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ARTICLE

Allometric Scaling and Proportion Regulation in the Freshwater Planarian *Schmidtea mediterranea*

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The regulation of scale and proportion in living organisms is an intriguing and enduring problem of biology. Regulatory mechanisms for controlling body size and proportion are clearly illustrated by the regeneration of missing body parts after amputation, in which the newly regenerated tissues ultimately attain a size that is anatomically congruent with the size of the rest of the organism. Understanding the molecular processes underpinning scaling would have deep consequences for our comprehension of tissue regeneration, developmental ontogeny, growth, and evolution. Although many theories have been put forward to explain this process, it is interesting that no satisfactory mechanistic explanation is currently available to explain scalar relationships. We chose to investigate the freshwater planarian, a commonly used model system for the study of metazoan regeneration, to delineate a strategy for the molecular dissection of scale and proportion mechanisms in metazoans. Here, we report on the cloning and discrete expression pattern of a novel planarian gene, which shares homology with the DEG/ENaC super-family of sodium channels. We have named H.112.3c *cintillo* ("head ribbon" in Spanish) and present a strategy for using the expression of this gene to monitor scale and proportion regulation during regeneration, growth and degrowth in the freshwater planarian *Schmidtea mediterranea*. *Developmental Dynamics 226:326–333, 2003.* © 2003 Wiley-Liss, Inc.

Key words: planarian; regeneration; allometry; scale and proportion; DEG/ENaC

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Tyger! Tyger! burning bright
In the forests of the night,
What immortal hand or eye
Could frame thy fearful symmetry?

William Blake, 1757–1827
Songs of Experience

INTRODUCTION

Through the centuries, the scale and proportion of living organisms have been subjects studied and admired by both artists and scientists alike. This is evidenced by Leonardo da Vinci's Vitruvian man (Richter, 1998) and Dürer's empirical work on human proportions (Dürer, 1591), as well as by the writings of Darwin (1859), Thompson (1917), and Huxley (1932) on this subject. In fact, contemporary research continues to grapple with this problem, as illustrated by the recent application of fractal geometry (West et al., 1997) and transportation networks (Banavar, 1999) to explain the nature of scalar relationships. As eclectic as the literature on scale and proportion may appear, it is interesting to note that the work of artists and scientists alike has independently uncovered rules of proportion in living organisms that differ significantly from the isometric principles of proportion dictated by Euclidian geometry (Schmidt-Nielsen, 1993). Such nonisometric scaling is referred to as allometric scaling, and for the most part obeys the simple equation $y = aM^b$, where y is a biological variable, a is a proportionality coefficient, M is body mass, and the exponent b is called the body-mass exponent (McMahon and Bonner, 1983).

Unlike geometric objects such as cubes and pyramids in which b scales by the third power, most biological phenomena scale as quarter powers of body mass (Huxley, 1932; Schmidt-Nielsen, 1993). For instance,

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metabolic rates in primates and other mammals have been shown to scale as $M^{3/4}$ (Benedict, 1938), respiratory variables as $M^{1/4}$ (Stahl, 1967), and heartbeat as $M^{-1/4}$ (West et al., 1997), relative to body size. In invertebrates, similar quarter-power scaling properties have also been observed, for example, between most measurable aspects of insect anatomy and body size (Emlen and Nijhout, 2000). In fact, quarter-power scaling can be found across a wide spectrum of animals, plants, and even microbes (West et al., 1997; Stern and Emlen, 1999; Darveau et al., 2002; Gillooly et al., 2002). Even though understanding the regulation of allometry would have broad implications on furthering our knowledge of developmental ontogeny, regeneration, population growth, and evolutionary processes, it is intriguing that no general molecular model exists to mechanistically explain scaling laws.

Although many molecular insights into biological processes have been obtained by studying simple organisms such as *Drosophila melanogaster* and *Caenorhabditis elegans*, their determinate growth does not make these animals particularly well-suited for studying scale and proportion relationships. Laboratory strains of *Drosophila* and *C. elegans* fail to display the typical variations in body size and shape arising in response to changes in the environment that are commonplace to many organisms in the wild. In fact, even when genetic manipulations are carried out to disrupt allometries in *Drosophila* and *C. elegans*, the results have basically confirmed the scalar rigidity of these animals. In *Drosophila*, for example, the deletion (Garcia-Bellido et al., 1973; Morata and Ripoll, 1975) or addition (Weigmann et al., 1997) of cells by genetic means to wing imaginal discs has no effect on either the shape or the size of the resulting wings. Even those existing mutations affecting body size in *Drosophila* and *C. elegans* do so by changing cell size rather than cell number (Montagne et al., 1999; Nystrom et al., 2002). This is in marked contrast to the countless "exaggerated" morphologies found in many animals and plants in the wild. Such changes typically arise not as a result of genetic alterations (mutations) but as a direct response of the individual's genotype to variations in environmental factors (Emlen and Nijhout, 2000). This finding is clearly illustrated by the horn length dimorphism displayed in animals of a clonal line of the beetle *Onthophagus acuminatus*. By responding to changing environmental variables, the genotype of this clone is capable of generating the full range of possible horn dimensions (Emlen, 1994). Therefore, the developmental rigidity of the better understood model organisms, along with the standardized laboratory conditions in which they are reared, make it difficult to unveil experimentally the molecular mechanisms responsible for variations in size and shape that occur normally in nature. Ultimately, these changes form the basis of scaling relationships in living organisms and, thus, need to be understood to identify the molecular basis of size and scale regulation.

