

# Breathing matters

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## Supplementary Box S1 | The quest for the primordial respiratory oscillator

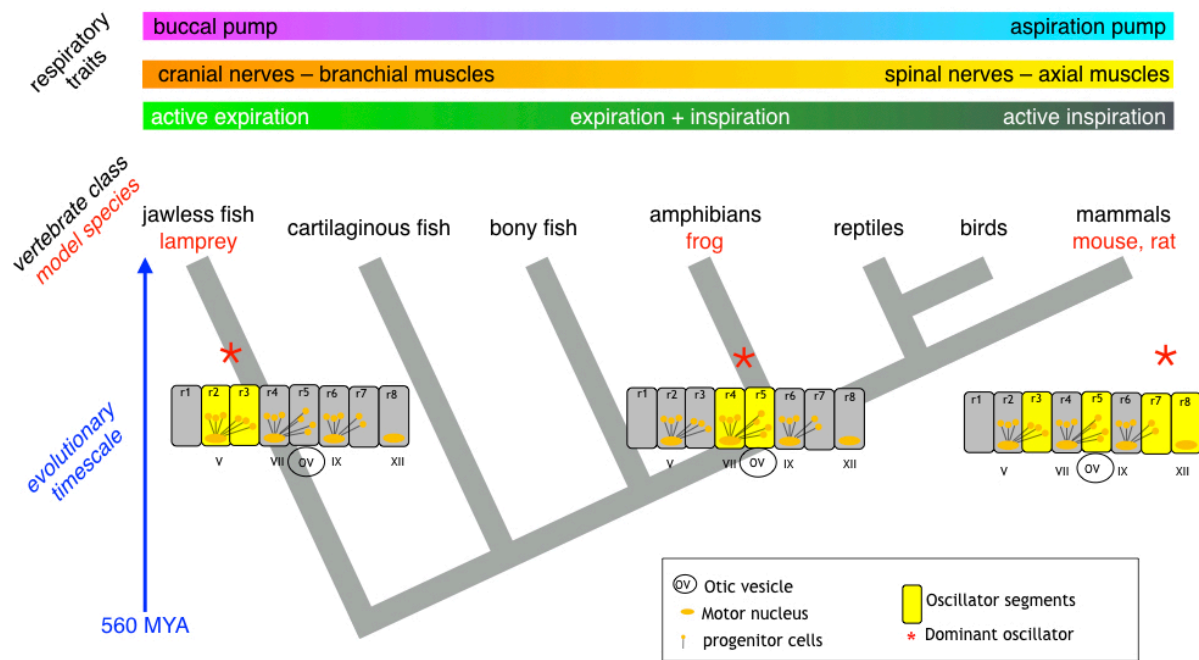
Phylogenetically, a dominant inspiratory oscillator for breathing is brand new; only mammals (at rest) breathe via active inspiration and passive expiration. In the remaining six lower vertebrate classes, either expiration (jawless fish and cartilaginous fish) or expiration and inspiration (bony fish, amphibians, reptiles, birds) are both active (see figure). These diverse ventilatory control systems originated from a common ancestor, but the primordial breathing oscillator has yet to be identified, particularly in the absence of abundant comparative data from extant vertebrates. Whereas small mammals (mice and rats) are well studied, only sparse data are available from non-mammalian vertebrates, with these primarily pertaining to only two classes: jawless fish (lamprey) and amphibians (tadpoles and frogs).

Vertebrate breathing circuits assemble in the developing hindbrain from eight rhombomeres: segments of the rostral neural tube that each contain precursor cells that give rise to local networks that drive motor rhythms in cranial and spinal nerves<sup>1-3</sup>. In lamprey (a jawless fish), a rostral paratrigeminal oscillator derived from rhombomeres 2–3 generates tidal breathing via expiratory contraction of branchial gill pouches, and a caudal oscillator from rhombomeres 4–7 generates a cough-like expiratory rhythm that principally clears debris from the gill pouches<sup>4</sup>. Being CO<sub>2</sub>-sensitive and utilizing axial muscles, the caudal site is a candidate for the primordial air breathing oscillator<sup>5</sup>.

