Developmental Biology 366 (2012) 64–73

Contents lists available at SciVerse ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/developmentalbiology

Review

The enteric nervous system

Valentina Sasselli [a], Vassilis Pachnis [a], Alan J. Burns [b,*]

[a] Division of Molecular Neurobiology, MRC National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK
[b] Neural Development Unit, UCL Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK

ARTICLE INFO

Article history:
Received for publication 12 January 2012
Accepted 13 January 2012
Available online 24 January 2012

Keywords:
Neural crest cells
Enteric nervous system
Gut
Ret
Endothelin-3

ABSTRACT

The enteric nervous system (ENS), the intrinsic innervation of the gastrointestinal tract, consists of numerous types of neurons, and glial cells, that are distributed in two intramuscular plexuses that extend along the entire length of the gut and control co-ordinated smooth muscle contractile activity and other gut functions. All enteric neurons and glia are derived from neural crest cells (NCC). Vagal (hindbrain) level NCC provide the majority of enteric precursors along the entire length of the gut, while a lesser contribution, that is restricted to the hindgut, arises from the sacral region of the neuraxis. After leaving the dorsal neural tube NCC undergo extensive migration, proliferation, survival and differentiation in order to form a functional ENS. This article reviews the molecular mechanisms underlying these key developmental processes and highlights the major groups of molecules that affect enteric NCC proliferation and survival (Ret/Gdnf and EdnrB/Et-3 pathways, Sox10 and Phox2b transcription factors), cell migration (Ret and EdnrB signalling, semaphorin 3A, cell adhesion molecules, Rho GTPases), and the development of enteric neuronal subtypes and morphologies (Mash1, Gdnf/neurturin, BMPs, Hand2, retinoic acid). Finally, looking to the future, we discuss the need to translate the wealth of data gleaned from animal studies to the clinical area and thus better understand, and develop treatments for, congenital human diseases affecting the ENS.

The enteric nervous system

The enteric nervous system (ENS) is the largest and most complex division of the peripheral and autonomic nervous systems (PNS and ANS) in vertebrates. It contains numerous different types of neurons comparable in number to that of the spinal cord and an array of neurotransmitters and neuromodulators similar to those found in the central nervous system (CNS). The ENS is organised into an interconnected network of neurons and glial cells that are grouped into ganglia located in two major plexuses: the myenteric (Auerbach's) plexus and the submucosal (Meissner's) plexus. ENS components form an integrated circuitry that controls motility of the intestine, exchange of fluids across the mucosal surface, blood flow and secretion of gut hormones. Although the gut also receives extrinsic parasympathetic and sympathetic innervation, the intrinsic neuronal circuits of the ENS are able to generate reflex gut contractile activity independent from any CNS intervention, setting the ENS apart from other components of the ANS (Furness, 2006).

Pre-enteric neural crest cells

The neural crest origin of the ENS was first established by Yntema and Hammond who showed that upon ablation of the vagal (hindbrain) region of the neural crest in avian embryos, enteric ganglia failed to form along the length of the gastrointestinal tract (Yntema and Hammond, 1954). These results were subsequently confirmed and extended by the use of isotopic and isochronic grafts of quail premigratory neural crest into chick embryos (Le Douarin, 1973). Fate mapping of the transplanted quail cells revealed a dual neural crest origin for the ENS in which neural crest cells (NCC) emigrating from the vagal region adjacent to somites 1–7 colonised the entire length of the gut forming the majority of the ENS, whereas NCC arising caudal to the 28th somite level (sacral NCC) contributed a smaller number of cells to the post-umbilical gut only (Le Douarin and Teillet, 1973). Cell lineage studies have subsequently revealed a similar origin for the ENS in mammals (Anderson et al., 2006a; Durbec et al., 1996; Kapur, 2000; Serbedzija et al., 1991; Wang et al., 2011).

Vagal NCC emerge from the neural tube around embryonic day 8.5 (E8.5) in the mouse and migrate ventro-medially, reaching the foregut at E9–E9.5 (Anderson et al., 2006a; Durbec et al., 1996). After this stage NCC are termed enteric neural crest cells (ENCC), as they contain ENS-restricted progenitor cells that migrate rostrocaudally to colonise the entire length of the developing gut, a process that is completed in approximately 5 days (by E14.5–E15.5). Sacral NCC delaminate from the neural tube at E9–E9.5 (Serbedzija et al., 1991), migrate ventrally to form extrinsic pelvic ganglia adjacent to the hindgut, then migrate from there into the gut to give rise to enteric neurons and glia. In the chick, sacral NCC also initially form an extrinsic nerve, the nerve of Remark, where they reside for a number of days before entering the hindgut (Burns and Douarin, 1998). Sacral

* Corresponding author. Fax: +44 (0)20 7831 4366.
E-mail address: alan.burns@ucl.ac.uk (A.J. Burns).

0012-1606/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2012.01.012

NCC in mouse and chick only migrate into the hindgut upon arrival of their vagal counterparts (Burns and Douarin, 1998; Kapur, 2000). However, they do not rely on vagal ENCC for hindgut colonisation to occur as shown by the fact that sacral ENCC still migrate into the gut, giving rise to enteric neurons and glia in the most distal regions of the intestine even after ablation of the vagal neural crest (Anderson et al., 2006a; Burns et al., 2000; Durbec et al., 1996; Kapur, 2000).

Several studies have addressed cell autonomous properties of pre-enteric vagal and sacral NCC in order to determine whether these cells are pre-specified towards the ENS lineage, or whether their fate depends on microenvironmental cues encountered along migratory pathways. Transplantation of quail vagal and sacral NC into new axial levels of chick embryos suggested that both of these scenarios are valid to some extent. For example, heterotopically grafted vagal and sacral NCC are able to migrate to sites characteristic of their new axial level (Erickson and Goins, 2000; Le Douarin and Teillet, 1974; Le Douarin et al., 1975); nevertheless, they seem to retain some of their original properties and to be able, to a certain extent, to reach the original target tissue (i.e. gut) (Barlow et al., 2008; Burns et al., 2002; Le Douarin and Teillet, 1974; Smith et al., 1977). In addition, a rapidly expanding number of markers have been discovered that identify undifferentiated NCC prior to their entry into the gut, which might point towards the existence of a pre-enteric specified subset of NCC. Vagal and sacral NCC express the transcription factor Sox10 before the onset of migration and they maintain this expression until they reach the gut (Anderson et al., 2006a; Southard-Smith et al., 1998). In avian embryos, pre-migratory vagal NCC have also been shown to express the endothelin receptor B (EDNRB) (Nataf et al., 1996). Shortly after emigration from the neural tube, vagal NCC express the low-affinity nerve growth receptor p75 (Anderson et al., 2006a; Wilson et al., 2004) and, while they are migrating in proximity to the dorsal aorta, they also induce the expression of the transcriptional regulator Phox2b (Pattyn et al., 1999) and the receptor tyrosine kinase RET (Anderson et al., 2006a; Durbec et al., 1996; Pachnis et al., 1993). Interestingly, recent findings have suggested a similar phenotypic profile exists in sacral NCC (Anderson et al., 2006a; Burns et al., 2002). However, it is not clear whether all cells express all the markers or whether different phenotypic classes exist. An attempt to identify "pre-enteric" NCC on the basis of concomitant or differential expression of markers has been made by Anderson et al., who identified a possible co-expression of $Sox10^+$ $p75^+$ in NCC located around the rostral foregut, with a subpopulation of them also expressing Phox2b and RET (Anderson et al., 2006a). Nevertheless, when cells enter the gut, phenotypic restriction seems to be in place and undifferentiated ENCC have a relatively defined expression of molecules as outlined below and as summarised in Fig. 1.

Enteric neural crest cells

Vagal ENCC enter the embryonic mouse gut at E9.5 and undergo a single rostrocaudal wave of migration to sequentially colonise all regions of the developing gut (Kapur et al., 1992; Young et al., 1998a). The mode of migration of ENCC along the mouse gut has been examined by time-lapse imaging (Druckenbrod and Epstein, 2005, 2007, 2009; Young et al., 2004). ENCC migrate through the gut predominantly in strands or chains of interconnected cells. Although the net movement of the cell population has a clear rostrocaudal directionality, individual cells at the migration wavefront have complex and unpredictable trajectories (Young et al., 2004). While ENCC are migrating through the gut mesenchyme, a subpopulation of cells starts to differentiate into neurons and express pan-neuronal markers (Baetge and Gershon, 1989; Young et al., 1999). Recent studies have shown that many of these immature neurons are also migrating caudally, although at a lower speed and for limited distances compared to undifferentiated vagal ENCC (Hao et al., 2009).

A secondary wave of migration occurs when a subset of ENCC, which had migrated initially through the outer half of the mesenchyme (the presumptive myenteric plexus), moves centripetally to colonise the submucosal region and subsequently forms the submucosal plexus (Jiang et al., 2003). However, in avian embryos, the converse occurs; ENCC first migrate within the submucosa then migrate outwards to contribute to the myenteric plexus (Burns and Douarin, 1998).

During gut colonisation (E10.5-E13.5 in the mouse) the most caudal cells are undifferentiated ENCC, and co-express Sox10, RET, p75 and Phox2b (Young et al., 1998a, 1999, 2003). Migrating ENCC also express EDNRB (Barlow et al., 2003; Gariepy et al., 1998) and the transcriptional regulator Mash1 (Blaugrund et al., 1996; Lo et al., 1991). Furthermore, chick sacral ENCC share gene expression markers including Sox10, EDNRB and RET (Delalande et al., 2008). Behind the migratory front, ENCC are at different stages of differentiation, with neurons appearing before glial cells (Young et al., 2003). Thus, differentiation of vagal-derived ENCC into neurons takes place shortly after invasion of the foregut and is accompanied by downregulation of Sox10 and p75, maintenance of RET and Phox2b expression and upregulation of pan-neuronal markers such as the ubiquitin hydrolase PGP9.5, neurofilament protein (NF), neuronal class III β-tubulin and the RNA-binding proteins HuC and HuD (Sato and Heuckeroth, 2008; Young et al., 2002, 2003, 2005) (see Fig. 1). A subset of $Mash1^+$ neuronal differentiating cells also transiently expresses the catecholaminergic marker tyrosine hydroxylase (TH) (Baetge and Gershon, 1989; Blaugrund et al., 1996; Young et al., 1999). All these neuronal-committed cells are considered to be progenitors at this stage, since they lack neuron-subtype specific markers and are still mitotically active (Baetge and Gershon, 1989; Sato and Heuckeroth, 2008; Young et al., 2003, 2005). Glial progenitors are first identified at E11.5 by expression of B-FABP and are located mainly in the foregut and midgut, a long way behind the migratory wavefront (Young et al., 2003). These cells also maintain Sox10 and p75 expression and upregulate S100 and GFAP only at E14.5 and E16.5, respectively (Rothman et al., 1986; Young et al., 2003).

