Chase-and-run between adjacent cell populations promotes directional collective migration

Eric Theveneau¹, Benjamin Steventon¹,²,⁴, Elena Scarpa¹, Simon Garcia¹,³, Xavier Trepat³, Andrea Streit² and Roberto Mayor¹,⁵

Collective cell migration in morphogenesis and cancer progression often involves the coordination of multiple cell types. How reciprocal interactions between adjacent cell populations lead to new emergent behaviours remains unknown. Here we studied the interaction between neural crest (NC) cells, a highly migratory cell population, and placodal cells, an epithelial tissue that contributes to sensory organs. We found that NC cells chase placodal cells by chemotaxis, and placodal cells run when contacted by NC. Chemotaxis to Sdf1 underlies the chase, and repulsion involving PCP and N-cadherin signalling is responsible for the run. This chase-and-run requires the generation of asymmetric forces, which depend on local inhibition of focal adhesions. The cell interactions described here are essential for correct NC migration and for segregation of placodes in vivo and are likely to represent a general mechanism of coordinated migration.

Cell migration is a fundamental process in morphogenesis¹,² and cancer metastasis¹,³, and often involves the coordinated movement of different cell types. However, how such coordinated behaviour is achieved remains unknown. Here we investigate this problem in two embryonic cell types: NC and placodes. NC is a highly migratory cell population⁴,⁵ likened to cancer⁶,⁷; placodes are epithelial and contribute to sensory organs⁸,⁹. Their derivatives interact to form several cephalic structures¹⁰–¹³; their precursors lie adjacent to each other and are already typical epithelial and mesenchymal tissues. Interaction of these precursors has not been investigated, but may provide a robust model to study cellular properties emerging through mutual interaction of tissues with different migratory capabilities, such as epithelial cancer and mesenchymal stromal fibroblast¹⁴.

Here we show that placodes form by local cell rearrangements within the epithelium in response to migrating NC. Surprisingly, NC and placode cells engage in a chase-and-run behaviour, with NC cells chasing placode cells by chemotaxis, and placode cells run as they are contacted by NC. We establish the molecular mechanisms underlying these behaviours and demonstrate the importance of this process for the coordinated morphogenesis of the NC and placodes in vivo.

RESULTS

Interaction between NC and placode cells

To study the interaction between placode and NC cells, we focused on cephalic NC and epibranchial placode precursors⁸, which are in direct apposition (Fig. 1a,b and Supplementary Fig. S1a–h). Analysing their movements by time-lapse cinematography in embryos with fluorescently labelled NC and placodes, we found that both populations undergo directional migration (Fig. 1c–i and Supplementary Fig. S1i–l). Double labelling revealed that gaps within the placode region formed precisely where NC cells migrated, suggesting that placodes (Fig. 1j, red) move away from NC (Fig. 1j, green; Supplementary Movies S1 and S2). Indeed, further cell tracking revealed that placode cells move randomly before NC migration (Fig. 1k,m) with a low directionality (Supplementary Movie S4). When cultured next to each other, NC cells move randomly (Fig. 2a) whereas placode cells undergo directional migration (Fig. 2b) with a net increase of directionality and displacement (Fig. 1p,q, brown bars). However, as NC cells arrive, placodal cells switch to directional migration (Fig. 1l,n) with a net increase of directionality and displacement (Fig. 1p,q, black bars, and Supplementary Movie S3). Directional migration is lost when NC cells are ablated (Fig. 1o–q, purple bars, and Supplementary Movie S3), suggesting that this interaction is crucial for placode cell behaviour.

To analyse NC–placode interaction in the absence of surrounding tissues, we set up an in vitro system where NC and placodes are cultured next to each other. Surprisingly, NC and placodal cells engage in a chase-and-run behaviour (Supplementary Movie S4). When cultured separately, NC cells move randomly (Fig. 2a) whereas placode cells hardly move (Fig. 2b). However, like in vivo (Fig. 1a–i), in the presence of NC, placodes switch to directional migration, away from the NC (Fig. 2c and Supplementary Movie S4). Conversely, NC cells seem to be attracted by placodes, which express Sdf1 (Supplementary Fig. S2a–e),

¹Department of Cell and Developmental Biology, University College London, London WC1E 6BT, UK. ²Craniofacial Development and Stem Cell Biology, King’s College London, London SE1 9RT, UK. ³Institut de Bioenginyeria de Catalunya (IBEC), ICREA, and Facultat de Medicina—Universitat de Barcelona, 08028 Barcelona, Spain. ⁴Present address: Institut Pasteur, 75724 Paris cedex 15, France. ⁵Correspondence should be addressed to R.M. (e-mail: r.mayor@ucl.ac.uk)

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a cytokine previously implicated in NC chemoattraction\textsuperscript{15–19}. Indeed, normal NC chemotaxis towards placodes placed at a distance was inhibited by morpholinos (MOs) against Sdf1 or its receptor\textsuperscript{19} (Fig. 2h–k and Supplementary Movie S3). Sdf1 is required only in tissues surrounding the NC (Supplementary Fig. S2h,i), and inhibiting Sdf1/Cxcr4 expression or blocking placode development using an Eya1 MO (ref. 20) equally blocked NC migration. Thus, NC migration requires both placodes and Sdf1 (Supplementary Fig. S2f,g) and blocking chemotaxis impairs the chase-and-run behaviour (Fig. 1d–f and Supplementary Movie S4). Interestingly, inhibition of chemotaxis by Cxcr4MO leads to transient contact between NC and placodes (Fig. 2d), which is however insufficient to promote directional placode movement (Fig. 2g, blue line), suggesting that continued contact between these two cell populations is required. To investigate whether chemotaxis alone accounts for the chase-and-run behaviour, we placed NC cells next to non-placodal ectoderm expressing endogenous Sdf1 (Supplementary Fig. S3 and Movie S6). NC cells were attracted to the ectoderm, but invaded it and no ectodermal run behaviour was observed. Together, these results identify placode precursors as the source of Sdf1 in vivo and establish a chase-and-run interaction between NC and placodes during which NC cells actively chase Sdf1-positive placode precursors and simultaneously repel them.

