A MEDIAN FIN DERIVED FROM THE LATERAL PLATE MESODERM AND THE ORIGIN OF PAIRED FINS

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INTRODUCTION

The lateral fin fold hypothesis is a prominent theory which proposes the evolution of paired fins, known to be derived from the lateral plate mesoderm (LPM), as a result of the subdivision of unpaired median fins, (1,2). Several studies have looked at the cellular origins in multiple organisms, including the zebrafish and xenopus (3,4,5). However, all adult and larval median fins were shown to be derived from the paraxial mesoderm (PM) rather than the LPM (3). This could suggest a transfer from the PM to the LPM in the median fin programme (3), of which its timing and mechanism is unknown. Similar to most gnathosomes, zebrafish contain 2 median unpaired fins. The caudal fin fold and pre-anal fin fold (PAFF) of zebrafish are transient developmental structures replaced by PM derived adult median fins.
The study aims to characterise the origin of median fins in zebrafish, in particular, investigate possible LPM origins to PAFF as opposed to a PM-lineage which may establish the PAFF as a developmental intermediate between median and paired fins.

METHODOLOGY

Animal Models
Zebrafish (*Danio rerio*), Medaka (*Oryzias latipes*), American paddlefish (*polyodon spathula*), Sea lamprey (*Petromyzon marinus*), African clawed frog (*Xenopus laevis),* Western clawed frog (*Xenopus tropicalis*), Twin-tailed goldfish (*Carassius auratus,* Ranchu strain) and Epaulette shark (*Hemiscyllium ocellatum*) were selected to provide a broad phylogenetic representation to allow tracing of the evolutionary history of PAFF.

In situ hybridisation
Chromogenic and fluorescent in situ hybridisation methods were used to visualise the spacial distribution specific transcription factors, mainly *hand2,* to investigate the LPM origin of the PAFF mesenchyme across different vertebrate lineages.

Transplantation
Transplantation of morpholino cells into wild-type host embryos were done to asses whether loss of specific TF affects PAFF development in a cell-autonomous manner

Dil injection of medaka
lipophilic dil dye was microinjected into the posterior LPM of medaka embryos to confirm the contribution of this region to the PAFF mesenchyme

Immunostaining
Antibody staining was executed using either zebrafish embryos or sections. The primary antibodies, sources and dilutions used were anti-Col2A1, anti-eGFP, zns-5 and and anti-SM22 alpha/Transgelin and fluorescent secondary antibodies from Invitrogen were used to target the primary antibodies.

Microscopy
A variety of microscopic images were taken from confocal images, high and low magnification of bright-field or Nomarski images, light-sheet fluorescence microscopy with sample preparation following manufacturer's manuals and image processing was done.

High resolution X-ray computer tomography
Shark samples were fixed in paraformaldehyde, dehydrated, stained with ethanol for X-ray computed tomography scans and images were software processed.

Photoconversion
To track cell lineage, green fluorescence expressing embryos were mounted in cellulose or agarose and imaged by confocal microscopy. Selected region of interest were photoconverted to express red fluorescence and imaged immediately to confirm successful conversion then re-imagedin various high power fields

Tamoxifen Therapy
The Tg(drl:creERT2, hsp70l:Switch) system utilised Tamoxifen-inducable Cre recombinase (CreERT2) under the control of *drl* promoter to label cells derived from the LPM. Through heat shock treatment, eGFP expression was induced specifically in LPM-derived cells that underwent Cre-mediated recombination, by "switching on" the *hsp70l* promoter. The embryos were then fixed, stained with DAPI and imaged using confocal microscopy.

Statistical analysis
Statistical analysis was conducted using Prism v.9, with two-tailed Mann–Whitney tests for comparisons. Sample size was not predetermined, and experiments were repeated minimally thrice with different biological samples. Experiments were non-randomized, and consistent labeling was verified before imaging.

RESULTS

- Kaede-labelled PM appeared in the Caudal Median Fin Fold (CMFF) but not the PAFF, and presence of LPM-specific *Hand2* in PAFF only demonstrates LPM origin of PAFF (Fig 1a, b)
- Unique contribution to PAFF of LPM demonstrated by Hand2 knockouts, which showed LPM derivative defects (e.g. heart) and reduced fin height of PAFF (Figure 2a-c)
- During early to mid-somitogenesis (8–10-somite stage (ss)), drl:H2B-Dendra2 is expressed in the nuclei of the LPM
- Functional overlap between PAFF and CMFF are seen despite distinct developmental origins (Figure 3)
- LPM-seeded median PAFF is a shared ancestral feature, seen in Medaka, sea lamprey, sarcopterygian lungfish and amphibian tadpoles, due to staining of *Hand2* in pre-anal fins
- Bifurcation of LPM-seeded PAFF can arise from reducing *Chordin*, demonstrating transition of PM-derived median fins to LPM-derived paired fins
- Multiple PAFFs can be a natural variation, observed in twin-tail goldfish strain, Ranchu, with bifurcated PAFFs due to loss-of-function mutation in a *chordin* paralogue (Figure 4)

