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Review

The sympathoadrenal cell lineage: Specification, diversification, and new perspectives

Katrin Huber *

Department of Neuroanatomy and Interdisciplinary Center for Neurosciences, University of Heidelberg, INF 307, D-69120 Heidelberg, Germany

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Abstract

During the past years considerable progress has been made in understanding the generation of cell diversity in the neural crest (NC). Sympathoadrenal (SA) cells constitute a major lineage among NC derivatives; they give rise to sympathetic neurons, neuroendocrine chromaffin cells, and the intermediate small intensely fluorescent (SIF) cells. The classic perception of how this diversification is achieved implies that (i) there is a common progenitor cell for sympathetic neurons and chromaffin cells, (ii) NC cells are instructed to a SA cell fate by signals derived from the wall of the dorsal aorta, especially bone morphogenetic proteins (BMP), and (iii) the local environments of secondary sympathetic ganglia and adrenal gland, respectively, are crucial for inducing differentiation of SA cells into sympathetic neurons and adrenal chromaffin cells. However, recent studies have suggested that the adrenal cortex is dispensable for the acquisition of a chromaffin cell fate. This review summarizes the current understanding of the development of SA cells. It covers the specification of SA cells from multipotent NC crest cells, the role of transcription factors during their development, the classic model of their subsequent diversification as well as alternative views for explaining the generation of endocrine versus neuronal SA derivatives.
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Introduction

One of the major challenges in developmental biology is the identification of mechanisms that lead to the diversification of multipotent progenitor cells. The neural crest (NC), a transient structure during vertebrate development, has been profitably exploited as a model system to study how extracellular signals and cell-intrinsic programs cooperate to generate a wide spectrum of different cell types. The NC emerges from the dorsal surface of the neural tube and generates a population of multipotent embryonic cells. They migrate along defined routes within the developing embryo and give rise to diverse cell types, like peripheral neurons and glial cells, the chromaffin cells of the adrenal gland and the C-cells of the thyroid, cells forming parts of the skull, vascular smooth muscles, and other cell types (for a comprehensive review see Le Douarin and Kalcheim, 1999). The developmental fate of NC cells is determined by an interplay of intrinsic and extrinsic factors before, during and after their migration (for review see LaBonne and Bronner-Fraser, 1999). NC cells from the trunk region of the neural tube develop into sensory and sympathetic neurons, chromaffin cells of the adrenal medulla and extra-adrenal paraganglia, peripheral glial cells, and melanocytes. It is widely assumed that sympathetic neurons and chromaffin cells are derived from a common fate-restricted sympathoadrenal (SA) progenitor, which develops from NC cell that have aggregated at the dorsal aorta (Anderson and Axel, 1986; Anderson et al., 1991). In response to the local environment these cells acquire neuronal and catecholaminergic properties. BMPs derived from the wall of the dorsal aorta have been shown to be essentially required for the specification of SA cells (Reissmann et al., 1996; Schneider et al., 1999; Shah et al., 1996). A variety of transcription factors including, MASH-1/CASH-1, Phox2a/b, Hand2 and GATA2/3 promote the development of SA cells. (Guillemot et al., 1993; Pattyn et al., 1999; Howard et al., 2000; Tsarovina et al., 2004). According to classic perception, SA

* Fax: +49 6221 545604.
E-mail address: katrin.huber@ana.uni-heidelberg.de.

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cells subsequently undertake a second migratory step from the dorsal aorta to their final destination, i.e. the definite (secondary) sympathetic ganglia and the adrenal gland, where they finally differentiate into sympathetic neurons or chromaffin cells, respectively (Anderson and Axel, 1986; Anderson et al., 1991). SA cells, that adopt an endocrine chromaffin cell fate, downregulate the expression of neuronal markers (for review see Langley and Grant, 1999). For decades it has been assumed that glucocorticoids provided by the adrenal cortex are essential for this first step towards chromaffin cell differentiation as well as for the induction of the adrenaline synthesizing enzyme phenylethanolamine N-methyltransferase (PNMT) (Anderson and Axel, 1986; Bohn et al., 1981; Doupe et al., 1985a, Seidl and Unsicker, 1989; Unsicker et al., 1978). However, the analysis of glucocorticoid receptor (GR) knockout mice has revealed that glucocorticoid signalling is dispensable for most aspects of chromaffin cell differentiation, with the exception of PNMT and secretogranin II expression (Finotto et al., 1999). Thus, the issue of chromaffin cell development from SA cells remains unsolved. Furthermore, the putative close lineage relationship between sympathetic neurons and chromaffin cells has never been proven, and there is emerging evidence that both cell types may be more distantly related than originally assumed. This review summarizes the current understanding of the specification of SA cells as well as their subsequent diversification. Finally, it will be discussed whether chromaffin cells may originate from an alternative source of NC cells other than the classical TH positive SA progenitor.

