SUPPLEMENTARY MATERIAL

From spiral cleavage to bilateral symmetry: The developmental cell lineage of the annelid brain

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The supplementary material contains Supplementary discussion, Supplementary materials and methods and supplementary figures 1-5:

1) Supplementary figure 1 – Comparing multiple embryos
2) Supplementary figure 2 – The consensus tree of the lineage
3) Supplementary figure 3 – In situ atlas
4) Supplementary figure 4 – More details about establishing bilateral symmetry
5) Supplementary figure 5 – The dynamics of early *otx* expression

In addition, not included in this file is the supplementary package 1 that is deposited as a separate archive file.

6) Supplementary package 1 (separate file):

   1a) Supplementary movie1 (the z-projection of the Reference lineage), a . tif file.
   1b) The track of the reference lineage, a .txt file.
   1c) The Macro package (visualizing), an .ijm file.
   1d) The documentation to the Plugin, a .pdf file

Supplementary discussion

Comparison of the cell lineage of the differentiated cell types with other spiralians

For the first time present the lineage of all cells including differentiated neurons of the larval nervous system, also integrating the previous reports of the cell lineage of the primary prototroch cells (Dorresteijn, 1990).

We fully addressed the lineage of the accessory trochoblasts, traditionally reported as cells 1m-12 (Ackermann et al., 2005; Dorresteijn, 1990). The descendants of 1m-12 form accessory trochoblasts (1a-c-122 and 1a-c-*1212*) characterized by a tight association with primary trochoblasts. Interestingly, their cell lineage is different from accessory trochoblasts in the polychaetes *Amphitrite* and *Podarke* (1a-2222-1c-1222) but partially similar to the mollusk *Dentalium*. Similarly to other polychaetes, the progeny of 1d-12 migrates posteriorly and probably gives rise to the antero-median part of the dorsal hyposphere (Nielsen, 2004) and references therein).

The lineage of the cells (1cd-11221) migrating laterally and forming the “head kidneys” posteriorly to the prototroch agrees with previous reports from *Nereis* (Wilson, 1892). Another population of migratory cells (1a-12111/1c-12111 and 1a-1122112/1b-1122112) leaving the episphere posteriorly might contribute to the trunk domains of 1a/b/c origin observed by (Ackermann et al., 2005).

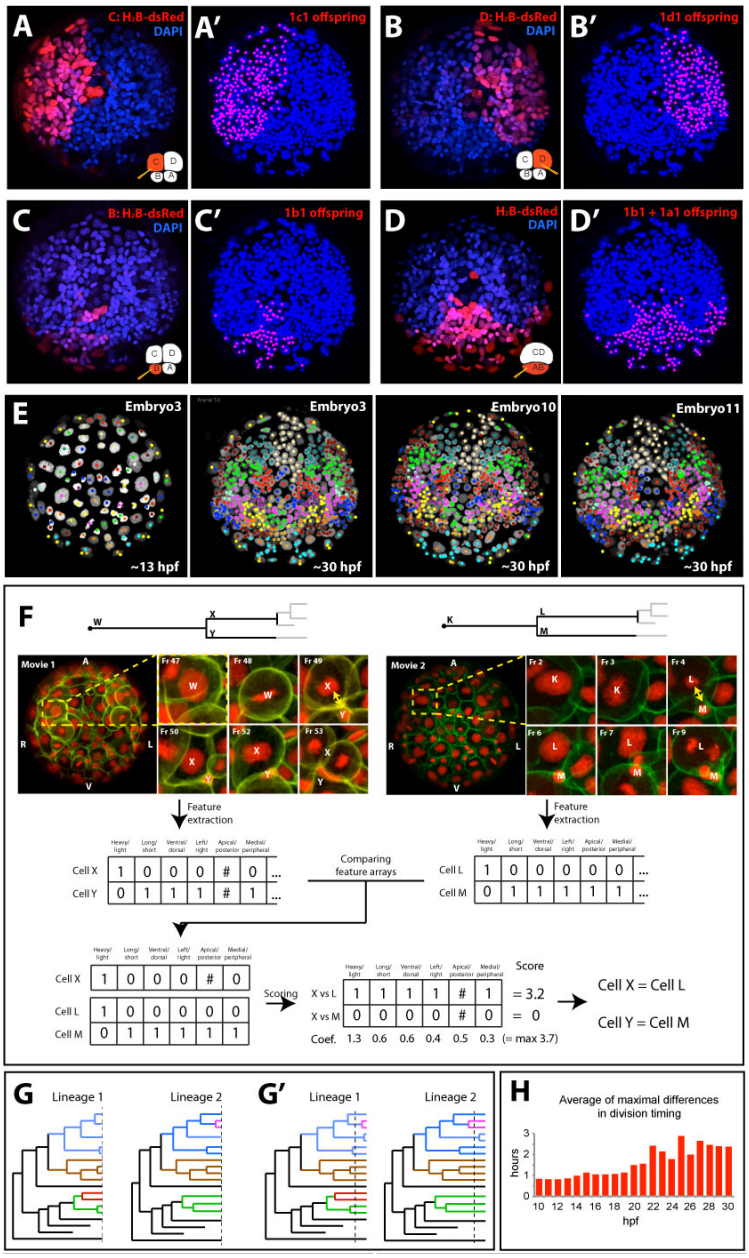
The apical organ is assumed to be derived from the apical rosette cells, although actual cell lineage studies are mostly lacking (Nielsen, 2005a). The cells forming the apical tuft in *Platynereis* are 1c-1111/1c-1112 (the ampullary cells) and 1d-1111/1d-1112 (the crescent cells). Similarly, in the mollusk *Dentalium*, the $1a^{111}$–$1d^{111}$ and $1a^{1121}$–$1b^{1121}$ become the apical organ, with the apical tuft developing from $1c^{1111}$ and $1d^{1111}$ (Nielsen, 2004).

