Somitogenesis in amphibia

IV. The dynamics of tail development

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SUMMARY

Following neurulation, the frog segments c. 40 somites and concurrently undergoes a striking elongation along the anteroposterior axis. This elongation (excluding the head) is largely the result of a presegmental extension of posterior tissue with a lesser contribution from the extension of segmented tissue.

Presegmental extension is entirely the result of activity within a narrow zone of extension that occupies the central region in the tail bud. Within the zone of extension, a minimum of six prospective somites undergo an eight- to ten-fold extension along the axis. The zone passes posteriorly across the tissue of the tail tip. The anterior of the tail bud contains three extended prospective somites in the course of segmentation. The anterior boundary of the zone of extension coincides in space exactly with the anterior boundary of the zone of abnormal segmentation that results from temperature shock. This means that extension ceases immediately before the sudden tissue change associated with segmentation.

INTRODUCTION

There are thirteen paired myotomes in the adult frog, and some forty pairs in the tadpole. Two thirds of the somites segmented in the embryo are therefore tail somites.

Previous accounts of tail development have been concerned with the origins of the parts of the tail in early development and the fate map on the early gastrula (Pasteels, 1939). The developmental mechanics of the tail has received scant attention. Nevertheless the rapid development of a tail and a regular segmental pattern from a relatively unstructured bud is not a trivial exercise (Cooke, 1975) and we do not know how it is done. To understand segmentation, we need a dynamic description of tail-bud development.

MATERIALS AND METHODS

Clutches of Rana embryos were collected from natural habitats and reared in pond water.

Operations were performed in modified Slacks medium (Slack & Forman, 1980): NaCl (105 mM), KCl (2·1 mM), Na HPO₄ (6·2 mM), KH PO₄ (1·1 mM),

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MgSO (1·0 mm), CaCl (1·0 mm), Ethylenediaminetetraacetic acid (EDTA) (0·1 mm), and NaHCO (0·5 mm).

1 to 3 h after operations, specimens were transferred to one-tenth strength medium and reared at 10 or 15 °C.

The techniques of stripping the skin from the embryo to reveal the somites, and of temperature shock have been described (Pearson & Elsdale, 1979; Elsdale & Pearson, 1979).

Measurements on embryos were made at 12·5 times or 25 times magnification using a centimetre ocular scale divided into 100 units. Measurements were made within 15 min of fixation, before shrinkage.

To throw into relief the shallow contours indicating the somite pattern on the surface of the living embryo, the following procedure was employed. Embryos were placed in 50 mm plastic dishes under shallow water. Fibre optics in flexible mounts were arranged so as to throw almost horizontal beams along the main axis of the embryo. To obtain the best result, one plays with four variables: the lamp positions, the angle of the beam, the position of the embryo in the dish (close to the side is best), and the direction of the beams – from the head, from the tail, or both. The procedure cannot be used to obtain an accurate somite count, because the most recently formed somites (counted after stripping) are not discriminated on the living embryo. Also not discriminated reliably are the posterior somites behind about somite 25. The procedure is ideal for the purpose for which it was here used, to make repeated measurements over the same somites.

Histological sections were prepared from material fixed in 2·5 % glutaraldehyde (buffered with cacodylate (0·1 M) to pH 7·4) and embedded in Araldite. Sections, 3 μm thick, were stained in toluidine blue and examined under a Zeiss Universal microscope.

RESULTS

1. Morphological description of the tail bud on the 13-somite Rana embryo (Figs 3, 6, 8)

The embryo is c.4·5 mm long. The length of the tail bud posterior to the last-formed somite is close to 1·2 mm. The blastopore provides a useful marker
Figs 1–5
located at the apex of a shallow concave curve on the silhouette of the embryo; a section through the blastopore normal to the dorsal surface passes immediately behind the last formed somite.

The epidermis over the tail bud is two layered. The outer, pigmented layer is a substantial structure formed of a monolayer of cuboidal cells rising to columnar in the keel, the precursor of the tail fin. The inner, unpigmented layer is a slight structure of flattened cells rising to cuboidal in the keel. The skin of the dorsal bud adheres to the underlying tissue, the skin over the ventral bud is loose. In *Bufo* there is a large space beneath the ventrolateral skin.