**Cell adhesion complex between NC and placodes**

Then we looked for the molecular effectors of the NC–placode interaction. Both NC and placodes express N-cadherin whereas E-cadherin is found only in placodes (Fig. 3a–d). *In vitro*, NC and placodes make repeated transient contacts lasting on average 4 min (Fig. 3e,f, first column, Supplementary Movie S7), during which N-cadherin, p120-catenin and α-catenin accumulate at the junction (Fig. 3f–I and Supplementary Movie S8). This indicates the formation of transient cell–cell adhesion complexes and contrasts with the stable accumulation of N-cadherin and p120-catenin between placode cells (Fig. 3m–o). To assess whether these transient NC–placode junctions were functional and able to transmit force we used traction-force microscopy to measure the tension produced at the NC–placodes interface\textsuperscript{21}. Indeed, a net force of 12 nN±2.25 is generated between the two explants. Together, these results show that NC and placodes form transient, but functional, cell–cell adhesion complexes.

**Asymmetric distribution of forces and focal adhesions at the NC–placode interface**

To assess whether local effects at the NC–placode interface promote a break of symmetry that could explain directional placode cell migration, we analysed the distribution of traction forces generated by the placode explant (Fig. 4a–d). When cultured alone, placode cells show radial distribution of traction forces pointing inwards (Fig. 4a,c,e), whereas, when co-cultured with NC cells, their traction forces are mostly aligned with the direction of migration and point towards the NC (Fig. 4b,d,e). This asymmetric distribution of traction forces in placodes is consistent with the direction of their migration. Traction forces require adhesion to the substrate and the size of the focal adhesions correlates with the force generated\textsuperscript{22}. Thus, to explain how the asymmetric forces are generated we analysed the distribution of focal adhesions using phospho-paxillin antibodies (Fig. 4f–r). Indeed, the number of focal adhesions was markedly reduced where placodes contact NC cells (Fig. 4f–h) or other placode cells (Fig. 4i), generating an asymmetric focal adhesion distribution in relation to the cell contact. This process is N-cadherin dependent: focal adhesion asymmetry is lost in the presence of N-cadherin MOs (Fig. 4j,k). To examine whether exposure to N-cadherin alone mimics the effect of placode–NC or placode–placode interaction on focal adhesion distribution, we plated placodes on fibronectin or fibronectin containing N-cadherin. On fibronectin, placode cells formed normal protrusions with large focal adhesions (Fig. 4l), whereas on fibronectin + N-cadherin the average focal adhesion size is markedly reduced, especially at the leading
edge of cells, and their contact-dependent distribution is abolished (Fig. 4m–r). These observations suggest that N-cadherin interferes with focal adhesion maturation rather than their formation. Together, our results show that N-cadherin-dependent cell–cell contacts between placode cells and between placode and NC cells locally inhibit plaque adhesion to the matrix and maturation of focal adhesions. This results in the restriction of traction forces to the free edge of the placode population. In addition, contact between NC and placodes leads to restriction of focal adhesions to the opposite side of the placode cluster, generating traction forces in the direction of placode movement.

**Contact with NC promotes collapse of cell protrusions in placodes**

Placode cells move directionally only after contact with NC cells, suggesting that direct contact somehow polarizes the entire placode cluster and may promote the formation or stabilization of protrusions away from the contact region. We compared the formation and stability of cell protrusion between NC and placode clusters in control conditions and during the chase-and-run (Fig. 5a,b). NC cells facing placodes have stable protrusions (Fig. 5a,b; bars 1 and 2) due to a local increase of Rac1 activity downstream of Cxcr4 in NC cells20. Importantly, no difference in protrusion stability was observed in placode cells away from NC cells or during the chase-and-run (Fig. 5a,b; bars 1 and 2) due to a contact inhibition of locomotion (CIL). This repolarization significantly biases the movement of cells away from the region of cell–cell interactions and thus may account for the placode cell behaviour observed after contact with NC cells. NC cells exhibit CIL for each other19,27, but this behaviour has not been assessed in placode cells. We analysed CIL in collision assays between isolated NC and placodal cells and measured the angle between the directions of migration before and after collision and the average distance between two colliding cells after a given time (Fig. 6a). We used NC–NC collisions as an internal control for a typical CIL response (Fig. 6c, green angles, and e). NC and placodal cells establish only transient contact on collision and move away from each other. After collision the new directionality is biased away from the site of contact (Fig. 6b,c; NC response: green angles; placode response: red angles; Supplementary Movie S11). As a consequence the distance between NC and placodes increases (Fig. 6b, controls; 6d, CTL bar).
Figure 3 NC and placodes form transient, but functional, adherens junctions. (a–d) Double immunostaining for N- and E-cadherin on histological sections through the cephalic regions of *Xenopus* embryos at stage 25. N-cadherin (a) is expressed in NC (arrows) and epibranchial placodes (asterisks) as well as the eye and the otic vesicle (ov). E-cadherin (b) is expressed only in epibranchial placodes and the superficial ectoderm. (c) Merged picture of the green and red channels. (d) Summary of cadherins distribution in NC and placodes. (e) Diagram representing the experimental set-up. (f) From left to right: duration of individual NC-placodes (PL) contact at the interface between the two tissues during the chase-and-run; duration of N-cadherin, p120-catenin and β-catenin accumulations during NC-placodes physical contacts (data collected from 3 independent experiments, error bars: s.d.). (g–l) Dynamics of the formation of transient adherens junctions between NC and placodes. (g, i, k) Confocal images. (g) NC and placodes express N-cadherin–GFP. (i) NC cells express p120-catenin–GFP; placodes are labelled with membrane-mCherry. (k) NC cells express β-catenin–GFP; placodes are labelled with membrane-mCherry. (h, j, l) Variation of fluorescence intensity over time of GFP-bound molecules shown in g, i, k; after background subtraction and normalization. Average from 5 independent cell–cell junctions (error bars, s.e.m.). (m–o) Localization and dynamics of N-cadherin–GFP (m–n) and p120-catenin–GFP (m, o) between placodal cells. Average from 4 independent cell–cell junctions; error bars, s.e.m.
Interestingly, placodes also exhibit CIL when colliding with each other but fail to separate after repolarization (Fig. 6f–h, Supplementary Movies S11 and S12). This failure in plaque separation seems to be due to the expression of E-cadherin, which is absent in NC. Consistently, when E-cadherin expression is forced into NC cells, cells remain attached to each other as observed for placode cells (Fig. 6i). CIL between NC cells requires N-cadherin expression, both in NC and placodes, becomes localized to the cell–cell contact, and that inhibition of plaque protrusions is N-cadherin dependent.