Supplementary materials and methods

Comparing the lineage of multiple embryo

Since the tree topology is given by the track, identifying corresponding cells requires walking through the tree from the root and assigning the corresponding cell among movies at each cell division. As the rooting point, the corresponding cells need to be identified by the user in the first frame of the 4D-stack. At each cell division, the algorithm extracts multiple “features” (relative spatial position of the daughter cells, future cell cycle length, number of offspring), creates feature matrix for each daughter cell and based on matrix similarity assigns the corresponding daughter cells between two tracks. Then the algorithm continues along the tree.

Our recordings cover the first ~34 hours of development and there are still many cell divisions to come after the last frame of the recording. Due to the increasing asynchrony between division timing (Supplementary Fig. 1H) corresponding cell divisions can happen before the end of one recording but after the last frame of another recording, leading to a false-positive differences in the comparison (Supplementary Fig. 1G-G’). A solution to this problem is to compare the recordings at earlier timepoint and use the remaining data as “known divisions to come”. We estimated a safe timepoint to compare the recordings at 30hpf (~3 hours before the last frames), since the average of maximum difference in cell division across the three time-lapse recordings increases with developmental time and reaches about 2.5 hours between 30-34hpf (Supplementary Fig. 1H).



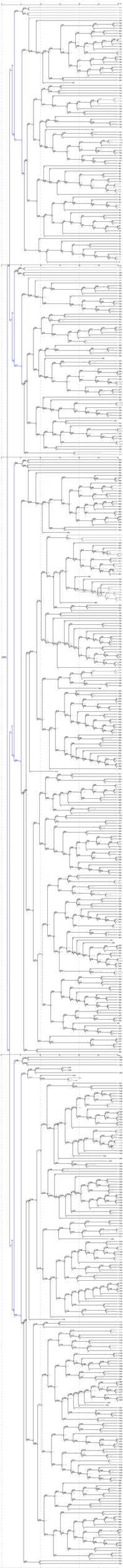
Supplementary figure 1: Comparing the cell lineage between multiple embryos.

