E, and G) is completely missing in $Phox2B^{LacZ/LacZ}$ mice. Scale bar: 100 μm.

We next investigated the expression of the catecholaminergic marker enzyme TH and the transcription factors dHand and Phox2A in the developing adrenal gland of Phox2B-deficient mice (Fig. 4). While in E14.5 wild-type adrenal glands all these markers are expressed, none of them could be detected in Phox2B-deficient mice at this age or other ages investigated (E14.5: Figs. 4C, F, I, and L; not shown are E13.5, E15.5, and E16.5). Adrenal medullary cells of MASH-1-deficient mice, in contrast, express the transcription factors Phox2A and dHand as shown in Figs. 4E and H, and a subpopulation expresses TH (Fig. 4K). In Phox2B-deficient mice, expression of MASH-1 could be detected at E12.5 and E13.5 (Figs. 5B and D) but had disappeared by E14.5 (Fig. 5F).

We have recently shown that chromaffin cells of MASH-1-deficient mice are arrested at the early neuroblast stage (Huber et al., 2002). The cells display a neuroblast-like ultrastructure and fail to downregulate neuronal markers, as e.g., neurofilament 68 and c-Ret. In order to compare the differentiation status of adrenal medullary cells of MASH-1- and Phox2B-deficient mice, we investigated the expression of neurofilament 68 at E14.5. At this age very few adrenal medullary cells, most likely the scarce adrenal neuronal cells express neurofilament in wild-type mice (Fig. 6A). No expression of neurofilament could be detected in adrenal SA cells of Phox2B-deficient mice (Fig. 6C). In contrast, adrenal medullary cells of MASH-1-deficient mice exhibit strong expression of neurofilament 68 (Fig. 6B). To clarify whether the adrenal SA cells in Phox2B-deficient mice downregulate expression of neurofilament, like wild-type adrenal chromaffin cells, or whether they never express neurofilament 68, we investigated the expression of neurofilament 68 in E12.5 wild-type and Phox2B-deficient mice. As shown in Fig. 7A, neurofilament 68 is expressed in developing adrenal chromaffin cells of E12.5 wild-type mice, when SA cells have just begun to colonize the adrenal gland. However, neurofilament 68 mRNA could not be detected in the adrenal gland of Phox2B-deficient mice at this age (Fig. 7B), suggesting that developing adrenal SA cells in Phox2B-deficient mice fail to initiate neurofilament 68 expression.

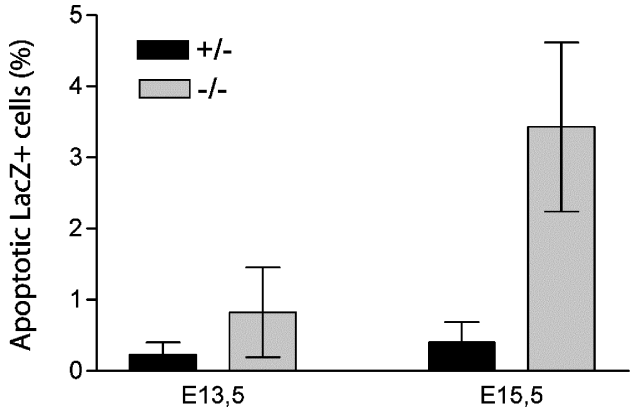

Fig. 2. Apoptotic cells (percentage of LacZ-positive cells) in the adrenal gland of Phox2B$^{+/LacZ}$ and Phox2B$^{LacZ/LacZ}$ at E13.5 and E15.5. Note that apoptosis is strongly enhanced in the adrenal glands of Phox2B$^{LacZ/LacZ}$ mice at E15.5. Every fifth section per adrenal gland was analyzed. Data are presented as mean ± SD (n = 4).

Similar to neurofilament, expression of c-Ret and neuron-specific tubulin was enhanced in adrenal medullary cells of MASH-1-deficient mice (Huber et al., 2002; not shown here) but was completely absent in Phox2B-deficient mice (not shown).

The lack of formation of a centrally located adrenal medulla in Phox2B-deficient mice prompted us to ask whether developing adrenal SA cells in Phox2B-deficient mice might also fail to express cell adhesion molecules, as e.g., the neural cell adhesion molecule NCAM, which is expressed by adrenal chromaffin cells (Leon et al., 1992). Fig. 8 shows that NCAM is expressed in the adrenal glands of E14.5 wild-type and MASH-1-deficient mice. In contrast, NCAM expression could not be detected in the adrenal gland of Phox2B-deficient mice at any age investigated (E13.5 through E16.5).