In addition, non-canonical Wnt/PCP signalling mediated by Dishevelled and Wnt11 has been implicated in CIL of NC (refs 28–32). Thus, we investigated the role of N-cadherin and Wnt/PCP in CIL of placode cells. Cells injected with N-cadherin MOs or with a dominant-negative form of Dsh (DshDep+) to inhibit PCP signalling remain in close contact (Fig. 6d) and exhibit random angles of migration after collision as compared with the systematic reorientation in controls (Fig. 6b,c and Supplementary Movie S13). Placodes express the Wnt receptor Fz4 (ref. 33) whereas migratory NC cells express the PCP ligand Wnt11 (Supplementary Fig. S4a–c), which is known to localize to cell–cell contacts to promote Wnt/PCP signalling and CIL between NC cells (refs 34, 35). Blocking Wnt11 in NC cells using a dominant-negative form is sufficient to randomize the response of placode cells to a collision with NC cells in vitro (Fig. 6b,c and Supplementary Movie S13). We then analysed a possible link between N-cadherin and Wnt/PCP and find that although cell–cell contacts are required for PCP signalling, they are not sufficient to trigger it (Supplementary Fig. S4d–m).

These results show that placode cells are repolarized by N-cadherin–Wnt/PCP-dependent CIL when colliding with NC, suggesting that CIL causes placode cell movement away from NC cells during the chase-and-run behaviour.

To investigate this possibility we performed chase-and-run assays and analysed the effect of blocking N-cadherin and Wnt/PCP (Fig. 6j and Supplementary Movie S14). Both treatments markedly impair the
Whereas control NC cells remain in close proximity and progressively 
progression (Fig. 7b, Cxcr4MO) or Wnt/PCP in placode cells (Fig. 7b, DshDep+).

To investigate the importance of the NC-protrusions when growing or collapsing. Asterisks mark the protrusions 
mediated by N-cadherin and Wnt/PCP.

Moreover, our data indicate that both chemotaxis and CIL are 
required for coordinated migration to emerge. As NC cells have CIL 
for each other, we investigated whether overexpression of Sdf1 in one 
NC explant co-cultured with a control NC explant reproduces the 
chase-and-run behaviour (Fig. 6n–s and Supplementary Movie S15). 
Whereas control NC cells remain in close proximity and progressively 
disperse (Fig. 6n,p,q), Sdf1 overexpression in one of the NC explants 
sustains coordinated migration of both groups for several hours 
(Fig. 6o,r,s) in a behaviour akin to NC-placode interaction.

Chase-and-run is required in vivo for NC migration and 
placode patterning

To investigate the importance of the NC-placode interaction in vivo, we performed time-lapse movies of placode cells in control conditions (Fig. 7a,b) and after blocking Sdf1 chemotaxis in NC cells (Fig. 7b, Cxcr4MO) or Wnt/PCP in placode cells (Fig. 7b, DshDep+). Cell tracking (Fig. 7b) shows that control cells undergo directional migration, but this is abolished under experimental conditions as seen 
that collapse after contact between NC and placode cells. (e) Over-time variation of protrusion area in placodal cells with or without contact with NC cells (t) (indicated = 5, n = 50 for 12 time points each; error bars, s.d.). Arrowhead indicates the moment of contact between NC and 
placode cells. (f–h) Stills from time-lapse movies of placodal cells on fibronectin (f), fibronectin + N-cadherin (g) and fibronectin + N-cadherin in low calcium/magnesium solution (h). Arrowheads indicate stable 
or growing protrusions. Red asterisks mark collapsing protrusions. 
(i) Duration of protrusion in placodal cells (n = 6 independent experiments; non-parametric ANOVA (Kruskal–Wallis), P < 0.0001, individual comparisons, **P < 0.001; error bars, s.d.). NCD2, blocking antibody against N-cadherin. Time is in minutes.

Finally, as interfering with CIL in placodal cells affected NC cell in-
vasion (Fig. 6j, DshDep+, NCD2). Inhibition of N-cadherin or Wnt/PCP leads to invasion of placode cells by NC cells as evidenced by the increased overlap between both cell populations (Fig. 6k). Blocking N-cadherin and Wnt/PCP also abolishes the overall response of placodes to NC cells. Placodes move randomly (Fig. 6j, displacement maps), with reduced directionality (Fig. 6l) and do not escape from NC cells (Fig. 6m). Blocking E-cadherin, however, has no impact on NC-placode interactions (Fig. 6j–m, green bars). These data show that coordinated migration of NC and placode cells relies on CIL 
meditated by N-cadherin and Wnt/PCP.

Outgrowth

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on NC migration \textit{in vivo} using different strategies. Control embryos or embryos with a homotypic, homochronic graft of control placodes show normal NC migration (Fig. 7l-m). In contrast, when placodes are replaced by a non-placodal Sdf1-negative ectoderm (Fig. 7n) or by placodes expressing DshDep+ (Fig. 7o) NC migration was clearly inhibited (Fig. 7s-t). When placodes are replaced by non-placodal Sdf1-positive ectoderm, NC cells migrate ventrally but are not organized into streams (Fig. 7p-t). These results show that CIL between NC and placodal cells favours directional NC cell migration \textit{in vivo}. However, in the absence of chemotaxis, CIL does not promote NC cell migration whereas chemotaxis in the absence of CIL is not sufficient to pattern NC migration.