Specification of sympathoadrenal cells

NC cells that develop into SA cells take the ventral migration route, passing mainly through the anterior parts of the somites (Loring and Erickson, 1987). During their migration they are exposed to signals from the somites, the ventral neural tube and the notochord. They aggregate in the vicinity of the dorsal aorta, where they form the so-called primary sympathetic ganglia and undergo neuronal and catecholaminergic differentiation (Figs. 1A, B). The cells initiate the expression of enzymes that are required for the synthesis of noradrenaline, i.e. tyrosine hydroxylase (TH) and dopamine β-hydroxylase (DBH) (Cochard et al., 1978; Ernsberger et al., 1995, 2000). In addition, they acquire a neuronal fate and start to express neurofilament (NF), SCG10, neuron specific tubulin, and other neuronal markers (Cochard and Paulin, 1984; Groves et al., 1995; Schneider et al., 1999; Sommer et al., 1995). A wealth of data suggests that bone morphogenetic proteins (BMPs) derived from the dorsal aorta play a central role for the differentiation of SA cells. This notion is supported by the expression of BMPs in the wall of the dorsal aorta of avian (BMP-4/7, Reissmann et al., 1996) and mouse embryos (BMP-2/4, Shah et al., 1996). NC cells that have aggregated at the dorsal aorta of the chick embryo express BMP-receptor IA and BMP-receptor IB (McPherson et al., 2000). Furthermore, BMP-2/4/7 have been shown to increase the number of catecholaminergic and neuronal cells in cultures of avian NC cells (Bilodeau et al., 2001; Reissmann et al., 1996; Varley et al., 1995). Over-expression of BMP-4 in chick embryos induced ectopic cells, expressing SA specific markers (Reissmann et al., 1996). The implantation of beads releasing noggin, a specific inhibitor of BMP-4/7, abolished the expression of catecholaminergic and neuronal markers in NC cells that had aggregated at the dorsal aorta (Schneider et al., 1999). Together, these data demonstrate that, at least in the chick embryo, BMPs are required in vivo for the development of SA cells. In mammalian embryos the role of BMPs for SA development has not been investigated in vivo, since such an analysis is limited by the fact that multiple BMPs and different type I receptors may be involved in the process and that some of the relevant mouse mutants, BMP-4,7 or BMP-RIA knockout mice (Luo et al., 1995; Mishina et al., 1995; Winnier et al., 1995) die at very early stages of their development.

Despite the unquestionable fundamental role of BMPs for the specification of SA cells, it seems likely that additional factors are required, since BMPs are also crucial for the development of other autonomic NC derivatives, which are clearly distinct from SA cells, as e.g. parasympathetic neurons (Muller and Rohrer, 2002). Furthermore, there is evidence to suggest that NC cells that have arrived at the dorsal aorta may already be pre-specified by signals other than BMPs. In the chick embryo it has been shown that the expression of CASH-1, a marker distinctly expressed in the autonomic sublineage of the NC, precedes the expression of BMP-4 in the wall of the dorsal aorta and its receptors in NC cells (Ernsberger et al., 1995; McPherson et al., 2000). Following the application of noggin in chick embryos CASH-1 expression is reduced, but not completely lacking (Schneider et al., 1999). Thus, it is conceivable that BMPs may not be absolutely crucial for the initial expression of CASH-1/MASH-1, but only for its maintenance, which depends on Phox2b (Pattyn et al., 1999; Huber et al., 2005). It should be noted that the expression of Phox2b is completely abrogated upon noggin treatment (Schneider et al., 1999).

Signals from the ventral neural tube and the notochord have also been implicated in the differentiation of SA cells. Early experiments from various groups have shown that the ablation or rotation of the neural tube/notochord abrogates catecholaminergic differentiation of NC cells that have assembled at the dorsal aorta (Groves et al., 1995; Stern et al., 1991; Teillet and Le Douarin, 1983). However, the relevant signals have not been identified. In addition, it has been proposed that catecholaminergic differentiation of NC cells is regulated by noradrenaline transporter (NET) promoted uptake of noradrenaline, provided by the notochord (Ren et al., 2001). NET is expressed in NC cells, and its expression is regulated by neurotrophin-3 and TGF-β1 (Ren et al., 2001). In summary, signals from the somites, the ventral neural tube, the notchord, and BMPs derived from the dorsal aorta may cooperate to specify SA cells.

