Genetic Analysis of Isometric Growth Control Mechanisms in the Zebrafish Caudal Fin

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ABSTRACT

The body and fins of the zebrafish grow rapidly as juveniles and slower as they reach maturation. Throughout their lives, the fins grow isometrically with respect to the body. Growth of individual fin rays is achieved by the distal addition of bony segments. We have investigated the genetic control of mechanisms that initiate new segments or control size of newly initiated segments. We find that both segment initiation and segment length are regulated during fin growth in wild-type fish. We examined the growth properties of *lof* and *sof* fin length mutants for effects on the number and length of fin ray segments. Fins of *lof* mutants continue to grow rapidly even after wild-type fin growth slows, resulting in positive allometric growth and additional fin ray segments. We suggest that *lof* mutants bypass mechanisms that limit segment initiation. Isometric growth is retained in *sof* mutants, resulting in shorter fins one-half the length of wild-type fins. The primary defect in *sof* mutants is that fin ray segments are shorter than wild-type segments, although segment number is also diminished. Double mutants for *sof;lof* reveal that segment length and segment number are controlled in different pathways. Our findings suggest that the *lof* gene product regulates segment initiation and the *sof* gene product regulates segment length.

MOST organs and limbs develop and grow to a size proportionate to the total body size of an individual. This process is not understood. It is likely that multiple growth control mechanisms are required to achieve the final form. Classically, biologists tend to think of isometric growth (constant proportion with respect to body size) and allometric growth (varied proportion with respect to body size) of organs and limbs (Gould 1966). The mature form of animals is the result of isometric growth of some tissues and allometric growth of other tissues. A fundamental question is how isometric and allometric growth control mechanisms are regulated to achieve the final form of the individual.

At one level, form depends on the number of cells and the size of cells within the organ, limb, or individual (Conlon and Raff 1999; Leevers 1999; Weinkove *et al.* 1999). Considerable research has therefore focused on the processes of cell proliferation and growth of individual cells (Leevers 1999). However, the number and size of cells within an organ presumably represents a response to higher level mechanisms that instruct cells to divide or grow, thereby controlling the size and morphology of the final tissue (Conlon and Raff 1999). Furthermore, these higher level mechanisms must be coupled to mechanisms controlling total body size in order to develop the final form (Stern and Emlen 1999).

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Most fish, including the zebrafish, grow throughout their lives, offering different perspectives on the role of growth control mechanisms on form and size of mature animals. Remarkably, the body and fins of the zebrafish appear to retain proportionality as juveniles and adults, suggesting that zebrafish utilize isometric growth mechanisms throughout their lifetime. Therefore, the zebrafish fin may provide an opportunity to understand isometric growth control mechanism(s) regulating size and shape.

In teleosts such as the zebrafish, fin growth is thought to be largely controlled by the fin ray. Each fin ray grows autonomously, and the anatomy of fin rays has been described (Nabrit 1929; Haas 1962; Santamaria and Becerra 1991). Fin rays are composed of multiple bony segments. Each comprises two apposed hemirays that surround nerves, blood vessels, and mesenchymal cells (Santamaria *et al.* 1992). The bony parts of the fin ray are referred to as lepidotrichia. The addition of bone occurs at the distal end of the growing fin ray (Goss and Stagg 1957; Haas 1962). Undifferentiated mesenchyme (or fibroblasts) in the medial part of the fin condense laterally and differentiate as osteoblasts. These osteoblasts then secrete the bone matrix of the developing segment (Goss and Stagg 1957; Haas 1962). Segments are separated by an osteoblast-free region (or joint) and, once established, do not increase in length (Haas 1962; M. Iovine, unpublished results). Although the process of segment and joint formation is recognizable histologically, it is not clear what controls the overall length of each segment or how often seg-

ments are initiated as the fin ray grows. Since the fins are not essential for life in the laboratory, it is possible to identify mutations that affect fin length (Johnson and Weston 1995; van Eeden *et al.* 1996; Amsterdam *et al.* 1999), but not viability. Such mutations may reveal how fin length is achieved. Recessive *short fin* mutants (*sof*^{b123}; Johnson and Bennett 1999), for instance, have shorter fins than wild type. In contrast, dominant mutations for *long fin* (*lof*^{Dt2}; Tresnake 1981; Johnson and Weston 1995), *another long fin* (*alf*; van Eeden *et al.* 1996), and the hiD862 mutation (Amsterdam *et al.* 1999) each cause overgrowth of the fins. These mutations may result in fins that are too short or too long, while retaining isometric growth. Alternatively, these mutations may abolish isometric growth, resulting in fins that grow with either positive allometry (resulting in increasingly longer fins with respect to the body) or negative allometry (resulting in increasingly shorter fins with respect to the body). Such mutations may bypass normal growth control mechanisms. Indeed, interaction of *lof* with the regeneration mutation *reg6* suggests that the *lof* mutation disrupts or bypasses developmental checkpoints during fin growth and development (Johnson and Weston 1995).