Molecular mechanisms controlling ENCC colonisation of the gut

ENS development is a complex and asynchronous process. As ENCC migrate along the gut, they respond to proliferative signals which increase their number at the migratory front enabling them to invade and colonise further posterior regions as well as rapidly populate growing gut colonised behind the wavefront. Concomitantly, subsets of ENCC undergo lineage restriction to generate enteric neurons and glial cells. Carefully controlled orchestration of these processes is essential for the formation of a complete functional ENS and involves many molecular mechanisms, some of which recently have begun to be elucidated.

Cell numbers

The population size of pre-enteric NCC plays an important role in determining the extent of gut colonisation. This has been demonstrated by reducing the initial number of vagal NCC in avian embryos *in vivo*, which leads to incomplete colonisation of the gut (Burns et al., 2000; Peters-van der Sanden et al., 1993; Yntema and Hammond, 1954). It is now clear that a minimal number of NCC is necessary for the complete rostrocaudal colonisation of the gastrointestinal tract (Barlow et al., 2008). The pool size of ENCC is also important for governing the speed of wavefront progression along the gut and the successful colonisation of the entire organ. When cells at the leading edge of the migratory front are isolated from the more rostral ENCC population, the rate of migration is dramatically reduced (Young

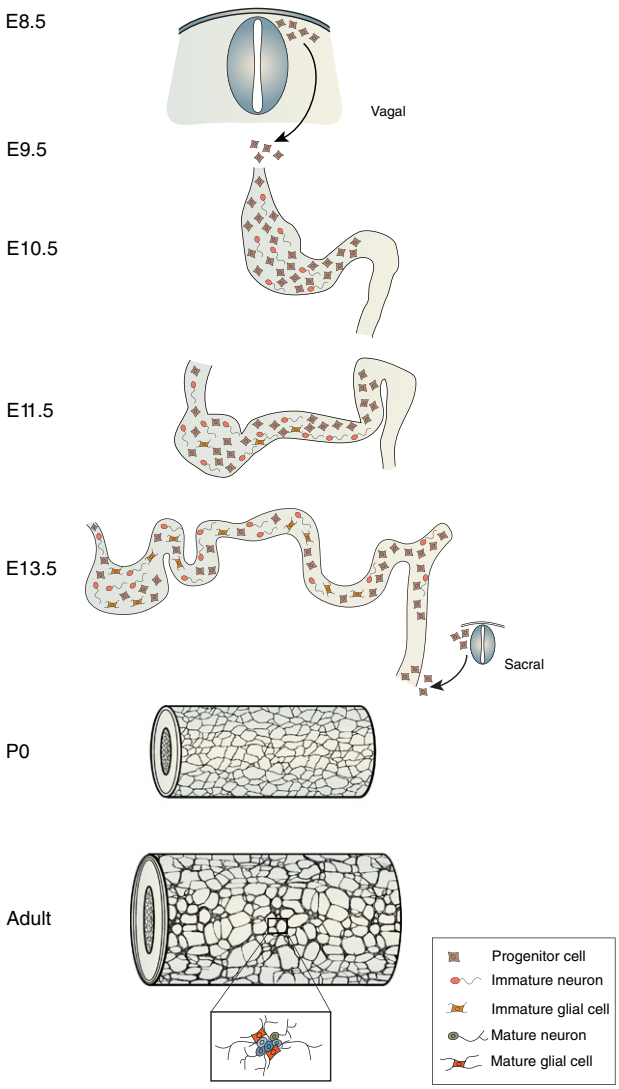

Fig. 1. Summary of murine enteric nervous system development from neural crest cells (NCC) showing the migration of NCC along the gut, key cellular events, and the molecular markers expressed by the various cell types.

Stage	Cellular events	Cell types	Markers
E8.5	Delamination from vagal region of neural tube	Vagal NCCs	Sox10/p75
E9.5	Invasion of the embryonic foregut by vagal progenitor cells	Pre-enteric NCCs	Sox10/p75 ± RET/Phox2b
E10.5	Rostro-caudal migration of progenitor cells	ENCCs (progenitor cells)	Sox10/p75/RET/Phox2b; EDNRB; Mash1
	Proliferation of progenitor cells	Immature neurons	RET/Phox2b/PGP9.5/HuC-D/TuJ1 ± Mash1/TH
	Start of neuronal differentiation		
E11.5	Rostro-caudal migration of progenitor cells	ENCCs (progenitor cells)	Sox10/p75/RET/Phox2b; EDNRB; Mash1
	Proliferation of progenitor cells	Immature neurons	RET/Phox2b/PGP9.5/HuC-D/TuJ1 ± Mash1/TH; ± NOS; ± Calb
	Neuronal differentiation (appearance of first neurotransmitters)	Immature glial cells	Sox10/p75/B-FABP
	Start of glial differentiation		
E13.5	Completion of rostro-caudal migration of vagal progenitor cells	Sacral NCCs	Sox10/p75 ± RET/Phox2b
	Invasion of the embryonic hindgut and caudo-rostral migration	ENCCs (progenitor cells)	Sox10/p75/RET/Phox2b; EDNRB
	of sacral progenitor cells	Immature neurons	RET/Phox2b/PGP9.5/HuC-D/TuJ1 ± NOS; ± Calb; ± VIP; ± NPY
	Proliferation of progenitor cells	Immature glial cells	Sox10/p75/B-FABP
	Neuronal differentiation		
	Glial differentiation		
P0 (to adult)	Proliferation of progenitor cells	ENCCs (progenitor cells)	Sox10/p75 ± RET/Phox2b
	Differentiation of mature neuronal phenotypes	Neurons	RET/Phox2b/PGP9.5/HuC-D/TuJ1 ± NOS; ± Calb; ± VIP; ± NPY;
	Differentiation of mature glial phenotype/s		± SubP; ± CGRP; ± 5HT; ± ChAT; ± Calret
	Gangliogenesis	Glial cells	Sox10/p75/Phox2b/B-FABP/S100β/GFAP
	Formation of functional neuronal circuits		
	(i.e. onset of coordinate intestinal motility)		

et al., 2004). Recent mathematical modelling and grafting experiments in embryonic avian gut *in vitro* have shown that unidirectional colonisation of the gut is accomplished when cells at the wavefront act as a proliferative source to generate enough motile ENCC to invade previously unoccupied regions, while those behind the front are essentially non proliferative and do not participate directly in the invasion of unoccupied tissues (frontal expansion model) (Simpson et al., 2007).

Proliferation and survival

Three major groups of molecules have been implicated to date in the control of ENCC proliferation and survival during ENS development, namely RET-GDNF, Endothelin-3(ET-3)-EDNRB and transcription factors such as Sox10 and Phox2b. RET is a transmembrane tyrosine kinase which acts as a receptor for the glial cell line-derived neurotrophic factor (GDNF) family of ligands including GDNF, neurturin, artemin and persephin (Manié et al., 2001). Each ligand is able to activate RET *via* binding to a preferred high-affinity glycosylphosphatidylinositol (GPI)-anchored co-receptor GFRα (GDNF family receptor α-component), which is GFRα1 in the case of GDNF (Baloh et al., 2000; Jing et al., 1996). RET is expressed in migratory ENCC and, later, is maintained exclusively in the neuronal population (Durbec et al., 1996; Pachnis et al., 1993; Young et al., 2003). GFRα1 expression is found both in ENCC and gut mesoderm (Chalazonitis et al., 1998; Schiltz et al., 1999), whereas GDNF is specifically expressed by the gut mesoderm (Natarajan et al., 2002; Young et al., 2001). In mouse, mutation in the RET, GFRα1, or GDNF genes leads to total intestinal aganglionosis, where ENCC fail to colonise the gastrointestinal tract beyond the rostral stomach (Cacalano et al., 1998; Enomoto et al., 1998; Moore et al., 1996; Pichel et al., 1996; Sanchez et al., 1996; Schuchardt et al., 1994). *In vitro* and *in vivo* investigations on the effects of the targeted mutation of the gene encoding RET (*c-Ret*) have revealed that the activation of the receptor by GDNF is required at early stages of gut colonisation for survival of the majority of ENCC. In the absence of RET, progenitors undergo extensive apoptosis, which dramatically depletes the pool of cells able to progress along the gut (Taraviras et al., 1999). In addition to its role in cell survival, RET-GDNF signalling generates a strong proliferative signal for ENCC in a time-dependent manner. *In vitro* studies have shown that GDNF enhances cell proliferation and induces an increase in the number of neuronal progenitors in early migratory ENCC (Chalazonitis et al., 1998; Hearn et al., 1998; Heuckeroth et al., 1998; Taraviras et al., 1999). In accordance with these studies, depletion of GDNF *in vivo*, as reported in mice heterozygous for mutation in the *Gdnf* gene ($Gdnf^{+/-}$), leads to a reduced number of enteric neurons along the intestine (hypoganglionosis) as a result of the diminished proliferative capacity of progenitor ENCC (Gianino et al., 2003). However, at later post-migratory stages, GDNF seems mainly to promote survival and neuronal differentiation (Chalazonitis et al., 1998; Taraviras et al., 1999; Uesaka et al., 2007; Wang et al., 2010).

EDNRB is a G protein-coupled receptor for the small peptides endothelin-1 (ET-1), ET-2 and ET-3 (Inoue et al., 1989). In the embryonic mouse gut, ET-3 is expressed by the gut mesenchyme, with its highest levels in the caecum (Barlow et al., 2003; Leibl et al., 1999), while EDNRB is expressed by both ENCC and non-neural crest gut tissue (Barlow et al., 2003; Woodward et al., 2000; Wu et al., 1999). Spontaneous or targeted mutations of the *Ednrb* or *Et-3* locus lead to identical phenotypes, characterised by the absence of enteric neurons from the terminal region of the gut (colonic aganglionosis) (Baynash et al., 1994; Hosoda et al., 1994; Kapur et al., 1995). *In vitro* studies in mouse and avian showed that ET-3 inhibits or delays neuronal differentiation of ENCC (Hearn et al., 1998; Wu et al., 1999), with little or no effect on proliferation (Chalazonitis et al., 1998; Heuckeroth et al., 1998; Kruger et al., 2003; Wu et al., 1999). Further studies on clonogenic cultures of isolated ENS progenitor cells (EPCs) have confirmed that ET-3 is reversibly able to inhibit the commitment of these cells towards neuronal and glial lineages, maintaining them in an uncommitted state (Bondurand et al., 2006). Therefore, it has been suggested that ET-3-EDNRB signalling inhibits ENCC differentiation, instead promoting the maintenance of a pool of undifferentiated and uncommitted progenitors that are able to colonise all regions of the developing gut. In accordance with this hypothesis, absence of ET-3 *in vivo* results in migratory delay in colonisation of the gut (Barlow et al., 2003), associated with a reduced pool of proliferative progenitors and with premature neuronal differentiation at the front of migration (Bondurand et al., 2003). In addition, several interactions of ET3-EDNRB signalling in cooperation with other signalling pathways have been reported to control proliferation and/or differentiation of ENCC. For example, ET-3 is able to synergistically increase the proliferative effect induced by RET-GDNF signalling *in vitro* (Barlow et al., 2003), while *in vivo*, mutations in *Et-3* or *EdnrB* increase the severity of aganglionosis in $Sox10^{Dom/+}$ mice (Cantrell et al., 2004; Stanchina et al., 2006) and in mice carrying only the monoisoformic *c-Ret* allele Ret^{51} (Barlow et al., 2003).