To explore the molecular basis of scale and proportion, organisms with plastic ontogenies that are also amenable to molecular manipulations need to be identified. The ability of planarians to regulate their allometry during the process of regeneration (Morgan, 1898; Newmark and Sánchez Alvarado, 2002), as well as to change their size according to environmental conditions (Abeloos, 1930) provides an intriguing paradigm to study scale and proportion regulation. Planarians are free-living, bilaterally symmetric members of the phylum Platyhelminthes, possessing derivatives of all three germ layers (ectoderm, mesoderm, and endoderm). These organisms are best known for their remarkable regenerative properties and have been the subject of developmental biology studies for more than 200 years (Newmark and Sánchez Alvarado, 2002). The recent characterization in our laboratory of nearly 3,000 cDNAs from the freshwater diploid planarian *Schmidtea mediterranea* (Sánchez Alvarado et al., 2002), as well as the introduction of RNAi-based loss-of-function assays (Sánchez Alvarado and Newmark, 1999), whole-mount in situ hybridization (Umesono et al., 1997), bromodeoxyuridine labeling of the planarian stem cells (Newmark and Sánchez Alvarado, 2000), and immunohistochemical reagents (Robb and Sánchez Alvarado, 2002) have allowed us to begin systematic molecular characterizations of the remarkable biological properties of these animals.

Here, we report the cloning and expression pattern of a novel planarian gene (clone H.112.3c; accession no. AY067542), which shares homology with the degenerin/epithelial sodium channel (DEG/ENaC) superfamily of sodium channels. We have named H.112.3c *cintillo* ("head ribbon" in Spanish) because of its expression in putative chemoreceptive neurons located along the anterior margin of the planarian head. We present a detailed characterization of the dynamics of expression of this gene during growth, degrowth, and regeneration and use *cintillo* to lay a quantitative and molecular foundation for the study of scale and proportion regulation during regeneration, growth, and degrowth in the planarian *S. mediterranea*.

RESULTS AND DISCUSSION

cintillo Is Similar to Members of the DEG/ENaC Superfamily of Sodium Channels, and Its Expression Is Restricted to a Subset of Cells in the Planarian Head

A cDNA with homology to *C. elegans* degenerin (*deg-1*; Fig. 1A) was isolated in an EST screen recently performed in our laboratory (Sánchez Alvarado et al., 2002). In *C. elegans*, the degenerin family (DEG) is composed of ~20 members and includes genes encoding proteins involved in mechanosensation such as *deg-1*, *mec-4*, and *mec-10* (Mano and Driscoll, 1999). Because DEG members share extensive similarities with subunits of epithelial sodium channel (ENaC) of rats and humans (Garcia-Anoveros et al., 1995), members of these two gene families have been organized into the DEG/ENaC superfamily of sodium chan-