In an effort to identify the nature of growth control mechanisms in the fin, we have begun an analysis of fin ray growth. We find that isometric growth of the fin ray is mediated by mechanisms controlling segment length and segment number. Analysis of fin length mutants indicates that mutants for *lof* are defective in mechanisms that limit segment initiation (resulting in excess segments), and that mutants for *sof* are defective for both segment initiation and segment length. Furthermore, *sof;lof* double mutants display both mutant phenotypes, suggesting that segment length and segment number are controlled independently. We propose a model in which fin ray growth combines alternating phases of growth (when segment length is controlled) and rest (the duration of time before initiating subsequent segments) in order to achieve the proper length.

MATERIALS AND METHODS

Stocks and fish maintenance: Wild-type fish stocks used in this study include C32 (Streisinger *et al.* 1981; Johnson and Zon 1999) and AB (Chakrabarti *et al.* 1983). *lof*^{Dt2} (Tresnake 1981; Johnson and Weston 1995) and the *sof*^{b123} (generous gift of C. Walker) have been maintained in mostly C32 backgrounds. However, markers that are tightly linked to the *lof* locus exhibit different haplotypes from C32. Markers that are tightly linked to the *sof* locus exhibit identical haplotypes to C32. All *lof* mutants in this study are heterozygous for the *lof*^{Dt2} mutation. All *sof* mutants are homozygous for the *sof*^{b123} mutation. Fish were reared at a constant temperature of 25° with a 14L:10D photo period (Westerfield 1993).

Measurements: Fish were anesthetized for several minutes and then fixed in 3.7% formaldehyde/PBS. The body of the fish was measured from the tip of the mouth to the caudal peduncle using a ruler (Figure 1A). For fin length and segment measurements, the skin and muscle surrounding the most proximal ends of the fin rays were dissected away before mounting in 100% glycerol under a coverslip. Measurements were facilitated with a 10 × 10 reticule in the eyepiece of the dissecting microscope (stemiscope 2000, Zeiss, Thornwood, NY) at low magnification. Unless otherwise stated, only the third fin ray (and typically the longest fin ray) from the ventral lobe of the caudal fin (referred to here as the V + 3 fin ray) was measured (Figure 1B).

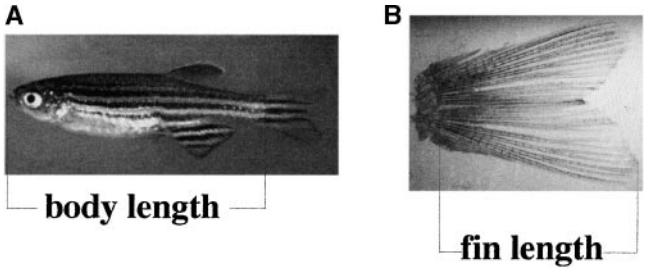

Figure 1.—Body and fin length measurements in zebrafish. (A) The body of the fish is measured from the tip of the mouth to the caudal peduncle (end of the body wall muscle). (B) The body muscle surrounding the proximal end of the fin rays is dissected away so that both proximal and distal ends of the fin ray can be visualized and measured.

Segment lengths were similarly determined, except at a higher magnification. Segments were measured as the distance between adjacent joints. Thus, the most distal segment was measured as the distance between the two distal-most joints. Only segments that were bound both proximally and distally by visible joints were counted for considerations of the total number of segments within the fin ray. Finally, only segments in the V + 3 fin ray were measured or counted.

Quantitative analysis: All slopes were generated using the least-square curve-fitting functions in Cricket Graph 1.3. Statistical analyses were performed with the JMP statistical analysis package for the Macintosh (SAS Institute, Cary, NC).

Identification of *sof*/*sof*;*lof*/+ mutant fish: The *sof*/*sof*;*lof*/+ double mutant fish were generated in two crosses. The first cross was between homozygous *sof* and homozygous *lof* individuals. The F_1 *sof*/+;*lof*/+ progeny were subsequently intercrossed. In the event that either mutation is completely epistatic to the other, only three phenotypic categories are expected: wild-type, *sof*, and *lof*. However, we identified a fourth phenotypic class that represents the double mutants. Since the *sof* mutation is recessive, this fourth class must carry two *sof*^{b123} alleles. Individuals that were heterozygous for the *lof* mutation were identified by the presence of both C32 and *lof* haplotypes for microsatellite markers tightly linked to *lof*. DNA preps of individual fish were prepared from the dorsal fins. Fins were placed in cold DNA extraction buffer (10 mm Tris, pH 8.2, 10 mm EDTA, 20 mm NaCl, 0.5% SDS) and heated to 95° for 5 min prior to digestion with 200 μg/ml proteinase K for 60 min at 55°. After vortexing and a subsequent 5-min incubation at 95°, the final solution was diluted 1:20 for use as PCR template.

