An Fgf–Shh positive feedback loop drives growth in developing unpaired fins

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An Fgf–Shh positive feedback loop drives growth in developing paired fins using chemical genetics, a mutually agonistic interaction between Fgf and Shh genes in the developing dorsal fin of the channel catfish, Ictalurus punctatus. We also find that Fgf8 and Shh orthologs are expressed in the apical ectodermal ridge and zone of polarizing activity, respectively, in the median fins of representatives from other major vertebrate lineages. These findings demonstrate the importance of this feedback loop in median fins and offer developmental evidence for a median fin-first scenario for vertebrate paired appendage origins.

The origin and diversification of appendage types is a central question in vertebrate evolution. Understanding the genetic mechanisms that underlie fin and limb development can reveal relationships between different appendages. Here we demonstrate, using chemical genetics, a mutually agonistic interaction between Fgf and Shh genes in the developing dorsal fin of the channel catfish, Ictalurus punctatus. We also find that Fgf8 and Shh orthologs are expressed in the apical ectodermal ridge and zone of polarizing activity, respectively, in the median fins of representatives from other major vertebrate lineages. These findings demonstrate the importance of this feedback loop in median fins and offer developmental evidence for a median fin-first scenario for vertebrate paired appendage origins.

Results and Discussion

The dorsal fin of the channel catfish, Ictalurus punctatus, displays striking morphological anterior–posterior polarity due to the presence of anterior fin spines associated with enlarged proximal radials (Fig. 1 B and C). We find that fgf8a and shha are expressed in the dorsal fin in their respective AER and ZPA domains typical of paired appendages beginning at the first morphological indication of the fin bud at stage 37 (Fig. 1D). We treated larvae with 50 μM SU5402, an inhibitor of Fgf signaling, and assessed shha expression by in situ hybridization (Fig. 24). We found ZPA shha expression was diminished in most treated embryos (6 of 10, 60%, absent in 3, reduced in 3) relative to controls (10 of 10, 100%). Reciprocally, when catfish were treated with 50 μM cyclopamine, a Hedgehog (Hh) signaling inhibitor, expression of fgf8a was diminished in the developing AER (7 of 9, 78%, absent in 5, reduced in 2) compared to controls (10 of 10, 100%). These experiments demonstrate that the mutually agonistic nature of Fgf8 and Shh signaling observed in paired appendages is also found in the median dorsal fin.

To determine the effect of Fgf–Hh feedback loop perturbation on dorsal fin morphology, we repeated the pharmacological experiments using lower drug dosages that permit survival to later stages. We found that treatment with 25 μM SU5402 caused reduction or absence of the dorsal fin endoskeleton (three of eight, 38%) compared to controls (five of five, 100%) at 11 dpf post fertilization (dpf) (Fig. 2B). However, SU5402-treated animals examined at 15 dpf displayed normal dorsal fin development (five of five, 100%) similar to controls (three of three, 100%), possibly due to catch up growth. Treatment with 10 μM cyclopamine resulted in reduced size of endoskeletal elements (seven of seven, 100%) relative to controls (five of five, 100%) at 11 dpf. Interestingly, cyclopamine-treated animals also exhibited a reduced number of proximal radials along the anterior–posterior axis, forming only six elements instead of seven (five of seven, 71%), while control animals develop the typical seven elements (five of five, 100%). This reduction is similar to that seen when Hh signaling is perturbed in the paired appendages (10). At 15 dpf, reductions in element...
length and number were still detected in cyclopamine-treated animals (four of four, 100%) but not in controls (three of three, 100%)

Next, we asked if Fgf8 and Shh orthologs are expressed in the developing median fins of species from other fish lineages. We examined the expression of these genes in a representative of the Chondrostei, a group comprising sturgeons and paddlefishes. In the American paddlefish, *Polyodon spathula*, we detected expression of *fgf8* in the AER as well as *shh* in the ZPA in the dorsal fin at stage 45 (Fig. 2C). Among elasmobranchs, a group that includes sharks, rays, and skates, *Shh* is expressed in the ZPA in the developing dorsal fins of the little skate, *Raja erinacea* (9). We find that *Fgf8* is also expressed in the AER of the dorsal fin of the little skate at stage 30 (Fig. 2D). These results suggest that the interaction of Fgf8 and Shh in median fins is phylogenetically wide-spread and likely represents the ancestral condition for jawed vertebrates.

Previous studies have searched for common patterning mechanisms between paired fins and more ancient structures,
such as gill rays (5), the axial skeleton (12), and median fins (3). The presence of an Fgf–Shh positive feedback loop in unpaired fins removes an objection to the median fin hypothesis of paired appendage origins and suggests that this mechanism arose early in the prototypical vertebrate appendage, before the diversification of different fin types. Similar scenarios have been proposed for the origins of collinear Hox expression (3), Shh genomic regulation (10), and Gli3–Shh interactions (11) in vertebrate appendage patterning. Together these results indicate that a rather complete developmental program was already in place in early unpaired fins prior to the emergence and divergence of additional appendage types.

Materials and Methods
Catfish and paddlefish embryos were purchased from Osage Catfisheries. Skate embryos were purchased from the Marine Biological Laboratory. Catfish were treated with pharmacological inhibitors for 8 h beginning at stage 39. Experiments were assessed and approved by the University of Colorado at Boulder Institutional Animal Care and Use Committee. Details of animal care, in situ hybridization, staining, and pharmacological treatments are provided in SI Appendix.

Data Availability. All study data are included in the main text and SI Appendix.

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