PHILOSOPHICAL
TRANSACTIONS
OF
THE ROYAL
SOCIETY B

Phil. Trans. R. Soc. B (2009) **364**, 2477–2483
doi:10.1098/rstb.2009.0085

Review

Breathing with *Phox2b*

Véronique Dubreuil[1,2], **Jacques Barhanin**[3], **Christo Goridis**[1,2,*] and **Jean-François Brunet**[1,2,*]

[1]*Ecole normale supérieure, Département de Biologie, 75005 Paris, France*
[2]*CNRS UMR8542, 75005 Paris, France*
[3]*CNRS-FRE3093, Université de Nice-Sophia Antipolis, 06108 Nice, France*

In the last few years, elucidation of the architecture of breathing control centres has reached the cellular level. This has been facilitated by increasing knowledge of the molecular signatures of various classes of hindbrain neurons. Here, we review the advances achieved by studying the homeodomain factor *Phox2b*, a transcriptional determinant of neuronal identity in the central and peripheral nervous systems. Evidence from human genetics, neurophysiology and mouse reverse genetics converges to implicate a small population of *Phox2b*-dependent neurons, located in the retrotrapezoid nucleus, in the detection of CO_2, which is a paramount source of the 'drive to breathe'. Moreover, the same and other studies suggest that an overlapping or identical neuronal population, the parafacial respiratory group, might contribute to the respiratory rhythm at least in some circumstances, such as for the initiation of breathing following birth. Together with the previously established *Phox2b* dependency of other respiratory neurons (which we review briefly here), our new data highlight a key role of this transcription factor in setting up the circuits for breathing automaticity.

Keywords: *Phox2b*; breathing; retrotrapezoid nucleus; visceral nervous system

1. INTRODUCTION: *PHOX2B* AND THE CORE REFLEX CIRCUITS OF THE VISCERAL NERVOUS SYSTEM

Phox2b is a homeobox gene specifically expressed in a limited set of neuronal types during development and for most of them, throughout adult life (Pattyn *et al.* 1997; Dauger *et al.* 2003; Tiveron *et al.* 2003; reviewed in Brunet & Goridis 2008). Unlike the vast majority of neural-type specific transcription factors, which are expressed either regionally or in discrete but unrelated sets of neurons, the expression of *Phox2b* matches a set of neurons related to each other based on their connectivity and function. These are the visceral neurons, afferent and efferent, that regulate the cardio-vascular, respiratory and digestive organs (Blessing 1997*a*) (figure 1). Indeed, the majority of *Phox2b*-expressing neurons make up the sensory and motor arms of the visceral reflex circuits. On sensory pathways, *Phox2b* is expressed in the three epibranchial sensory ganglia (geniculate, petrosal and nodose), which monitor blood pressure and the chemical composition of the vascular and digestive contents, and in their targets in the CNS, namely the sensory neurons of the nucleus of the solitary tract (nTS). *Phox2b* is also expressed in the two main chemosensitive organs: the carotid body (CB) (innervated by the petrosal ganglion and specialized in sensing blood oxygen and sugar levels) (Gonzalez *et al.* 1994; Pardal & Lopez-Barneo 2002) and the area postrema (AP) (appended to the nTS and which senses toxins in the bloodstream and cephalo-spinal fluid) (Miller & Leslie 1994). The visceral motor pathways also consist of *Phox2b*-positive neurons: this includes all autonomic ganglia (sympathetic, parasympathetic and enteric), the adrenal medulla and 'general visceral' motor (VM) or pre-ganglionic neurons to the enteric and parasympathetic ganglia (in the dorsal motor nucleus of the vagus nerve (dmnX), external formation of the nucleus ambiguus (nA) and salivatory nuclei). The only exceptions are the sympathetic pre-ganglionic neurons of the spinal cord that do not express *Phox2b* and appear more related by their transcriptional code to somatic motoneurons (Thaler *et al.* 2004). *Phox2b* is also expressed in the 'special visceral' or branchial motor (BM) neurons (in the trigeminal (nV) and facial (nVII) nuclei, the nA and the spinal accessory (nXI) nucleus) that share with VM neurons many developmental features: they are born at the same dorsoventral level in the hindbrain neuroepithelium, they have the same transcriptional code (*Phox2a/b*$^+$, *Lhx3*$^-$, *HB9*$^-$, *Tbx20*$^+$), they undergo a similar dorsal migration and like them send their axons to dorsal exit points. In terrestrial vertebrates, the visceral function of BM neurons is somewhat obscured and they are accordingly excluded from standard anatomical accounts of the visceral nervous system (VNS). However, in the aquatic relatives and ancestors of terrestrial vertebrates, the sole

---

*Authors for correspondence (goridis@biologie.ens.fr, jfbrunet@biologie.ens.fr)

One contribution of 17 to a Discussion Meeting Issue 'Brainstem neural networks vital for life'.

