

Development of the lateral line system in *Xenopus laevis*

IV. Pattern formation in the supraorbital system

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SUMMARY

The periodic pattern of the supraorbital lateral line organs forms in the epidermis of *Xenopus* by the subdivision of a streak-like primordium into a linear series of small cell groups. In normal development, each such organ initially contains about 8 cells (Winklbauer & Hausen, 1983*a,b*). To see whether this initial organ size depends on the size of the streak-like primordium at the time of organ segregation, primordium size was reduced experimentally before the onset of pattern formation. In such small primordia, the size of the primary organs formed is not adjusted so as to allow the formation of a normal number of organs. Instead, the initial organ size is kept approximately normal, and the number of organs is correspondingly reduced, i.e. the pattern forming mechanism is not capable of 'size regulation'.

INTRODUCTION

The supraorbital (SO) lateral line system of *Xenopus* is an example of a periodic pattern, that is, a regular array of identical or developmentally homologous elements. During the development of the SO lateral line, a narrow streak of cells which extends within the epidermis along the dorsal margin of the eye is fragmented into small clusters of cells, the individual SO organs. These are initially of equal size, but they grow at very different rates, thus forming primary organs of greatly varying sizes (Winklbauer & Hausen, 1983*a,b*).

One may ask what rules and principles govern the subdivision of the streak-like primordium into a series of equal-sized groups of cells. With triploid embryos, it can be shown that despite a marked increase in cell size, the absolute size of the forming organs remains normal. This is achieved by a proportional reduction of the number of cells per organ (accompanying communication). The question then arises whether other perturbations, e.g. a size reduction of the whole system, also leave the absolute size of the individual SO organs unchanged. Alternatively, size regulation could occur, leading to the formation of a normal number of proportionally smaller organs. Our results demonstrate that in embryos in which the size of the SO primordium is experimentally reduced well before organ

Key words: *Xenopus laevis*, lateral line system, supraorbital system, pattern formation.

segregation, the initial size of the SO organs is kept normal, and the number of organs formed is correspondingly reduced as a consequence.

MATERIALS AND METHODS

Xenopus laevis embryos and larvae were obtained, kept and staged, and the skin preparations were made as described (Winklbauer & Hausen, 1983a; accompanying communication). Extirpation of the SO primordium at stages 26–35/36 was carried out in full-strength Modified Barth's Solution (MBS-H) (Elsdale, Gurdon & Fischberg, 1960) containing MABA (Sandoz) as an anaesthetic. The SO primordium on the left side was always extirpated, while the primordium on the right side served as a control. Electrolytically sharpened tungsten needles were used to excise a large, nearly rectangular piece of epidermis (along with some underlying attached material) from the region between the ear vesicle and the posterior margin of the eye (Fig. 1). Extirpation of a still larger piece of epidermis had no effect on the result. After an hour, the wound was nearly closed by the contraction of the wound margins. Operated animals were reared in 1/10-strength MBS-H without feeding at 22°–23°C until fixation.

RESULTS

The SO system after early extirpation of the primordium

In the SO system, which consists of the SO line (comprising the anterior auditory group of organs) and the parietal line of organs, primary organ formation is completed at about stage 47½. In normal SO systems of that stage the SO line of organs extends from the anterior margin of the ear vesicle to a point near the nasal pit, where it is inflected ventrad. To a less and more variable degree, the posterior end of the line is also drawn ventrad, forming the anterior auditory group of organs. Dorsal to the SO line, the parietal line extends in a rostrocaudal direction. It is formed from the anteriormost part of the SO primordium, which turns dorsad during normal development (Winklbauer & Hausen, 1983a).

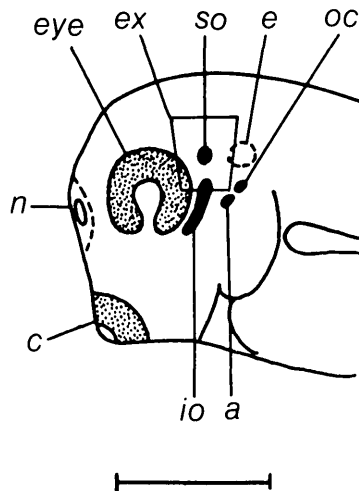


Fig. 1. Operation scheme of extirpation experiments. Supraorbital (*so*), infraorbital (*io*), occipital (*oc*) and aortic (*a*) lateral line primordia, and cement gland (*c*), nasal pit (*n*), eye, and ear vesicle (*e*) of stage-33/34 embryo indicated, together with area of skin extirpated (*ex*). Bar = 500 µm.

After extirpation of the SO primordium early in development, a wide variety of results is obtained, which can be ordered into a series of progressive truncations beginning from the anterior end of the SO line system. In a few cases, the SO system appears normal after extirpation (not shown, identical to Fig. 2A). With moderate truncation, only the parietal line is missing (Fig. 2B). Further shortening affects the SO line. It becomes progressively deleted from the anterior end (e.g. Fig. 2C) until the SO system is completely absent (Fig. 2D).

