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Saltatory control of isometric growth in the zebrafish caudal fin is disrupted in *long fin* and *rapunzel* mutants

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Abstract

Zebrafish fins grow by sequentially adding new segments of bone to the distal end of each fin ray. In wild type zebrafish, segment addition is regulated such that an isometric relationship is maintained between fin length and body length over the lifespan of the growing fish. Using a novel, surrogate marker for fin growth in conjunction with cell proliferation assays, we demonstrate here that segment addition is not continuous, but rather proceeds by saltation. Saltation is a fundamental growth mechanism shared by disparate vertebrates, including humans. We further demonstrate that segment addition proceeds in conjunction with cyclic bursts of cell proliferation in the distal fin ray mesenchyme. In contrast, cells in the distal fin epidermis proliferate at a constant rate throughout the fin ray growth cycle. Finally, we show that two separate fin overgrowth mutants, *long fin* and *rapunzel*, bypass the stasis phase of the fin ray growth cycle to develop asymmetrical and symmetrical fin overgrowth, respectively.

Keywords: Zebrafish; *Danio rerio*; Fin; Growth; Saltatory growth; Overgrowth; Cell proliferation; Isometric growth; *longfin*; *rapunzel*

Introduction

Animals display remarkable variety in size and shape, differences that are found both within and between species. Elucidation of the genetic mechanisms regulating growth and form remains a fundamental problem challenging developmental biologists. Growth may be achieved either through increases in extracellular content or, more frequently, increases in total cell mass. In the latter case, cell mass can increase through increases in cell size and cell number. There is good evidence in invertebrate systems for growth control mechanisms that operate through the regulation of cell size (Prout and Barker, 1989; Saucedo and Edgar, 2002); however, in vertebrates, growth control is largely regulated at the level of cell number (Conlon and Raff, 1999; Raff, 1996).

Zebrafish offer a unique opportunity to investigate the fundamental mechanisms controlling growth and size. In addition to being a genetically tractable species, they have properties of growth uniquely suited to investigation, including: (1) indeterminate growth (Iovine and Johnson, 2000; Jordan, 1905); (2) isometric growth of their fins relative to their bodies (Johnson and Bennett, 1999); and (3) regenerative growth of their fins following amputation (Johnson and Weston, 1995). Furthermore, because fins are anatomically simple structures that are unessential for life in the relatively pampered environs of a laboratory fish, they are easily amputated for detailed study without needing to sacrifice the fish. Finally, mutations that affect fin growth can be developed without impacting on viability or fecundity (Iovine and Johnson, 2000).

Fin rays are composed of multiple segments, each segment comprised of two hemirays (lepidotrichia) of dermal bone in apposition, surrounding intraray mesenchyme (Santamaria et al., 1992). Lepidotrichia are covered on both surfaces by a monolayer of bone-forming osteoblasts (scleroblasts) that form as undifferentiated mesenchymal

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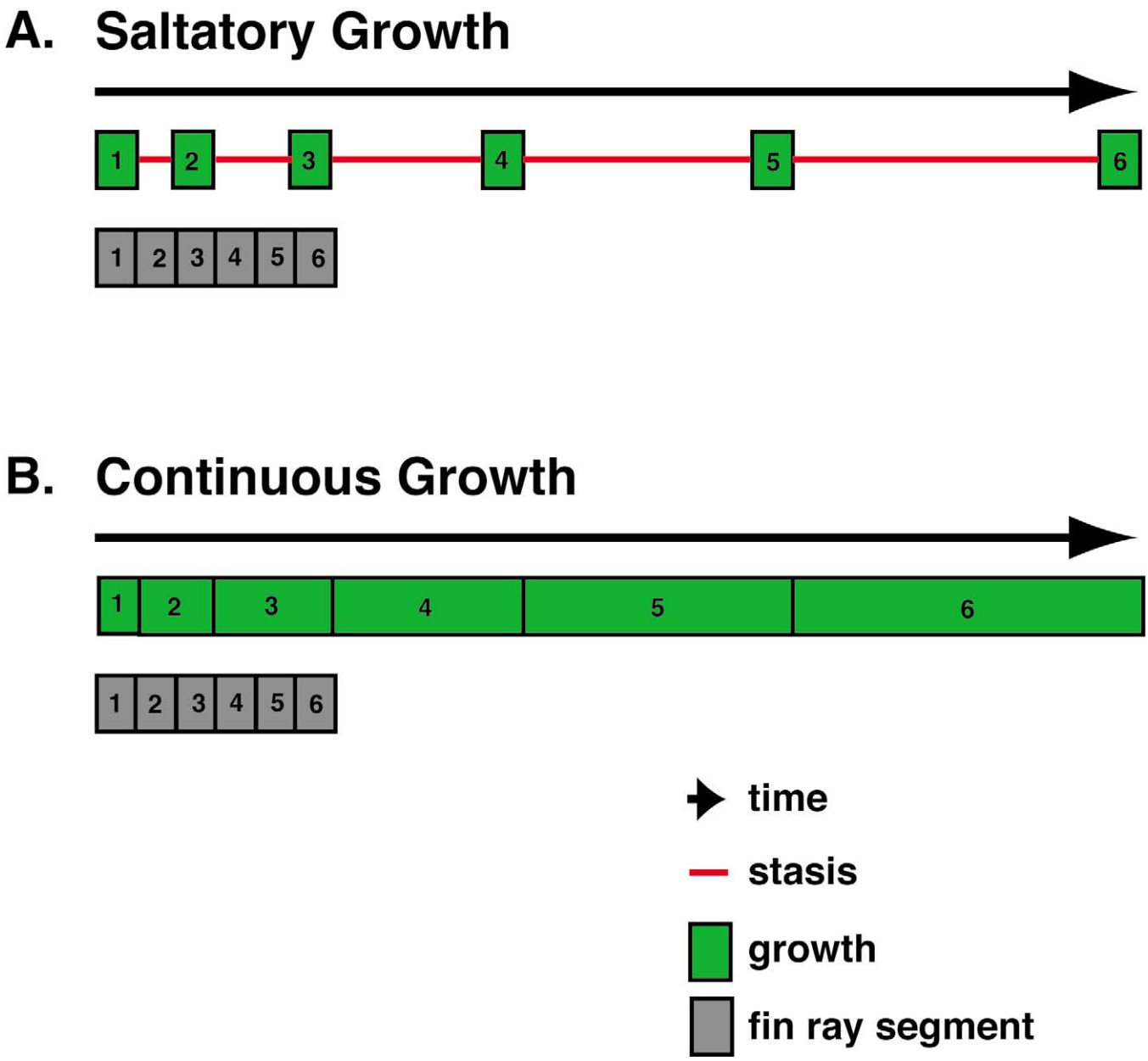


Fig. 1. Modeling growth in the zebrafish caudal fin. Two alternative models, saltatory growth and continuous growth, could explain proportionate growth in the zebrafish caudal fin. In both models, the black arrow depicts time with the left side of the arrow representing younger fish and the right side of the arrow representing older fish. Green boxes denote periods of segment formation. Gray boxes illustrate segments. In saltatory growth (A), segment formation is a finite, episodic, and interspersed by variable periods of stasis (red lines). In continuous growth (B), the rate of segment formation is variable and periods of stasis are absent.

cells in the central portion of the intraray condense laterally along actinotrichia, differentiate, and begin secreting bone matrix (Goss, 1978; Haas, 1962). While some histological details of these events have been described, little is known about the pathways that regulate segment formation and fin growth.

In teleosts, including zebrafish, fin growth occurs via the sequential, distal addition of new segments of bone to each fin ray (Haas, 1962; Nabrit, 1929; Santamaria and Becerra, 1991). We can imagine two models that could satisfactorily explain proportionate growth in zebrafish fins. One model is growth by saltation, wherein periods of segment addition alternate with periods of rest, or stasis (Fig. 1A). In saltatory growth, segment addition is a finite event, occurring in discrete, uniform stages. Proportionality is achieved by regulating the timing of segment addition following varying periods of stasis. The second model posits that growth occurs continuously, wherein segment formation is uninterrupted (periods of stasis are by definition, absent); however, the rate of segment formation changes to reflect the overall growth velocity of the fish (Fig. 1B). Genetic analysis (Iovine and Johnson, 2000) has supported the saltation model, although direct evidence was lacking.