**DISCUSSION**

Our results show that NC cells chase placode cells by chemotaxis in an Sdf1-dependent manner, and placodal cells run as they are contacted by NC, in a mechanism that involves PCP and N-cadherin signalling. This interdependence between NC and placode cells is reminiscent of the popular image of the donkey and a carrot (Fig. 8a). Placodes...
produce a NC cell chemoattractant (Fig. 8h). Physical NC–placode contact directly controls the direction of placodal cell displacement by locally inhibiting cell protrusions (Fig. 8c). In turn, the escape or run behaviour prolongs the directional motion of NC cells by displacing the source of the attractant (Fig. 8d). This phenomenon relies on N-cadherin and Wnt/PCP most probably by inhibiting Rac1 (Fig. 8e–g) and locally increasing RhoA activity19,27. This is a highly original mechanism that ensures a persistent directional migration, which depends on the source of the chemoattractant (placodes) being modified by the attracted cells (NC).

The run phase during the chase-and-run behaviour corresponds to the collective migration of a placode cluster, reminiscent of the migration of Drosophila border and zebrafish lateral line cells23,24. It is well known that cell protrusions such as lamellipodia play an important role in establishing the directionality in single cells as well as in collective cell migration25,26. However, our findings show that unlike border or lateral line cells, placodes move forward not by stabilizing protrusions at the front, but by collapsing protrusions at the back of the cluster. In Xenopus, local rearrangements of placode cells without large-scale cell migration40 have been described previously and we confirm this

Figure 7 Interaction between NC and placodes through CIL and chemotaxis is required for placode and NC migration in vivo. (a,b) Cell tracking analysis of placodal cells (PL) in vivo in controls, after inhibition of chemotaxis in NC cells (Cxcr4MO) or Wnt/PCP in placodal cells (DshDep+) from 3 independent experiments. (c,d) Directionality, (one-way ANOVA: $P < 0.0001$, individual comparisons, **$P < 0.01$; error bars, s.d.) and net displacement (one-way ANOVA: $P < 0.0001$, individual comparisons, **$P < 0.01$; error bars, s.d.) of placodal cells extracted from the tracks shown in (b). (e–k) Zebrafish embryos. (e) Diagrams of the two stages of zebrafish development shown hereafter. (f,g) Sdf1 is expressed in placodal cells (Sox3). (h) Placodal cell distribution in an embryo injected with a control MO (h) or Sdf1MO (i) to block NC cell migration (14 animals analysed, 65% showed a fusion of placodes). A 3D reconstruction of Sox3 staining in (h) and (i) is provided and summarized in the diagram. Dotted lines indicate the placodes. White arrows highlight the distance between the placodes and the neural tube. (j) Distance between placodal cells and the neural tube; 19 animals from 3 independent experiments were analysed; Student’s t-test (two-tailed): **$P = 9 \times 10^{-7}$; error bars, s.d. (k) Average size of the individual placodal domains; 42 animals from 3 independent experiments were analysed; Student’s t-test (two-tailed): **$P = 0.0023$; error bars, s.d. (l–r) NC cell migration after interfering with placodes. (l) Control embryo provided for reference. (m) A homotypic, homochronic graft of control placodes. (n) Placodes replaced by a non-placodal Sdf1-negative ectoderm. (o) Placodes replaced by placodes expressing DshDep+. (p) Placodes replaced by a non-placodal Sdf1-positive ectoderm. Black arrowheads indicate the NC streams migrating normally; the red arrowheads mark the NC streams that stopped prematurely. (q–t) Sections of an embryo with a graft similar to that presented in (p). (s) Summary of the different treatments presented in (l–p). (t) Ratio of NC migration along the dorsal-ventral axis on the grafted side versus the control side for (l–p); 44 animals from 3 independent experiments were analysed; one-way ANOVA: $P < 0.0001$; all conditions compared with the first column; **$P < 0.01$; error bars, s.d.
NC Placodes
Attraction (Fig)
Methods and any associated references are available in the online version of the paper.

Note: Supplementary Information is available in the online version of the paper.

Figure 8 CIL and chemotaxis between NC and placodal cells drives coordinated migration of both cell populations. (a) The overall behaviour of NC and placodal cells is reminiscent of the popular image of the donkey and the carrot, where the donkey (NC) is attracted to the carrot (placodes) but the carrot moves away because of the donkey’s progression. (b) NC cells are attracted to placodal cells owing to Sdf1-dependent chemotaxis. (c) Contact between NC and placodal cells induces CIL. Protrusions are inhibited in placodal cells at the region of contact with NC cells. This breaks the symmetry of the placodal tissue, thus promoting directional movement. (d) The system self-sustains owing to chemotaxis and CIL. The Sdf1 gradient is shown as shades of grey. NC cells are in green; placodal cells are in red. (e–g) Molecular pathways involved in the chase-and-run between NC and placodes (PL). (e) Sdf1 released by the placodes acts on NC cells promoting an increase in Rac activity, which stabilizes protrusions and focal adhesions. NC moves towards placodes (grey arrow). (f) NC moves forward contacting placode cells and triggering a CIL response in both cell types. A transient collapse of cell protrusions and disassembly of focal adhesions. This localized response within the placode cluster generates an asymmetry that leads to directional migration of the cluster away from NC (grey arrow from placodes). (g) During this run phase, placodes continue secreting Sdf1, which will attract NC cells (grey arrow from NC), with the consequent coordinated migration of both cell populations.

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AUTHOR CONTRIBUTIONS
Experiments were designed by E.T., B.S. and R.M., with the participation of X.T and A.S. All experiments were performed in the laboratory of R.M. E.T and B.S. performed most of the experiments. E.S. performed the repolarization and clustering assays. S.G. E.T. and X.T. performed the traction force experiments. E.T., B.S. and R.M. analysed and interpreted data, with the participation of X.T. and A.S. E.T., B.S., A.S. and R.M. wrote the manuscript. All authors commented on the manuscript.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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**METHODS**

**Cell labelling.** For cell tracking, *Xenopus laevis* embryos were injected at the 2-cell stage with messenger RNA for nuclear mCherry (300 pg). For invasion assays, embryos were injected at the 2-cell stage with either fluorescein–dextran or rhodamine–dextran. For analysis of cell–cell adhesion dynamics, embryos were injected at the 2-cell stage with mRNA for either N-cadherin–GFP (50 pg), p120–GFP (50 pg) or α-catenin–GFP (50 pg).