In the SO system, the range of organ sizes at the end of primary organ formation is strikingly large, and the fluctuation of organ size within the system is essentially random (Winklbauer & Hausen, 1983*b*). However, superimposed upon this random fluctuation is a position-dependent modulation of organ size (Fig. 3). On the average, a normal SO line begins posteriorly with an organ of a size well above the mean. Characteristically, the second and third organ in the line fall well below the average size. With increasing distance from the posterior end, the size of the organs, on the average, increases again fluctuating around the mean value. Although the range of organ sizes for a given organ position is very large, the overall pattern is nevertheless reproducible from experiment to experiment or often even from line to line. Experimentally truncated SO systems show the same characteristic size modulation at the posterior end as untreated controls (Fig. 3). Thus, in addition to their characteristic position they appear to represent the posterior portions of normal SO systems also in this respect.

Cell and organ number in operated and control SO systems

In previous experiments, it was shown that primary organ formation is completed in the SO system between stages 47 and 48. At this time, development is arrested in starving larvae, and the growth of the SO system ceases (Winklbauer & Hausen, 1983*b*). In the present experiments, including those with triploid larvae (accompanying article), the final cell number attained in growth-arrested control SO systems is about 500 cells, the number of organs formed is 17.3, and the number of cells per organ is therefore 29 (Table 1). There is a slight difference in these values to those obtained in our previous investigation (Winklbauer & Hausen, 1983*b*). This difference may be due to the fact that different batches of females were used in the respective experiments to produce embryos. Since the data are consistent within the present series of experiments and those of the accompanying article, the data for the controls of the extirpation and the triploidy experiments were pooled when appropriate.

The SO primordium or its precursor, the VII placode, was extirpated at various stages between its formation at stage 24 (Nieuwkoop & Faber, 1967) and the initial phase of primordial elongation at stage 35/36. No systematic effect of the time of extirpation upon the final cell number, organ number, or organ size could be observed (Table 1).

A comparison of the total number of operated SO systems and the controls from the non-operated side of the same animals shows an average reduction of the total cell number to less than half of normal, and a reduction of the average organ size

