Figures and figure supplements

Distribution of neurosensory progenitor pools during inner ear morphogenesis unveiled by cell lineage reconstruction

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Figure 1. Expansion of the neuroblast delamination domain and formation of the SAG rudiment. (a) Overview of the imaging and image processing strategy: inner ears of zebrafish embryos stained for cell membrane, nucleus and cell fate markers were imaged between 14-42 hpf. Image datasets were processed by nucleus center detection, cell tracking and cell shape segmentation. Data were validated and curated (Figure 1—figure supplement 1). (b–d) Time-lapse stills showing the posterior expansion of the neuroblast delamination domain over time; 3D-rendering of segmented epithelial neuroblasts (green) in context of the otic structure (plasma membranes in magenta) at indicated times; insets display only the segmented delamination domain with the otic vesicle contour in white. ID Dataset: 140210aX; see Figure 1—figure supplement 2d for additional analyses. (e–g). Time-lapse stills showing a segmented delaminating neuroblast (red; Video 2); (e’–g’) magnifications of framed regions in (e–g). ID Dataset: 140426aX. (h–i) Still images from Video 1 displaying: otic tissue architecture (h), and cellular distribution (i) upon SAG formation. Reconstructed cell centers are color-coded according to cell position/identity (see legend). ID Dataset: 140423aX. SAG/ALLg, statoacoustic/anterior lateral line ganglia. AM/PM, anterior/posterior maculae.

DOI: 10.7554/eLife.22268.003
Figure 1—figure supplement 1. 3D+time image analysis pipeline. Information about plasma membranes, nuclei and cell fates was collected upon imaging the inner ears of zebrafish embryos for several hours (14-42 hpf; Table 1) under a Zeiss Lightsheet Z.1 microscope (3D+t SPIM imaging). The acquired data were preprocessed to generate the high-resolution datasets to be launched in BioEmergences platform (Faure et al., 2016, Olivier et al., 2010) for cell center detection and automatic tracking. Data were validated, curated and analyzed using an ad-hoc strategy based on Mov-IT, a custom-made graphical interface (Faure et al., 2016), which offers the tools for segmentation and tracking of cells to accurately reconstruct their positions, movements and divisions. The high-resolution datasets and reconstructed lineages were used for qualitative and quantitative studies of the indicated biological processes (Table 2). The cohort of embryos used in the study can be found in Table 1.

DOI: 10.7554/eLife.22268.004
Figure 1—figure supplement 2. Posterior expansion of the otic neuroblast delamination domain. Tg[neuroD:GFP] embryos were injected with lyn-TdTomato mRNA at 1cell-stage and imaged from 14.5 hpf onwards. Embryos express GFP (green) in neuronal progenitors and differentiating neuroblasts, and TdTomato in all cell membranes (magenta). In the case of the inner ear, GFP is expressed in epithelial neuroblasts just prior to delamination and in the SAG neuroblasts. (a–c) Still image views of the ventral otic vesicle at the indicated time points showing the quick expansion of the delamination domain in the otic epithelium within 2 hr from anterolateral to posteromedial regions; note that at this stage the rudiment of the adjacent ALLg is already visible. (a’–c’) Transverse views are digital reconstructions along the lines indicated in (a–c) and illustrate that the onset of neuroblasts’ delamination progresses from lateral to medial domains (see arrowheads). ALLg, anterior lateral line ganglion; SAG, statoacoustic ganglion; nt, neural tube. The otic vesicle contour is depicted in white. ID Dataset: 140210aX. (d) Plot depicting the posterior expansion of the neuroblast delamination domain as assessed by neuroD-GFP expression. The position of posterior-most neuroD-GFP expressing cells in the otic epithelium, and the anterior and posterior edge of the otic vesicle (dorsal view) were assessed over time (see scheme). The plot displays the position of posterior-most GFP epithelial cells as a percent of the AP otic vesicle length (ID datasets: 140306aX, 140125aX, 140210aX).