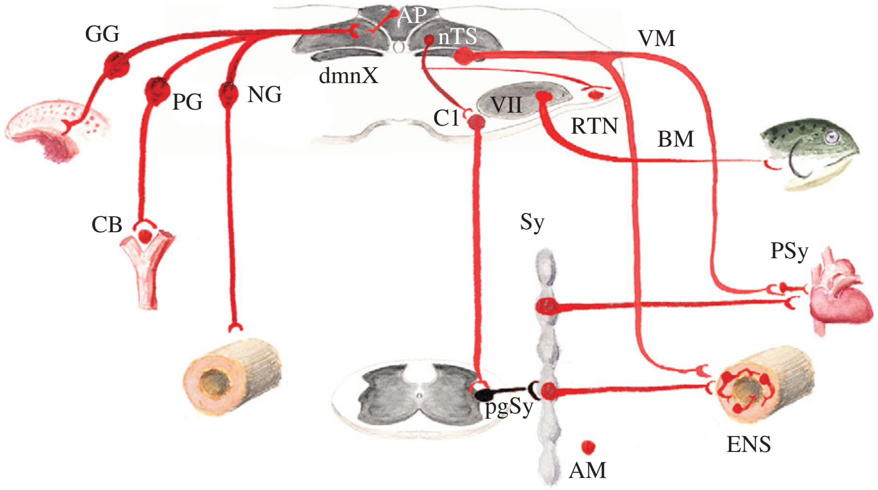

Figure 1. Functional anatomy of the visceral reflex circuits. All neurons shown in red express *Phox2b* and depend on it for their differentiation. VII, facial nucleus; AM, adrenal medulla; AP, area postrema; BM, branchiomotor neuron; C1, adrenergic centre; CB, carotid body; dmnX, dorsal motor nucleus of the vagus nerve; ENS, enteric nervous system; GG, geniculate ganglion; NG, nodose ganglion; nTS, nucleus of the solitary tract; PG, petrosal ganglion; RTN, retrotrapezoid nucleus; pgSy, pre-ganglionic sympathetic neuron; PSy, parasympathetic ganglion; Sy, sympathetic ganglion; VM, visceromotor neuron; see text for details.

functions of BM neurons is digestive (for nV) and respiratory (for nV, nVII and nA) (see evolutionary discussion below). Beyond these core component of the VNS, *Phox2b* is expressed in only three other discrete sets of hindbrain neurons: (i) four atypical efferent nuclei of the hindbrain that share the transcriptional code of VM and BM neurons: vestibular and cochlear efferents in the medulla (Tiveron *et al.* 2003), and occulomotor and trochlear motoneurons in the isthmus (Pattyn *et al.* 1997); (ii) noradrenergic and adrenergic centres of the hindbrain (respectively A1–A7 and C1–C3) (Pattyn *et al.* 2000*a*)—some of which are *bona fide* parts of the VNS, such as the C1 pre-motor sympathetic neurons (Guyenet 2006); and (iii) a population of interneurons in the medulla and pons distributed in the ventrolateral medulla (VLM), intermediate reticular formation and paratrigeminal region (Kang *et al.* 2007; J.-F. Brunet, unpublished data). The latter await attribution of a physiological function. In light of the overwhelming visceral theme of the *Phox2b* expression pattern, it seems a safe working hypothesis that at least some of them will turn out to participate in the control of visceral circuits and function. As this review will illustrate, this prediction has just started to be born out.

In all classes of *Phox2b*-positive neurons, expression of the gene starts either before or just after mitotic arrest (Pattyn *et al.* 1997). In *Phox2b* knock-outs, most *Phox2b* neurons fail either to appear or to differentiate (Pattyn *et al.* 1999, 2000*a*,*b*; Dauger *et al.* 2003; Huber *et al.* 2005), with the exception of the oculomotor and trochlear motoneurons, which depend on *Phox2a*—a paralogue of *Phox2b* (Pattyn *et al.* 1997), and possibly some hindbrain interneurons (Brunet & Goridis 2008; J.-F. Brunet, unpublished data). Thus, *Phox2b* can be viewed as a master regulator of core visceral circuits. The *raison d'être* for this unusual foreshadowing of connectivity among different neuronal types by a single transcriptional determinant is still elusive and speculations lie outside the scope of this review. Here, we will discuss recent insights into the first group of hindbrain *Phox2b* interneurons to be characterized. We show that they further illustrate the intriguing correlation of *Phox2b* with the VNS and have an essential role in both the neural control of breathing at birth and central chemoreception in the adult mammal.

2. MUTATIONS IN *PHOX2B* AND THE DRIVE TO BREATHE

Until recently, the role of *Phox2b*$^+$ neurons in the circuits that control breathing seemed patchier than in those that control digestion and blood circulation. On the afferent side of respiratory reflexes, *Phox2b* is required in the nodose ganglion, which contains the cell bodies of pulmonary stretch receptors responsible for the Herring–Breuer reflexes, in the CB, which contains oxygen and CO_2 sensors, in the petrosal ganglion, which innervates the CB, and in the nTS, which integrates of chemosensory and barosensory information. On the other hand, the motoneurons for the diaphragm and parietal muscles that ensure breathing never express *Phox2b* (but see phylogenetic discussion below). The best characterized central component of the respiratory rhythm generator (RRG)—the pre-Bötzinger complex (preBötC) (Smith *et al.* 1991; Rekling & Feldman 1998)—contains few, if any, *Phox2b*-positive neurons (Blanchi *et al.* 2003). Finally, some pontine or medullary nuclei thought to provide positive or negative influences on the respiratory rhythm are *Phox2b* positive (e.g. noradrenergic nuclei A6 and A5; Hilaire *et al.* 2004) while others are not (e.g. the parabrachial and Kölliker–Fuse nuclei; Kang *et al.* 2007; J.-F. Brunet, unpublished data).