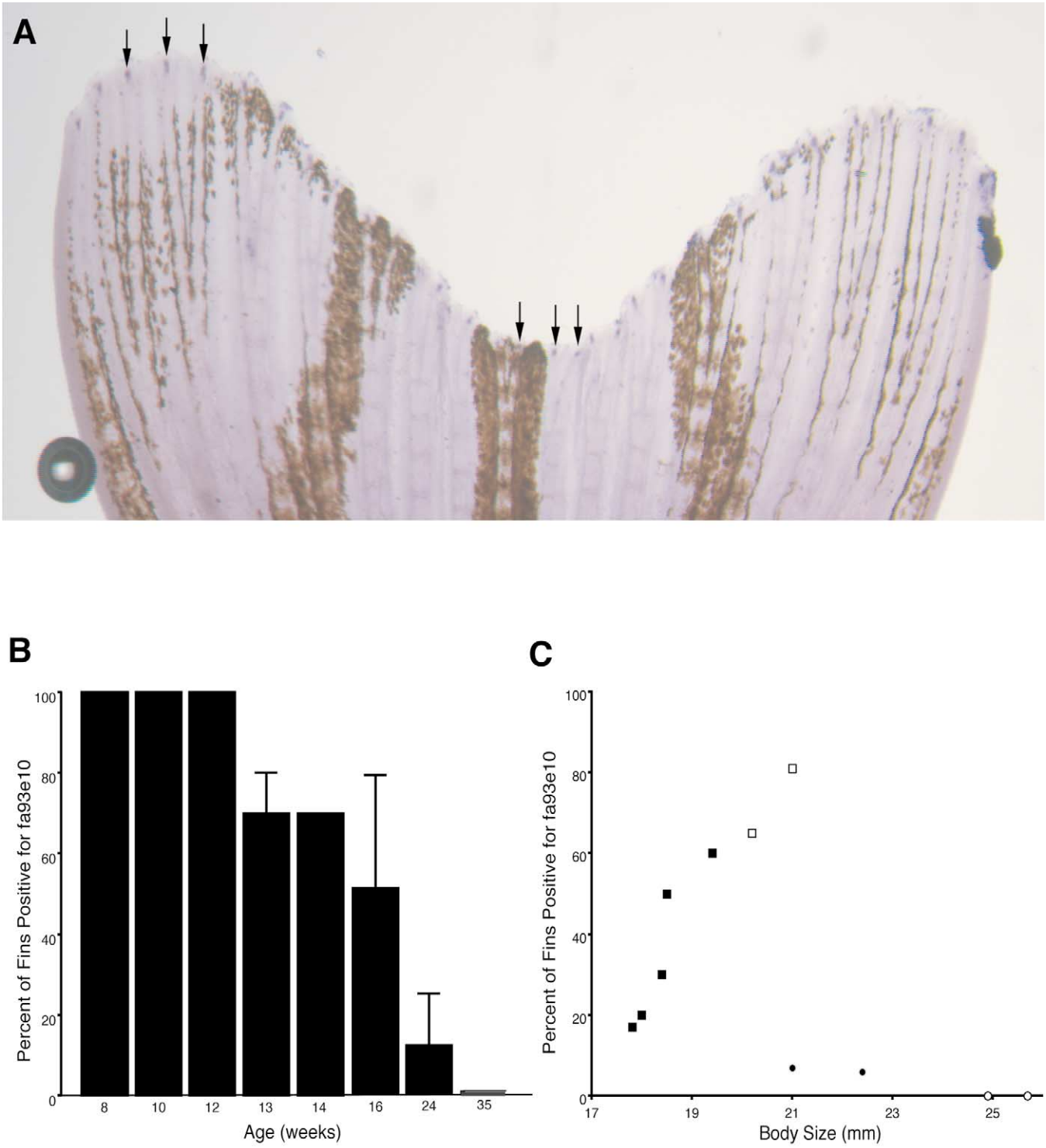


Fig. 2. fa93e10 expression cycles during ontogenetic fin growth. (A) Whole-mount in situ hybridization illustrating fa93e10 expression in an 8-week-old zebrafish caudal fin. fa93e10 expression (purple staining) is clearly seen at the distal tip of every fin ray (highlighted in six fin rays with black arrows). (B) fa93e10 expression was assessed by whole-mount in situ hybridization in 8- to 35-week-old zebrafish caudal fins (n = 10–150 fins for each age group). (C) In 11 groups of fish from (B) (n = 200), aged 13 weeks (open squares), 16 weeks (solid squares), 24 weeks (solid circles), and 35 weeks (open circles), fa93e10 expression is plotted relative to body size. Each data point represents mean body size (mm) and percent of fins expressing the growth marker fa93e10 for a single group of fish (n = 14–24).

In this report, we tested the saltation and continuous growth models by in situ hybridization (ISH) with fin growth markers and by bromodeoxyuridine (BrdU) incorporation to directly assess the role of cell division in fin ray growth. An ISH screen of fin ESTs was performed in regenerating fins, identifying several genes as candidate growth markers. As predicted by the saltation model, expression of growth markers in zebrafish caudal fins cycled, with the majority of fins being growth marker-positive in young fish and fewer fins being growth marker-positive in older fish. Subsequently, we used ISH with one such growth marker, in conjunction with BrdU-labeling of proliferating cells, to show that growth marker expression correlates tightly with cell proliferation. Careful analysis of BrdU-labeled cells demonstrated not only the episodic nature of cell proliferation predicted by the saltation model but also that the growth-associated burst in cell proliferation was restricted to the mesenchymal compartment of the fin. In contrast, fin epithelium appeared to proliferate independently of segment formation. Finally, we describe growth marker expression and BrdU-labeling in two dominant fin overgrowth mutants, *long fin (lof)* (Iovine and Johnson, 2000; Tresnake, 1981) and *rapunzel (rap).* Both *lof* and *rap* mutants demonstrate allometric fin growth (fins size increases out of proportion with body size over time), bypassing the normal mechanisms regulating stasis. Interestingly, *lof* and *rap* mutants demonstrate dissimilar patterns of fin overgrowth. In *lof* heterozygotes, overgrowth is asymmetrical (most pronounced in the ventral lobe of the caudal fin), whereas overgrowth in the *rap* caudal fin is symmetrical. These data raise the possibility that at least two distinct pathways converge to regulate growth and stasis: one, acting locally, perhaps involving *lof* function, and a second, acting globally, perhaps involving *rap* function.

Materials and methods

Fish husbandry and general methods

Wild-type fish stocks used for this study were from the C32 strain (Streisinger et al., 1981). *Long fin*^{dt2} (Johnson and Weston, 1995; Tresnake, 1981) and *rapunzel*^{c14} were maintained in a mostly C32 background. Fish were reared at a constant temperature of 25°C with a 14L: 10D photoperiod (Westerfield, 1993). Microscopy was performed with either an Olympus SZX12 stereomicroscope or a Zeiss Axioscope compound microscope. Photography was performed with either a Spot II digital camera (Diagnostics, Inc.) or a ProgRes C14 CCD camera (Jenoptik Jena, Germany). Images were captured by using either Adobe Photoshop or ProgRes C14 camera software and processed with Adobe Photoshop 7.0. Chemicals were from Sigma unless otherwise specified.

Morphometry

For fin length and segment length measurements (as well as fin amputations), fish were anesthetized for several minutes in Tricaine (Westerfield, 1993). Measurement of body length, fin length, and segment length has been described previously (Iovine and Johnson, 2000). All measurements were made by using the Olympus SZ40 stereomicroscope fitted with a calibrated eyepiece reticule.

In situ hybridization screen

Abundantly expressed ESTs from fin regeneration libraries (part of the Washington University Zebrafish Genome Resources EST project that will be published elsewhere) were used to generate riboprobes for an ISH screen. To obtain regenerating fin tissue, fish were anesthetized as described above and approximately 50% of the length of their caudal fins was amputated. Fins were reamputated at various stages of regeneration (Johnson and Weston, 1995), and candidate growth markers were selected for further evaluation.

Preparation of riboprobes and ISH

Riboprobe synthesis and ISH were performed as described elsewhere (Poss et al., 2000). ISH was either performed manually or with the assistance of an automated ISH robot (Abimed In Situ Pro, Intavis AG, Bergisch Gladbach, Germany). All color development was performed manually.

Detection of proliferating cells using BrdU

Fish were labeled with BrdU in vivo by allowing them to swim for 6 h in fresh water containing BrdU (50 μg/ml). Fins were harvested as described above. Detection of BrdU incorporation was done on both whole mounts and sectioned fins (Nechiporuk and Keating, 2002). For BrdU detection on sections, fins were washed following the postfixation step and embedded in agarose–sucrose (Johnson and Weston, 1995). Agarose blocks containing fins were allowed to equilibrate for several days in 30% sucrose prior to sectioning in a Leica cryostat (12-μm sections). After sectioning, slides were fixed for 30 min in EtOH containing 50 mM glycine (pH 2), then processed for BrdU detection using a Roche BrdU Detection Kit and following the manufacturer's instructions. On fin sections where ISH was also performed, ISH was carried out as described above immediately following BrdU labeling. For whole-mount BrdU detection, fins were harvested as described above, then fixed overnight in Carnoy's solution (60% EtOH, 30% chloroform, 10% acetic acid) followed by dehydration in 100% MeOH. Fins were subsequently rehydrated into PBS containing 0.3% Triton X-100 (PBTx), washed in PBTx, rinsed twice in 2 N HCl in PBTx, and then incubated for 30 min in 2 N HCl/PBTx at room temperature. After two more PBTx

washes, fins were blocked for several hours in PBTxB (PBTx containing 0.25% BSA), then incubated overnight at 4°C in anti-BrdU (Roche, 1:50 dilution in PBTxB). Fins were then washed extensively (4-24 h) in multiple changes of PBTx, blocked for 30 min in PBTxB, then incubated overnight at 4°C in secondary antibody (Molecular Probes; goat anti-mouse conjugated to either Alexa-488 of Alexa-546, 1:200 dilution in PBTxB). After 4-24 h of additional washes in PBTx, fins were stored in the dark in Vectashield (Vector Laboratories) until ready for mounting. For whole-mount fins processed for both ISH and BrdU detection, ISH was performed first as outlined above. Following color development, fins were washed in PBTx and then processed as above. Microscopy was performed as described above.

Results

fa93e10 expression cycles in wild type zebrafish caudal fins

To identify potential markers of fin ray segment growth, we first screened EST clone collections for genes expressed in regenerating fins in patterns suggestive of growth (see http://www.genetics/sjlab/RW/listall.shtml for a list of ESTs screened and images from a partial set of in situs from growing and regenerating fins). Antisense probes generated from EST clone fa93e10 had expression suggestive of a useful marker for growth (data not shown). The reading frame from the EST assembly containing fa93e10 (EST assembly wz2529.3; S.L.J and R.W., unpublished, but see http://fisher.wustl.edu/fish_lab/cgi-bin/display.cgi?type=wz&value=2529) has no clear homology relationship with genes identified in either human or fugu sequence (Aparicio et al., 2002; Lander et al., 2001; Larsen et al., 2000), thus we will refer to this gene here as fa93e10. In addition, the predicted protein for fa93e10 has no homologous domains that allow us to speculate on its function. Based on our expression data (fa93e10 is expressed in the basal layer of the distal fin epithelium), we chose to further explore fa93e10 as a surrogate marker for growth status.

Our prediction for the model of saltatory fin ray growth was that genes could be identified that are expressed frequently in young, rapidly growing fins but rarely in old, slowly growing fins. To test whether fa93e10 fit this prediction, we assessed its expression by whole-mount ISH in caudal fins from populations of fish ranging between 8 and 35 weeks of age (Fig. 2B). At 8, 10, and 12 weeks of age, all fins examined were fa93e10-positive ($n = 10$ for each group). At 13 (3 groups, $n = 10–20$ for each group, 46 total fins examined) and 14 ($n = 10$) weeks, fa93e10 was expressed in 70% of the fins examined. At 16 weeks, fa93e10 was expressed in 51% of the fins examined (6 groups, $n = 10–20$ for each group, 100 total fins examined). At 24 weeks, fa93e10 was expressed in 12% of the fins examined (5 groups, $n = 9–100$ for each group, 150 total fins examined). Finally, at 35 weeks, no fa93e10 expression was observed in 30 fins (2 groups).

