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From proliferation to target innervation: signalling molecules that direct sympathetic nervous system development

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Introduction

This review will concentrate on the embryonic development of sympathetic para- and prevertebral ganglia, the adrenal medullae and extra-adrenal chromaffin cells. All of these tissues are derived from neural crest cells (Le Douarin and Kalcheim, 1999). Neural crest cells (NCC) originate from the neuroectoderm and migrate throughout the developing embryo to generate a wide range of cell types (Dupin and Le Douarin, 2014). Subsequent articles in this issue discuss in detail a range of disorders that can result from errors in the development of this subset of NCC.

Sympathetic ganglia

Sympathetic ganglia exist as two chains of paravertebral ganglia running from the cervical into the pelvic region and as prevertebral ganglia associated with the junction of the celiac and superior and inferior mesenteric arteries with the aorta in the abdomen. Sympathetic neurons are predominantly noradrenergic in phenotype, that is, they release noradrenaline as a neurotransmitter. A small number of sympathetic neurons are cholinergic in phenotype, releasing acetylcholine as a neurotransmitter.

Paraganglia

Paraganglia consist of catecholamine-containing neuroendocrine cells of neural crest origin (Le Douarin and Teillet, 1971, Le Douarin and Teillet, 1973). Some paraganglia are chemoreceptors associated with branches of the vagus and glossopharyngeal nerves in the head and neck and will not be considered in detail in this review. The remaining paraganglia are endocrine in function and work closely with the autonomic nervous system by secreting catecholamines to support homeostasis in response to environmental stresses (Jänig, 1989, Kannan, 1986). They form the adrenal medullae and extra-adrenal chromaffin cells. Extra-adrenal chromaffin cells appear in clusters from the level of the superior cervical ganglion down to the pelvis, often in association with sympathetic ganglia, the abdominal aorta and the urogenital organs (McNicol, 2004).

There are two types of chromaffin cell, in the adrenal medullae; one (the majority) that synthesizes predominantly adrenaline (adrenergic chromaffin

cells) and the other type that synthesizes only noradrenaline (noradrenergic chromaffin cells). In rats, 65–85% of medullary chromaffin cells are adrenergic while the remaining 15–35% are noradrenergic (Allmendinger, et al., 2003, Coupland and Weakley, 1970a, El-Maghraby and Lever, 1980, Eränkö, 1955). Adrenergic chromaffin cells are characterized by expression of phenylethanolamine N-methyltransferase (PNMT), which catalyses methylation of noradrenaline to form adrenaline (Coupland and Weakley, 1970a, El-Maghraby and Lever, 1980, Eränkö, 1955). Both cell types release their catecholamine into the bloodstream under the control of sympathetic preganglionic neurons.

Extra-adrenal chromaffin cells are mainly noradrenergic (Ahonen, et al., 1987, McNicol, 2004). They are believed to be an important source of circulating catecholamines during fetal development (Thomas, et al., 1995, Zhou, et al., 1995). The organ of Zuckerkandl is the largest cluster of extra-adrenal chromaffin cells and is located close to the adrenal glands, around the origin of the inferior mesenteric artery (McNicol, 2004, Schober, et al., 2013). It disappears postnatally.

The role of nerves in the function of endocrine extra-adrenal chromaffin cells is uncertain. Abdominal extradrenal ganglia lack innervation in some species (Coupland and Weakley, 1970b, Mascorro, et al., 1994, Mascorro and Yates, 1977), including the mouse, (Mascorro and Yates, 1971), but nerve fibres of unknown origin are present in others (Coupland, et al., 1982, Mascorro and Yates, 1974, Partanen, et al., 1984a, Partanen, et al., 1984b) where they appear much less dense than those associated with adrenal chromaffin cells. In all these cases it remains to be established that any innervation is motor rather than sensory. However, it has been suggested that the release of catecholamines from the extra-adrenal tissues of the guinea pig parallels that from the adrenal medullae, and that this is due to nerve activity (Coupland, et al., 1982). Otherwise, it is possible that extra-adrenal chromaffin cells release catecholamines in response to humoral factors, or hypoxia (Hervonen and Korkala, 1972, Hervonen and Korkala, 1973).

Early development of NCC

The neural crest appears to have arisen in the chordate lineage leading to the vertebrates (Muñoz and Trainor, 2015) and arises from the lateral margins of the neuroepithelium forming the neural plate as it invaginates to form the neural tube. The cells from the dorsal part of the neural tube undergo an epithelial to mesenchymal transition (EMT,) delaminate and migrate throughout the developing embryo. The EMT and migratory properties of NCC have been likened to that of metastasizing tumor cells (Kerosuo and Bronner-Fraser, 2012, Lim and Thiery, 2012). The early stages in the development of NCC, including induction and delamination from the neural tube, have been extensively reviewed (Krispin, et al., 2010a, Le Douarin, et al., 2008, Le Douarin and Kalcheim, 1999, Mayanil, 2013, McKinney, et al., 2013, Newbern, 2015, Noisa and Raivio, 2014, Sauka-Spengler and Bronner-Fraser, 2006, Vega-Lopez, et al., 2017).

When are NCC specified? Multipotent versus lineage-restricted

NCC give rise to a wide range of cell types, including bone, cartilage and connective tissues of the face and jaws, the peripheral nervous system and skin pigment cells (Dupin and Le Douarin, 2014). Trunk NCC that give rise to chromaffin cells and sympathetic nervous system are at least, *in vivo*, more restricted than cranial NCC as they give rise to only one mesenchymal cell type, the epineurial fibroblasts of the peripheral nerves (Joseph, et al., 2004). However, trunk NCC give rise to sensory and sympathetic neurons, satellite cells and myelinating and non-myelinating Schwann cells, paraganglion cells and to melanocytes (Le Douarin and Kalcheim, 1999).

A key question about NCC is whether the different lineages to which they give rise are specified early, for instance prior to delamination from the neural tube, or whether NCC in the neural tube and during migration represent multipotent cells whose final fate is determined by the environment of their final destination. Many studies have attempted to answer this question, using a number of approaches, in zebrafish (Raible and Eisen, 1994, Schilling and Kimmel, 1994) avians (Baroffio, et al., 1988, Bronner-Fraser and Fraser, 1989, Bronner-Fraser and Fraser, 1988, Dupin, et al., 2010, Frank and Sanes, 1991, Henion and Weston, 1997, Krispin, et al., 2010b, McKinney, et al., 2013,

Shtukmaster, et al., 2013) and mice (Baggiolini, et al., 2015, Serbedzija, et al., 1990, Stemple and Anderson, 1992). These studies have demonstrated that many individual NCC within the neural tube or as they delaminate can give rise to clones of cells that include multiple cell types, the exact combinations being strongly influenced by the stage of development examined. However, nearly all studies also record instances of individual NCC giving rise to only one cell type, thus appearing to be fate restricted. The ratio of multipotent to unipotent NCC may depend on the species. Certainly zebrafish appear to have very few multipotent NCC prior to delamination (Raible and Eisen, 1994, Schilling and Kimmel, 1994) but avians and mammals both seem to show a mixture of unipotent and multipotent NCC in the neural tube. Whatever the frequency of multipotency in NCC prior to delamination, there is compelling evidence (see below) that once delaminated, subpopulations of migrating NCC rapidly start to express different combinations of molecules that correlate with their fate and which likely represents significant fate restriction as they migrate.

Migration of NCC

NCC cells are generated along the full length of the embryo, with the exception of the most anterior part of the embryo. Cells that give rise to the most cranial sympathetic ganglia in the chick, the superior cervical ganglion (SCG), arise from the trunk neural crest that forms between somites 5 and 10 (Le Douarin and Kalcheim, 1999), while in the mouse, the SCG forms from cells from somites 1-5 (Durbec, et al., 1996), with other sympathetic ganglia formed by NCC from more caudal levels. In chickens, chromaffin cells of the adrenal medullae arise from NCC between somites 18 and 24 (Le Douarin and Kalcheim, 1999).

The first requirement for migration of NCC is that they undergo an EMT in order to leave the epithelium of the neural tube. The EMT appears to be under the control of Wnt/β catenin and bone morphogenetic protein (BMP) signaling (Lim and Thiery, 2012). For trunk NCC, the EMT requires the concordant activation of SRY-related HMG-Hox 9 (*Sox9)*, snail family transcriptional repressor 2 (*Snai2*) and forkhead box D3 (*FoxD3*) genes to regulate the competence to respond to the EMT initiation signals, start the EMT program and

co-ordinate the necessary cell adhesion molecules for migration respectively (Cheung, et al., 2005).

In mice, the *initial* wave of delaminating NCC moves ventrally along blood vessels between the somites (Schwarz, et al., 2009b). These cells are relatively few in number. Shortly after, delaminating NCC of the *intermediate* wave move ventrally through the anterior part of the somite in the sclerotome, while the first NCC of the *final* wave move dorsolaterally between the epidermis and the dermomyotome (Schwarz, et al., 2009b). In mice, the timing of the three waves overlap significantly (Schwarz, et al., 2009a, Serbedzija, et al., 1990). In avians, the ventral pathway is active first (Bronner-Fraser, 1986, Rickmann, et al., 1985) and cells are only present in the dorsolateral pathway later (Erickson and Goins, 1995, Krispin, et al., 2010b, Serbedzija, et al., 1989).

The initial and intermediate waves give rise to dorsal root ganglion neurons and, more ventrally, to sympathetic neurons and to adrenal chromaffin cells as well as all of the support cells for each of these cell types. Cells of the final wave give rise to melanocytes of the dorsolateral skin.

Molecular heterogeneity of migrating NCC

Following delamination, *FoxD3* expression is maintained in the early migrating trunk NCC that take the ventral pathway ("neural" NCC), but later emigrating NCC that take the lateral pathway ("melanocytic" NCC) lose *FoxD3* expression (Kos, et al., 2001). *FoxD*3 seems critical to the difference between the two lineages as overexpression of *FoxD3* increases the number of neural NCC at the expense of melanocytic NCC (Nitzan, et al., 2013, Thomas and Erickson, 2009). The SoxE transcription factors, *Sox8*, *Sox9* and *Sox10* are expressed by premigratory NCC (Hong and Saint-Jeannet, 2005). *Sox10* expression is maintained in migrating NCC and is critical for NCC survival, multiipotency and differentiation into specific lineages (Kelsh, 2006, Wegner and Stolt, 2005).

The first NCC to populate the sympathetic ganglia express the chemokine receptor, Cxcr4 (Kasemeier-Kulesa, et al., 2010). The ligand, SDF-1 is present around the dorsal aorta and in the ventral migratory pathway. NCC of the ventral pathway migrate towards beads soaked in SDF-1 and inhibition of *Cxcr4* expression lead many early migrating NCC to end up in the DRG (Kasemeier-

Kulesa, et al., 2010). Cxcr4-positive NCC form the core of the developing sympathetic ganglia and represent the first wave of NCC to leave the neural tube(Kasemeier-Kulesa, et al., 2010). The periphery of the ganglia forms from Cxcr4-negative NCC that emigrate from the neural tube with the later emigrating NCC, suggesting that another system is responsible for their homing to the ganglia.

The EGF-like factor, neuregulin 1 (Nrg1), and its tyrosine-kinase receptors, ErbB2 and ErbB3, play a role in migration to the developing sympathetic ganglia and knock-out of any of the components of this pathway perturbs sympathetic ganglion formation, but leaves DRG unaffected (Britsch, et al., 1998). Nrg1 is present in the mesenchyme around the dorsal aorta and migrating NCC express *ErbB2* and *ErbB3*. In mice in which *Nrg1* or its receptors are deleted, NCC are reduced in number ventral to the DRG and appear arrested in more dorsal parts of the embryo (Britsch, et al., 1998). In the *ErbB3* knockout, which survives past mid-gestation, the adrenal gland is missing and the sympathetic chain much reduced in size (Britsch, et al., 1998).

Saito *et al* (2012) have investigated the regulation of both Nrg1 and SDF-1 in chicken embryos. They showed that migration towards the aorta depends on the induction of both *Nrg1* and *Cxcl12*, the gene encoding SDF-1, by BMPs from the aorta. In addition, they showed that combined knockdown of both *Nrg1* and *Cxcl12* to be more disruptive compared to single gene knockdown, and that both SDF-1 and neuregulin are chemoattractants to NCC (Saito, et al., 2012)

Neuropilin 1 and 2 (Nrp1 and Nrp2), receptors for class 3 semaphorins, are also present on NCC of the ventral pathway (Schwarz, et al., 2009a, Schwarz, et al., 2009b). High levels of Nrp1, and low levels of Nrp2, are present on the intermediate wave of NCC that give rise to sympathetic ganglia, while high levels of both Nrp1 and Nrp2 are expressed on NCC that give rise to cells of the DRG (Lumb, et al., 2014, Schwarz, et al., 2009a, Schwarz, et al., 2009b, Schwarz and Ruhrberg, 2010). Knockout of *Nrp1* leads to ectopic localization of sympathetic neurons (Kawasaki, et al., 2002, Schwarz, et al., 2009b). *Sema3a* knockout leads to similar disruptions in the location of sympathetic neurons and it appears that *Sema3a* plays a crucial role as a repulsive cue guiding migration of the intermediate wave of *Nrp1*-expressing NCC into the anterior somite, as it is

expressed in both the intersomitic furrow and posterior somite (Schwarz, et al., 2009b, Schwarz and Ruhrberg, 2010). Of note is that, although small in number, the initial wave of NCC that migrates through the intersomitic furrow and which lacks Nrp1 goes onto to seed formation of the sympathetic ganglia (Schwarz, et al., 2009b). This means that sympathetic ganglia are composed of both Nrp1-positive and negative NCC.

Trunk NCC that populate the sympathetic ganglia can also be defined by the *absence* of certain markers. The neurogenin family of basic helix-loop-helix transcription factors are expressed by migrating trunk NCC that are destined to populate the dorsal root ganglia, but not by those that populate the sympathetic ganglia (Ma, et al., 1996, Perez, et al., 1999, Sommer, et al., 1996). Thus the absence of neurogenin in NCC in the ventral pathway defines a population of cells likely to give rise to sympathetic ganglia and perhaps cells of the adrenal medulla. However, the division is not absolute, as a few NCC with neurogenin do end up as sympathetic neurons (Zirlinger, et al., 2002).

Premigratory trunk NCC that are destined to give rise to sympathetic neurons, glia and chromaffin cells also lack expression of the receptor tyrosine kinase, *Kit*, which encodes for the receptor for stem cell factor (Wilson, et al., 2004). Kit-positive NCC give rise to melanocytes, but not to sympathetic ganglia (Luo, et al., 2003, Reid, et al., 1995, Wilson, et al., 2004).

Differentiation of sympathetic neuroblasts from NCC

Once migrating NCC arrive in the vicinity of the aorta, they start to differentiate under the influence of the local environment. When they arrive, all NCC express *Sox10*. As they differentiate into sympathoblasts, they suppress *Sox10* expression (Kim, et al., 2006) and start to express a characteristic suite of transcription factors which induce expression of general neuronal markers, including neurofilament proteins, and the catecholamine synthetic enzymes, tyrosine hydroxylase (TH) and dopamine β-hydroxylase (DBH) (Stubbusch, et al., 2013).

The key cells directing NCC differentiation at the aorta are likely the vascular smooth muscle cells. In zebrafish, loss of vascular smooth muscle cells leads to a failure in differentiation of NCC to sympathetic neuroblasts (Fortuna,

et al., 2015). The critical signal(s) released by vascular smooth muscle are likely to be bone morphogenetic proteins (BMPs). BMPs 4 and 7, members of the TGF-β super family are expressed by smooth muscle cells of chicken (McPherson, et al., 2000, Reissmann, et al., 1996) and mouse (Saito, et al., 2012, Shah, et al., 1996) and BMP2, BMP4 and BMP7 can induce sympathetic neuronal differentiation in NCC *in vitro* (Reissmann, et al., 1996, Shah, et al., 1996, Varley and Maxwell, 1996, Varley, et al., 1995). The effect of BMPs can be blocked *in vivo* in the chicken using noggin, a BMP antagonist (Saito, et al., 2012, Schneider, et al., 1999). In mice, the receptor involved is likely the Bmpr1a-type, as conditional knockout of this receptor prevents differentiation of NCC that reach the site of sympathetic ganglia and leads to their subsequent death (Morikawa, et al., 2009). Similarly, over-expression of *Bmpr1a* in avians leads to differentiation of NCC into sympathetic neuroblasts *in vitro* (Varley, et al., 1998). Knockout of *Smad4*, part of the canonical TGFbeta-signalling pathway, is without effect on sympathetic neuroblast differentiation, revealing that the action of BMPs must be through a non-canonical pathway (Buchmann-Moller, et al., 2009, Morikawa, et al., 2009).

Small interfering RNA (siRNA) and microRNA (miRNA) are two forms of RNA that modify expression of genes by binding to complementary or near complementary RNA sequences respectively on mRNA and activating the RNA-induced silencing complex to destroy mRNA via RNases. Hence, they are part of a post-transcriptional gene regulation system. Dicer1 is an RNase that cleaves siRNA and miRNA from larger forms of RNA. Knock-out of *Dicer1* therefore disturbs siRNA and miRNA production. In mice in which there is loss of *Dicer1* from TH-expressing cells, sympathetic neurons show disturbed induction of neuronal markers and increased cell death in late embryonic ages (Stubbusch, et al., 2015), indicating that siRNA and/or miRNA are important. (Stubbusch, et al., 2013). One such microRNA, miRNA 124, is present in sympathetic mouse neuroblasts and causes neurite elongation when over-expressed in cultured chromaffin cells and is up-regulated during the induction of neurites by NGF in PC12 cells (Shtukmaster, et al., 2016).