RESULTS

Growth control in wild-type zebrafish: *Proportionate growth of fin ray and body:* To begin to identify the fin growth mechanisms in wild-type fish, we analyzed the body length and the fin length of zebrafish at various

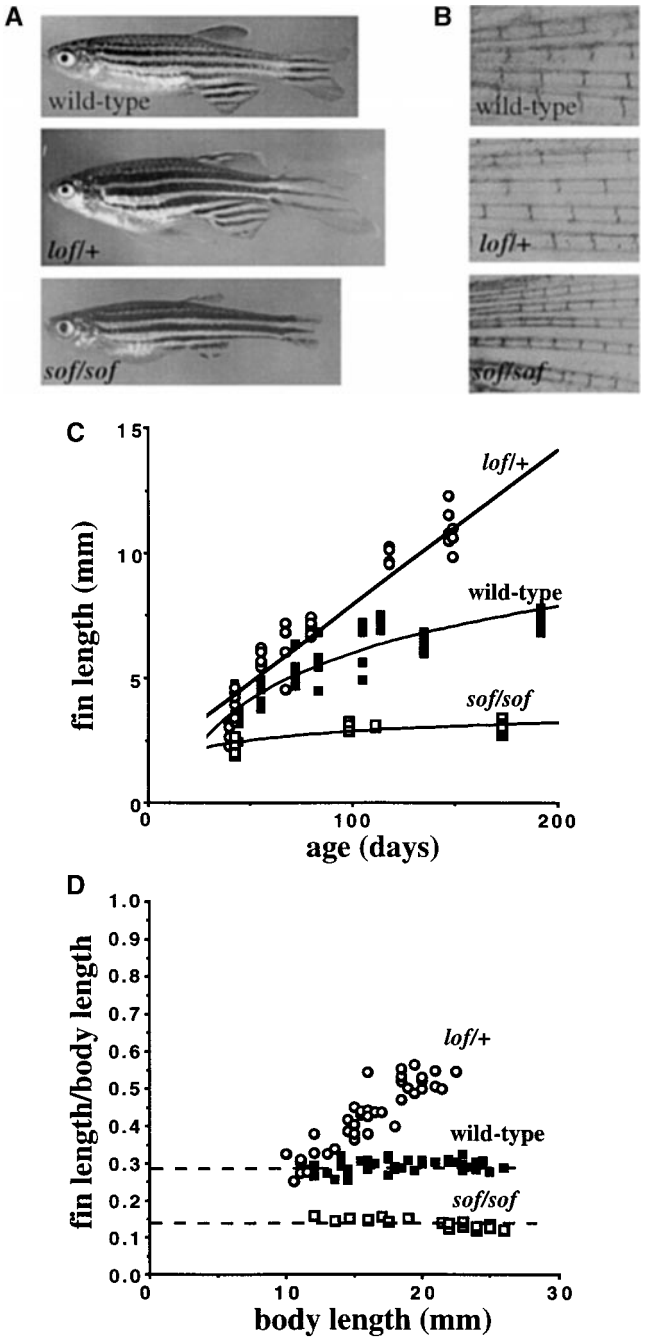

Figure 2.—Wild-type and fin growth mutant growth properties. (A) Wild-type, *lof*/+, and *sof*/*sof* zebrafish are shown. (B) Higher magnification views of the segments in wild-type, *lof*/+, and *sof*/*sof* mutants. (C) Growth rates of wild type, *lof*/+, and *sof* mutants. The wild-type and *sof* fin growth data were best fit with a logarithmic curve, and the *lof* data were best fit as a first-order line. (D) The fins of wild-type and *sof* mutants grow isometrically throughout their lives. Wild-type fish exhibit a proportionality constant of ~0.28, whereas *sof* mutants exhibit a proportionality constant of ~0.14. Fins of *lof* mutants do not grow proportionately. Rather, the fins grow with positive allometry.

ages. For consistency, we limited our analyses to the third fin ray of the ventral lobe of the caudal fin (this fin ray will be referred to as the V + 3 fin ray). We found that the body (data not shown) and fin ray grow rapidly when the fish are young and more slowly upon maturation (10–12 weeks at 25°, Figure 2C). Although the rate of fin ray growth slows as the fish matures, the V + 3 fin ray maintains a constant proportion with respect to the body (Figure 2D), indicating that fin length increases isometrically with respect to the body. We refer to the proportion of fin ray length / body length as the proportionality constant. For wild-type V + 3 fin ray, the proportionality constant is ~0.28 (Figure 2D). Presumably, the shape and form of the fin is achieved by each fin ray utilizing different proportionality constants.

Fin growth is regulated by the rate of initiation of new segments: Haas (1962) showed that fin growth occurs by the distal addition of new fin ray segments. However, the mechanisms controlling segment addition are unclear. We suggest two models. In the first model, segments are added periodically. This model predicts that the number of segments is proportional to the age of the fish. In an alternative model, segments are added in response to body growth. This model predicts that the number of segments is proportional to the size of the fish.

We reasoned that we could distinguish between these models by comparing segment number in fish grown to similar sizes under conditions that promote fast growth (few fish per tank) or slow growth (many fish per tank). Thus, we compared the number of segments in 8-week-old rapidly growing fish to similarly sized 8-month-old slowly growing fish. If segments are produced at fixed timepoints and therefore reflect the age of the fish, then older fish should have many more fin ray segments than younger fish of the same size. In contrast, if segments are added in response to body growth, then older fish should have a similar number of fin ray segments as younger fish of the same size. We found that the number of segments of 8-month old fish (Figure 3, open circles) and the number of segments in 8-week-old fish (Figure 3, solid squares) do not differ significantly in fish of similar body lengths (analysis of covariance, $P = 0.067$). Thus, segment number increases as a function of body size and only indirectly as a function of time.

