Growth Control and Pattern Regulation in the Lateral Line Systems of *Xenopus*

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In *Xenopus,* the supraorbital lateral line system consists of a periodic pattern of lateral line organs which is formed by the regular fragmentation of a streak-like primordium. The pattern forming mechanism which subdivides the primordium into individual organs is not capable of adjusting to the variable size of the system. Nevertheless, the number of organs per supraorbital system tends to be held constant. This is achieved by regulating the growth of the system in an appropriate manner.

Introduction

The lateral line system consists of small mechanoreceptive organs which are arranged in characteristic rows in the epidermis of fishes and aquatic amphibians. Each of these organs contain a small number of cells which are of two types: sensory hair cells and supporting cells [1]. The individual organs of a given lateral line row are formed during development by the fragmentation of a streak-like primordium into the periodic pattern of organs [2]. In *Xenopus,* the initial size of these repeated pattern elements is fixed and is not adjusted to the overall size of the primordium, *i.e.,* from a primordium artificially reduced in size, a smaller number of organs is formed [3]. Nevertheless, the number of organs formed tends to be held constant during normal development by the regulation of primordium size itself. A mechanism is described below which achieves pattern regulation through an appropriate regulation of the growth of the system.

The growth of the supraorbital (SO) system of lateral line organs was analyzed. The development of this organ system has been studied recently [3–6]. The SO system in *Xenopus* (Fig. 1) develops from a small primordium in the epidermis at the anterior margin of the ear vesicle. After stage 33/34 (about 2 days post fertilization), it begins to elongate in the direction of the nasal pit along the dorsal margin of the eye. At stage 39½ (2½ days p.f.), the streak-like primordium becomes fragmented into a linear series of small cell groups, the primary lateral line organs [4]. These organs subsequently grow to their final size which is attained at stage 47½ (6½ days p.f.). The total number of cells increases steadily and linearly with time throughout the development of the system. However, different organs, although of equal size initially, grow at very different rates, so that the final organ sizes are rather variable [5]. Surprisingly, a high degree of regularity is found in the frequency distribution of final organ sizes. When the organs are classified according to the number of cells they contain and the frequency of occurrence of each size class is plotted on a histogram, a series of regularly-spaced peaks is revealed. The peaks lie at 8, 15, 22, 29, 36, 43, 50, 57 and 64 cells per organ, *i.e.* at values of $8+7\,n$ where $n=0, 1, 2$ etc. When the relative size of these peaks is compared, it turns out that they fit approximately a nearly symmetrical binominal distribution. The probability of getting a given value of n is the same as the probability for n successes in 8 independent trials when the probability of success is about ½ in any given trial [5]. This can be explained as follows. The SO system is thought to grow by the asymmetric division of stem cells. Each stem cell produces 8 nondividing, terminal cells in successive divisions before becoming terminal itself. Only stem cells are present at the beginning of development. The stem cells begin to produce terminal cells as the primordium elongates. After about one round of cell divisions, when stem cells and terminal cells are present in nearly equal numbers, organ segregation occurs, and stem cells and terminal cells are randomly allocated to the nascent organs which contain 8 cells apiece. The further growth of these organs depends on the number of stem cells they obtain by chance. For this reason, the frequency distribution of