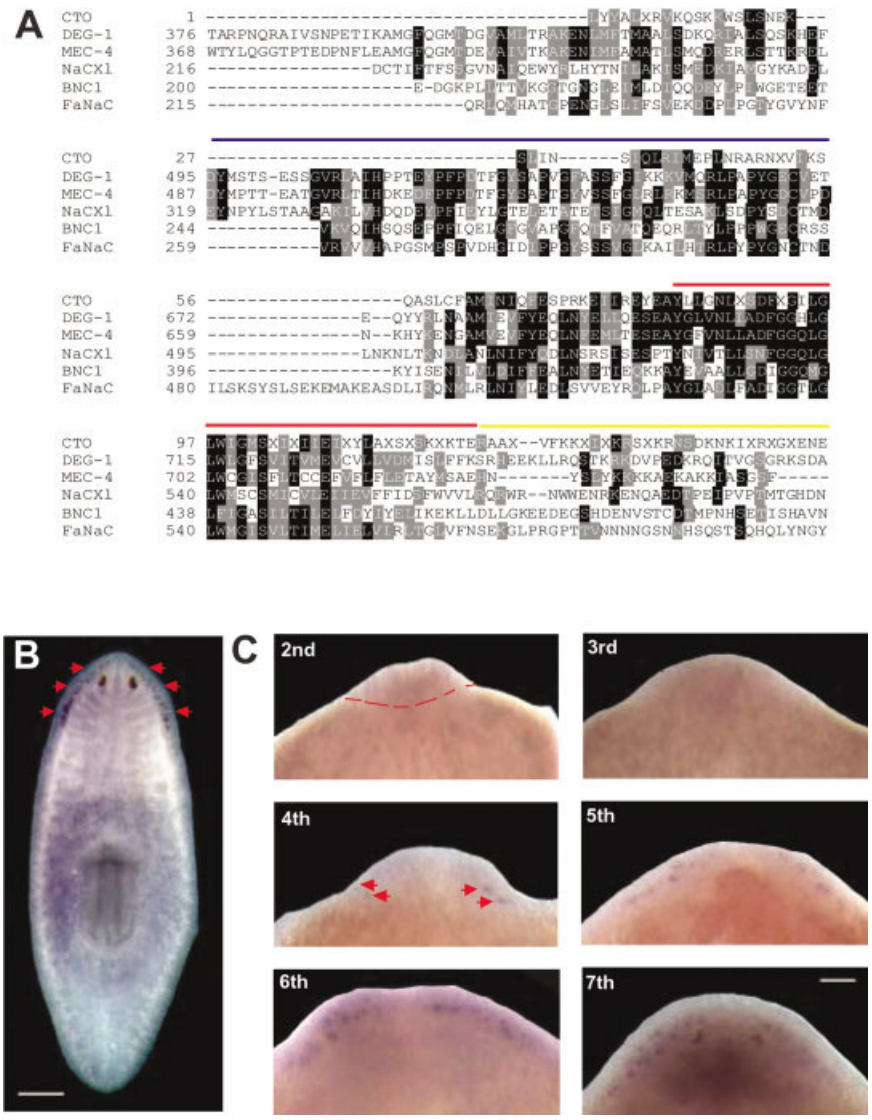


Fig. 1. Sequence analysis and spatial expression of *cintillo*. **A:** Deduced protein sequence of CTO and alignment with the following members of the DEG/ENaC superfamily: *C. elegans* degenerins DEG-1 (accession no. P24585) and MEC-4 (accession no. T29859), sodium channel *X. laevis* NaCXI (accession no. P51171), human brain sodium channel-1 BNC1 (accession no. U50352), and the snail (*Helix aspersa*) FMRF-amide–gated sodium channel (FaNaC; accession no. Q25011). Identical residues are boxed in black, and conservative substitutions are in gray. Colored lines indicate conserved domains as follows: blue, extracellular domain; red, transmembrane region; yellow, intracellular domain. Given the partial sequence of clone H.112.3c, the amino acid numbering of CTO is relative and not absolute as is the case for the DEG/ENaC family members. **B:** Representative expression pattern of *cto* visualized by whole-mount in situ hybridization. Expression of *cto* is limited to the anterodorsal margin of the animals (red arrows). **C:** *cto* expression during regeneration. Cephalic regeneration was followed for 7 days after amputation, and in situ hybridizations performed for each day. The blastema boundary is designated by the dashed red line. *cto* expression is first detected between days 3 and 4 of cephalic regeneration (red arrows). Scale bars = 300 μm in B, 100 μm in C.

nels, which includes subunits of the snail FMRF-amide gated channel (FaNaC) and at least 14 members in the *D. melanogaster* genome and approximately a dozen members in the human genome, including brain sodium channel 1 (BNC1), which may contribute to pain perception (Hong et al., 2000; Gillespie and Walker, 2001). Recent work on the gene products of DEG/ENaC superfamily members suggests a common participation (directly and/or indirectly) in mechanical signal transduction among different species (Garcia-Anoveros and Corey, 1997; Gillespie and Walker, 2001), suggesting that *cintillo* (*cto*) may be playing similar roles in planarians.

A possible role of *cto* in sensory transduction is also supported by the cellular distribution of its transcript. The spatial expression pattern of *cto* is restricted to a subset of cells located in the anterodorsal margin of the planarian head (Fig. 1B). These cells occupy a position that is similar to previously described chemoreceptive neurons in planarians (Farnesi and Tei, 1980). During regeneration, *cto* is detected relatively late (3–4 days after amputation; Fig. 1C). At this stage, the cerebral ganglia anlagen have already been established and begun their differentiation (Cebrià et al., 2002b), indicating that the *cto*-positive cells arise relatively late during central nervous system (CNS) ontogeny. Because the expression of *cto* is restricted to an easily quantifiable subset of cells (Sánchez Alvarado et al., 2002), we surmised that this gene would provide a unique opportunity to measure changes in cell number as planarians underwent growth and degrowth, as well as during the remodeling of body proportions known to occur during regeneration.

Number of *cintillo* Expressing Cells Changes During Growth and Degrowth

To better understand the dynamics of growth and degrowth in *S. mediterranea,* we first set out to measure changes in body length over time during feeding (growth) and starvation conditions (degrowth) at a constant temperature of 20°C. Planarians ~2 mm in length were used for the feeding experiments, whereas animals of ~8 mm in length were followed during the starvation experiments. Body lengths of fed and starving animals were measured weekly while alive (Fig. 2A; n = 20 per time point) and collected and fixed (n = 6 for each size point) whenever 2 mm changes in body length were recorded. The fixed specimens were processed for in situ hybridization and the *cto*-positive