Fin growth is regulated by segment size: The above experiment indicates that fin ray growth may be controlled by the rate of segment initiation. However, the length of segments likely also contributes to fin ray growth. For example, in the zebrafish, the most proximal segments in the V + 3 fin ray (typically the longest ray of the caudal fin) are ~0.270 ± 0.004 mm ($n = 10$) in length, whereas the most proximal segments in the medial fin ray (the shortest ray of the caudal fin) are only 0.225 ± 0.007 mm ($n = 10$). Segment length also decreases in a proximal to distal fashion within an individual fin ray (discussed below, see Figure 4). These data

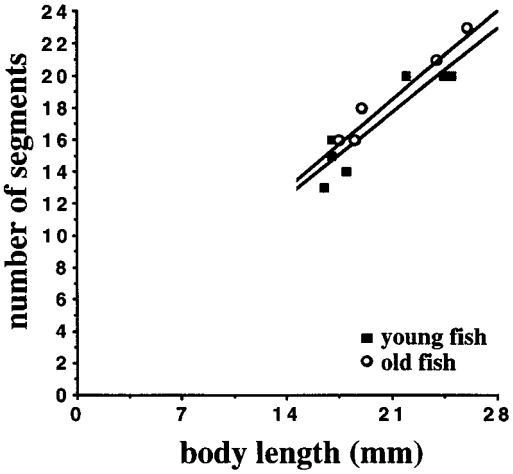

Figure 3.—Segment number is a function of body size and not of fish age. The number of segments in similarly sized fish of 8 weeks (solid squares) and 8 month (open circles) are similar, indicating that segment addition is a function of body size (analysis of covariance, $P = 0.067$ when age is varied). Both sets of data were fitted as first order lines.

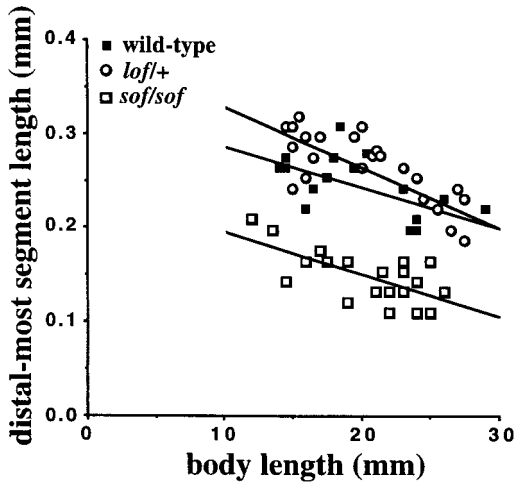

Figure 4.—The lengths of the most distal segments are similar in wild-type and *lof* fish, but not in *sof* fish. All data were fitted with a first order line, and the slopes for *lof* and *sof* are each not significantly different from the slope for wild-type fish, indicating that neither *lof* nor *sof* is defective for the gradual shortening of fin ray segments proximodistally (analysis of covariance). However, the lengths of segments in *sof* (open squares) mutants are considerably shorter than the segments in wild type (solid squares) and *lof* (open circles) mutants.

are supported by observations made in other fishes. In the caudal fin of *Trichogaster sumatranus* and *Carassius auratus*, segment length decreases along the proximal-distal axis within a single fin ray, and furthermore, segment length differs from one fin ray to another (Haas 1962). Thus, different fin rays within the same fin regulate growth by controlling segment length and segment number.

Note that this analysis relies on the permanence of individual segments, but not on the permanence of individual cells. For example, it is possible that cellular turnover contributes to outgrowth of the fin ray. Since we examine fin ray growth at the level of the segments (which are static once produced), the processes of cell death (or cell division) are not described here.

Growth control in fin length mutants: The preceding analysis suggests that fin ray growth may be regulated by two types of mechanisms: (1) mechanisms controlling how often fin ray segments are initiated and (2) mechanisms controlling segment length. Insights into these mechanisms may come from analyses of mutations affecting either or both properties of new segment addition. Here, we focus on two fin growth mutations, *lof* and *sof* (Figure 2A). Since these genes have been mapped to different linkage groups (S. Johnson, unpublished data), they are not alleles of the same gene. The dominant fin length mutant, *lof*, develops long fins that are first detectably different from wild-type upon maturation. At early ages (<10 weeks), little or no difference is observed between *lof* and wild-type individuals (Figure 2C). After 10 weeks, as fin growth slows in wild-type fish, *lof* fins continue to grow rapidly as a function of age rather than a function of body size (Figure 2, C and D). Thus, isometric growth is lost in *lof* mutants (Figure 2D), and instead, *lof* mutant fins grow with positive allometry. In contrast, the recessive fin length mutant, *sof*, develops fins that are shorter than wild-type fins at all ages. Fin growth is slower than wild-type fin growth throughout the life of *sof* mutants (Figure 2C), yet *sof* mutants retain isometric growth (Figure 2D). The proportionality constant of the V + 3 fin ray in *sof* mutants is ~0.14 (Figure 2D), one-half that of the V + 3 fin ray in wild-type fish. We conclude that *sof* mutants retain proportionate growth, but alter the proportionality constant.