**Embryology.** For *in vitro* experiments, explants of NC and placodes were dissected at stage 18. The superficial ectoderm was first removed and explants of plaques and NC were then dissected and placed on a fibronectin-coated dish. Briefly, non-treated plastic Petri dishes were incubated with a 10 mg ml⁻¹ fibronectin solution for 1 h at 37°C and 30 min in phosphate buffer saline containing 0.1% bovine serum albumin. Explants were transferred into the coated dishes containing modified Danilchick’s medium (2) and allowed to adhere for 20 min before the beginning of the time-lapse recordings.

For *in vivo* experiments, grafts of placodal cells were performed at Stage 13, grafts of NC cells were performed at stage 16 and the embryos were left to recover before time-lapse recordings. For placode grafts, the whole region including the superficial layer was dissected from a fluorescently labelled embryo and grafted onto a control embryo. The embryos were left to heal, the superficial layer was carefully peeled off and the unlabelled superficial layer from the host allowed to heal over the grafted area. This procedure ensures that only cells from the deep layer of the ectoderm (placodal cells) are labelled. For double grafts of NC and placode cells onto a single host embryo, the placode cells were grafted first, followed by NC graft. For the accuracy of the grafting procedures, compare the distribution of NC and placode markers with the distribution of grafted cells shown in Supplementary Fig. S1.

**In vivo cell tracking.** A custom mould was prepared by melting agarose (1%) in normal amphibian medium (NAM). While the agarose was still hot, a wooden grid made of 1-mm-wide wooden sticks was placed floating on top of the melted agarose. This was allowed to cool and solidify; the grid was then carefully removed. The grooves were covered with 4% methylcellulose and the dish was filled with NAM before placing the embryos, grafted side up, on top of the methylcellulose layer. The dish can be left open to be used with water-immersion lenses or filled with an excess of liquid and closed with a lid lined with silicone grease to be used with dry lenses. Cell tracks were made and analysed using the Manual Tracking and Chemotaxis Tool plug-ins from ImageJ or commercial automatic cell tracking software. As the size of the grafted placode tissue can vary from one experiment to another, the region of interest was defined as a 250 × 150 μm region located directly posterior to the eye and halfway along the dorso-ventral axis (Fig. 1 and Supplementary Fig. S1). The selection of placode cells to be tracked was based on three parameters: the size of the grafted placode tissue can vary from one experiment to another, the shape of the grafted area and the orientation of the grafted area. The procedure ensures that only cells from the deep layer of the ectoderm (placodal cells) are labelled. For double grafts of NC and placode cells onto a single host embryo, the placode cells were grafted first, followed by NC graft. For the accuracy of the grafting procedures, compare the distribution of NC and placode markers with the distribution of grafted cells shown in Supplementary Fig. S1.

**Collision analysis and invasion assays.** Angles for each cell were measured between three positions: 9 min before contact, during contact, 9 min after cells have separated. The distance between the cell centroids was measured 30 min after cells have separated. Invasion assays were performed as previously described (23). Briefly, two explants labelled with different fluorescent markers (that is, fluorescein and rhodamine) were placed on a fibronectin-coated dish in direct contact with one another and left to migrate. The fluorescence micrographs were then thresholded and the area of overlap between the green and red channels was measured.

**Traction forces.** Traction force measurements were performed as previously described (24).

The preparation of the polyacrylamide substrate containing fluorescent beads was adapted from previously published protocols (25,26). The concentrations of crosslinker and polymer were adjusted for a Young’s modulus of 600 Pa. The gels were casted with covalently bound fibronectin to allow attachment of NC and placode cells. Cells were then imaged on an inverted microscope with an ×10 lens and micrographs of the uppermost layer of the gel and the cells were taken at 5 min intervals. Images of the gels in a relaxed state were taken after removing the cells by trypsinization. The algorithm used for traction microscopy has been previously published (24). Importantly, placode cells were injected with fluorescein–dextran and the fluorescence signal was used as a reference such that traction forces were analysed only underneath the placodal region.

**Statistical analysis.** Angles after collision were compared with a random distribution using custom-made Matlab scripts implementing the modifications in ref. 47 to Rayleigh’s test as described previously (27) and the Excel plug-in for directional statistics StatistiXL. Comparison of percentages was performed using contingency tables as described previously (28). Two data sets were considered significantly different (null hypothesis rejected) if T > 3.841 (α = 0.05, *), T > 6.635 (α = 0.01, **), or T > 10.83 (α = 0.001, ***). Normality of data sets was tested using Kolmogorov–Smirnov’s test, d’Agostino and Pearson’s test and Shapiro–Wilk’s test using Prism4 (GraphPad). A data set was considered normal if found as normal by all three tests. Data sets following a normal distribution were compared with Student’s t-test (two-tailed, unequal variances) in Excel or a one-way analysis of variance (ANOVA) with a Dunnett’s multiple comparisons post-test in Prism4 (GraphPad). Cross-comparisons were performed only if the overall P value of the ANOVA was < 0.05.