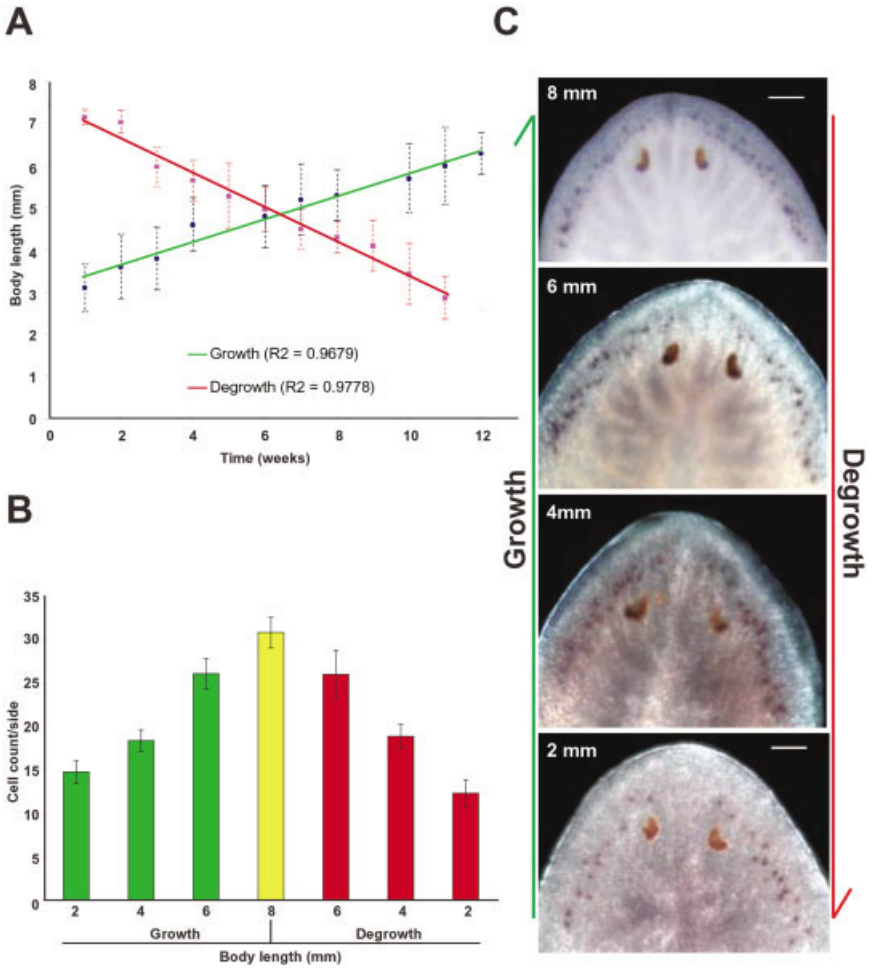
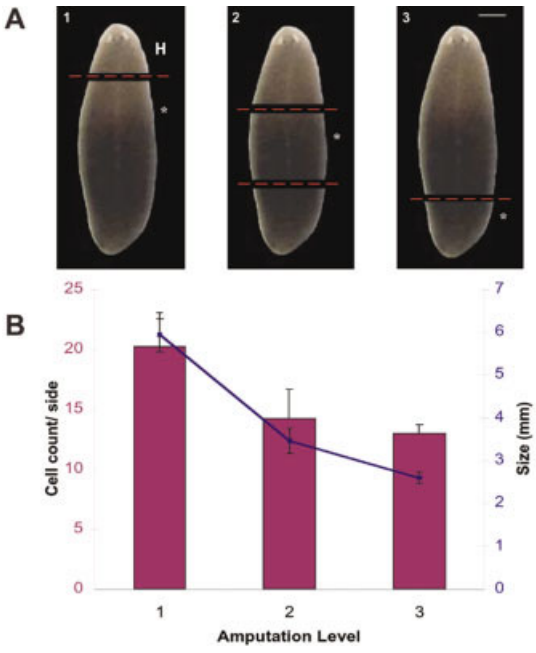


Fig. 2. Growth and degrowth in *S. mediterranea*. Green represents growth and red represents degrowth. **A:** Growth (feeding) and degrowth (starvation) curves. Each point represents mean of length of 20 worms measured weekly. Error bars are standard deviations (SD). The lines are fit by using least-squares linear regression to the mean values. R2 indicates correlation coefficients. **B:** Average number of cells per side expressing *cto* during growth or degrowth. Starting with planarians of ~2 mm of length, green bars indicate the number of cells per side expressing *cto* as planarians increase ~2 mm in length as a consequence of feeding. Red bars represent the number of cells expressing *cto* per side as planarians decrease ~2 mm in length during starvation. The yellow bar shows the combined number of cells per side expressing *cto* at 8 mm of length in samples at the start of degrowth conditions and samples at the end of the growth conditions ($P = 0.91$). Each point ($n = 6$) represents mean of cells per side expressing *cto* (± SD). **C:** Representative results of whole-mount in situ hybridizations, showing distribution of cells expressing *cto* at different sizes during growth and degrowth. The images illustrate the relative ease with which *cto* expressing cells can be quantified. Each sample was examined at different focal planes under the compound microscope and scored for *cto*-positive cells. In very flat animals such as the 2-mm animal shown here, the cells can be counted in the photographic image (13 and 14 cells on the left and right sides, respectively). Scale bar = 50 μm in the 2-mm animal (applies for the 2-, 4-, and 6-mm animals), 100 μm in 8-mm sample.