To understand the effects of the *lof* and *sof* mutations on segment initiation and segment size, we examined these properties in *lof* and *sof* mutants.

Segment initiation is affected in lof mutants: To determine if the *lof* mutation alters segment size, we compared the length of newly initiated segments in *lof* and wild-type fins (Figure 4). We find that the length of newly initiated segments is similar in similarly sized fish. On average, the length of the most distal segments in wild-type fish of 18–21 mm is 0.229 ± 0.011 mm ($n = 10$), and the length of the most distal segments in similarly sized *lof* mutants is 0.246 ± 0.017 mm ($n = 10$). This small but significant difference in segment length (~7%) cannot account for the large (up to 60%) difference in fin length between *lof* and wild-type fins. In addition, we observe a small decrease in newly initiated segment length with increasing body size in both wild-type and *lof* mutants (Figure 4), suggesting that there is not a significant difference in segment size observed between wild-type and *lof* mutants over the entire growth history

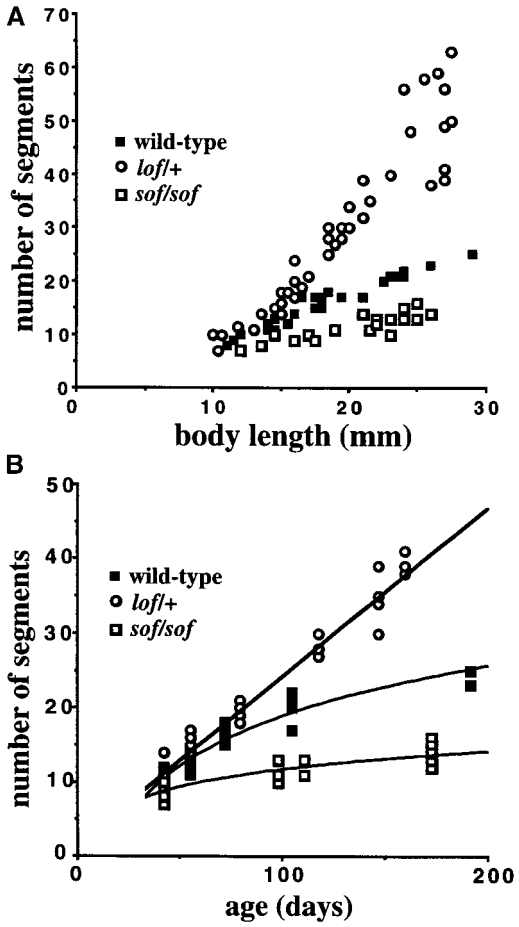

Figure 5.—*lof* and *sof* mutants affect the number of segments in the growing fin ray. (A) *lof* mutants have an increased number of fin ray segments when compared with wild-type segments in fish of the same size (A) or of the same age (B). *sof* mutants have a decreased number of fin ray segments when compared with wild-type segments in fish of the same size (A) or of the same age (B). In B, wild-type and *sof* data were best fit with a logarithmic curve, and the *lof* data were best fit with a first-order line.

of the fin (analysis of covariance, $P > 0.38$, see also Table 1 and Figure 2B).

In contrast to our analysis showing that the *lof* mutation does not dramatically affect segment size, we find that the *lof* mutation does cause an increase in segment number (Figure 5). Comparing the segment number in similarly sized wild-type and *lof* fish reveals that *lof* mutant fins have increasingly more segments as body size increases (Figure 5A). This difference in the number of fin ray segments is also apparent when fish are compared by age, rather than by body size (Figure 5B). Thus, the excess of segments in *lof* mutants becomes increasingly apparent as body size increases or as the fish ages (Figure 5, A and B). We conclude that *lof* mutants initiate new segments rapidly even after reaching maturation.

sof mutations affect the size and number of segments: The size of new segments was also examined in *sof* mutant fish (Figure 4). The average size of the distal segments in *sof* mutants (0.152 ± 0.013 mm, $n = 10$) is significantly shorter than the segments of wild-type fish (0.229 ± 0.007 mm, $n = 10$). Although the newly formed segments in *sof* mutant fin rays decrease in length with body size, similar to the decreased distal segment size in wild-type fins, the length of newly initiated segments is consistently shorter than in similarly sized wild-type fish. Thus, a significant deficit in segment length is observed over the entire growth history of the fin ray (see also Table 1 and Figure 2B). Interestingly, the decrease in length of newly added segments in *sof* mutants is not significantly different from the decrease in segment length of wild-type mutants (analysis of covariance, $P > 0.83$), suggesting that *sof* mutants are not defective in the gradual shortening of newly added segments.

In *sof* mutants, the caudal fin ray is approximately one-half the length of the wild-type caudal fin ray. Since the length of the segments represents a difference of only ~35%, it remains possible that *sof* also causes a defect in the initiation of new segments. Figure 5, A and B, shows that *sof* mutants produce ~20% fewer fin ray segments when compared with the number of segments from similarly sized or similarly aged wild-type fish. For example, in fish of ~21 mm, *sof* mutants have ~14 segments in the V + 3 fin ray, and wild-type fish have ~17 segments in the V + 3 fin ray. Together, the 35% decrease in segment size and the 20% decrease in segment number account for the ~50% decrease in length of the third fin ray in *sof* mutants. Therefore, the *sof* mutation causes both decreased segment size and decreased initiation of fin ray segments.