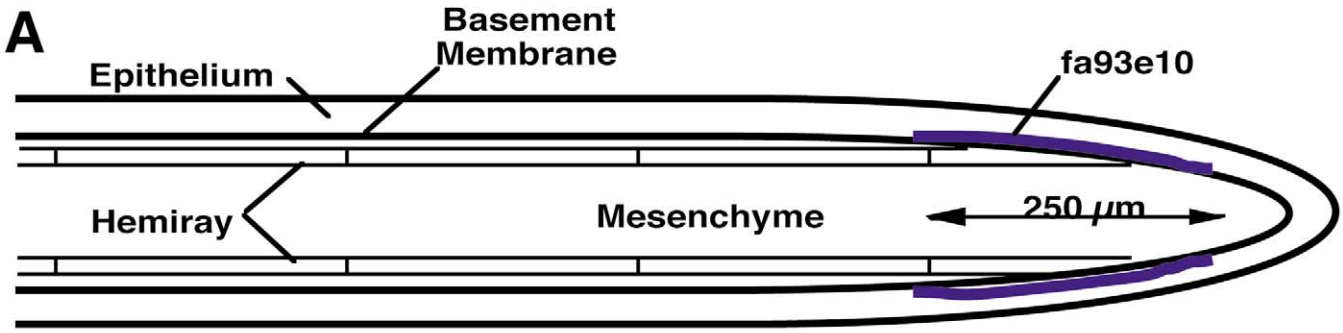
This analysis suggests that growth status (fa93e10 expression) is loosely correlated with age (Fig. 2B). We asked whether growth status better correlates with the size of the fish. fa93e10 expression was plotted against size for a subset of fish used in Fig. 2B (11 groups, 13–35 weeks, 200 fins total) (Fig. 2C). We find that, in these surveyed populations, the smaller, 16-week-old fish were less often in the fin ray segment growth cycle than the younger, larger 13-week-old fish sampled here. We conclude that fish size is a poor predictor of growth status.

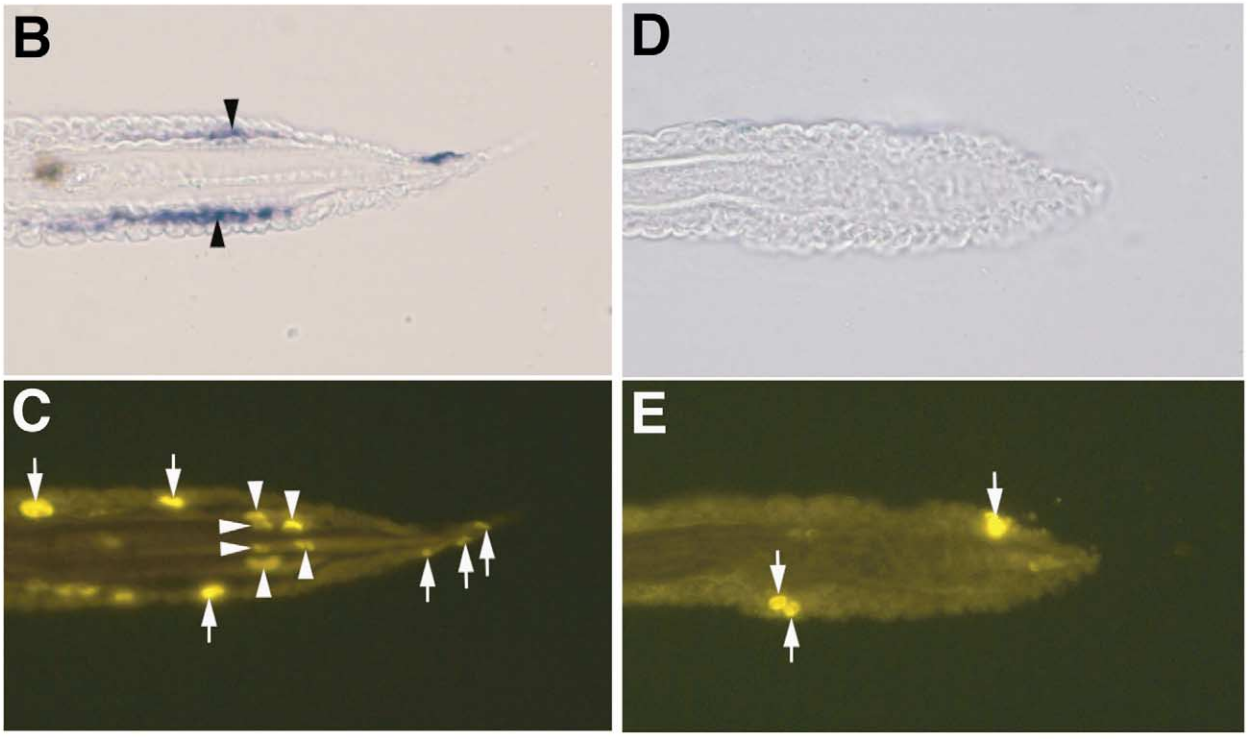
Presence or absence of fa93e10 expression is critical to the interpretation of these results; because 4-day fin regenerates faithfully express fa93e10 in a robust manner, every group of fins was concurrently hybridized with 4-day fin regenerates to provide positive controls. Color development was stopped only when fin regenerates became intensely labeled. Of note, when fa93e10 expression was observed in wild-type fins, the tip of every fin ray expressed the marker (Fig. 2A), irrespective of the age or size of the fish. Thus, fa93e10 expression meets our initial criteria for a gene expression marker that identifies cycles of fin ray segment growth.

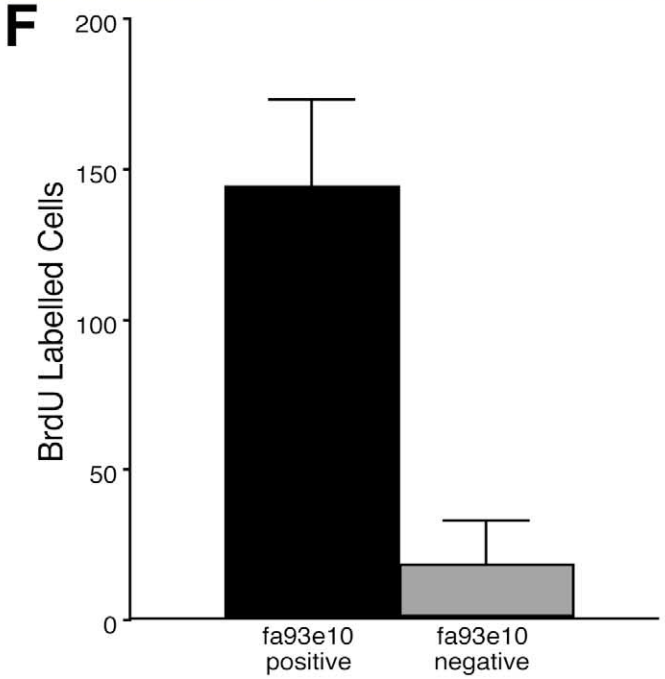
fa93e10 expression delimits periods of cell proliferation

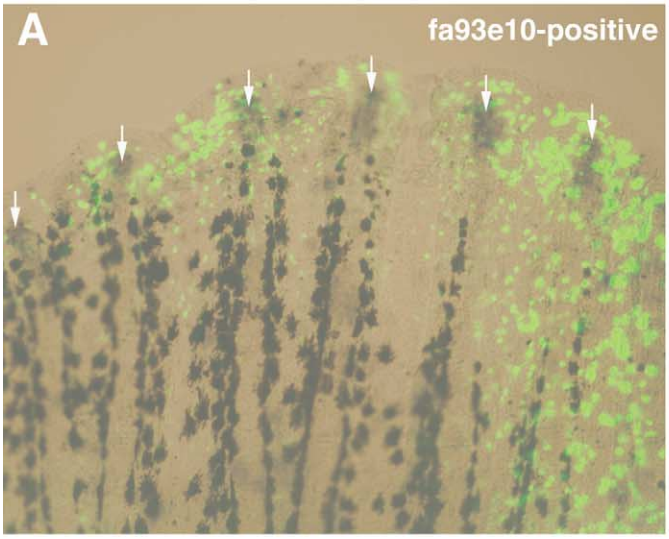
The notion that fa93e10 expression delimits periods of fin ray growth predicts that its expression will also correlate with bursts of cell proliferation. Adult fish are easily labeled

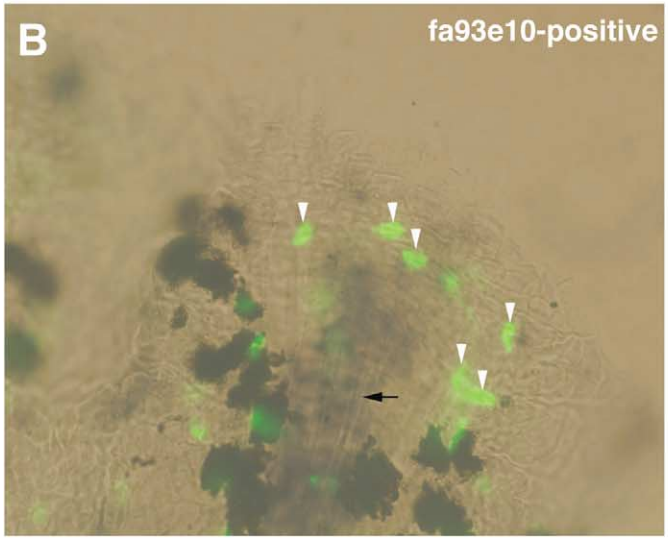
Fig. 3. fa93e10 expression delimits cycles of cell proliferation. (A) A diagrammatic representation of a longitudinal section through a zebrafish caudal fin. Two hemirays are seen enclosing the intraray fin mesenchyme. The mesenchymal compartment is surrounded by a basement membrane and an epithelium, fa93e10 is expressed in the basal layer of the epidermis, illustrated in dark blue. For the purposes of counting proliferating cells (see F), the intraray mesenchyme was divided into a distal portion (distal most 250 μm, equivalent to one segment length) and a proximal portion (everything proximal to the distal portion). (B–E) Brightfield and fluorescence microscopy of longitudinal sections through a fa93e10-positive (B, C) and a fa93e10-negative (D, E) fin ray. (B) Brightfield illumination demonstrates fa93e10 expression in the basal layer of the epithelium (black arrowheads). (C) Fluorescence illumination of the same section (B) demonstrates 6 BrdU-labeled cells in the distal intraray fin mesenchyme (white arrowheads). Six epithelial cells are also highlighted (white arrows). (D) A representative fa93e10-negative section. (E) Fluorescence illumination of the same section (D) illustrates 3 BrdU-labeled cells in the epithelium (white arrows) but none in the mesenchyme. (F) Fifty contiguous longitudinal sections were cut from 6-month-old fa93e10-positive ($n = 4$) and fa93e10-negative ($n = 5$) fins that had been in vivo labeled with BrdU. Following BrdU immunohistochemistry, labeled cells in the distal intraray mesenchyme were counted. Labeled cells in the proximal intraray mesenchyme and epithelium were excluded. Values represent the mean number of BrdU labeled cells per 50 sections ± SD.