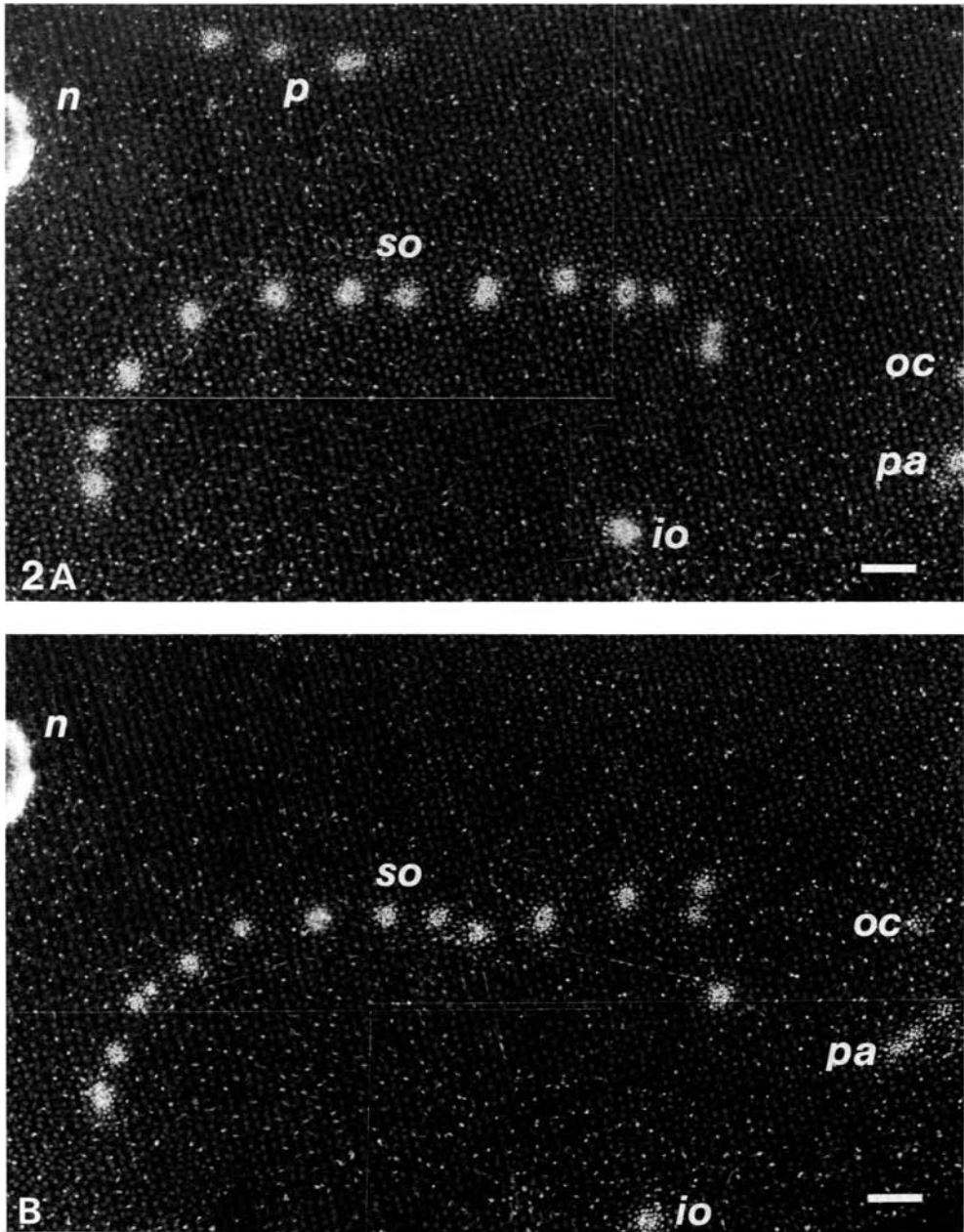
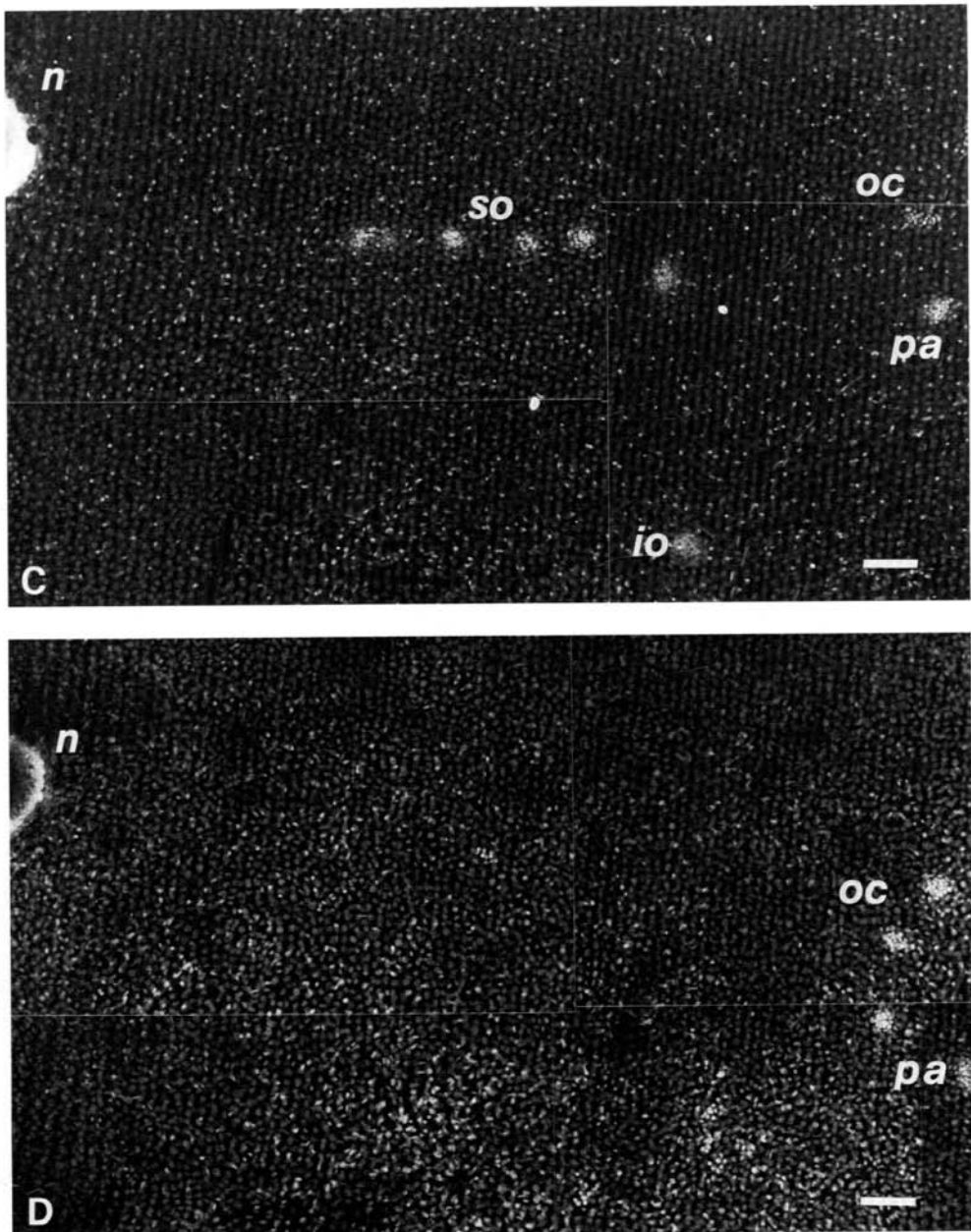


Fig. 2. DAPI-stained skin preparations of growth-arrested stage-47½ embryos showing control (A) and operated (B,C,D) supraorbital systems. In each specimen, the nasal pit (*n*) and parts of the occipital (*oc*) and posterior auditory (*pa*) groups of lateral line organs can be recognized. In (A), the supraorbital line (*so*) and the parietal line (*p*) of organs are also present. The position of the ear vesicle would be between the occipital



and posterior auditory groups of organs and the posterior end of the supraorbital line. The eye would be situated ventral to the supraorbital line of organs. In (B) and (C), the supraorbital line is completely (B) or partially (C) present, but the parietal line is always absent. In (D), both the supraorbital and the parietal line are missing. The first organ of the infraorbital line (*io*) is visible in (A), (B), and (C). Bar = 100 μ m.

to about three quarters of normal on the operated side of the growth-arrested larvae (Table 1). The great variability in the effects produced by the extirpations is reflected in the greater standard deviations for the total cell number and the organ number as compared to the controls (Table 1).