Three axial structures are present in the bud: nerve tube, chorda, and postanal endoderm. The attenuated neural canal follows the curve of the bud and ends about the level of the chorda and some 200 μm from the posterior extremity. The connection with the blastopore has been lost. Moreover, the postanal endoderm is lumenless and there is no neurenteric canal.

The chorda occupies a central position and can be distinguished in horizontal section to within 300 μm of the extremity of the bud. A narrow space separates chorda from flanking presomitic mesoderm. The cells of the posterior chorda are neither vacuolated nor distinctively arranged.

Postanal endoderm originates by a backward extension, from the posterior dorsal angle of the archenteron roof, commenced around the 9-somite stage. In the 13-somite embryo the tissue forms a substantial, ventraxial wedge; the apex lies immediately beneath the chorda, the base underlies the ventral keel. The extension thus occupies an equivalent position in the ventral bud to that taken by the nerve tube in the dorsal bud. The cells are uniquely large and yolk laden. It appears to us on the evidence of sections that the tissue breaks up to contribute the ventral fin mesenchyme, analogous to the neural-crest-derived fin mesenchyme in the dorsal bud.

The transverse section of the tail bud is an ellipse. The horizontal axis is inflated by massive, paired, presomitic, mesodermal shields that encase the axial


Figs 6 and 7: Horizontal, longitudinal sections through the notochord. Figs 8 and 9: transverse sections about mid-way along the bud. Figs 6 and 8: 13-somite stage. Figs 7 and 9: 20-somite stage.

These figures illustrate the shape of the bud and the location of the mesoderm and postanal endoderm. Comparison of the 13-somite stage with the 20-somite stage shows the gross changes which accompany development, especially the flattening in the frontal plane. The two layers of the epidermis are readily distinguished, especially in the keel in Fig. 9. The posterior notochordal rudiment is everywhere discrete in Fig. 7, this is not the case in Fig. 6. One pair of somitic furrows is visible on the right of Fig. 6, three furrows are visible at the top right of Fig. 7. Notice how much slimmer the mediolateral profile has become by the 20-somite stage. The dark material ventral to the notochord in the transverse sections is postanal endoderm. Neural crest cells located dorsal to the nerve tube in Fig. 9 will contribute the dorsal fin mesenchyme.
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Figs 6–9
structures. The shields extend to the base of the dorsal keel, ventrad, their extension is curtailed by the endodermal wedge.

All the presumptive tissues are visibly discrete except in the tip of the bud. There, in the final 300 μm, nerve tube, chorda, and presomitic mesoderm appear to derive from a common pool of cells. About half a century ago there was controversy over the issue whether this region of the bud represented a blastema of multipotential cells. Those who took this view believed the tail bud to be the site of an independent ‘secondary morphogenesis’. This idea fell out of favour as evidence accumulated to show that tail development was essentially a continuation of trunk development, and the differences were quantitative rather than qualitative, see Pasteels (1939) for a discussion of the issues and references, also Bijtel (1931). The involutory movements of gastrulation continue in the posterior part of the embryo long after the ‘gastrulation stage’ is past (Keller, 1976). At what stage these movements are completed in the frog is unknown.

2. Axial elongation

i. Elongation of the embryo and mode of extension of the tail (Figs 1–5)

Throughout development to the neurula stage the embryo has remained roughly spherical. Following the closure of the neural folds, and with the commencement of somitogenesis, the embryo elongates. All parts of the embryo eventually share in this elongation. In Urodeles there is a considerable elongation of the head and trunk before tail extension. In our frogs the head elongates little, the trunk to an intermediate extent, and the greatest contribution to the elongation of the embryo is provided by the extension of the tail. The frog embryo at the neural fold stage is a sphere about 2·5 mm in diameter. Already at the 6-somite stage, about half a day later in the case of embryos reared at 15 °C, the shape has deformed to an ellipse 3·5 mm long. Over the ensuing 4 days, during which the segmentation of 40+ pairs of somites is completed, the length extends to 11 mm.