Ultrastructurally, developing wild-type adrenal chromaffin cells analyzed at E14.5 exhibit numerous large secretory "chromaffin" granules (core diameter >100 nm; Fig. 9A), the morphological hallmark of chromaffin cells, which distinguishes them from sympathetic neurons (cf. Coupland, 1972; Coupland and Tomlinson, 1989; Eränkö, 1972). In addition, chromaffin cells at this age possess an elaborate Golgi network and rough endoplasmatic reticulum (ER). Numerous axons and axon terminals, some of which contain small (approximately 50 nm in diameter) synaptic vesicles, can be seen abutting chromaffin cells. In E14.5 Phox2B mutant mice, two ultrastructurally distinct cell types can be distinguished in the adrenal gland (Fig. 9B): the more numerous ones exhibit typical features of steroid producing cells, i.e., mitochondria of the tubular type, and smooth-surfaced ER. The other cell type has very sparse organelles, few mitochondria of the crista-type, many free ribosomes and protruding irregular processes typical of migratory cells. We interpret this second cell type as a very immature SA progenitor cell. Axons, none with the structure of axon terminals, can be found.

Discussion

As shown by a series of gene ablation studies, the development of SA cells depends on a variety of transcriptions factors, including Phox2B, MASH-1, and GATA3 (Guillemot et al., 1993; Lim et al., 2000; Pattyn et al., 1999). However, their precise roles and putative interactions during the development of the distinct SA cell types, i.e., para- and prevertebral sympathetic neurons, intra- and extraadrenal chromaffin, and small intensely fluorescent (SIF) cells, are only rudimentarily understood. Studies concerning roles of Phox2B and MASH-1 in SA development have mainly focused on (paravertebral thoracic) sympathetic neurons, while chromaffin cells, which are supposed by many to share a common SA progenitor with sympathetic neurons, have been largely ignored.

The present study has revealed both differences and similarities of sympathetic neuronal and adrenal chromaffin progenitor cells concerning their dependencies on Phox2B. Adrenal chromaffin progenitor cells seem to be less severely affected by the Phox2B mutation in terms of their survival but are apparently similar in their dependency on Phox2B for the induction or maintenance, respectively, of Phox2A, dHand, TH, and MASH-1. Thus, in adrenal chromaffin progenitor cells, as in secondary sympathetic ganglionic anlagen, maintenance of MASH-1 is also dependent on

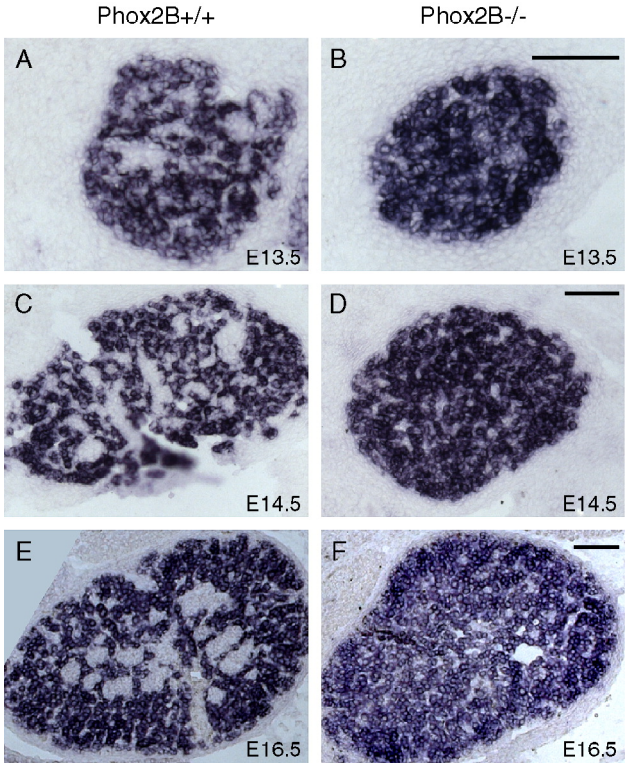

Fig. 3. The size of the adrenal cortex appears unaltered in Phox2B$^{LacZ/LacZ}$ embryos as compared to wild-type mice. In situ hybridization for the adrenal cortex-specific marker SF-1 in Phox2B$^{+/+}$ (A, C, and E) and Phox2B$^{LacZ/LacZ}$ (B, D, and F) embryos at E13.5 (A, B), E14.5 (C, D), and E16.5 (E, F). Scale bars: 100 μm.