Frequency distribution of organ sizes in operated SO systems

After growth arrest, the frequency distribution of organ sizes from operated SO systems forms a series of distinct peaks (Fig. 4A). The cell numbers of adjacent peaks differ by 6 cells, and the first peak is at an organ size of 7 cells. If the peaks are superimposed to equalize random irregularities, a highly symmetrical peak is in fact obtained when superposition is according to this frame (Fig. 4C).

From previous results (Winklbauer & Hausen, 1983*b*; accompanying communication), it can be deduced that when the first peak corresponds to an organ size of 7 cells, the empirical distribution should approximate a binominal distribution with $n=7$, and the number of peaks should be 8. Indeed, the

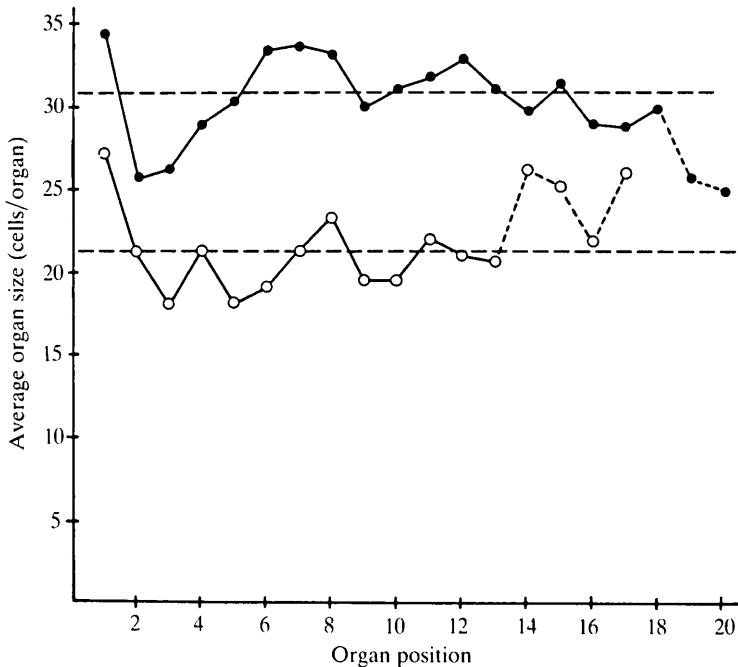


Fig. 3. Position-dependent modulation of organ size. The average organ size is drawn as a function of organ position for control (closed circles) and operated (open circles) SO systems. Organs are numbered successively, beginning from the posterior end of the supraorbital line and ending with the anteriormost organ of this line or, if present, with the most caudal organ of the parietal line (which corresponds to the previous anterior end of the supraorbital primordium). Since the organ numbers of normal and of operated SO systems vary, the number of organs at a given position diminishes towards the right-hand end of the curves, which is indicated by dashed lines in that region. The dashed horizontal lines represent the average organ sizes of all control and of all operated SO systems examined, respectively.

Table 1. Cell number, organ number, and organ size in operated and control SO systems at completion of primary organ formation

experiment (stage of extirpation)	extirpated side			control side		
	total cell number	organ number	cells/ organ	total cell number	organ number	cells/ organ
st. 35/36 (n = 7/7)	180 ± 60	8.9 ± 3.2	20.4 ± 2.5	501 ± 45	17.1 ± 1.9	29.2 ± 3.7
st. 33/34 (n = 5/6)	243 ± 82	11.6 ± 3.6	20.9 ± 1.9	521 ± 27	17.7 ± 1.5	29.5 ± 3.3
st. 33/34 (n = 9/7)	171 ± 145	7.6 ± 5.9	21.6 ± 4.9	489 ± 46	17.4 ± 2.2	28.1 ± 4.5
st. 32 (n = 6/6)	295 ± 134	12.7 ± 4.6	23.3 ± 4.0	528 ± 85	17.0 ± 1.7	31.1 ± 4.1
st. 26 (n = 8/9)	228 ± 127	11.3 ± 6.0	20.3 ± 1.7	447 ± 57	16.7 ± 1.3	26.8 ± 3.0
controls triploids (n = 6)	—	—	—	538 ± 39	18.0 ± 1.8	29.9 ± 2.1
mean (n = 35/41) ± S.D.	217 ± 120	10.2 ± 5.1	21.3 ± 3.2	499 ± 59	17.3 ± 1.7	28.9 ± 3.6

Cells and organs were counted in DAPI-stained skin preparations. The left-hand side of an embryo was operated, the right-hand side primordium serving as a control.

frequency distribution of organ sizes approximates very closely an asymmetrical binominal distribution with $n = 7$ and $P = 0.33$ (Fig. 4B). The absence of a peak at the theoretical maximum organ size of 49 cells can readily be explained by the fact that the probability for an organ of that size to occur among the 300 organs examined is very low. One would expect less than one organ of this size among 2000, given the estimated parameters of the distribution.