Concomitant with the axial elongation of the tail bud there is a marked compression in the horizontal plane; the section of the early tail bud is almost circular, whereas the mature tail is leaf shaped. Furthermore, the cells and tissues of the bud are closely packed and there is little free space: in the tail, there is much free space especially in the bases of the fins where the mesenchymes are very diffuse.

The presomitic mesoderm of the frog neurula forms two bands of tissue a little more than 1·5 mm long in the anterior posterior axis. The first three somites appear almost simultaneously at the fold stage; thereafter, new somite boundaries appear at regular intervals of 2 h 20 min at 15 °C. Newly formed somites in the anterior embryo are 0·15 mm in the anterior posterior axis. It follows that if the process of segmentation were like slicing a loaf, then the length of the unsliced portion would reduce by 0·15 mm for each somite segmented, and the
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Fig. 10. Lengths of unsegmented mesoderm on embryos of different ages. Each bar represents the length of the unsegmented paraxial mesoderm measured on a fixed embryo immediately after it was stripped. The figures on the right are means of the lengths in mm of ten embryos from the same batch measured at the 5-, 15-, 25-, and 35-somite stages.

Loaf could be cut into no more than 10 or 11 slices. In fact, the length of the unsegmented mesoderm reduces by very much less as successive somites are cut from it, (Fig. 10). Thus, after 12 somites have formed the length of the unsegmented mesoderm has reduced by a mere 0.3 mm to 1.2 mm. The 30-somite embryo has 0.5 mm of unsegmented tip. These measurements indicate that normal development involves the extension of material in the unsegmented tip. Mechanisms apart, the process of tail extension is similar to the playing out of a rolled-up tape.

Table 1. Presegmental extension

<table>
<thead>
<tr>
<th>Somite block</th>
<th>Length in mm</th>
<th>No. of embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>2–6</td>
<td>0.80</td>
<td>5</td>
</tr>
<tr>
<td>7–11</td>
<td>0.70</td>
<td>5</td>
</tr>
<tr>
<td>12–16</td>
<td>0.70</td>
<td>10</td>
</tr>
<tr>
<td>17–21</td>
<td>0.65</td>
<td>10</td>
</tr>
<tr>
<td>22–26</td>
<td>0.65</td>
<td>8</td>
</tr>
<tr>
<td>27–31</td>
<td>0.60</td>
<td>6</td>
</tr>
<tr>
<td>32–36</td>
<td>0.55</td>
<td>4</td>
</tr>
<tr>
<td>37–41</td>
<td>0.35</td>
<td>5</td>
</tr>
</tbody>
</table>

Clearly, the material for new somites must come from the presegmental tail-bud tissue. The extension of this tissue is given by summation of the lengths of the somites measured before their subsequent expansion. For this purpose it is sufficient to measure blocks of five somites starting with the second somite (the first is quickly obscured). In order to ensure that measurements were made prior to somite expansion, embryos were fixed at eight stages, and the last completed block of five somites alone was measured on each embryo. Hence, no embryo had segmented more than four somites posterior to the last somite included in a measurement. Measurements contributing to any one entry were within 0.7 mm of one another.

On the basis of these figures one can calculate the contribution of presegmental extension to the length of the embryo at any particular stage, by summation of the appropriate number of entries in the table. For example, the contribution to the 21-somite embryo is provided by summation of the lengths of the first four blocks in the table up to and including somite 21: 

\[(0.80 + 0.70 + 0.70 + 0.65) \text{ mm} = 2.85 \text{ mm} \]

The overall length of the 21-somite embryo is 6 mm.
ii. The two contributions to tail elongation

How much tape is played out? In order to quantitate the extension of the unsegmented tail-bud tissue, it is not sufficient to compare the lengths of the paraxial mesoderm before and after somitogenesis. Such a comparison would lead to an overestimation, for the formed somites expand too. There are thus two spatially distinct contributions to elongation of the tail during somitogenesis: (1) Presegmental extension, by which the tail bud extends to make room for the segmentation of additional somites, and, (2) Somite expansion.