We propose that the *lof* and *sof* mutations affect two independent aspects of fin ray growth: control of segment initiation and control of segment length, respectively. It is possible to test this model by generating a *sof*;*lof* double mutant. If the proposed growth control mechanisms act independently of one another, then we might expect to see both the *lof* segment initiation defect and the *sof* segment length defect expressed in the *sof*;*lof* double mutant. Alternatively, if segment initiation and segment length are controlled by the same mechanism or pathway, then we might expect the double mutant to exhibit either the *sof* phenotype or the *lof* phenotype.

We find that the V + 3 fin rays of *sof*;*lof* double mutants develop similarly sized segments as *sof* single mutants (Table 1) and similar numbers of segments as *lof* single mutants (Figure 6, analysis of covariance, $P < 0.008$). Thus, proximal segment length in *sof*;*lof* is 0.181 + 0.012 ($n = 10$), similar to that of *sof* mutants, and segment number in 88-day *sof*;*lof* mutants is ~23 ($n = 8$), similar to similarly aged *lof* mutants. Interestingly, overall fin ray length of the double mutant is intermediate between *sof* and *lof* single mutants (not shown). This is accounted for by the simultaneous expression of both the short segment phenotype of *sof*

TABLE 1

Proximal segment length in zebrafish fin length mutants

Genotype	Most proximal segment length (mm)
Wild type	0.270 ± 0.004
lof/+	0.269 ± 0.009
sof/*sof*	0.175 ± 0.008
sof/*sof*;*lof*/+	0.181 ± 0.012

The most proximal segment from the third fin ray of the caudal fin was measured from 10 individual fish for each genotype.

mutants and the increased segment initiation phenotype of *lof* mutants. We note, however, that *sof*;*lof* double mutants add fin ray segments at the same rate as *lof* single mutants, indicating that *lof* is epistatic to the defect in segment initiation of the *sof* single mutant. We conclude that the *lof* mutation affects segment initiation and the *sof* mutation affects segment growth by independent mechanisms that regulate the size and shape of the zebrafish fin.

DISCUSSION

We have begun a genetic dissection of growth control mechanisms that regulate isometric growth in the zebrafish fin ray. Our analysis has identified two independent pathways controlling fin length. One pathway controls the initiation of segments, and another pathway controls the length of segments. Both are required for isometric growth. The *sof* mutation, which changes fin length without disrupting isometric growth, decreases both segment initiation and segment length. In contrast, the *lof* mutation results in positive allometric growth of the fins by bypassing mechanisms that slow the rate of segment initiation.

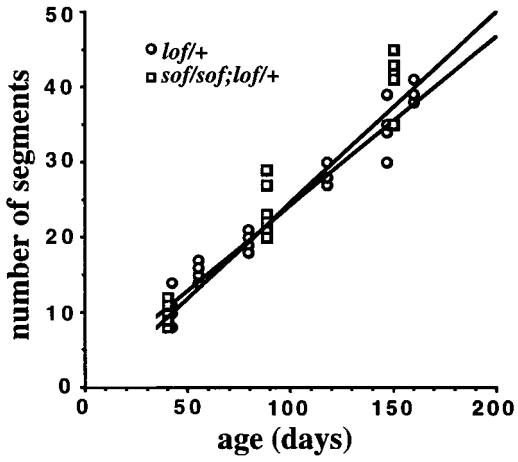

Figure 6.—*lof* and *sof*;*lof* double mutants produce segments at similar rates. *lof* and *sof*;*lof* double mutants of the same age have similar numbers of segments in the fin ray (analysis of covariance, $P < 0.008$ when genotype is varied), indicating that segments are initiated with similar frequencies. Note that the data points for *lof*/+ are identical to the data points shown for *lof*/+ in Figure 5B.

We favor a model for fin growth that integrates these mechanisms into phases of growth (G) and rest (R; Figure 7). In this model, the length of segments is controlled during G phase, and the initiation (or number) of segments is determined by the length of R phase. Thus, we suggest that *sof* mutants have shorter fins primarily because of a defect during G phase, but also because the R phase is extended. Furthermore, we suggest that *lof* mutants have longer fins because the R phase is diminished or abolished. Growth control mechanisms affecting G and R phases may be regulated independently, demonstrated by the finding that double mutants for *sof*;*lof* exhibit both the *lof* defect in limiting the initiation of segments and the *sof* defect resulting in shorter segments.

Does *sof* cause smaller segments by decreasing the duration of G phase or instead by decreasing the rate of growth during G phase? If we assume that the *lof* mutation uniformly abolishes the R phase, we may now use the *sof*;*lof* double mutant to address this question. If the length of G phase is shorter in *sof* mutants, such that segment growth stops prematurely, then we expect a more increased rate of segment addition in the *sof*;*lof* double mutant than in the *lof* single mutant. Alternatively, if the growth rate during G is slower, such that segments are produced for the same amount of time in *sof*;*lof* double mutants as in *lof* single mutants, then we expect the same rate of segment addition in *lof* and in *sof*;*lof* fin rays. We found that there are the same number of segments in similarly aged *lof* and *sof*;*lof* mutants (Figure 6), suggesting that new segments are initiated at similar rates in each. Thus, for *sof*;*lof* mutants to produce the same number of segments as *lof* mutants, the effect of the *sof* mutation is likely to cause a slower rate of segment growth.