Comparison of operated and control SO primordia

During normal development, organ formation occurs around stage $39\frac{1}{2}$ in the SO system (Winklbauer & Hausen, 1983a,b). The cell nuclei in the streak-like

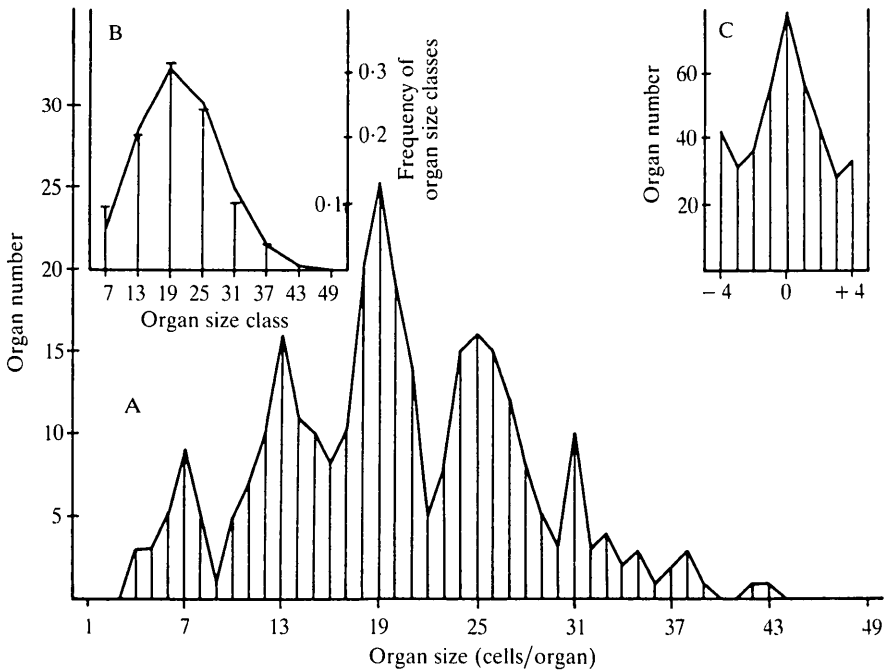


Fig. 4. Frequency distribution of organ sizes of operated SO systems. (A) The cell number per organ from 299 SO organs was determined from DAPI-stained skin preparations. Growth-arrested larvae (starvation) from five different matings (all the experiments in Table 1) were used. When regeneration of the SO system was (nearly) complete and the average organ size was larger than 25 cells/organ (three cases), organs were not included into the frequency distribution. (B) Frequency of organ size classes. According to the frame indicated in (C), organ size classes can be defined, each ranging over an organ size difference of 6 cells/organ, with 7, 13, 19, etc. cells/organ as the medians of the respective classes. The frequency of the organ size classes is indicated by bars. This empirical distribution approximates a binominal distribution with number of trials $n = 7$ and probability of success $P = 0.33$ (solid line). (C) Superposition of peaks. It is assumed that the first peak is at 7 cells/organ and that the difference between neighbouring peaks is 6 cells/organ. Organ numbers at 7, 13, 19, etc. cells/organ of (A) are summed to give the organ number at zero in (C). Organ numbers at 8, 14, 20, etc. cells/organ are added to give the organ number at +1 in (C), etc. This superposition yields a symmetrical peak.

primordia appear tightly packed in DAPI-stained skin preparations (Fig. 5A). In contrast to this, operated SO primordia appear only faintly outlined at stage 39½. A streak of small, bright nuclei is always present at the expected position in skin preparations, but the nuclei are far less densely packed, and the primordia are not so clearly marked off from the surrounding epidermis (Fig. 5B). Thus, the cell number and the shape and extent of the operated primordia cannot be accurately determined at that stage. At stage 40½ operated and control SO primordia appear equally distinct for the first time (Fig. 6A,B).

A comparison of the operated SO primordia at stage 40½ and their controls from the same embryos reveals that in both series the degree of fragmentation, and hence the progression of organ formation, is about the same (Fig. 7). It is only the length of the primordia which is different. The control primordia have already reached a stage where their anteriormost part turns dorsad to form the parietal line. The operated primordia, although varying greatly in size, are always shorter than the controls, and no signs of parietal line formation are detectable (Fig. 7).

The ratio of total cell numbers in operated as compared to control SO systems is the same in stage-40½ SO primordia and in SO systems at the end of primary organ formation (Table 2). The length of the operated primordia is reduced in proportion to the diminished cell number, while the width of operated and control