To quantitate presegmental extension it is necessary to measure the lengths of newly formed somites before they expand. It is sufficient for this purpose to measure blocks of five somites as described in the legend in Table 1. By summation

![Graph showing somite expansion](image)

**Fig. 11. Somite expansion.** Five sets of measurements were made on a single embryo during the course of development from the 13-somite to the 31-somite stage. The vertical axis is the age of the embryo in terms of the number of somites segmented. Each point represents the length on a block of five somites. Repeat measurements made on the same block are connected. This data is representative of measurements made on ten embryos.
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of successive entries in the table the figure of 5 mm is obtained for presegmental extension to the 41-somite stage.

To follow the course of somite expansion advantage has been taken of the remarkable view obtained of intact, live embryos under oblique illumination by fibre optics (Figs 12, 13). The skin contours faithfully reproduce the underlying segmental pattern. This method of observation has allowed us to make several measurements over a period of time on the same block of five somites, Fig. 11. The figure shows that the first formed somites about double their length in the time taken to segment a further 25 somites.

Table 2 presents the partition of the file lengths of six late embryos between the two contributions. More than a third of the length of the files in embryos completing somitogenesis is the result of somite expansion, presegmental extension accounts for hardly two thirds.

Table 2. Contributions to the length of the somite file

<table>
<thead>
<tr>
<th>Somite number</th>
<th>Length of file</th>
<th>Contributions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Somite expansion</td>
</tr>
<tr>
<td>1</td>
<td>36</td>
<td>8·0</td>
</tr>
<tr>
<td>2</td>
<td>36</td>
<td>7·5</td>
</tr>
<tr>
<td>3</td>
<td>36</td>
<td>8·0</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>8·0</td>
</tr>
<tr>
<td>5</td>
<td>41</td>
<td>8·0</td>
</tr>
<tr>
<td>6</td>
<td>31</td>
<td>6·5</td>
</tr>
</tbody>
</table>

Six late embryos were fixed and stripped and the files counted and measured. The contribution of presegmental extension was estimated using the data in Table 1. Thus, Embryo 1 of Table 2 has 36 somites. The contribution of presegmental extension to the length of the 36-somite file is given by the summation of all the lengths of the somite blocks up to and including somites 32–36 in Table 1. The cumulative length is 4·5 mm. The somite counts were thus used to determine how many entries of Table 1 were to be summed. The length of the files in excess of the contribution from presegmental extension is a measure of somite expansion.

iv. Pre-segmental extension and segmentation are separable processes

Although extension and segmentation go hand in hand in normal development, the following observations show how unwise it would be to assume mutual dependence.

Anyone who has experimented with amphibian embryos, particularly Xenopus, is likely to have encountered stunted embryos. These embryos occur spontaneously and are more commonly found among embryos that have developed under adverse conditions. Stunted embryos show a failure of elongation; all degrees of the condition occur (Fig. 14). Even in severely stunted embryos the segmental pattern may be essentially normal. The somites are short in the anteroposterior axis and long in the dorsoventral axis; they are straight.
instead of V shaped. In embryos that have extended hardly at all one can often count upwards of 30 pairs of somites. The existence of stunted embryos indicates that segmentation does not depend on extension.

Another observation indicates that extension can occur in the absence of normal segmental patterning. The embryo illustrated in Fig. 15 suffered a temperature shock at 43°C. There is an inhibition of the segmental pattern over most of the normally extended tail.

3. Development of the isolated tail bud

This experiment was undertaken to test the self sufficiency of the tail bud.

Tail buds were removed from 13-somite embryos by a cut normal to the axis and passing through the blastopore. Trunks and tails were allowed to heal in full-strength medium for several hours and maintained in 0-1 medium. Fifty isolated tails were reared.