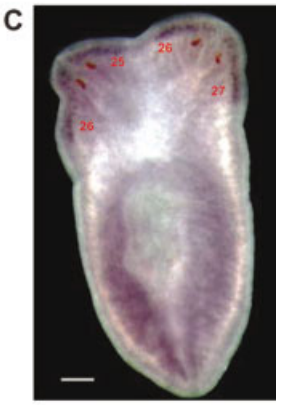


Fig. 3. Regulation of *cto* expression in cephalic regenerates. **A:** Schematic representation of the three different amputation levels (red dashed line). The asterisks denote the fragments used to monitor *cto*-positive cells 10 days after amputation. H indicates the head fragments analyzed for *cto*-positive cell number modulation during tissue remodeling. 1: Decapitation, in which the trunk piece regenerates the missing head. 2: Pre- and postpharyngeal amputations to generate bipolarly regenerating fragments. 3: Caudal amputations to obtain tail pieces regenerating cephalic structures. **B:** Number of cells expressing *cto* per side and the corresponding size of the regenerating planarians 10 days after amputation. For each size point, $n = 10$. Error bars are ± SD. **C:** Expression of *cto* in a bicephalic regenerate produced by improper wound healing after fission. The numbers (in red) correspond to the *cto*-positive cells detected on the lateral margins of each of the two heads. Scale bars = 1 mm in A, 300 μm in C.

cells were then counted as described in the experimental procedures (Fig. 2B,C). Under our culture conditions, growth and degrowth in the clonal line CIW4 of *S. mediterranea* are, within statistical limits, linear processes with strikingly similar rates of body length change (Fig. 2A), since it takes approximately the same time (~2 weeks) to increase or decrease 1 mm in size (slope values of 0.3 and −0.4, respectively). Of interest, when whole-mount in situ hybridizations were performed (Fig. 2C), we also found that a linear relationship was maintained between the number of *cto*-positive cells and body length, because their number increases or decreases at similar rates during both growth and degrowth ($P = 0.93$, i.e., difference is not statistically significant) with an average rate-of-change of approximately 3 cells/side per mm (Fig. 2B).

These results are in contrast with basal and activated metabolic rates measured in other organisms, in which animals at rest or undergoing starvation display low or basal metabolic rates (BMR), while displaying higher or maximal levels (MMR) under conditions of high nutrient availability (Suarez et al., 1997; Darveau et al., 2002). Under the experimental conditions tested, the data indicate that the BMR and MMR may closely approximate each other in *S. mediterranea* (Fig. 2A). If so, this finding may explain why degrowth occurs in these animals, because their metabolism under starvation conditions does not appear to slow down. Once an exogenous carbon source (food) is no longer available, similar resting and active metabolisms could necessitate the catabolism of cells to generate a source of carbon and metabolites to sustain the metabolic rate of those cells maintaining the form and function of the organism. How the animal "decides" which cells to sacrifice for catabolic purposes and which ones to maintain for anabolic functions is unknown, but may be dependent, among other possible variables, on the relative age of the individual cells.

It will be necessary to obtain more direct measurements of metabolic activity to determine whether the growth and degrowth curves directly correlate with metabolic rates. Given that planarians are devoid of a circulatory system, yet some terrestrial species can grow as long as 1 m in length, oxygen consumption measurements to gauge metabolic activity may not be appropriate, because it is unlikely that the diffusion-based, oxygen-delivery methods will be a rate-limiting metabolic step. Instead, measuring ATP production during growth and degrowth should provide a more accurate measurement of metabolic activity, because the overall metabolic rate at any given time should correspond to total ATP turnover (Darveau et al., 2002). If BMR and MMR are equivalent in planarians, then ATP production during growth and degrowth would be expected to closely resemble each other.

Even though planarians are well-known for their plasticity (Newmark and Sánchez Alvarado, 2002), the data in Figure 2 clearly demonstrate that these animals have rigorous mechanisms in place to control cell number and, consequently, scale and proportion. The increase or decrease of *cto*-positive cells (Fig. 2C) corroborates, at the molecular level, that changes in planarian body size are in fact due to changes in cell number rather than cell size (Baguñà and Romero, 1981). This finding is in direct contrast to *Drosophila* and *C. elegans*, in which most mutants causing size changes are effected by changes in cell size. It is conceptually possible to picture how a fixed number of cells can maintain phenotypic information to maintain form and function (Stern and Emlen, 1999). Yet, in animals such as planarians that are constantly adding and removing cells according to environmental conditions, it is difficult to imagine how cells are being counted during growth and degrowth and how proper organ function is maintained during these changes. The present work does not address these questions per se but provides quantifiable traits and a methodologic framework in which to begin a systematic, molecular characterization of scale and proportion regulation in planarians.

Number of Cells Expressing *cintillo* After Regeneration Is Determined by the Size of the Regenerating Fragment

The discrete spatial expression of *cto* also provides us with a useful marker to monitor scale and proportion regulation during planarian regeneration—after amputation, which scale is obeyed by the regenerating head: that of the intact animal, the fragment, or a determinate scale dictated by the regenerating tissues? To answer this question, we amputated animals 8 mm in length at various levels along their anterior/posterior axis as shown in Figure 3A and monitored *cto* expression in the resulting fragments.