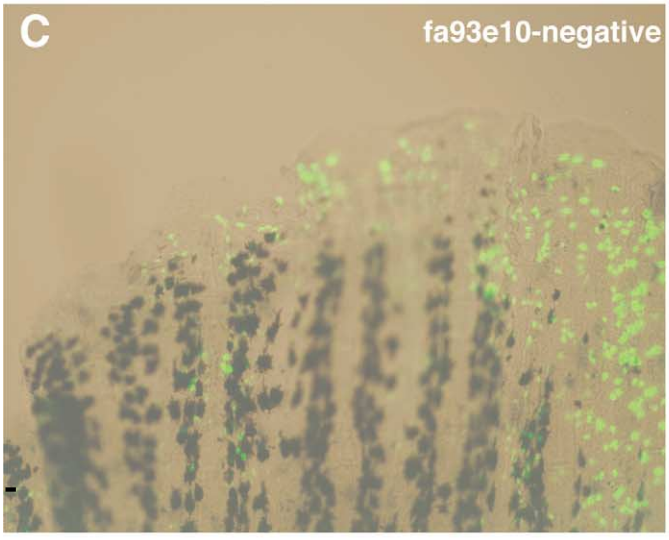

A
Basement Membrane
Epithelium
fa93e10
Hemiray
Mesenchyme
250 μm

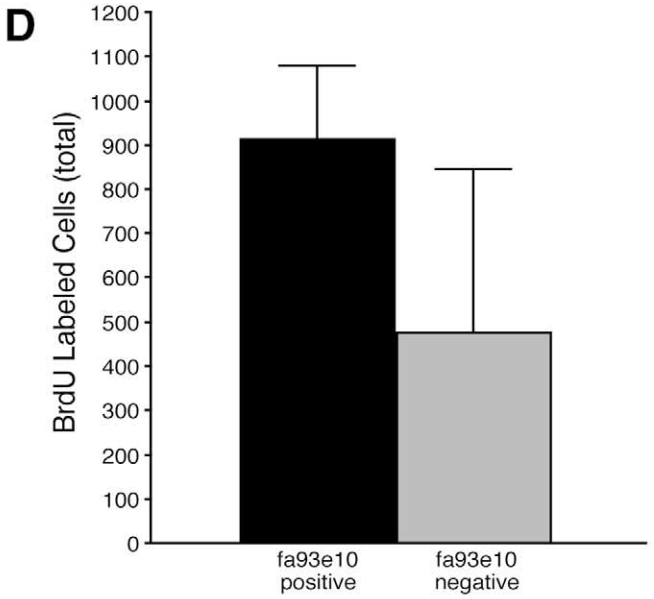

B
D
C
E

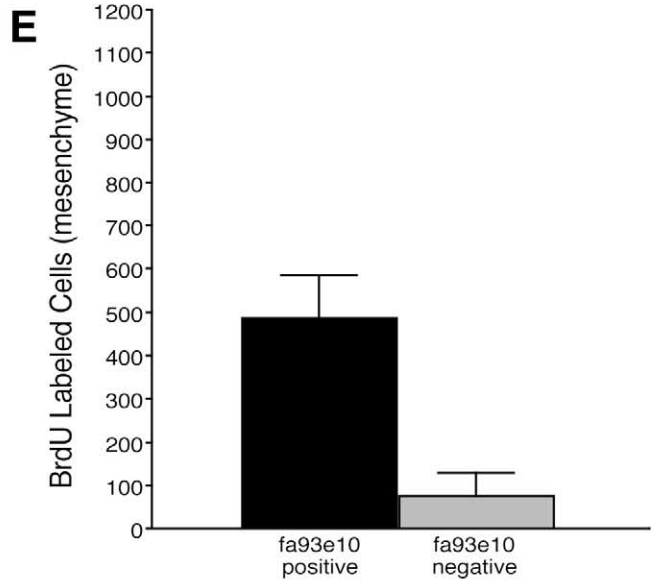

F
200
150
100
50
0
BrdU Labelled Cells
fa93e10 positive
fa93e10 negative

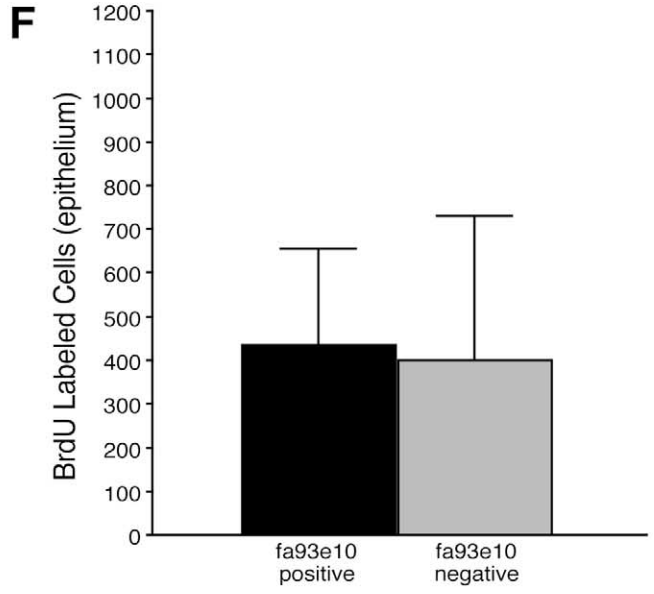

A
fa93e10-positive


B
fa93e10-positive


C
fa93e10-negative


D
BrdU Labeled Cells (total)
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
fa93e10 positive
fa93e10 negative


E
BrdU Labeled Cells (mesenchyme)
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
fa93e10 positive
fa93e10 negative


F
BrdU Labeled Cells (epithelium)
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
fa93e10 positive
fa93e10 negative

in vivo with BrdU (Nechiporuk and Keating, 2002), a thymidine analogue that is incorporated into DNA during S-phase and can subsequently be detected immunohistochemically. One hundred 24-week-old fish were labeled in vivo with BrdU, their fins amputated and first probed for fa93e10 expression. Four percent of the fins were found to be fa93e10-positive and 96% of the fins were fa93e10-negative (data from Fig. 2B). From the fa93e10-positive fins and 5 randomly selected fa93e10-negative fins, 50 contiguous sections were cryosectioned, starting from either the most dorsal or most ventral fin ray, then processed for BrdU detection. BrdU-labeled cells were counted if they were in the distal fin mesenchyme, within 1 segment length (250 μm) of the tip of the fin ray (Fig. 3A). Both fa93e10-positive and fa93e10-negative fins contained BrdU-labeled cells; however, fa93e10-positive fins had approximately 8 times as many labeled cells (142.75 $\pm$ 28.8 cells) compared with fa93e10-negative fins (17 $\pm$ 14.6 cells) ($P < 0.05$, Fig. 3D). Taking these results together, we conclude that fin rays grow by saltation, resulting from episodic periods of mesenchymal cell division and that these episodes of mesenchymal growth are marked by fa93e10 expression in the overlying basal epithelium.

Epithelial and mesenchymal cell proliferation are regulated independently

Although the analysis above (Fig. 3) describes cell proliferation in the mesenchymal compartment of the caudal fin, careful examination of those fins clearly identified proliferating cells in the fin epithelium (Fig. 3C and E). We used fa93e10 expression and whole-mount BrdU labeling to ask whether cell proliferation in the distal fin epithelium occurs in saltatory bursts, mirroring the proliferative burst in the intraray fin mesenchyme that accompanies segment formation. Fourteen-week-old fish ($n = 10$) were labeled in vivo with BrdU. Following fin amputation and whole-mount ISH, fa93e10-positive ($n = 7$) and fa93e10-negative ($n = 3$) fins were processed for BrdU detection as whole mounts. For each fin, all BrdU-labeled cells within one segment length (250 μm) of the distal tip (epithelium and mesenchyme) were counted. Both fa93e10-positive (Fig. 4A and B) and fa93e10-negative (Fig. 4C) fins contained BrdU-labeled cells. There was no significant difference ($P > 0.05$) between the total number (mesenchymal plus epithelial) of labeled cells from fa93e10-positive and fa93e10-negative fins (910 $\pm$ 166 and 476 $\pm$ 370 cells, respectively) (Fig. 4D). In contrast, when cell counts were restricted to the intraray fin mesenchyme (cells in the intraray region sharing the same focal plane as the actinotrichia; Fig. 4B), fa93e10-positive fins contained significantly more dividing cells (479 $\pm$ 100 cells) than fa93e10-negative fins (76 $\pm$ 52 cells) ($P < 0.05$; Fig. 4E), similar to the results obtained in 24-week-old fish using fin sections (see Fig. 3). Subtracting dividing mesenchymal cells from total dividing cells reveals that fa93e10-positive and fa93e10-negative fins contained almost equal numbers of proliferating cells (430 $\pm$ 213 and 400 $\pm$ 330 cells, respectively) in the distal fin epithelium ($P > 0.05$, Fig. 4F). From this analysis, we conclude that cell proliferation in the distal fin epithelium is not tightly coupled to the cell proliferation responsible for formation of new fin ray segments. Similar results in 50 fin sections (from Fig. 3) were found when BrdU incorporation was assessed in epithelial and mesenchymal compartments (142 $\pm$ 29 and 124 $\pm$ 57 cells, respectively; $P > 0.05$), tending to confirm our results from whole-mount analysis.

Fin overgrowth mutations

long fin

long fin (lof) is a dominant fin overgrowth mutant first identified in the pet store trade (Iovine and Johnson, 2000; Tresnake, 1981). Iovine and Johnson (2000) originally postulated the saltation model of fin growth based on genetic analyses of *lof* and *short fin* (*sof*) single mutants and *lof; sof* double mutants. A further prediction of this model is that the *lof* mutation causes a bypass of the stasis phase of the fin ray growth cycle. Availability of the growth marker fa93e10 and BrdU protocols for assessing cell division now make a test of this model possible.

We used fa93e10 expression ($n = 43$ fins) or BrdU-labeling ($n = 33$ fins) to ask whether *lof* mutants (*lof*/+) bypass stasis (ISH and BrdU-labeling were performed in separate groups). This analysis was performed in 24-week-old mutants and limited to the longest fin rays in the ventral lobe of the caudal fin. Whole-mount BrdU labeling in wild type caudal fins (see above) was used to estimate the number of BrdU-labeled cells in the distal intraray mesenchyme of fa93e10-positive fin rays (9.6 $\pm$ 2) and fa93e10-negative fin rays (1.5 $\pm$ 1). We used either BrdU-labeled mesenchymal cells (>10 per fin ray) or fa93e10 expression (assessed by ISH) as criteria to determine whether an individual fin ray was in the growth phase of the segment addition cycle. We found that 95% (41/43) of the fins in the ISH group and 97% (32/33) of the fins in the BrdU group had fin rays that were in the segment addition phase of the fin ray growth

Fig. 4. Independent mesenchymal and epithelial regulation of cell proliferation. Proliferating cells (green), identified by whole-mount BrdU immunohistochemistry, can be seen in both fa93e10-positive (A, B) (purple staining, highlighted by white arrows in A) and fa93e10-negative (C) fins. At higher magnification (B), BrdU-labeled cells in the distal intraray mesenchyme (white arrowheads) are easily identified as they lie in the same focal plane as the actinotrichia (black arrow). (D) Total BrdU-labeled cells in distal fin (mesenchymal and epithelial) were counted for fa93e10-positive ($n = 7$) and fa93e10-negative ($n = 10$) fins. (E) fa93e10-positive fins contained significantly more BrdU-labeled cells in the distal intraray mesenchyme than fa93e10-negative fins. (F) To estimate the number of proliferating epithelial cells for fa93e10-positive and fa93e10-negative fins, we subtracted the number of proliferating cells in the intraray mesenchyme (E) from the total number of proliferating cells (D).

cycle (Fig. 5H and I, ventral lobe). This percentage is far in excess of 12% observed for wild-type fins that are in the segment addition phase at any given time in populations of 24-week-old fish that we examined (see above).