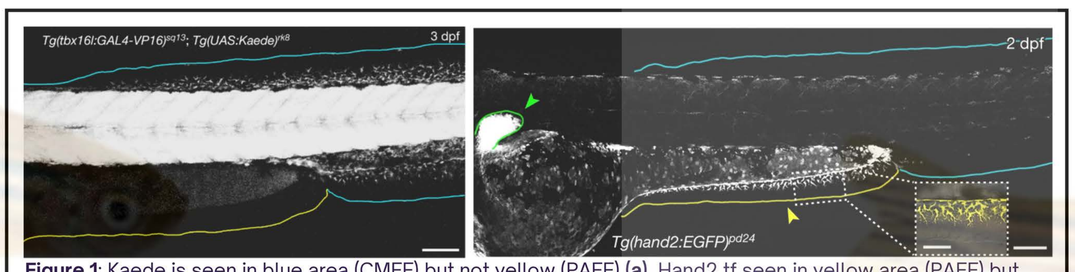


Figure 1: Kaede is seen in blue area (CMFF) but not yellow (PAFF) **(a)**, Hand2 tf seen in yellow area (PAFF) but not blue (CMFF) **(b)**

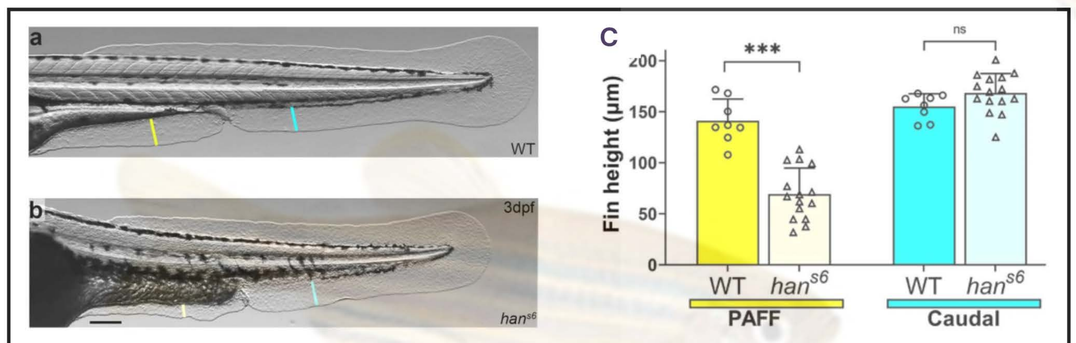


Figure 2 WT PAFF (yellow, a) is taller than hans6 (knockout) PAFF (yellow, a). Height of CMFF (cyan) is similar in both WT and knockout **(a-b)** Barchart depicting fin height differences in PAFF and CMFF between WT and hans6 zebrafish **(c-d)**

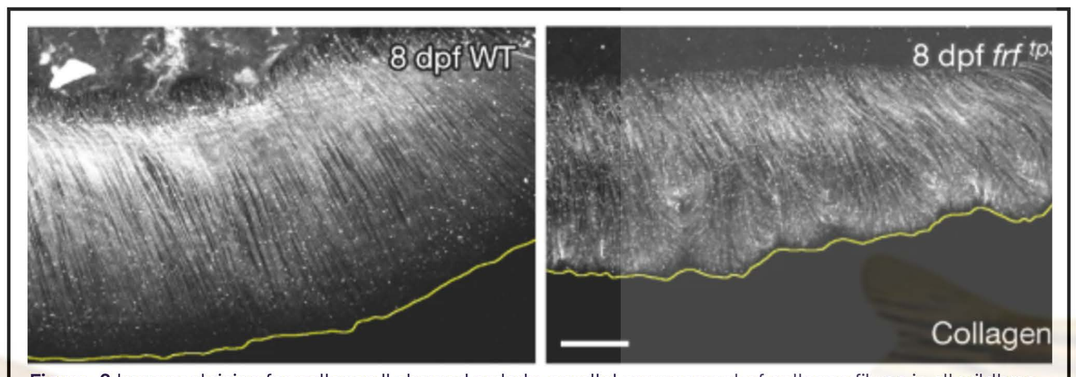


Figure 3 Immunostaining for collagen II showed orderly, parallel arrangement of collagen fibres in all wildtype fins **(a)** but loss of fibril organization was seen in *frf* mutants which expresses loss-of-function mutation in Bmp1a