Sox10 is a member of the *S*RY-b*ox* containing (Sox) family of transcription factors (Bowles et al., 2000). *Sox10* is expressed in NCC as they emigrate from the neural tube and continues to be expressed as they migrate towards their target tissues (Kuhlbrodt et al., 1998). In post-migratory target tissues such as dorsal root ganglia (DRG) and the ENS, Sox10 expression is present in undifferentiated multipotent progenitors and, upon lineage segregation, is maintained only by the glial cell lineage (Anderson et al., 2006a; Kuhlbrodt et al., 1998; Paratore et al., 2001; Southard-Smith et al., 1998; Young et al., 2003). In mouse, a spontaneous (*dominant megacolon, $Sox10^{Dom}$*) and a targeted ($Sox10^{LacZ}$) mutation of *Sox10* lead to colonic aganglionosis in heterozygosity and to total intestinal aganglionosis in homozygosity in addition to several other defects in multiple neural crest derivatives (Britsch et al., 2001; Herbarth et al., 1998; Lane and Liu, 1984; Southard-Smith et al., 1998). From *in vivo* studies, Sox10 is thought to play a primary role in sustaining the survival of multipotent undifferentiated NCC. In the absence of Sox10, a dramatic increase in apoptotic death has been reported both in vagal NCC prior to their entry into the rostral foregut (Kapur, 1999) and in undifferentiated trunk NCC within the DRG (Paratore et al., 2001). Premature death seems therefore to preclude the entrance of ENCC into the gut, since investigations using progenitor markers failed to find any positive cells during the early stages of gut colonisation (Kapur, 1999). A combination of *in vivo* and *in vitro* studies has also implicated Sox10 in maintenance of ENCC in an undifferentiated state. Paratore et al. (2002) reported that, in the case of Sox10 haploinsufficiency, as seen in $Sox^{LacZ/+}$ mice, the pool of ENCC expressing progenitor markers such as Sox10 and p75 is reduced along the gastrointestinal tract, with concomitant increase in cells expressing neuronal precursor markers (Paratore et al., 2002). On the other hand, Bondurand et al. (2006) showed that overexpression of Sox10 is able to reduce the number of neurons and glial cells differentiated from ENS progenitors *in vitro* (Bondurand et al., 2006). Thus, these findings demonstrate that Sox10 levels are indeed critical for maintenance of the ENS progenitor pool. Furthermore, Sox10 is part of a molecular cascade that controls spatial and temporal commitment and differentiation of the ENS by interacting with other signalling pathways. In fact, genetic studies have shown that Sox10-binding sites exist at the *Ednrb* enhancer and these are required for appropriate spatiotemporal regulation of the gene in the developing ENS (Zhu et al., 2004). In addition, other studies have implicated Sox10 in the regulation of *c-Ret* expression (Lang and Epstein, 2003; Lang et al., 2000) and *Phox2b* (Elworthy et al., 2005; Kim et al., 2003).

Phox2b is a paired-homeodomain transcription factor which is widely expressed in the developing autonomic nervous system (Pattyn et al., 1997). Phox2b expression in vagal NCC occurs after the expression of Sox10 and is maintained by all ENCC within the embryonic mouse gut, including both neuronal and glial precursors (Anderson et al., 2006a; Young et al., 1998a). Recently, a transgenic line driving the cerulean fluorescent protein (CFP) under control of the Phox2b promoter has revealed differential levels of Phox2b expression at the front of migrating ENCC as well as showing that Phox2b expression persists both in mature enteric neurons and glial cells (Corpening et al., 2008). Mice with targeted mutation of the *Phox2b* locus ($Phox2b^{LacZ}$) lack autonomic ganglia, including the

ENS in all regions of the gastrointestinal tract (Pattyn et al., 1999). In the absence of Phox2b, vagal NCC enter the foregut but fail to express markers such as RET, Mash1 and TH and they coalesce around the esophageal–gastric junction before undergoing extensive apoptotic death (Pattyn et al., 1999). These studies suggest a role for Phox2b in control of ENCC survival by direct regulation of RET expression.

Cell migration

Several processes involving responses to diffusible molecules, extracellular matrix interactions, cell–cell interactions and intracellular reorganisation control motility and directionality of ENCC migrating through the gut mesenchyme. *In vitro* studies have shown that GDNF acts as a chemoattractant for vagal NCC prior to their entry into the gut and to ENCC within the gut. Thus the RET–GDNF signalling pathway seems to play a key role in retaining NCC within the gut mesenchyme, and also in inducing their rostro-caudal migration (Natarajan et al., 2002; Young et al., 2001). *In vivo*, GDNF mRNA is expressed by the gut mesenchyme in a time-dependent fashion, with high levels initially in the foregut and later in the caecum, which precede the colonisation of those regions by ENCC (Natarajan et al., 2002). However, attempts to identify a GDNF gradient within the pre-cecal gut have been unsuccessful (Anderson et al., 2007). In fact, it is not known how ENCC are able to pass the caecum and proceed through the hindgut, since no other sources of GDNF have been found in the most caudal regions of the gut. Experimental evidence points to a GDNF-opposing role of ET-3, which is specifically expressed in the caecum prior to the arrival of ENCC in this region (Barlow et al., 2003). Addition of ET-3 *in vitro* enables ENCC present in the proximal hindgut of ET-3 deficient mice to fully colonise the distal aganglionic regions (Wu et al., 1999) and specifically reduces the chemoattractive effect of GDNF on ENCC migrating in a collagen matrix from gut explants (Barlow et al., 2003; Kruger et al., 2003). In addition, Druckenbrod and Epstein (2009) have recently shown, by pharmacological inhibition of the EDNRB receptor in organ cultures, that the migratory behaviour of ENCC is directly affected, and results in retraction of cellular processes and loss of chain migration (Druckenbrod and Epstein, 2009). Thus, ET-3 might be able to negate GDNF attraction and enhance the migratory properties of ENCCs within the caecal region, allowing the cells to progress further instead of stalling.

As outlined above, sacral ENCC are delayed in their entry to the gut, and once in the gut they migrate caudo-rostrally, unlike the rostro-caudal migration of vagal ENCC. Some studies suggested that sacral NCC colonisation of the gut might be inhibited by the temporally regulated expression of molecules in the hindgut, such as semaphorins. In avian embryos, Sema3A is transiently expressed in the hindgut and it acts as a chemorepellent for axons of neurons present in the nerve of Remark (Shepherd and Raper, 1999). When Sema3A expression decreases during development, axons enter the hindgut and sacral NCCs migrate along with them. Therefore it has been suggested that Sema3A might account indirectly for the delayed entry of the sacral-derived ENCC into the gut (Burns, 2005). In mouse, Sema3A also is expressed in the most caudal regions of the gut and, when absent, some sacral ENCC and extrinsic axons enter the distal hindgut prematurely (Anderson et al., 2007). However, it is still not clear if Sema3A acts directly on ENCC, and it is likely that other molecular mechanisms are involved in the directed migration of sacral NCC into the gut mesenchyme. A recent study in chick embryos from Delalande et al. (2008) reported that sacral ENCC express mRNA transcript for RET almost 4-fold less than vagal ENCC. When RET is overexpressed in sacral ENCC, increased number of cells enter the hindgut earlier in development, thus suggesting differential control of a similar molecular mechanism underlies directed migration in vagal and sacral ENCC (Delalande et al., 2008).

ENCC migration involves the control of cell adhesion to extracellular matrix (ECM) components as well as between migrating cells themselves. The intestinal ECM is a dynamic environment, with temporally regulated expression of several molecules affecting the migratory behaviour of ENCC during development. Thus, altered expression of defined ECM components or impairment in adhesive properties of ENCC might lead to incomplete colonisation of the gastrointestinal tract. Studies from Breau et al. (2009) have reported the existence of a gradient of ECM components in the developing gut. For instance, fibronectin (FN) and tenascin C (TNC) are expressed by the mesenchyme of E11.5 mouse gut more abundantly in the caecum and hindgut compared to the midgut, whereas other components, such as vitronectin, found in all pericellular spaces around epithelial and mesenchymal cells, or laminin α1, laminin α5 and collagen V, found mainly in the basal laminae, are expressed at similar levels in all regions of the gut (Breau et al., 2009). The high levels of expression of FN and TNC in the caecum and proximal hindgut coincide with the timing of crossing of these regions by ENCC, which show opposing migratory responses to them. *In vitro* tenascin C inhibits ENCC migration (Breau et al., 2009), which correlates with the temporary arrest of migration observed when ENCC reach the base of the caecum (Druckenbrod and Epstein, 2005). Conversely, FN promotes migration, cell adhesion and spreading through a mechanism involving β1 integrins expressed on the surface of ENCC (Breau et al., 2006, 2009). Consistent with this, *in vivo* ablation of β1 integrin leads to a delay in ENCC migration with an altered colonisation of the caecum and hindgut, which ultimately results in colonic aganglionosis (Breau et al., 2006, 2009). These findings suggest that expression of β1 integrin is essential for ENCC to interact with fibronectin and overcome the TNC-mediated migratory inhibition during the invasion of the caecum and proximal hindgut and suggest that alteration of adhesive properties of ENCC might represent *per se* a molecular basis for incomplete colonisation of the gut. In addition to these findings, recent studies have shown how physiological or pathological changes occurring during development in the structure of the intestinal wall might account for defects in colonisation of distal segments of the gut reported in several mutants. In fact, the intestinal mesenchyme undergoes dramatic changes during embryonic development (Young, 2008), such as differentiation into specific cells types (i.e. smooth muscle, mucosal and epithelial cells) and production of specific ECM components. *In vitro* recombination of E11.5 caecum grafts into aganglionic hindgut explants of different developmental ages demonstrated an age-dependent efficiency in colonisation of the host gut, with the oldest (E16.5) explant the least colonised by E11.5 donor ENCC (Hotta et al., 2010). Accordingly, other investigations showed decreased permissiveness to ENCC migration in the hindgut between E13.5 and E14.5, a change possibly connected to an increase in ECM deposition of laminin, a potent cue for differentiation of enteric neurons (Druckenbrod and Epstein, 2009). It has also been suggested that this change in the gut environment, which normally occurs during development, might create a physical obstacle to a delayed wavefront of ENCC, as reported by the same authors for *Ednrb* mutant ENCC (Druckenbrod and Epstein, 2009). On the other hand, studies have shown that ET-3 mutations directly alter the expression of laminin by the gut mesoderm and, along with the cell-autonomous effects of the mutation, lead to terminal aganglionosis (Rothman et al., 1996; Tennyson et al., 1986; Wu et al., 1999).