**In situ hybridization.** In *in situ* hybridization on *Xenopus* embryos was performed as previously described (29). Briefly, embryos were fixed in MEMFA overnight at 4°C and dehydrated in 100% methanol and kept overnight at −20°C. Embryos were then rehydrated with a series of methanol/PBS solutions of decreasing methanol concentration diluted 1 in 4 until PBS was reached. Embryos were then bleached with mild hydrogen peroxide solution, post-fixed in 3.7% formaldehyde, washed with PBS with 0.1% Tween and pre-incubated in hybridization buffer (HB) for 2 h at 65°C. HB was replaced by HB containing a digoxigenin-labelled RNA probe, in which embryos were incubated overnight at 65°C. Embryos were washed in formamide-based washing solutions, washed in PBS with 0.1% Tween, followed by TBSTX and then incubated in TBSIX with 10% serum for 1 h at room temperature before being incubated in TBSIX with 10% serum containing the anti-digoxigenin antibody overnight at 4°C. Embryos were then thoroughly washed in TBSTX with 10% serum for one day and night before being transferred into NTMT for three short washes. The last wash was then replaced by NTMT containing NBT and BCIP (3.5 μl ml⁻¹). Embryos were monitored under a dissecting microscope every 30 min until satisfactory staining was achieved.

**Confocal imaging of in situ hybridization.** NBT/BCIP staining from *in situ* hybridization was imaged as previously described (31). The NBT/BCIP precipitate fluoresces at wavelengths above 700 nm. Zebrafish injected with control MO or Sdf1lMO were labelled by *in situ* hybridization against Sox3 and then imaged under a confocal microscope. Stacks of fluorescent images were used to generate a 3D reconstruction of the placodal region.

**Histology.** Cryosections and immunostainings were performed as previously described (25). Briefly, embryos were fixed in MEMFA for 30 min at room temperature, transferred into phosphate buffer (PB) with 15% sucrose overnight at 4°C. Embryos were then passed into PB with 15% sucrose/7.5% gelatine for two hours at 42°C and mounted in the PB with 15% sucrose/7.5% gelatine at room temperature, allowed to set in the fridge for 1 h and then cut into cubes before being frozen in isopentane at −80°C and stored until sectioning.

**Immunostaining and antibodies, antisense MOs and dominant-negative constructs.** Immunostainings were performed as previously described (25). Briefly, cryosections were incubated in PBSIX at 37°C for 30 min to remove the gelatine. Sections were blocked in PBS 2% serum, incubated in primary antibody overnight at 4°C, washed in PBS, incubated with the secondary antibody for 2 h at room temperature, washed in PBS, mounted in Mowiol and stored in a cool dry place, in an opaque container before imaging. The following antibodies were used: N-cadherin antibody for staining (rat IgG, clone MNC2D, DSHB; supernatant diluted 1 in 5), E-cadherin antibody (mouse IgG, clone SD3, DSHB; concentrate diluted 1 in 100); N-cadherin antibody for blocking purposes (rat IgG, clone NCID2, Invitrogen, 1:3000; diluted 1 in 5). See the following references for N-cadherin MO (ref. 53), E-cadherin MO (ref. 53), Sdf1aMO (ref. 54), Dehisabled dominant-negative DshDep+ (refs 27, 31), Wnt11 dominant-negative (27). The following secondary antibodies were used: donkey anti-mouse–Alexa 555 (Invitrogen, A31570; 1 in 500), goat anti-rat–FITC (Sigma, F6258; 1 in 200). DAPI was used to stain nuclei (diluted 1 in 1000 in the secondary antibody solution).
Reagents and solutions. The following were used: agarose (Fischer Scientific, BP1356-100), bovine serum albumin (Sigma, A4503), fibronectin (Sigma, F1141), fluorescein–dextran (Sigma, D1821), gelatin (Sigma, G1890), methylcellulose (Sigma, M0387-100), Mowiol 40-88 (Fluka, 81386), penicillin–streptomycin (Sigma, P4458), rhodamine–dextran (Sigma, D1824), sucrose (VWR, 27480.294), Danilchick’s medium 1X: NaCl (53 mM), Na₂CO₃ (5 mM), K gluconate (4.5 mM), Na gluconate (32 mM), MgSO₄·7H₂O (1 mM), CaCl₂ (1 mM) and BSA 0.1%. MEMFA: MOPS (1 mM), EGTA (2 mM), MgSO₄ (1 mM) and formaldehyde (3.7%). NAM: NaCl (110 mM), KCl (2 mM), CaCO₃ (1 mM), MgSO₄ (1 mM), EDTA (0.1 mM), NaHCO₃ (1 mM) and sodium phosphate (2 mM). NTMT: NaCl (0.1 M), Tris–HCl at pH 9.5 (0.1 M), MgCl₂ (50 mM) and Tween 0.1%. Phosphate buffered saline 1X: NaCl (137 mM), KCl (2.7 mM), Na₂HPO₄ (10 mM), KH₂PO₄ (1.8 mM), CaCl₂–H₂O (137 mM), KCl (2.7 mM), Na₂HPO₄ (10 mM), KH₂PO₄ (1.8 mM), CaCl₂–H₂O (137 mM) and MgCl₂·6H₂O (0.5 mM).