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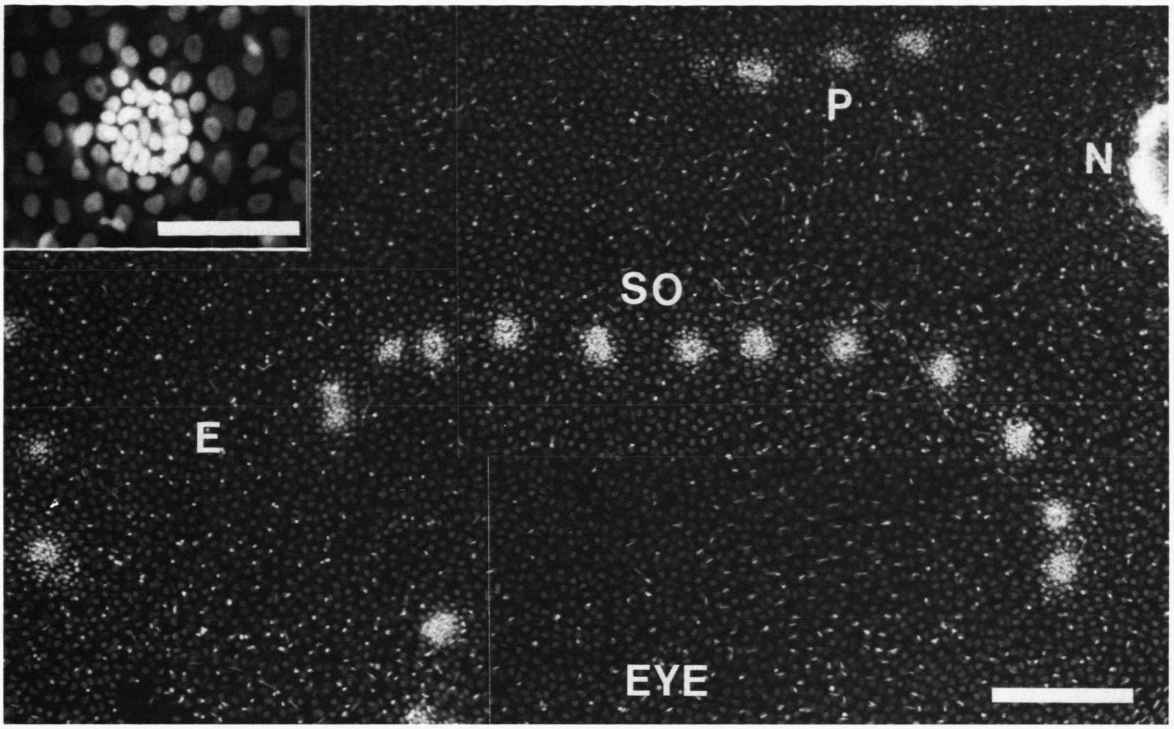

Fig. 1. DAPI-stained skin preparation from the head of a stage 47½ [8] *Xenopus* larva. The supraorbital (SO) and the parietal (P) line of organs together form the SO system of lateral line organs, the parietal line being derived from the anterior most part of the elongating SO primordium, which migrates dorsal and detaches from the rest of the line during development. The nasal pit (N), and the position of the ear vesicle (E) and the eye are indicated. Bar = 200 μm. Inset: single primary lateral line organ at higher magnification. Bar = 50 μm.

the final organ sizes (in cells/organ) exhibits 9 discrete peaks (corresponding to 0, 1, 2, ..., 8 stem cells/organ), with the first peak at 8 cells/organ (no stem cell contained in organ). The distance between neighbouring peaks is 7 cells/organ because each stem cell produces another 7 terminal cells after the first round of cell divisions. The random allocation of stem cells to the nascent organs is reflected in the fact that the relative sizes of the peaks fit approximately a binominal distribution [5]. In agreement with this developmental scheme are several independent observations; *e.g.* the linear increase in cell number, and the constant number of cells in the S-phase of the cell cycle at all times during the growth of the system [5]. Furthermore, the polyclonal origin of individual lateral line organs, and the presence of non-dividing cells within growing organs has been directly demonstrated [5]. In addition, the altered frequency distributions of the final organ sizes of experimentally manipulated systems [3, 6] are consistent with this scheme. In the present article, the relationship between the growth rate and the size of the system, and the number of pattern elements which are eventually formed, will be analyzed.

Materials and Methods

Larvae of *Xenopus laevis* were kept in tapwater at 22–23 °C without feeding. Under these conditions, larval development ceases between stages 47 and 48, and the growth of the lateral line system becomes arrested at about the same time. Embryos and larvae were fixed in 4% formaldehyde, and the skin was peeled off and stained with the fluorescent dye 4,6-diamidino-2-phenylindoledihydrochloride (DAPI, Boehringer Mannheim) to visualize cell nuclei. The nuclei of the lateral line organ cells appear bright and tightly packed. The numbers of cells/organ and the numbers of organs/SO system were determined from such DAPI-stained skin preparations.

To produce experimentally diminished SO systems, the SO primordium was extirpated completely before the onset of SO system development. A primordium almost always regenerates which is smaller than normal to varying degrees. Small, truncated SO systems develop in the normal way and time course from these primordia [3].