Transient cell–cell interactions play a key role in migration of NCC. Directional migration of cranial NCC has been shown to rely on contact inhibition of locomotion (CIL), a cellular mechanism for which, within a population of migrating cells, a cell becomes polarised and initiates sustained directional migration away from contact with another NCC (Carmona-Fontaine et al., 2008). In this system, therefore, multiple transient cell interactions are necessary for collective migration of the entire population of cells, whereas loss of contact and cell isolation results in diminished chemotaxis

and directionality (Carmona-Fontaine et al., 2008; Theveneau et al., 2010). In the developing ENS, it is likely that a similar mechanism acts on migrating ENCC. *In vitro* blocking of the cell adhesion molecule L1 expressed on ENCC leads to partial disruption of the chains of cells at the wavefront with increased numbers of isolated cells and consequent incomplete colonisation of the distal gut, thus suggesting that L1 dependent cell–cell contact is important for migration of ENCC (Anderson et al., 2006b). However, *in vivo* deletion of L1 only results in a transient delay of ENCC, which does not lead to aganglionosis (Anderson et al., 2006b), indicating that other cell adhesion molecules might account for cell–cell interactions within chains of migrating ENCC. It has been suggested that addition of polysialic acid (PSA) to the neural cell adhesion molecule 1 (NCAM1) might affect the migration of ENCC into the hindgut in E11.5 gut explants *in vitro*. Expression of PSA-NCAM1 is found in both ENCC and the underlying muscle layer during foetal life, with a consistent increase in maturing neurons and neurites as development proceeds (Fu et al., 2006).

Cell migration requires polarisation and coordinated activation of proteins, which ultimately control cytoskeleton remodelling. There are some indirect data showing that inhibition of polarisation of ENCC leads to delayed colonisation of the hindgut in organotypic cultures of E11.5 guts (Vohra et al., 2007). *In vitro*, retinoic acid has been shown to facilitate ENCC migration by reducing the levels of the phosphatase and tensin homolog PTEN, whose function affects the polarised and coordinated activity of proteins that regulate the actin cytoskeleton (Fu et al., 2010). Candidate molecules suggested to regulate the intracellular actin dynamics in response to chemotactic signals such as GNDF include the Rho GTPases. Early biochemical studies have shown that the phosphorylation of the serine residue at codon 696 (S696) of RET is required for Rac1-GEF activation *in vitro* (Fukuda et al., 2002) and that targeted mutation of S696 leads to abnormal colonisation of the gut by ENCC *in vivo* (Asai et al., 2006). More direct evidence of the involvement of Rho GTPases in ENS development was reported by Stewart et al. (2007), who pharmacologically inhibited the activity of Rac1/Cdc42 and Rho kinases, Rock-I and Rock-II in intact gut explants (Stewart et al., 2007). The authors found that inhibition of both Rac1/Cdc42 and Rho signalling led to retarded migration of ENCC, without affecting cell proliferation and differentiation. In addition, Rac1/Cdc42 also led to reduced protrusions and cell–cell contacts in ENCC at the migratory wavefront. These effects were shown to be possibly RET-dependent, since ENCC in explants of guts from embryos that were heterozygous for RET were more sensitive to the inhibitors (Stewart et al., 2007).

Development of enteric neurons

Neuronal subtype specification

Enteric neurons are born and mature throughout foetal life and in early postnatal stages. As early as E10–E10.5, neuronal progenitors can be identified in the developing gut by expression of pan-neuronal markers, along with maintenance or increase in RET expression (Baetge and Gershon, 1989; Young et al., 1999). Differentiation into distinct neuronal subtypes is an asynchronous and heterogeneous process. Subtype-specific neuronal progenitors exit the cell cycle during defined developmental windows; however, immunoreactivity for the related neurotransmitters or subtype markers might not appear until several days later. Moreover, as in other parts of the nervous system, immature enteric neurons may express combinations of neurotransmitters and neurotransmitter synthetic enzymes that are not observed in mature neurons, while the full spectrum of enteric chemical coding is only developed after birth. For example, transient expression of the catecholamine synthetic enzyme tyrosine hydroxylase (TH) is found in the majority of developing neurons between E10 and E12.5 (Baetge and Gershon, 1989; Young et al., 1999). These cells, which also co-express Mash1 and are still proliferative (Baetge and Gershon, 1989; Blaugrund et al., 1996), have been suggested to be neuronal progenitors of serotonergic neurons (Blaugrund et al., 1996) and NOS (nitrergic) neurons (Young et al., 2002), but this issue still remains controversial. Certainly, transiently expressing TH^+ progenitors do not seem to generate the small population of catecholaminergic neurons present in the adult mouse (Li et al., 2004). The first enteric neurons to express a neurotransmitter synthetic enzyme present in mature enteric neurons are the NOS neurons around E11.5–E12.5 (Baetge and Gershon, 1989; Hao et al., 2010; Young et al., 2002). In addition, calbindin-positive (Hao et al., 2010) and serotonergic (Rothman and Gershon, 1982) neurons are also believed to develop around the same time. Analogously, cholinergic neurons are thought to develop around E10 and E12, when conversion of radioactive choline into acetylcholine (ACh) can be recorded within the ENS (Rothman and Gershon, 1982). However, only at E18.5 have the synthetic enzyme and the vesicular transporter for ACh been detected by immunolabeling (Vannucchi and Faussone-Pellegrini, 1996). Furthermore, VIP and NYP immunoreactivity are first found around E13.5 (Branchek and Gershon, 1989; Rothman et al., 1984), substance P at E14–E14.5 (Rothman et al., 1984), CGRP at E17 (Branchek and Gershon, 1989), while calretinin has been first reported only at P0 (Young et al., 1998b).

Two major signalling pathways have been shown to regulate the relative abundance of neuronal classes within the developing ENS by controlling proliferation of neuronal progenitors: GDNF and BMP. A recent study has shown that temporal control of GDNF expression regulates the proliferation of specific precursors and thus the abundance of specific subclasses of postmitotic enteric neurons (Wang et al., 2010). When GDNF is over-expressed at late stages of development under control of the GFAP promoter (from ~E17 onwards), the number of neurons expressing NADPH-diaphorase, a marker for NOS activity, is increased, whereas neurons expressing ChAT/Substance P remain unaffected. Since NOS progenitors proliferate up to P1, in contrast to choline acetyltransferases (ChAT) progenitors that undergo cell cycle exit by E15, this result suggests a selective mitogenic effect of GDNF based on the stage that enteric neuronal precursors exit the cell cycle (Wang et al., 2010). Partially consistent with these findings, *in vivo* GDNF haploinsufficiency leads to a reduction in the number of myenteric neurons (Gianino et al., 2003), with some studies indicating concomitant reduction in the proportion of NOS and ChAT myenteric neurons in the small intestine (Wang et al., 2010), and others suggesting unaffected proportion of NOS myenteric neurons in the colon (Roberts et al., 2008). Bone morphogenetic proteins (BMPs) play a role in enteric neuronal development by possibly acting on several molecular processes similar to those controlled by GDNF. *In vitro* concentration-dependent activity of BMP-2 and BMP-4 increases neuronal differentiation and affects neuronal survival of purified enteric progenitors (Chalazonitis et al., 2004). *In vivo*, overexpression of the BMP inhibitor noggin under control of the neuron specific enolase (NSE) is associated with a significant increase in the number of enteric neurons in both enteric plexi with different effects on specific neuronal subtypes. Noggin-mediated BMP antagonism leads to an increase in number of neurons derived from precursors that exit the cell cycle early in neurogenesis (serotonin, calretinin and calbindin neurons), whereas those that exit the cell cycle late (CGRP, GABA, TH, DAT neurons) were decreased, and NOS neurons remained unaffected (Chalazonitis et al., 2004, 2008). Thus, BMP signalling might contribute to enteric neuronal phenotypic diversity by promoting cell cycle exit of neuronal precursors at appropriate developmental time points.

Neuronal commitment of ENCC progenitors is a process that is strictly connected to maintenance of their undifferentiated state. Thus, the function of transcription factors such as Sox10, which have been found to inhibit neuronal differentiation, must be down regulated during neuronal specification (Bondurand et al., 2006).

Therefore, it has been proposed that Mash1, a transcription factor that promotes several aspects of neurogenesis in the CNS, suppresses Sox10 expression in some of the enteric progenitors that give rise to neurons (Okamura and Saga, 2008). Another bHLH transcription factor, Hand2, has been found to modulate neuronal development by promoting terminal differentiation of enteric neuronal progenitors (D'Autreaux et al., 2007; Hendershot et al., 2007). In fact, conditional ablation of Hand2 affects neuronal differentiation of ENCC *in vitro* and *in vivo* and in the absence of subtype-specific markers such as NOS and VIP in the gut of embryonic mice *in vivo* (D'Autreaux et al., 2007; Hendershot et al., 2007).

Additionally, some studies have also implicated ET-3 in late events of neuronal differentiation. In fact, in ET3 null mice, an increase in the proportion of NOS^+ myenteric neurons has been reported in the region rostral to the aganglionic colon (Roberts et al., 2008) and in the small intestine (Sandgren et al., 2002), thus suggesting a subtle effect of this mutation on the phenotype of enteric neurons.

Genetic control of neuronal subtype specification is still a key issue in ENS development. To date, few mutations result in loss of specific neuronal subtypes, suggesting that neuronal differentiation might be the result of the combined activity of one or more genetic factors. Mash1 is the only transcription factor that has been unequivocally associated with the development of a defined neuronal population such as the serotonergic subtype. Targeted ablation of *Mash1* leads to lack of neurons in the oesophagus in addition to a failure to develop TH^+ progenitors and 5-HT neurons along the entire gastrointestinal tract (Blaugrund et al., 1996). Similarly, mice lacking the tyrosine kinase receptor C (TkrC) or its ligand neurotrophin-3 (NT-3) show reduction in myenteric neuron number and have a dramatic decrease in CGRP positive submucosal neurons (Chalazonitis et al., 2001).