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Figure 1 Early Neural Crest and placodes are in direct apposition and NC migration triggers placodes migration. (a-b) In situ hybridization showing early NC cells (a, Slug) and early placodal cells (b, Eya1) in close proximity. (c-f) These two regions can be easily dissected and grafted into unlabelled host embryos. After 12 hours grafted NC cells (e) and placodes (f) distribute as expected in a control embryo. (g-h) In situ of Twist (g) and Eya1/Foxi1c (h) showing the distribution of NC and placodes at later stages to compare with the distribution obtained from the grafts. (i-l) In vivo cell migration of NC and placodes. Cells were labelled with nuclear-mCherry prior to the graft. Note that placodal cells move away from the NC but that the actual direction of movement depends on the relative position of NC and placodes. NC cells from the third NC stream (j) and placodes from the third epibranchial region (k). An overlay of both datasets is presented in l. See supplementary Movies 1 and 2.
Figure 2 Neural Crest cell migration requires Sdf1-positive placodes. (a-e) NC cells follow Sdf1-positive placodes. (a) Diagram of a Xenopus head showing the orientation of the sections shown in b-e. NC cells are located dorsally to a region of deep ectoderm co-expressing the placodal marker FoxI1c and Sdf1. (b, d) Onset of NC cell migration. (c, e) Late stages of NC migration. Note that placodes and NC cells are moving ventrally and that placodal cells accumulate at the tip of the NC stream. Blue arrowheads indicate the leading edge of the NC stream. Purple arrowheads indicate the dorsal limit of placodal marker and Sdf-1 expression. (f) Injection of Sdf1MO, Cxcr4MO and Eya1MO all inhibit NC cells migration in vivo. Eya1 is expressed in the placodes and required for their development. (g) Sections from embryos shown in f. Note that blocking chemotaxis (Cxcr4MO) or placode development (Eya1MO) equally blocks NC migration, arrowheads indicate the front of NC migration on control and injected side. (h) Graft of NC cells injected with Sdf1MO into a control host, NC migration proceeds normally, indicating that Sdf-1 is not required in the NC. (i) Graft of control NC cells (Red) into a host injected with Sdf1MO, NC migration is impaired. This indicates that Sdf1 is not required in the NC cells but only in the surrounding tissues.
Figure 3 Co-culture of NC cells and placodal cells or Sdf1-positive non-placodal ectoderm. Invasion assay with control NC (green) and control placode (red) explants (a, 25 explants from 3 independent experiments) or control NC (green) and Sdf1-positive non-placodal ectoderm (red) (b, 21 explants from 3 independent experiments). (c) Average overlap for each condition. Note that both ectoderm explants endogenously express Sdf1 but that NC cells invade only the non-placodal tissue (b) and that directional movement is seen only with NC cells and placodes.
Figure 4 Wnt/PCP between NC and placodes. (a-c) Twist (a, NC marker) and Wnt11 (b) show similar expression patterns. (c) Diagram summarizing the distribution of Wnt11 and that of Frizzled4 which has been described in cranial placodes in Xenopus (Shi and Boucaut, 2000) and Zebrafish (Nikaido et al., 2013). (d-h) Localization of Dishevelled-GFP in placodal cells. (d) Diagram showing the orientation of the sections shown in e-h. (e-f) Dsh is at the cell membrane in control placodes adjacent to migratory NC cells, indicating an activation of the Wnt/PCP signalling in these placode cells. (g-h) Dsh becomes localized in the cytoplasm of placodes located next to NC cells injected with NCadherin MO, indicating a loss of Wnt/PCP signalling. (i-m) Dishevelled-GFP localization in animal caps. (i) Diagram indicating the stages used for injections and analysis. (j) Dsh-GFP injected alone localizes in the cytoplasm. (k) Co-injection of Frizzled7 and Dsh-GFP triggers membrane localization of Dsh-GFP. (l) Placing cells injected as in k in a low calcium culture medium for 10 minutes prior to confocal imaging abolishes membrane localization of Dsh-GFP suggesting that cell-cell adhesions are required for Wnt/PCP signalling. (m) Co-injection of Dsh-GFP and N-Cadherin are not sufficient to target Dsh at the cell membrane.
Figure 5 NC-placodes interactions via CIL and chemotaxis are required for discrete placodes formation and NC cell migration in vivo. (a-c) Diagrams showing the orientation of sections in d-h. (d) Dorsal level of a control embryo, placodes accumulate in between the NC streams (arrows). (e) Ventral level of a control embryo, NC cells have not reached this region yet. Placodes remain superficial. Ablation of NC (f), inhibition of Wnt/PCP signalling in the placodes (g) and inhibition of chemotaxis in the NC (h) leads to impairment in placode segregation. (i) Percentages of embryos from 2 independent experiments (46 animals were analyzed) with discrete or fused placodes for the different treatments shown in d–h (Parametric approach for percentages, two-sided test, $T_{NCablation} = 12.69$, $\alpha = 0.001 (***)$; $T_{PLdep+} = 11.37$, $\alpha = 0.001 (***)$; $T_{NCcxcr4MO} = 9.54$, $\alpha = 0.01 (**))$. 

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Supplementary Video Legends

Supplementary Video 1. In vivo cell migration of Neural Crest and placodal cells after a double graft. Neural crest cells are in green, placodal cells in red. Note that a gap is generated in the placodal region where NC cells are migrating as marked by red dots. Green arrow corresponds to ventral limit of the migrating NC. 1 picture every 5 minutes. 10x lens. 5 hours.

Supplementary Video 2. In vivo cell migration of Neural Crest and placodal cells. Panel 1: placodes from the third epibranchial domain located posterior to the third NC stream. Panel 2: NC cells from the third NC stream. Panel 3: NC cells from the second NC stream. Panel 4: placodes from the first epibranchial domain located ventral and anterior to the second NC stream. Note that placodes move away from the migratory NC cells but that the actual direction of their movement depends on the relative position of placodes and NC cells. Diagrams at the top indicate which regions are monitored. Blue squares mark the NC streams whereas yellow squares indicate the placodes. 1 picture every 5 minutes, 10x lens. 1h30min.

Supplementary Video 3. In vivo placodal cell migration. Placodal cells express nuclear-RFP. Time-lapse movie of the placodal region depicted in Supplementary Figure 1i-k. Left panel: placodal cells in a control embryo before NC cell migration. Middle panel: placodal cell movements during NC cell migration. Right panel: placodes in an embryo where NC cells were removed. Note that directionality is observed only in the control situation (middle panel) in cells that are located to a gap (second NC stream, green circle). 1 picture every 5 minutes. 10x lens. 3 hours.

Supplementary Video 4. Chase-and-Run: co-culture of NC cells and placodes First row: control NC cells Second row: control placodal cells Third row: co-culture of NC (green) and placodes (red). Note that NC and placodes undergo coordinated cell migration (chase-and-run). Fourth row: co-culture of Cxcr4MO NC (green) and placodes (red). Note that inhibiting Sdf1 chemotaxis impairs the coordinated migration. 1 picture every 5 minutes. 5 hours.

Supplementary Video 5. Attraction assay: control NC cells and control placodes (left), Cxcr4MO NC cells and control placodes (middle), control NC cells and Sdf1MO placodes (right). Note that control placodal cells endogenously express Sdf1. Lower panels show the automated tracks for NC cells for each condition. 1 picture every 5 minutes. 5 hours.