The differentiation of NCC down the sympathetic neuron line is regulated by a network of transcription factors induced as a consequence of BMP signalling

that include achaete-scute family BHLH transcription factor 1 (Ascl1), paired-like homeobox 2A (Phox2a) and paired-like homeobox 2B (Phox2b), insulinoma-associated 1 (Insm1), heart and neural crest derivatives expressed 2 (Hand2), GATA binding protein 3 (Gata3), Sox4 and Sox11, insulin gene enhancer protein 1 (Isl1) and activating enhancer-binding protein 2 (AP-2) family members (Apostolova and Dechant, 2009, Cane and Anderson, 2009, Chan, et al., 2016b, Ernsberger and Rohrer, 2009, Howard, 2005, Rohrer, 2011, Young, et al., 2011). This complex of transcription factors confers a neuronal phenotype and the expression of catecholamine synthesizing enzymes, proliferation and survival in differentiating sympathetic neuroblasts. While the transcription factors may appear sequentially, particularly in chicken where differentiation is more drawn out than in mice, they do not form a simple hierarchy, as more complicated interrelationships are apparent (Rohrer, 2011). In particular, late-appearing transcription factors may well play crucial feedback roles in regulating upstream transcription factors, as is seen in the case of Gata3 (Moriguchi, et al., 2006) and Insm1 (Wildner, et al., 2008) effects in Ascl1. Over-expression studies also suggest that Phox2a, Hand2 or Ascl1 may interact in similar ways (Howard, et al., 2000, Lo, et al., 1998, Stanke, et al., 1999, Stanke, et al., 2004), although the expression levels in these models are above biological levels.

Phox2b, along with *Ascl1*, is expressed coincident with differentiation of NCC at sites of sympathetic gangliogenesis. *Phox2b* expression initially overlaps with that of *Sox10* in both chicken (Tsarovina, et al., 2008) and mouse (Callahan, et al., 2008, Gonsalvez, et al., 2013) sympathoblasts. *Sox10* is then quickly down-regulated coincident with the appearance of neuronal markers (Kim, et al., 2003). *Sox10* has been shown to maintain the "stemness" of NCC because it maintains the cell in the cell cycle, but simultaneously is needed to allow the induction of Phox2b and Ascl1 and subsequent neuronal differentiation, the different behaviours perhaps being regulated by the levels of *Sox10* expression (Kim, et al., 2003). The mechanism by which *Sox10* expression is down-regulated is unclear. Forced expression of *Phox2b* and *Ascl1* can repress expression of *Sox10 in vitro* in mouse NCC (Kim, et al., 2003), but more slowly than seen *in vivo*.

The individual roles and target genes of the transcription factors induced by BMPs are not understood in great detail. *Phox2b* is critical because its loss in

mice leads to apoptosis of sympathetic progenitors prior to NCC reaching the aorta (Pattyn, et al., 1999) and a failure to express the other transcription factors in the network (Hendershot, et al., 2008, Tsarovina, et al., 2004, Wildner, et al., 2008). If knockout of *Phox2b* in the mouse is made conditional on the expression of Isl1 by NCC, marking the onset of neuronal differentiation , then transcription factors characteristic of neuronal differentiation do appear, but the sympathetic chain is atrophic and the incorporation of bromodeoxyuridine (BrDU) is much reduced with no increase in apoptosis, (Coppola, et al., 2010).

Ascl1 is the only transcription factor that appears independently in the absence of Phox2b and is insufficient to prevent cell death (Hirsch, et al., 1998, Pattyn, et al., 2006). Loss of *Ascl1* delays, but does not prevent, noradrenergic differentiation (Guillemot and Joyner, 1993, Hirsch, et al., 1998, Pattyn, et al., 2006) and also decreases proliferation (Morikawa, et al., 2009, Pattyn, et al., 2006), with the consequence that mature sympathetic ganglia are present but reduced in size.

The roles of the other transcription factors have been summarized in detail by Rohrer (2011). *Phox2a*, a paralogue of *Phox2b,* is induced by Phox2b, but cannot replace it (Coppola, et al., 2005). The action of *Phox2a* seems limited to amplifying the induction of other transcription factors downstream of *Phox2*b (Coppola, et al., 2010). Knockout of *Insm1*, a downstream target of *Ascl1*, has a similar effect to loss of *Ascl1* (Wildner, et al., 2008) and Rohrer (2011) has suggested that the effect is on precursor cells rather than on the dividing sympathoblasts.

Hand2 expression is crucial for generating a catecholaminergic phenotype in sympathetic noradrenergic neurons, as its loss leads to an absence of a noradrenergic phenotype in sympathetic neurons of mice (Hendershot, et al., 2008, Morikawa, et al., 2007) and zebrafish (Lucas, et al., 2006). Loss of Hand2 also reduces proliferation of sympathoblasts (Reiff, et al., 2010). Of the remaining transcription factors, the loss of Gata2 in the chicken, or it equivalent in the mouse, *Gata3*, leads to reduction in expression of catecholamine markers, apoptosis and consequent reduction in ganglion size (Lim, et al., 2000, Tsarovina, et al., 2004). *Gata3* is also required for the survival of adult sympathetic neurons (Tsarovina, et al., 2010). *Sox11* appears to promote proliferation of sympathetic

neuroblasts while *Sox4* is most important for survival of more mature neurons (Potzner, et al., 2010).

The LIM-homeodomain transcription factor, Isl1, appears in mouse early (E9-E10), possibly under the control of *Phox2b* because *Isl1* is not induced in NCC in *Phox2b* knockout mice (Huber, et al., 2013). Knockout of *Isl1* results in smaller sympathetic ganglia, the presence of more apoptotic cells and a decrease in the expression of mRNA for Gata2, among other transcription factors (Huber, et al., 2013).

NCC forming sympathetic ganglia also express AP-2 transcription factors (Mitchell, et al., 1991, Moser, et al., 1997). There are five AP-2 transcription factors in mice and three in chickens, with AP-2α and AP-2β expressed in developing mouse sympathetic ganglia (Mitchell, et al., 1991, Moser, et al., 1997, Schmidt, et al., 2011). In mice deficient for both transcription factors, sympathetic ganglia are reduced to rudiments (Schmidt, et al., 2011). Knockout of *Tfap2b*, the gene for AP-2β, reduces sympathetic ganglion size by 40% but spares the DRG. The major action of its loss is on survival of the NCC and sympathetic neurons neuroblasts, rather than on differentiation (Schmidt, et al., 2011).

In addition to transcription factors, differentiation of sympathetic neurons is also influenced by a number of cell signalling systems. The Notch/Delta signaling system is important in regulating the transition from neural progenitor to sympathetic neuroblast. In developing chicken sympathetic ganglia, the gene encoding Delta1, *Dll1*, is up-regulated (Tsarovina, et al., 2008) in cells that already express the neural marker SCG10 (a stathmin-family protein and marker of the neuronal lineage). SCG10-positive cells have differentiated from their NCC identity toward a neuronal identity and no longer express *Sox10* (Tsarovina, et al., 2008). *Dll1*, expressed in immature neuroblasts, activates Notch signalling in the cells yet to acquire a neuronal phenotype and prevents their differentiation (Rohrer, 2011, Tsarovina, et al., 2008). In the chick, *in vivo* inhibition of Notch signalling depletes the progenitor pool and leads to an increase in the proportion of neuroblasts (Tsarovina, et al., 2008). Consistent with this is the fact that over-expression of Notch results in the maintenance of undifferentiated NC progenitors at the expense of the neuronal SCG10-positive population

(Tsarovina, et al., 2008). It likely that Notch/Delta signalling controls the balance between progenitor/glial cell maintenance and differentiation that leads to a neuronal phenotype (Tsarovina, et al., 2008) and allows some NCC to differentiate as satellite glia in sympathetic ganglia, instead of all NCC in ganglia becoming neurons.

Glial cell line-derived neurotrophic factor (GDNF) family ligands (GFLs) also play a role in sympathetic ganglion development. *Gdnf*-knock-out mice have fewer SCG neurons and deficient sympathetic innervation of targets in the head (Granholm, et al., 1997, Moore, et al., 1996), but the mechanism is unclear, as loss of the relevant receptor, GDNF family receptor α1, has no effect on sympathetic development (Cacalano, et al., 1998, Enomoto, et al., 1998).

Development of adrenal chromaffin cells

Adrenal chromaffin cells first appear among TH-expressing cells ventrolateral to the dorsal aorta (Figure 1a-b) when a subset of cells suppress the expression of neuronal markers but retain expression of catecholaminergic markers (Anderson and Axel, 1986, Stubbusch, et al., 2013). In mice, the anatomical segregation of developing chromaffin cells from differentiating prevertebral sympathetic neuroblasts can be clearly observed at E13.5 (Figure 1c-e) when chromaffin cells have migrated laterally to coalesce at the centre of the mass of adrenal cortical cells to form the adrenal medulla (Bocian-Sobkowska, et al., 1996, Huber, et al., 2009, Kameda, 2014, Waring, 1936). The chromaffin cells can then be readily identified by their anatomical location, embedded among the steroidogenic factor 1 (SF1, gene name *Nr5a1*)-expressing adrenal cortical cells (Gut, et al., 2005, Lohr, et al., 2006). Only by E14.5 in the mouse (Figure 1f) do a sub-population of chromaffin cells express PNMT (Chan, et al., 2016a, Lohr, et al., 2006).

It was long believed that adrenal chromaffin cells and sympathetic neuroblasts shared a common progenitor cell, the sympathoadrenal cell (Landis and Patterson, 1981). This belief was based on the readiness of chromaffin cells *in vivo* and *in vitro* to adopt a sympathetic neuron-like phenotype when stimulated by NGF (Anderson and Axel, 1986, Doupe, et al., 1985, Unsicker, et al., 1978). It was also noted that developing chromaffin cells and sympathetic

neuroblasts were indistinguishable in the E11.5 mouse with the available techniques, as both appear to express a similar suite of neuronal markers and cell surface antigens (Anderson, et al., 1991).

In this model, the exact timing of the separation of adrenal chromaffin cells from sympathetic neuroblasts was uncertain. A recent lineage tracing experiment (Shtukmaster, et al., 2013) confirmed that a common progenitor for adrenal chromaffin cells and sympathetic neuroblasts is present among premigratory NCC of chickens. The lineage separation could occur as late as the time the sympathoadrenal precursor arrived ventrolateral to the aorta (E12.5 in the mouse and E5.5/Stage 27 in the chick) (Chan, et al., 2016a, Ernsberger, et al., 2005, Gut, et al., 2005, Huber, 2006). However, based on differences in expression of markers including neurofilament M (Ernsberger, et al., 2005) and cocaine and amphetamine regulated transcript (CART) peptide (Chan, et al., 2016a), and on differences in their proliferation rates (Chan, et al., 2016a) between chromaffin cell precursors and sympathetic neuroblasts, it was thought possible that lineage separation may occur earlier, while NCC were still migrating along the ventral pathway. However, a recent study (Furlan, et al., 2017) has demonstrated that the majority of chromaffin cells have a different origin to sympathetic neuroblasts, as they arise from Schwann cell precursors.

Schwann cell precursors are NCC that give rise to myelinating and non-myelinating Schwann cells of peripheral nerves. Schwann cell precursors are present within nerves in the embryo and express markers such as proteolipid protein (Plp) under the control of Nrg1 released from axons acting on ErbB3 receptors (Jessen, et al., 2015). Recent studies have shown that Schwann cell precursors also give rise to a wide range of other cell types, including endoneurial fibroblasts in peripheral nerves (Joseph, et al., 2004), melanocytes of the limbs and ventrolateral skin. (Adameyko, et al., 2009), parasympathetic neurons of the cranial, pelvic and cardiac ganglia (Dyachuk, et al., 2014, Espinosa-Medina, et al., 2014), tooth pulp cells and odontoblasts (Kaukua, et al., 2014) and enteric neurons (Uesaka, et al., 2015).

Furlan et al. (2017) used lineage tracing in the mouse to show that activation of YFP from the *Plp1* promoter (a marker of Schwann cell precursors) at E11.5 resulted in many YFP-positive chromaffin cells at E17.5, but negligible

labelling of sympathetic neuroblasts in the coeliac/superior mesenteric/suprarenal ganglion complex. They also identified that sympathetic preganglionic axons had penetrated into the vicinity of the forming adrenal medullae and were the likely source of Schwann cell precursors, as using diphtheria toxin driven from the *Isl1* locus to destroy spinal motor neurons, including sympathetic preganglionic neurons, resulted in the loss of 78% of chromaffin cells by E14.5.

Furlan et al. (2017) were also able to independently confirm the origin of chromaffin cells from Schwann cell precursors using single cell RNAseq. They identified cells of neural crest origin using *Wnt1*cre to drive Tomato expression and then isolated individual NCC cells. Analysis of the transcriptomes of each cell allowed them to be classified into Schwann cell precursors, sympathetic neuroblasts and adrenal chromaffin cells (Figure 2a-b) based on pathway and gene set over-dispersion analysis (PAGODA). Significantly, cells intermediate in transcriptomes between Schwann cell precursors and chromaffin cells ("bridge cells") were also identified. Bridge cells fell into two classes, one characterized by many genes indicative of active cycling and the other lacking such genes (Figure 2a). Further pseudotime analysis positioned cells along a "differentiation trajectory" and identified genes that varied systematically along this trajectory between Schwann cell precursors and adrenal chromaffin cells (Figure 2d). Bridge cells expressing many cell-cycle genes were located at the start of this trajectory, while bridge cells lacking cell-cycle genes were positioned close to the chromaffin cells (Figure 2b, c).

A key question left unanswered by Furlan et al. (2017) is whether <u>all</u> chromaffin cells arise from Schwann cell precursors. Both reporter genes and diptheria toxin ablation studies only affected a maximum of around 80% of the chromaffin cells in in any experiment. While this could well represent a limitation on the penetrance of the genetic techniques used it may alternatively mean, as acknowledged by Furlan et al. (2017), that some chromaffin cells come directly from early NCC, as do the sympathetic neuroblasts. Arguing against this is the absence of cells that "bridge" between sympathetic neuroblasts and adrenal chromaffin cells, although such "bridge"cells would be expected to be present prior to E12.5 (see below) and involve NCC rather than sympathetic

neuroblasts. If some chromaffin cells do arise from sources other than Schwann cell precursors then the next question is whether these have a specific fate amongst the chromaffin cells, for instance as the minority of cells that are noradrenergic rather than adrenergic in phenotype.

Furlan et al. (2017) also showed differences in timing between the differentiation of chromaffin cells and sympathetic neuroblasts. Sox10 is expressed by all NCC and is down-regulated as the NCC differentiate. When YFP expression driven from the Sox10 promoter was activated on E11.5, only chromaffin cells were labelled, indicating that substantial numbers of Sox10-expressing Schwann cell precursors were actively differentiating into chromaffin cells at this age. In contrast, few neuroblasts were labelled, indicating their Sox10 expressing progenitors had already completed Sox10 down-regulation on E10.5. Figure 3 summarises current knowledge of the development of sympathetic neuroblasts and adrenal chromaffin cells.

Control of adrenal chromaffin cells development

The RNAseq analysis by Furlan et al. (2017) provides an insight into what genes and signalling pathways are active during the differentiation of Schwann cell precursors into chromaffin cells. Across the differentiation trajectory from Schwann cell precursor to chromaffin cell, 139 transcription factors and 60 other molecules varied systematically. Gene ontology analysis suggested that signalling by Notch TGFbeta, canonical Wnt and Sonic-signalling pathways were active during this time.

Each stage of differentiation was characterized by different patterns of gene expression (Figure 2e). Schwann cell precursors were characterized by *Foxd3*, *Erbb3*, and *Sox10*, the initial stage of differentiation along the chromaffin cell trajectory by *Tcf3*, *Smo*, *Id3*, *Ybx1*, *Sox4*, *Notch1*, *Fzd2* and *Ptk7*, while later in the trajectory *Hand1* and *Hand2*, *Phox2a*, *Eya1*, *Thra*, *Gata3*, *Insm1*, *Tbx20*, *Tlx2*, *PlxnA3*, *Dll4*, *Amer2* and *Cxxc4* were prominent. Final differentiation into chromaffin cells saw the expression of *Chga*, *Th*, *Foxq1*, *Egr1*, *Elf4* and *Nrp2*.

Many of these genes have not previously been associated with chromaffin cell differentiation and it remains for their expression to be confirmed and a role established. However, many of the same BMP-induced transcription factors that

characterize sympathetic neuroblast differentiation are present and their roles in chromaffin cell differentiation have been investigated.

Ascl1 is expressed for much longer in adrenal precursors than in sympathetic neuroblasts, and in *Ascl1* knockouts, most TH-expressing chromaffin cell progenitors have differentiation arrested at an early stage (Huber, et al., 2002a). Furlan et al. (2017) showed that, in the absence of Ascl1, the arrested cells show either the Schwann cell precursor markers Sox10 and S100β or else Phox2b, although no catecholamine phenotype is seen.