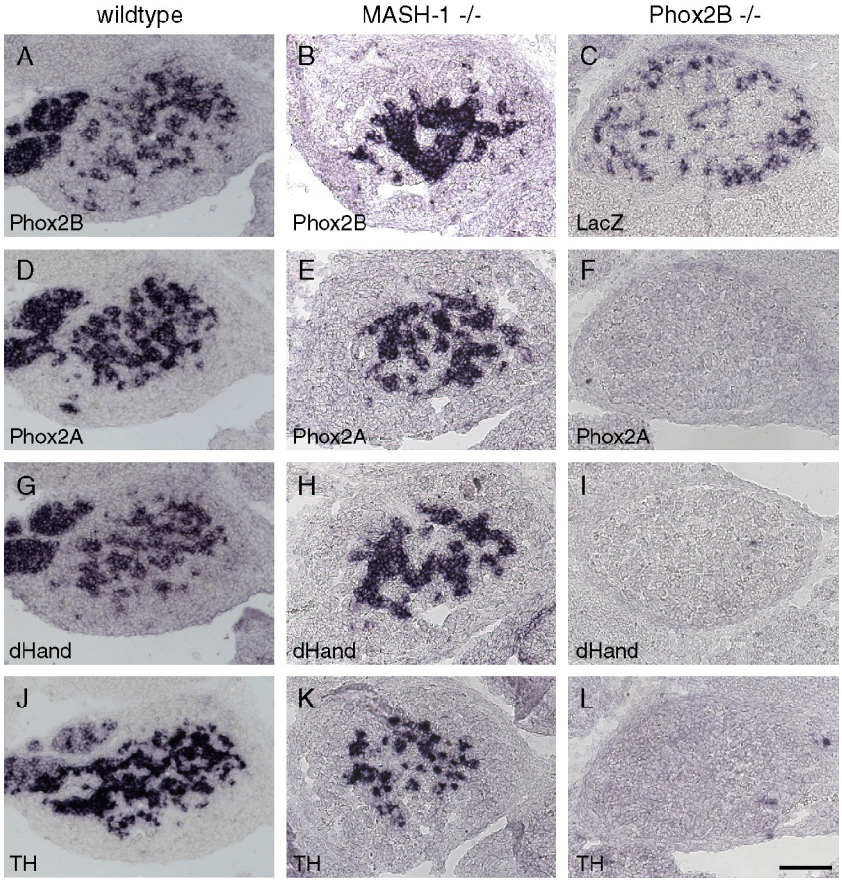

Fig. 4. In situ hybridization for Phox2B (A and B), LacZ (C), Phox2A (D–F), dHand (G–I), and TH (J–L) on sections of the adrenal glands of E14.5 wild-type mice (A, D, G, and J), MASH-1$^{-/-}$ (B, E, H, and K) mice, and Phox2B$^{LacZ/LacZ}$ (C, F, I, and L) mice. Expression of Phox2A, dHand, and TH is completely lacking in Phox2B$^{LacZ/LacZ}$ mice. Scale bar: 100 μm.

Phox2B (Pattyn et al., 1999). The difference in survival of Phox2B deprived sympathetic neuronal and chromaffin progenitor cells may reflect the different survival promoting capacities of the local environments of sympathetic neurons and adrenal chromaffin cells. Adrenal chromaffin progenitor cells may benefit from growth factors provided by the developing cortex, as e.g., IGF-1, FGF, glucocorticoids, and others (Seidl and Unsicker, 1989; Unsicker, 1993). Alternatively, longer persistence of chromaffin progenitor cells might also reflect ongoing immigration of SA cells into the adrenal gland (cf. Coupland, 1965). Thus, LacZ-positive cells in the adrenal gland of Phox2B mutant mice might represent newly immigrated SA progenitors cells, which may not survive substantially longer than sympathetic neuronal progenitors (Pattyn et al., 1999). In support of this view, the LacZ-expressing cells in the adrenal gland of Phox2B-deficient mice occupy mostly the periphery of the adrenal gland, where SA cells enter the adrenal anlagen rather than its central portion, where SA cells maturate (cf. Figs. 1D, F, and H). However, at E15.5 substantial apoptosis of LacZ-positive cells was observed in the adrenal glands of Phox2B-deficient mice, suggesting that LacZ-positive cells that have colonized the adrenal anlagen eventually die as well.