A direct assessment of the role of *Phox2b* in the neural control of breathing in mouse has been largely hindered because of the embryonic death of *Phox2b* null mutants from cardiovascular failure (Pattyn *et al.* 1999, 2000*a*). It was at first limited to the study of

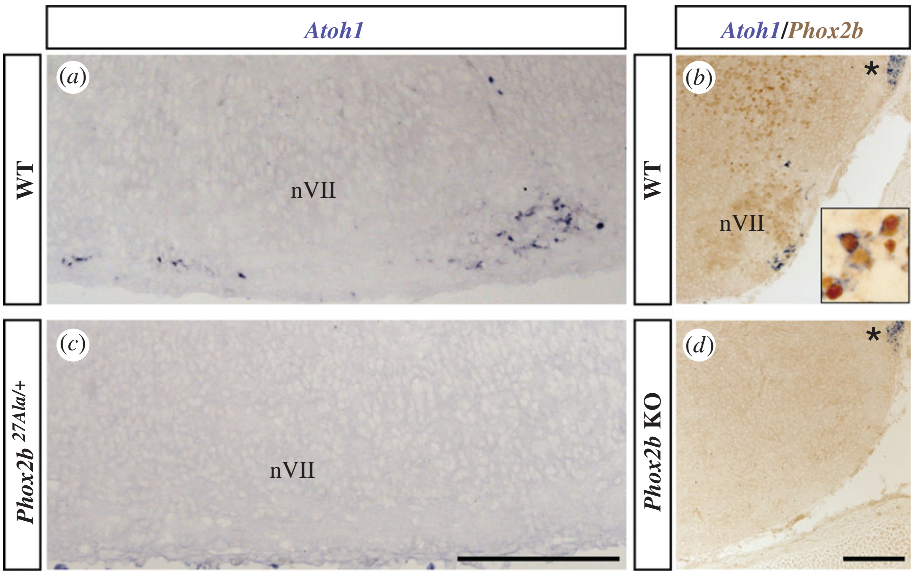

Figure 2. Expression of *Atoh1* by RTN neurons and sensitivity to the $Phox2b^{27Ala/+}$ and $Phox2b^{lacZ/lacZ}$ mutations. (*a*,*b*) *In situ* hybridization showing (in blue) expression of *Atoh1* combined (*b*) with Phox2b immunohistochemistry (in brown) on parasagittal (*a*, anterior to the left) and transverse (*b*) sections through the hindbrain of a wild-type hindbrain at E15.5 Inset: close-up of $\text{Phox2b}^{+}/Atoh1^{+}$ in the RTN. (*c*) Parasagittal section through the hindbrain of a $Phox2b^{27Ala/+}$ mutant at an equivalent position as in (*a*), showing the disappearance of *Atoh1* expression in the RTN. (*d*) Transverse section through the hindbrain of a homozygous *Phox2b* null mutant at an equivalent position and treated as in (*b*) showing the disappearance of the *Atoh1* signal in the RTN but not in pre-cerebellar neurons (asterisk). nVII, facial nucleus. Bars, 100 μm.

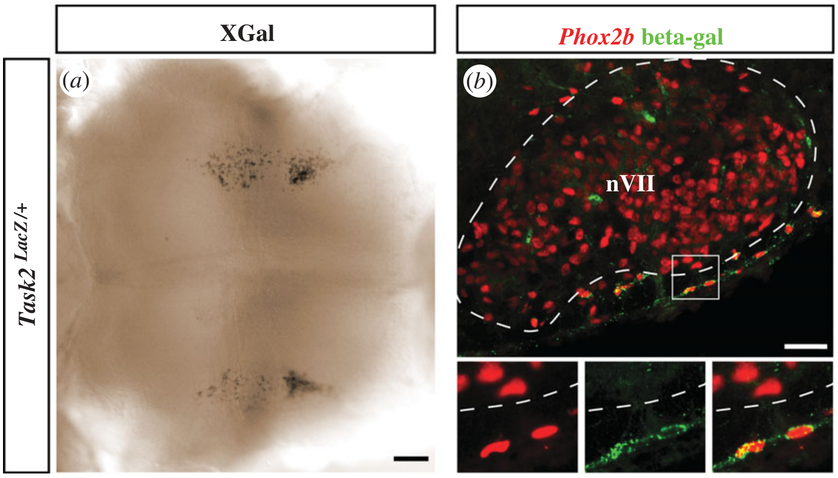

Figure 3. Expression of the leak potassium channel TASK2 in RTN cells. (*a*) Ventral view of the hindbrain of E17.5 $Task2^{LacZ/+}$ mouse embryo ($Task2^{LacZ/+}$ mouse line; adapted from W. C. Skarnes) stained by XGal for LacZ expression (rostral to the left). A bilateral population of positive cells is seen at the level of the facial nucleus. (*b*) Transverse section through the facial nucleus of an E15.5 embryo immunostained for Phox2b (in red) and beta-galactosidase (in green) showing coexpression of Phox2b and beta-gal in RTN cells at the ventral medullary surface. The square window delineated in white is enlarged in the three panels below. nVII, facial nucleus. Bars, 100 μm in (*a*) and 50 μm in (*b*).

heterozygous knock-out pups who present a complex, mild and regressive ventilatory phenotype (reviewed in Gallego & Dauger 2008).