Results and Discussion

The strongest argument for a dynamic relationship between the rate of cell division and the size of SO systems comes from a comparison of the frequency distributions of the final organ sizes of normally sized (Fig. 2a) and experimentally diminished (Fig. 2b) SO systems. The latter were produced by extirpation of the SO primordium followed by the regeneration of a smaller primordium some time before the onset of primordial elongation and stem cell division. Comparing the two frequency distributions reveals that in both cases a stem cell divides 8 times, but that the timing of the divisions is different. The distance between neighbouring peaks in experimentally diminished SO systems is only 6 cells/organ, instead of the 7 cells/organ in normal SO systems. This implies that the number of stem cell divisions after organ segregation is only 6 times in the experimental case

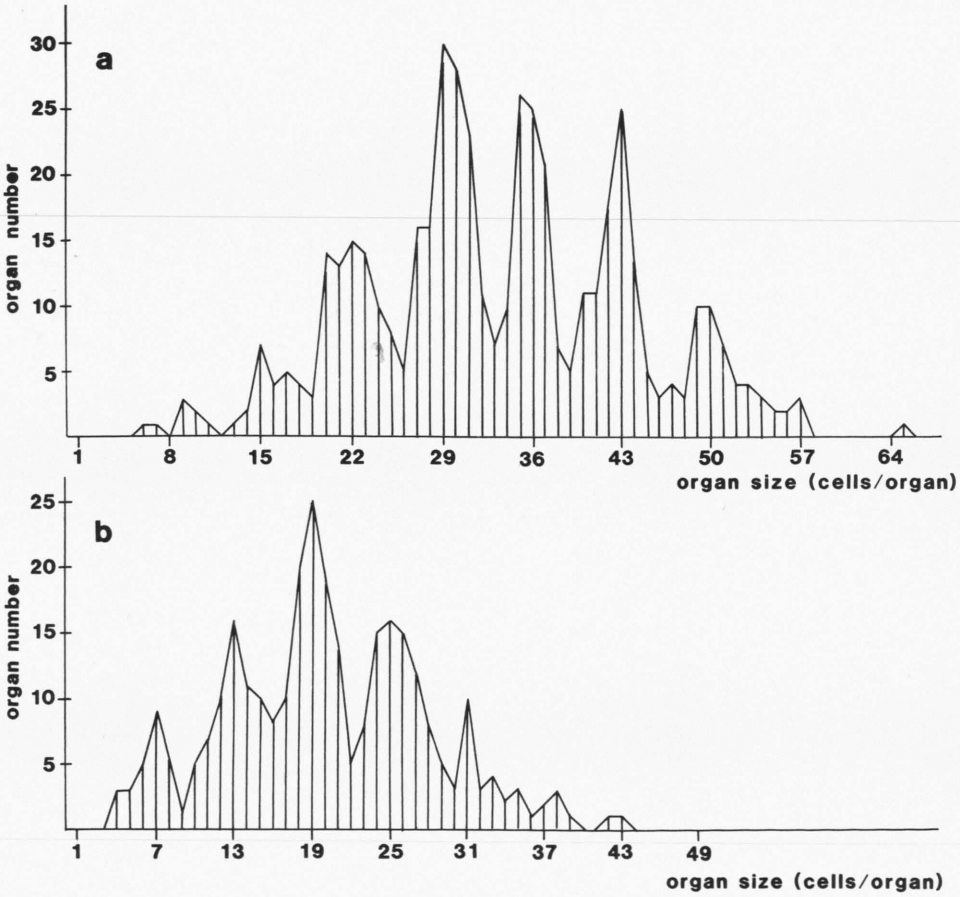

Fig. 2. Frequency distribution of primary lateral line organ sizes after completion of growth. A) Organ size distribution of large, normally developing SO systems. The average distance between neighbouring peaks is 7 cells/organ. The relative size of the peaks fits approximately into a binominal distribution with $n = 8$ and $p = 0.45$. B) Organ size distribution of small, experimentally diminished SO systems. The distance between neighbouring peaks is 6 cells/organ. The relative size of the peaks approximates a binominal distribution with $n = 7$ and $p = 0.33$.