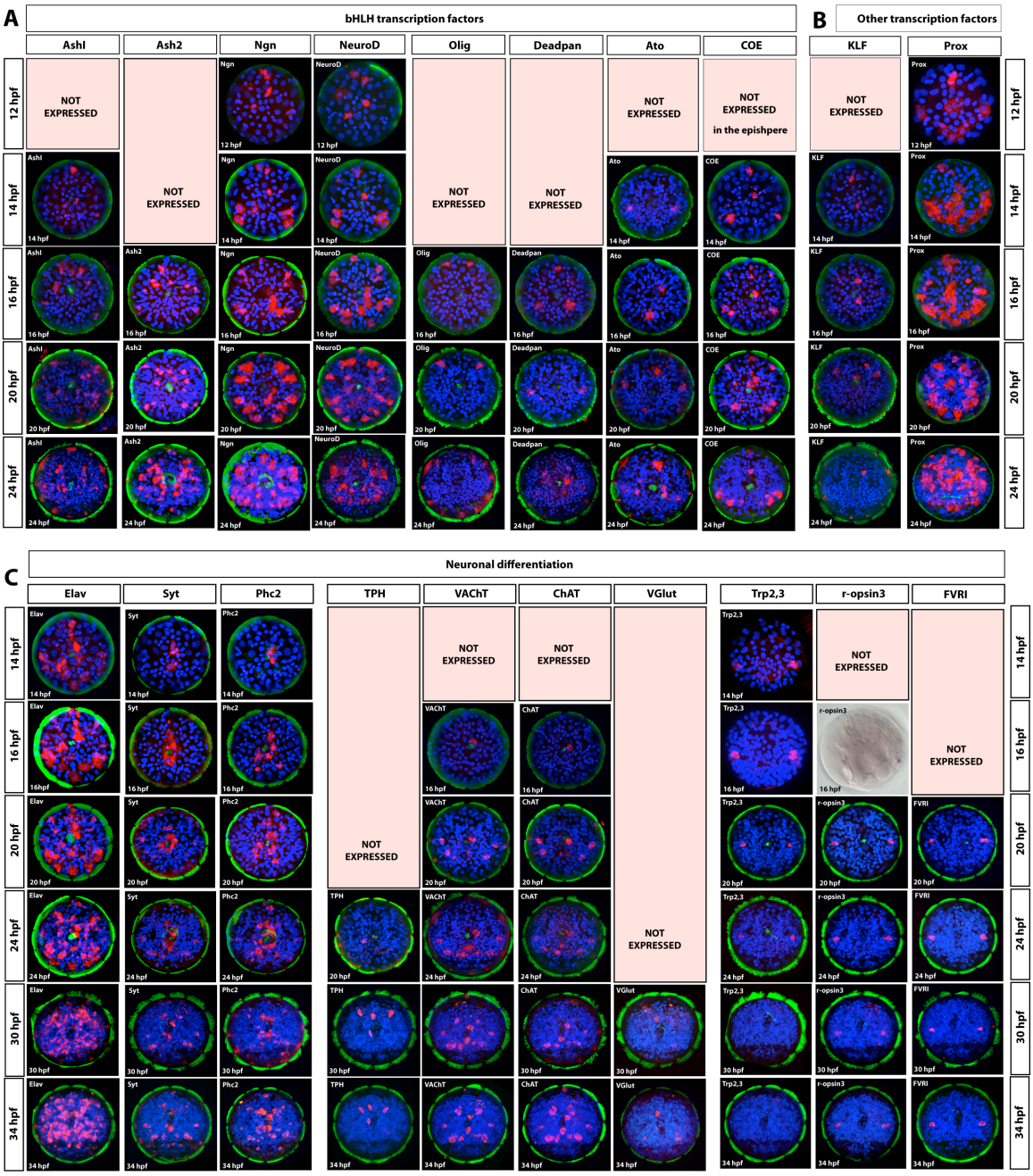
(A-D') The comparison between the clonal domains revealed by injections of H2B-mCherry mRNA into a blastomere and the clonal domain of the reference lineage movie. (E) The comparison of the clonal domains originating from ~13hpf in three different embryos. (F) Multiple "features" of each cell division are extracted from the tracking information and the features are compared against each other to create a truth table for each cell (eg. long/short – the cell having longer (1) or shorter (0) cell cycle etc. for more details, see supplementary materials and methods). The feature arrays are compared between the movies to score the similarity and assign the correspondig cells. (G-G') The problem of assessing the differences between incomplete lineage trees: Due to the asynchrony in cell division timing, some of the corresponding divisions happen after the last frame of a given movie (dashed line) - *eg* the magenta lineage within the blue domain in Lineage1 divides only later and therefore does not represent a real difference in cell division pattern. In contrast, the red cells within the green sublineage do not divide in Lineage2 and therefore represents a real difference. (H) The differences in cell division timing increase with time, reaching around 2.5 hours at 30hpf.