DOI: 10.7554/eLife.22268.005
Figure 2. The organization of cells within the SAG relies on specific temporal and spatial cues. (a) Flat representation of the neuroblast lineage tree with branches indicating cell divisions. The x-axis displays the time of embryonic development in hours post-fertilization (hpf). Neuroblast lineages are displayed from the moment of delamination onwards and ordered and color-coded according to delamination timing (intervals: 18–20 hpf white, 20–22 hpf yellow, 22–24 hpf orange, 24–30 hpf red). Some cells were not tracked until the end of the sequence, and are depicted as interrupted lines. The extensive cell loss during the early stages of delamination (18–22 hpf) was verified in a second embryo; in both cases, about 25% (23.2% and 26.8%) of the otic epithelial cells at 18 hpf exit by delamination in the consecutive four hours. (c–c’, e–e’, g–g’) Neuroblasts within the SAG (n = 144 of roughly total n = 250) were backtracked to their progenitor state in the epithelium (n = 98; b,d,f; Videos 3 and 5). Cell lineages were color-coded for: time of delamination (b–c’; same intervals as in (a)), position in the epithelium along the AP (d–e’), or ML (f–g’) axes. Note that ML organization of neuroblasts within the SAG (c–c’) relies on their delamination order, and that the blue/white/red epithelial pattern (d–e’, neuroblasts AP position) but not the green/white/red one (f–g’, neuroblasts ML position) is maintained in the SAG over this time period (18–30 hpf). Reconstructed cell centers were displayed as colored-dots together with the corresponding raw images (plasma membranes in grey level). (b,d,f) dorsal views; (c,e,g) ventral views; (c’,e’,g’) lateral views. Anterior is always to the left. For this analysis, Tg[cldnb:lynGFP] Tg[Isl3:GFP] line was injected with H2B-mCherry mRNA at 1 cell-stage (Tables 1–2). ID Dataset: 140426aX; see Figure 2—figure supplement 1 for additional analysis.

DOI: 10.7554/eLife.22268.010
Figure 2—figure supplement 1. Time of delamination and position of epithelial neuroblasts prefigure their location within the SAG. Tg[cldnb:lynGFP] Tg[Brn3c:GFP] embryo was injected with H2B-mCherry at 1cell-stage, imaged and analyzed from 16 hpf (Table 1). Reconstructed cell centers from neuronal progenitors were color-coded for time of delamination (a), or position along the AP axis in the otic epithelium (c), and followed from 18 hpf to 24 hpf. Note that: (i) among the delaminated neuroblasts from 18 hpf to 24 hpf, those delaminating earlier (white cell centers in a-b) are more medially located in the SAG than those delaminating later (purple cell centers in a-b); and (ii) the relative position of neuronal precursors along the AP axis in the otic epithelium is conserved within the SAG (see cyan anterior cells vs. red posterior cells in c-d'). ID dataset: 140423aX.
DOI: 10.7554/eLife.22268.011
Figure 3. Clonal analysis of neuroblasts. (a) Lineages of neuroblasts (epithelial: colored; delaminated: white) ordered by time of division and grouped according to the division behavior - dividing before (red, top) or after (orange, bottom) delamination. Each line corresponds to a single neuroblast. Discontinued lines represent cells that were not tracked further. The x-axis displays the corresponding time of embryonic development (hpf). (b) Box plot illustrating the temporal delay in delamination between sister cells (Figure 3—figure supplement 1d). (c) Illustration of neuroblast division behavior colored as in (a). (d) Dynamic map of neuronal progenitors (orange circles) and their epithelial neighboring cells (grey circles) in the context of the whole otic vesicle (grey dots) over time; see Video 8 for the 24 hpf animation. The color intensity of cell centers depicts the position of cells along the dorsoventral axis of the otic vesicle. The map was built after following the lineages from 18 hpf to 26 hpf of all encircled cells. Note how neuroblast delamination impacts on the size and position of the progenitor domain (orange cell centers) over time. Tether cells are depicted as black circles. For this analysis, Tg[cldnb:lynGFP] Tg[isl3:GFP] line was injected with H2B-mCherry mRNA at 1cell-stage (Tables 1–2). ID Dataset: 140426aX.
DOI: 10.7554/eLife.22268.015
Figure 3—figure supplement 1. Spatial distribution of epithelial neuroblasts according to division behavior or delamination time. Tg[cldnb::lynGFP] Tg[Isl3:GFP] embryo was injected with H2B-mCherry at 1cell-stage, imaged and analyzed from 18 hpf to 36.2 hpf (Table 1). Images of (a) nuclei as Maximal Intensity Projection of few orthoplanes of the ventral otic vesicle, and (b) plasma membranes as 3D-rendering are displayed. (c) Spatial distribution of neuroblasts, whose reconstructed cell centers were color-coded according to their division behavior -before or after delamination- (Figure 3). Out of 116 tracked epithelial neuroblasts, 42 divide before delamination (red), 40 do so after delamination (orange), and 34 do not divide within this time window (blue). Note that there is no preferential spatial distribution of cells for these features. (d) Reconstructed cell centers of neuroblasts (n = 131) were color-coded according to time of delamination: 18–20 hpf white, 20–22 hpf yellow, 22–24 hpf orange, 24–30 hpf red. Neuroblasts giving rise to two sister cells falling into distinct delamination intervals are shown as bicolored cell centers (n = 11). ID Dataset: 140426aX.