Phox2b knockout mice show a similar phenotype to *Ascl1* knockouts, as differentiation of TH-expressing progenitors is arrested even earlier and, consequently, there is an absence of adrenal chromaffin cells in the adrenal medulla (Huber, et al., 2005). In mice, *Insm1* knockout results in the down-regulation of *Pnmt* and *Chra*, the gene for chromagranin A and up-regulation of neurofilament genes (Wildner, et al., 2008). Conditional knockout of *Hand2* results in a reduction of *Th* and *Pnmt* gene expression in postnatal adrenal glands (VanDusen, et al., 2014). .

AP-2β is also strongly expressed in embryonic adrenal chromaffin cells (Hong, et al., 2011). Knockout of *Tfap2b* significantly reduced the expression of *Dbh* and *Pnmt* as well as *Phox2b*, suggesting a role in the acquisition of an adrenergic phenotypic (Hong, et al., 2011). Moreover, *Isl1* is also critical in the acquisition of an adrenergic phenotype. In addition to a reduction of TH-immunoreactive adrenal chromaffin cells by 40% at E16.5, the expression of TH and DBH, PNMT and chromagranin A protein expression are also much reduced, as is expression of *Gata2*, *Hand1* and *Tfap2b* (Huber, et al., 2013).

In chicks, NCC cells that aggregate at the aorta respond to BMP-signalling by phosphorylating Smad to regulate the gene response to BMPs (Saito, et al., 2012), however, phosphorylated Smad disappears by stage 21, but reappears in developing chromaffin cells, but not sympathetic neuroblasts. When BMP-signalling is inhibited by expression of a dominant-negative Bmpr1a receptor at the time of chromaffin cell/sympathetic neuroblast segregation, cells with impaired Bmpr1a signaling fail to give rise to chromaffin cells. The BMP inhibitor, noggin, applied at the time of segregation, also inhibits generation of

chromaffin cells (Saito, et al., 2012). Aorta-derived BMPs may therefore contribute to the induction of chromaffin cell differentiation.

Dicer1, via the siRNA and miRNA it generates (see above) may also play a role in the segregation of neuronal and chromaffin cell lineages. Conditional knockout of *Dicer1* leads to a maintenance of the neuronal markers that are normally rapidly down-regulated in developing chromaffin cells (Stubbusch, et al., 2013) and leads to increased postnatal death of the cells (Stubbusch, et al., 2015).

The role of the adrenal cortex in the development of chromaffin cells through glucocorticoid signalling has been investigated (Anderson and Axel, 1986, Doupe, et al., 1985, Huber, et al., 2009). Glucocorticoids produced from the adrenal cortex were initially suggested to down-regulate neuronal markers and up-regulate *Pnmt* expression (Anderson and Axel, 1985, Unsicker, et al., 1978, Wurtman and Axelrod, 1966). However, later studies showed that chromaffin cells are still found in the right location in glucocorticoid receptor gene (*Nr3c1)* knockout in mice as well as in *Nr5a1*-deficient mice, which lack SF-1 and the adrenal cortex (Finotto, et al., 1999, Gut, et al., 2005, Huber, et al., 2002b, Luo, et al., 1994). Although these studies show that the adrenal cortex might not be crucial for chromaffin cell formation, the adrenal cortex appears important for maintaining an adequate number of PNMT-expressing chromaffin cells in the medulla (Finotto, et al., 1999, Gut, et al., 2005).

Development of extra-adrenal chromaffin cells

The development of extra-adrenal chromaffin cells has always been assumed to be closely related to that of adrenal medullary chromaffin cells, as they share most phenotypic features during embryonic stages, except the extra-adrenal chromaffin cells are not embedded within the adrenal cortex (Bocian-Sobkowska *et al*. 1996). Although there are no studies of the transcriptional control of extra-adrenal chromaffin cell development, it is likely that intra- and extra- adrenal chromaffin are influenced by a common set of factors. Extra-adrenal chromaffin cells appear by week 25 in the human foetus and around E13.5 in mouse embryos (McNicol, 2004). During embryonic development, the largest cluster, the organ of Zuckerkandl is more prominent and mature than the

chromaffin cells of the adrenal medullae and the organ of Zuckerkandl is believed play a role in maintaining catecholamines during foetal development (Subramanian and Maker, 2006). In human, most of the extra-adrenal chromaffin cell clusters continue to grow in size during postnatal stages up to 3 years of age and are present in adult, with the exception of the organ of Zuckerkandl, which degenerates after 3 years of age with its role being replaced by the mature adrenal medulla (Coupland, 1954). The organ of Zuckerkandl totally disappears by 14 years of age in human and by P20 in mice (Coupland, 1954, Schober, et al., 2013). Glucocorticoid has been suggested to have a physiological role in the maintenance of extra-adrenal chromaffin cells as mice that lack the glucocorticoid receptor showed accelerated cell loss by autophagy in the organ of Zuckerkandl (Schober, et al., 2013).

One question that arises in light of the study by Furlan et al. (2017) is the origin of extra-adrenal chromaffin cells. If extra-adrenal chromaffin cells lack innervation in some species, as has been reported (Coupland and Weakley, 1970b, Mascorro, et al., 1994, Mascorro and Yates, 1971, Mascorro and Yates, 1977), then can they arise from Schwann cell precursors?

Origin of sympathetic glia

Glial cells support both neurons in peripheral ganglia as satellite glia (Hanani, 2010) and axons in peripheral nerves as Schwann cells (Jacob, 2015, Jessen, et al., 2015) and nearly all are of neural crest origin. The exceptions are a small number of Schwann cells and satellite glia derived from boundary cap cells directly derived from the neuroepithelium of the neural tube (Hjerling-Leffler, et al., 2005, Maro, et al., 2004). Nerve associated Schwann cell precursors are also the precursor cells for a wide variety of NCC-derived tissues (see above). However, within peripheral nerves they give rise to myelinating and non-myelinating Schwann cells as well as endoneurial fibroblasts.

NCC give rise to nerve-associated Schwann cell precursors around E11.0 in the mouse when myelin protein 0 (P0/Mpz) and brain fatty acid binding protein (Bfabp/Fabp7) are first expressed (Britsch, et al., 2001, Hagedorn, et al., 1999, Kurtz, et al., 1994). *FoxD3* is required for Schwann cell differentiation and absence of FoxD3 causes NCC to differentiate down a neuronal or melanocyte

line (Nitzan, et al., 2013). *Sox10* expression is maintained in Schwann cells (Kuhlbrodt, et al., 1998) and is essential for the development of Schwann cells from NCC (Britsch, et al., 2001, Paratore, et al., 2001). Sox10 maintains *ErbB3* expression (Britsch, et al., 2001) and the ErbB3 ligand, Nrg1, reinforces gliogenesis (Birchmeier and Nave, 2008, Shah, et al., 1994). Schwann cell precursors cannot survive in the absence of Nrg1 (Dong, et al., 1995).

Another signaling pathway that regulates glial development consists of the secreted protein, leucine-rich repeat LGI family member 4 (Lgi4), acting on A disintergrin and metalloproteinase domain 22 (Adam22). Schwann cell precursors secrete Lgi4 that acts on Adam22 in an autocrine manner (Ozkaynak, et al., 2010). In peripheral ganglia, the same system is important in satellite cell differentiation, where satellite cells secrete Lgi4 to interact with Adam22 (Nishino, et al., 2010). Deletion of either *Lgi4* or *Adam22* results in greatly diminished numbers of Schwann cells and satellite glia (Nishino, et al., 2010, Ozkaynak, et al., 2010). It is not clear whether Lgi4 acts in an autocrine manner in sympathetic ganglia, but DRG neurons do express *Adam22* (Ozkaynak, et al., 2010).

Notch ligands are also involved in interactions between Schwann cell precursors and axons. Jagged 1 on NCC interacts with Notch on axons and promotes the formation of immature Schwann cells from Schwann cell precursors, (Woodhoo, et al., 2009). Immature Schwann cells are characterized by up-regulation of markers such as glial fibrillary acid protein and S100β and a major reorganization of the structure of the nerve (Jacob, 2015, Jessen, et al., 2015). At the same time, the transcription factor AP-2α is down-regulated, and *Ap2a* expression slows the transition to immature Schwann cells (Stewart, et al., 2001).

Phox2b may also play a role in the specification of Schwann cells. Both mature and developing Schwann cells of the enteric nervous system express Phox2b (Corpening, et al., 2008, Young, et al., 2003). In addition, many NCC in cranial nerves express both Sox10 and Phox2b prior to the formation of cranial ganglia (Espinosa-Medina, et al., 2014). A proportion of these cells give rise to both neurons and glia of cranial ganglia such as the otic ganglion (see below). However, using lineage tracing, it appears that a proportion of mature Schwann

cells in cranial nerves arise from cells that have expressed *Phox2b*, as do a smaller proportion of mature Schwann cells in limb nerves (Espinosa-Medina, et al., 2014). Finally, NCC that initially form sympathetic ganglia all express both Sox10 and Phox2b, suggesting that satellite glia generated in the ganglia arise from *Phox2b*-expressing NCC (Gonsalvez, et al., 2013, Tsarovina, et al., 2008). The significance of *Phox2b* expression in NCC-derived glial cells remains to be determined, as does any difference between Schwann cells that once expressed *Phox2b* versus those that have never expressed *Phox2b*.

Satellite glia support sympathetic neurons in ganglia (Hanani, 2010). Mature satellite glia show some differences from Schwann cells. For instance, satellite glia express the Ets domain transcription factor, *Erm*, (Hagedorn, et al., 2000). They also lack Schwann cell myelin protein, whichis present even in non-myelinating Schwann cells (Dulac, et al., 1988). However, satellite glia can readily adopt Schwann cell-like characteristics in culture. (Cameron-Curry, et al., 1993, Le Douarin, et al., 1991, Murphy, et al., 1996).Within sympathetic ganglia, the differentiation of satellite glia from NCC tends to lag behind the differentiation of neurons (Callahan, et al., 2008, Lawson and Biscoe, 1979) so gliogenesis follows neurogenesis in sympathetic ganglia as it does in the CNS. It seems likely that satellite glia arise from Schwann cell precursors. In Furlan et al. (2017), lineage tracing based on expression of the Schwann cell marker gene, Plp1, labels satellite glia in the suprarenal ganglion (Fig. 1A in Furlan et al, (2017). Satellite glia in cranial parasympathetic also arise from Plp1-expressing Schwann cell precursors (Dyachuk, et al., 2014, Espinosa-Medina, et al., 2014).

The adrenal medulla also contains the glial-like sustentacular cells, expressing the glial markers S100 and BFABP (Kameda, 2007, Pakkarato, et al., 2015). These cells are generated from the neural crest cell and retain Sox10 expression. Sustentacular cells in adult are mainly located in the noradrenergic region of adrenal medulla (Suzuki and Kachi, 1994). These cells form sheet-like structures partially enveloping groups of noradrenergic chromaffin cells (Ahmed, 2017, Pakkarato, et al., 2015). The function of sustentacular cells in the adrenal is still unclear, however it is believed that they play a role in supporting and regulating catecholamine secretion (Ahmed, 2017, Pakkarato, et al., 2015, Rodriguez, et al., 2007).

Mature sustentacular cells appear to maintain stem-cell like properties. They express nestin, a marker of neural stem cells (Rubin de Celis, et al., 2015) and when isolated form spheres under appropriate culture conditions. Lineage tracing based on nestin expression shows they can differentiate into a chromagranin A-expressing cell *in vitro*, when the animal is under isolation stress (Rubin de Celis, et al., 2015).

Measuring proliferation

NCC must undergo massive proliferation to allow the relatively low number of starting cells within each tissue to provide enough mature cells. A method widely used to measure proliferation is the visualization of the uptake of ^{3}H thymidine or analogues like BrDU by dividing cells (eg. (Rohrer and Thoenen, 1987, Rothman, et al., 1978). A single pulse of ^{3}H thymidine or BrDU is taken up by cells actively synthesizing new DNA to replicate chromosomes, i.e. cells in S-phase of the cell cycle. Even if the all of the cells that are in S-phase at the time of the pulse are correctly identified (the instantaneous labeling index), the information that such an approach gives is limited. The number of cells labelled in a BrDU pulse depends on the fraction of cells in the total population that are in the cell cycle (the growth fraction, GF), the length of time a cell takes to move through the cell cycle (the cell cycle length, T_c) and the time it spends in S-phase, T_s (Nowakowski, et al., 1989). The proportion of cells that are labeled by BrDU or 3H thymidine can vary due to changes in either the GF, T_s or T_c and it is impossible to tell which from the labelling index alone. To obtain a full understanding of proliferation, it is necessary to measure all four of these parameters. Methods to measure cell cycle dynamics were originally developed for studies on the developing cortex (Nowakowski, et al., 2002, Takahashi, et al., 1996, Takahashi, et al., 1997), and have been modified to use 5 ethynyl 2 deoxyuridine instead of ^{3}H thymidine and also multilabel immunohistochemistry in the peripheral nervous system (Gonsalvez, et al., 2013). The following discussion is based largely on our own studies (Chan, et al., 2016a, Gonsalvez, et al., 2013, Gonsalvez, et al., 2015).

Proliferation of migrating neural crest cells

The dorsal part of the neural tube containing the neural crest is highly proliferative (Kahane and Kalcheim, 1998) and within the neuroepithelium, cells undergo interkinetic nuclear migration (Burstyn-Cohen and Kalcheim, 2002). It has been suggested that delamination of NCC occurs during S-phase (Burstyn-Cohen and Kalcheim, 2002), although this has not been seen in other studies (Ridenour, et al., 2014, Theveneau, et al., 2007).

In the mouse, nearly all migratory trunk NCC are in the cell cycle and express Sox10 without any neuronal markers (Gonsalvez, et al., 2013, Gonsalvez, et al., 2015). At the level of the somite, the cell cycle length of E9.5 trunk NCC is 8.5 hours with an S-phase of 5 hours (Gonsalvez, et al., 2015). This is comparable to estimates of cell cycle lengths within the avian neuroepithelium of around 8 hours (Langman, et al., 1966, Smith and Schoenwolf, 1987, Smith and Schoenwolf, 1988). The short cell cycle length and relatively long S-phase is characteristic of embryonic stem (ES) cells or early embryonic cells (Mac Auley, et al., 1993, White and Dalton, 2005). NCC in the mouse that have migrated past the somite and reached the aorta at E9.5 also maintain a short cell cycle length (10.6 hours) and long S-phase of 7.2 hours (Gonsalvez, et al., 2013). At the same time, around 20% of the NCC show Phox2b expression but none express a neuronal marker.

Proliferation of sympathetic neuroblasts

By E10.5 in the mouse, at the level of the forelimbs, trunk NCC cells aggregate into paravertebral sympathetic ganglia (Figure 4a-d) and nearly all NCC now express Phox2b (Gonsalvez, et al., 2013). Half of the Phox2b-immunoreactive cells express neuronal markers such as TH and Tuj1 and in these cells Sox10 expression is weak (Figure 4 and disappears by E11.5 (Gonsalvez, et al., 2013). The transition from a Sox10-immunoreactive NCC lacking Phox2b via a Sox10/Phox2b immunoreactive cell to a sympathetic neuroblast lacking Sox10 (Figure 4b, d) takes around 24 hours in the mouse. In avians, this transition takes 2 to 3 days, from E3 to E5 (Tsarovina, et al., 2008). As NCC differentiate into sympathetic neuroblasts and lose Sox10 expression, the proliferative behavior of NCC undergoes a major change. All of the Phox2b/Tuj1

cells in the stellate ganglion of E10.5 mice transiently stop dividing (Figure 4d), as judged by the absence of KI67, BrDU-uptake and phosphohistone 3 staining, (Gonsalvez, et al., 2013). However, the few Sox10/Phox2b cells lacking Tuj1 in the ganglia are still in the cell cycle but now have a cell cycle length of 38 hours with an S-phase nearly 10 hours (Gonsalvez, et al., 2013). In neuroblasts in the CNS, differentiation also coincides with cell cycle withdrawal (Takahashi, et al., 1996). However, unlike in the CNS, the cell cycle withdrawal of sympathoblasts is only temporary. In E11.5 mice, TH+ sympathetic neuroblasts in the stellate ganglion have all re-entered the cell cycle (4e-h), with a cell cycle length of around 20 hours and an S-phase less than 5 hours. In neural progenitors of the CNS (Takahashi, et al., 1996), the DRG (Gonsalvez, et al., 2015) and parasympathetic ganglia (Rohrer, 2011), neuronal differentiation coincides with permanent cell cycle withdrawal. Sympathetic neurons are therefore unique as neurons because they divide after neuronal differentiation (Rohrer and Thoenen, 1987, Rothman, et al., 1978), although differentiated retinal horizontal cells can also be induced to re-enter the cell cycle, but only after down-regulation of retinoblastoma protein (Ajioka, et al., 2007). In the mouse stellate ganglion from E12.5 on, progressively fewer neuroblasts remain in the cell cycle (Figure 4i) and those that do maintain a cell cycle length of around 20 h. By E18.5, virtually all sympathetic neuroblasts in the stellate ganglion have left the cell cycle (Figure 4j), although a handful of dividing sympathetic neurons are still present in neonatal mouse sympathetic ganglia (Shi, et al., 2008). The exact timing of cell cycle withdrawal of a sympathetic neuroblast has been linked to the target tissue they eventually innervate (Chubb and Anderson, 2010).