The role of transcription factors during SA development

Differentiation of SA cells is promoted by a cross-regulatory network of transcription factors, which is at least in part

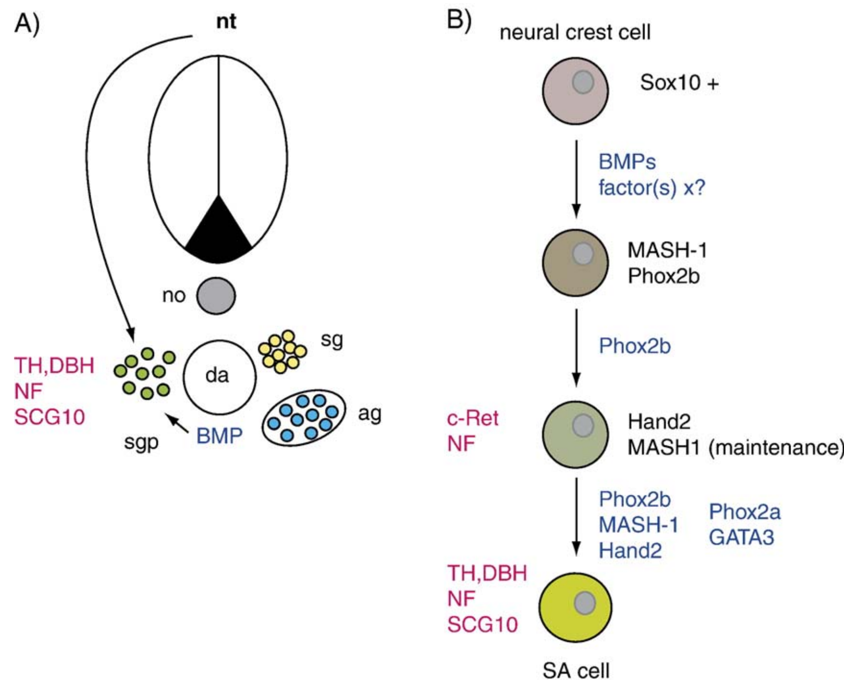

Fig. 1. (A) SA cells develop from NC cells that have aggregated at the dorsal aorta, where they form the primary sympathetic ganglia (sgp). In response to BMPs, which are provided by the wall of the dorsal aorta (da), the cells acquire a catecholaminergic neuronal fate and initiate the expression of neuronal markers and the enzymes for noradrenaline synthesis TH and DBH. According to the classic perception SA cells re-migrate from the location of the primary sympathetic ganglia to populate the definite sympathetic ganglia (sg) or the adrenal primordia (ag). In these locations the cells are considered to undergo terminal differentiation into sympathetic neurons or chromaffin cells respectively. no: notochord. (B) The development of SA cells from multipotent NC cells may involve several steps. Sox10 positive neural crest cells receive a BMP signal and start to express MASH-1 and Phox2b. It is not clear, whether the initial expression of MASH-1 is dependent on BMPs. Analyses of mouse mutants have suggested that Phox2b, but not MASH-1, is required for the earliest events of SA differentiation. This includes the induction of early neuronal markers like neuron specific tubulin, neurofilament 68, c-Ret and the transcription factor Hand2. Phox2b is also required for the maintenance of MASH-1. Further differentiation of SA cells appears to be promoted by the interaction of MASH-1, Hand2, Phox2b and finally Phox2a and GATA2/3.

activated by BMPs. This includes the mammalian achaete–scute homolog 1 (MASH-1), the paired homeodomain proteins Phox2a and Phox2b, Hand2 (dHAND) and GATA 2/3 (Guillemot et al., 1993; Howard et al., 2000; Pattyn et al., 1999; Lim et al., 2000; Stanke et al., 1999). In the primary sympathetic ganglia of chick embryos the expression of CASH-1 (the chick homologue of MASH-1) and Phox2B is followed by that of Hand2, Phox2a and GATA2 (Ernsberger et al., 1995, 2000; Howard et al., 2000; Tsarovina et al., 2004). The order of appearance of CASH-1 and Phox2b has not been assessed. Gain- and loss-of-function experiments have been employed to unravel the role of these transcription factors during development (Figs. 1B and 2).

MASH-1 is a helix–loop–helix transcription factor and the mammalian homologue of Asc genes in Drosophila (Johnson et al., 1990). MASH-1 and its chick homologue CASH-1 are transiently expressed in the whole autonomic progeny of the neural crest, i.e. sympathetic, parasympathetic and enteric neurons, adrenal chromaffin cells, as well as in specific subsets of central neurons (Guillemot and Joyner, 1993; Lo et al., 1991). Mice with a targeted null mutation of MASH-1 die during the first 24 h after birth, probably due to feeding problems (Guillemot et al., 1993). In the absence of MASH-1, the development of the autonomic nervous system is almost completely abrogated. However, the primary sympathetic ganglia assemble normally at the dorsal aorta, and the cells undergo parts of their normal differentiation program, before they eventually die (Guillemot et al., 1993; Hirsch et al., 1998; Sommer et al., 1995). MASH-1 activates Phox2a, a positive regulator of noradrenergic traits, and it is required for the acquisition of the noradrenaline biosynthetic enzymes TH and DBH (Hirsch et al., 1998; Lo et al., 1998). Furthermore, some neuronal markers, as e.g. SCG10 and peripherin, are lacking in