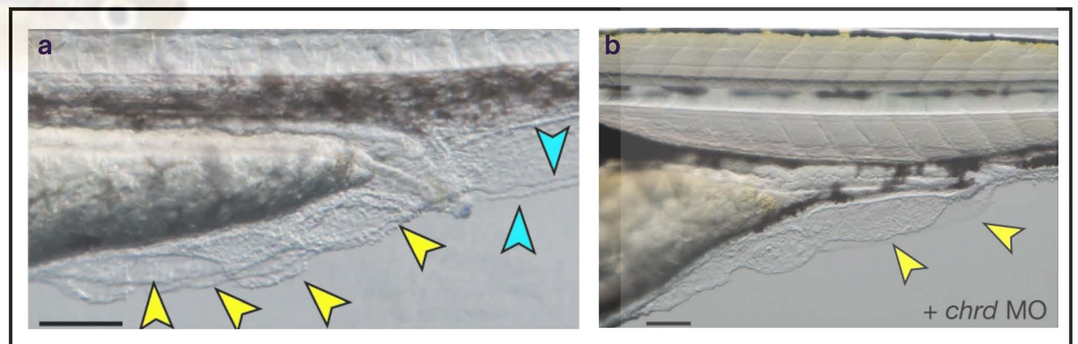


Figure 4 Bifurcation of PAFF is seen in Ranchu goldfish **(a)** the chrd mutant zebrafish **(b)** , demonstrating multiple PAFFs as a possible natural variation

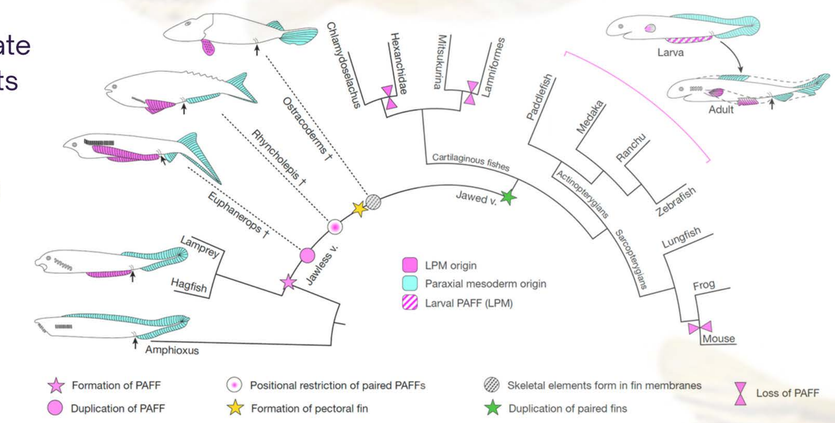
DISCUSSION

- LPM-derived PAFF is a transient developmental structure which bridges the evolutionary gap between PM-derived median fins and LPM-paired fins
- LPM-derived PAFF is a preserved ancestral feature common to cyclostomes and gnathostomes, which supports the notion that is a trait ubiquitous to vertebrates
- Paired fins may have arose following natural variation, which is supported by BMP-stimulated PAFF bifurcation, Ranchu goldfish fin morphology and fossil records
- Despite the ephemerality of PAFF, pre-anal fins are seen in fossilized vertebrates like Haikouella and Haikouichthys, suggesting its developmental importance
- We propose an updated fin model that positions LPM-derived PAFF as an intermediate structure to paired fins such as pectoral and pelvic fins following duplication and regionalization.

CONCLUSION

The study positions the PAFF as a critical intermediate in the evolution of paired appendages, highlighting its LPM-derived origin and evolutionary conservation.

Findings of this study supports a model of paired fin evolution in vertebrates where larval median fin programs were co-opted into the lateral plate mesoderm (LPM), followed by fin duplication and regionalization into pectoral and pelvic fins. Future studies involving other vertebrates can help to broaden phylogenetic comparisons, expanding the knowledge of conserved and divergent developmental mechanisms across vertebrates .



The proposed model posits that the PAFF originated as a small ectodermal fin fold and later gained LPM contributions through changes in LPM structure, such as persistence of the somatopleure or lateral mesodermal divisions. These adaptations likely facilitated PAFF elongation, duplication, and subsequent specialization into paired fins.

As the PAFF exhibits traits of both unpaired and paired fins, it may represent a novel evolutionary module or demonstrate developmental mechanisms underlying paired appendage evolution.

RELATED LITERATURE

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