First, we determined the effect of amputation on the *cto*-positive cells present in head fragments (H, Fig. 3A). In planarians, head fragments produced by decapitation are known to remodel themselves to match the scale and size of the regenerating tail which, in the end, results in a properly proportioned animal (Morgan, 1898). The remodeling of head fragments to produce a fully formed and well-proportioned planarian continues for several months. We measured the number of *cto*-positive cells in head fragments that have been undergoing remodeling for 4 weeks and recorded a change from 31 (± 1.6 SD) to 19.4 (± 1.1 SD). Based on the data presented in Figure 2, the reduction of cells observed during remodeling are similar to the cell reduction levels observed when intact, 8-mm-long planarians have been degrowing for 4 weeks (19 ± 1.4 SD). This finding corresponds to ~3 cells/side per mm. Thus, the data indicate that at least for the cephalic fragments, the rate of reduction of *cto*-expressing cells during tissue remodeling closely resembles that of degrowing animals. We do not know, however, if the final number of *cto*-positive cells will correspond allometrically to the final size of the regenerated worm. We addressed this question, instead, by monitoring *cto*-positive cells in fragments of different sizes engaged in cephalic regeneration.

Three different types of fragments (marked with an asterisk in 1, 2, and

TABLE 1. Measured and Predicted Values of *cto*-expressing Cells Relative to Body Size

		cto-positive cell numbers	
Size (mm)	Mass (μg)	Observed	Predicted[a]
2	16.5 ± 0.6	24.7 ± 1.53	24.5
4	29.8 ± 0.6	37.8 ± 1.38	38.2
6	44.5 ± 0.5	52.3 ± 2.75	51.7
8	56.6 ± 0.3	61.7 ± 1.77	66.1

[a]The equation $y = 3.0\ M^{0.75}$ (see text for derivation) was used to obtain the predicted *cto*-positive cell values. Standard deviations are shown.

3, Fig. 3A) were analyzed 10 days after amputation. In all cases, planarians of approximately 8 mm in length were used. Ten days after amputation, group 1 had body lengths of 5.9 ± 0.4 mm, group 2 of 3.4 ± 0.3 mm, and group 3 of 2.6 ± 0.3 mm (Fig. 3B). Of interest, the number of cells expressing *cto* in the regenerated heads (1 = 20.3 ± 2.8; 2 = 14.3 ± 2.4; 3 = 13 ± 0.7, respectively) coincides with the numbers seen in intact animals of similar dimensions (compare Fig. 3B with Fig. 2B). Even in those cases in which regeneration resulted in two-headed animals, the number of *cto*-positive cells (red numbers in Fig. 3C) is regulated according to the size of the regenerating fragment. In this organism, all lateral margins of the two cephalic regions are populated by virtually the same number of *cto*-positive cells. Altogether, these results suggest that the scale and proportion information of the original, 8-mm animal does not perdure after amputation and that such information is re-codified during regeneration by as yet unknown mechanisms to reflect the new, smaller size of the amputated fragments.

Do Planarians Obey the Allometric Equation?

It has been suggested in the literature that "nearly two-dimensional" animals such as planarians may not obey quarter-power scaling (West et al., 1997). To test this assumption, we decided to use the *cto* expression data to determine the scaling component of this population of cells relative to body size. One way to determine the scaling exponent (*b* in $y = aM^b$) is to use the log form of the allometric equation, i.e., $\log y = \log a + b \log M$, where *b* now becomes the slope of the graphed function. To determine the body size (weight), animals of 2, 4, 6, and 8 mm were weighed as described in experimental procedures. When the *log* of *cto*-positive cells (*y*) and body weight are calculated and plotted, and linear regression is applied to the data set, the equation resolves into $\log y = 0.48 + 0.75 \log M$ (correlation coefficient = 0.99). This results in an allometric equation of *cto*-positive cells to body mass of $y = 3.0\ M^{0.75}$. The scaling exponent thus calculated is clearly three quarters and suggests that even with their remarkably plastic characteristics and "nearly two-dimensional" body plan, planarians do obey quarter-power scaling (Table 1).

CONCLUSION

One clear implication of the observations reported here is that allometry in planarians is a dynamic process unlike most other model systems in which static allometry is observed (Stern and Emlen, 1999). Such dynamism is not stochastic, however, because at no time during growth or degrowth are animals observed to lose the form and/or function of their bodies. Therefore, mechanisms capable of integrating information on the size of the animal with the size of its individual components (CNS, gastrovascular system, pharynx, body wall musculature, etc.) must exist in planarians. The precise modulation of *cto* cells in *S. mediterranea* (Figs. 2, 3) and the recent identification of a gene in planarians (*nou-darake*), which when abrogated by RNAi, results in a caudal expansion of the brain without affecting the scale and proportion of other organs such as the pharynx (Cebrià et al., 2002a), suggests that discrete mechanisms for regulating organ size are operating in these animals. Understanding how metabolic rate, cell loss, and cell proliferation are regulated in planarians should shed light on the mechanisms responsible for this animal's dynamic allometry. It should be interesting, for example, to determine whether RNAi abrogation (Sánchez Alvarado and Newmark, 1999) of the phosphatase-tensin homologue (PTEN) would have an effect on planarian allometry as it has been reported for mouse (Li et al., 2002) and *Drosophila*; Oldham et al., 2002).