A new phase in our understanding of the respiratory role of *Phox2b* started when mutations in *PHOX2B* were discovered in humans with congenital central hypoventilation syndrome (CCHS; Amiel *et al.* 2003). *PHOX2B* mutations are now detected in 96 per cent of CCHS patients and considered causal for this condition (Weese-Mayer *et al.* 2005). The idea to look for *PHOX2B* mutations in CCHS sprung from the long list of symptoms associated with the disease (Chen & Keens 2004), each relating to a site of known *Phox2b* expression: for example, partial agenesis of the enteric nervous system (Hirschprung disease), tumours of the sympathetic ganglia or adrenal medulla (neuroblastomas), dysmotility of the intrinsic and extrinsic muscles of the eye (that receive innervation from the superior cervical and ciliary ganglia, and the occulomotor and trochlear nuclei, respectively), reduction of cardiac rhythm variability (which is under dual sympathetic and parasympathetic influence), dysphagia (which depends on both intrinsic innervation of the oesophagus and an extrinsic reflex loop passing through the nTS and the nucleus ambiguus). The diagnostic symptom of CCHS, however, is respiratory: alveolar hypoventilation with no underlying muscular or cardiovascular deficit, and apneic episodes, typically during sleep. The severity of the disease ranges from respiratory arrests occurring late in life (in mild cases, which originally defined the syndrome (Mellins *et al.* 1970), and are now classified as 'late-onset CCHS' (Antic *et al.* 2006)), to the absence of spontaneous breathing at birth (Gozal 1998). In all cases, functional evaluation reveals the underlying defect: abrogation or a great reduction of the sensitivity to hypercapnia, which normally maintains pCO_2 within strict limits. Thus, the implication of *Phox2b* in CCHS not only paved the way for an understanding of a devastating disease but offered fresh insight into the long-standing quest for the anatomical site of CO_2 sensitivity. Two theories have dominated the field over the past decades (see Guyenet (2008) for a review). The 'distributed chemoreception theory' states that the exquisite CO_2 sensitivity of the respiratory network results from the summation of the lesser sensitivities of its parts (Feldman *et al.* 2003). Conversely, the 'specialized chemoreceptor theory' (Guyenet 2008) originated from the work of Loeschcke and Mitchell (Loeschcke 1982), who favoured dedicated sites for CO_2 sensing restricted to discrete regions of the ventral medullary surface. The two best-documented candidates for the latter role are raphe serotonergic nuclei (Richerson 2004) and the retrotrapezoid nucleus (RTN), a group of glutamatergic neurons located in the marginal layer of the VLM beneath the facial motor nucleus (Mulkey *et al.* 2004). The fact that mutations in *Phox2b*, a neuronal-type specific transcription factor, can abolish CO_2 sensitivity without affecting many other aspects of the respiratory behaviour pleads for the specialized chemoreceptor theory. Indeed, it implies (barring an unlikely widespread non-cell autonomous effect of the mutation) that the CO_2 response passes through at least one obligatory *Phox2b*$^{+}$ neuronal relay. The simplest scenario is that the central CO_2 sensor consists in a *Phox2b*$^{+}$ population of neurons sensitive to the CCHS mutations.

The mutations found in CCHS patients are mostly expansions of a stretch of 20 Alanines by 4 to 13 residues in the C-terminal part of *PHOX2B*, but frame shifts and mis-sense mutations are also found (Weese-Mayer *et al.* 2005; Repetto *et al.* 2008). Three lines of evidence argue that they are not null. First, *Phox2b*$^{+/-}$ mouse mutants display a much more subtle phenotype than CCHS patients (Dauger *et al.* 2003; Cross *et al.* 2004; Durand *et al.* 2005). Second, patients with heterozygous deletions of the *PHOX2B* region do not have CCHS (reviewed in Weese-Mayer *et al.* 2005); and third, different *PHOX2B* mutations are associated with different combinations and frequencies of symptoms (Gaultier *et al.* 2005; Weese-Mayer *et al.* 2005). Rather, the mutant protein may cause CCHS by a dominant-negative mechanism or by a toxic gain of function (Bachetti *et al.* 2005; Trochet *et al.* 2005). In man, an argument for a dominant-negative activity or cellular toxicity of the mutated protein is that a fraction of CCHS patients have strabismus (Goldberg & Ludwig 1996), implying a dysfunction of the *Phox2a*-dependent third and fourth motor nuclei, where *Phox2b* is expressed but not required (see §1).

In an attempt to model CCHS, the most frequent mutation, a 7-residue expansion of the poly-Alanine stretch, was introduced in mice (Dubreuil *et al.* 2008). Transmitting chimeras produced heterozygous pups (*Phox2b*$^{+/27Ala}$) that died soon after birth from respiratory failure (Dubreuil *et al.* 2008). Plethysmography performed prior to death revealed an absence of response to hypercapnia. *Phox2b*$^{27Ala/+}$ newborns are models of human CCHS in which to look for the cellular locus and developmental mechanism of the physiological defects of the disease.

3. PHOX2B AND THE RETROTRAPEZOID NUCLEUS

Two independent lines of research have now established a link between *Phox2b* and the RTN, one of the two main contenders for the role of CO_2/pH sensor in the CNS (§2). First, neurons of the adult rat RTN, defined physiologically by their responsiveness to CO_2 *in vivo* (Mulkey *et al.* 2004; reviewed in Guyenet 2008), and phenotyped as *Vglut2*$^{+}$ (but negative for *glutamate decarboxylase* and *tyrosine hydroxylase*) and located at the ventral medullary surface under the facial nucleus and extending approximately 500 μm caudal to it, were all found to express *Phox2b* (Stornetta *et al.* 2006). Second, inspection of the hindbrain of *Phox2b*$^{27Ala/+}$ newborn mice that have no response to hypercapnia (§2) showed that there was an 85 per cent depletion of the *Phox2b*$^{+}$/*Vglut2*$^{+}$ RTN neurons (Dubreuil *et al.* 2008). This observation held true for embryonic day (E)15.5 embryos, ruling out post-natal hypoxic injury as a cause. This defect contrasted with several other *Phox2b*$^{+}$ neuronal populations involved in breathing (such as the CB, the petrosal and nodose ganglia, the noradrenergic centres and the nTS) that were present in normal numbers in the mutants, suggesting that among *Phox2b*$^{+}$ neurons the mutation affects preferentially the RTN (Dubreuil *et al.* 2008) and that the RTN atrophy is causal to the lack of CO_2 sensitivity. Notably, the raphe nuclei were present and anatomically intact.