Morphological development of enteric neurons

Most classes of enteric neurons possess a characteristic morphology with the presence of one or more axons, different shape of dendrites and several degrees of branching, as originally described by Dogiel in the late 1800s. Development of specific neuronal morphologies involves growth of neurites and specification of axons and dendrites. From *in vivo* studies involving the use of the lipophilic dye, DiI, to retrogradely label neuronal projection and cell bodies, Young et al. (2002) found that the majority of neurons present in E11.5–E16.5 mouse gut had one single long process and multiple short neurites, suggesting the establishment of neuronal polarity at early stages of ENS development (Young et al., 2002). Few studies have addressed the molecular mechanisms underlying neuritogenesis and determination of neuronal polarity in the developing ENS. Evidence emerging from the work of Vohra et al. (2007) demonstrates a role for protein kinase C zeta (PKCζ) and glycogen synthase kinase 3β (GSK3β) in axon specification of enteric neurons. In culture, the majority of neurons developing from immunoselected rat ENCC produce a single axon. PKCζ and GSK3β transiently localise at the tip of growing axons and pharmacological inhibition of their activity resulted in an increase in both the number of multi-axonal neurons and the number of neurons without any axons, indicating that PKCζ and GSK3β are able to influence neuronal polarity and are likely to influence the number of axons in enteric neurons. Furthermore, the authors also implicated PKCζ and GSK3β activity in neurite growth, *via* a mechanism that most likely involves recruitment of SMURF1 protein, which, in turn, promotes RhoA degradation (Vohra et al., 2007). Conversely, retinoic acid (RA) reduces neurite length in cultured enteric neurons by decreasing the levels of expression of *smurf1* and, consequently, increasing the abundance of RhoA protein, especially at the tip of neurites (Sato and Heuckeroth, 2008). Nevertheless, there is currently no information on the relevance of genetic programmes and environmental cues on the morphological maturation of enteric neurons and on the expression of intracellular components and regulators of cytoskeleton dynamics during development.

Axon guidance

Mature enteric neurons differ in their axon projection patterns and cell targets. Some classes of neurons project orally, others project anally, circumferentially and locally. Very little is known about the mechanisms controlling directional navigation of enteric axons to their correct targets. DiI tracing of neuronal projections in E11.5–E12.5 mouse gut revealed that many of the first differentiating neurons have a long longitudinal process which projects anally, whereas oral projecting neurons appear to develop only at later stages (Young et al., 2002). By immunofluorescence analysis, it is possible to identify many of the caudally projecting neurons as TH and NOS positive (at E11.5 and E12.5 respectively), with cell bodies located between tens to hundreds of microns behind the wavefront and their processes often in contact with migrating ENCC at the leading edge (Breau et al., 2006; Young et al., 1999, 2002). Furthermore, the longitudinal processes of individual neurons form prominent bundles of fibres shortly after each gut region is colonised by ENCC (Hao and Young, 2009; Young et al., 2002). Thus, there must be some neural guidance cues allowing growing axons to sense the polarity of the gut and also some molecular mechanisms that mediate interaction of axons for the formation of longitudinal fibres. In co-culture experiments in which vagal ENCC were forced to migrate caudo-rostrally, most of the neurons projected rostrally, suggesting that migration and axon guidance are associated at least during early stages of colonisation of the gut (Young et al., 2002). However, the molecular mechanism linking neuronal processes to undifferentiated ENCC is not yet clear; moreover, this hypothesis cannot explain the onset of oral/circumferential projecting neurons or the patterning of projections after ENCC migration is concluded.

To date, there have been no defects in enteric axon targeting described in mice lacking any of the major neural guidance molecules. Nevertheless, it has been reported that mice, in which neurturin (NRTN, one of the members of the GDNF family of ligands) or GFRα2 (NRT high affinity co-receptor) have been genetically ablated, have a drastic reduction of cholinergic fibres projecting to the circular muscle, whereas there are no changes in NOS-containing nerve fibres (Heuckeroth et al., 1999; Rossi et al., 1999). Since density of neurons is unaffected by the mutations (Gianino et al., 2003), it has been suggested that NRTN produced by the circular muscle layer acts as a chemoattractant or a trophic cue for axons of a sub-population of myenteric neurons. Recently, transgenic mice overexpressing GDNF have shown an increase in NADPH-diaphorase fibres surrounding and connecting ganglia, without similar changes in TH or ChAT positive fibres. This accumulation matched the distribution of GFAP-expressing glial cells, thus suggesting that the spatial expression of GDNF can attract specific subsets of neuronal processes (Wang et al., 2010).

Future directions

It is evident from the above review of the literature that a considerable body of evidence exists concerning the mechanisms that regulate the proliferation, survival, migration and differentiation of the NCC-derived precursors that form the ENS. Although numerous questions remain to be addressed, many of the major genes and signalling pathways controlling these processed have been identified in laboratory animals and their involvement in ENS development in humans has been largely confirmed. However, our knowledge of the development of gut motility is more rudimentary (Burns et al., 2009) and our understanding of the aetiopathology of enteric neuropathies is also lacking (possibly with the exception of Hirschsprung's disease). Thus, unravelling the links between ENS development, ENS physiology, and ENS disease is likely to be a priority for the field for the next

decade and should ultimately lead to better treatments for gut motility disorders.

Development of gut motility

In the developing human gut, ENCC colonisation, differentiation of the muscle layers and the development of interstitial cells of Cajal (ICC) occur in a similar sequence to that described in laboratory animals. ENCCs complete their rostro-caudal migration between weeks 4 and 7 of development (Wallace and Burns, 2005), and by week 24 a full complement of enteric neurotransmitters is present (Rauch et al., 2006; Timmermans et al., 1994; Walters et al., 1993). However, the development of gut motility does not occur until late gestation and after birth (Berseth, 1996; Burns et al., 2009) indicating that there is a significant time lag between the appearance of neurons within the gut, and the establishment of neural control of gut motility. Since little is known about the development of functional neuronal subtypes, and even less about how circuits underlying motility reflexes are formed during this time window, the relationship between ENS development and the neurally-mediated control of gut motility ("ENS developmental physiology") will be a key area of research in the coming years.

Better understanding of congenital diseases affecting the ENS

Current evidence indicates that enteric neuropathies arise from loss, degeneration, and/or functional impairment of enteric neurons resulting from congenital developmental defects of the ENS, the action of known agents (i.e. toxic, infectious), or secondary to pathological conditions such as chronic inflammation, diabetic neuropathy, and Parkinson's disease. Nevertheless, for many of the primary enteric neuropathies, the aetiology is still unclear and may involve multiple causes (Di Nardo et al., 2008). The best known enteric neuropathy is Hirschsprung's disease (HSCR), a congenital condition associated with a failure of ENCC to colonise the entire length of the gut resulting in the absence of myenteric and submucosal neurons in the variable lengths of the colon and rectum. Although the genetics and aetiology of HSCR are now relatively well understood and are reviewed extensively elsewhere (Amiel et al., 2008; Kenny et al., 2010), other common intestinal disorders have also shown some genetic inheritance, but the lack of defined neuropathological features and few animal models in which specific motility disturbances can be investigated (Margolis et al., 2011), have hindered research of their aetiology. Current and future work, in which patients with specific motility defects are grouped together and genotype/phenotype can be established, should help to identify the mechanisms underlying such enteric neuropathies.

Cell therapies for the treatment of motility disorders

Although gut motility disorders represent a significant challenge in clinical management with little in the way of definitive cures, a number of recent studies (Lindley et al., 2008; Metzger et al., 2009; Tsai et al., 2010) have suggested that stem cells (mainly sourced from the gut) have the potential to replace/restore missing or defective ENS cells in aganglionic gut, thus offering hope for the development of novel therapies for neuropathies such as HSCR. Although the work in animal models has been exciting and encouraging, the next challenges will be to isolate and characterise ENS stem cells from the human gut, demonstrate that transplanted ENS stem cells have the capacity to influence/restore gut motility, and progress to "first in man" studies whereby cell delivery methods, safety and efficacy can be assessed.

References

Amiel, J., Sproat-Emison, E., Garcia-Barcelo, M., Lantieri, F., Burzynski, G., Borrego, S., Pelet, A., Arnold, S., Miao, X., Griseri, P., Brooks, A.S., Antinolo, G., de Pontual, L., Clement-Ziza, M., Munnich, A., Kashuk, C., West, K., Wong, K.K., Lyonnet, S., Chakravarti, A., Tam, P.K., Ceccherini, I., Hofstra, R.M., Fernandez, R., 2008. Hirschsprung disease, associated syndromes and genetics: a review. J. Med. Genet. 45, 1–14.

Anderson, R.B., Stewart, A.L., Young, H.M., 2006a. Phenotypes of neural-crest-derived cells in vagal and sacral pathways. Cell Tissue Res. 323, 11–25.

Anderson, R.B., Turner, K.N., Nikonenko, A.G., Hemperly, J., Schachner, M., Young, H.M., 2006b. The cell adhesion molecule l1 is required for chain migration of neural crest cells in the developing mouse gut. Gastroenterology 130, 1221–1232.

Anderson, R.B., Bergner, A.J., Taniguchi, M., Fujisawa, H., Forrai, A., Robb, L., Young, H.M., 2007. Effects of different regions of the developing gut on the migration of enteric neural crest-derived cells: a role for Sema3A, but not Sema3F. Dev. Biol. 305, 287–299.

Asai, N., Fukuda, T., Wu, Z., Enomoto, A., Pachnis, V., Takahashi, M., Costantini, F., 2006. Targeted mutation of serine 697 in the Ret tyrosine kinase causes migration defect of enteric neural crest cells. Development 133, 4507–4516.

Baetge, G., Gershon, M.D., 1989. Transient catecholaminergic (TC) cells in the vagus nerves and bowel of fetal mice: relationship to the development of enteric neurons. Dev. Biol. 132, 189–211.

Baloh, R.H., Enomoto, H., Johnson Jr., E.M., Milbrandt, J., 2000. The GDNF family ligands and receptors — implications for neural development. Curr. Opin. Neurobiol. 10, 103–110.

Barlow, A., de Graaff, E., Pachnis, V., 2003. Enteric nervous system progenitors are coordinately controlled by the G protein-coupled receptor EDNRB and the receptor tyrosine kinase RET. Neuron 40, 905–916.

Barlow, A.J., Wallace, A.S., Thapar, N., Burns, A.J., 2008. Critical numbers of neural crest cells are required in the pathways from the neural tube to the foregut to ensure complete enteric nervous system formation. Development 135, 1681–1691.