The majority of isolated tail buds segmented upwards of 25 somites of normal appearance at the same rate as controls (Figs 16, 17). In only one particular was isolated tail development visibly different from that of intact controls: isolated tails were frequently but not invariably shorter than controls. This difference could be eliminated by including extra ventral skin with the isolate. A small minority of tail buds did not develop a normal complement of somites, failed to increase in length, and disintegrated early; these failures were probably due to injury to the skin and poor healing.
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The result allows two conclusions: (1) all the material necessary to grow a tail is present in the bud, (2) the bud can control its development independently of the rest of the embryo.

4. The pre-somite mesoderm of the 13-somite embryo tail bud is a highly determined system

Histology has shown the tail bud to be a mosaic of presumptive tissues clearly separate from one another except at the tip. To investigate tissue determination

Figs 12–17
further, the state of the cells within the shields of presomitic mesoderm has been tested in deletion experiments. Cuts were made to excise parts of the shields along with accompanying skin etc. Operated embryos were reared to a stage at which the normality of the somite pattern could be assessed.

Fig. 18 presents sketches illustrating some of the excisions made on the 13-somite embryo tail bud. Generally, excision of a half, a quarter, or one eighth of the bud resulted in the segmentation of a reduced number of somites. This is not true of all cases, however: excision of the dorsal posterior quarter or of the lower ventral one eighth resulted in no loss of somites although some somites were reduced. In no case was tail morphogenesis entirely normal after an excision.

The smallest amount of tissue removed in the above experiment was a little less than one eighth of the bud. A small supplementary experiment was performed
to see if pattern deficiencies resulted from even smaller deletions. A vertical slice was removed from the posterior tail bud according to Fig. 18. Around 1/16th to 1/20th of the bud was removed. The results illustrate the limitations that emerge as more stringent tests of the state of determination are attempted. Three embryos showed dorsal deficiencies in several somites on either side of and including somite 28. This result suggests a failure in pattern regulation. The remaining four embryos showed no such dorsal deficiencies, but some irregularities in the pattern. One is reluctant however to say that pattern regulation had unquestionably occurred in these embryos for the following reason. In order to create the most favourable conditions for obtaining regulation, deletions are made on the posterior bud, in a region of immature tissue not due to segment for some time; thus one allows plenty of time for regulation to occur. One is then committed however to scoring deficiencies in posterior somites of small size among which spontaneous irregularities are commonplace. The most favourable conditions for obtaining regulation are not, unfortunately, the most favourable for demonstrating it.

The conclusion drawn from these experiments is that the presomitic mesoderm of the bud retains little if any capacity for regulation following deletions; indeed adjacent parts may be unable to substitute for an excision within the same presumptive somite. The paraxial mesoderm is viewed, therefore, as a mosaic of small groups of cells already differentiated from their neighbours in the 13-somite embryo. It does not follow however that the cells wear labels identifying themselves as assigned to a particular part of a particular somite. No such somite prepattern need be established at the 13-somite stage. The results demand only that the cells be differentiated from one another in some way upon which their eventual assignation depends. The cells have acquired positional values (Wolpert, 1969).

5. Packing of prospective somites in the bud and the dynamics of extension

The non-regulative behaviour of the paraxial mesoderm implies that, in principle, prospective somites can be located in the tissue unambiguously. On the basis of this conclusion, the previous results can now be reconsidered from the standpoint of the clues they offer concerning the packing of the prospective somites in the bud.

Removal of the dorsal posterior quarter of the bud does not reduce the number of somites segmented. Removal of the ventral posterior quarter results in the further segmentation of around 10–12 somites only and a loss of c.15 posterior somites. This means that the dorsal anterior quarter contains representative portions of somites 14 to c.21 only, and c.20 posterior somites are located entirely within the ventral posterior quarter. This quarter can be further divided into upper and lower portions according to Fig. 18. Removal of the upper part gives the same result as removal of the quarter, whereas the lower part can be removed without reduction in the somite number.
The conclusion drawn from these experiments is that the majority of the prospective tail somites are accommodated in the posterior tip of the bud, in the ventral portion, centred upon the upper 1/8th.