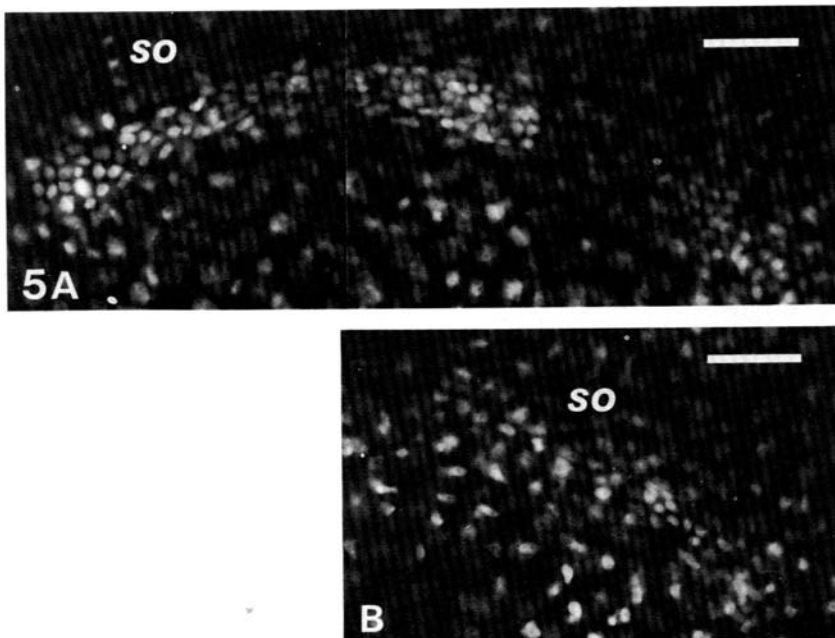


Fig. 5. DAPI-stained skin preparations of stage-39½ embryo. (A) Normal supraorbital (*so*) primordium from right-hand (control) side of embryo. (B) Faintly indicated supraorbital (*so*) primordium from operated (left-hand) side of embryo. Both primordia point to the left with their anterior ends (5B printed reversed relative to 5A). Bars = 50 μ m.

primordia remains equal (Table 2). Thus, the operated SO primordia of stage-40½ embryos essentially appear as truncated posterior ends of normal stage-40½ SO primordia, in the same way and to the same degree as operated SO systems appear as truncations of normal SO systems after completion of primary organ formation (Figs 2, 3).

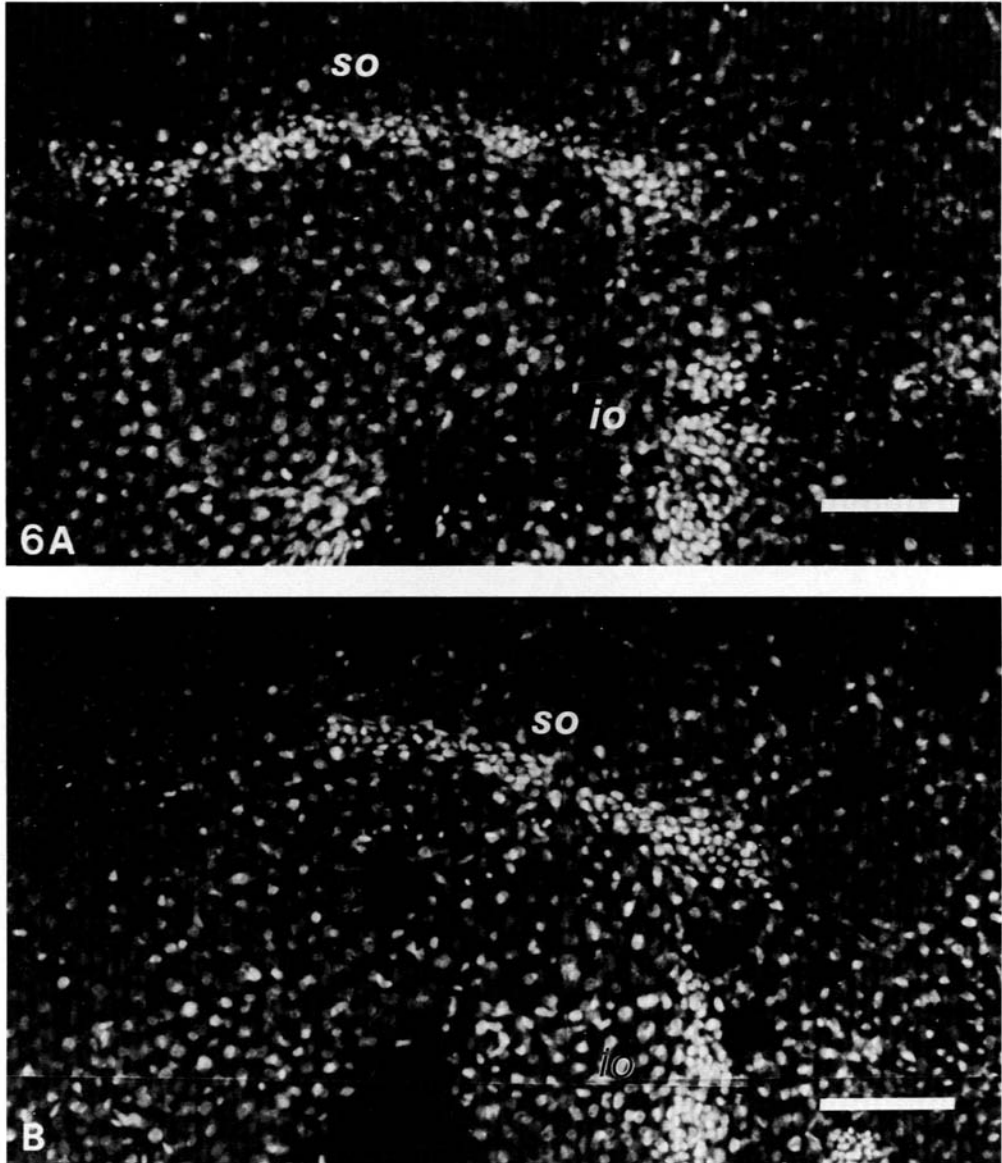


Fig. 6. DAPI-stained skin preparations of stage-40½ embryo. (A) Normal supraorbital (*so*) primordium from right-hand (control) side of embryo. (B) Short supraorbital (*so*) primordium from operated (left-hand) side of embryo, being as distinct as control primordium. Part of the infraorbital lateral line (*io*) is visible. Both primordia point to the left with their anterior ends (6B printed reversed relative to 6A). Bars = 100 μ m.