DOI: 10.7554/eLife.22268.016
Figure 4. Spatiotemporal pattern of hair cell differentiation and map of sensory progenitors. Differentiated hair cells were tracked during 18 hr in control and MO-neurog1, and reconstructed cell centers were color-coded according to the differentiation time displayed in the legend (Video 6). (a–b, f–g) Spatiotemporal pattern of hair cell differentiation of the anterior/posterior maculae (AM/PM); reconstructed colored cell centers overlaid with the corresponding raw images (hair cell fate in grey level) from Tg[Brn3c:GFP] embryos; (c, h) reconstructed colored cell centers in lateral view. Note how the temporal but not the spatial development is altered in the MO-neurog1 PM (see Figure 4—figure supplement 1). (d, i) Map of hair cell progenitors in the whole otic vesicle (Videos 7–8); the maps were generated by backtracking the differentiated PM hair cells (e, j). ID Datasets: 140507aX for control, 140519aX for MO-neurog1; see Figure 4—figure supplement 1 for additional analyses.

DOI: 10.7554/eLife.22268.017
Figure 4—figure supplement 1. Temporal pattern of hair cell differentiation in AM and PM. Tg[Bmn3c:GFP] embryos injected with H2B-mCherry mRNA at 1-cell-stage (with/without MO-neurog1) were imaged from 24 hpf to Figure 4—figure supplement 1 continued on next page.
42 hpf. (a) Graphs showing the increase of differentiated hair cells in the anterior (AM) and posterior (PM) maculae over time; the final number of differentiated hair cells at 42 hpf is indicated. Each line corresponds to a differentiated hair cell plotted from and color-coded for time of differentiation (see legend). Tether cells are depicted as white lines. Note the temporal differences in the development of the PM between control and MO-neurog1 embryos, with no major changes in the spatial pattern (Figure 4). Asterisk depicts an apoptotic hair cell.