Excision experiments give us a general idea of somite packing in the bud: they are, however, biased. Sloughing of cells from the wound faces prior to healing may effectively increase the quantity of excised tissue. Although this source of error can be controlled by careful technique, excision experiments are no substitute for the marking experiment now to be described.

To reveal the dynamics of bud extension, an experiment was carried out in which marks placed on the tail bud of the 13-somite embryo were located on the tail of the 35-somite embryo. Because even minor irregularities of the repeated pattern are conspicuous, we used simple, surgical marks in preference to chemical markers.

Each embryo tail bud received a single, vertical cut transverse to the axis at a measured distance from the posterior extremity. The cuts extended from the dorsal surface to below the notochord. Using a very fine and freshly cleaned tungsten needle, minimal disturbance was created; there was almost no loss of cells and healing started immediately. Within minutes, it was difficult to see where cuts had been made, and certainly after 3 h, when operated embryos were transferred to dilute medium, they were indistinguishable from controls. Nevertheless, fixed and stripped at the 35-somite stage, the majority of embryos showed a single, irregularity in the somite pattern. Only in a few of the embryos that received cuts on the posterior bud had the marks entirely disappeared. In order to be sure that the irregularity was indeed the surgical mark, a triangular piece of the putative dorsal fin was removed at the end of the cut on some embryos. In these cases the irregularity in the somite pattern occurred in a fixed relation to a fan-shaped deficiency in the dorsal fin. In fact, the experiment revealed a posterior extension of the somite mesoderm on the skin, for the fin deficiency was always 2 to 4 somites anterior to the somite irregularity. Turning to the extent of the irregularity, anterior cuts resulted in one, or at most two, abnormal somites, whereas posterior cuts resulted in an irregularity stretching over three to six somites. In the latter cases the position of the irregularity was taken to be its middle point.

The results are presented in the three columns on the left side of Table 3. The right side of the table gives values of a Packing Index, calculated from the data as explained in the legend. The index quantitates the crowding of the prospective somites along the axis.

The data suggest how the bud can be conceptually divided into three zones: a posterior stack, an anterior zone of presumptive somites about to segment, and a zone of extension between the two (Fig. 19). The packing index calculated for the posterior third of the bud is high, about 55, and some twenty presumptive posterior somites are packed into this region. In contrast, the packing index for the anterior third is low, 7, the same indeed as that calculated for the segmented
Table 3. Location and packing of prospective somites in the bud

<table>
<thead>
<tr>
<th>Location of mark: distance in mm from tip</th>
<th>No. of embryos</th>
<th>Position of mark along somite file at advanced stage: nth somite</th>
<th>Packing index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>1·15</td>
<td>5</td>
<td>12·00</td>
<td>11–13</td>
</tr>
<tr>
<td>0·70</td>
<td>8</td>
<td>15·00</td>
<td>13–16</td>
</tr>
<tr>
<td>0·60</td>
<td>9</td>
<td>17·30</td>
<td>16–19</td>
</tr>
<tr>
<td>0·45</td>
<td>4</td>
<td>21·30</td>
<td>20–23</td>
</tr>
<tr>
<td>0·30</td>
<td>4</td>
<td>29·80</td>
<td>28–32</td>
</tr>
</tbody>
</table>

A single mark was made on each embryo by a single vertical cut across two thirds of the bud. The wound was rapidly effaced. Embryos were reared on and fixed and stripped at the 35-somite stage. Each mark resulted in a single, localized irregularity along the file centred on a particular somite (the nth somite). By making marks at different distances from the tip and determining the subsequent location of the mark in terms of the nth somite, one discovers the position of the nth somite on the 13-somite-stage bud.

Marks were made at the five locations given in Column 1. The number of embryos receiving marks at each of these locations is given in Column 2. Column 3 gives the means of the nth somites, and Column 4 the range for each class of mark.

These results have been used to calculate a packing index expressing the density of prospective somites per unit length of bud.