We recently found that a subset of *Vglut2*$^{+}$/*Phox2b*$^{+}$ neurons in the RTN region expressed the bHLH transcription factor *Atoh1* (figure 2*a*,*b*, and data not shown). This, incidentally, points to the RTN as a potential culprit in the *Atoh1* KO neonatal lethal respiratory phenotype (Ben-Arie *et al.* 1997). The very discrete expression of *Atoh1* in the RTN, which disappears in *Phox2b*$^{27Ala/+}$ pups (figure 2*c*), allowed us to assess the fate of RTN neurons in a *Phox2b* null background where *Phox2b* is no longer available as a marker. Similar to *Phox2b*$^{27Ala/+}$ mutants, *Atoh1* expression was abolished in *Phox2b*$^{-/-}$ E15.5 embryos (figure 2*d*), suggesting that the differentiation of the RTN (at least its *Atoh1*/*Phox2b* component) in addition to being sensitive to the *Phox2b*$^{27Ala/+}$ mutation, depends on *Phox2b*. However, we cannot, at this stage, exclude the possibility that this dependency may be non-cell autonomous and, for example, mediated by the disappearance of the nearby facial nucleus.

Combined with the abundant physiological data concerning the RTN, the phenotype of the *Phox2b*$^{27Ala/+}$ mutants suggests that RTN neurons are essential for the sensitivity to CO_2 of the respiratory circuitry. Moreover, these cells receive direct projections from a subpopulation of *Phox2b*$^{+}$ nTS neurons that themselves relay PaO_2 responsiveness by virtue of an input from the CB, via the petrosal ganglion (Stornetta *et al.* 2006), which are all dependent on *Phox2b*. Thus, the circuitry for monitoring blood gases, which provides a major drive to breathe, might consist of an uninterrupted *Phox2b*-dependent four-neuron circuit.

It remains that the *Phox2b*$^{27Ala/+}$ mouse model, in which it is impossible to rule out subtle or purely functional deficits in *Phox2b*$^{+}$ neuronal populations other than the RTN, does not amount to a specific genetic deletion of that nucleus. Definitive proof that the RTN ensures CO_2 sensitivity will require triggering deleterious alterations of gene expression specifically in that nucleus. Co-expression of *Phox2b* and *Atoh1* (see above) or the potassium leak channel *TASK2* (figure 3) in at least a subset of RTN neurons might help devise strategies to that effect. This could be by recombining a conditional *Phox2b* allele with an *Atoh1*-driven Cre recombinase, for example.

Parallel to the progressive elucidation of the functional role of the RTN in respiration, other studies have highlighted another group of respiratory neurons located below and just caudal to nVII, designated as the parafacial respiratory group (pFRG). Cells of the pFRG are defined in the neonatal rat where, unlike RTN neurons of the adult rat, they display a phasic pre-inspiratory (pre-I) firing pattern (Onimaru & Homma 2003). It is proposed that the pFRG is a respiratory oscillator that either would entrain the preBötC (Onimaru & Homma 2006), be merely coupled to the preBötC and responsible for active expiration (Janczewski & Feldman 2006) or together with the preBötC form a degenerate rhythmogenic network (Mellen 2008). Two lines of evidence argue for a possible overlap between the RTN and pFRG populations, despite their distinct firing patterns. First is the fact that pFRG cells are sensitive to CO_2 in newborn rats (Kawai *et al.* 2006). Second, a collection of rhythmic cells at the same location as the RTN/pFRG, but recorded in the mouse embryo, also express *Phox2b* and are responsive to CO_2 (Thoby-Brisson *et al.* in press). Thus, it is conceivable that the RTN and the pFRG represent overlapping, if not identical groups of cells that would change properties between the perinatal and adult stages of life. Together, these data point to the possibility that *Phox2b* cells control aspects of respiratory rhythm generation, at least at birth. This is compatible with the lack of spontaneous breathing at birth in severe cases of CCHS (Gozal 1998). It could also explain the abnormalities of the respiratory rhythm in *Phox2b*$^{27Ala/+}$ newborns, which range from a slow rhythm to gasping (Dubreuil *et al.* 2008). Very recently, RTN/pFRG *Phox2b* cells, which are derived from neuronal precursors expressing the transcription factor *Lbx1* (Pagliardini *et al.* 2008), were found to be missing in *Lbx1*$^{-/-}$ mice (Pagliardini *et al.* 2008) whose CO_2 sensitivity was not explored but that die at birth from extreme bradypnoea detectable from E15.5 on. These data are compatible with a role for the RTN in setting the neonatal respiratory pace, although massive abnormalities in other respiratory centres of the *Lbx1* null mutants, such as the nTS and (nor)adrenergic centres (but not the preBötC) were also present and could play a role.

4. *PHOX2B* AND THE HOMEOSTATIC SIDE OF BREATHING

Breathing in terrestrial animals is a complex behaviour that combines an obvious homeostatic function—maintaining pO_2 and pCO_2 in extracellular fluids—with voluntary or ‘somatic’ functions such as sniffing, vocalizing and sighing (Blessing 1997*b*). It is striking that *Phox2b*-dependent respiratory neurons, in line with the visceral roles of other *Phox2b* neurons, are preferentially associated with homeostatic or automatic control of breathing, which is evolutionary speaking, more ancient. Those parts of respiratory circuitry that have evolved more recently and that allow somatic functions involve *Phox2b*$^{-}$ neurons.

On the afferent pathways, as has been developed throughout this review, the homeostatic control of blood oxygen and carbon dioxide involves an uninterrupted chain of four *Phox2b*-dependent neuronal relays: the CB, the petrosal ganglion, the nTS and the RTN. As to the efferent pathways, a major change has occurred at the transition from aquatic to terrestrial life, which was accompanied by a shift from gills to lungs for gas exchange and a corresponding shift from gill, opercular and buccal muscles to axial muscles (e.g. intercostal, abdominal and the diaphragm) for respiratory pumping (Liem 1985; Brainerd 1999). Accordingly, the motor control of breathing shifted from *Phox2b*-positive neurons (BM neurons) to *Phox2b*-negative neurons (e.g. somatic motoneurons of the spinal cord such as phrenic motoneurons). In turn, the original visceral function of branchial muscles and motoneurons, while still apparent in a number of respiratory and feeding-related behaviours such as coughing, suckling or swallowing, has been obscured by the voluntary functions for which they have been recruited (e.g. facial expression or the motorization of the vocal cords). Work on the ascidian *Ciona intestinalis*, a representative of the tunicates—the closest relatives of vertebrates (Delsuc *et al.* 2006)—has shown that BM neurons in their original visceral avatar predate the advent of vertebrates. Indeed, the branchial basket of these sessile animals, which serves as both food collector and site of gas exchange, is motorized by *CiPhox2*$^{+}$ neurons. Moreover, these neurons express the T-box transcription factor *Tbx20*, a specific marker in the vertebrate CNS for both BM and VM neurons (Dufour *et al.* 2006). BM neurons with dual respiratory and digestive functions could represent one of the most primitive parts of the vertebrate visceral circuitry.