Supplementary figure 2: **The consensus tree of the episphere development from fertilization until 30 hpf.**

The previously described early cell lineage is highlighted in blue (Dorresteijn, 1990). The black branches represent a consensus of three embryos. If one embryo differs from the remaining two, the tree topology based of the two embryos is shown in gray. The horizontal error bars at cell division time points represent the minimal and maximal time point observed for that cell division. The numbers rNNN (eg. r214) above the branch represent the unique cell ID that can be used to find/label the cell within the FIJI plugins. The full lineage name can be read from the characters (1, 2, a, b) below the branches: Following the cell lineage trajectory starting from one of the micromere names (1a, 1b, 1c and 1d) and subsequently adding these characters at each cell division the full lineage name is obtained. For example the developmental trajectory of the cell r215 (posterior prototroch cell at 4 o'clock, #4 in Table I) is *1a*-r210(*2*)-r214(*2*)-r215(*1*) resulting lineage name *1a-221*. The annotations of the known differentiated cell types from Table I are shown at the end points of the branches.

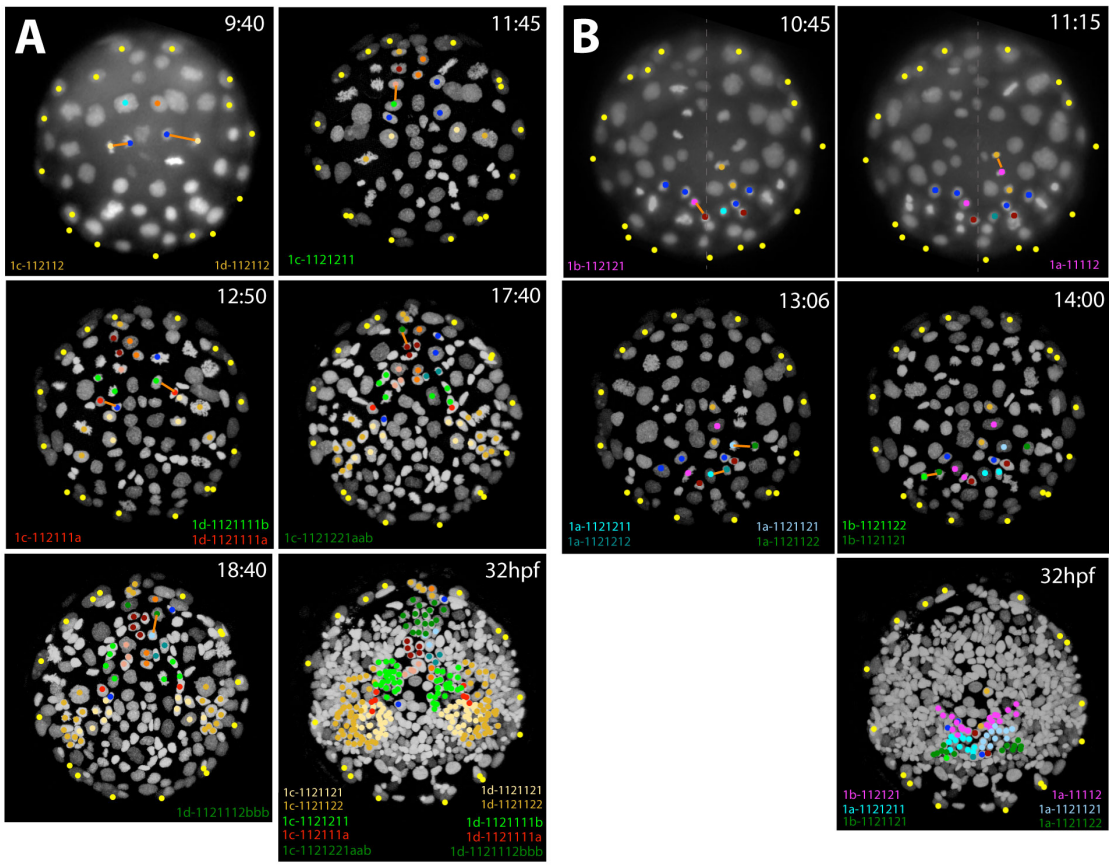
Supplementary figure 2



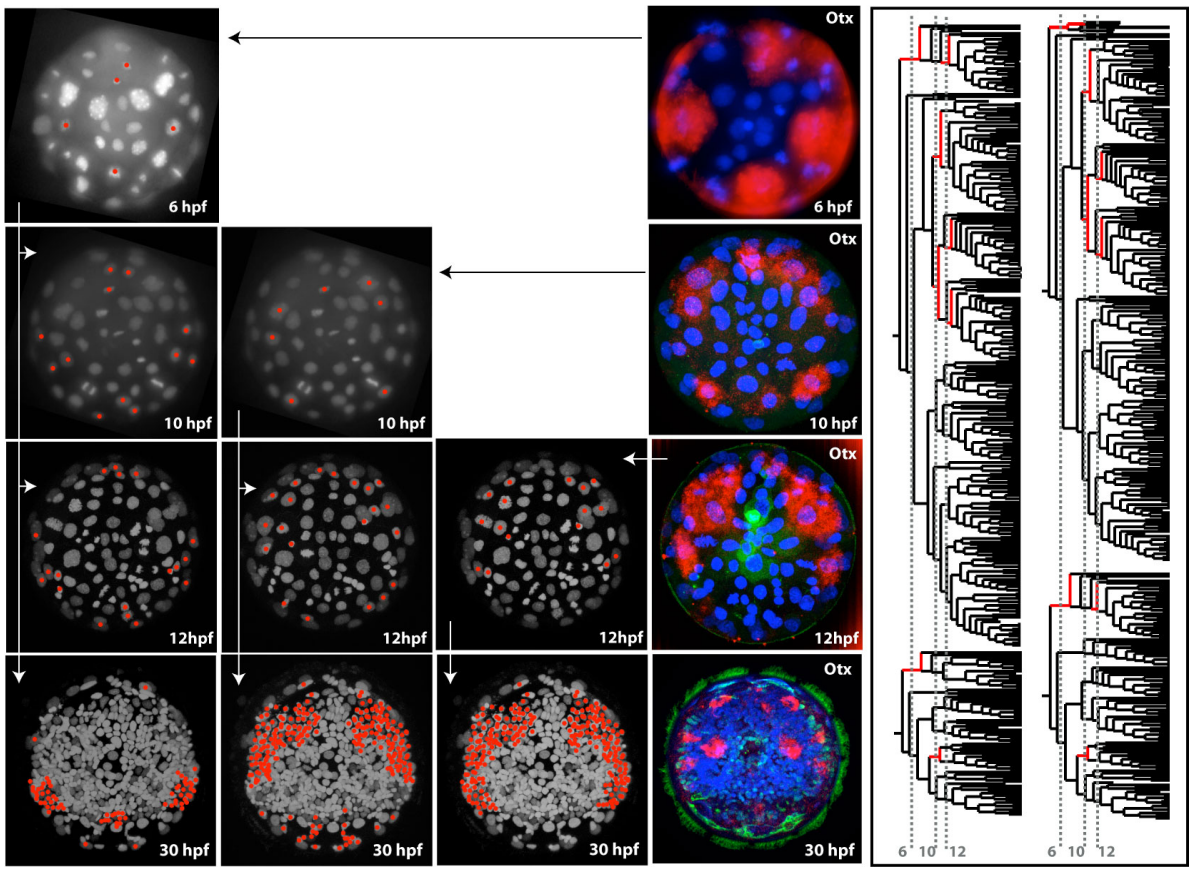


Supplementary figure 3: A gene expression atlas of the episphere between 12-34 hpf.