**Worked example.** A mark made at 1·15 mm from the tip of the tail (13-somite stage), results in an irregularity centred on the 12th somite of the 35-somite embryo. The adjacent mark at 0·70 mm results in an irregularity centred on the 15th somite. Thus (15–12) = 3 prospective somites are located between these two marks situated (1·15–0·70) mm = 0·45 mm apart. Index = 3/0·45 = 7. This value appears as the second entry in Column 5 set between the lines of the other columns. The first entry of Column 5, also of value 7, refers to the Index of newly segmented somites. It is calculated from the data in Table 1 as follows: reference to Column 2 of Table 1 shows that the five-somite block nos. 7 to 11 measures 0·70 mm. Index = 5/0·70 = 7. The final entry, 55, in Column 5 of Table 3 is the Index of the remaining tissue posterior to the mark at 0·30 mm, and is calculated as follows. On the basis of completed files of 41 pairs of somites, this tissue contains 11 prospective somites. The distance between the mark and the tail tip, 0·3 mm, included the thickness of the skin covering, about 0·1 mm. Index = 11/(0·3–0·1) = 55.
Fig. 19. The order and approximate dimensions of the zones proposed in the text are drawn on an outline of the tail bud of an embryo at the 13-somite stage. To the left the last three somites are outlined and labelled. Adjacent to the last somite, the prepatterned zone occupies the anterior third of the bud. The figure (3) indicates that three prospective somites occupy this region; these somites are in the process of segmentation, but their boundaries are not yet visible. The figure 7 above indicates the Packing Index. Posterior to the prepatterned zone follows the zone of extension. This zone contains at least six prospective somites. This is the zone where prospective somites undergo an eight-fold extension. In the posterior third of the bud c.20 pairs of somites are densely packed (Index = c.57) prior to their extension. This static, waiting zone is called the packing zone or somite stack.

DISCUSSION

These results portray the frog tail bud as an independent, highly determined system. We have identified four zones in the paraxial mesoderm: (1) the segmented zone, (2) the anterior, unsegmented zone, (3) the zone of extension, and (4) the posterior, ventral stack of presumptive somites. Attention is focused in this discussion on the dynamic relations between these zones and the implications of this organization for the mechanisms of somitogenesis.
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The boundaries between the zones

i. Between the visibly segmented region and the anterior, unsegmented mesoderm

Unpublished time-lapse observations made with Dr Bard indicate that segmentation is not an abrupt, once and for all process. The segmental boundaries are initially transient and changeable in their appearance as if the definitive boundary were the culmination of repeated efforts. The somite boundaries in the frog are slow to stabilize, even after the visible pattern has emerged (Elsdale & Pearson, 1979). The chick segmental plate, homologous with the anterior tail bud of the frog, already shows some spatial organization, and the future somites are prefigured by poorly defined bodies termed ‘somitomeres’ (Meier, 1979). Somitomeres have not been invoked in Amphibia: nevertheless, the staging of segmentation on the basis of SEM observations tends to be earlier than previously recognized. Where you draw the line between segmented and unsegmented tissue is largely a matter of how carefully you look, the techniques employed, and what you consider to be a well-formed somite. The distinction between the zones is one of appearance only.

ii. Between the anterior unsegmented mesoderm and the zone of extension

Temperature-shock experiments have revealed a cellular change occurring prior to segmentation (Elsdale, Pearson & Whitehead, 1976; Pearson & Elsdale, 1979). This change is precisely located in time and is abrupt. The basic observation is as follows. After a temperature shock is applied to an embryo undergoing somitogenesis, development continues for a time at the normal rate during which three to four normal somites are segmented. The length of this period and the number of normal somites segmented is invariant and does not depend on the severity of the shock. Following this period of normal development, the embryo suffers an arrest, the length of which depends on the severity of the shock. Development resumes at the normal rate, but the somite pattern is at first grossly disturbed. The disturbance to the pattern is greatest at the beginning of the zone of abnormal segmentation where there is a sharp transition from normal to abnormal tissue: indeed, the transition often occurs within a somite. The result indicates that a moving wave of sudden change passes down the axis precisely three to four prospective somites tailwards of the last well-formed somite. The quantitation is inherently accurate by virtue of the sharp transition just mentioned.