On a more speculative note, another shift from *Phox2b*$^{+}$ to *Phox2b*$^{-}$ neurons might have occurred at the advent of air breathing and is implicit in recent discussions on the evolution of the RRG (Vasilakos *et al.*

2005; Feldman & Del Negro 2006; Wilson *et al.* 2006). The 'buccal oscillator' of amphibians, which may derive from a fish-type respiratory oscillator, has been proposed as an evolutionary forerunner of the pFRG. Conversely, the preBötC would be an invention of terrestrial vertebrates, possibly homologous to the 'lung oscillator' of amphibians. A cautionary note here is that the anatomical positions of the buccal and lung oscillators of frogs are not readily reconcilable with those of the pFRG and preBötC, respectively (Vasilakos *et al.* 2005). When we have a cellular definition of the respiratory oscillator(s) of fish or amphibians, testing for *Phox2b* expression might provide an argument for or against homology. As things stand, it is only a seductive hypothesis that the pFRG would represent an ancestral RRG driving the purely homeostatic gill breathing, and that it would have been superseded by the preBötC, which is better suited for the flexible integration of automatic breathing with many other behaviours observed in terrestrial vertebrates.

As mentioned at the beginning of this review, many *Phox2b*$^+$ interneurons of yet unknown function are found in the VLM. The study of these neurons may shed further light on the architecture of visceral circuits, including those regulating respiration.

This work was supported by grants from the Agence Nationale pour la Recherche and the Fondation pour la Recherche Médicale.

REFERENCES

Amiel, J. *et al.* 2003 Polyalanine expansion and frame shift mutations of the paired-like homeobox gene *PHOX2B* in congenital central hypoventilation syndrome (Ondine's curse). *Nat. Genet.* **33**, 459–461. (doi:10.1038/ng1130)

Antic, N. A. *et al.* 2006 PHOX2B mutation-confirmed congenital central hypoventilation syndrome: presentation in adulthood. *Am. J. Respir. Crit. Care Med.* **174**, 923–927. (doi:10.1164/rccm.200605-607CR)

Bachetti, T., Matera, I., Borghini, S., Di Duca, M., Ravazzolo, R. & Ceccherini, I. 2005 Distinct pathogenetic mechanisms for PHOX2B associated polyalanine expansions and frameshift mutations in congenital central hypoventilation syndrome. *Hum. Mol. Genet.* **14**, 1815–1824. (doi:10.1093/hmg/ddi188)

Ben-Arie, N., Bellen, H. J., Armstrong, D. L., McCall, A. E., Gordadze, P. R., Guo, Q., Matzuk, M. M. & Zoghbi, H. Y. 1997 Math1 is essential for genesis of cerebellar granule neurons. *Nature* **390**, 169–172. (doi:org/10.1038/36579)

Blanchi, B. *et al.* 2003 MafB deficiency causes defective respiratory rhythmogenesis and fatal central apnea at birth. *Nat. Neurosci.* **6**, 1091–1100. (doi:10.1038/nn1129)

Blessing, W. W. 1997*a* Anatomy of the lower brainstem. In *The lower brainstem and bodily homeostasis* (ed. W. W. Blessing), pp. 29–99. New York, NY: Oxford University Press.

Blessing, W. W. 1997*b* Breathing. In *The lower brainstem and bodily homeostasis*, pp. 101–164. New York, NY: Oxford University Press.

Brainerd, E. L. 1999 New perspectives on the evolution of lung ventilation mechanisms in vertebrates. *Exp. Biol. Online* **4**, 1–28. (doi:org/10.1007/s00898-999-0002-1)

Brunet, J.-F. & Goridis, C. 2008 Phox2b and the homeostatic brain. In *Genetic basis for respiratory control disorders* (ed. C. Gaultier), pp. 25–44. New York, NY: Springer.

Chen, M. L. & Keens, T. G. 2004 Congenital central hypoventilation syndrome: not just another rare disorder. *Paediatr. Respir. Rev.* **5**, 182–189. (doi:10.1016/j.prrv.2004.04.009)

Cross, S. H., Morgan, J. E., Pattyn, A., West, K., McKie, L., Hart, A., Thaung, C., Brunet, J.-F. & Jackson, I. J. 2004 Haploinsufficiency for *Phox2b* in mice causes dilated pupils and atrophy of the ciliary ganglion: mechanistic insights into human congenital central hypoventilation syndrome. *Hum. Mol. Genet.* **13**, 1433–1439. (doi:10.1093/hmg/ddh156)

Dauger, S., Pattyn, A., Lofaso, F., Gaultier, C., Goridis, C., Gallego, J. & Brunet, J.-F. 2003 *Phox2b* controls the development of peripheral chemoreceptors and afferent visceral pathways. *Development* **130**, 6635–6642. (doi:10.1242/dev.00866)

Delsuc, F., Brinkmann, H., Chourrout, D. & Philippe, H. 2006 Tunicates and not cephalochordates are the closest living relatives of vertebrates. *Nature* **439**, 965–968. (doi:10.1038/nature04336)