Because all tissues found in planarians are derived from a mesenchymal population of totipotential stem cells known as neoblasts (Newmark and Sánchez Alvarado, 2000, 2002), it is very likely that some of the components dictating allometry in these animals will be associated with the regulation of proliferation and differentiation of neoblasts. Therefore, planarians offer a unique opportunity not only to study the molecular events guiding size and scale but also to monitor in the whole animal the cell biology effecting this process. The results and methodologic framework presented here will serve as a point of departure for more detailed analyses aimed at elucidating the regulation of scale and proportion in this remarkable metazoan.

EXPERIMENTAL PROCEDURES

Planarian Culture

Clonal line CIW4 (Sánchez Alvarado et al., 2002) of the diploid, asexual strain of *Schmidtea mediterranea* (Benazzi et al., 1972) was used for all experiments. Animals were maintained at 20°C as previously described (Newmark and Sánchez Alvarado, 2000).

Growth, Degrowth, and Regeneration Experiments

During growth experiments, planarians of ~2 mm (n = 100) were fed

homogenized calf liver twice a week, and their length measured weekly immediately before feeding. After each 2-mm increase in length, 20 animals were fixed as previously described (Sánchez Alvarado and Newmark, 1999). During degrowth experiments, planarians measuring ~8 mm in length (n = 100) were starved, their length measured once a week, and specimens fixed (n = 20) as above. The weight of animals was obtained by measuring the length of animals (2, 4, 6, 8 mm), placing them in small petri dishes according to size, removing all of the water and then adding 2 ml of water to each dish. A petri dish with water but no planarians was used for taring purposes. Each size group contained at least 50 animals, and the combined mass was weighed several times. The final individual weight was calculated by dividing the net weight obtained by the number of animals present in each petri dish. In regeneration experiments, animals of approximately 8 mm in length were cut with a sterile razor blade perpendicularly to and at different levels along the anterior/posterior axis. The specimens were evaluated daily, and fixed 10 days after amputation. In all cases, cells expressing *cto* were counted under differential interference contrast by using a Zeiss Axiovert S-100 microscope. To determine whether the *cto*-positive cells counted per side (lateral margins) did or did not belong statistically to the same distribution, a standard, paired *t*-test was carried out. When comparisons between *cto*-positive cells from different organisms were required, two sample *t*-tests for independent samples with unequal variances were performed. All images are representatives of at least three different experiments and were captured with a Zeiss AxioCam digital camera and Improvision image-acquisition software.

Cloning *cintillo*

A cDNA for *cto* was obtained as described in Sánchez Alvarado et al. (2002). The partial cDNA was directionally cloned into pBluescript II/ SK^+, and the sequence has been deposited in GenBank under accession no. AY067542. Further information on this and other cDNAs can be found at http://planaria.neuro.utah.edu.

Whole-Mount In Situ Hybridizations

Samples used during growth or degrowth experiments were processed for whole-mount in situ hybridization as previously described (Umesono et al., 1997). Regeneration samples were similarly processed 10 days after amputation. All animals were starved for at least 1 week before in situ hybridizations. Digoxigenin-labeled RNA probes for *cto* were prepared as described (Sánchez Alvarado and Newmark, 1999). Whole-mount in situ hybridizations were performed with some modifications to accommodate the liquid handling characteristic of an in situ Pro liquid-handling robot (Intavis, Germany; Sánchez Alvarado et al., 2002).

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REFERENCES

Abeloos M. 1930. Recherches expérimentales sur la croissance et la régénération chez les planaires. Bull Biol 1:1–140.

Baguñà J, Romero R. 1981. Quantitative analysis of cell types during growth, degrowth and regeneration in the planarians *Dugesia mediterranea* and *Dugesia tigrina*. Hydrobiologia 84:181–194.

Banavar J. 1999. Size and form in efficient transportation networks. Nature 399: 130–132.

Benazzi M, Ballester R, Baguña J, Puccinelli I. 1972. The fissiparous race of the planarian *Dugesia lugubris* S.L. Found in Barcelona (Spain) belongs to the biotype g: comparative analysis of the karyotypes. Caryologia 25:59–68.

Benedict F. 1938. Vital energetics: a study in comparative basal metabolism. Washington DC: Carnegie Institution of Washington. p 215.

Cebrià F, Kobayashi C, Umesono Y, Nakazawa M, Mineta K, Ikeo K, Gojobori T, Itoh M, Taira M, Sánchez Alvarado A, Agata K. 2002a. FGFR-related gene *nou-darake* restricts brain tissues to the head region of planarians. Nature 419: 620–624.

Cebrià F, Nakazawa M, Mineta K, Gojobori T, Agata K. 2002b. Dissecting planarian central nervous system regeneration by the expression of neural-specific genes. Dev Growth Differ 44: 135–146.

Darveau CA, Suarez RK, Andrews RD, Hochachka PW. 2002. Allometric cascade as a unifying principle of body mass effects on metabolism. Nature 417:166–170.

Darwin C. 1859. On the origin of species. Cambridge, MA: Harvard University Press.

Dürer A. 1591. Della simmetria de i corpi humani, libri quattro/di Alberto Durero. Venetia: Presso Domenico Nicolini.