Interestingly, the *lof* heterozygote (*lof*/+) develops an asymmetrically overgrown caudal fin (Fig. 5A). We reasoned that only the longest caudal fin rays (ventral lobe) would persistently bypass rest phase (shown above). Fin rays in the dorsal lobe (intermediate length) and in the cleft (shortest length) should show some evidence of cycling; however, even the shortest fin rays from the cleft should be in growth phase more frequently than wild-type fish of the same size, since the shortest caudal fin rays from a *lof*/+ mutant have more segments than their wild type counterparts (data not shown). Further examination of the longest 3 fin rays from each of dorsal lobe and clefts in 24-week-old, *lof*/+ mutants revealed that only 51 or 66% of dorsal lobe fin rays (BrdU and ISH groups, respectively), and 22 or 46% of cleft fin rays (BrdU and ISH groups, respectively) are in the segment addition phase of the fin ray growth cycle, in contrast to 95–97% observed for the ventral lobe (Fig. 5H and I). In fins from 24-week-old *lof* mutants, the number of rays designated cycling in the BrdU-assessed group was lower than in the ISH-assessed group. This may reflect a stringent threshold for observing BrdU-labeled cells. Ten cells is approximately the number of BrdU-labeled cells seen in an ISH-positive (fa93e10) fin ray. Photomicrographs (Fig. 5B–G) clearly demonstrate that, in some *lof*/+ fins, even neighboring fin rays may not be in the same phase of the segment growth cycle. Thus, unlike the wild-type caudal fin, wherein all fin rays cycle synchronously between growth and stasis, synchrony is lost in the *lof*/+ mutant. A separate analysis of younger *lof*/+ mutants bolsters this conclusion. In 16-week-old *lof*/+ mutants, fa93e10 expression was observed in 76, 54, and 91% of fin rays from the dorsal lobe, cleft, and ventral lobe, respectively.

rapunzel

rapunzel (rap) is a dominant, ENU-induced fin overgrowth mutation (Fig. 6A); however, the *rap* phenotype is, in many ways, dissimilar to that of *lof*. *rap*, unlike *lof*, is a recessive lethal mutation, developing midface hypoplasia, jaw abnormalities, pericardial edema, abnormal hematopoiesis, and fin fold defects in homozygous embryos (data not shown). Also unlike *lof*, the adult phenotype of *rap* (*rap*/+) is not limited to fin overgrowth. *rap* adults have craniofacial abnormalities and a generalized defect in bone formation (not shown), in addition to fin overgrowth (below).

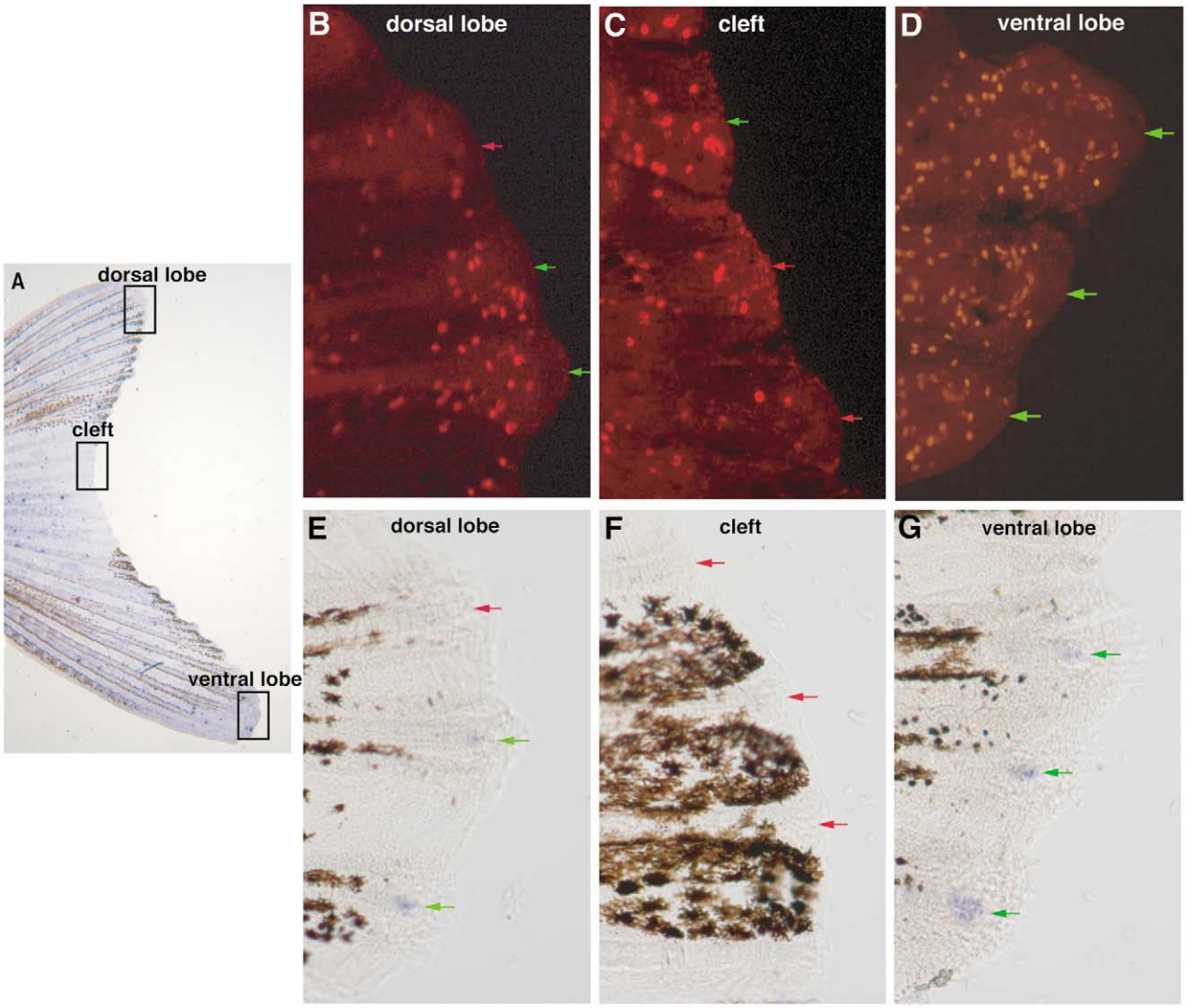
To determine the anatomical basis of the *rap* overgrowth defect, we analyzed fin ray segment length and segment number. Similar to observations for *lof* mutants (Iovine and Johnson, 2000), segment size in the *rap* fin ray is normal (Fig. 6C). Instead (also like *lof*), all of the fin overgrowth can be accounted for by an increase in segment number (Fig. 6C). Indeed, when fin length:body length ratio is plotted as a function of body size for *rap* (Fig. 6B), growth is clearly allometric, unlike the isometric growth described for wild-type zebrafish (Iovine and Johnson, 2000). The synchronous fin overgrowth seen in *rap* mutants predicts that all fin rays of the *rap* caudal fin should demonstrate fa93e10 gene expression and contain abundant proliferating cells in the distal intraray mesenchyme. We performed whole-mount ISH with fa93e10 (Fig. 6D) and whole-mount BrdU-labeling (Fig. 6E) of *rap* caudal fins from 8- to 24-week-old fish (total $n = 40$). In no case did *rap* fins fail to express fa93e10 or lack abundant dividing cells in the distal intraray fin mesenchyme. This result demonstrates that all fin rays of *rap* caudal fins are always in the segment addition phase of the fin ray growth cycle and never in the stasis phase.

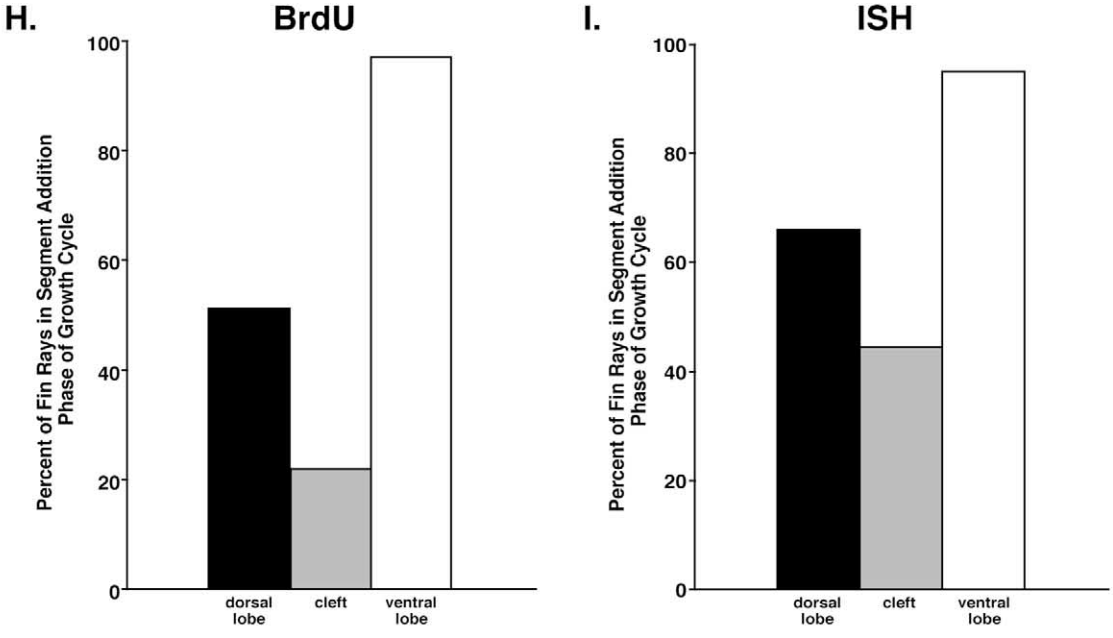
Discussion

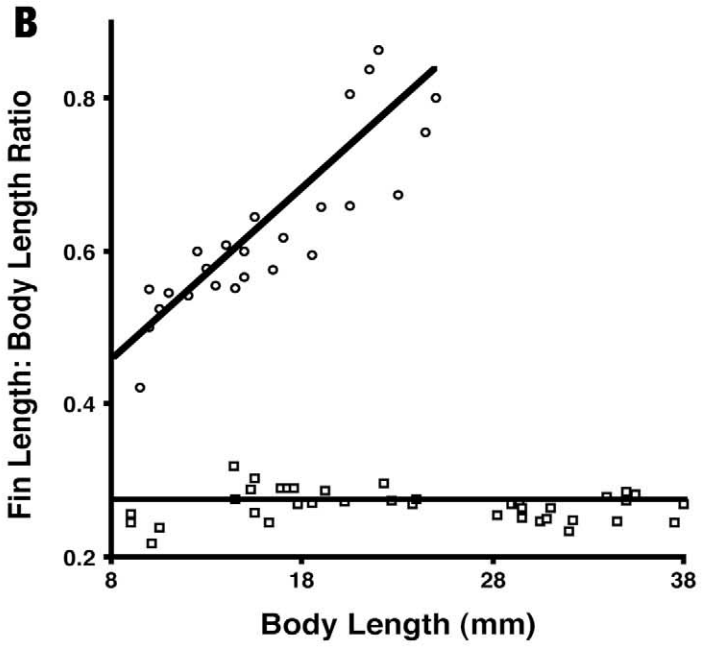
The zebrafish caudal fin: a powerful vertebrate model of saltatory growth