A similar pattern of transient cell cycle withdrawal described above for the mouse stellate ganglion is also seen in the mouse suprarenal ganglion, part of the prevertebral ganglion complex (Chan, et al., 2016a). However, compared to the stellate ganglion, timing is delayed by around one day due to the rostro-caudal lag in development along the length of the embryo (Le Douarin and Kalcheim, 1999).

Avian sympathetic neuroblasts also withdraw from the cell cycle as they differentiate at E3 (Holzmann, et al., 2015). By E5, all of the sympathetic neuroblasts are once again cycling, but the proportion of cycling neuroblasts has

dropped to 10% by E11 (Holzmann, et al., 2015) and only very low numbers of dividing sympathetic neuroblasts have been described at hatching in chickens (Rothman, et al., 1978).

Relationship between proliferation and differentiation

The short cell cycle length seen in E9.5 mouse trunk NCC, in particular a short Gap 1 phase (G1), is likely to isolate cells from differentiation signals (Blomen and Boonstra, 2007, Calder, et al., 2013, Pfeuty, et al., 2008, Ruiz, et al., 2011). The trigger for the differentiation into sympathetic neuroblasts at E10.5 might be the increase of the cell cycle length seen in NCC expressing both Sox10 and Phox2b . It is possible that the increase in G1 allows time for a differentiation signal to accumulate and trigger the change (Orford and Scadden, 2008, Salomoni and Calegari, 2010, Takahashi, et al., 1997), but this may also occur with an increase in S-phase length (Arai, et al., 2011). It remains to be established whether exposure to the BMPs in the vicinity of the aorta or another signal triggers the change in cell cycle length.

Proliferation in postnatal sympathetic ganglia

While nearly all sympathetic neuroblasts are out of the cell cycle by birth in the mouse, sympathetic ganglia still contain dividing cells of unknown provenance (Hansford, et al., 2004). Clusters of small, TH and βIII-tubulin-negative cells ("hyperplastic cells") are present in 25% of sympathetic ganglia of wild-type newborn mice. The clusters have all disappeared by apoptosis by postnatal day 14. The hyperplastic cells are significant because in TH-MYCN transgenic mice, where *Mycn* is overexpressed under the TH promoter, the clusters of hyperplastic cells increase in size in the first week of life, rather than regressing (Hansford, et al., 2004). In heterozygous TH-MYCN mice, the hyperplastic clusters are present up to six weeks postnatal and neuroblastomas later appear in 30% of sympathetic ganglia. In homozygous TH-MYCN mice, nearly 60% of ganglia still have hyperplastic clusters at six weeks and nearly all sympathetic ganglia already have tumors (Hansford, et al., 2004). Cells in hyperplastic clusters in TH-MYCN animals are mostly Phox2b-immunoreactive, lack the glial marker BFABP and most of them are cycling (Alam, et al., 2009). A few of the hyperplastic cells lack Phox2b but express nestin (Alam, et al., 2009).

While the TH-MYCN mice never develop neuroblastoma in the adrenal gland, similar hyperplastic clusters of cell are present in a minority of the adrenal glands of newborn wild-type mice (Chan and Anderson, pers. obs.). As in the sympathetic ganglia, they have small dense nuclei and little cytoplasm and appear not to be TH-immunoreactive. Significantly, most are Ki67 immunoreactive, indicating they are cycling. Cells with many of the histological characteristics of hyperplastic cells have also been reported in normal human adrenal medullae of stillborn and neonate infants up to three months of age (Guin and Gilbert, 1968, Ikeda, et al., 1981, Shanklin and Soteloav, 1969).

The hyperplastic cell in mouse and human sympathetic ganglia and adrenal medullae is a candidate for the cell of origin of neuroblastoma, particularly as they appear to generate the neuroblastoma-like tumors that appear in TH-MYCN mice (Hansford, et al., 2004). A cell with an unknown relationship to the hyperplastic cells can be isolated from adult human (Santana, et al., 2012) and neonatal mouse adrenal (Saxena, et al., 2013). It has the properties of a stem cell as, in culture, it forms "chromospheres" containing cells expressing Phox2b and Sox10 among other neural crest markers. It can differentiate down both sympathetic neuronal and chromaffin cell lineages (Santana, et al., 2012, Saxena, et al., 2013).

One question about neuroblastoma that arises from the recent study of Furlan et al. (2017) is how can the same tumor arise from both lineages, given that sympathetic neuroblasts and adrenal chromaffin cells develop independently from the time of delamination from the neural tube. It may prove that neuroblastoma is a disorder in the genetic programs that remain common to the two cell types, like the catecholamine handling pathways. Alternatively, if the neuroblast lineage does give rise to a minority of chromaffin cells, perhaps it is these chromaffin cells only that can give rise to neuroblastoma. A final alternative is that neuroblastoma is really multiple diseases with characteristics that reflect its different origins.

Regulation of proliferation

Roles for a number of transcription factors and signaling pathways in regulating proliferation of sympathetic neuroblasts and their precursors have

been proposed. When Frizzled (Fzd) 3, a receptor for Wnt, or β catenin, a down-stream target of Wnt signaling, is knocked out in mice, proliferation of sympathetic neuroblasts decreases and there is premature cell cycle exit (Armstrong, et al., 2011).

In chicks, IGF-I and IGF-II are present in sympathetic ganglia and exogenous IGF *in vitro* and *in vivo* increases sympathetic neuroblast proliferation, while neutralizing antibodies against IGFs reduced neuronal proliferation and subsequent neuronal numbers (Zackenfels, et al., 1995).

The anaplastic leukemia kinase receptor (Alk), which is mutated in some cases of familial neuroblastoma (Janoueix-Lerosey, et al., 2008), and its putative ligand, midkine, stimulates proliferation of sympathetic neuroblasts (Reiff, et al., 2011). Developing chick sympathetic ganglia express midkine and *Alk* overexpression or constitutive activation both increase the rate of proliferation of sympathetic neuroblasts in culture (Reiff, et al., 2011). Inhibition of *Alk* or its knockdown decrease proliferation, as does knock-down of endogenous midkine (Reiff, et al., 2011).

Artemin is the receptor for neurturin, a member of GDNF family of ligands, and exogenous artemin increases the proliferation of cultured mouse sympathetic neuroblasts (Andres, et al., 2001). Knockout of the binding partner for artemin, GFRα3, leads to reduced size of the SCG and reduction in the number of neuroblasts in S-phase (Andres, et al., 2001). Artemin signaling through the receptor, Ret, and loss of *Ret* also affects sympathetic neuron development (Enomoto, et al., 2001). Sympathetic ganglion size is reduced on E16.5 and apoptosis increased at birth in *Ret* null mutants. However, *Ret* is only expressed by sympathetic neuroblasts up until E14.5 (Callahan, et al., 2008, Enomoto, et al., 2001) and it was suggested that the effects were mediated by the loss of artemin signalling to sympathetic axons growing along blood vessels (see below) prior to E14.5 (Enomoto, et al., 2001). Loss of *Ret* also increases the cell cycle length of mouse sympathetic neuroblasts at E16.5, which is after Ret protein disappears from sympathetic neuroblasts, suggesting a delayed effect of its loss or some indirect action (Gonsalvez, et al., 2013). For instance, *Ret* knockout mice lack kidneys and some kidney-derived circulating factor that drives sympathetic proliferation may be absent.

The transcriptional network that underlies sympathetic neuron differentiation is also likely to regulate proliferation. Deficits in Phox2b (Coppola, et al., 2010), Ascl1 (Morikawa, et al., 2009, Pattyn, et al., 2006), Insm1 (Wildner, et al., 2008), AP-2β (Schmidt, et al., 2011), Hand2 (Hendershot, et al., 2008, Schmidt, et al., 2009), Gata2 (Rohrer, 2011) and Sox11 (Potzner, et al., 2010) have all been reported to affect proliferation. Each of these transcription factors is likely to affect multiple genes and the regulation of proliferation may be via effects on the cell cycle network, either directly or through intermediate genes. However, as the network of transcription factors is likely to affect genes in many pathways, the effect on proliferation could be secondary to some major disturbance in an unrelated cellular process, such as metabolism or cellular homeostasis.

To interpret the actions of transcription factors on proliferation in sympathetic neuroblasts, the target genes of the transcription factors need to be identified. We are only in the very early stages of being able to do this. For instance, cell cycle gene network is a major target of Ascl1 in the mouse cortex (Castro, et al., 2011, Raposo, et al., 2015). Loss of Ascl1 in NCC leads to a reduction in the number of sympathetic neurons (Pattyn, et al., 2006), which may be caused by decreased proliferation due to effects on cell cycle genes. However, Ascl1 is only transiently expressed early in sympathetic neuroblast differentiation (Ernsberger, et al., 1995, Groves, et al., 1995, Tsarovina, et al., 2010) and so appears not to regulate later changes in proliferation.

Proliferation of adrenal chromaffin cells

The proliferative behavior of chromaffin cells has been examined in the mouse (Chan, et al., 2016a). The earliest age when sympathetic neuroblasts could be distinguished from chromaffin cell precursors was E12.5, when sympathetic neuroblasts expressed CART but adrenal chromaffin cell precursors did not (Chan, et al., 2016a). Prior to this age, 90% of NCC in the vicinity of the abdominal aorta were not in the cell cycle. Whereas the majority of NCC that differentiate as sympathetic neuroblasts of the prevertebral ganglia reenter the cell cycle on E12.5, only 20% of adrenal chromaffin cell precursors were in the cell cycle at E12.5. They maintained this proportion through E16.5. Furlan et al.

(2017) have shown that the Schwann cell precursors that give rise to adrenal chromaffin cells are strongly proliferative, and that the decrease in cycling intensity occurs during in the transitional stages between the two cell types. Cell cycle lengths and S-phase lengths are similar to those of sympathetic neuroblasts over the period E12.5-E16.5 (Chan, et al., 2016a). Rodent chromaffin cells are known to maintain this proportion of cycling cells into postnatal life (Tischler, et al., 1989). PNMT-immunoreactive (adrenergic) chromaffin cells appeared on E14.5, and these behaved in a similar way to PNMT negative (noradrenergic) chromaffin cells.

Sympathetic axon pathfinding

Sympathetic neurons, must extend their axons into the periphery, often over long distances, and form functional connections with a wide range of targets. The initial task is therefore extension along an appropriate route, while the final task is innervation of the appropriate target tissue.

Sympathetic axons leave ganglia via three main routes. One is via the *rami communicantes* into spinal nerves, from where they access all tissues served by the spinal nerves. The second route is via nerves arising directly from sympathetic ganglia separate from the *rami communicantes*. Examples include the splanchnic, cardiac and carotid nerves. Other small nerves may also arise directly from sympathetic ganglia. The third route is between sympathetic ganglia along the sympathetic chain in the interganglionic connectives, before the axons exit by the first and second routes.

Sympathetic axon extension into the spinal nerves is via the *rami communicantes*, which carries sympathetic preganglionic and sensory axons. Once within the spinal nerve, which contains both spinal motor and somatosensory axons, outgrowing sympathetic neurons appear to be directly influenced by sensory axons, as removal of the somatosensory neurons leads to a failure of sympathetic axons to extend along peripheral nerves (Wang, et al., 2014).

In addition to sensory nerves, blood vessels that accompany most major nerves also appear to drive growth of sympathetic axons. The glial-derived neurotrophic factor family ligand, artemin (Baloh, et al., 2000), appears to be one

factor involved. Loss of the gene for artemin, *Artn* (Enomoto, et al., 2001, Honma, et al., 2002), or Ret (Enomoto, et al., 2001) or *Gfra3* (Honma, et al., 2002) leads to defects in sympathetic outgrowth along blood vessels. Artemin is most highly expressed in proximal mouse blood vessels at E14.5 when sympathetic axons are growing within the paravascular nerves, and then most heavily in more peripheral vessels two days later (Enomoto, et al., 2001). Another signal that regulates the growth of axons of mouse SCG neurons into the external carotid nerve is vascular endothelium-derived endothelin-3 acting on neuronal EdrnA receptors (Makita, et al., 2008).

It is less clear whether growth of sympathetic axons into the nerves that arise directly from the sympathetic ganglia also depends on prior formation of the nerve by other axons. Such nerves when mature will contain only sensory axons in addition to autonomic motor axons (not spinal motor axons), and so it may be that growth into these nerves requires that the nerve first form by growth of sensory axons through the ganglia via the rami communicantes.

It is also not clear whether growth of sympathetic axons in interganglionic connectives also requires that the connective be established by sensory axons. Interganglionic connectives contain sensory axons in mature animals but whether their presence precedes that of sympathetic axons is unknown.

Innervation of targets

After artemin and interactions with sensory axons have promoted the growth of sympathetic axons along major peripheral nerves, they must then enter and ramify within their target tissue. This process is regulated by multiple factors that may be target specific.

Blood vessels and the heart are major targets for sympathetic axons. While sympathetic axons are present in the peripheral nerves that accompany major blood vessels from embryonic ages, in mice they do not innervate the vascular smooth muscle until postnatal ages, when the vascular wall matures (Brunet, et al., 2014). Up until that age, sympathetic axons are found in the paravascular bundles running with blood vessels. The ingrowth of sympathetic axons into the wall of the blood vessel at P2 is triggered by netrin-1 in vascular smooth muscle acting on Deleted in colorectal cancer (Dcc) receptors on sympathetic terminals

(Brunet, et al., 2014). Loss of netrin-1 in vascular smooth muscle or Dcc receptors in sympathetic axons leads to a significant loss of sympathetic terminals in resistance vessels, without affecting sympathetic axons in paravascular axon bundles accompanying large blood vessels (Brunet, et al., 2014). As netrin-1 signalling was not completely blocked in any of the studies, the authors could not rule out additional signals that might also regulate vascular innervation.

Nerve growth factor (NGF) promotes sympathetic axon extension and branching in some tissues, in addition to its role as a sympathetic neuron survival factor (Levi-Montalcini, 1976). If cell death of sympathetic neurons is prevented by deleting *Bax,* a pro-apoptotic Bcl-2 family member, then the role of NGF in the innervation of target tissues can be examined (Glebova and Ginty, 2004). In *Bax*-null animals, loss of NGF does not affect the projection of sympathetic axons into and along major peripheral nerves, but does prevent the projection of sympathetic fibres into peripheral target tissues (Glebova and Ginty, 2004). However, not all targets are equal, with the heart and salivary glands most severely affected. The effect of NGF on sympathetic axon branching may be mediated through effects on Wnt signaling. *Wnt5a* is expressed by sympathetic neurons around the time they contact target tissues and NGF up-regulates the expression of *Wnt5a* in cultured sympathetic neurons (Bodmer, et al., 2009). *Wnt5a* null mutant mice have deficits in sympathetic target innervation *in vivo* and in axonal branching and elongation *in vitro* (Bodmer, et al., 2009). The effects of NGF-induced Wnt5a secretion are presumably autocrine. Wnt ligands act through Fzd receptors but the Fzd receptor involved in sympathetic axon pathfinding has not yet been definitively identified. However, Fzd3 may have a role. *Fzd3* knockout mice exhibit greatly diminished numbers of sympathetic neurons in the sympathetic chain (Armstrong, et al., 2011) due to an effect on proliferation. However, surviving sympathetic neurons that project to the gastrointestinal tract fail to branch into the organ (Armstrong, et al., 2011), a phenotype akin to *Wnt5a* null mutant mice (Bodmer, et al., 2009).

Innervation of the heart is also affected by factors other than NGF. Endothelin-1 has been reported to act on EdnrA receptors on mouse stellate ganglion neurons to guide their axons towards the heart over the surface of large

veins (Manousiouthakis, et al., 2014). It is not clear how NGF and endothelin-1 interact in the heart.

Sympathetic innervation of the heart is also regulated by neuropilins and their ligands. Sympathetic axons expressing Nrp1 and Nrp2 receptors are guided to the heart by the patterning of the repellant molecules, Sema3A and Sema3F. Knockout of either receptor/ligand pair in mice results in disordered organization of paravertebral ganglia and Nrp1/Sema3A knockout results in disrupted sympathetic innervation of the heart and aorta (Maden, et al., 2012). Presumably other, unidentified factors regulate the projection into organs not affected by loss of NGF, endothelin-1 or Sema3a.

Conclusion

Most embryonic cells face challenges as they balance proliferation and differentiation and competing extrinsic and intrinsic signals. Neural crest cells are no exception and their challenges may be amongst the most extreme of all embryonic cells as they include a fundamental cellular reorganization by EMT, extensive migration, differentiation into a wide range of cell types and rapid proliferation from a small founding population. In addition, sympathetic neurons are unique amongst neurons in continuing to divide after differentiation into neurons. Disorders in NCC development result in a number of neurocristopathies, including neuroblastoma. While we have made progress in understanding the basic biology of NCC development and the processes that give rise to the sympathetic ganglia and chromaffin cells, many questions remain. Recently developed genetic approaches, like the ability to analyse transcriptomes of single cells, are likely to revolutionise our understanding of these processes.

Conflict of Interest: The authors declare that they have no conflict of interest.