as opposed to 7 times during normal development (see Introduction). At the same time, the skewness of the distribution, which gives the ratio of stem cells to terminal cells at the time of organ segregation (see above [5]), changes from 0.45 to 0.33. So at the time of organ formation there is 1 terminal cell for every stem cell in normally developing systems, whereas there are 2 terminal cells for every stem cell in experimentally diminished systems. This implies that there has been one round of stem cell divisions before organ segregation in the large SO systems and two in the smaller ones. So during normal development, a stem cell divides once before and seven times after organ segregation, whereas in diminished systems 2 divisions are performed before organ segregation and 6 after it. What has changed is the timing of the divisions relative to the process of organ segregation. As the time course of morphogenetic development is essentially the same in both cases [3], the rate of cell division must be accelerated in small SO systems so as to allow for an extra round of stem cell divisons before the time of organ formation. As a consequence of this, the average number of stem cells per forming organ is reduced, leading to a reduction of the average final organ size. At the same time, relatively more organs of normal initial size can be formed due to the increased primordial growth before organ segregation. In the following, these consequences will be analyzed in more detail.

If the rate of cell division in the SO system is permanently affected by the initial size of the primordium and not just during the first divisions within the unfragmented primordium, then this should become apparent when one compares the proliferation kinetics of SO systems of different sizes, *e.g.* SO systems from different batches of embryos, and experimentally diminished systems (Fig. 3).

The increase in cell number is always linear until growth ceases. The characteristics of the regression lines drawn are a) for normally developing SO sys-

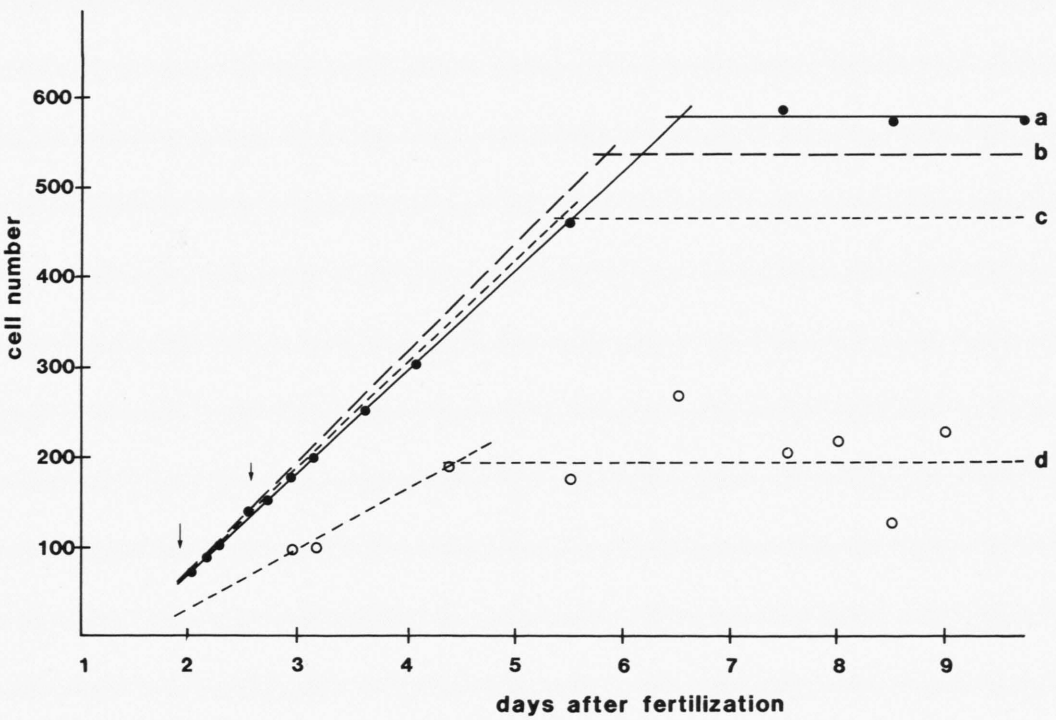

Fig. 3. Cell kinetics for SO systems of varying sizes. Since curves a) to c) lie very close together, data points are only indicated for curve a) (closed circles) and d) (open circles). Each point represents the mean value from about 6 (a) or about 7 (d) measurements. The average standard deviation for each point is about 10% for a), b) and c); and due to the strong variation in size of the regenerated systems, about 60% for d). A total of 270 skin preparations were evaluated.