We suggest that *lof* mutants bypass normal growth control mechanisms as the result of bypassing the R phase during fin growth. Thus, the length of R phase may partially control the rate of fin ray growth. For example, in wild-type fish the rate of segment initiation decreases with time (Figure 5B) while segment length decreases only slightly. Furthermore, recall that the rate of fin growth in *lof* fins is similar to the rate of fin growth in wild-type fins when the fish are young and growth is rapid. We suggest that the duration of R phase (and therefore the rate of segment initiation, Figure 5B) between *lof* and wild-type fish is similar when the fish are young. At later stages, the duration of R likely increases in wild-type fins (and therefore the rate of segment initiation slows), but remains short or absent in *lof* fins (and therefore the rate of segment initiation is constant). Thus, the difference in fin length between wild-type and *lof* fins becomes more apparent as the fish age

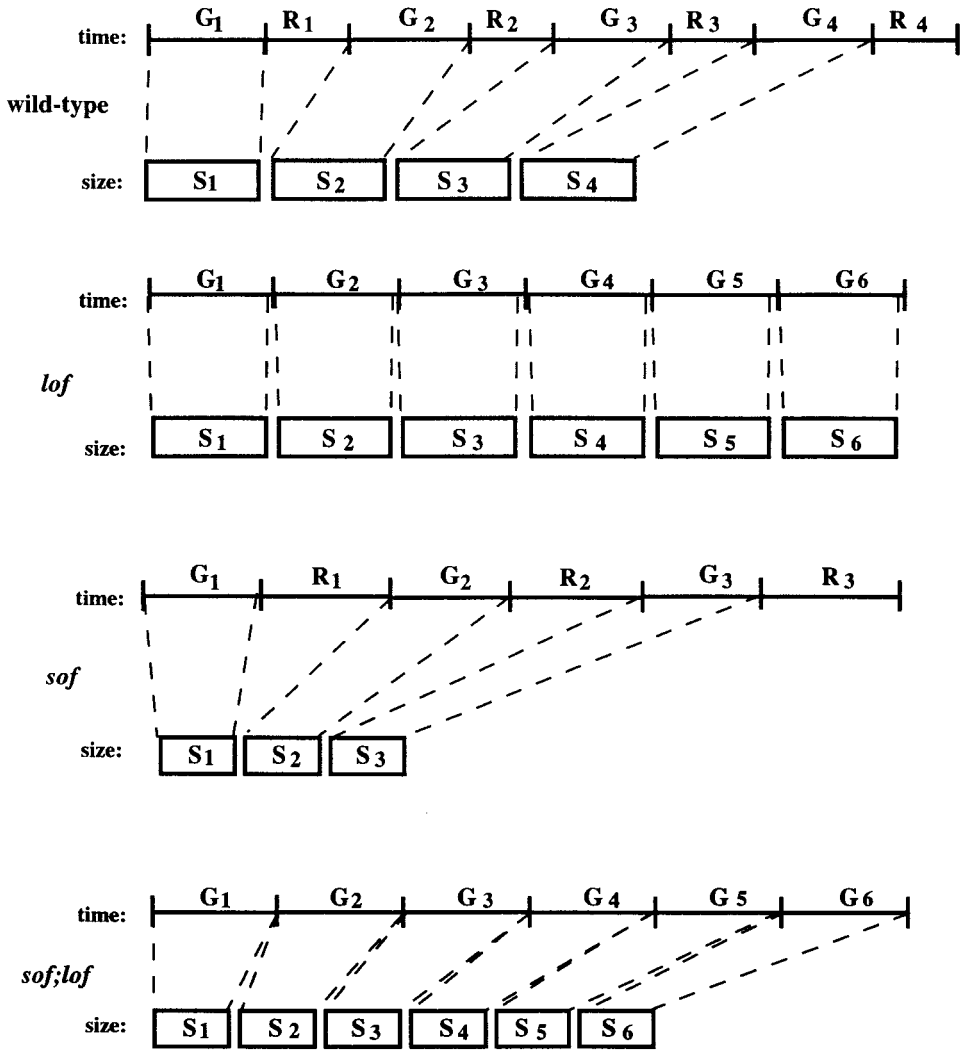

Figure 7.—A model for fin growth. We propose that there are two phases required for segment addition: growth (G) and rest (R). We suggest that segment length is determined during the G phase, and rate of segment addition is controlled by the duration of the R phase. These two phases help to regulate isometric growth. Isometric growth is maintained in *sof* mutants; however, the fins are half the length of wild-type fins. The *sof* phenotype is the result of decreasing the rate of growth during G phase and lengthening the duration of the R phase. Therefore, *sof* mutants produce shorter and fewer fin ray segments. Isometric growth is lost in *lof* mutants. The *lof* phenotype is the result of abolishing the R phase and therefore initiating segments more frequently. Since the *sof;lof* double mutant exhibits both the short segment phenotype and the additional segment phenotype, we suggest that segment length and segment number are controlled by independent pathways.

and is the result of bypassing growth control mechanisms that limit the rate of segment initiation.