Figure legends

Figure 1

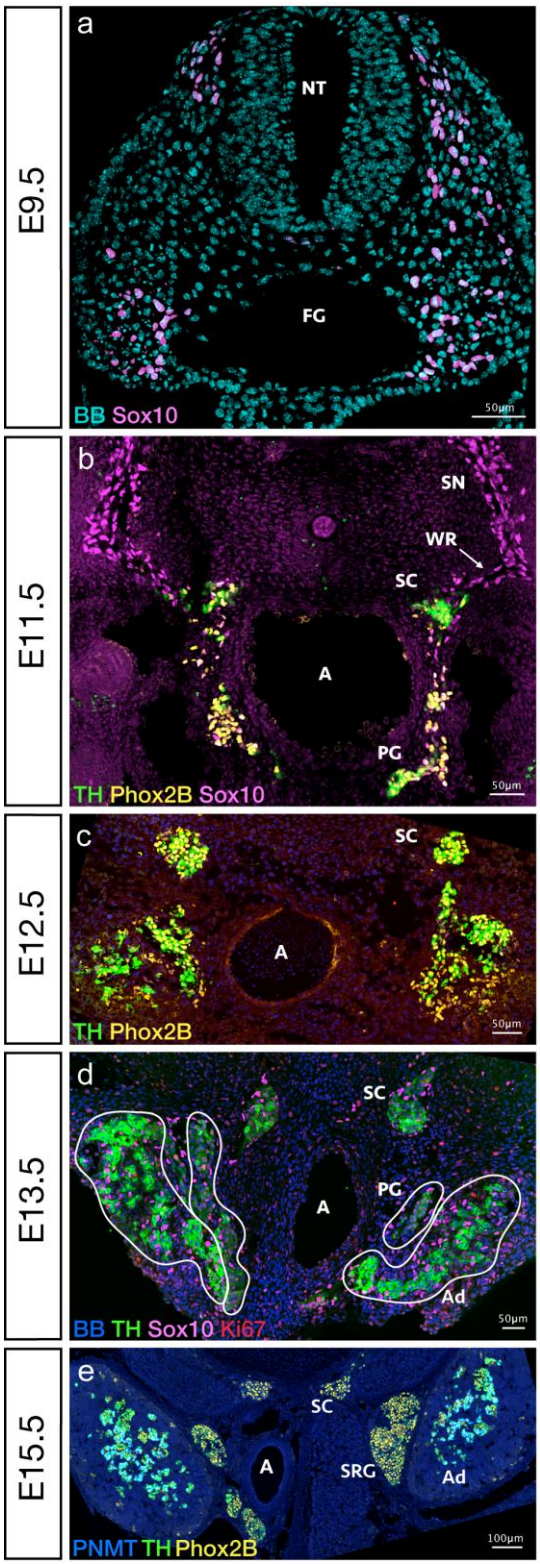

Figure 1a-e. All images are transverse sections through embryonic mice. 1a. On E9.5, early migrating Sox10-immunoreactive cells (magenta) are leaving the dorsal neural tube (NT) and migrating ventrolaterally through the developing somites and lateral to the foregut (FG). Other cells are labelled with a DNA stain, bisbenzimide (cyan). 1b. On E11.5, Sox10-immunoreactive cells (magenta) are present in the spinal nerves (SN) and white ramus (WR) as well as lateral to the aorta (A). TH-immunreactive cells (green) are present in the sympathetic chains (SC) and ventrolateral to the aorta in developing prevertebral ganglia (PG). Many other neural crest cells lacking TH-immunoreactivity lateral to the aorta have started to express Phox2b immunoreactivity (yellow). 1c. On E12.5, sympathetic chains (SC) contain prominent TH and Phox2b-immunoreactive cells, which are also present extensively lateral to the aorta where they represent the developing adrenal medullae and prevertebral ganglia. 1d. On E13.5, TH-immunoreactive cells (green) are identifiable as either sympathetic neuroblasts of the Sympathetic chain (SC) and prevertebral ganglia (PG) or chromaffin cells of the adrenals (Ad). The

developing adrenal medullae (lateral) and developing prevertebral ganglia (medially) are outlined. Sox10-immunoreactive cells (magenta) are present around and throughout all ganglia and adrenal medullae. 1e. On E15.5, the adrenal medullae (Ad) lie within well-formed adrenal glands with the suprarenal ganglia (SRG) present medially. The adrenal glands contain many PNMT-immunoreactive cells (cyan). Phox2b-immunoreactivity (yellow) is present in all TH-immunoreactive cells (green), including in the sympathetic chains (SC).

Figure 2

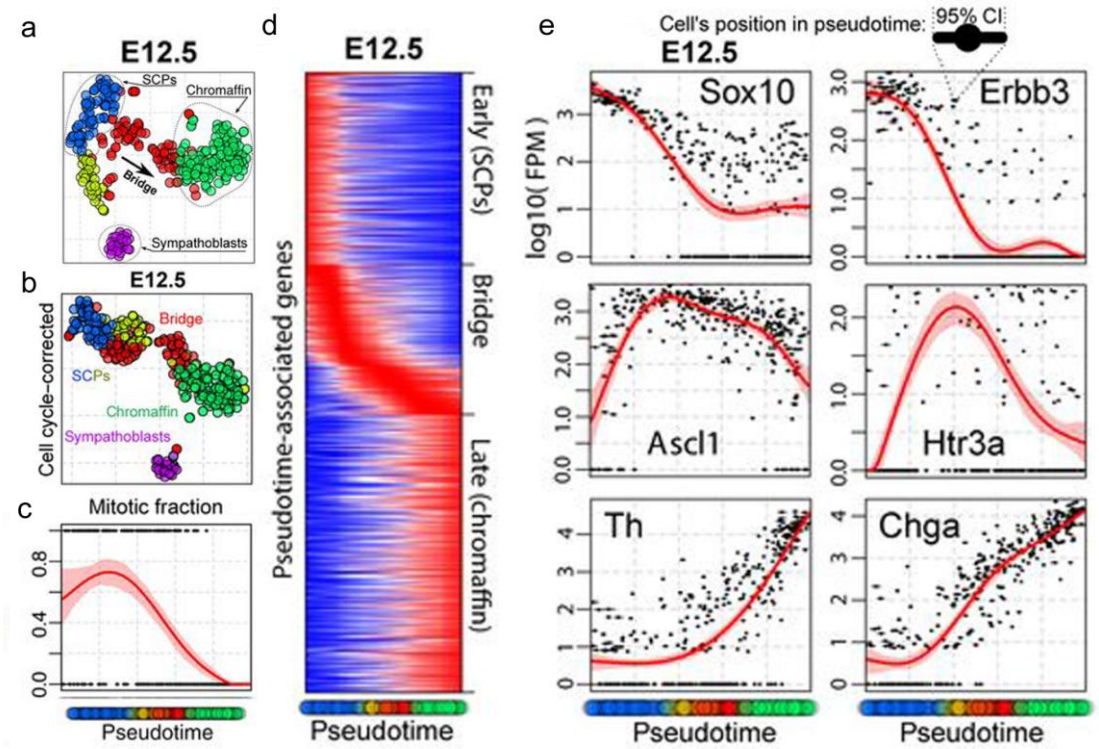

Figure 2a. Clustering analysis using t-SNE of single cell RNAseq of E12.5 mouse abdominal NCC assigns individual cells to one of five classes (purple - sympathetic neuroblasts, blue – Schwann cell precursors, yellow - cycling bridge cells, red – non-cycling bridge cells, green- chromaffin cells). 2b. t-SNE plot as in in 2a, normalised for cell cycle genes. It shows cycling and non-cycling bridge cells are otherwise similar and that cycling characterises bridge cells early in the sequence. 2c. Change in proportion of mitotic cells across pseudotime for bridge cells from Schwann cell precursors (blue) to chromaffin cells (green). 2d. Nearly 1500 genes that are significantly associated with NCC cells as they differentiate from Schwann cell precursors to chromaffin cells (right Y axis), each gene

represented as a horizontal line colour-coded from high-expression (red) to low expression (blue). The x-axis is the change with time ("pseudotime") colour-coded for cell type as in 2a. There is a clear transition of the genes expressed as cells move from Schwann cell precursor through bridge cell to chromaffin cell. 2e. Representative patterns of gene expression across pseudotime from Schwann cell precursors to chromaffin cells, (left to right, X-axis). Sox10 and Erbb3 are expressed by Schwann cell precursors and then down regulated, Ascl1 and Htr3a are expressed most strongly by bridge cells and Th and Chga by chromaffin cells. (From Furlan et al. Multipotent peripheral glial cells generate neuroendocrine cells of the adrenal medulla. DOI 10.1126/scienceaal3753, 2017, reprinted with permission from AAAS and the authors)

Figure 3.

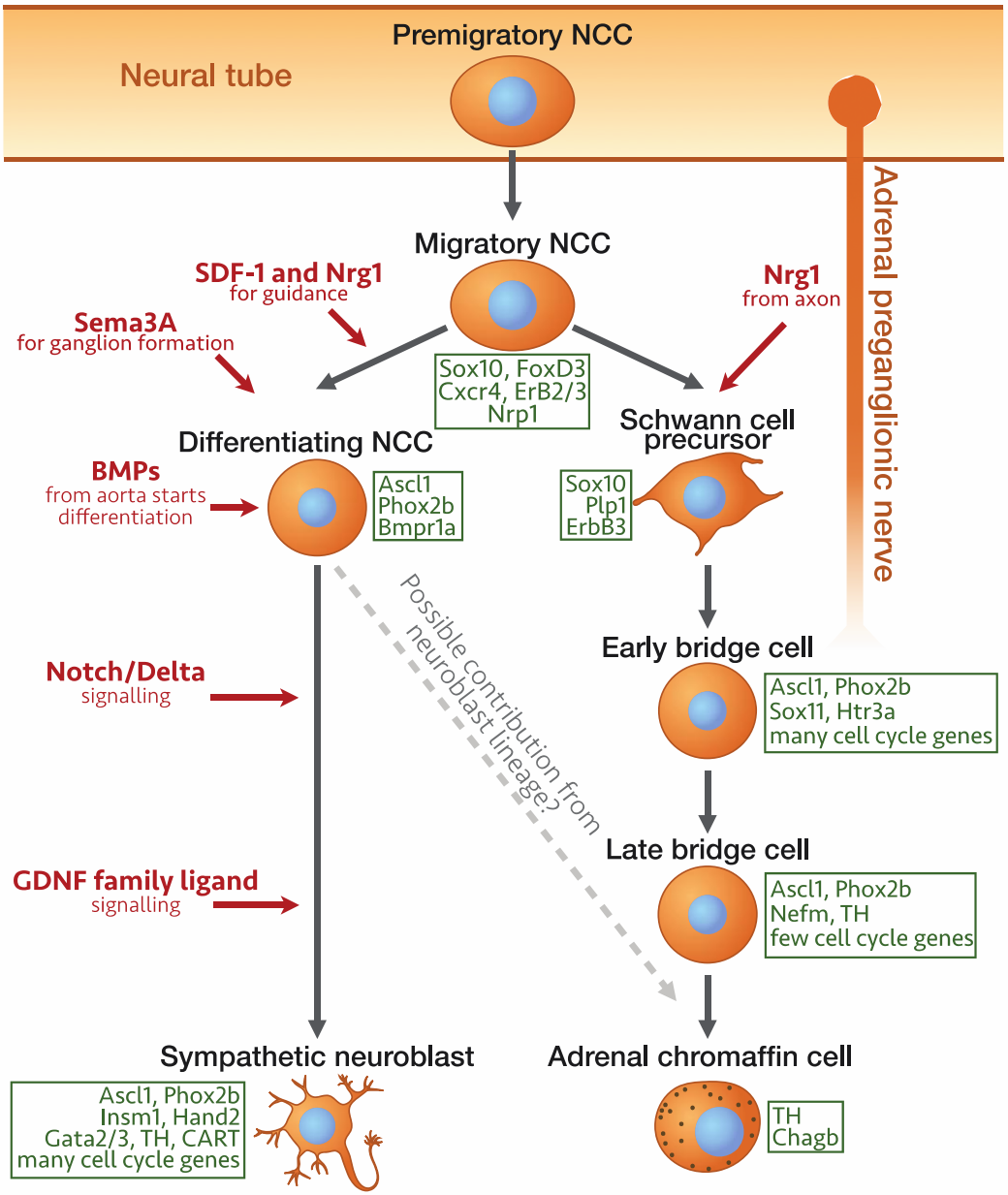

Figure 3. Summary of the development of sympathetic neuroblasts and adrenal chromaffin cells. On delamination from the neural tube, NCC either move directly to a position around the dorsal aorta (sympathetic neuroblast lineage) or associate with nerve trunks, where, under the influence of axonal Nrg1, they become Schwann cell precursors. In the neuroblast lineage, differentiation is initiated by BMPs which induce multiple transcription factors, starting with Ascl1 and Phox2b. Schwann cell precursors from the adrenal preganglionic nerve populate the area lateral to the aorta with chromaffin cells which differentiate to express many of the same transcription factors as the neuroblast lineage. Some of the other known influences influencing the two lineages are also shown.

Figure 4

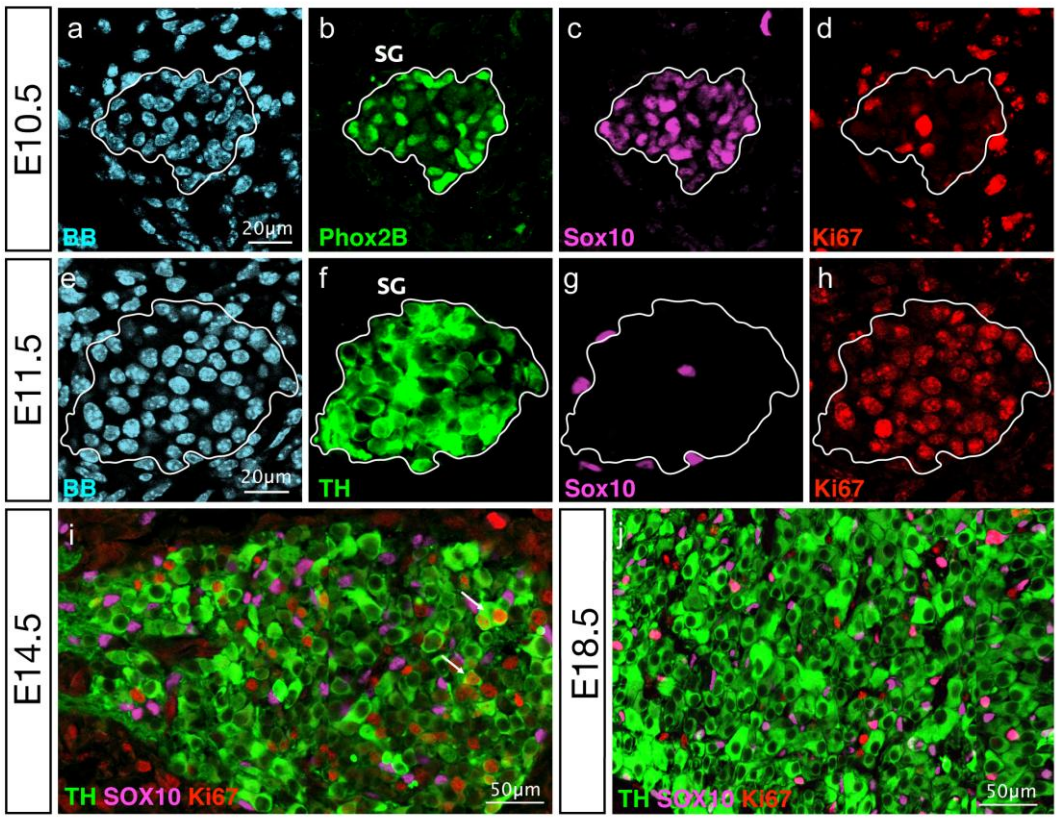

Figure 4a-j. All images are transverse sections through mouse embryos stained with immunofluorescence to reveal characteristics of developing sympathetic neuroblasts in sympathetic chain ganglia. 4a-d. E10.5 sympathetic ganglion stained with bisbenzimide to label all nuclei (a) showing most developing neuroblasts are immunoreactive for both Phox2b (b) and Sox10 (c), but few of them are Ki67-immunoreactive, indicating they have temporarily withdrawn from the cell cycle at this age. 4e-h. On E11.5, most of the cells in the ganglion are TH-immunoreactive (4Ee,f) but few are Sox10 immunoreactive (g). All of the neuroblasts have now re-entered the cell cycle, as judged by Ki67-immunoreactivity (h). 4I. By E14.5, a substantial proportion of sympathetic neuroblasts have left the cell cycle for the last time so that few are Ki67 immunoreactive (red, examples arrowed). Among the closely packed sympathetic neuroblasts immunoreactive for TH (green) are scattered Sox10-immunoreactive immature satellite glia (magenta). 4j. By E18.5, hardly any sympathetic neuroblasts are Ki67, indicating they are not cycling. Sox10-immunoreactive satellite glia (magenta) are present between neuroblasts. A

minority of the satellite cells are Ki67 immunoreactive (red), indicating they are still cycling. Other cycling cells are non-neural cells in the still growing ganglion.

