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Dermal fin rays and scales derive from mesoderm, not neural crest

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Neural crest cells disperse throughout the embryonic head to generate diverse cell types of two classes: non-ectomesenchymal (including melanocytes, peripheral neurons and glia) and ectomesenchymal (skeletogenic, odontogenic, cartilaginous and connective tissue cell fates). In contrast to cranial neural crest, trunk neural crest of amniotes generates only non-ectomesenchymal cell types. Anamniote trunk neural crest, however, has been assumed to generate derivatives of both classes, including osteoblasts of dermal skeletal elements, which includes scales and fin rays. Through genetic lineage tracing in zebrafish, we present the first test of this assumption and find that trunk neural crest does not generate fin osteoblasts; rather, these derive from a late emerging population of paraxial mesoderm. Similarly we show that the mineralising cells of the scales are mesodermally derived, with no contribution from neural crest. Our data suggest that trunk/tail exoskeletal structures evolved through deployment of mesodermally derived mesenchyme, rather than neural crest.

Post-cranial dermal exoskeletal elements such as the bony fin rays (lepidotrichia), scales, bony armour and turtle plastrons have been believed to derive from neural crest, based on the fact that they are composed of odontogenic tissues and/or dermal bone [1–4]. It had long been assumed that in chick, such tissues were generated exclusively by cranial neural crest, which had led many to conclude that any dermal skeletal elements of the trunk must derive from trunk ectomesenchymal neural crest [2,3]. Supporting this notion were experiments showing that trunk neural crest, upon manipulation, can exhibit skeletogenic potential [5]. Further analysis in mammals, however, identified a significant mesodermal contribution to dermal bone of the neurocranium [6]. To test for the origin of trunk exoskeletal elements, we generated a transgenic line expressing Cre recombinase under the control of the *sox10* promoter, which drives expression in the premigratory neural crest [7] and crossed this to the *ubi:switch* Lox reporter line [8], thus permanently labelling *sox10* expressing