Growth in zebrafish is indeterminate (Iovine and Johnson, 2000; Jordan, 1905). Although others have disputed this idea in its extremis, (Mommsen, 2001), 6- to 8-week-old juvenile zebrafish clearly grow with a greater overall growth velocity than 16- to 24-week-old adult zebrafish, that grow faster than 9- to 12-month-old adult zebrafish (Iovine and Johnson, 2000). Amidst this background of decreasing growth velocity, proportionate (isometric) growth of the caudal fin with respect to the body is maintained. Fins grow via the distal addition of segments, an anatomical unit of fixed length within any given fin ray. Thus, proportionate growth demands either a model wherein segment formation is episodic, finite, and interspersed with variable periods of stasis (saltatory growth), or a model wherein segment formation is continuous but the rate of segment growth is variable (continuous growth).

Fig. 5. Asynchronous saltation in *long fin*. In separate experiments, interray growth synchrony in *long fin* was assessed by using either BrdU to label dividing cells or fa93e10 expression (ISH) to identify fin rays in the segment addition phase of the growth cycle. (A) A whole-mount caudal fin from a *long fin* heterozygote demonstrating asymmetric overgrowth. Black boxes highlight the three regions (dorsal lobe, cleft, ventral lobe) where growth was assessed. (B–G) Higher magnification views from the dorsal lobe (B, E), cleft (C, F), and ventral lobe (D, G) demonstrating BrdU-labeling (B–D) and fa93e10 expression (E–G). Three fin rays are shown in each photomicrograph. Green arrows identify fin rays in growth phase (>10 BrdU-labeled cells in the distal intraray mesenchyme or expressing the growth marker fa93e10). Red arrows identify fin rays in stasis (<10 BrdU-labeled cells in the distal intraray mesenchyme or not expressing the growth marker fa93e10). (H, I) Using BrdU-labeling (H, $n = 33$ fins/99 fin rays) or fa93e10 expression (I, $n = 43$ fins/129 fin rays), three fin rays from each of the dorsal lobe, cleft, and ventral lobe were scored as being either in growth phase or stasis.

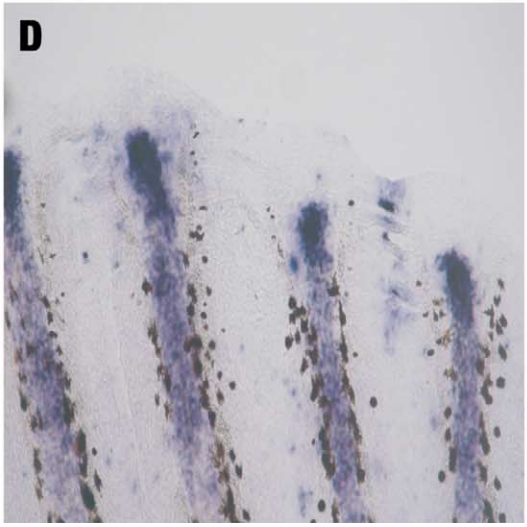

A
dorsal lobe
cleft
ventral lobe
B
dorsal lobe
C
cleft
D
ventral lobe
E
dorsal lobe
F
cleft
G
ventral lobe


H.
BrdU
I.
ISH
Percent of Fin Rays in Segment Addition Phase of Growth Cycle
100
80
60
40
20
0
dorsal lobe
cleft
ventral lobe



C

	wild type	*rap*
average number of segments	26±2	52.2±3
average segment length (μm)	300±21	300±30



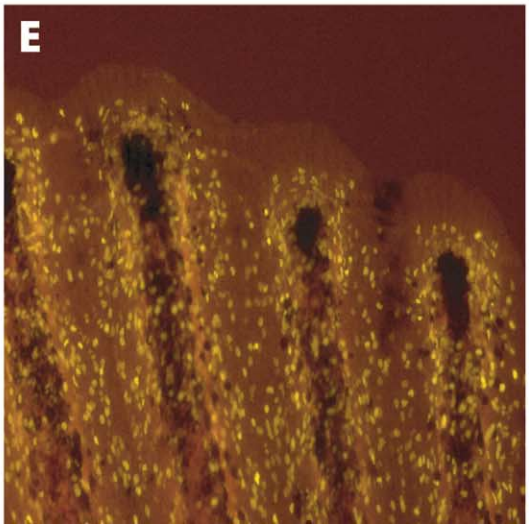


Fig. 6. Synchronous overgrowth in *rapunzel*. (A) The *rapunzel* heterozygote has symmetrically fin overgrowth. (B) Fin length: body length ratio plotted against body length for *rap* (circles) and wild type (squares) clearly demonstrates allometric fin growth in *rap* in contrast to isometric growth seen in the wild type caudal fin. Lines represent approximate growth curves. (C) Fin overgrowth in *rap* is due to increased segment number ($n = 4$ fin rays for each of *rap* and wild type; $P < 0.05$). There is no significant difference in fin ray segment length between *rap* and wild type ($n = 30$ segments for each of *rap* and wild type; $P > 0.05$). Growth was assessed in 6-month-old *rapunzel* caudal fins via fa93e10 gene expression (D) and BrdU labeling (E).

Here, we directly demonstrate, using a growth marker (fa93e10) and analysis of cell proliferation, that fin growth is saltatory, consistent with the previous genetic model (Iovine and Johnson, 2000). Proportionate growth of the wild-type zebrafish caudal fin occurs through episodic but finite periods of saltation, alternating with variable periods of stasis.

fa93e10, a robust surrogate growth marker delimits cycles of mesenchymal cell proliferation

We used fa93e10, an orphan EST, as a surrogate marker for fin growth. fa93e10 is ideally suited in this role because its robust expression delimits bursts of cell proliferation in the distal intraray fin mesenchyme. fa93e10's surrogacy as a growth marker cannot be overlooked, as a specific function for fa93e10 with respect to fin growth remains to be demonstrated. Notwithstanding fa93e10's unknown function with respect to fin growth, this gene is not unique amongst candidate genes that we have explored whose expression pattern cycles in a manner consistent with the saltation model of fin growth. For example, fa99c12, an EST corresponding to *colla1,* is expressed in a pattern remarkably similar to fa93e10, albeit not as robust (data not shown). fa93e10 is expressed in the basal layer of the distal fin epithelium (although not at the most distal tip of the fin ray), mirroring the spatial expression of other molecules postulated to play a role in fin growth, including *sonic hedgehog (shh), patched* (the receptor for *shh*), and *bmp2* (Laforest et al., 1998; Quint et al., 2002). Interestingly, fa93e10 expression in the basal epidermis only partially overlies the distal mesenchyme where most cell division is observed. fa93e10 expression also extends proximally, overlying the zone where mesenchymal cells condense laterally and differentiate into bone-forming osteoblasts (Goss and Stagg, 1957; Haas, 1962). While it is intriguing to postulate a role for fa93e10 in helping to recruit osteoblasts from their mesenchymal cell precursors, there are no data to support such speculation. For example, fa93e10 may play a structural role in the epithelium during growth of the nascent fin ray segment.

fa93e10 is also a reliable marker for the growth status of the fin ray. One indication that it reliably marks the growth status of the fin ray is that fa93e10-positive fin rays have many more mesenchymal cell divisions than fa93e10-negative rays. One might argue that this represents a threshold for fa93e10 detection, masking graded expression levels of fa93e10, corresponding to different levels of cell division. The fact that the number of mesenchymal cell divisions observed in a fa93e10-negative fin ray is always many fold lower than that observed for fin rays with the least number of mesenchymal cell divisions in the fa93e10-positive class tends to argue against this threshold model (not shown). Additionally, our observations of heterogeneous fa93e10 expression in *lof* mutants support the all-or-nothing model. The all-or-nothing pattern of fa93e10 expression in individual *lof* fin rays persisted even when fins were 20-fold overdeveloped (not shown). Thus, we conclude that the difference in fa93e10 expression between positive and negative fin rays reliably identifies their growth status in the segment addition cycle.

Growth status of the fish or fin is not strictly a function of the age or size of the fish. It is important to realize that our graphs showing fa93e10 expression data for fish of different ages (Fig. 2B) or sizes (Fig. 2C) cannot be construed as a reliable predictor of fin growth status for all populations of zebrafish. Many factors may affect the instantaneous growth status of the fish fin, including age and size, but also temperature, recent nutritional history, and genetic background.

In addition, because ISH and BrdU-labeling of excised fins cannot directly demonstrate an individual fin ray progressing through successive cycles of saltation and stasis, one might argue that our data support a continuous model of growth wherein individual fish (and fins) are terminating growth as they reach a final, mature size. We do not believe this to be the case. First, we find that between groups of fish of different ages, growth status does not correlate with size (Fig. 2C). Second, even within individual groups of fish of the same age, we find that some smaller fish are negative for expression of fa93e10, while some larger fish are positive for the marker (data not shown). Because of the indeterminate nature of zebrafish growth, these smaller fish will continue to grow and continue to add segments. A direct demonstration of fins cycling between saltation and stasis must await the generation of transgenic lines of fish containing a vital reporter (e.g., GFP) driven by a growth-regulated promoter in the fin.

Cell proliferation in the distal fin epithelium does not proceed in saltatory bursts concomitant with segment addition

It was clear from the cell proliferation analyses of fin sections that dividing cells were present, albeit in fewer numbers, in the distal fin during stasis (fa93e10-negative fins). We hypothesized that most of the cells that continue to divide during stasis were not contributing to growth of the fin ray compartment. We demonstrated that cells proliferate in the distal fin epithelium during both saltation and stasis and that the number of epithelial cells proliferating during saltation is not significantly different from the number of epithelial cells proliferating during stasis.