(A) The expression of bHLH transcription factors. (B) The expression of KLF and Prox transcription factors. (C) The expression of neuronal differentiation markers. Embryos were counterstained with DAPI to reveal the nuclei, anti-Acetylated-Tubulin to visualize axonal projections and ciliary band.



Supplementary figure 4: Establishment of bilateral clonal domains in the medial region. The snapshots of the cell divisions producing bilaterally symmetric clones within the apico-ventral region (A) and the apico-dorsal region (B). Note that multiple cells producing bilaterally symmetric clones at 32hpf originate at different developmental times from different cell divisions (orange lines). Rather than similar lineage, the position of the cell seems to be important for adopting bilateral behavior in the medial region.



Supplementary figure 5: The dynamics of early Otx expression
Otx expression visualised by whole-mount in situ hybridization (column next to the lineage trees) was mapped on lineage movie and lineage tree at corresponding stages. The comparison of theoretical clonal expansion (down the columns) of Otx-expressing cells at different stages to real expression pattern at a given stage shows that Otx expression is not clonal and that the gene is dynamically switched on/off between cell cycles.

Supplementary references:

Dorresteijn, A.W.C. (1990). Quantitative-Analysis of Cellular-Differentiation during Early Embryogenesis of Platynereis-Dumerilii. Rouxs Archives of Developmental Biology *199*, 14-30.