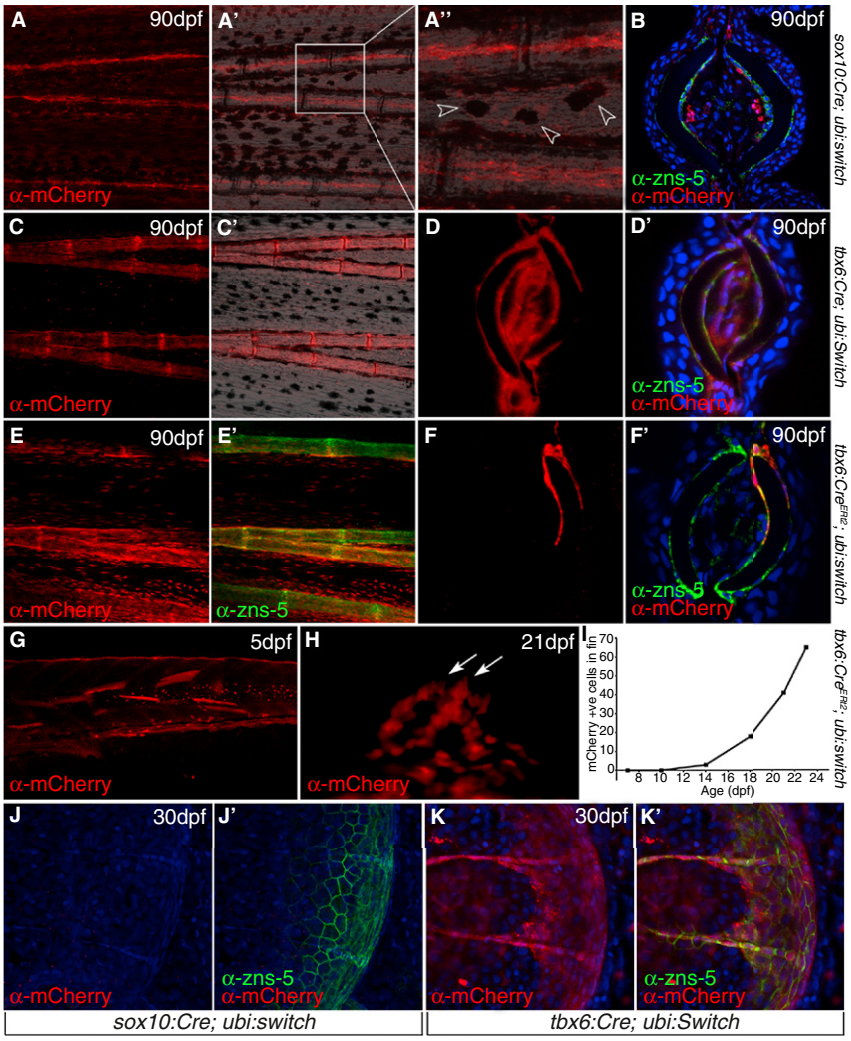

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Figure 1. Fin osteoblasts derive from late immigrating paraxial mesoderm.
(A,B) Adult (90 dpf) *sox10:Cre; ubi:switch* transgenics showing derivatives in the fin derived from neural crest and sox10 expressing cells. Lateral view of wholemount fin, immunostained to detect mCherry, indicates labelled cells running along the fin rays (A). Superimposing on a bright-field image demonstrates that these run within the lepidotrichia (A'). Magnified view of part of A' highlights the position within the ray and also mCherry-positive melanophores (arrowheads; A''). Transverse cryosection of a *sox10:Cre; ubi:switch* fin immunostained for mCherry (red) and zns-5 (green) and counterstained with DAPI (blue). mCherry positive cells are concentrated in two bundles within the fin ray and are zns-5 negative (B). (C–F') Adult (90 dpf) fins of *tbx6:Cre; ubi:switch* (C–D') and *tbx6:Cre^{ERt2}; ubi:switch* (E–F') imaged as lateral wholemounts (C–C'; E–E') or as transverse cryosections (D–D'; F–F'). Fins have been immunostained for mCherry (red; C–F') and zns-5 (green; D',E',F') and counterstained with DAPI (blue; D',F'). (C) is also shown superimposed on the Nomarski image (C'). In both lines, osteoblasts are mCherry positive. (G–H) Lateral images of the trunk/tail (G) and dorsal medial fin (H) of 5 dpf (G) and 21 dpf (H) *tbx6:Cre^{ERt2}; ubi:switch* transgenics treated with 4-hydroxytamoxifen and immunostained for mCherry. Larvae with no labelled cells in the fins at larval stages (G) often were observed to have chains of cells, aligned to forming bony rays, within the fins at 21 dpf (arrows; H). (I) Counts of mCherry-positive cells in the fins during postembryonic development. Secondary immigration was noted from 14 dpf. (J–K') Lateral images of scales in 30 dpf *sox10:Cre; ubi:switch* (J–J') and *tbx6:Cre; ubi:switch* (K–K') transgenics immunostained for mCherry (red; J–K') and zns-5 (green; J',K'). Co-labelling is only seen in the *tbx6:Cre; ubi:switch* line.

cells with the red fluorescent protein mCherry. mCherry was expressed widely in migratory neural crest from 24 hours post-fertilisation (hpf) and maintained expression in all expected neural crest derivatives (Supplemental data). We additionally observed expression of mCherry in cells running the length of the lepidotrichia (Figure 1A–A"). However co-immunostaining of cryosections and wholemounts for mCherry, with either the osteoblast marker zns-5, myelin basic protein or acetylated tubulin, identified these cells as labelling axon-associated Schwann cells in the ray interior, and never osteoblasts (Figure 1B; Supplemental data). Screening the fins of 5 individuals from three independent *sox10:cre; ubi:switch* transgenic lines failed to identify any discernible neural crest contribution to fin ray osteoblasts.

Aside from neural crest, cells with osteogenic potential able to migrate into the zebrafish tail might also derive from the somitic mesoderm. To determine if this is the source of lepidotrichial osteoblasts, we generated a *tbx6:cre* transgenic line which drives Cre recombinase expression in the embryonic paraxial mesoderm [9], and crossed this to the *ubi:Switch* line. At three months of age, we noted extensive labelling of the lepidotrichia (Figure 1C–C'), suggesting that paraxial mesoderm contributes cells to the adult fin rays. We confirmed that these included osteoblasts by co-immunofluorescent staining of transverse cryosections of these fins with antibodies against mCherry and zns-5, but also noted more widespread contribution of paraxial mesoderm to cells in the interior of the fin ray (Figure 1D–D'). We analysed fins from six individuals and the lepidotrichia of all were fully labelled, suggesting they derive solely from the paraxial mesoderm. This fin ray labelling reflects true paraxial mesoderm origin from larval (rather than adult) *tbx6* promoter activity, as we also generated a transgenic line expressing the tamoxifen-inducible Cre recombinase, Cre^{ERt2}, under the *tbx6* promoter and crossed it to the *ubi:switch* line. Doubly transgenic embryos were treated with 4-hydroxytamoxifen from 8 hpf to 48 hpf, when the *tbx6* promoter drives expression in paraxial mesoderm. We noted the presence of mCherry-positive paraxial mesoderm derivatives at larval stages, notably muscle fibres (Figure 1G). We raised these larvae and also found mCherry-positive cells distributed along the fin rays at three months (Figure 1E–E). Both in whole-mount fins and transverse sections, we observed significant co-labelling of zns-5-positive cells with the mCherry lineage label (Figure 1E–F), thus confirming a paraxial mesodermal origin of fin ray osteoblasts.