Our evidence for a segment development cycle composed of distinct rest and growth phases is largely genetical, depending heavily on the finding that in *sof;lof* double mutants both the excess segment phenotype of *lof* and the shorter segment phenotype of *sof* are expressed. We imagine that there is a relatively fixed period of segment development and that growth in *sof* mutants is slower in this period, resulting in shorter segments. An alternate model is that growth is continuous and that *sof* causes slower, but continuous growth. This model predicts that in the *sof;lof* double mutant fin, which grows faster than the *sof* single mutant, we might expect the segment length to increase as well. The finding that segment length remains that of the *sof* single mutants would cause us to postulate an additional role for the *sof* gene, that of determining segment length independently of controlling the rate of growth. The model that we present of periodic growth is more parsimonious in that it requires a single role for *sof*, that of controlling rate of growth during the segment growth phase.

These two models make additional predictions that may distinguish between them as well. First, the periodic model that we favor suggests that cell division responsible for growth may be periodic as well, whereas the continuous growth model predicts no periodicity in cell division. Consistent with the former model, Haas (1962) has reported periodic cell division in the rays of regenerating goldfish fins. Second, the periodic growth model predicts we might find molecular markers for segment development that are also periodically expressed, at decreasing frequency as fins age. Consistent with this model, preliminary results from *in situ* surveys of fin growth and regeneration cDNAs have identified genes that are expressed continuously in the distal growth zone in young, rapidly growing fins, but relatively rarely in older, slow growing fins (R. Waterman and S. Johnson, unpublished results).

Our model for isometric growth suggests that fins grow to achieve a "target" size that defines their proper proportion with respect to the body and that *lof* mutants disregard this target. An alternate possibility is that the *lof* mutation causes an inflated target size. Indeed, we

have observed *lof* mutants with fins as long as their bodies (suggesting a target fin length/body length of 1.0 or greater, rather than the 0.28 observed for wild type). However, we do not find that fin length plateaus in *lof* mutants (even past 1 year of measurements, data not shown). A second way to explore the possibility that *lof* mutants have an inflated target fin size comes from fin regeneration studies. Typically, when fins are amputated, the fin rapidly and precisely regenerates to replace the excised portion (Morgan 1901; Tassava and Goss 1966; S. Johnson, unpublished results). If *lof* mutant fins have an inflated target size (for instance, greater than body length), we expect *lof* mutant fins to regenerate rapidly to replace the excised portion. However, Geraudie *et al.* (1995) report that *lof* mutant fins do not regenerate to replace the excised length of the fin, but instead regenerate to a size appropriate for wild-type fish. These findings suggest that *lof* mutants indeed have a notion of the target size, and that this target size is the same as wild type. During ontogenetic growth, however, mechanisms to achieve final length are ignored in *lof* mutants, resulting in uncontrolled fin growth.

The *lof* mutation may affect tissues and aspects of fin growth control other than fin rays and rate of bone segment addition. Evidence for this comes from the combination of the *lof* mutation with a temperature-sensitive fin regeneration mutation, *reg*6, that affects the intermediate stages of fin regeneration (Johnson and Weston 1995). At the restrictive temperature, *reg*6 mutants regenerate amputated fins with disorganized lepidotrichia, blood blisters that appear as swollen blood vessels, and overgrowth of the epidermis. Single mutants for *reg*6 have little or no effect on ontogenetic fin growth at the restrictive temperature. Yet, when this mutation is combined with *lof*, the *reg*6;*lof* double mutant develops blood blisters during ontogenetic growth similar to that observed during regeneration. Johnson and Weston (1995) interpreted this result to indicate that the *reg*6 mutation caused defects in angiogenesis during ontogeny that are normally identified by developmental checkpoints and subsequently repaired. But, in the presence of the *lof* mutation, these checkpoints are bypassed and therefore the *reg*6-induced defects are not repaired, resulting in catastrophic blood blisters. Thus, we suggest that *lof* may act as a developmental checkpoint in several tissues during the outgrowth of fin rays.

Segment length and segment number vary among individual fin rays. We believe growth mechanisms identified for the V + 3 fin ray will be applicable to each of the fin rays in the caudal fin. Thus, the final shape and form of the caudal fin may be the result of the differential response of each fin ray to the mechanisms affecting segment length and number. The identification of the *sof* and *lof* genes will be a first step in the identification of the mechanisms involved. For example, identification of the *sof* gene product and its interacting factors may lend insight as to how segment length is achieved, and identification of the *lof* gene product and its interacting factors may lend insight as to how segment addition is achieved. Modulation of these pathways may result in the different fin shapes and sizes in the zebrafish. Furthermore, the morphologies of fins vary among species, suggesting that analysis of *sof* and *lof* in zebrafish may shed light on the mechanisms regulating isometric and allometric growth in other species as well. Recent progress in the development of high density maps for zebrafish (Knapik *et al.* 1998; Geisler *et al.* 1999; Hukriede *et al.* 1999) will help to accelerate the identification of these genes.

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