Is it surprising that the epithelium covering the fin ray does not demonstrate bursts of cell proliferation in conjunction with segment addition? Several possibilities could explain our results. Unlike fin mesenchyme, the epithelium is under constant assault from the external environment. One possibility is that the high rate of epithelial cell proliferation during both saltation and stasis reflects a large, continual demand for cell renewal to maintain epidermal integrity. This high rate of epithelial cell proliferation may either be

sufficient for the needs of both growth and epithelial maintenance or, alternatively, may mask a small but real proliferative burst during saltation. We cannot distinguish between these two possibilities at the present time. A separate possibility is that we missed the burst of epithelial cell proliferation occurring during saltation because we only examined cell proliferation in the distal fin epithelium. Perhaps segment addition puts mechanical traction on the epithelium, inducing cell proliferation at more proximal levels in much the same way that a plastic surgeon uses tissue expanders to induce skin growth for coverage of large wounds (Malata et al., 1995). In the fin, such traction could conceivably induce epithelial cell proliferation in the more proximal fin epithelium, some distance from the region of segment addition. Qualitative examination of BrdU labeling in the more proximal caudal fin epithelium (not shown) demonstrates that the rate of cell proliferation is very high, irrespective of whether fins are in the segment addition phase of the fin ray growth cycle or not.

long fin and rapunzel: asynchronous and synchronous overgrowth suggest disparate mechanisms orchestrating growth control

Growth is undoubtedly regulated by many different kinds of signals. Some of these signals will likely act locally, at the level of individual organs or tissues, while others may act globally, instructing or permitting growth at the level of the whole organism. Orchestration of this complex hierarchy of signals acts to bring about growth and the ultimate form of the organism. This orchestration is beautifully illustrated when one examines allometric growth in the developing human (Moore and Persaud, 1998). For example, the head of a human embryo is exceedingly large with respect to the body. Following the embryonic period, growth of the head slows relative to the growth of the torso, arms, and legs. Such examples of allometric growth do not end at birth. For example, growth of the brain (mass) is rapid during infancy and early childhood, while the reproductive organs do not grow significantly until adolescence (Ulijaszek et al., 1998).

Isometric growth in the fin seems to be maintained by coordinating synchronous development of new segments. Such growth occurs by saltations, separated by little or no rest (stasis) in younger fish, and increasing periods of rest (stasis) as the fish matures. The finding that in wild-type fins, fin rays across the fin are either all in growth, or all in stasis, suggests that growing segments proceed synchronously through the growth phase of the segmentation cycle. Direct evidence will require identification of markers for different stages within this phase of the growth cycle. Using ISH (fa93e10) and BrdU-labeling, we demonstrate here that synchronous saltation is lost in *lof* (*lof*/+) mutants. Loss of synchronous saltation was seen in our evaluation of both 16- and 24-week-old *lof* mutants, although at 16 weeks the percentage of dorsal lobe and cleft fin rays expressing fa93e10 was somewhat greater than at 24 weeks (76% dorsal lobe/54% cleft vs 66% dorsal lobe/46% cleft, respectively). This finding is consistent with the model of the *lof* phenotype. *lof* is thought to bypass the stasis phase of the fin ray growth cycle (Iovine and Johnson, 2000), thus the *lof* phenotype is less apparent in young fish that spend proportionately more time in the segment addition phase of the fin ray growth cycle. Indeed, very young fish (i.e., less than 6 weeks old) cannot be scored for the *lof* mutation (data not shown). The finding of heterogeneous expression of fa93e10 and incorporation of BrdU from fin ray to fin ray in older *lof* mutants suggests that entry into the segment growth phase can be controlled at level of the individual fin ray. Whether this is controlled by the *lof* mutant gene in a fin ray-autonomous fashion will require analysis of chimeric individuals. In contrast, we find that *rap* mutants are always in the segment addition phase of the fin ray growth cycle. We find that the number of fin ray segments added in postjuvenile development is uniform from fin ray to fin ray in *rap* mutants (K. Iovine and M.I.G., unpublished results), raising the possibility that these fin rays are adding segments synchronously. As above, direct evidence for synchronous segment initiation in *rap* mutants will require the identification of markers specific for different stages of segment growth phase. Nevertheless, these observations lead us to speculate that global or systemic mechanisms that coordinate segment initiation are abrogated in *rap* mutants. The possible systemic nature of this defect is reinforced by finding of growth defects in other bones of *rap* mutant fish, including vertebrae and craniofacium (not shown). Molecular identification of the *rap* and *lof* genes will help elucidate their disparate roles in growth control.

Saltatory growth: a fundamental growth regulatory mechanism conserved between humans and teleosts

If saltatory growth was restricted to structures containing anatomically discrete, repetitive units of growth such as a fin ray segment, then the broader significance of our findings would be questionable. Saltatory growth has been demonstrated for multiple bone types in human growth and development. Studies of human growth during infancy, when overall growth velocity is greatest, and during adolescence, when overall growth velocity exceeds all other times aside from infancy, clearly demonstrate that human growth is also by saltation (Lampl and Johnson, 1993; Lampl et al., 1992). In these studies, length (infants) or stature (adolescents) was measured daily for more than a year. During 95 and 97% of these 24-h epochs in infants and adolescents, respectively, no growth occurred. All growth could be accounted for by a small number of saltations, each lasting less than 24 h. Interestingly, evidence supporting saltatory growth in humans is not limited to instances wherein growth occurs at physes, the unique growth centers found in endochondral bone (Favus, 1999; Olsen et al., 2000). Vertebrate skulls consist primarily of dermal bone,

which like the fin ray, develop and grow via the direct condensation of mesenchymal cells into bone-forming osteoblasts rather than through the replacement of a cartilaginous template (endochondral ossification) (Favus, 1999; Olsen et al., 2000). During gestation, head circumference also increases in a saltatory manner (Bernstein et al., 1996), indicating that bones that do not contain specialized growth zones, or physes, may respond to similar cues that regulate long bone growth. Our findings in zebrafish advance the exciting and important notion that fundamental growth control mechanisms are conserved amongst disparate members of the vertebrate phylum, including zebrafish and humans.

The importance of a tractable model of growth control towards understanding the genetics of human growth dysregulation

Growth problems, both genetic and acquired, are a ubiquitous feature of pediatric pathophysiology. Indeed, growth is the defining patient characteristic of pediatric medicine. Genetic growth disorders in humans span a breadth of problems that include both overgrowth (Cohen, 1999; Sotos, 1997a,b) and undergrowth. Furthermore, human growth disorders may affect growth symmetrically or alternatively, may act regionally, and thereby affect the normal proportionality of the human body. One example of a symmetrical overgrowth syndrome is Beckwith–Wiedemann syndrome (BWS), which causes pre- and postnatal macrosomia, malformations, and an increased risk of embryonal tumors (Beckwith, 1969; Wiedemann, 1964). The genetics of BWS is heterogeneous, complex, and may involve imprinting of the 11p15 locus that includes the candidate genes such as IGF2 (Li et al., 1998). Asymmetric overgrowth syndromes are also well described. Sotos syndrome (Opitz et al., 1998; Sotos, 1997a,b) results in cerebral gigantism, while Marfan syndrome results in a disproportionate increase in arm span relative to height (Pyeritz, 2000). Interestingly, mutations in PTEN account for some patients with Sotos syndrome (Marsh et al., 1997) and mutations in PTEN also cause abnormal size phenotypes in Drosophila (Goberdhan et al., 1999; Oldham et al., 2002; Stocker et al., 2002). Marfan syndrome is a clinically heterogeneous syndrome resulting from mutations in the gene encoding fibrillin-1 (Robinson and Booms, 2001; Robinson and Godfrey, 2000). Undergrowth syndromes are also encountered in pediatrics. One well-characterized class of growth factors/growth factor receptors, the fibroblast growth factor (fgf)/fgf receptor family, is involved in a heterogeneous collection of disorders that result in disproportionate limb undergrowth, or dwarfism (Naski et al., 1996; Ornitz, 2001). Activating FGFR3 mutations in mice recapitulate the human phenotype (Wang et al., 2001), while a murine FGFR3 knockout causes the opposite phenotype of overgrowth (Colvin et al., 1996). Many of these genes have now been identified by the zebrafish EST and genome sequencing projects, and placed on the zebrafish genetic map (Clark et al., 2001; Hukriede et al., 2001; Woods et al., 2000; S.L.J., unpublished). The finding that *lof* maps to LG2 (Iovine and Johnson, 2002) and *rap* to LG16 (S.L.J., unpublished), unlinked or distant from each of the above candidate genes, raises the possibility that *lof* and *rap* identify new pathways or molecules controlling vertebrate bone growth.

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References

Aparicio, S., Chapman, J., Stupka, E., Putnam, N., Chia, J.-m., Dehal, P., Christoffels, A., Rash, S., Hoon, S., Smit, A., Gelpke, M.D.S., Roach, J., Oh, T., Ho, I.Y., Wong, M., Detter, C., Verhoef, F., Predki, P., Tay, A., Lucas, S., Richardson, P., Smith, S.F., Clark, M.S., Edwards, Y.J.K., Doggett, N., Zharkikh, A., Tavtigian, S.V., Pruss, D., Barnstead, M., Evans, C., Baden, H., Powell, J., Glusman, G., Rowen, L., Hood, L., Tan, Y.H., Elgar, G., Hawkins, T., Venkatesh, B., Rokhsar, D., Brenner, S., 2002. Whole-genome shotgun assembly and analysis of the genome of fugu rubripes. Science 297, 1301–1310.

Beckwith, J., 1969. Macroglossia, omphalocele, adrenal cytomegaly, gigantism, and hyperplastic visceromegaly. Birth Defects Orig. Art. Ser. 5, 188–196.

Bernstein, I.M., Blake, K., Wall, B., Badger, G.J., 1996. Evidence that normal fetal growth can be noncontinuous. Obstet. Gynecol. Surv. 51, 213–214.

Clark, M.D., Hennig, S., Herwig, R., Clifton, S.W., Marra, M.A., Lehrach, H., Johnson, S.L., Group t, W., 2001. An oligonucleotide fingerprint normalized and expressed sequence tag characterized zebrafish cDNA library. Genome Res. 11, 1594–1602.

Cohen Jr., M.M., 1999. Overgrowth syndromes: an update. Adv. Pediatr. 46, 441–491.

Colvin, J.S., Bohne, B.A., Harding, G.W., McEwen, D.G., Ornitz, D.M., 1996. Skeletal overgrowth and deafness in mice lacking fibroblast growth factor receptor 3. Nat. Genet. 12, 390–397.

Conlon, I., Raff, M., 1999. Size control in animal development. Cell 96, 235–244.

