



**Figure 6 | Dorsoventral patterning and CNS evolution. a**, Gene expression summary. Dotted lines indicate that the expression does not extend along the entire trunk. **b**, Proposed scenario for the evolution of neuroectodermal patterning systems in Bilateria. A nerve net, and the anteroposterior (AP; OA, oral–aboral) and BMP axial patterning predate the Cnidaria–Bilateria split, and were present in the Bilateria ancestor.

*nkx2.1* paralogues, *nkx2.2*, and *pax6* are all in distinct brain domains of the juvenile rotifer (Fig. 5a). Only the gene *nkx6* is detected in two posterior trunk cells (Fig. 5a). As in brachiopods and nemerteans, the trunk CNS comprises two VNCs, and additional paired dorsolateral nerves (Fig. 5b). The trunk expression of *nkx6* probably corresponds to the vesicle ganglia<sup>1</sup>, but it is not related to motor neurons, as inferred by the expression of *Hb9* and *ChAT* (Extended Data Fig. 9a). Therefore, spiralian with paired VNCs deploy the dorsoventral transcription factors without a consistent association with their trunk neuroanatomies.

### Dorsoventral patterning in Annelida

To investigate the conservation of the dorsoventral patterning in Annelida, the only spiralian lineage with a medially condensed VNC<sup>1,5</sup>, we studied the annelid *O. fusiformis*, which belongs to the sister lineage to all remaining annelids<sup>33</sup>. Remarkably, this annelid deploys the dorsoventral transcription factors differently from *P. dumerilii*<sup>12,34</sup>. Besides the gut-related expression of *nkx2.1* (ref. 30), *nkx2.2*, and *nkx6* in embryos and larvae, the ventral ectodermal midline expresses *nkx6*, *pax3/7*, and two *msx* paralogues (Fig. 5c and Extended Data Fig. 9b). Additionally, *pax6* and *pax3/7* show more lateral larval expression domains (Fig. 5c). However, the ventral ectoderm of the juvenile only expresses *nkx6* and *msx-b* (Fig. 5c and Extended Data Fig. 9c). As in most other annelids<sup>1</sup>, the adult CNS includes a VNC in *O. fusiformis*, which is not yet present in the early larva<sup>35</sup> (Fig. 5d). In the juvenile, only the expression of *nkx6* and *msx-b* relates to the location of serotonin (Fig. 5d) and motor neuronal markers (Extended Data Fig. 9d). Therefore, the dorsoventral patterning system also varies among annelids with a homologous condensed VNC, and between larval<sup>12</sup> and adult stages<sup>34</sup> (Extended Data Fig. 10a).

### Discussion

Our study provides compelling evidence that the genes involved in the dorsoventral patterning of vertebrate, *Drosophila*, and *P. dumerilii*

The ancestral nephrozoan neuroanatomy remains unclear (question mark). The dorsoventral (DV) patterning system is not tied to the CNS arrangement in Bilateria (as in Chordata and Annelida). In red, lineages analysed in this study. The green circle with red border indicates that there are annelids with and without the dorsoventral patterning.

nerve cords do not show a similar staggered expression in the nerve cords of xenacoelomorphs and many spiralian lineages (Fig. 6a and Extended Data Fig. 10a, b). Although dorsoventral transcription factors define ectodermal domains in the larval brachiopod trunks and the nemertean juvenile (Fig. 6a), these do not necessarily correlate with the trunk CNS and the location of neuronal markers (Fig. 6a). Indeed, the cell lineage relationships between the early ectodermal expression domains and specific neuronal cell types<sup>4,9,12</sup> are unclear, even in *Drosophila*<sup>9,32</sup>, and still need to be broadly and functionally tested. Our findings demonstrate that the expression of dorsoventral transcription factors not only differs between species with multiple nerve cords but also between spiralian that share a medially condensed homologous VNC. A similar case is observed among chordates, where the cephalochordate<sup>36</sup> and tunicate<sup>37</sup> neural plates only partly show the vertebrate molecular arrangement (Extended Data Fig. 10b and Supplementary Table 2), which is probably not a secondary loss given the absence of the dorsoventral patterning in Hemichordata<sup>10,11</sup>. Therefore, the expression of dorsoventral transcription factors evolved independently from the trunk neuroanatomy at least in certain bilaterian lineages, which restricts the use of this patterning system to homologize CNS anatomies<sup>4,7,13</sup> and neuronal cell types<sup>2,4</sup>.

The similarities in the expression of anteroposterior and BMP patterning systems in Cnidaria and Bilateria<sup>7,20,24</sup> suggest that these mechanisms predate the Cnidaria–Bilateria split (Fig. 6b). However, these systems are deployed in organisms within these clades with diffuse nerve nets and/or centralized nervous systems, which indicates that their ancient role was probably general body plan regionalization<sup>8</sup>, and not CNS patterning and neurogenesis<sup>2,7</sup>. This also limits their use to homologize CNS anatomies. However, the evolution of the dorsoventral patterning of the nerve cords is more complicated (Extended Data Fig. 10c). If the similarities in dorsoventral CNS patterning between vertebrates, flies, and *P. dumerilii* are homologous and thus reflect the ancestral bilaterian (or nephrozoan) state<sup>4,7,12,13</sup>, then this patterning