Given speculation that larval fin mesenchyme is the source of skeletogenic cells generating the adult lepidotrichia [2], we next asked if lepidotrichial osteoblasts are deposited early in larval fins, or if they populate the fins at a later timepoint. During our analysis of the *tbx6:*Cre^{ERt2}*; ubi:switch* larvae following treatment with 4-hydroxytamoxifen, we often noted mosaic larvae with mCherry-labelled cells in the somite region, but none in the larval fins (Figure 1G). We raised such larvae and asked if these fish contained zns-5-positive osteoblasts in the adult fin rays. In four of 20 individuals we could observe mCherry-positive lepidotrichial osteoblasts, suggesting that fin osteoblasts derive from a secondary source, not present in the larval fins at the stage we sorted. To determine when these cells were populating the fins, we repeated the experiment but counted the number and locations of mCherry-positive cells in the fins at time-points up to 23 days post-fertilisation (dpf). We noted a significant increase in the numbers of mCherry-positive cells in the fins at 2–3 weeks of age (Figure 1G–I), with chains of cells aligned to sites of nascent lepidotrichia ossification first identified at 18–21 dpf (Figure 1H). Finally, we tested if the odontogenic cells contributing to the mineralised scales were derived from neural crest [1,4], or were also mesodermal in origin. We could identify cells on the scales co-labelled with zns-5 and mCherry in 30 dpf *osterix:mCherry* transgenics (Supplemental data). These zns-5-positive cells were unlabelled by mCherry in *sox10:cre; ubi:switch* transgenics (Figure 1J–J'; 30 scales analysed), but were always positive for mCherry in *tbx6:cre; ubi:switch* transgenics (Figure 1K–K'; 40 scales analysed), indicating a mesodermal, and not neural crest, origin.

We here provide the first evidence of mesodermal contribution to vertebrate post-cranial exoskeleton, suggesting that the dermal bones and odontogenic tissues in the trunk and tail evolved through deployment of mesodermal cells. Whilst it may be able to adopt skeleto-odontogenic fates upon manipulation, trunk neural crest of anamniotes, like that of amniotes, appears not skeleto-odontogenic *in situ*, implying that skeleto-odontogenic neural crest arose exclusively in the 'new head' during vertebrate evolution [10].

Supplemental Information

Supplemental Information including experimental procedures and two figures can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.02.055.

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References

1. Sire, J.Y., and Akimenko, M.A. (2004). Scale development in fish: a review, with description of sonic hedgehog (shh) expression in the zebrafish (*Danio rerio*). Int. J. Dev. Biol. *48*, 233–247.
2. Smith, M., Hickman, A., Amanze, D., Lumsden, A., and Thorogood, P. (1994). Trunk neural crest origin of caudal fin mesenchyme in the zebrafish Brachydanio rerio. Proc. R. Soc. Lond. B Biol. Sci. *256*, 137–145.
3. Smith, M.M., and Hall, B.K. (1990). Development and evolutionary origins of vertebrate skeletogenic and odontogenic tissues. Biol. Rev. Camb. Philos. Soc. *65*, 277–373.
4. Vickaryous, M.K., and Sire, J.Y. (2009). The integumentary skeleton of tetrapods: origin, evolution, and development. J. Anat. *214*, 441–464.
5. McGonnell, I.M., and Graham, A. (2002). Trunk neural crest has skeletogenic potential. Curr. Biol. *12*, 767–771.
6. Gross, J.B., and Hanken, J. (2008). Review of fate-mapping studies of osteogenic cranial neural crest in vertebrates. Dev. Biol. *317*, 389–400.
7. Carney, T.J., Dutton, K.A., Greenhill, E., Delfino-Machin, M., Dufourcq, P., Blader, P., and Kelsh, R.N. (2006). A direct role for Sox10 in specification of neural crest-derived sensory neurons. Development *133*, 4619–4630.
8. Mosimann, C., Kaufman, C.K., Li, P., Pugach, E.K., Tamplin, O.J., and Zon, L.I. (2011). Ubiquitous transgene expression and Cre-based recombination driven by the ubiquitin promoter in zebrafish. Development *138*, 169–177.
9. Szeto, D.P., and Kimelman, D. (2004). Combinatorial gene regulation by Bmp and Wnt in zebrafish posterior mesoderm formation. Development *131*, 3751–3760.
10. Gans, C., and Northcutt, R.G. (1983). Neural crest and the origin of vertebrates: a new head. Science *220*, 268–273.

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