Dubreuil, V., Ramanantsoa, N., Trochet, D., Vaubourg, V., Amiel, J., Gallego, J., Brunet, J.-F. & Goridis, C. 2008 A human mutation in Phox2b causes lack of CO_2 chemosensitivity, fatal central apnea, and specific loss of parafacial neurons. *Proc. Natl Acad. Sci. USA* **105**, 1067–1072. (doi:10.1073/pnas.0709115105)

Dufour, H. D., Chettouh, Z., Deyts, C., de Rosa, R., Goridis, C., Joly, J.-S. & Brunet, J.-F. 2006 Pre-craniate origin of cranial motoneurons. *Proc. Natl Acad. Sci. USA* **103**, 8727–8732. (doi:10.1073/pnas.0600805103)

Durand, E., Dauger, S., Pattyn, A., Gaultier, C., Goridis, C. & Gallego, J. 2005 Sleep-disordered breathing in newborn mice heterozygous for the transcription factor Phox2b. *Am. J. Respir. Crit. Care Med.* **172**, 238–243. (doi:10.1164/rccm.200411-1528OC)

Feldman, J. L. & Del Negro, C. A. 2006 Looking for inspiration: new perspectives on respiratory rhythm. *Nat. Rev. Neurosci.* **7**, 232–242. (doi:10.1038/nrn1871)

Feldman, J. L., Mitchell, G. S. & Nattie, E. E. 2003 Breathing: rhythmicity, plasticity, chemosensitivity. *Annu. Rev. Neurosci.* **26**, 239–266. (doi:10.1146/annurev.neuro.26.041002.131103)

Gallego, J. & Dauger, S. 2008 PHOX2B mutations and ventilatory control. *Respir. Physiol. Neurobiol.* **17**, 17.

Gaultier, C., Trang, H., Dauger, S. & Gallego, J. 2005 Pediatric disorders with autonomic dysfunction: what role for PHOX2B? *Pediatr. Res.* **58**, 1–6. (doi:10.1203/01.PDR.0000166755.29277.C4)

Goldberg, D. S. & Ludwig, I. H. 1996 Congenital central hypoventilation syndrome: ocular findings in 37 children. *J. Pediatr. Ophthalmol. Strabismus* **33**, 175–180.

Gonzalez, C., Almaraz, L., Obeso, A. & Rigual, R. 1994 Carotid body chemoreceptors: from natural stimuli to sensory discharges. *Physiol. Rev.* **74**, 829–898.

Gozal, D. 1998 Congenital central hypoventilation syndrome: an update. *Pediatr. Pulmonol.* **26**, 273–282. (doi:10.1002/(SICI)1099-0496(199810)26:4<273::AID-PPUL7>3.0.CO;2-C)

Guyenet, P. G. 2006 The sympathetic control of blood pressure. *Nat. Rev. Neurosci.* **7**, 335–346. (doi:10.1038/nrn1902)

Guyenet, P. G. 2008 The 2008 Carl Ludwig Lecture: retrotrapezoid nucleus, CO_2 homeostasis, and breathing automaticity. *J. Appl. Physiol.* **105**, 404–416. (doi:10.1152/japplphysiol.90452.2008)

Hilaire, G., Viemari, J. C., Coulon, P., Simonneau, M. & Bevengut, M. 2004 Modulation of the respiratory rhythm generator by the pontine noradrenergic A5 and A6 groups in rodents. *Respir. Physiol. Neurobiol.* **143**, 187–197. (doi:10.1016/j.resp.2004.04.016)

Huber, K., Karch, N., Ernsberger, U., Goridis, C. & Unsicker, K. 2005 The role of Phox2B in chromaffin cell development. *Dev. Biol.* **279**, 501–508. (doi:10.1016/j.ydbio.2005.01.007)

Janczewski, W. A. & Feldman, J. L. 2006 Distinct rhythm generators for inspiration and expiration in the juvenile rat. *J. Physiol.* **570**, 407–420.

Kang, B. J., Chang, D. A., Mackay, D. D., West, G. H., Moreira, T. S., Takakura, A. C., Gwilt, J. M., Guyenet, P. G. & Stornetta, R. L. 2007 Central nervous system distribution of the transcription factor Phox2b in the adult rat. *J. Comp. Neurol.* **503**, 627–641. (doi:10.1002/cne.21409)

Kawai, A., Onimaru, H. & Homma, I. 2006 Mechanisms of CO_2/H^+ chemoreception by respiratory rhythm generator neurons in the medulla from newborn rats *in vitro*. *J. Physiol.* **572**, 525–537. (doi:10.1113/jphysiol.2005.102533)

Liem, K. F. 1985 Ventilation. In *Functional vertebrate morphology* (eds M. Hildebrand, D. M. Bramble, K. F. Liem & D. Wake), pp. 185–209. Cambridge, MA: Harvard University Press.

Loeschcke, H. H. 1982 Central chemosensitivity and the reaction theory. *J. Physiol.* **332**, 1–24.

Mellen, N. M. 2008 Belt-and-suspenders as a biological design principle. *Adv. Exp. Med. Biol.* **605**, 99–103. (doi:10.1007/978-0-387-73693-8)

Mellins, R. B., Balfour Jr, H. H., Turino, G. M. & Winters, R. W. 1970 Failure of automatic control of ventilation (Ondine's curse). Report of an infant born with this syndrome and review of the literature. *Medicine* (*Baltimore*) **49**, 487–504.