References

Adameyko I, Lallemend F, Aquino JB, Pereira JA, Topilko P, Muller T, Fritz N, Beljajeva A, Mochii M, Liste I, Usoskin D, Suter U, Birchmeier C, Ernfors P (2009) Schwann cell precursors from nerve innervation are a cellular origin of melanocytes in skin. Cell 139:366-379

Ahmed AM (2017) Immunohistochemical study of sustentacular cells in adrenal medulla of neonatal and adult rats using an antibody against S-100 protein. Folia Morphol 76:246-251

Ahonen M, Soinila S, Joh TH (1987) Pre- and postnatal development of rat retroperitoneal paraganglia. J Auton Nerv Syst 18:111-120

Ajioka I, Martins RA, Bayazitov IT, Donovan S, Johnson DA, Frase S, Cicero SA, Boyd K, Zakharenko SS, Dyer MA (2007) Differentiated horizontal interneurons clonally expand to form metastatic retinoblastoma in mice. Cell 131:378-390

Alam G, Cui H, Shi H, Yang L, Ding J, Mao L, Maltese WA, Ding HF (2009) MYCN promotes the expansion of Phox2B-positive neuronal progenitors to drive neuroblastoma development. Am J Pathol 175:856-866

Allmendinger A, Stoeckel E, Saarma M, Unsicker K, Huber K (2003) Development of adrenal chromaffin cells is largely normal in mice lacking the receptor tyrosine kinase c-Ret. Mechanisms of Development 120:299-304

Anderson DJ, Axel R (1985) Molecular probes for the development and plasticity of neural crest derivatives. Cell 42:649-662

Anderson DJ, Axel R (1986) A bipotential neuroendocrine precursor whose choice of cell fate is determined by NGF and glucocorticoids. Cell 47:1079-1090

Anderson DJ, Carnahan JF, Michelsohn A, Patterson PH (1991) Antibody markers identify a common progenitor to sympathetic neurons and chromaffin cells in vivo and reveal the timing of commitment to neuronal differentiation in the sympathoadrenal lineage. J Neurosci 11:3507-3519

Andres R, Forgie A, Wyatt S, Chen Q, de Sauvage FJ, Davies AM (2001) Multiple effects of artemin on sympathetic neurone generation, survival and growth. Development 128:3685-3695

Apostolova G, Dechant G (2009) Development of neurotransmitter phenotypes in sympathetic neurons. Auton Neurosci 151:30-38

Arai Y, Pulvers JN, Haffner C, Schilling B, Nusslein I, Calegari F, Huttner WB (2011) Neural stem and progenitor cells shorten S-phase on commitment to neuron production. Nat Commun 2:154

Armstrong A, Ryu YK, Chieco D, Kuruvilla R (2011) Frizzled3 is required for neurogenesis and target innervation during sympathetic nervous system development. J Neurosci 31:2371-2381

Baggiolini A, Varum S, Mateos JM, Bettosini D, John N, Bonalli M, Ziegler U, Dimou L, Clevers H, Furrer R, Sommer L (2015) Premigratory and migratory neural crest cells are multipotent in vivo. Cell Stem Cell 16:314-322

Baloh RH, Enomoto H, Johnson EM, Jr., Milbrandt J (2000) The GDNF family ligands and receptors - implications for neural development. Curr Opin Neurobiol 10:103-110

Baroffio A, Dupin E, Le Douarin NM (1988) Clone-forming ability and differentiation potential of migratory neural crest cells. Proc Natl Acad Sci U S A 85:5325-5329
Birchmeier C, Nave KA (2008) Neuregulin-1, a key axonal signal that drives Schwann cell growth and differentiation. Glia 56:1491-1497
Blomen VA, Boonstra J (2007) Cell fate determination during G1 phase progression. Cell Mol Life Sci 64:3084-3104
Bocian-Sobkowska J, Wozniak W, Malendowicz LK, Ginda W (1996) Stereology of human fetal adrenal medulla. Histol Histopathol 11:389-393
Bodmer D, Levine-Wilkinson S, Richmond A, Hirsh S, Kuruvilla R (2009) Wnt5a mediates nerve growth factor-dependent axonal branching and growth in developing sympathetic neurons. J Neurosci 29:7569-7581
Britsch S, Goerich DE, Riethmacher D, Peirano RI, Rossner M, Nave KA, Birchmeier C, Wegner M (2001) The transcription factor Sox10 is a key regulator of peripheral glial development. Genes Dev 15:66-78
Britsch S, Li L, Kirchhoff S, Theuring F, Brinkmann V, Birchmeier C, Riethmacher D (1998) The ErbB2 and ErbB3 receptors and their ligand, neuregulin-1, are essential for development of the sympathetic nervous system. Genes Dev 12:1825-1836
Bronner-Fraser M (1986) Analysis of the early stages of trunk neural crest migration in avian embryos using monoclonal antibody HNK-1. Dev Biol 115:44-55
Bronner-Fraser M, Fraser S (1989) Developmental potential of avian trunk neural crest cells in situ. Neuron 3:755-766
Bronner-Fraser M, Fraser SE (1988) Cell lineage analysis reveals multipotency of some avian neural crest cells. Nature 335:161-164
Brunet I, Gordon E, Han J, Cristofaro B, Broqueres-You D, Liu C, Bouvree K, Zhang J, del Toro R, Mathivet T, Larrivee B, Jagu J, Pibouin-Fragner L, Pardanaud L, Machado MJ, Kennedy TE, Zhuang Z, Simons M, Levy BI, Tessier-Lavigne M, Grenz A, Eltzschig H, Eichmann A (2014) Netrin-1 controls sympathetic arterial innervation. J Clin Invest 124:3230-3240
Buchmann-Moller S, Miescher I, John N, Krishnan J, Deng CX, Sommer L (2009) Multiple lineage-specific roles of Smad4 during neural crest development. Dev Biol 330:329-338
Burstyn-Cohen T, Kalcheim C (2002) Association between the cell cycle and neural crest delamination through specific regulation of G1/S transition. Dev Cell 3:383-395
Cacalano G, Farinas I, Wang LC, Hagler K, Forgie A, Moore M, Armanini M, Phillips H, Ryan AM, Reichardt LF, Hynes M, Davies A, Rosenthal A (1998) GFRalpha1 is an essential receptor component for GDNF in the developing nervous system and kidney. Neuron 21:53-62
Calder A, Roth-Albin I, Bhatia S, Pilquil C, Lee JH, Bhatia M, Levadoux-Martin M, McNicol J, Russell J, Collins T, Draper JS (2013) Lengthened G1 phase indicates differentiation status in human embryonic stem cells. Stem Cells Dev 22:279-295
Callahan T, Young HM, Anderson RB, Enomoto H, Anderson CR (2008) Development of satellite glia in mouse sympathetic ganglia: GDNF and GFR alpha 1 are not essential. Glia 56:1428-1437

Cameron-Curry P, Dulac C, Le Douarin NM (1993) Negative regulation of Schwann cell myelin protein gene expression by the dorsal root ganglionic microenvironment. Eur J Neurosci 5:594-604
Cane KN, Anderson CR (2009) Generating diversity: Mechanisms regulating the differentiation of autonomic neuron phenotypes. Auton Neurosci 151:17-29
Castro DS, Martynoga B, Parras C, Ramesh V, Pacary E, Johnston C, Drechsel D, Lebel-Potter M, Garcia LG, Hunt C, Dolle D, Bithell A, Ettwiller L, Buckley N, Guillemot F (2011) A novel function of the proneural factor Ascl1 in progenitor proliferation identified by genome-wide characterization of its targets. Genes Dev 25:930-945
Chan WH, Gonsalvez DG, Young HM, Southard-Smith EM, Cane KN, Anderson CR (2016a) Differences in CART expression and cell cycle behavior discriminate sympathetic neuroblast from chromaffin cell lineages in mouse sympathoadrenal cells. Dev Neurobiol 76:137-149
Chan WH, Stamp LA, Hirst CS, McKeown SJ, Anderson CR, Young HM (2016b) Development of the autonomic nervous system. In: Meyers RA (ed) Reviews in Cell Biology and Molecular Medicine, vol 2, pp 23-65
Cheung M, Chaboissier MC, Mynett A, Hirst E, Schedl A, Briscoe J (2005) The transcriptional control of trunk neural crest induction, survival, and delamination. Dev Cell 8:179-192
Chubb DP, Anderson CR (2010) The relationship of the birth date of rat sympathetic neurons to the target they innervate. Developmental Dynamics 239:897-904
Coppola E, d'Autreaux F, Rijli FM, Brunet JF (2010) Ongoing roles of Phox2 homeodomain transcription factors during neuronal differentiation. Development 137:4211-4220
Coppola E, Pattyn A, Guthrie SC, Goridis C, Studer M (2005) Reciprocal gene replacements reveal unique functions for Phox2 genes during neural differentiation. Embo J 24:4392-4403
Corpening JC, Cantrell VA, Deal KK, Southard-Smith EM (2008) A Histone2BCerulean BAC transgene identifies differential expression of Phox2b in migrating enteric neural crest derivatives and enteric glia. Dev Dyn 237:1119-1132
Coupland R, Weakley B (1970a) Electron microscopic observations on the adrenal medulla and extra adrenal chromaffin tissue of the postnatal rabbit. J Anat 106:213-231
Coupland RE (1954) Post-natal fate of the abdominal para-aortic bodies in man. Journal of Anatomy 88:455-464
Coupland RE, Kent C, Kent SE (1982) Normal function of extra-adrenal chromaffin tissues in the young rabbit and guinea-pig. J Endocrinol 92:433-442
Coupland RE, Weakley BS (1970b) Electron microscopic observation on the adrenal medulla and extra-adrenal chromaffin tissue of the postnatal rabbit. J Anat 106:213-231
Dong Z, Brennan A, Liu N, Yarden Y, Lefkowitz G, Mirsky R, Jessen KR (1995) Neu differentiation factor is a neuron-glia signal and regulates survival, proliferation, and maturation of rat Schwann cell precursors. Neuron 15:585-596

Doupe AJ, Landis SC, Patterson PH (1985) Environmental influences in the development of neural crest derivatives: glucocorticoids, growth factors, and chromaffin cell plasticity. J Neurosci 5:2119-2142
Dulac C, Cameron-Curry P, Ziller C, Le Douarin NM (1988) A surface protein expressed by avian myelinating and nonmyelinating Schwann cells but not by satellite or enteric glial cells. Neuron 1:211-220
Dupin E, Calloni GW, Le Douarin NM (2010) The cephalic neural crest of amniote vertebrates is composed of a large majority of precursors endowed with neural, melanocytic, chondrogenic and osteogenic potentialities. Cell Cycle 9:238-249
Dupin E, Le Douarin NM (2014) The neural crest, a multifaceted structure of the vertebrates. Birth Defects Res C 102:187-209
Durbec P, Marcos-Gutierrez CV, Kilkenny C, Grigoriou M, Wartiowaara K, Suvanto P, Smith D, Ponder B, Costantini F, Saarma M, et al. (1996) GDNF signalling through the Ret receptor tyrosine kinase. Nature 381:789-793
Dyachuk V, Furlan A, Shahidi MK, Giovenco M, Kaukua N, Konstantinidou C, Pachnis V, Memic F, Marklund U, Muller T, Birchmeier C, Fried K, Ernfors P, Adameyko I (2014) Neurodevelopment. Parasympathetic neurons originate from nerve-associated peripheral glial progenitors. Science 345:82-87
El-Maghraby M, Lever JD (1980) Typification and differentiation of medullary cells in the developing rat adrenal. A histochemical and electron microscopic study. J Anat 131:103-120
Enomoto H, Araki T, Jackman A, Heuckeroth RO, Snider WD, Johnson EM, Jr., Milbrandt J (1998) GFR alpha1-deficient mice have deficits in the enteric nervous system and kidneys. Neuron 21:317-324
Enomoto H, Crawford PA, Gorodinsky A, Heuckeroth RO, Johnson EM, Jr., Milbrandt J (2001) RET signaling is essential for migration, axonal growth and axon guidance of developing sympathetic neurons. Development 128:3963-3974
Eränkö O (1955) Distribution of adrenaline and noradrenaline in the adrenal medulla. Nature 175:88-89
Erickson CA, Goins TL (1995) Avian neural crest cells can migrate in the dorsolateral path only if they are specified as melanocytes. Development 121:915-924
Ernsberger U, Esposito L, Partimo S, Huber K, Franke A, Bixby JL, Kalcheim C, Unsicker K (2005) Expression of neuronal markers suggests heterogeneity of chick sympathoadrenal cells prior to invasion of the adrenal anlagen. Cell Tissue Res 319:1-13
Ernsberger U, Patzke H, Tissier-Seta JP, Reh T, Goridis C, Rohrer H (1995) The expression of tyrosine hydroxylase and the transcription factors cPhox-2 and Cash-1: evidence for distinct inductive steps in the differentiation of chick sympathetic precursor cells. Mech Dev 52:125-136.
Ernsberger U, Rohrer H (2009) Development of the autonomic nervous system: New perspectives and open questions. Auton Neurosci 151:1-2
Espinosa-Medina I, Outin E, Picard CA, Chettouh Z, Dymecki S, Consalez GG, Coppola E, Brunet JF (2014) Neurodevelopment. Parasympathetic ganglia derive from Schwann cell precursors. Science 345:87-90

Finotto S, Krieglstein K, Schober A, Deimling F, Lindner K, Bruhl B, Beier K, Metz J, Garcia-Arraras JE, Roig-Lopez JL, Monaghan P, Schmid W, Cole TJ, Kellendonk C, Tronche F, Schutz G, Unsicker K (1999) Analysis of mice carrying targeted mutations of the glucocorticoid receptor gene argues against an essential role of glucocorticoid signalling for generating adrenal chromaffin cells. Development 126:2935-2944

Fortuna V, Pardanaud L, Brunet I, Ola R, Ristori E, Santoro MM, Nicoli S, Eichmann A (2015) Vascular mural cells promote noradrenergic differentiation of embryonic sympathetic neurons. Cell Rep 11:1786-1796

Frank E, Sanes JR (1991) Lineage of neurons and glia in chick dorsal root ganglia: analysis in vivo with a recombinant retrovirus. Development 111:895-908

Furlan A, Dyachuk V, Kastriti ME, Calvo-Enrique L, Abdo H, Hadjab S, Chontorotzea T, Akkuratova N, Usoskin D, Kamenev D, Petersen J, Sunadome K, Memic F, Marklund U, Fried K, Topilko P, Lallemend F, Kharchenko PV, Ernfors P, Adameyko I (2017) Multipotent peripheral glial cells generate neuroendocrine cells of the adrenal medulla. Science 357:

Glebova NO, Ginty DD (2004) Heterogeneous requirement of NGF for sympathetic target innervation in vivo. J Neurosci 24:743-751

Gonsalvez DG, Cane KN, Landman KA, Enomoto H, Young HM, Anderson CR (2013) Proliferation and cell cycle dynamics in the developing stellate ganglion. J Neurosci 33:5969-5979

Gonsalvez DG, Li-Yuen-Fong M, Cane KN, Stamp LA, Young HM, Anderson CR (2015) Different neural crest populations exhibit diverse proliferative behaviors. Dev Neurobiol 75:287-301

Granholm AC, Srivastava N, Mott JL, Henry S, Henry M, Westphal H, Pichel JG, Shen L, Hoffer BJ (1997) Morphological alterations in the peripheral and central nervous systems of mice lacking glial cell line-derived neurotrophic factor (GDNF): immunohistochemical studies. J Neurosci 17:1168-1178

Groves AK, George KM, Tissier-Seta JP, Engel JD, Brunet JF, Anderson DJ (1995) Differential regulation of transcription factor gene expression and phenotypic markers in developing sympathetic neurons. Development 121:887-901

Guillemot F, Joyner AL (1993) Dynamic expression of the murine Achaete-Scute homologue Mash-1 in the developing nervous system. Mech Dev 42:171-185

Guin GH, Gilbert EF (1968) Incidental neuroblastomas in infants. American Journal of Clinical Pathology 49:261-&

Gut P, Huber K, Lohr J, Bruhl B, Oberle S, Treier M, Ernsberger U, Kalcheim C, Unsicker K (2005) Lack of an adrenal cortex in Sf1 mutant mice is compatible with the generation and differentiation of chromaffin cells. Development 132:4611-4619

Hagedorn L, Paratore C, Brugnoli G, Baert JL, Mercader N, Suter U, Sommer L (2000) The Ets domain transcription factor Erm distinguishes rat satellite glia from Schwann cells and is regulated in satellite cells by neuregulin signaling. Dev Biol 219:44-58