Baynash, A.G., Hosoda, K., Giaid, A., Richardson, J.A., Emoto, N., Hammer, R.E., Yanagisawa, M., 1994. Interaction of endothelin-3 with endothelin-B receptor is essential for development of epidermal melanocytes and enteric neurons. Cell 79, 1277–1285.

Berseth, C.L., 1996. Gastrointestinal motility in the neonate. Clin. Perinatol. 23, 179–190.

Blaugrund, E., Pham, T.D., Tennyson, V.M., Lo, L., Sommer, L., Anderson, D.J., Gershon, M.D., 1996. Distinct subpopulations of enteric neuronal progenitors defined by time of development, sympathoadrenal lineage markers and Mash-1-dependence. Development 122, 309–320.

Bondurand, N., Natarajan, D., Thapar, N., Atkins, C., Pachnis, V., 2003. Neuron and glia generating progenitors of the mammalian enteric nervous system isolated from foetal and postnatal gut cultures. Development 130, 6387–6400.

Bondurand, N., Natarajan, D., Barlow, A., Thapar, N., Pachnis, V., 2006. Maintenance of mammalian enteric nervous system progenitors by SOX10 and endothelin 3 signalling. Development 133, 2075–2086.

Bowles, J., Schepers, G., Koopman, P., 2000. Phylogeny of the SOX family of developmental transcription factors based on sequence and structural indicators. Dev. Biol. 227, 239–255.

Branchek, T.A., Gershon, M.D., 1989. Time course of expression of neuropeptide Y, calcitonin gene-related peptide, and NADPH diaphorase activity in neurons of the developing murine bowel and the appearance of 5-hydroxytryptamine in mucosal enterochromaffin cells. J. Comp. Neurol. 285, 262–273.

Breau, M.A., Pietri, T., Eder, O., Blanche, M., Brakebusch, C., Fassler, R., Thiery, J.P., Dufour, S., 2006. Lack of beta1 integrins in enteric neural crest cells leads to a Hirschsprung-like phenotype. Development 133, 1725–1734.

Breau, M.A., Dahmani, A., Broders-Bondon, F., Thiery, J.P., Dufour, S., 2009. Beta1 integrins are required for the invasion of the caecum and proximal hindgut by enteric neural crest cells. Development 136, 2791–2801.

Britsch, S., Goerich, D.E., Riethmacher, D., Peirano, R.I., Rossner, M., Nave, K.A., Birchmeier, C., Wegner, M., 2001. The transcription factor Sox10 is a key regulator of peripheral glial development. Genes Dev. 15, 66–78.

Burns, A.J., 2005. Migration of neural crest-derived enteric nervous system precursor cells to and within the gastrointestinal tract. Int. J. Dev. Biol. 49, 143–150.

Burns, A.J., Douarin, N.M., 1998. The sacral neural crest contributes neurons and glia to the post-umbilical gut: spatiotemporal analysis of the development of the enteric nervous system. Development 125, 4335–4347.

Burns, A.J., Champeval, D., Le Douarin, N.M., 2000. Sacral neural crest cells colonise aganglionic hindgut *in vivo* but fail to compensate for lack of enteric ganglia. Dev. Biol. 219, 30–43.

Burns, A.J., Delalande, J.M., Le Douarin, N.M., 2002. In ovo transplantation of enteric nervous system precursors from vagal to sacral neural crest results in extensive hindgut colonisation. Development 129, 2785–2796.

Burns, A.J., Roberts, R.R., Bornstein, J.C., Young, H.M., 2009. Development of the enteric nervous system and its role in intestinal motility during fetal and early postnatal stages. Semin. Pediatr. Surg. 18, 196–205.

Cacalano, G., Farinas, I., Wang, L.C., Hagler, K., Forgie, A., Moore, M., Armanini, M., Phillips, H., Ryan, A.M., Reichardt, L.F., 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.

Cantrell, V.A., Owens, S.E., Chandler, R.L., Airey, D.C., Bradley, K.M., Smith, J.R., Southard-Smith, E.M., 2004. Interactions between Sox10 and EdnrB modulate penetrance and severity of aganglionosis in the Sox10Dom mouse model of Hirschsprung disease. Hum. Mol. Genet. 13, 2289–2301.

Carmona-Fontaine, C., Matthews, H.K., Kuriyama, S., Moreno, M., Dunn, G.A., Parsons, M., Stern, C.D., Mayor, R., 2008. Contact inhibition of locomotion *in vivo* controls neural crest directional migration. Nature 456, 957–961.

Chalazonitis, A., Rothman, T.P., Chen, J., Gershon, M.D., 1998. Age-dependent differences in the effects of GDNF and NT-3 on the development of neurons and glia from neural crest-derived precursors immunoselected from the fetal rat gut: expression of GFRalpha-1 *in vitro* and *in vivo*. Dev. Biol. 204, 385–406.

Chalazonitis, A., Pham, T.D., Rothman, T.P., DiStefano, P.S., Bothwell, M., Blair-Flynn, J., Tessarollo, L., Gershon, M.D., 2001. Neurotrophin-3 is required for the survival-differentiation of subsets of developing enteric neurons. J. Neurosci. 21, 5620–5636.

Chalazonitis, A., D'Autreaux, F., Guha, U., Pham, T.D., Faure, C., Chen, J.J., Roman, D., Kan, L., Rothman, T.P., Kessler, J.A., Gershon, M.D., 2004. Bone morphogenetic protein-2 and −4 limit the number of enteric neurons but promote development of a TrkC-expressing neurotrophin-3-dependent subset. J. Neurosci. 24, 4266–4282.

Chalazonitis, A., Pham, T.D., Li, Z., Roman, D., Guha, U., Gomes, W., Kan, L., Kessler, J.A., Gershon, M.D., 2008. Bone morphogenetic protein regulation of enteric neuronal phenotypic diversity: relationship to timing of cell cycle exit. J. Comp. Neurol. 509, 474–492.

Corpening, J.C., Cantrell, V.A., Deal, K.K., Southard-Smith, E.M., 2008. A Histone2BCerulean BAC transgene identifies differential expression of Phox2b in migrating enteric neural crest derivatives and enteric glia. Dev. Dyn. 237, 1119–1132.

D'Autreaux, F., Morikawa, Y., Cserjesi, P., Gershon, M.D., 2007. Hand2 is necessary for terminal differentiation of enteric neurons from crest-derived precursors but not for their migration into the gut or for formation of glia. Development 134, 2237–2249.

Delalande, J.M., Barlow, A.J., Thomas, A.J., Wallace, A.S., Thapar, N., Erickson, C.A., Burns, A.J., 2008. The receptor tyrosine kinase RET regulates hindgut colonization by sacral neural crest cells. Dev. Biol. 313, 279–292.

Di Nardo, G., Blandizzi, C., Volta, U., Colucci, R., Stanghellini, V., Barbara, G., Del Tacca, M., Tonini, M., Corinaldesi, R., De Giorgio, R., 2008. Review article: molecular, pathological and therapeutic features of human enteric neuropathies. Aliment. Pharmacol. Ther. 28, 25–42.

Druckenbrod, N.R., Epstein, M.L., 2005. The pattern of neural crest advance in the cecum and colon. Dev. Biol. 287, 125–133.

Druckenbrod, N.R., Epstein, M.L., 2007. Behavior of enteric neural crest-derived cells varies with respect to the migratory wavefront. Dev. Dyn. 236, 84–92.

Druckenbrod, N.R., Epstein, M.L., 2009. Age-dependent changes in the gut environment restrict the invasion of the hindgut by enteric neural progenitors. Development 136, 3195–3203.

Durbec, P.L., Larsson-Blomberg, L.B., Schuchardt, A., Costantini, F., Pachnis, V., 1996. Common origin and developmental dependence on c-ret of subsets of enteric and sympathetic neuroblasts. Development 122, 349–358.

Elworthy, S., Pinto, J.P., Pettifer, A., Cancela, M.L., Kelsh, R.N., 2005. Phox2b function in the enteric nervous system is conserved in zebrafish and is sox10-dependent. Mech. Dev. 122, 659–669.

Enomoto, H., Araki, T., Jackman, A., Heuckeroth, R.O., Snider, W.D., Johnson Jr., E.M., Milbrandt, J., 1998. GFR alpha1-deficient mice have deficits in the enteric nervous system and kidneys. Neuron 21, 317–324.

Erickson, C.A., Goins, T.L., 2000. Sacral neural crest cell migration to the gut is dependent upon the migratory environment and not cell-autonomous migratory properties. Dev. Biol. 219, 79–97.

Fu, M., Vohra, B.P., Wind, D., Heuckeroth, R.O., 2006. BMP signaling regulates murine enteric nervous system precursor migration, neurite fasciculation, and patterning *via* altered Ncam1 polysialic acid addition. Dev. Biol. 299, 137–150.

Fu, M., Sato, Y., Lyons-Warren, A., Zhang, B., Kane, M.A., Napoli, J.L., Heuckeroth, R.O., 2010. Vitamin A facilitates enteric nervous system precursor migration by reducing Pten accumulation. Development 137, 631–640.

Fukuda, T., Kiuchi, K., Takahashi, M., 2002. Novel mechanism of regulation of Rac activity and lamellipodia formation by RET tyrosine kinase. J. Biol. Chem. 277, 19114–19121.

Furness, J.B., 2006. The Enteric Nervous System. Blackwell Publishing, Oxford.

Gariepy, C.E., Williams, S.C., Richardson, J.A., Hammer, R.E., Yanagisawa, M., 1998. Transgenic expression of the endothelin-B receptor prevents congenital intestinal aganglionosis in a rat model of Hirschsprung disease. J. Clin. Invest. 102, 1092–1101.

Gianino, S., Grider, J.R., Cresswell, J., Enomoto, H., Heuckeroth, R.O., 2003. GDNF availability determines enteric neuron number by controlling precursor proliferation. Development 130, 2187–2198.

Hao, M.M., Young, H.M., 2009. Development of enteric neuron diversity. J. Cell Mol. Med. 13, 1193–1210.

Hao, M.M., Anderson, R.B., Kobayashi, K., Whitington, P.M., Young, H.M., 2009. The migratory behavior of immature enteric neurons. Dev. Neurobiol. 69, 22–35.

Hao, M.M., Moore, R.E., Roberts, R.R., Nguyen, T., Furness, J.B., Anderson, R.B., Young, H.M., 2010. The role of neural activity in the migration and differentiation of enteric neuron precursors. Neurogastroenterol. Motil. 22, e127–e137.

Hearn, C.J., Murphy, M., Newgreen, D., 1998. GDNF and ET-3 differentially modulate the numbers of avian enteric neural crest cells and enteric neurons *in vitro*. Dev. Biol. 197, 93–105.