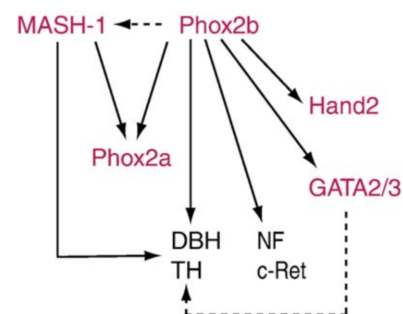

Fig. 2. Transcriptional regulation of the development of SA cells. This scheme summarizes data obtained from gene knockout studies (for details and references see text). Dashed lines indicate a requirement for maintenance. The expression of GATA3 in the absence of MASH-1 has not been assessed. For Hand2 loss of function data are not available yet. Note that overexpression of any of these transcription factors in chick embryos (apart from GATA2) can induce the generation of ectopic sympathetic neurons (Howard et al., 2000; Tsarovina et al., 2004; Stanke et al., 1999, 2004).

SA cells following the inactivation of MASH-1 (Sommer et al., 1995). However, the expression of early markers, like Phox2b, Hand2, c-Ret, neurofilament 68 and 160, and neuron specific tubulin is not affected (Guillemot et al., 1993; Howard et al., 2000; Huber et al., 2002; Sommer et al., 1995). These findings, along with a series of in vitro studies (Sommer et al., 1995), suggested that MASH-1 is not required for NC cells to adopt a neuronal fate, but rather promotes the further development of early precursors that are already committed to a neuronal fate. Some sympathetic neurons seem to escape MASH-1 dependency and undergo at least parts of their normal differentiation program, including the expression of Phox2a and DBH and TH (Guillemot et al., 1993; Hirsch et al., 1998). Originally, it was reported that adrenal medullary chromaffin cells develop largely normal in MASH-1 mutant mice, which would have implied that the neuronal and endocrine progenies of the SA lineage differ regarding their molecular requirements already at early developmental stages. However, our re-analysis of MASH-1 deficient mice has revealed that no more than about 20% of chromaffin cells differentiate normally in the absence of MASH-1 (Huber et al., 2002). The vast majority of mutant adrenal medullary cells, identified by the expression of Phox2b, does not initiate the expression of TH and DBH and, unlike wildtype chromaffin cells, maintain the expression of neurofilament 68 and c-Ret. Ultrastructurally, adrenal medullary cells of MASH-1−/− mice resemble immature neuroblasts and do not contain the typical chromaffin granules. This result indicates that, in principle, chromaffin cells, like sympathetic neurons, depend on MASH-1 to transit from an early precursor stage to a mature cell. However, it must be noted that there are also differences between chromaffin cells and sympathetic neurons concerning their dependency on MASH-1. This includes the timing of cell death and the fact that, in contrast to sympathetic ganglia, Phox2a expression appears to be unaltered in chromaffin cells of MASH-1 deficient mice (Huber et al., 2002).

The homeodomain Phox2b is expressed in all central and peripheral noradrenergic neurons as well as in a majority of neurons that are integrated in autonomic reflex pathways (Dauger et al., 2003; Pattyn et al., 1997, 1999). In SA cells Phox2b is expressed independently of MASH-1 (Hirsch et al., 1998; Huber et al., 2002). Phox2b deficient mice die at midgestational stages, but can be rescued until birth by the application of noradrenaline agonists to the drinking water of the mother (Pattyn et al., 2000). Autonomic neurogenesis is completely abolished in the absence of Phox2b and neural crest cells assembling at the dorsal aorta at E10.5 or colonizing the adrenal gland at E13.5 are devoid of any autonomic markers, apart from MASH-1 (Huber et al., 2005; Pattyn et al., 1999). Even MASH-1 is prematurely downregulated in these cells, indicating that Phox2b is required for the maintenance of MASH-1. A whole battery of gene products, which are not affected by the MASH-1 mutation, like neuron–specific tubulin, neurofilament 68, c-Ret or the transcription factor Hand2 (Guillemot et al., 1993; Sommer et al., 1995; Huber et al., 2002) are lacking in the absence of Phox2b (Pattyn et al., 1999, 2000; Howard et al., 2000; Huber et al., 2005). Altogether, the defects observed in MASH-1 and Phox2b mutant mice suggest that Phox2b plays a more fundamental role for SA development than MASH-1.