Our study has also revealed substantial differences in the phenotypes of adrenal chromaffin cells comparing Phox2B and MASH-1 mutant mice. These differences may help to shed light on the distinct importance of these transcription factors for chromaffin cell development. Loss of MASH-1 only partially abrogates the acquisition of a differentiated chromaffin phenotype. Thus, most adrenal chromaffin progenitor cells acquire expression of Phox2B, Phox2A, and dHand, and about 10% of the chromaffin progenitors have started to express TH at birth. Approximately half of the TH-positive adrenal cells even show immunoreactivity for PNMT, the adrenaline-synthesizing enzyme. However, persistence of neurofilament and c-Ret expression and the typical ultrastructural features of neuroblasts rather than immature chromaffin cells strongly suggest that adrenal chromaffin progenitor cells lacking MASH-1 in their majority cannot convert to terminally differentiated chromaffin cells and die (Huber et al., 2002). In contrast, SA cells populating the adrenal gland in Phox2B-deficient mice are phenotypically apparently more severely compromised. They fail to gain competence for the expression of Phox2A, dHand, and TH and only briefly express MASH-1. Ultrastructurally, the cells appear even more immature than in MASH-1 knockout mice.

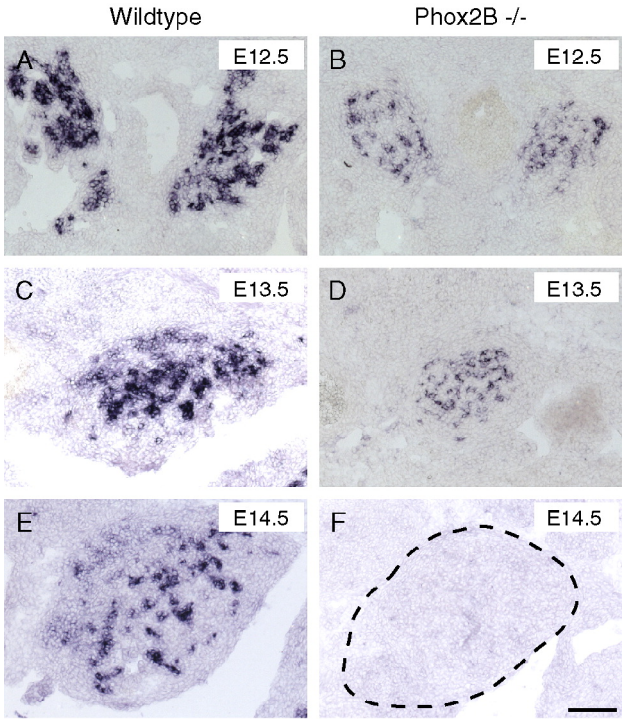

Fig. 5. Expression of MASH-1 mRNA in the adrenal glands of wild-type mice (A, C, and E) and Phox2B$^{LacZ/LacZ}$ mice (B, D, and F). Mash-1 is expressed in the adrenal glands of E12.5 (B) and E13.5 (D) Phox2B-deficient mice but has disappeared at E14.5 (F). The border of the adrenal gland is demarcated. Scale bar: 100 μm.

Together, the comparison of the MASH-1 and Phox2B knockouts suggests that Phox2B is more crucial for chromaffin cell development than MASH-1. While the lack of MASH-1 can apparently be compensated by other factors, possibly including Phox2B, lack of Phox2B fundamentally and very early impairs chromaffin cell development. Recent gain-of-function experiments overexpressing either MASH-1 or Phox2B in peripheral nerve precursors of chick embryos (Stanke et al., 2004) have suggested that Phox2B coordinates generic and noradrenergic gene expression, recruiting