Hendershot, T.J., Liu, H., Sarkar, A.A., Giovannucci, D.R., Clouthier, D.E., Abe, M., Howard, M.J., 2007. Expression of Hand2 is sufficient for neurogenesis and cell type-specific gene expression in the enteric nervous system. Dev. Dyn. 236, 93–105.

Herbarth, B., Pingault, V., Bondurand, N., Kuhlbrodt, K., Hermans-Borgmeyer, I., Puliti, A., Lemort, N., Goossens, M., Wegner, M., 1998. Mutation of the Sry-related Sox10 gene in Dominant megacolon, a mouse model for human Hirschsprung disease. Proc. Natl. Acad. Sci. U. S. A. 95, 5161–5165.

Heuckeroth, R.O., Lampe, P.A., Johnson, E.M., Milbrandt, J., 1998. Neurturin and GDNF promote proliferation and survival of enteric neuron and glial progenitors *in vitro*. Dev. Biol. 200, 116–129.

Heuckeroth, R.O., Enomoto, H., Grider, J.R., Golden, J.P., Hanke, J.A., Jackman, A., Molliver, D.C., Bardgett, M.E., Snider, W.D., Johnson Jr., E.M., Milbrandt, J., 1999. Gene targeting reveals a critical role for neurturin in the development and maintenance of enteric, sensory, and parasympathetic neurons. Neuron 22, 253–263.

Hosoda, K., Hammer, R.E., Richardson, J.A., Baynash, A.G., Cheung, J.C., Giaid, A., Yanagisawa, M., 1994. Targeted and natural (piebald-lethal) mutations of endothelin-B receptor gene produce megacolon associated with spotted coat color in mice. Cell 79, 1267–1276.

Hotta, R., Anderson, R.B., Kobayashi, K., Newgreen, D.F., Young, H.M., 2010. Effects of tissue age, presence of neurones and endothelin-3 on the ability of enteric neurone precursors to colonize recipient gut: implications for cell-based therapies. Neurogastroenterol. Motil. 22, 331–e386.

Inoue, A., Yanagisawa, M., Kimura, S., Kasuya, Y., Miyauchi, T., Goto, K., Masaki, T., 1989. The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. Proc. Natl. Acad. Sci. U. S. A. 86, 2863–2867.

Jiang, Y., Liu, M.T., Gershon, M.D., 2003. Netrins and DCC in the guidance of migrating neural crest-derived cells in the developing bowel and pancreas. Dev. Biol. 258, 364–384.

Jing, S., Wen, D., Yu, Y., Holst, P.L., Luo, Y., Fang, M., Tamir, R., Antonio, L., Hu, Z., Cupples, R., Louis, J.C., Hu, S., Altrock, B.W., Fox, G.M., 1996. GDNF-induced activation of the ret protein tyrosine kinase is mediated by GDNFR-alpha, a novel receptor for GDNF. Cell 85, 1113–1124.

Kapur, R.P., 1999. Early death of neural crest cells is responsible for total enteric aganglionosis in Sox10(Dom)/Sox10(Dom) mouse embryos. Pediatr. Dev. Pathol. 2, 559–569.

Kapur, R.P., 2000. Colonization of the murine hindgut by sacral crest-derived neural precursors: experimental support for an evolutionarily conserved model. Dev. Biol. 227, 146–155.

Kapur, R.P., Yost, C., Palmiter, R.D., 1992. A transgenic model for studying development of the enteric nervous system in normal and aganglionic mice. Development 116, 167–175.

Kapur, R.P., Sweetser, D.A., Doggett, B., Siebert, J.R., Palmiter, R.D., 1995. Intercellular signals downstream of endothelin receptor-B mediate colonization of the large intestine by enteric neuroblasts. Development 121, 3787–3795.

Kenny, S.E., Tam, P.K., Garcia-Barcelo, M., 2010. Hirschsprung's disease. Semin. Pediatr. Surg. 19, 194–200.

Kim, J., Lo, L., Dormand, E., Anderson, D.J., 2003. SOX10 maintains multipotency and inhibits neuronal differentiation of neural crest stem cells. Neuron 38, 17–31.

Kruger, G.M., Mosher, J.T., Tsai, Y.H., Yeager, K.J., Iwashita, T., Gariepy, C.E., Morrison, S.J., 2003. Temporally distinct requirements for endothelin receptor B in the generation and migration of gut neural crest stem cells. Neuron 40, 917–929.

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.

Lane, P.W., Liu, H.M., 1984. Association of megacolon with a new dominant spotting gene (Dom) in the mouse. J. Hered. 75, 435–439.

Lang, D., Epstein, J.A., 2003. Sox10 and Pax3 physically interact to mediate activation of a conserved c-RET enhancer. Hum. Mol. Genet. 12, 937–945.

Lang, D., Chen, F., Milewski, R., Li, J., Lu, M.M., Epstein, J.A., 2000. Pax3 is required for enteric ganglia formation and functions with Sox10 to modulate expression of c-ret. J. Clin. Invest. 106, 963–971.

Le Douarin, N., 1973. A biological cell labeling technique and its use in experimental embryology. Dev. Biol. 30, 217–222.

Le Douarin, N.M., Teillet, M.A., 1973. The migration of neural crest cells to the wall of the digestive tract in avian embryo. J. Embryol. Exp. Morphol. 30, 31–48.

Le Douarin, N.M., Teillet, M.A., 1974. Experimental analysis of the migration and differentiation of neuroblasts of the autonomic nervous system and of neurectodermal mesenchymal derivatives, using a biological cell marking technique. Dev. Biol. 41, 162–184.

Le Douarin, N.M., Renaud, D., Teillet, M.A., Le Douarin, G.H., 1975. Cholinergic differentiation of presumptive adrenergic neuroblasts in interspecific chimeras after heterotopic transplantations. Proc. Natl. Acad. Sci. U. S. A. 72, 728–732.

Leibl, M.A., Ota, T., Woodward, M.N., Kenny, S.E., Lloyd, D.A., Vaillant, C.R., Edgar, D.H., 1999. Expression of endothelin 3 by mesenchymal cells of embryonic mouse caecum. Gut 44, 246–252.

Li, Z.S., Pham, T.D., Tamir, H., Chen, J.J., Gershon, M.D., 2004. Enteric dopaminergic neurons: definition, developmental lineage, and effects of extrinsic denervation. J. Neurosci. 24, 1330–1339.

Lindley, R.M., Hawcutt, D.B., Connell, M.G., Almond, S.L., Vannucchi, M.G., Faussone-Pellegrini, M.S., Edgar, D.H., Kenny, S.E., 2008. Human and mouse enteric nervous system neurosphere transplants regulate the function of aganglionic embryonic distal colon. Gastroenterology 135 (205–216), e206.

Lo, L.C., Johnson, J.E., Wuenschell, C.W., Saito, T., Anderson, D.J., 1991. Mammalian achaete-scute homolog 1 is transiently expressed by spatially restricted subsets of early neuroepithelial and neural crest cells. Genes Dev. 5, 1524–1537.

Manié, S., Santoro, M., Fusco, A., Billaud, M., 2001. The RET receptor: function in development and dysfunction in congenital malformation. Trends Genet. 17, 580–589.

Margolis, K.G., Stevanovic, K., Karamooz, N., Li, Z.S., Ahuja, A., D'Autréaux, F., Saurman, V., Chalazonitis, A., Gershon, M.D., 2011. Enteric neuronal density contributes to the severity of intestinal inflammation. Gastroenterology 141 (588–598), 598.e1–598.e2.

Metzger, M., Caldwell, C., Barlow, A.J., Burns, A.J., Thapar, N., 2009. Enteric nervous system stem cells derived from human gut mucosa for the treatment of aganglionic gut disorders. Gastroenterology 136 (2214–2225), e2211–e2213.

Moore, M.W., Klein, R.D., Farinas, I., Sauer, H., Armanini, M., Phillips, H., Reichardt, L.F., Ryan, A.M., Carver-Moore, K., Rosenthal, A., 1996. Renal and neuronal abnormalities in mice lacking GDNF. Nature 382, 76–79.

Nataf, V., Lecoin, L., Eichmann, A., Le Douarin, N.M., 1996. Endothelin-B receptor is expressed by neural crest cells in the avian embryo. Proc. Natl. Acad. Sci. U. S. A. 93, 9645–9650.

Natarajan, D., Marcos-Gutierrez, C., Pachnis, V., de Graaff, E., 2002. Requirement of signalling by receptor tyrosine kinase RET for the directed migration of enteric nervous system progenitor cells during mammalian embryogenesis. Development 129, 5151–5160.

Okamura, Y., Saga, Y., 2008. Notch signaling is required for the maintenance of enteric neural crest progenitors. Development 135, 3555–3565.

Pachnis, V., Mankoo, B., Costantini, F., 1993. Expression of the c-ret proto-oncogene during mouse embryogenesis. Development 119, 1005–1017.

Paratore, C., Goerich, D.E., 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.

Paratore, C., Eichenberger, C., Suter, U., Sommer, L., 2002. Sox10 haploinsufficiency affects maintenance of progenitor cells in a mouse model of Hirschsprung disease. Hum. Mol. Genet. 11, 3075–3085.

Pattyn, A., Morin, X., Cremer, H., Goridis, C., Brunet, J.F., 1997. Expression and interactions of the two closely related homeobox genes Phox2a and Phox2b during neurogenesis. Development 124, 4065–4075.

Pattyn, A., Morin, X., Cremer, H., Goridis, C., Brunet, J.F., 1999. The homeobox gene Phox2b is essential for the development of autonomic neural crest derivatives. Nature 399, 366–370.

Peters-van der Sanden, M.J., Kirby, M.L., Gittenberger-de Groot, A., Tibboel, D., Mulder, M.P., Meijers, C., 1993. Ablation of various regions within the avian vagal neural crest has differential effects on ganglion formation in the fore-, mid- and hindgut. Dev. Dyn. 196, 183–194.

Pichel, J.G., Shen, L., Sheng, H.Z., Granholm, A.C., Drago, J., Grinberg, A., Lee, E.J., Huang, S.P., Saarma, M., Hoffer, B.J., Sariola, H., Westphal, H., 1996. Defects in enteric innervation and kidney development in mice lacking GDNF. Nature 382, 73–76.

Rauch, U., Klotz, M., Maas-Omlor, S., Wink, E., Hansgen, A., Hagl, C., Holland-Cunz, S., Schafer, K.H., 2006. Expression of intermediate filament proteins and neuronal markers in the human fetal gut. J. Histochem. Cytochem. 54, 39–46.

Roberts, R.R., Bornstein, J.C., Bergner, A.J., Young, H.M., 2008. Disturbances of colonic motility in mouse models of Hirschsprung's disease. Am. J. Physiol. Gastrointest. Liver Physiol. 294, G996–G1008.