tems (data of Winklbauer and Hausen [5]) slope $a = 4.6$ cells/h, regression coefficient $r = 0.99$, b) for normally developing SO systems from a different batch of embryos $a = 5.0$, $r = 0.94$, c) for normally developing systems from still another batch (control side SO systems of embryos from extirpation experiments) $a = 4.7$, $r = 0.96$, and d) for SO systems developing from small, regenerated primordia (same embryos as in c), operated side) $a = 2.8$, $r = 0.46$. The frequency distribution of Fig. 2a corresponds to curve a and the distribution shown in 2b corresponds to curve d.

With a smaller number of stem cells, the final cell number is proportionally lower, since each stem cell always produces 8 terminal cells [3, 5, 6]. The plateaus in Fig. 3 are all significantly different from each other at $\alpha = 0.01$, except for a)–b), which are different only at $\alpha = 0.1$. If the length of the cell cycle were constant and independent of the size of the system, the final cell numbers would always be reached at identical times with the slope of the growth curves being smaller for smaller systems. However, in all four cases shown in Fig. 3, the final cell numbers are reached earlier in smaller systems. The slopes of the growth curves are moderately ($\alpha = 0.1$ for b) and $\alpha = 0.15$ for d)) or highly ($\alpha = 0.001$ for c)) significantly larger than expected under the assumption that the length of the cell cycle were constant at 4.6 cells/h (curve a). So apparently, the fewer stem cells there are in a given system, the faster they divide.

Curve d represents the cell kinetics of experimentally diminished SO systems from the left-hand sides of animals in which the right-hand sides provided normally developing SO systems as controls (curve c). Even in this case, the plateau is reached about one day earlier in the smaller systems. The growth of SO systems is apparently controlled independently for each side of the embryo. Therefore, this control is probably local rather than global.

Fig. 4a shows the relationship between stem cell number and cell cycle length in the SO system. Each stem cell produces 8 terminal cells and then becomes terminal itself. Thus, the number of stem cells/SO system is calculated by dividing the final cell number/SO system by 9. The length of the cell cycle is calculated from the number of stem cells/SO system, and the slope of the regression line of the cell kinetics. The solid line represents a theoretical line predicted upon a constant organ number per SO system. It was constructed under the assumption that at the time of organ segregation the same number of cells should always be present, irrespective of the initial size of the SO primordium. Obviously, the fewer stem cells there are, the faster they would have to divide to give the same number of cells at the time of organ segregation. With a fixed initial organ size of 8 cells/organ, the same number of organs could then always be formed, irrespective of the initial (and hence the final) size of the system. The observed values for normally developing SO systems (open circles) lie close to this theoretical curve. One would expect, however, that the length of the cell cycle cannot be reduced below a certain minimum. Indeed, the value for the experimentally diminished SO systems (triangle) lies well above the theoretical curve, and 8 h is perhaps the shortest cycle time possible for the primordial stem cells.

The value for triploid SO systems is also indicated (filled square). The cell volume is $1.5\times$ normal in triploids. This increase is generally compensated for by a corresponding reduction in cell number [7]. The SO primordium is of normal size in triploids, but consists of a smaller number of larger cells [6]. When the length of the cell cycle in triploid SO systems is related to the number of stem cells present, it seems that there is no compensation for the reduced stem cell number. However, when the length of the cell cycle is related to the total stem cell mass, expressed as the equivalent number of diploid cells which could be formed from this cell mass, then the value for triploid systems (open square) is consistent with those for normally developing diploid SO systems. We may conclude, therefore, that the length of the cell cycle is not determined by the number, but by the total mass of stem cells present, or by any equivalent parameter.