SA cells also express Phox2a, a homeodomain transcription factor closely related to Phox2b (Valarché et al., 1993; Morin et al., 1997; Pattyn et al., 1997). Gain-of-function experiments have indicated that Phox2a is sufficient to promote autonomic neurogenesis in vivo and to activate Phox2b (Stanke et al., 1999). In contrast to this, loss of Phox2a function does not impair the development of sympathetic neurons or chromaffin cells. The only reported alteration in the sympathetic nervous system, following inactivation of Phox2a, is a morphological anomaly of the superior cervical ganglion (Morin et al., 1997). This implies that Phox2a is largely dispensable for the development of cells of the SA lineage or its function may be compensated by Phox2b. However, it is required for the differentiation of parasympathetic neurons as well as for some noradrenergic centres of the brain (Morin et al., 1997). Very recently, reciprocal gene replacements of the Phox2 genes have revealed that Phox2a and Phox2b are functionally not equivalent. During development of SA cells Phox2a, which is normally downstream of Phox2b, cannot replace Phox2b function, even if it is expressed from the Phox2b locus (Coppola et al., 2005).

The basic helix loop helix transcription factor Hand2 has been identified as a noradrenergic co-determinant. It is initiated subsequently to Phox2b, but prior to Phox2a (Howard et al., 1999, 2000). Hand2 expression depends on Phox2b but is independent of MASH-1 (Howard et al., 2000; Huber et al., 2002, 2005; Morikawa et al., 2005). Forced expression of Hand2 induces the expression of noradrenergic as well as pan-neuronal markers in NC cells (Howard et al., 2000; Morikawa et al., 2005). Although these findings imply that Hand2 has a broad range of functions during SA development regulating generic neuronal as well as subtype specific aspects, it has been speculated that Hand2 may specifically be required for the maintenance of a noradrenergic phenotype in sympathetic neurons. Hand2 is lacking in the cholinergic parasympathetic ciliary ganglion and overexpression of Hand2 in ciliary neurons leads to persistent expression of TH and DBH (Muller and Rohrer, 2002). However, more recently it has been reported that other parasympathetic ganglia like the sphenopalatine ganglion, the submandibular ganglion and the sacral ganglionic complex express Hand2 (Dai et al., 2004; Morikawa et al., 2005), suggesting a broader function of Hand2 during the development of autonomic neurons. Unfortunately, loss of function data are not available yet, due to early embryonic lethality of Hand2 deficient mice (Srivastava et al., 1997).

In addition to the above transcription factors, the zinc finger transcription factors GATA2 and GATA3 are expressed in SA cells of mouse embryos, while in the chick only GATA2 is present (George et al., 1994; Lim et al., 2000; Tsarovina et al., 2004). Overexpression of GATA2 in chick embryos generates ectopic neurons with a non-autonomic identity (Tsarovina et al., 2004). Inactivation of GATA3 in mice leads to a loss of GATA2 in SA cells. Apart from this, at E10.5 primary sympathetic ganglia seemed to be largely intact. They express c-ret, neuron specific β-tubulin, TH, albeit at reduced levels, and DBH as

well as the transcription factors Phox2a, Phox2b and Hand2 (Tsarovina et al., 2004). However, at later stages sympathetic neurons downregulate DBH and lose TH expression. In addition, enhanced apoptosis has been observed in sympathetic ganglia (Tsarovina et al., 2004). Adrenal medullary chromaffin cells have not been investigated in detail in mice lacking GATA3, but it was reported that they lack TH expression at E14.5 (Lim et al., 2000). It has been controversely discussed whether GATA2/3 transcription factors are specifically required for the establishment/maintenance of noradrenergic traits in autonomic neurons, or whether they have a broader range of functions in their development. The fact that sympathetic neurons undergo apoptosis in the absence of GATA3 (Tsarovina et al., 2004), however, may corroborate the latter notion.

Taken together, the transcription factors that have been identified to contribute to the specification of SA cells may be ranked in their relative importance for SA development, as Phox2b > MASH-1 > GATA3. Nevertheless, it is still unclear how Hand2 should be integrated into this scheme, due to the lack of loss-of-function data. Phox2b and MASH-1 appear to promote the development of all autonomic NC lineages, while GATA2/3 may be more specifically required for establishing a sympathoadrenal phenotype that is characterized by the maintenance of noradrenergic traits.

At this stage, it should be noted that only few direct targets of these transcription factors have been identified so far, e.g. DBH and the cell adhesion molecule NCAM for the Phox2 transcription factors (Valarché et al., 1993; Kim et al., 1998; Yang et al., 1998), and that the molecular cascades that lead to the differentiation of SA cell are still only poorly understood. However, there has been some recent progress in that it has been shown that BMP-4 supports neurogenesis by a canonical pathway, involving smad1/4 translocation as well as a non-canonical pathway dependent on PKA (Liu et al., 2005). CAMP was implicated in the coordination of both pathways, and it has been demonstrated that it regulates Phox2a transcription and activity (Chen et al., 2005; Liu et al., 2005). The cAMP effect on Phox2a activity has been shown to involve the dephosphorylation of Phox2a (Adachi and Lewis, 2002). It was reported before that the induction of catecholaminergic traits by BMPs in rat NC cultures requires the activation of a cAMP-dependent transduction pathway in addition to BMPs (Lo et al., 1999).