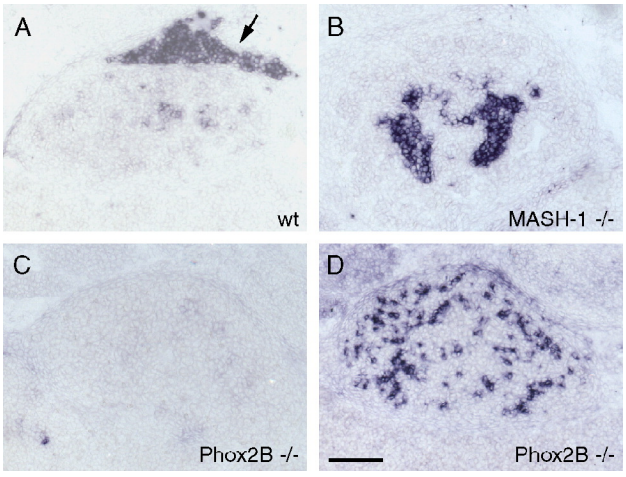

Fig. 6. In situ hybridization for neurofilament 68 mRNA on sections of the adrenal glands of an E14.5 wild-type mouse (A), MASH-1$^{-/-}$ mouse (B), and a Phox2B$^{LacZ/LacZ}$ (C) mouse. LacZ in situ hybridization (D) on a section adjacent to panel C shows the presence of LacZ+ cells in the adrenal gland of the Phox2B$^{LacZ/LacZ}$ embryo. Expression of neurofilament 68 is detectable in the suprarenal ganglion (arrow in A) and in very few cells, presumably neurons, within the adrenal gland of wild-type mice. Many adrenal medullary cells of MASH-1$^{-/-}$ mice express neurofilament 68, while it is not detectable in the adrenal glands of Phox2B$^{LacZ/LacZ}$ mice. Scale bar: 100 μm.

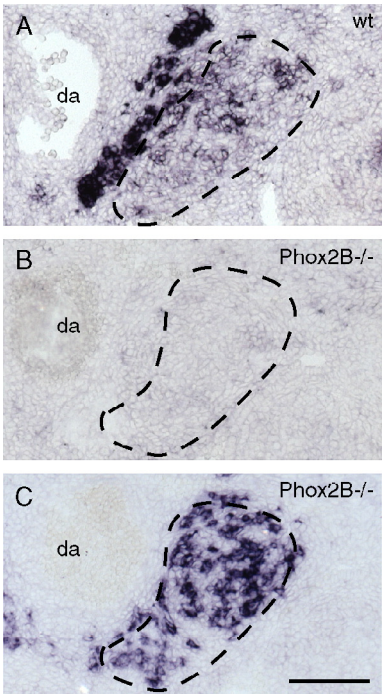

Fig.7. Neurofilament 68 mRNA is not expressed in the adrenal glands of E12.5 Phox2B$^{LacZ/LacZ}$ mice (B). Wild-type chromaffin cell progenitors (A) express neurofilament 68 at this age. The border of the adrenal gland is demarcated. (C) LacZ in situ hybridization on an adjacent section to panel B shows the presence of LacZ+ cells in the Phox2B$^{LacZ/LacZ}$ embryo. Scale bar: 100 μm.

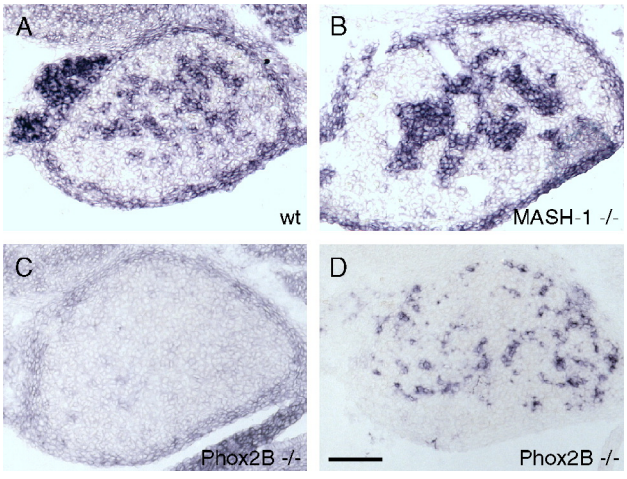

Fig. 8. Expression of NCAM mRNA is lacking in the adrenal gland of E14.5 Phox2B$^{LacZ/LacZ}$ mice (C), while adrenal medullary cells of wild-type (A) and MASH-1$^{-/-}$ mice (B) express NCAM. LacZ in situ hybridization (D) on a section adjacent to panel C shows the presence of LacZ+ cells in the adrenal gland of the Phox2B$^{LacZ/LacZ}$ embryo. Scale bar: 100 μm.