Emlen DJ. 1994. Environmental control of horn length dimorphism in the beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). Proc R Soc Lond Ser B 256:131–136.

Emlen DJ, Nijhout HF. 2000. The development and evolution of exaggerated morphologies in insects. Annu Rev Entomol 45:661–708.

Farnesi RM, Tei S. 1980. *Dugesia lugubris* s.l. auricles: research into the ultrastructure and on the functional efficiency. Riv Biol 73:65–77.

Garcia-Anoveros J, Corey DP. 1997. The molecules of mechanosensation. Annu Rev Neurosci 20:567–594.

Garcia-Anoveros J, Ma C, Chalfie M. 1995. Regulation of *Caenorhabditis* elegans degenerin proteins by a putative extracellular domain. Curr Biol 5:441–448.

Garcia-Bellido A, Ripoll P, Morata G. 1973. Developmental compartmentalization of the wing disk of *Drosophila*. Nature 245:251–253.

Gillespie PG, Walker RG. 2001. Molecular basis of mechanosensory transduction. Nature 413:194–202.

Gillooly JF, Charnov EL, West GB, Savage VM, Brown JH. 2002. Effects of size and temperature on developmental time. Nature 417:70–73.

Hong K, Mano I, Driscoll M. 2000. In vivo structure-function analyses of *Caenorhabditis* elegans MEC-4, a candidate mechanosensory ion channel subunit. J Neurosci 20:2575–2588.

Huxley J. 1932. Problems of relative growth. London: Methuen and Co. Ltd.

Li G, Robinson GW, Lesche R, Martinez-Diaz H, Jiang Z, Rozengurt N, Wagner K-U, Wu D-C, Lane TF, Liu X, Hennighausen L, Wu H. 2002. Conditional loss of PTEN leads to precocious development and neoplasia in the mammary gland. Development 129:4159–4170.

Mano I, Driscoll M. 1999. DEG/ENaC channels: a touchy superfamily that watches its salt. Bioessays 21:568–578.

McMahon T, Bonner J. 1983. On size and life. New York: Scientific American Library.

Montagne J, Stewart M, Stocker H, Hafen E, Kozma S, Thomas G. 1999. *Drosophila* S6 kinase: a regulator of cell size. Science 285:2126–2129.

Morata G, Ripoll P. 1975. Minutes: mutants of *Drosophila* autonomously affecting cell division rate. Dev Biol 42: 211–221.

Morgan TH. 1898. Experimental studies of the regeneration of *Planaria maculata*. Arch Entw Mech Org 7:364–397.

Newmark PA, Sánchez Alvarado A. 2000. Bromodeoxyuridine specifically labels the regenerative stem cells of planarians. Dev Biol 220:142–153.

Newmark PA, Sánchez Alvarado A. 2002. Not your father's planarian: a classic model enters the era of functional genomics. Nat Rev Genet 3:210–219.

Nystrom J, Shen Z, Aili M, Flemming A, Leroi A, Tuck S. 2002. Increased or decreased levels of *Caenorhabditis elegans lon-3,* a gene encoding a collagen, cause reciprocal changes in body length. Genetics 161:83–97.

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.

Richter JP, editor. 1998. The notebooks of Leonardo da Vinci. Toronto, Ontario: General Publishing Company. p 182–183.

Robb SMC, Sánchez Alvarado A. 2002. Identification of immunological reagents for use in the study of freshwater planarians by means of whole-mount immunofluorescence and confocal microscopy. Genesis 32:293–298.

Sánchez Alvarado A, Newmark PA. 1999. Double-stranded RNA specifically disrupts gene expression during planarian regeneration. Proc Natl Acad Sci U S A 96:5049–5054.

Sánchez Alvarado A, Newmark PA, Robb SMC, Juste R. 2002. The *Schmidtea mediterranea* database as a molecular resource for studying platyhelmintes, stem cells and regeneration. Development 129:5659–5665.

Schmidt-Nielsen K. 1993. Scaling: why is animal size so important? Cambridge: Cambridge University Press.

Stahl W. 1967. Scaling of respiratory variables in mammals. J Appl Physiol 22: 453–460.

Stern D, Emlen D. 1999. The developmental basis for allometry in insects. Development 126:1091–1101.

Suarez RK, Staples JF, Lighton JRB, West GB. 1997. Relationship between enzymatic flux capacities and metabolic flux rates: non-equilibrium reactions in muscle glycolysis. Proc Natl Acad Sci U S A 94:7065–7069.

Thompson D. 1917. On growth and form. (abridged edition, J. T. Bonner, ed.) Cambridge: Cambridge University Press. p 1961.

Umesono Y, Watanabe K, Agata K. 1997. A planarian orthopedia homolog is specifically expressed in the branch region of both the mature and regenerating brain. Dev Growth Differ 39:723–727.

Weigmann K, Cohen S, Lehner C. 1997. Cell cycle progression, growth and patterning in imaginal discs despite inhibition of cell division after inactivation of *Drosophila* Cdc2 kinase. Development 124:3555–3563.

West G, Brown J, Enquist B. 1997. A general model for the origin of allometric scaling laws in biology. Science 276:122–126.