Miller, A. D. & Leslie, R. A. 1994 The area postrema and vomiting. *Front. Neuroendocrinol.* **15**, 301–320. (doi:10.1006/frne.1994.1012)

Mulkey, D. K., Stornetta, R. L., Weston, M. C., Simmons, J. R., Parker, A., Bayliss, D. A. & Guyenet, P. G. 2004 Respiratory control by ventral surface chemoreceptor neurons in rats. *Nat. Neurosci.* **7**, 1360–1369. (doi:10.1038/nn1357)

Onimaru, H. & Homma, I. 2003 A novel functional neuron group for respiratory rhythm generation in the ventral medulla. *J Neurosci* **23**, 1478–1486.

Onimaru, H. & Homma, I. 2006 Point:counterpoint: the parafacial respiratory group (pFRG)/pre-Botzinger complex (preBotC) is the primary site of respiratory rhythm generation in the mammal. Point: the PFRG is the primary site of respiratory rhythm generation in the mammal. *J. Appl. Physiol.* **100**, 2094–2095. (doi:10.1152/japplphysiol.00119.2006)

Pagliardini, S., Ren, J., Gray, P. A., Vandunk, C., Gross, M., Goulding, M. & Greer, J. J. 2008 Central respiratory rhythmogenesis is abnormal in *Lbx1*-deficient mice. *J. Neurosci.* **28**, 11 030–11 041. (doi:10.1523/JNEUROSCI.1648-08.2008)

Pardal, R. & Lopez-Barneo, J. 2002 Low glucose-sensing cells in the carotid body. *Nat. Neurosci.* **5**, 197–198. (doi:10.1038/nn812)

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. (doi:org/10.1038/20700)

Pattyn, A., Goridis, C. & Brunet, J.-F. 2000*a* Specification of the central noradrenergic phenotype by the homeobox gene *Phox2b*. *Mol. Cell Neurosci.* **15**, 235–243. (doi:10.1006/mcne.1999.0826)

Pattyn, A., Hirsch, M.-R., Goridis, C. & Brunet, J.-F. 2000*b* Control of hindbrain motor neuron differentiation by the homeobox gene *Phox2b*. *Development* **127**, 1349–1358.

Rekling, J. C. & Feldman, J. L. 1998 PreBotzinger complex and pacemaker neurons: hypothesized site and kernel for respiratory rhythm generation. *Annu. Rev. Physiol.* **60**, 385–405. (doi:10.1146/annurev.physiol.60.1.385)

Repetto, G. M., Corrales, R. J., Abara, S. G., Zhou, L., Berry-Kravis, E. M., Rand, C. M. & Weese-Mayer, D. E. 2008 Later-onset congenital central hypoventilation syndrome due to a heterozygous 24-polyalanine repeat expansion mutation in the PHOX2B gene. *Acta Paediatr.* **98**, 192–195. (doi:10.1111/j.1651-2227.2008.01039.X)

Richerson, G. B. 2004 Serotonergic neurons as carbon dioxide sensors that maintain pH homeostasis. *Nat. Rev. Neurosci.* **5**, 449–461. (doi:10.1038/nrn1409)

Smith, J. C., Ellenberger, H. H., Ballanyi, K., Richter, D. W. & Feldman, J. L. 1991 Pre-Botzinger complex: a brainstem region that may generate respiratory rhythm in mammals. *Science* **254**, 726–729. (doi:10.1126/science.1683005)

Stornetta, R. L., Moreira, T. S., Takakura, A. C., Kang, B. J., Chang, D. A., West, G. H., Brunet, J. F., Mulkey, D. K., Bayliss, D. A. & Guyenet, P. G. 2006 Expression of Phox2b by brainstem neurons involved in chemosensory integration in the adult rat. *J. Neurosci.* **26**, 10 305–10 314. (doi:10.1523/JNEUROSCI.2917-06.2006)

Thaler, J. P., Koo, S. J., Kania, A., Lettieri, K., Andrews, S., Cox, C., Jessell, T. M. & Pfaff, S. L. 2004 A postmitotic role for Isl-Class LIM homeodomain proteins in the assignment of visceral spinal motor neuron identity. *Neuron* **41**, 337–350. (doi:10.1016/S0896-6273(04)00011-X)

Thoby-Brisson, M., Karlén, M., Wu, N., Charnay, P., Champagnat, J. & Fortin, G. In press. Genetic identification of an embryonic parafacial oscillator coupling to the preBötzinger complex. *Nature Neuroscience*.

Tiveron, M.-C., Pattyn, A., Hirsch, M.-R. & Brunet, J.-F. 2003 Role of Phox2b and Mash1 in the generation of the vestibular efferent nucleus. *Dev. Biol.* **260**, 46–57. (doi:10.1016/S0012-1606(03)00213-6)

Trochet, D., Hong, S. J., Lim, J. K., Brunet, J.-F., Munnich, A., Kim, K. S., Lyonnet, S., Goridis, C. & Amiel, J. 2005 Molecular consequences of PHOX2B missense, frameshift and alanine expansion mutations leading to autonomic dysfunction. *Hum. Mol. Genet.* **14**, 3697–3708. (doi:10.1093/hmg/ddi401)

Vasilakos, K., Wilson, R. J., Kimura, N. & Remmers, J. E. 2005 Ancient gill and lung oscillators may generate the respiratory rhythm of frogs and rats. *J. Neurobiol.* **62**, 369–385. (doi:10.1002/neu.20102)

Weese-Mayer, D. E., Berry-Kravis, E. M. & Marazita, M. L. 2005 In pursuit (and discovery) of a genetic basis for congenital central hypoventilation syndrome. *Respir. Physiol. Neurobiol.* **149**, 73–82. (doi:10.1016/j.resp.2005.06.010)

Wilson, R. J., Vasilakos, K. & Remmers, J. E. 2006 Phylogeny of vertebrate respiratory rhythm generators: the Oscillator Homology Hypothesis. *Respir. Physiol. Neurobiol.* **154**, 47–60. (doi:10.1016/j.resp.2006.04.007)