Rossi, J., Luukko, K., Poteryaev, D., Laurikainen, A., Sun, Y.F., Laakso, T., Eerikainen, S., Tuominen, R., Lakso, M., Rauvala, H., Arumae, U., Pasternack, M., Saarma, M., Airaksinen, M.S., 1999. Retarded growth and deficits in the enteric and parasympathetic nervous system in mice lacking GFR alpha2, a functional neurturin receptor. Neuron 22, 243–252.

Rothman, T.P., Gershon, M.D., 1982. Phenotypic expression in the developing murine enteric nervous system. J. Neurosci. 2, 381–393.

Rothman, T.P., Nilaver, G., Gershon, M.D., 1984. Colonization of the developing murine enteric nervous system and subsequent phenotypic expression by the precursors of peptidergic neurons. J. Comp. Neurol. 225, 13–23.

Rothman, T.P., Tennyson, V.M., Gershon, M.D., 1986. Colonization of the bowel by the precursors of enteric glia: studies of normal and congenitally aganglionic mutant mice. J. Comp. Neurol. 252, 493–506.

Rothman, T.P., Chen, J., Howard, M.J., Costantini, F., Schuchardt, A., Pachnis, V., Gershon, M.D., 1996. Increased expression of laminin-1 and collagen (IV) subunits in the aganglionic bowel of ls/ls, but not c-ret −/− mice. Dev. Biol. 178, 498–513.

Sanchez, M.P., Silos-Santiago, I., Frisen, J., He, B., Lira, S.A., Barbacid, M., 1996. Renal agenesis and the absence of enteric neurons in mice lacking GDNF. Nature 382, 70–73.

Sandgren, K., Larsson, L.T., Ekblad, E., 2002. Widespread changes in neurotransmitter expression and number of enteric neurons and interstitial cells of Cajal in lethal spotted mice: an explanation for persisting dysmotility after operation for Hirschsprung's disease? Dig. Dis. Sci. 47, 1049–1064.

Sato, Y., Heuckeroth, R.O., 2008. Retinoic acid regulates murine enteric nervous system precursor proliferation, enhances neuronal precursor differentiation, and reduces neurite growth *in vitro*. Dev. Biol. 320, 185–198.

Schiltz, C.A., Benjamin, J., Epstein, M.L., 1999. Expression of the GDNF receptors ret and GFRalpha1 in the developing avian enteric nervous system. J. Comp. Neurol. 414, 193–211.

Schuchardt, A., D'Agati, V., Larsson-Blomberg, L., Costantini, F., Pachnis, V., 1994. Defects in the kidney and enteric nervous system of mice lacking the tyrosine kinase receptor Ret. Nature 367, 380–383.

Serbedzija, G.N., Burgan, S., Fraser, S.E., Bronner-Fraser, M., 1991. Vital dye labelling demonstrates a sacral neural crest contribution to the enteric nervous system of chick and mouse embryos. Development 111, 857–866.

Shepherd, I.T., Raper, J.A., 1999. Collapsin-1/semaphorin D is a repellent for chick ganglion of Remak axons. Dev. Biol. 212, 42–53.

Simpson, M.J., Zhang, D.C., Mariani, M., Landman, K.A., Newgreen, D.F., 2007. Cell proliferation drives neural crest cell invasion of the intestine. Dev. Biol. 302, 553–568.

Smith, J., Cochard, P., Le Douarin, N.M., 1977. Development of choline acetyltransferase and cholinesterase activities in enteric ganglia derives from presumptive adrenergic and cholinergic levels of the neural crest. Cell Differ. 6, 199–216.

Southard-Smith, E.M., Kos, L., Pavan, W.J., 1998. Sox10 mutation disrupts neural crest development in Dom Hirschsprung mouse model. Nat. Genet. 18, 60–64.

Stanchina, L., Baral, V., Robert, F., Pingault, V., Lemort, N., Pachnis, V., Goossens, M., Bondurand, N., 2006. Interactions between Sox10, Edn3 and Ednrb during enteric nervous system and melanocyte development. Dev. Biol. 295, 232–249.

Stewart, A.L., Young, H.M., Popoff, M., Anderson, R.B., 2007. Effects of pharmacological inhibition of small GTPases on axon extension and migration of enteric neural crest-derived cells. Dev. Biol. 307, 92–104.

Taraviras, S., Marcos-Gutierrez, C.V., Durbec, P., Jani, H., Grigoriou, M., Sukumaran, M., Wang, L.C., Hynes, M., Raisman, G., Pachnis, V., 1999. Signalling by the RET receptor tyrosine kinase and its role in the development of the mammalian enteric nervous system. Development 126, 2785–2797.

Tennyson, V.M., Pham, T.D., Rothman, T.P., Gershon, M.D., 1986. Abnormalities of smooth muscle, basal laminae, and nerves in the aganglionic segments of the bowel of lethal spotted mutant mice. Anat. Rec. 215, 267–281.

Theveneau, E., Marchant, L., Kuriyama, S., Gull, M., Moepps, B., Parsons, M., Mayor, R., 2010. Collective chemotaxis requires contact-dependent cell polarity. Dev. Cell 19, 39–53.

Timmermans, J.P., Barbiers, M., Scheuermann, D.W., Bogers, J.J., Adriaensen, D., Fekete, E., Mayer, B., Van Marck, E.A., De Groodt-Lasseel, M.H., 1994. Nitric oxide synthase immunoreactivity in the enteric nervous system of the developing human digestive tract. Cell Tissue Res. 275, 235–245.

Tsai, Y.H., Murakami, N., Gariepy, C.E., 2010. Postnatal intestinal engraftment of prospectively selected enteric neural crest stem cells in a rat model of Hirschsprung disease. Neurogastroenterol. Motil. 23, 362–369.

Uesaka, T., Jain, S., Yonemura, S., Uchiyama, Y., Milbrandt, J., Enomoto, H., 2007. Conditional ablation of GFRalpha1 in postmigratory enteric neurons triggers unconventional neuronal death in the colon and causes a Hirschsprung's disease phenotype. Development 134, 2171–2181.

Vannucchi, M.G., Faussone-Pellegrini, M.S., 1996. Differentiation of cholinergic cells in the rat gut during pre- and postnatal life. Neurosci. Lett. 206, 105–108.

Vohra, B.P., Fu, M., Heuckeroth, R.O., 2007. Protein kinase Czeta and glycogen synthase kinase-3beta control neuronal polarity in developing rodent enteric neurons, whereas SMAD specific E3 ubiquitin protein ligase 1 promotes neurite growth but does not influence polarity. J. Neurosci. 27, 9458–9468.

Wallace, A.S., Burns, A.J., 2005. Development of the enteric nervous system, smooth muscle and interstitial cells of Cajal in the human gastrointestinal tract. Cell Tissue Res. 319, 367–382.

Walters, J.R., Bishop, A.E., Facer, P., Lawson, E.M., Rogers, J.H., Polak, J.M., 1993. Calretinin and calbindin-D28k immunoreactivity in the human gastrointestinal tract. Gastroenterology 104, 1381–1389.

Wang, H., Hughes, I., Planer, W., Parsadanian, A., Grider, J.R., Vohra, B.P., Keller-Peck, C., Heuckeroth, R.O., 2010. The timing and location of glial cell line-derived neurotrophic factor expression determine enteric nervous system structure and function. J. Neurosci. 30, 1523–1538.

Wang, X., Chan, A.K.K., Sham, M.-H., Burns, A.J., Chan, W.Y., 2011. Analysis of the sacral neural crest cell contribution to the hindgut enteric nervous system in the mouse embryo. Gastroenterology 141, 992–1002.

Wilson, Y.M., Richards, K.L., Ford-Perriss, M.L., Panthier, J.J., Murphy, M., 2004. Neural crest cell lineage segregation in the mouse neural tube. Development 131, 6153–6162.

Woodward, M.N., Kenny, S.E., Vaillant, C., Lloyd, D.A., Edgar, D.H., 2000. Time-dependent effects of endothelin-3 on enteric nervous system development in an organ culture model of Hirschsprung's disease. J. Pediatr. Surg. 35, 25–29.

Wu, J.J., Chen, J.X., Rothman, T.P., Gershon, M.D., 1999. Inhibition of *in vitro* enteric neuronal development by endothelin-3: mediation by endothelin B receptors. Development 126, 1161–1173.

Yntema, C.L., Hammond, W.S., 1954. The origin of intrinsic ganglia of trunk viscera from vagal neural crest in the chick embryo. J. Comp. Neurol. 101, 515–541.

Young, H.M., 2008. On the outside looking in: longitudinal muscle development in the gut. Neurogastroenterol. Motil. 20, 431–433.

Young, H.M., Hearn, C.J., Ciampoli, D., Southwell, B.R., Brunet, J.F., Newgreen, D.F., 1998a. A single rostrocaudal colonization of the rodent intestine by enteric neuron precursors is revealed by the expression of Phox2b, Ret, and p75 and by explants grown under the kidney capsule or in organ culture. Dev. Biol. 202, 67–84.

Young, H.M., Torihashi, S., Ciampoli, D., Sanders, K.M., 1998b. Identification of neurons that express stem cell factor in the mouse small intestine. Gastroenterology 115, 898–908.

Young, H.M., Ciampoli, D., Hsuan, J., Canty, A.J., 1999. Expression of Ret-, p75(NTR)-, Phox2a-, Phox2b-, and tyrosine hydroxylase-immunoreactivity by undifferentiated neural crest-derived cells and different classes of enteric neurons in the embryonic mouse gut. Dev. Dyn. 216, 137–152.

Young, H.M., Hearn, C.J., Farlie, P.G., Canty, A.J., Thomas, P.Q., Newgreen, D.F., 2001. GDNF is a chemoattractant for enteric neural cells. Dev. Biol. 229, 503–516.

Young, H.M., Jones, B.R., McKeown, S.J., 2002. The projections of early enteric neurons are influenced by the direction of neural crest cell migration. J. Neurosci. 22, 6005–6018.

Young, H.M., Bergner, A.J., 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, H.M., Bergner, A.J., Anderson, R.B., Enomoto, H., Milbrandt, J., Newgreen, D.F., Whitington, P.M., 2004. Dynamics of neural crest-derived cell migration in the embryonic mouse gut. Dev. Biol. 270, 455–473.

Young, H.M., Turner, K.N., Bergner, A.J., 2005. The location and phenotype of proliferating neural-crest-derived cells in the developing mouse gut. Cell Tissue Res. 320, 1–9.

Zhu, L., Lee, H.O., Jordan, C.S., Cantrell, V.A., Southard-Smith, E.M., Shin, M.K., 2004. Spatiotemporal regulation of endothelin receptor-B by SOX10 in neural crest-derived enteric neuron precursors. Nat. Genet. 36, 732–737.