Diversification of SA cells

According to the classic view of SA development, SA cells, after acquisition of neuronal and catecholaminergic traits, undergo a second migration step away from the dorsal aorta to form the secondary sympathetic ganglia, the prevertebral ganglia, the adrenal medulla and extra-adrenal chromaffin tissues, as e.g. the organ of Zuckerkandl. In these locations the cells undergo their final differentiation into sympathetic neurons or chromaffin cells, respectively. Mature sympathetic neurons and adrenal chromaffin cells share many characteristics, but are very distinct in other aspects. Both cell types harbor the enzymes for synthesizing noradrenaline, i.e. TH and DBH. In contrast to chromaffin cells, sympathetic neurons extend long neurites and maintain the expression of typical neuronal markers, as e.g. neurofilament 68 and SCG10, which are downregulated in chromaffin cells at early developmental stages (for review see Langley and Grant, 1999). In the mouse adrenal gland neurofilament 68 expression is detectable at E12.5 (Huber et al., 2005), but it is virtually extinguished at E14.5. The most striking morphological hallmark of chromaffin cells are the typical large chromaffin granules required for the storage and release of catecholamines (Coupland, 1972; Coupland and Tomlinson, 1989). A subpopulation of chromaffin cells harbors the adrenaline synthesizing enzyme phenylethanolamine N-methyltransferase (PNMT).

For decades it was assumed that the local environment, i.e. the site of the secondary sympathetic ganglia or the adrenal cortex, is essential to determine whether SA cells differentiate into sympathetic neurons or chromaffin cells. Based on in vitro studies it was postulated that SA cells require high doses of glucocorticoids, provided by the adrenal cortex, to differentiate into chromaffin cells. Glucocorticoids were believed to promote two steps of chromaffin cell differentiation (i) the downregulation of neuronal markers and (ii) the induction of the adrenaline synthesizing enzyme PNMT (Anderson and Axel, 1986; Bohn et al., 1981; Doupe et al., 1985a, Unsicker et al., 1978). However, the analysis of mice deficient for the glucocorticoid receptor has revealed that glucocorticoid signalling is dispensable for the generation of chromaffin cells (Finotto et al., 1999).

Differentiation of chromaffin cells is almost normal in GR knockout mice, with the exception of the lack of secretogranin II and PNMT (Finotto et al., 1999). Moreover, even the complete absence of an adrenal cortex does not abrogate chromaffin cell development. In mice deficient for steroidogenic factor-1 (SF-1), which lack an adrenal cortex (Luo et al., 1994), chromaffin cells accumulate and differentiate at the precise site, where the adrenal anlage is located in wildtype mice, i.e. at the upper pole of the kidney in close association with the suprarenal ganglion (Gut et al., 2005). Like their counterparts in wildtype mice, they develop the typical large chromaffin granules and downregulate neuronal markers (NF68 and SCG10). However, in SF-1 mutant mouse embryos chromaffin cells lack the expression of PNMT, which is known to depend on high levels of glucocorticoids and their numbers are reduced to about 50% as early E13.5, suggesting that the adrenal cortex is essential for assembling the full number of chromaffin cells to the correct site. Since neither glucocorticoid signalling nor an adrenal cortex appear to be an absolute prerequisite for most aspects of specific chromaffin cell differentiation, mechanisms that generate chromaffin cells from SA progenitors remain unclear. An early study has suggested that FGF-2 influences the development of SA cells to adopt a neuronal fate and induces NGF dependency (Stemple et al., 1988). Thus it is possible that SA cells, which do not receive such signals, undergo chromaffin cell differentiation by a default pathway. The notion that chromaffin cells probably have occurred earlier in evolution may support this hypothesis (see next chapter). Taking the endocrine function of chromaffin cells

into consideration, the most obvious requirement on the local environment a chromaffin cell may have is the presence of a well-developed network of capillaries, venules, and large veins to release their products. The adrenal gland provides such an environment, and extra-adrenal chromaffin cells are also preferentially found in close association with large veins, like the inferior caval or the renal veins (cf. Coupland, 1965). Whether signals specifically derived from blood vessels contribute to the attraction or even differentiation of chromaffin cells has not been investigated.