The excision experiment described in the present paper reveals an eight-fold extension of prospective somites in the course of their maturation, an extension which is complete in the three to four prospective somites immediately posterior to the last well-formed somite. This quantitation is accurate by virtue of the fact that where the packing index is lowest a small inaccuracy in the measured position of the cut will have little impact on the result.
We see here the convergence of two independent lines of enquiry. The anterior boundary of the zone of extension coincides with the wavefront of sudden change revealed by temperature shock. We conclude that an underlying critical transition in the state of the cells defines the invisible boundary between the two zones.

iii. Between the zone of extension and the stack

We have no independent characterization of this boundary. However, given that the zone is fairly narrow, and that within the zone the packing index is drastically reduced, it is difficult to see how extension could occur in the absence of an orderly recruitment in space and time of cells from the stack. We should expect, therefore, the boundary to be well defined.

The dynamics of tail development

In previous publications in this series, reasons have been given for the equation of the wavefront of sudden change revealed by temperature shock with the establishment of a somite pre-pattern. We envisage an invisible wave of determination sweeping across the paraxial mesoderm initiating changes taking 7 to 8 h (in the frog, at 15°C) to become apparent (Elsdale et al. 1976; Pearson & Elsdale, 1979). We proved that the wave of determination is not stopped by a cut across its path. Such a ‘kinematic’ wave (Zeeman, 1974) does not depend upon the propagation of a signal across the tissue, but results from the fact that the cells are timers laid out in the order in which they are preset to change – anterior cells are everywhere in advance of posterior cells. Viewed in time, the zones of the bud are sequential phases in the maturation of the tissue. In the light of the present results we can look back in time before the establishment of the prepattern to earlier stages in the maturation of the paraxial mesoderm. Our results indicate that immediately prior to the sudden change, and during a period stretching over many hours, the tissue undergoes an eight-fold extension. We cannot say with any precision how long this period lasts because the posterior bound of the zone of extension is not accurately determined by the marking experiment. The period of extension may be as short as 14 h (frog, 15°C), corresponding to six somites within the zone. The period could, however, be longer if the process of extension were non-linear and tissue initially extended only slowly.

The dynamic aspect of zonation, the fact that each prospective somite will be located within each zone in turn, can be expressed in either of two ways carrying mutually exclusive implications. One way is to say that the prospective somites pass through and out of each zone in turn as they progress to mature somites. This formulation would be appropriate where zones could be defined independently of the passing tissue, as for example, in the case of the progress zone under the influence of the apical ectodermal ridge in the chick limb bud (Summerbell, Lewis & Wolpert, 1973). Alternatively, one can say that the zones sweep across
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the tissue. This is the correct formulation where kinematicity has been proved. A non-technical example of a kinematic wave will make the point. Suppose chestnut trees flower earlier in the south; at a particular time of year there will be a zone of flowering across the country, with trees that have finished flowering in the south and trees yet to flower in the north. The zone of flowering sweeps the country from south to north as the season advances.

We have asked the question, does each zone reflect the separation in time between successive waves of change? The question focuses attention on the wavefronts of change, that is to say the boundaries between the zones. We show that the visible boundary between the segmented and non-segmented tissue is a boundary in appearance only and is without significance, whereas the invisible boundary between the anterior zone and the zone of extension does indeed mark a critical developmental transition.

What is the status of the material in the somite stack at the tail tip? We have shown that, following a deletion of all or part of this tissue, the system does not regulate and a deficiency results. No experiments were done to test the ability of small isolates of tip tissue to survive and develop. Thus the tip remains an unknown quantity. Our results have not ruled out the possibility that the tip may be a progress zone (Summerbell et al. 1973; Meinhardt, 1982).

We have pleasure in thanking Ms Allyson Ross for her expert help in preparing the histological sections.

REFERENCES


(Accepted 14 March 1983)