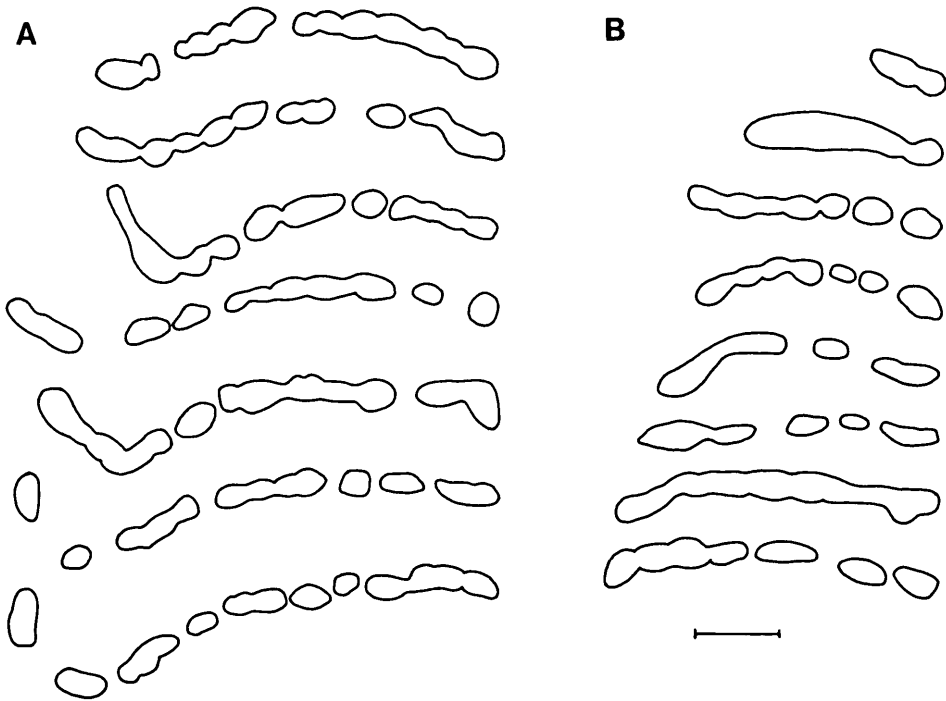


Fig. 7. Morphology of control (A) and operated (B) SO primordia at stage 40½. The outlines of these primordia were redrawn from photographs of skin preparations. All primordia point to the left with their anterior end. Bar = 100 µm.

DISCUSSION

Application of the proposed model of cell multiplication and organ segregation to operated SO systems

The analysis of the frequency distribution of organ sizes led to a model of normal growth and organ formation in the SO system (Winklbauer & Hausen, 1983b) which explains all of the data available at that time. Furthermore, precise quantitative predictions of the model can be verified experimentally (accompanying communication). Therefore, the frequency distribution of the organ sizes of truncated SO systems will now be analysed within the framework of our previous model (Winklbauer & Hausen, 1983b; accompanying article).

According to the model, the operated SO system grows by the asymmetric division of stem cells. At the beginning of development, only stem cells are present. Each stem cell is capable of producing 8 non-dividing terminal cells by successive unequal cell divisions before becoming terminal itself. After two rounds of cell divisions, when stem cells and terminal cells are present in a ratio of 1:2, organ segregation occurs, and the streak-like primordium is divided into a series of small cell groups consisting of 7 cells each. Stem cells and terminal cells are

Table 2. Cell number and size of operated and control SO primordia at stage 40½*

	cell number	length of primordium (μm)	width of primordium (μm)
extirpated (n = 8)	98 ± 35	311 ± 101	25.9 ± 4.3
control (n = 7)	182 ± 27	588 ± 67	25.0 ± 2.3
ratio extirpated/control	0.54	0.53	1.04
final ratio extirpated/control**	0.51	0.67†	—

* Same batch of embryos as in experiment 'stage 26' in Table 1.

** Ratio of the corresponding values of operated and control SO systems after completion of primary organ formation (experiment 'stage 26' in Table 1).

† Length of growth-arrested SO systems estimated as the number of organs/SO system. Distance between organs is the same in operated and control SO systems.

Cells were counted, and dimensions of primordia were measured in DAPI-stained skin preparations.

randomly allocated to these groups, which are the nascent SO organs. Since an individual organ may obtain either 0, 1, 2 . . . , 7 stem cells, since the probability for a given cell to be a stem cell is $P = 0.33$, and since each stem cell is capable of producing a further 6 terminal cells, the frequency distribution of organ sizes should exhibit eight discrete peaks, with an interval of 6 cells/organ between neighbouring peaks, with the first peak at 7 cells/organ, and with the size of the peaks fitting approximately into a binominal distribution with $P = 0.33$. These characteristics of the frequency distribution are actually observed, with the exception of the peak at the largest organ size of 49 cells/organ, which our model predicts as unlikely to occur in our sample of 299 organs.