Favus, M.J., 1999. Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism. Lippincott Williams & Wilkins, Philadelphia.

Goberdhan, D.C., Paricio, N., Goodman, E.C., Mlodzik, M., Wilson, C., 1999. Drosophila tumor suppressor PTEN controls cell size and number by antagonizing the Chico/PI3-kinase signaling pathway. Genes Dev. 13, 3244–3258.

Goss, R.J., 1978. The Physiology of Growth. Academic Press, New York.

Goss, R.J., Stagg, M.W., 1957. The regeneration of fins and fin rays in *Fundulus heteroclitus*. J. Exp. Zool. 136, 487–508.

Haas, H.J., 1962. Studies on mechanisms of joint and bone formation in the skeletal rays of fish fins. Dev. Biol. 5, 1–34.

Hukriede, N., Fisher, D., Epstein, J., Joly, L., Tellis, P., Zhou, Y., Barbazuk, B., Cox, K., Fenton-Noriega, L., Hersey, C., Miles, J., Sheng, X., Song, A., Waterman, R., Johnson, S.L., Dawid, I.B., Chevrette, M.,

Zon, L.I., McPherson, J., Ekker, M., 2001. The LN54 radiation hybrid map of zebrafish expressed sequences. Genome. Res. 11, 2127–2132.
Iovine, M.K., Johnson, S.L., 2000. Genetic analysis of isometric growth control mechanisms in the zebrafish caudal fin. Genetics 155, 1321–1329.
Iovine, M.K., Johnson, S.L., 2002. A genetic, deletion, physical, and human homology map of the long fin region on zebrafish linkage group 2. Genomics 79, 756–759.
Johnson, S.L., Bennett, P., 1999. Growth control in the ontogenetic and regenerating zebrafish fin. Methods Cell Biol. 59, 301–311.
Johnson, S.L., Weston, J.A., 1995. Temperature-sensitive mutations that cause stage-specific defects in zebrafish fin regeneration. Genetics 141, 1583–1595.
Jordan, D.S., 1905. A guide to the Study of Fishes. Henry Holt and Co., New York.
Laforest, L., Brown, C.W., Poleo, G., Geraudie, J., Tada, M., Ekker, M., Akimenko, M.A., 1998. Involvement of the sonic hedgehog, patched 1 and bmp2 genes in patterning of the zebrafish dermal fin rays. Development 125, 4175–4184.
Lampl, M., Johnson, M.L., 1993. A case study of daily growth during adolescence: a single spurt or changes in the dynamics of saltatory growth? Ann. Hum. Biol. 20, 595–603.
Lampl, M., Veldhuis, J.D., Johnson, M.L., 1992. Saltation and stasis: a model of human growth. Science 258, 801–803.
Lander, E.S., Linton, L.M., Birren, B., Nusbaum, C., Zody, M.C., Baldwin, J., Devon, K., Dewar, K., Doyle, M., FitzHugh, W., Funke, R., Gage, D., Harris, K., Heaford, A., Howland, J., Kann, L., Lehoczky, J., LeVine, R., McEwan, P., McKernan, K., Meldrim, J., Mesirov, J.P., Miranda, C., Morris, W., Naylor, J., Raymond, C., Rosetti, M., Santos, R., Sheridan, A., Sougnez, C., Stange-Thomann, N., Stojanovic, N., Subramanian, A., Wyman, D., Rogers, J., Sulston, J., Ainscough, R., Beck, S., Bentley, D., Burton, J., Clee, C., Carter, N., Coulson, A., Deadman, R., Deloukas, P., Dunham, A., Dunham, I., Durbin, R., French, L., Grafham, D., Gregory, S., Hubbard, T., Humphray, S., Hunt, A., Jones, M., Lloyd, C., McMurray, A., Matthews, L., Mercer, S., Milne, S., Mullikin, J.C., Mungall, A., Plumb, R., Ross, M., Shownkeen, R., Sims, S., Waterston, R.H., Wilson, R.K., Hillier, L.W., McPherson, J.D., Marra, M.A., Mardis, E.R., Fulton, L.A., Chinwalla, A.T., Pepin, K.H., Gish, W.R., Chissoe, S.L., Wendl, M.C., Delehaunty, K.D., Miner, T.L., Delehaunty, A., Kramer, J.B., Cook, L.L., Fulton, R.S., Johnson, D.L., Minx, P.J., Clifton, S.W., Hawkins, T., Branscomb, E., Predki, P., Richardson, P., Wenning, S., Slezak, T., Doggett, N., Cheng, J.F., Olsen, A., Lucas, S., Elkin, C., Uberbacher, E., Frazier, M., et al., 2001. Initial sequencing and analysis of the human genome. Nature 409, 860–921.
Larsen, F.S., Hansen, B.A., Blei, A.T., 2000. Intensive care management of patients with acute liver failure with emphasis on systemic hemodynamic instability and cerebral edema: a critical appraisal of pathophysiology. Can. J. Gastroenterol. 14, 105D–111D.
Li, M., Squire, J.A., Weksberg, R., 1998. Molecular genetics of Wiedemann-Beckwith syndrome. Am. J. Med. Genet. 79, 253–259.
Malata, C.M., Williams, N.W., Sharpe, D.T., 1995. Tissue expansion: an overview. J. Wound Care 4, 37–44.
Marsh, D.J., Dahia, P.L., Zheng, Z., Liaw, D., Parsons, R., Gorlin, R.J., Eng, C., 1997. Germline mutations in PTEN are present in Bannayan–Zonana syndrome. Nat. Genet. 16, 333–334.
Mommsen, T.P., 2001. Paradigms of growth in fish. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 129, 207–219.
Moore, K.L., Persaud, T., 1998. The Developing Human: Clinically Oriented Embryology. Saunders, Philadelphia.
Nabrit, S.M., 1929. The role of the fin rays in regeneration in the tail fins of fishes (*Fundulus* and goldfish). Biol. Bull. 56, 235–266.
Naski, M.C., Wang, Q., Xu, J., Ornitz, D.M., 1996. Graded activation of fibroblast growth factor receptor 3 by mutations causing achondroplasia and thanatophoric dysplasia. Nat. Genet. 13, 233–237.
Nechiporuk, A., Keating, M.T., 2002. A proliferation gradient between proximal and msxb-expressing distal blastema directs zebrafish fin regeneration. Development 129, 2607–2617.
Oldham, S., Stocker, H., Laffargue, M., Wittwer, F., Wymann, M., Hafen, E., 2002. The Drosophila insulin/IGF receptor controls growth and size by modulating PtdInsP3 levels. Development 129, 4103–4109.
Olsen, B.R., Reginato, A.M., Wang, W., 2000. Bone development. Annu. Rev. Cell Dev. Biol. 16, 191–220.
Opitz, J.M., Weaver, D.W., Reynolds Jr., J.F., 1998. The syndromes of Sotos and Weaver: reports and review. Am. J. Med. Genet. 79, 294–304.
Ornitz, D.M., 2001. Regulation of chondrocyte growth and differentiation by fibroblast growth factor receptor 3. Novartis Found. Symp. 232, 63–76discussion 76–80, 272–282..
Poss, K.D., Shen, J., Nechiporuk, A., McMahon, G., Thisse, B., Thisse, C., Keating, M.T., 2000. Roles for Fgf signaling during zebrafish fin regeneration. Dev. Biol. 222, 347–358.
Prout, T., Barker, J.S., 1989. Ecological aspects of the heritability of body size in *Drosophila buzzatii*. Genetics 123, 803–813.
Pyeritz, R.E., 2000. The Marfan syndrome. Annu. Rev. Med. 51, 481–510.
Quint, E., Smith, A., Avaron, F., Laforest, L., Miles, J., Gaffield, W., Akimenko, M.-A., 2002. Bone patterning is altered in the regenerating zebrafish caudal fin after ectopic expression of sonic hedgehog and bmp2b or exposure to cyclopamine. Proc. Natl. Acad. Sci. USA 99, 8713–8718.
Raff, M.C., 1996. Size control: the regulation of cell numbers in animal development. Cell 86, 173–175.
Robinson, P.N., Booms, P., 2001. The molecular pathogenesis of the Marfan syndrome. Cell Mol. Life Sci. 58, 1698–1707.
Robinson, P.N., Godfrey, M., 2000. The molecular genetics of Marfan syndrome and related microfibrillopathies. J. Med. Genet. 37, 9–25.
Santamaria, J.A., Becerra, J., 1991. Tail fin regeneration in teleosts: cell-extracellular matrix interaction in blastemal differentiation. J. Anat. 176, 9–21.
Santamaria, J.A., Mari-Beffa, M., Becerra, J., 1992. Interactions of the lepidotrichial matrix components during tail fin regeneration in teleosts. Differentiation 49, 143–150.
Saucedo, L., Edgar, B., 2002. Why size matters: altering cell size. Curr. Opin. Genet. Dev. 12, 565.
Sotos, J.F., 1997a. Overgrowth. Section V. Syndromes and other disorders associated with overgrowth. Clin. Pediatr. (Phila.) 36, 89–103.
Sotos, J.F., 1997b. Overgrowth. Section VI. Genetic syndromes and other disorders associated with overgrowth. Clin. Pediatr. (Phila.) 36, 157–170.
Stocker, H., Andjelkovic, M., Oldham, S., Laffargue, M., Wymann, M.P., Hemmings, B.A., Hafen, E., 2002. Living with lethal PIP3 levels: viability of flies lacking PTEN restored by a PH domain mutation in Akt/PKB. Science 295, 2088–2091.
Streisinger, G.C., Walker, C., Dower, N., Knauber, D., Singer, F., 1981. Production of clones of homozygous diploid zebrafish (*Brachydanio rerio*). Nature 291, 293–296.
Tresnake, I., 1981. The long-finned zebra Danio. Tropical Fish Hobby 29, 43–56.
Ulijaszek, S.J., Johnston, F.E., Preece, M., 1998. The Cambridge Encyclopedia of Human Growth and Development. Cambridge University Press, New York.
Wang, Q., Green, R.P., Zhao, G., Ornitz, D.M., 2001. Differential regulation of endochondral bone growth and joint development by FGFR1 and FGFR3 tyrosine kinase domains. Development 128, 3867–3876.
Westerfield, M., 1993. The Zebrafish Book: A Guide for the Laboratory Use of Zebrafish (*Brachydanio rerio*). University of Oregon Press, Eugene, OR.
Wiedemann, H., 1964. Complexe malformatif familial avec hernie ombilicale et macroglossie. J. Genet. Hum. 13, 223–232.
Woods, I.G., Kelly, P.D., Chu, F., Ngo-Hazelett, P., Yan, Y.L., Huang, H., Postlethwait, J.H., Talbot, W.S., 2000. A comparative map of the zebrafish genome. Genome Res. 10, 1903–1914.