ID Datasets: 140507aX for control, 140519aX for MO-neurog1. (b) Spatiotemporal pattern of hair cell differentiation of the AM and PM of both ears of Tg[Brn3c:GFP]Tg[Isl3:GFP] embryo injected with lyn-TdTomato and H2B-mCherry mRNA at 1cell-stage (imaged from 25 hpf to 36 hpf). Differentiated hair cells were tracked and reconstructed colored cell centers overlaid with the corresponding raw images (hair cell fate/SAG in grey level as 3D-rendering). Graphs showing the increase of differentiated hair cells in the AM and PM over time; the final number of differentiated hair cells at 36 hpf is indicated. ID Dataset: 140402aX. (c) Spatiotemporal pattern of hair cell differentiation of AM and PM. Tg[Brn3c:GFP] embryo injected with lyn-TdTomato mRNA at 1cell-stage and imaged from 25 hpf to 45 hpf. Graphs displaying the increase of differentiated hair cells in AM/PM. Hair cell progenitors and differentiated hair cells were tracked (except for the one encircled in blue), and reconstructed colored cell centers overlaid with the corresponding raw images (MIP of lynTomato and GFP signal of a few slices in grey level). ID Dataset: 130326aX. Legend in panel (a) applies to all plots and diagrams of the figure. DOI: 10.7554/eLife.22268.018
Figure 5. Heterogeneous cell behavior in the non-sensory and sensory domains. Neighboring cells in the non-sensory and sensory domains of control (a–f) and MO-neurog1 (g–l) were tracked and reconstructed cell centers were color-coded according to cell proliferation/differentiation status (see legend in (c); Video 9); they were plotted on the top of the corresponding raw images (a–b,d–e,g–h,j–k; nuclei in grey level), or in graphs over time (c,f,i,l) displaying the total number of cells in each domain and their status in the course of the video. Note the Figure 5 continued on next page.
differences in the graphs between non-sensory and sensory domains, but not between control and MO-neurog1 embryos. (m–n) Estimated local cell densities at 24 hpf are represented by color-coded cell centers across the whole otic epithelium (Video 10). Tg[Brn3c:GFP] embryos injected with H2B-mCherry and with/without MO-neurog1 at 1cell-stage were used for full lineage reconstruction (Tables 1–2). Anterior is always to the left. ID Datasets: 140507aX for control, 140519aX for MO-neurog1; see Figure 5—figure supplement 1 for additional analyses. (o) Graphic depicting the total number of cells in the otic vesicles for wild type (control, n = 3), neurog1mutant in the Tg[is13:GFP] background (n = 3), and MO-neurog1 embryos (n = 2) at 24 hpf. DOI: 10.7554/eLife.22268.022
Figure 5—figure supplement 1. Tissue architecture in sensory and non-sensory domains. Tg[Brn3c:GFP] injected with H2B-mCherry mRNA at 1 cell-stage were imaged and analyzed. (a) Plots at three time points showing the distribution of tracked neighboring otic cells within the non-sensory (blue) or sensory (green) domains in the context of the whole otic vesicle (grey dots). Color intensities represent the nearest neighbor distance (NN-distance, see legend); graph shows the median and quartiles of the NN-distance for each domain over time. Note that cells within the non-sensory territory are more spaced than cells in the sensory domain. ID Dataset: 140507aX. (b) Graph showing the differences in average NN-distances between the ventromedial and dorsolateral domains (top), or ventral and dorsal territories (bottom) of several ears. Note that cells within the dorsolateral/dorsal territories are more spaced than cells in the ventromedial/ventral domains. Cell selections in the context of the whole ear for 140426aX are shown in the left-hand side. ID Datasets: 140507aX, 140519aX, 140430aX, 140426aX. (c–e) Comparison of the volumes of otic vesicles of control and MO-neurog1 embryos at 24 hpf. (c–d) Lateral views of otic vesicle volumes depicting the epithelial surfaces: basal/outer (grey mesh) and apical/inner (green mesh); insets in (c–d) display the corresponding dorsal views. ID Datasets: 140507aX for control, 140519aX for MO-neurog1. (e) Graph showing the average volume of otic vesicles for control (n = 3) and MO-neurog1 (n = 2) embryos in cubic μm. Note the increase of the average volume in MO-neurog1. Tg [Brn3c:GFP] embryos were injected with H2B-mCherry and with/without MO-neurog1 at 1 cell-stage.

DOI: 10.7554/eLife.22268.023