Consistent with the results of Fig. 4a, the number of organs per SO system tends to be kept constant within the range of normal variation in SO system size (Fig. 4b). As expected from the existence of a lower limit to the length of the cell cycle (Fig. 4a), compensation for reduced primordial size comes to a limit in experimentally diminished SO systems, and organ number is directly proportional to the size of the system when there are less than 350–400 cells (corresponding to about 40 stem cells, Fig. 4b). For diminished systems, the slope of the regression line $a = 0.045$, and the regression coefficient $r = 0.96$; for normally developing systems, $a = 0.010$ and

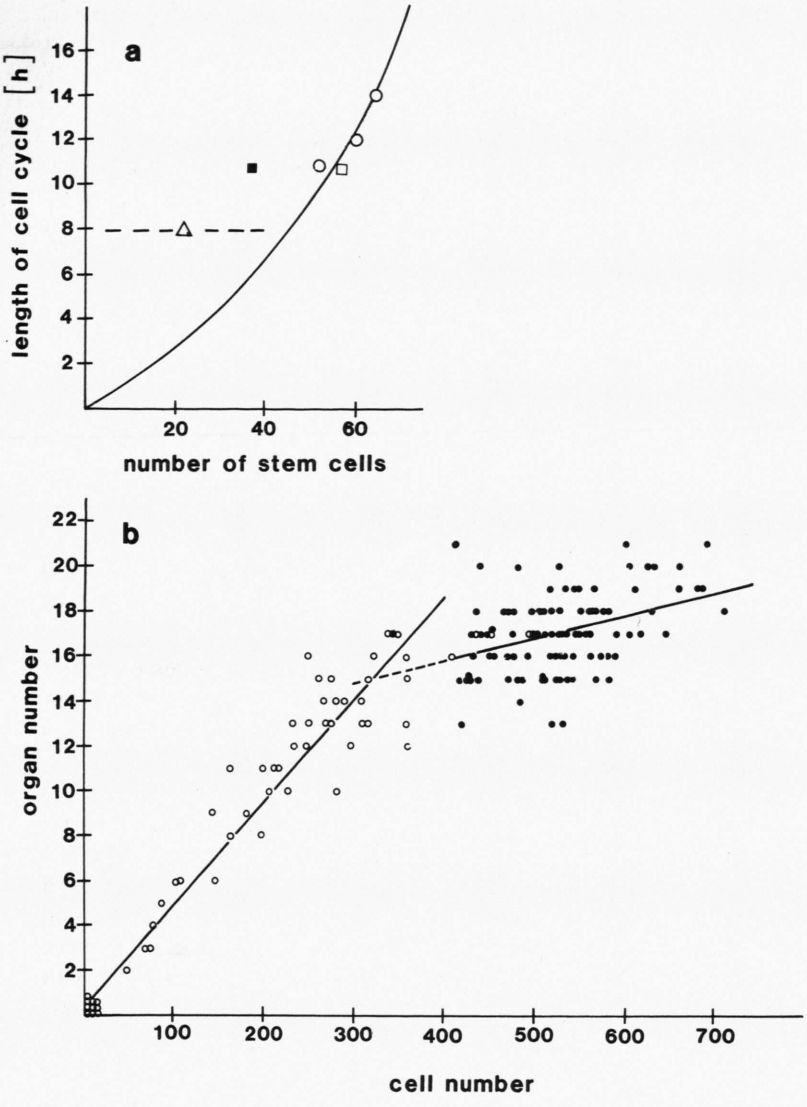

Fig. 4. Compensatory growth and pattern regulation in the SO system. A) Cell cycle length as a function of stem cell number/SO system. The line of constant organ number (solid line) was constructed as described in the text. The values for normally developing SO systems of different sizes (open circles, corresponding to curves a, b, and c in Fig. 3), and for triploid SO systems (squares, calculated from the data of ref. [6]) are indicated. No compensation for reduced primordial size occurs when the number of stem cells is less than about 40 (dashed line; see text and Fig. 4b). B) Relationship between cell number/SO system and organ number. The two regression lines indicated were calculated for cell number < 400 and > 400, respectively, which correspond in practice to experimentally diminished (open circles) and normally developing (closed circles) SO systems.

$r = 0.40$. The slope of the latter regression line is significantly smaller than the one for diminished SO systems ($\alpha = 0.001$), but it is larger than zero ($\alpha = 0.001$).

So, although the pattern forming mechanism which subdivides the streak-like lateral line primordium into individual organs is not capable of adjusting to the variable size of the system, there is a tendency to keep constant the number of organs per SO system; and this is achieved by regulating the growth of the system in an appropriate manner. This example demonstrates how the relatively poor regulative capability which is inherent to some classes of pattern forming mechanisms can be circumvented by a dynamic adaptation of growth parameters.

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