Hagedorn L, Suter U, Sommer L (1999) P0 and PMP22 mark a multipotent neural crest-derived cell type that displays community effects in response to TGF-beta family factors. Development 126:3781-3794
Hanani M (2010) Satellite glial cells in sympathetic and parasympathetic ganglia: in search of function. Brain Res Rev 64:304-327
Hansford LM, Thomas WD, Keating JM, Burkhart CA, Peaston AE, Norris MD, Haber M, Armati PJ, Weiss WA, Marshall GM (2004) Mechanisms of embryonal tumor initiation: distinct roles for MycN expression and MYCN amplification. Proc Natl Acad Sci U S A 101:12664-12669
Hendershot TJ, Liu H, Clouthier DE, Shepherd IT, Coppola E, Studer M, Firulli AB, Pittman DL, Howard MJ (2008) Conditional deletion of Hand2 reveals critical functions in neurogenesis and cell type-specific gene expression for development of neural crest-derived noradrenergic sympathetic ganglion neurons. Dev Biol 319:179-191
Henion PD, Weston JA (1997) Timing and pattern of cell fate restrictions in the neural crest lineage. Development 124:4351-4359
Hervonen A, Korkala O (1972) The effect of hypoxia on the catecholamine content of human fetal abdominal paraganglia and adrenal medulla. Acta Obstet Gynecol Scand 51:17-24
Hervonen A, Korkala O (1973) Effect of hypoxia on the fine structure of the catecholamine-storing cells of the human fetal paraganglia. Virchows Arch B Cell Pathol 13:341-349
Hirsch MR, Tiveron MC, Guillemot F, Brunet JF, Goridis C (1998) Control of noradrenergic differentiation and Phox2a expression by MASH1 in the central and peripheral nervous system. Development 125:599-608
Hjerling-Leffler J, Marmigere F, Heglind M, Cederberg A, Koltzenburg M, Enerback S, Ernfors P (2005) The boundary cap: a source of neural crest stem cells that generate multiple sensory neuron subtypes. Development 132:2623-2632
Holzmann J, Hennchen M, Rohrer H (2015) Prox1 identifies proliferating neuroblasts and nascent neurons during neurogenesis in sympathetic ganglia. Dev Neurobiol 75:1352-1367
Hong CS, Saint-Jeannet JP (2005) Sox proteins and neural crest development. Semin Cell Dev Biol 16:694-703
Hong SJ, Huh YH, Leung A, Choi HJ, Ding Y, Kang UJ, Yoo SH, Buettner R, Kim K-S (2011) Transcription factor AP-2β regulates the neurotransmitter phenotype and maturation of chromaffin cells. Mol Cell Neurosci 46:245-251
Honma Y, Araki T, Gianino S, Bruce A, Heuckeroth R, Johnson E, Milbrandt J (2002) Artemin is a vascular-derived neurotropic factor for developing sympathetic neurons. Neuron 35:267-282
Howard MJ (2005) Mechanisms and perspectives on differentiation of autonomic neurons. Dev Biol 277:271-286
Howard MJ, Stanke M, Schneider C, Wu X, Rohrer H (2000) The transcription factor dHAND is a downstream effector of BMPs in sympathetic neuron specification. Development 127:4073-4081
Huber K (2006) The sympathoadrenal cell lineage: specification, diversification, and new perspectives. Dev Biol 298:335-343

Huber K, Brühl B, Guillemot F, Olson EN, Ernsberger U, Unsicker K (2002a) Development of chromaffin cells depends on MASH1 function. Development 129:4729-4738
Huber K, Combs S, Ernsberger U, Kalcheim C, Unsicker K (2002b) Generation of neuroendocrine chromaffin cells from sympathoadrenal progenitors: beyond the glucocorticoid hypothesis. Ann N Y Acad Sci 971:554-559
Huber K, Kalcheim C, Unsicker K (2009) The development of the chromaffin cell lineage from the neural crest. Auton Neurosci 151:10-16
Huber K, Karch N, Ernsberger U, Goridis C, Unsicker K (2005) The role of Phox2B in chromaffin cell development. Dev Biol 279:501-508
Huber K, Narasimhan P, Shtukmaster S, Pfeifer D, Evans SM, Sun Y (2013) The LIM-Homeodomain transcription factor Islet-1 is required for the development of sympathetic neurons and adrenal chromaffin cells. Dev Biol 380:286-298
Ikeda Y, Lister J, Bouton JM, Buyukpamukcu M (1981) Congenital neuroblastoma, neuroblastoma in situ, and the normal fetal development of the adrenal. J Pediatr Surg 16:636-644
Jacob C (2015) Transcriptional control of neural crest specification into peripheral glia. Glia
Jänig W (1989) Autonomic nervous system. Human physiology 333-370
Janoueix-Lerosey I, Lequin D, Brugieres L, Ribeiro A, de Pontual L, Combaret V, Raynal V, Puisieux A, Schleiermacher G, Pierron G, Valteau-Couanet D, Frebourg T, Michon J, Lyonnet S, Amiel J, Delattre O (2008) Somatic and germline activating mutations of the ALK kinase receptor in neuroblastoma. Nature 455:967-970
Jessen KR, Mirsky R, Lloyd AC (2015) Schwann Cells: Development and Role in Nerve Repair. Cold Spring Harb Perspect Biol, vol 7, pp a020487
Joseph NM, Mukouyama YS, Mosher JT, Jaegle M, Crone SA, Dormand EL, Lee KF, Meijer D, Anderson DJ, Morrison SJ (2004) Neural crest stem cells undergo multilineage differentiation in developing peripheral nerves to generate endoneurial fibroblasts in addition to Schwann cells. Development 131:5599-5612
Kahane N, Kalcheim C (1998) Identification of early postmitotic cells in distinct embryonic sites and their possible roles in morphogenesis. Cell Tissue Res 294:297-307
Kameda Y (2007) Expression of glial progenitor markers p75NTR and S100 protein in the developing mouse parathyroid gland. Cell Tissue Res 327:15-23
Kameda Y (2014) Signaling molecules and transcription factors involved in the development of the sympathetic nervous system, with special emphasis on the superior cervical ganglion. Cell and tissue research 1-22
Kannan CR (1986) Anatomy of the adrenal glands. Essential Endocrinology: A Primer for Nonspecialists. Springer US, pp 233-234
Kasemeier-Kulesa JC, McLennan R, Romine MH, Kulesa PM, Lefcort F (2010) CXCR4 controls ventral migration of sympathetic precursor cells. J Neurosci 30:13078-13088
Kaukua N, Shahidi MK, Konstantinidou C, Dyachuk V, Kaucka M, Furlan A, An Z, Wang L, Hultman I, Ahrlund-Richter L, Blom H, Brismar H, Lopes NA, Pachnis V, Suter U, Clevers H, Thesleff I, Sharpe P, Ernfors P, Fried K,

Adameyko I (2014) Glial origin of mesenchymal stem cells in a tooth model system. Nature 513:551-554
Kawasaki T, Bekku Y, Suto F, Kitsukawa T, Taniguchi M, Nagatsu I, Nagatsu T, Itoh K, Yagi T, Fujisawa H (2002) Requirement of neuropilin 1-mediated Sema3A signals in patterning of the sympathetic nervous system. Development 129:671-680
Kelsh RN (2006) Sorting out Sox10 functions in neural crest development. Bioessays 28:788-798
Kerosuo L, Bronner-Fraser M (2012) What is bad in cancer is good in the embryo: importance of EMT in neural crest development. Semin Cell Dev Biol 23:320-332
Kim CH, Pennisi P, Zhao H, Yakar S, Kaufman JB, Iganaki K, Shiloach J, Scherer PE, Quon MJ, LeRoith D (2006) MKR mice are resistant to the metabolic actions of both insulin and adiponectin: discordance between insulin resistance and adiponectin responsiveness. Am J Physiol Endocrinol Metab 291:E298-305
Kim J, Lo L, Dormand E, Anderson DJ (2003) SOX10 maintains multipotency and inhibits neuronal differentiation of neural crest stem cells. Neuron 38:17-31
Kos R, Reedy MV, Johnson RL, Erickson CA (2001) The winged-helix transcription factor FoxD3 is important for establishing the neural crest lineage and repressing melanogenesis in avian embryos. Development 128:1467-1479
Krispin S, Nitzan E, Kalcheim C (2010a) The dorsal neural tube: a dynamic setting for cell fate decisions. Dev Neurobiol 70:796-812
Krispin S, Nitzan E, Kassem Y, Kalcheim C (2010b) Evidence for a dynamic spatiotemporal fate map and early fate restrictions of premigratory avian neural crest. Development 137:585-595
Kuhlbrodt K, Herbarth B, Sock E, Hermans-Borgmeyer I, Wegner M (1998) Sox10, a novel transcriptional modulator in glial cells. J Neurosci 18:237-250
Kurtz A, Zimmer A, Schnutgen F, Bruning G, Spener F, Muller T (1994) The expression pattern of a novel gene encoding brain-fatty acid binding protein correlates with neuronal and glial cell development. Development 120:2637-2649
Landis SC, Patterson PH (1981) Neural crest cell lineages. Trends in Neurosciences 4:172-175
Langman J, Guerrant RL, Freeman BG (1966) Behavior of neuro-epithelial cells during closure of the neural tube. J Comp Neurol 127:399-411
Lawson SN, Biscoe TJ (1979) Development of mouse dorsal root ganglia: an autoradiographic and quantitative study. J Neurocytol 8:265-274
Le Douarin N, Dulac C, Dupin E, Cameron-Curry P (1991) Glial cell lineages in the neural crest. Glia 4:175-184
Le Douarin N, Teillet MA (1971) Localization, by the method of interspecific grafts of the neural area from which adrenal cells arise in the bird embryo. C R Acad Sci Hebd Seances Acad Sci D 272:481-484
Le Douarin NM, Calloni GW, Dupin E (2008) The stem cells of the neural crest. Cell Cycle 7:1013-1019

Le Douarin NM, Kalcheim C (1999) The Neural Crest. Cambridge University Press, Cambridge
Le Douarin NM, Teillet MA (1973) The migration of neural crest cells to the wall of the digestive tract in avian embryo. Journal of embryology and experimental morphology 30:31-48
Levi-Montalcini R (1976) The nerve growth factor: its role in growth, differentiation and function of the sympathetic adrenergic neuron. Prog Brain Res 45:235-258
Lim J, Thiery JP (2012) Epithelial-mesenchymal transitions: insights from development. Development 139:3471-3486
Lim KC, Lakshmanan G, Crawford SE, Gu Y, Grosveld F, Engel JD (2000) Gata3 loss leads to embryonic lethality due to noradrenaline deficiency of the sympathetic nervous system. Nat Genet 25:209-212
Lo L, Tiveron MC, Anderson DJ (1998) MASH1 activates expression of the paired homeodomain transcription factor Phox2a, and couples pan-neuronal and subtype-specific components of autonomic neuronal identity. Development 125:609-620
Lohr J, Gut P, Karch N, Unsicker K, Huber K (2006) Development of adrenal chromaffin cells in Sf1 heterozygous mice. Cell Tissue Res 325:437-444
Lucas ME, Muller F, Rudiger R, Henion PD, Rohrer H (2006) The bHLH transcription factor hand2 is essential for noradrenergic differentiation of sympathetic neurons. Development 133:4015-4024
Lumb R, Wiszniak S, Kabbara S, Scherer M, Harvey N, Schwarz Q (2014) Neuropilins define distinct populations of neural crest cells. Neural Dev 9:24
Luo R, Gao J, Wehrle-Haller B, Henion PD (2003) Molecular identification of distinct neurogenic and melanogenic neural crest sublineages. Development 130:321-330
Luo XR, Ikeda YY, Parker KL (1994) A cell-specific nuclear receptor is essential for adrenal and gonadal development and sexual-differentiation. Cell 77:481-490
Ma Q, Kintner C, Anderson DJ (1996) Identification of neurogenin, a vertebrate neuronal determination gene. Cell 87:43-52
Mac Auley A, Werb Z, Mirkes PE (1993) Characterization of the unusually rapid cell cycles during rat gastrulation. Development 117:873-883
Maden CH, Gomes J, Schwarz Q, Davidson K, Tinker A, Ruhrberg C (2012) NRP1 and NRP2 cooperate to regulate gangliogenesis, axon guidance and target innervation in the sympathetic nervous system. Dev Biol 369:277-285
Makita T, Sucov HM, Gariepy CE, Yanagisawa M, Ginty DD (2008) Endothelins are vascular-derived axonal guidance cues for developing sympathetic neurons. Nature 452:759-763
Manousiouthakis E, Mendez M, Garner MC, Exertier P, Makita T (2014) Venous endothelin guides sympathetic innervation of the developing mouse heart. Nat Commun 5:3918
Maro GS, Vermeren M, Voiculescu O, Melton L, Cohen J, Charnay P, Topilko P (2004) Neural crest boundary cap cells constitute a source of neuronal and glial cells of the PNS. Nat Neurosci 7:930-938

Mascorro JA, Breaux TF, Yates RD (1994) Morphological observations of small granule-containing (chromaffin) cells in the celiac ganglion of the guinea pig, with emphasis on cell contacts. Microsc Res Tech 29:169-176
Mascorro JA, Yates RD (1971) Ultrastructural studies of the effects of reserpine on mouse abdominal sympathetic paraganglia. Anat Rec 170:269-279
Mascorro JA, Yates RD (1974) Innervation of abdominal paraganglia: an ultrastructural study. J Morphol 142:153-163
Mascorro JA, Yates RD (1977) The anatomical distribution and morphology of extraadrenal chromaffin tissue (abdominal paraganglia) in the dog. Tissue Cell 9:447-460
Mayanil CS (2013) Transcriptional and epigenetic regulation of neural crest induction during neurulation. Dev Neurosci 35:361-372
McKinney MC, Fukatsu K, Morrison J, McLennan R, Bronner ME, Kulesa PM (2013) Evidence for dynamic rearrangements but lack of fate or position restrictions in premigratory avian trunk neural crest. Development 140:820-830
McNicol AM (2004) Adrenal Medulla and Paraganglia. Humana Press, pp 227-243
McPherson CE, Varley JE, Maxwell GD (2000) Expression and regulation of type I BMP receptors during early avian sympathetic ganglion development. Dev Biol 221:220-232
Mitchell PJ, Timmons PM, Hebert JM, Rigby PW, Tjian R (1991) Transcription factor AP-2 is expressed in neural crest cell lineages during mouse embryogenesis. Genes Dev 5:105-119
Moore MW, Klein RD, Farinas I, Sauer H, Armanini M, Phillips H, Reichardt LF, Ryan AM, Carver-Moore K, Rosenthal A (1996) Renal and neuronal abnormalities in mice lacking GDNF. Nature 382:76-79
Moriguchi T, Takako N, Hamada M, Maeda A, Fujioka Y, Kuroha T, Huber RE, Hasegawa SL, Rao A, Yamamoto M, Takahashi S, Lim KC, Engel JD (2006) Gata3 participates in a complex transcriptional feedback network to regulate sympathoadrenal differentiation. Development 133:3871-3881
Morikawa Y, D'Autreaux F, Gershon MD, Cserjesi P (2007) Hand2 determines the noradrenergic phenotype in the mouse sympathetic nervous system. Dev Biol 307:114-126
Morikawa Y, Zehir A, Maska E, Deng C, Schneider MD, Mishina Y, Cserjesi P (2009) BMP signaling regulates sympathetic nervous system development through Smad4-dependent and -independent pathways. Development
Moser M, Ruschoff J, Buettner R (1997) Comparative analysis of AP-2 alpha and AP-2 beta gene expression during murine embryogenesis. Dev Dyn 208:115-124
Muñoz WA, Trainor PA (2015) Neural crest cell evolution: How and when did a neural crest cell become a neural crest cell. In: Paul AT (ed) Current Topics in Developmental Biology, vol 111. Academic Press, pp 3-26
Murphy P, Topilko P, Schneider-Maunoury S, Seitanidou T, Baron-Van Evercooren A, Charnay P (1996) The regulation of Krox-20 expression reveals important steps in the control of peripheral glial cell development. Development 122:2847-2857

Newbern JM (2015) Molecular control of the neural crest and peripheral nervous system development. In: Paul AT (ed) Current Topics in Developmental Biology, vol 111. Academic Press, pp 201-231
Nishino J, Saunders TL, Sagane K, Morrison SJ (2010) Lgi4 promotes the proliferation and differentiation of glial lineage cells throughout the developing peripheral nervous system. J Neurosci 30:15228-15240
Nitzan E, Pfaltzgraff ER, Labosky PA, Kalcheim C (2013) Neural crest and Schwann cell progenitor-derived melanocytes are two spatially segregated populations similarly regulated by Foxd3. Proc Natl Acad Sci U S A 110:12709-12714
Noisa P, Raivio T (2014) Neural Crest Cells: From Developmental Biology to Clinical Interventions. Birth Defects Research Part C-Embryo Today-Reviews 102:263-274
Nowakowski RS, Caviness VS, Jr., Takahashi T, Hayes NL (2002) Population dynamics during cell proliferation and neuronogenesis in the developing murine neocortex. Results Probl Cell Differ 39:1-25
Nowakowski RS, Lewin SB, Miller MW (1989) Bromodeoxyuridine immunohistochemical determination of the lengths of the cell cycle and the DNA-synthetic phase for an anatomically defined population. J Neurocytol 18:311-318
Orford KW, Scadden DT (2008) Deconstructing stem cell self-renewal: genetic insights into cell-cycle regulation. Nat Rev Genet 9:115-128
Ozkaynak E, Abello G, Jaegle M, van Berge L, Hamer D, Kegel L, Driegen S, Sagane K, Bermingham JR, Jr., Meijer D (2010) Adam22 is a major neuronal receptor for Lgi4-mediated Schwann cell signaling. J Neurosci 30:3857-3864
Pakkarato S, Chomphoo S, Kagawa Y, Owada Y, Mothong W, Iamsaard S, Sawatpanich T, Kondo H, Hipkaeo W (2015) Immunohistochemical analysis of sustentacular cells in the adrenal medulla, carotid body and sympathetic ganglion of mice using an antibody against brain-type fatty acid binding protein (B-FABP). Journal of Anatomy 226:348-353
Paratore C, Goerich DE, Suter U, Wegner M, Sommer L (2001) Survival and glial fate acquisition of neural crest cells are regulated by an interplay between the transcription factor Sox10 and extrinsic combinatorial signaling. Development 128:3949-3961
Partanen M, Linnoila I, Hervonent A, Rapoport SI (1984a) The effect of aging on extra-adrenal catecholamine storing cells of the rat. Neurobiol Aging 5:105-110
Partanen M, Rapoport SI, Reis DJ, Joh TH, Stolk JM, Linnoila I, Teitelman G, Hervonen A (1984b) Catecholamine-synthesizing enzymes in paraganglia of aged Fischer-344 rats. Immunohistochemistry and fluorescence microscopy. Cell Tissue Res 238:217-220
Pattyn A, Guillemot F, Brunet JF (2006) Delays in neuronal differentiation in Mash1/Ascl1 mutants. Dev Biol 295:67-75
Pattyn A, Morin X, Cremer H, Goridis C, Brunet JF (1999) The homeobox gene Phox2b is essential for the development of autonomic neural crest derivatives. Nature 399:366-370