Although there is a great variability in size, one can calculate some parameters of the average operated SO system. Since each stem cell divides eight times to produce an average final cell number of 217 (Table 1), there must be, on the average, 24 stem cells. On the average, 10.2 organs are formed, each containing 7 cells initially. At the time of organ segregation 10.2×7 , that is 71 cells, must be present, which also gives exactly the ratio of 1:2 for stem cells to terminal cells. At stage 40½, the average cell number per primordium is 98 cells. This means that organ segregation must occur well before that stage, which is also suggested by the morphology of stage-40½ primordia.

From the frequency distribution of organ sizes of the controls (including controls of experiment in accompanying article, not shown) and the data of Table 1, the corresponding parameter values for control SO systems can be extracted as follows: initial organ size of 8 cells/organ; 17.3 organs and 55 stem cells per SO system; 8 cell divisions per stem cell; total cell number at completion of primary organ formation 499 cells; and a ratio of terminal cells to stem cells of somewhat larger than 1:1 at organ segregation. Taken together, these data support the assumption that the mechanism of organ formation is identical in operated and normal SO systems, with only the exact values of some parameters being altered.

Extirpation of the SO primordium

In the extirpation experiments, a large piece of epidermis is excised which is presumed to include the whole SO primordium or its precursor (Fig. 1). Nevertheless, SO line systems of various lengths develop in most cases after the operation. In accordance with Stone (1922, 1928), who performed similar extirpation experiments in *Ambystoma*, we conclude that this is due to incomplete regeneration of the SO primordium. This notion implies that, after extirpation, the truncated SO line in fact develops from a regenerated primordium in the normal way and not, for example, directly from the epidermis. Strongly in favour of the first alternative is the fact that the frequency distribution of organ sizes is easily explained only when the model for normal SO system development is applied to operated SO systems. It would be difficult, indeed, to explain the binominal distribution of organ sizes in a simple way under the assumption that each organ directly develops from the epidermis. In support of this notion are the results of Wright (1947), who showed that under a variety of experimental

conditions lateral line organs could never be observed to develop directly from the epidermis in anurans.

When operated SO systems develop normally, SO primordia of reduced size should be present during early development, which is actually found. Unfortunately, however, the cell density of these primordia is less than normal initially, approaching the density of the surrounding epidermis for progressively earlier stages. This makes identification and counting of these primordial cells difficult. However, when the cell density of operated primordia has reached normal levels at stage $40\frac{1}{2}$, their morphology differs from control SO primordia of that stage only in the expected reduction of length, suggesting that they are in a state of development which corresponds to their normal counterparts.

A further indication of the normal development of operated SO systems lies in the fact that a position-dependent modulation of the average organ size is conserved, which is characteristic of the posterior end of a normal SO line. Whether this size modulation is an inherent property of the primordium, or whether it is imposed by the environment, conditions must have been restored after the operation which allowed the development of a normal posterior end in the primordium. Taken together, these arguments support the view that operated SO systems develop in the normal way and with a normal time course from SO primordia, which are only smaller than normal to varying degrees.

Pattern formation in the SO system

A periodic pattern can be characterized by the size and the number of its elements. When the size of the whole system is reduced prior to pattern formation, either the size or the number of elements or both must also be reduced proportionally. In SO systems developing from experimentally diminished primordia, it is obvious that the normal number of pattern elements, the primary organs, is not conserved. Moreover, from the frequency distribution of organ sizes one can accurately deduce the size of the nascent lateral line organs, which is 8 cells in normal development and 7 cells in operated SO systems. The development of triploid SO systems shows that an organ can be formed from a smaller number of cells as well, provided however that these cells are correspondingly larger (accompanying article). Thus, at pattern formation, it is the *initial* size of the pattern elements, measured for example as the total cell mass per organ, which is kept constant in primordia of reduced size, at the expense of the number of elements. It must be noted, however, that the *final* organ size is reduced to three quarters of normal in operated SO systems. Most of this reduction is due to the fact that the ratio of stem cells to terminal cells at organ segregation is 1:2 in operated primordia and about 1:1 in the controls, and that the number of stem cell divisions after organ segregation is 6 and 7, respectively. A more detailed analysis of this finding is in progress.

If the size of the nascent organs was determined by the total cell mass included per organ, the shape of the primordium would not be important in determining the size and number of pattern elements. Alternatively, the size of a forming organ

could be determined by a fixed length of primordium allotted to it, the number of organs segregated being proportional to the total length of the primordium. The length of primordium corresponding to a single organ would be about 26 μm . This is equivalent to about 3 cell diameters in diploid primordia and to about 2.5 cell diameters in triploids. At stage 40½, operated primordia containing fewer cells are seen to be proportionally shorter, whereas the average width is the same in operated and control primordia (Table 2). This is compatible with both alternatives. In order to decide conclusively whether the number of organs formed is proportional to the total primordial cell mass or to the length of the primordium, it would be necessary to alter systematically the width of SO primordia prior to pattern formation.

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