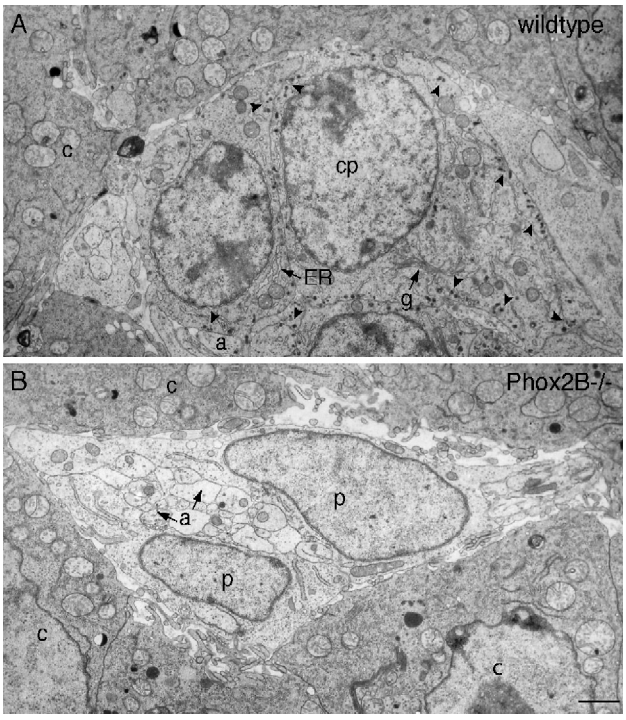

Fig. 9. Electron micrographs showing (A) normally differentiated chromaffin progenitors (cp) at E14.5 in an adrenal gland of a wild-type mouse. Note the specific large secretory "chromaffin" vesicles (arrowheads). (B) Adrenal glands of Phox2B$^{LacZ/LacZ}$ mice contain very immature process-bearing progenitor cells (p) that lack secretory granules. Axons (a), cortical cells (c), endoplasmatic reticulum (ER), Golgi apparatus (g). Scale bar: 1 μm.

MASH-1, which may have a major function in the control of pan-neuronal genes. For chromaffin progenitor cells our present data also suggest a more profound role for Phox2B than for MASH-1 concerning the generation of catecholaminergic traits.

In the avian embryo CASH-1 expression precedes the expression of Phox2B in the SA cells assembling at the dorsal aorta (Ernsberger et al., 1995, 2000). Although this temporal order might suggest a similar temporal sequence in the requirement of the two transcription factors, our data suggest that Phox2B regulates earlier steps in chromaffin progenitor development than MASH-1: chromaffin progenitor cells lacking MASH-1 undergo at least some steps of their normal differentiation program, while Phox2B-deficient progenitors lack all markers that may implicate development beyond the NC stage. Phox2B and MASH-1 appear to be initiated independently in NC cells (Guillemot et al., 1993; Huber et al., 2002). However, maintenance of MASH-1 expression requires Phox2B (cf. our data, and Pattyn et al., 1999). In support of our data, it has been shown that MASH-1 does not specify neural crest cells to develop into autonomic neurons but rather promotes the differentiation of predetermined neuronal progenitors into mature neurons (Sommer et al., 1995). Together, these data suggest that MASH-1 is not essentially required for regulating the earliest steps of autonomic differentiation (chromaffin cells as well as sympathetic neurons). In contrast, Phox2B appears to be essential, at least for chromaffin cell progenitors, to promote differentiation beyond the NC stage.

Interestingly, SA cells, despite their lack of differentiation, still migrate to the adrenal anlagen. The Phox2B-dependent development of catecholaminergic neuronal traits is considered to occur before the cells have migrated to their final destinations, i.e., the definitive (secondary) sympathetic ganglia or the adrenal anlagen. Apparently, molecules that may be postulated to guide SA cells into the adrenal gland are unaffected by the Phox2B mutation.

In summary, we have shown that Phox2B deficiency severely impairs the development of the chromaffin cell phenotype. Even though, a substantial number of SA cells migrates in the adrenal anlagen. As for sympathetic neurons, Phox2B is essential for the development of both catecholaminergic and generic traits, as e.g., the typical ultrastructure of chromaffin cells.

Acknowledgments

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