However, it has been hypothesized previously that signals from capillaries may influence the differentiation SIF cells (Hall and Landis, 1991). SIF cells are intermediate in morphology between chromaffin cells and sympathetic neurons (see Eränkö, 1978 for review). Similar to chromaffin cells they have small cell bodies packed with large dense core vesicles, but they also resemble sympathetic neurons in their ability to grow neurites. SIF cells may function as endocrine cells or interneurons (Elfvin et al., 1993). In sympathetic ganglia they are closely associated with fenestrated capillaries (Hall and Landis, 1991). Little is known about the signals that regulate the development of SIF cells versus that of sympathetic principal neurons. In vitro and in vivo experiments have suggested that the development of SIF cells, similar to that of chromaffin cells, is supported by glucocorticoids (Bohn, 1987; Doupe et al., 1985b; Eränkö et al., 1972). However, in light of the fact that chromaffin cell development is intact in GR-deficient mice it seems unlikely that SIF cells essentially require glucocorticoids for their differentiation. It has been speculated that SIF cells of sympathetic ganglia have escaped signals that promote neuronal differentiation (FGF/NGF) and that molecular cues from capillaries may further promote and/or stabilize SIF cell development (Hall and Landis, 1991). In contrast to sympathetic principal neurons and similar to adrenal chromaffin cells, SIF cells survive and differentiate in the absence of TrkA, the high affinity receptor for NGF (Fagan et al., 1996; Schober et al., 1997 and A. Schober personal communication).

Sympathetic neurons and chromaffin cells: evolutionary aspects

Generally speaking there is a shift from chromaffin cells to sympathetic neurons during evolution. In the most basal vertebrates, the clyclostomes, cardiovascular functions is mainly controlled by chromaffin cells, which are located on the wall of blood vessel as well as in the kidney and along the urogenital ducts (Botar, 1974). Cyclostomes lack sympathetic ganglia, but they exhibit small groups of neurons, which have been interpreted as peripheral autonomic neurons. The numbers of these neurons, however, are very low, as compared to those of chromaffin cells (Botar, 1974). Sympathetic ganglia first appear in elasmobranchs. They are segmentally arranged and closely associated with large groups of chromaffin cells. In teleosts the sympathetic ganglia have further developed into a well-organized paravertebral trunk and the major aggregation of chromaffin tissue is associated with the posterior cardinal vein. In tetrapods (with the exception of primitive urodels) the bulk of chromaffin tissue becomes closely associated with the steroidogenic interrenal tissue (adrenal cortical cells) to form an adrenal gland (see Gibbins, 1994 for an extensive review).

The lineage relationship between chromaffin cells and sympathetic neurons

The hypothesis that chromaffin cells and sympathetic neurons develop from a common fate-restricted SA progenitor that segregates as a separate lineage from the NC has predominantly been based on the examination of marker expression and the fact that SA progenitor cells isolated from embryonic or early postnatal sympathetic ganglia or adrenal glands can differentiate into sympathetic neurons or chromaffin cells depending on specific cues provided (Doupe et al., 1985a; Anderson and Axel, 1986; Seidl and Unsicker, 1989; Unsicker et al., 1978). In addition it was shown in rat embryos that cells populating the primary sympathetic ganglia co-express neuronal markers and the chromaffin cell specific markers SA1–5, in addition to the catecholaminergic marker enzyme TH (Anderson and Axel, 1986). Furthermore, SA cells colonizing the adrenal gland initially express neuron-specific markers like neurofilament and SCG10 (Anderson and Axel, 1986; Vogel and Weston, 1990). These findings lead to the hypothesis that chromaffin cells and sympathetic neurons originate from the same pool of precursors, which is located in the primary sympathetic ganglia (Figs. 1A, 3A). However, although this hypothesis is still widely accepted, a formal proof based on solid in vivo data is lacking to date. Moreover, there is emerging evidence arguing against the postulated close lineage relationship between sympathetic neurons and chromaffin cells. We found that the adrenal anlage of mouse embryos is colonized at E11.5 by cells that express MASH-1, but still lack TH expression, while nearby located sympathetic neurons are already TH positive (Gut et al., 2005). This finding suggests that chromaffin cells and sympathetic neurons may not share the same pool of TH-positive progenitors, but develop independently from the neural crest. However, it should be noted at this point, that sympathetic neurons at different axial levels also develop independently. The term lineage is commonly used for the progeny of equivalent progenitors and not only in its strict sense for the progeny of the same ancestor. Thus, sympathetic neurons and chromaffin cells may still be regarded as one lineage, in case their early precursors are equivalent. This question, however, has not been clarified to date.

Recent studies in chick embryos have pointed to the possibility that the precursors of chromaffin cells and sympathetic neurons may already be distinct at the earliest stages of their development. Prior to the formation of a discrete adrenal anlagen, catecholaminergic cells, positive for TH, but negative for neuronal markers like NF-M, prevailed at the position, where the adrenal anlagen become distinct later (Ernsberger et al., 2005). Neuronal markers may have been rapidly downregulated in these cells. However, it is also conceivable that undifferentiated precursors directly differentiate into TH-positive, NF-