Perez SE, Rebelo S, Anderson DJ (1999) Early specification of sensory neuron fate revealed by expression and function of neurogenins in the chick embryo. Development 126:1715-1728

Pfeuty B, David-Pfeuty T, Kaneko K (2008) Underlying principles of cell fate determination during G1 phase of the mammalian cell cycle. Cell Cycle 7:3246-3257

Potzner MR, Tsarovina K, Binder E, Penzo-Mendez A, Lefebvre V, Rohrer H, Wegner M, Sock E (2010) Sequential requirement of Sox4 and Sox11 during development of the sympathetic nervous system. Development 137:775-784

Raible DW, Eisen JS (1994) Restriction of neural crest cell fate in the trunk of the embryonic zebrafish. Development 120:495-503

Raposo AA, Vasconcelos FF, Drechsel D, Marie C, Johnston C, Dolle D, Bithell A, Gillotin S, van den Berg DL, Ettwiller L, Flicek P, Crawford GE, Parras CM, Berninger B, Buckley NJ, Guillemot F, Castro DS (2015) Ascl1 coordinately regulates gene expression and the chromatin landscape during neurogenesis. Cell Rep 10:1544–1556

Reid K, Nishikawa S, Bartlett PF, Murphy M (1995) Steel factor directs melanocyte development in vitro through selective regulation of the number of c-kit+ progenitors. Dev Biol 169:568-579

Reiff T, Huber L, Kramer M, Delattre O, Janoueix-Lerosey I, Rohrer H (2011) Midkine and Alk signaling in sympathetic neuron proliferation and neuroblastoma predisposition. Development 138:4699-4708

Reiff T, Tsarovina K, Majdazari A, Schmidt M, del Pino I, Rohrer H (2010) Neuroblastoma phox2b variants stimulate proliferation and dedifferentiation of immature sympathetic neurons. J Neurosci 30:905-915

Reissmann E, Ernsberger U, Francis-West PH, Rueger D, Brickell PM, Rohrer H (1996) Involvement of bone morphogenetic protein-4 and bone morphogenetic protein-7 in the differentiation of the adrenergic phenotype in developing sympathetic neurons. Development 122:2079-2088

Rickmann M, Fawcett JW, Keynes RJ (1985) The migration of neural crest cells and the growth of motor axons through the rostral half of the chick somite. J Embryol Exp Morphol 90:437-455

Ridenour DA, McLennan R, Teddy JM, Semerad CL, Haug JS, Kulesa PM (2014) The neural crest cell cycle is related to phases of migration in the head. Development 141:1095-1103

Rodriguez H, Filippa V, Mohamed F, Dominguez S, Scardapane L (2007) Interaction between chromaffin and sustentacular cells in adrenal medulla of viscacha (Lagostomus maximus maximus). Anat Histol Embryol 36:182-185

Rohrer H (2011) Transcriptional control of differentiation and neurogenesis in autonomic ganglia. Eur J Neurosci 34:1563-1573

Rohrer H, Thoenen H (1987) Relationship between differentiation and terminal mitosis: chick sensory and ciliary neurons differentiate after terminal mitosis of precursor cells, whereas sympathetic neurons continue to divide after differentiation. J Neurosci 7:3739-3748

Rothman TP, Gershon MD, Holtzer H (1978) The relationship of cell division to the acquisition of adrenergic characteristics by developing sympathetic ganglion cell precursors. Dev Biol 65:322-341
Rubin de Celis MF, Garcia-Martin R, Wittig D, Valencia GD, Enikolopov G, Funk RH, Chavakis T, Bornstein SR, Androutsellis-Theotokis A, Ehrhart-Bornstein M (2015) Multipotent glia-like stem cells mediate stress adaptation. Stem Cells 33:2037-2051
Ruiz S, Panopoulos AD, Herrerias A, Bissig KD, Lutz M, Berggren WT, Verma IM, Izpisua Belmonte JC (2011) A high proliferation rate is required for cell reprogramming and maintenance of human embryonic stem cell identity. Curr Biol 21:45-52
Saito D, Takase Y, Murai H, Takahashi Y (2012) The dorsal aorta initiates a molecular cascade that instructs sympatho-adrenal specification. Science 336:1578-1581
Salomoni P, Calegari F (2010) Cell cycle control of mammalian neural stem cells: putting a speed limit on G1. Trends Cell Biol 20:233-243
Santana MM, Chung KF, Vukicevic V, Rosmaninho-Salgado J, Kanczkowski W, Cortez V, Hackmann K, Bastos CA, Mota A, Schrock E, Bornstein SR, Cavadas C, Ehrhart-Bornstein M (2012) Isolation, characterization, and differentiation of progenitor cells from human adult adrenal medulla. Stem Cells Translational Medicine 1:783-791
Sauka-Spengler T, Bronner-Fraser M (2006) Development and evolution of the migratory neural crest: a gene regulatory perspective. Curr Opin Genet Dev 16:360-366
Saxena S, Wahl J, Huber-Lang MS, Stadel D, Braubach P, Debatin KM, Beltinger C (2013) Generation of murine sympathoadrenergic progenitor-like cells from embryonic stem cells and postnatal adrenal glands. PLoS One 8:e64454
Schilling TF, Kimmel CB (1994) Segment and cell type lineage restrictions during pharyngeal arch development in the zebrafish embryo. Development 120:483-494
Schmidt M, Huber L, Majdazari A, Schutz G, Williams T, Rohrer H (2011) The transcription factors AP-2beta and AP-2alpha are required for survival of sympathetic progenitors and differentiated sympathetic neurons. Dev Biol 355:89-100
Schmidt M, Lin S, Pape M, Ernsberger U, Stanke M, Kobayashi K, Howard MJ, Rohrer H (2009) The bHLH transcription factor Hand2 is essential for the maintenance of noradrenergic properties in differentiated sympathetic neurons. Dev Biol
Schneider C, Wicht H, Enderich J, Wegner M, Rohrer H (1999) Bone morphogenetic proteins are required in vivo for the generation of sympathetic neurons. Neuron 24:861-870
Schober A, Parlato R, Huber K, Kinscherf R, Hartleben B, Huber TB, Schutz G, Unsicker K (2013) Cell loss and autophagy in the extra-adrenal chromaffin organ of Zuckerkandl are regulated by glucocorticoid signalling. J Neuroendocrinol 25:34-47
Schwarz Q, Maden CH, Davidson K, Ruhrberg C (2009a) Neuropilin-mediated neural crest cell guidance is essential to organise sensory neurons into segmented dorsal root ganglia. Development 136:1785-1789

Schwarz Q, Maden CH, Vieira JM, Ruhrberg C (2009b) Neuropilin 1 signaling guides neural crest cells to coordinate pathway choice with cell specification. Proc Natl Acad Sci USA 106:6164-6169
Schwarz Q, Ruhrberg C (2010) Neuropilin, you gotta let me know: should I stay or should I go? Cell Adh Migr 4:61-66
Serbedzija GN, Bronner-Fraser M, Fraser SE (1989) A vital dye analysis of the timing and pathways of avian trunk neural crest cell migration. Development 106:809-816
Serbedzija GN, Fraser SE, Bronner-Fraser M (1990) Pathways of trunk neural crest cell migration in the mouse embryo as revealed by vital dye labelling. Development 108:605-612
Shah NM, Groves AK, Anderson DJ (1996) Alternative neural crest cell fates are instructively promoted by TGFbeta superfamily members. Cell 85:331-343
Shah NM, Marchionni MA, Isaacs I, Stroobant P, Anderson DJ (1994) Glial growth factor restricts mammalian neural crest stem cells to a glial fate. Cell 77:349-360
Shanklin DR, Soteloav C (1969) In situ tumors in fetuses, newborns and infants. Biologia Neonatorum 14:286-&
Shi H, Cui H, Alam G, Gunning WT, Nestor A, Giovannucci D, Zhang M, Ding HF (2008) Nestin expression defines both glial and neuronal progenitors in postnatal sympathetic ganglia. J Comp Neurol 508:867-878
Shtukmaster S, Narasimhan P, El Faitwri T, Stubbusch J, Ernsberger U, Rohrer H, Unsicker K, Huber K (2016) MiR-124 is differentially expressed in derivatives of the sympathoadrenal cell lineage and promotes neurite elongation in chromaffin cells. Cell Tissue Res 365:225-232
Shtukmaster S, Schier MC, Huber K, Krispin S, Kalcheim C, Unsicker K (2013) Sympathetic neurons and chromaffin cells share a common progenitor in the neural crest in vivo. Neural Dev 8:12
Smith JL, Schoenwolf GC (1987) Cell cycle and neuroepithelial cell shape during bending of the chick neural plate. Anat Rec 218:196-206
Smith JL, Schoenwolf GC (1988) Role of cell-cycle in regulating neuroepithelial cell shape during bending of the chick neural plate. Cell Tissue Res 252:491-500
Sommer L, Ma Q, Anderson DJ (1996) Neurogenins, a novel family of atonal-related bHLH transcription factors, are putative mammalian neuronal determination genes that reveal progenitor cell heterogeneity in the developing CNS and PNS. Mol Cell Neurosci 8:221-241
Stanke M, Junghans D, Geissen M, Goridis C, Ernsberger U, Rohrer H (1999) The Phox2 homeodomain proteins are sufficient to promote the development of sympathetic neurons. Development 126:4087-4094.
Stanke M, Stubbusch J, Rohrer H (2004) Interaction of Mash1 and Phox2b in sympathetic neuron development. Mol Cell Neurosci 25:374-382
Stemple DL, Anderson DJ (1992) Isolation of a stem cell for neurons and glia from the mammalian neural crest. Cell 71:973-985
Stewart HJ, Brennan A, Rahman M, Zoidl G, Mitchell PJ, Jessen KR, Mirsky R (2001) Developmental regulation and overexpression of the transcription factor AP-2, a potential regulator of the timing of Schwann cell generation. Eur J Neurosci 14:363-372

Stubbusch J, Narasimhan P, Hennchen M, Huber K, Unsicker K, Ernsberger U, Rohrer H (2015) Lineage and stage specific requirement for Dicer1 in sympathetic ganglia and adrenal medulla formation and maintenance. Dev Biol 400:210-223
Stubbusch J, Narasimhan P, Huber K, Unsicker K, Rohrer H, Ernsberger U (2013) Synaptic protein and pan-neuronal gene expression and their regulation by Dicer-dependent mechanisms differ between neurons and neuroendocrine cells. Neural Development 8:1-1
Subramanian A, Maker VK (2006) Organs of Zuckerkandl: their surgical significance and a review of a century of literature. Am. J. Surg., vol 192, pp 224-234
Suzuki T, Kachi T (1994) Differences between Adrenaline and Noradrenaline Cells in Cellular-Association with Supporting Cells in the Adrenal-Medulla of the Pig - an Immunohistochemical Study. Neuroscience Letters 176:217-220
Takahashi T, Nowakowski RS, Caviness VS, Jr. (1996) The leaving or Q fraction of the murine cerebral proliferative epithelium: a general model of neocortical neuronogenesis. J Neurosci 16:6183-6196
Takahashi T, Nowakowski RS, Caviness VS, Jr. (1997) The mathematics of neocortical neuronogenesis. Dev Neurosci 19:17-22
Theveneau E, Duband JL, Altabef M (2007) Ets-1 confers cranial features on neural crest delamination. PLoS One 2:e1142
Thomas AJ, Erickson CA (2009) FOXD3 regulates the lineage switch between neural crest-derived glial cells and pigment cells by repressing MITF through a non-canonical mechanism. Development 136:1849-1858
Thomas SA, Matsumoto AM, Palmiter RD (1995) Noradrenaline is essential for mouse fetal development. Nature 374:643-646
Tischler AS, Ruzicka LA, Donahue SR, DeLellis RA (1989) Chromaffin cell proliferation in the adult rat adrenal medulla. Int J Dev Neurosci 7:439-448
Tsarovina K, Pattyn A, Stubbusch J, Muller F, Van Der Wees J, Schneider C, Brunet JF, Rohrer H (2004) Essential role of Gata transcription factors in sympathetic neuron development. Development 131:4775-4786
Tsarovina K, Reiff T, Stubbusch J, Kurek D, Grosveld FG, Parlato R, Schutz G, Rohrer H (2010) The Gata3 transcription factor is required for the survival of embryonic and adult sympathetic neurons. J Neurosci 30:10833-10843
Tsarovina K, Schellenberger J, Schneider C, Rohrer H (2008) Progenitor cell maintenance and neurogenesis in sympathetic ganglia involves Notch signaling. Mol Cell Neurosci 37:20-31
Uesaka T, Nagashimada M, Enomoto H (2015) Neuronal differentiation in Schwann cell lineage underlies postnatal neurogenesis in the enteric nervous system. J Neurosci 35:9879-9888
Unsicker K, Krisch B, Otten U, Thoenen H (1978) Nerve growth factor-induced fiber outgrowth from isolated rat adrenal chromaffin cells: impairment by glucocorticoids. Proc Natl Acad Sci USA 75:3498-3502
VanDusen NJ, Vincentz JW, Firulli BA, Howard MJ, Rubart M, Firulli AB (2014) Loss of Hand2 in a population of Periostin lineage cells results in

pronounced bradycardia and neonatal death. Developmental biology 388:149-158
Varley JE, Maxwell GD (1996) BMP-2 and BMP-4, but not BMP-6, increase the number of adrenergic cells which develop in quail trunk neural crest cultures. Exp Neurol 140:84-94
Varley JE, McPherson CE, Zou H, Niswander L, Maxwell GD (1998) Expression of a constitutively active type I BMP receptor using a retroviral vector promotes the development of adrenergic cells in neural crest cultures. Dev Biol 196:107-118
Varley JE, Wehby RG, Rueger DC, Maxwell GD (1995) Number of adrenergic and islet-1 immunoreactive cells is increased in avian trunk neural crest cultures in the presence of human recombinant osteogenic protein-1. Dev Dyn 203:434-447
Vega-Lopez GA, Cerrizuela S, Aybar MJ (2017) Trunk neural crest cells: formation, migration and beyond. Int J Dev Biol 61:5-15
Wang L, Mongera A, Bonanomi D, Cyganek L, Pfaff SL, Nusslein-Volhard C, Marquardt T (2014) A conserved axon type hierarchy governing peripheral nerve assembly. Development 141:1875-1883
Waring H (1936) Development of the adrenal gland of the mouse. Quarterly Journal of Microscopical Science 78:329-336
Wegner M, Stolt CC (2005) From stem cells to neurons and glia: a Soxist's view of neural development. Trends Neurosci 28:583-588
White J, Dalton S (2005) Cell cycle control of embryonic stem cells. Stem Cell Rev 1:131-138
Wildner H, Gierl MS, Strehle M, Pla P, Birchmeier C (2008) Insm1 (IA-1) is a crucial component of the transcriptional network that controls differentiation of the sympatho-adrenal lineage. Development 135:473-481
Wilson YM, Richards KL, Ford-Perriss ML, Panthier JJ, Murphy M (2004) Neural crest cell lineage segregation in the mouse neural tube. Development 131:6153-6162
Woodhoo A, Alonso MB, Droggiti A, Turmaine M, D'Antonio M, Parkinson DB, Wilton DK, Al-Shawi R, Simons P, Shen J, Guillemot F, Radtke F, Meijer D, Feltri ML, Wrabetz L, Mirsky R, Jessen KR (2009) Notch controls embryonic Schwann cell differentiation, postnatal myelination and adult plasticity. Nat Neurosci 12:839-847
Wurtman RJ, Axelrod J (1966) Control of enzymatic synthesis of adrenaline in the adrenal medulla by adrenal cortical steroids. J Biol Chem 241:2301-2305
Young HM, Bergner AJ, Muller T (2003) Acquisition of neuronal and glial markers by neural crest-derived cells in the mouse intestine. J Comp Neurol 456:1-11
Young HM, Cane KN, Anderson CR (2011) Development of the autonomic nervous system: A comparative view. Auton Neurosci 165:10-27
Zackenfels K, Oppenheim RW, Rohrer H (1995) Evidence for an important role of IGF-I and IGF-II for the early development of chick sympathetic neurons. Neuron 14:731-741
Zhou QY, Quaife CJ, Palmiter RD (1995) Targeted disruption of the tyrosine hydroxylase gene reveals that catecholamines are required for mouse fetal development. Nature 374:640-643

Zirlinger M, Lo L, McMahon J, McMahon AP, Anderson DJ (2002) Transient expression of the bHLH factor neurogenin-2 marks a subpopulation of neural crest cells biased for a sensory but not a neuronal fate. Proc Natl Acad Sci U S A 99:8084-8089