Supplementary Video 6. Invasion assay. Control NC cells are co-cultured with control non-placodal ectoderm from stage 10 Xenopus embryo (left panel) or control placodes (right panel). Both tissues endogenously express Sdf1. Note that NC invade the ectoderm and do not undergo coordinated migration. White cross marks the centre of the placode explants at the beginning of the movie for reference. 1 picture every 5 minutes. 5 hours.

Supplementary Video 7. Cell-cell interaction at the NC-placodes interface. This movie shows two examples of the dynamic interactions of NC and placode cells at the interface. Left panel: NC (green) migrates towards placodes (red) making numerous transient contacts. Note that NC cells are far more active and migratory than placodes in accordance with their respective mesenchymal (NC) and epithelial (placodes) phenotypes. 1 picture every 30 seconds, 10X objective, 1h45minutes. Right panel: NC (green) and placodes (red) go through cycle of protrusion, contact and retraction. Note that NC cells are more readily reforming cell protrusions than placodes. 1 picture every 10 seconds, 40X objective, 35minutes. Both examples show how repeated contacts between NC cells progressively force placodes to retreat.

Supplementary Video 8. Transient accumulation of N-Cadherin-GFP between NC and placodes. Both NC and placodes were transplanted with N-Cadherin-GFP. Placodes were also injected with nuclear-mCherry. Top panel shows the merged green (N-Cadherin) and red (nuclear-mCherry) fluorescent channels. Middle panels show the green channel only. Bottom panels show the heat maps of the green channel. Regions of cell-cell contact are highlighted with a white square. Frames are from a single confocal z plan extracted from a stack. 63X objective, digital zoom 2x, 1 picture every 15 seconds, 6 minutes.

Supplementary Video 9. Placodal cells protrusion dynamics. Spinning disk confocal microscopy. Both cell types were injected with LifeActin-mCherry. NC cells were co-injected with membrane-GFP. Left panel, placodal cells alone; right panel, interface between NC and placodes explants. Asterisks mark the collapsing protrusions. 100x lens. 1 picture per minute. 30 minutes.

Supplementary Video 10. N-Cadherin affects Placodal cell protrusions. Panel 1: Placodal cells cultured on Fibronectin. Panel 2: Placodal cells on FN+1ug/mL of N-Cadherin. Panel 3: Placodal cells on FN+3ug/mL of N-Cadherin. Panel 4: Placodal cells on FN+3ug/mL of N-Cadherin in low Calcium/Magnesium conditions. Panel 5: Placodal cells on FN+3ug/mL of N-Cadherin with placodal cells preincubated in blocking antibody against N-Cadherin (NCD2). 10X lens, digital zoom 2X. 1 picture every 3 minutes. 1 hour.

Supplementary Video 11. Heterotypic collisions between control NC cells and control placodal cells. Left panel: single NC cell versus a placodes explant. Middle panel: single placodal cell versus a NC explant. Right panel: single placodal cells colliding. Note that collisions between NC and placodal cells lead to Contact-Inhibition of Locomotion with cells moving away from each other whereas collisions between placodal cells lead to cell clustering. 1 picture every 3 minutes. 10x lens. 30 minutes. Contact after collision. 1 picture every 5 minutes. 10x lens. 30 minutes.
**Supplementary Video 12.** Homotypic collisions between NC or placodes.
Top panel: Collisions between NC cells.
Bottom panel: Collisions between placodes.
Consecutive frames were subtracted and colour-coded such that protrusions and retraction appear red and blue respectively. Cell bodies are green. Note that cells collapse protrusions and repolarize upon contact with one another, making new protrusions opposite to the region cell-cell contact. NC cells subsequently move away from each other whereas placodes cluster.
1 picture every 3 minutes, 10x lens, 36 minutes.

**Supplementary Video 13.** Heterotypic collisions between NC and placodal cells upon inhibition of N-Cadherin, Dishevelled and dnWnt11.
Panel 1: Control cells.
Panel 2: Both cell types injected with N-Cadherin Morpholino.
Panel 3: Both cell types expressing Dishevelled dominant-negative (DshDep+).
Panel 4: control placodes (red) and NC cells expressing dominant-negative Wnt11 (dnWnt11, green).
Note that control NC and placodal cells move away from each other. When N-Cadherin, Dsh or dnWnt11 are inhibited NC and placodal cells remain in contact after collision. 1 picture every 5 minutes. 10x lens. 30 minutes.

**Supplementary Video 14.** Invasion assay with NC and placodal cells.
Panel 1: Control cells.
Panel 2: Both cell types injected with DshDep+.
Panel 3: Placodal explants treated with N-cadherin antibody (NCD2).
Panel 4: Placodal explants treated with E-Cadherin antibody.
Note that control conditions and E-Cadherin treated explants show a clear chase-and-run behaviour while Dsh and N-Cadherin inhibition lead to overlapping of both explants and no directional movement of the placodal cells. 1 picture every 5 minutes. 10x lens. 5 hours.

**Supplementary Movie 15.** Co-culture of NC explants
Left panel: Control NC cells alone.
Middle panel: Two control NC explants.
Right panel: A control NC explant (red, nuclear-mCherry) is co-cultured next to a NC explant injected with Sdf1 (green, cytoplasmic Fluorescein-dextran).
Note that overexpressing Sdf1 in one NC explant is sufficient to drive a coordinated migration of the two explants.
1 picture every 3 minutes, 10x lens, 4 hours.

**Supplementary Movie 16.** In vivo placodal cell migration.
Left panel: Control conditions
Middle and right panels: Placodal cells expressing Dishevelled dominant negative (DshDep+).
Note that coordinated movements can be seen in DshDep+ condition compared to the control placodes (left).
1 picture every 5 minutes. 10x lens. 3 hours.

**Supplementary Movie 17.** 3D reconstruction of Sox3 in situ hybridization in Zebrafish injected with a control MO or Sdf1 MO. Note that after blocking NC cell migration with Sdf1 MO (right panel) placodal cells remains organized as one domain instead of splitting into subgroups (control embryo, left panel).