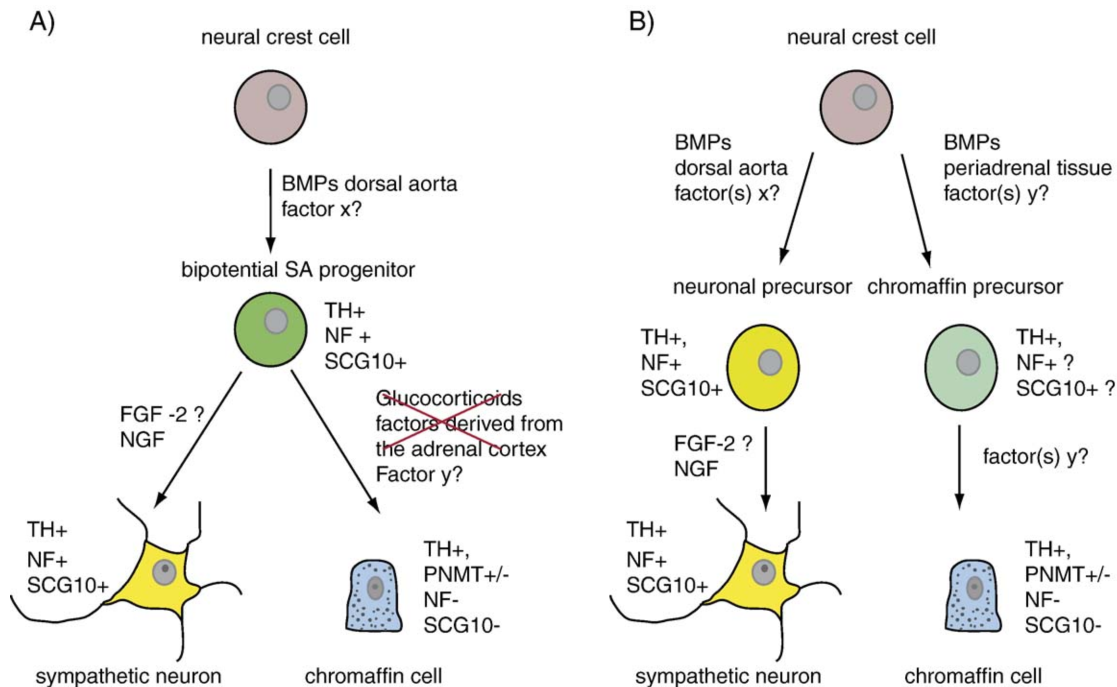

Fig. 3. The lineage relationship between sympathetic neurons and chromaffin cells is unclear. (A) The classic view implies that sympathetic neurons and chromaffin cells originate from a pool of bipotential TH+ positive SA progenitors that are initially located in the primary sympathetic ganglia. These cells re-migrate to the secondary sympathetic ganglia or the adrenal anlage, where they undergo final differentiation in response to the local environment. However, analyses of mice deficient for the GR-receptor or SF-1 have argued against an essential role of the adrenal cortex in the determination of chromaffin cell fate. (B) There is emerging evidence to suggest that sympathetic neurons and chromaffin cells are more distinctly related than originally assumed. Both cell types may develop independently from NC cells, following different maturation schedules. Thus, undifferentiated NC cells may migrate to the adrenal anlage, where they receive a BMP-4 signal provided by cells surrounding the primordium.

M-negative cells destined to become adrenal chromaffin cells (Ernsberger et al., 2005).

Chromaffin cells and sympathetic neurons seem to recruit the same set of transcription factors during early stages of their development and depend on them in a similar way. However, there are differences between chromaffin cells and sympathetic neurons concerning the timing of appearance of defects and cell death in the absence of MASH-1 or Phox2b (Huber et al., 2002, 2005). Chromaffin cells and sympathetic neurons may develop independently from NC cells following different maturation schedules and not, as hypothesized, via a common TH positive precursor (Fig. 3B). NC cells that migrate to the adrenal anlage could be instructed to develop into SA cells by BMP-4, which is expressed in cells surrounding the adrenal primordium (Gut et al., 2005). If it were true that chromaffin cells and sympathetic neurons are more distantly related then generally assumed, it cannot be excluded that the decision whether a SA cell will adopt an endocrine or neuronal fate is predetermined by yet unknown signals delivered before the cells arrive at the position of the definite sympathetic ganglia or the adrenal gland, or even prior to reaching the dorsal aorta. In this case the continuing use of the term “sympathoadrenal lineage” would not be appropriate.

Conclusion

Our understanding of how cell diversity among NC derivatives is achieved has been substantially improved during the past years. BMPs have been identified as key molecules in regulating the development of SA cells, and a variety of transcription factors have been identified that operate during their development. Other issues, however, including the question how endocrine vs. neuronal SA cells develop are unsolved. The classic perception that the generation of chromaffin cells from SA progenitors depends on the adrenal cortex is not correct. Moreover, there is increasing evidence to suggest that sympathetic neurons and chromaffin cells are more distantly related than originally assumed.

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