The expiratory pump is elaborated in fish and amphibians into a buccal-pharyngeal pump that generates both active expiration and inspiration. Tidally ventilated, air-breathing organs or lungs, key for terrestrial invasion, also emerged in bony fish, but the neural sources of buccal versus lung rhythms are unknown. Amphibians (frogs and tadpoles) have two (perhaps three<sup>1</sup>) coupled oscillators. They generate buccal (mouth) breaths (60–90 per minute) that originate from a caudal oscillator (rhombomeres 7–8) and ‘lung’ breaths (~6 per minute, at pH 7.4) that originate from a rostral oscillator (rhombomeres 4–5)<sup>1,6,7</sup>.

Terrestrial vertebrates recruited spinal motor neurons and axial muscles to power episodic (reptiles) or continuous (birds and mammals) aspiration breathing — so-called because negative pressure draws air in. Intercostal and abdominal muscles power the pump in reptiles and birds, but evolution of the mammalian diaphragm, a highly efficient inspiratory muscle relatively free of postural and locomotor constraints, marks the apotheosis of the aspiration pump. There is no consensus on the number of oscillators in reptiles and birds, but there are two (possibly three) oscillators in mammals (see main text). The preBötC is derived from rhombomeres 7–8, akin to the buccal oscillator of amphibians, whereas the lateral parafacial nucleus (pF<sub>L</sub>) oscillator and the chemosensing ventral parafacial nucleus (pF<sub>V</sub>) are derived from rhombomeres 3 and 5 (ref. <sup>8</sup>) (the involvement of rhombomere 4 remains unclear), similar to the ‘lung’ oscillator of amphibians. The evolution and development of the postinspiratory complex (PiCo)<sup>9</sup> have not been addressed.

In summary, at least two segmentally organized, coupled oscillators generate the vertebrate breathing rhythm. The dominant oscillator is not conserved, but varies phylogenetically from expiratory to inspiratory dominance, and shifts from a pontine site in jawless fish to progressively more caudal medullary positions in amphibians and mammals.



## References

1. Baghdadwala, M. I., Duchcherer, M., Trask, W. M., Gray, P. A. & Wilson, R. J. A. Diving into the mammalian swamp of respiratory rhythm generation with the bullfrog. *Respir. Physiol. Neurobiol.* **224**, 37–51 (2016).
2. Milsom, W. K. Evolutionary trends in respiratory mechanisms. *Adv. Exp. Med. Biol.* **605**, 293–298 (2008).
3. Champagnat, J. & Fortin, G. Primordial respiratory-like rhythm generation in the vertebrate embryo. *Trends Neurosci.* **20**, 119–124 (1997).
4. Martel, B. *et al.* Respiratory rhythms generated in the lamprey rhombencephalon. *Neuroscience* **148**, 279–293 (2007).
5. Hoffman, M., Taylor, B. E. & Harris, M. B. Evolution of lung breathing from a lungless primitive vertebrate. *Respir. Physiol. Neurobiol.* **224**, 11–16 (2016).
6. Torgerson, C. S., Gdovin, M. J. & Remmers, J. E. Ontogeny of central chemoreception during fictive gill and lung ventilation in an in vitro brainstem preparation of *Rana catesbeiana*. *J. Exp. Biol.* **200**, 2063–2072 (1997).
7. Vitalis, T. Z. & Shelton, G. Breathing in *Rana Pipiens*: the Mechanism of Ventilation. *J. Exp. Biol.* **154**, 537–556 (1990).
8. Dubreuil, V. *et al.* Defective respiratory rhythmogenesis and loss of central chemosensitivity in *Phox2b* mutants targeting retrotrapezoid nucleus neurons. *J. Neurosci. Off. J. Soc. Neurosci.* **29**, 14836–14846 (2009).
9. Anderson, T. M. *et al.* A novel excitatory network for the control of breathing. *Nature* **536**, 76–80 (2016).