The formation of somites in *Xenopus*

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The cell movements and contacts in somite formation in *Xenopus* are unusual. The vertebrate somite as seen in section is traditionally described as a rosette and is formed by pinching off a group of cells from the paraxial mesoderm; these cells then increase intercellular contacts. Typical rosettes with cells radiating from the myocoel may be seen in the Axolotl (Fig. 1A). Not all the cells of a rosette have the same fate. The lateral ones become dermis and spread out under the epidermis forming a continuous sheet with their counterparts from neighbouring somites. The majority of the somitic cells, which are myotomal, will elongate antero-posteriorly and fuse end to end with other cells of the same somite (Przybylski & Blumberg, 1966; Riabova, 1966). In this way myotubes are formed which stretch from one end of the somite to the other and obliterate the myocoel. Somites are formed in an orderly anterior-posterior succession.

In a pre-hatching *Xenopus* larva of stage 28 (Nieuwkoop & Faber, 1956) it can be observed that the myotomal cells are already elongated antero-posteriorly and that each cell stretches the length of the entire somite. This is even true in the most newly formed somites as can be seen in Fig. 1B and Fig. 2C. The cells of the dermis do not seem to pass through a segmental phase and no somite contains a myocoel. It thus appears that somite formation in *Xenopus* does not follow the typical vertebrate pattern. A fuller description of this process in *Xenopus* now follows.

**DESCRIPTION**

(a) Normal somite development in *Xenopus*

Throughout this paper the coelom is defined as a cavity surrounded by mesoderm. This may be subdivided into the myocoel, which is surrounded by somitic mesoderm and assumes its definitive nature when the somites are segmented, and the splanchnocoel, which is the main unsegmented body cavity. The term 'premyocoel' represents the most dorsal part of the coelom which is present before segmentation. 'Height', 'length' and 'width' refer to those

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Notochord; Somites; Nuclei; Myotome; Pre-myocoel; Myocoel; Dermatome
dimensios which are measured respectively: dorso-ventrally, antero-posteriorly and laterally.

*Xenopus* embryos at stages ranging from 12½ to 32 were fixed and prepared for microscopical examination. In order to facilitate a three-dimensional reconstruction, sections were cut both transversely and longitudinally and stained with Ehrlich's haematoxylin.

By stage 12½ the paraxial and lateral mesoderm is already double except where the upper and lower layers meet in the mid line (Fig. 1 C). The space between these layers is the coelom. The cells of the notochord, as well as those of the paraxial mesoderm, are orientated at right angles to the long axis of the embryo. The neural folds are barely visible.

When the neural folds rise there are slight changes in the arrangement of somitic cells such that by stage 18 there is a much sharper distinction between cells that will form myotome and those of the dermatome. The former become more spindle-shaped and are still cut longitudinally in transverse sections of the embryo. This arrangement is also clear in the unsegmented hinder portion of stage-23 embryos (Fig. 1 D), where the premyotomal cells point radially from the premyocoel to the gut, notochord and neural tube.

Since somites are formed sequentially in an anterior-posterior direction it is possible to see the several stages involved in segmentation in one animal, or in different animals at the same stage. It has already been shown that prior to segmentation the premyotomal cells lie at right angles to the long axis of the embryo. However, within the plane of the long axes of the cells the individual orientations may vary, since the cells appear to radiate from the premyocoel. The only premyotomal cells which will be cut longitudinally in a longitudinal horizontal section of the embryo are those adjacent to the notochord; the others would be sectioned obliquely or even transversely. For this reason the movements involved in somite segmentation are most clearly seen in horizontal sections of the embryo which pass through the notochord. Nevertheless the change in orientation of all the somitic cells is similar. Figure 2 A demonstrates this reorientation very clearly in an embryo with twelve somites (stage 23). It is here clear that even in early stages the somites are one cell long and that all the somitic cells are extended in an antero-posterior direction. No myocoel is visible. The cells of the unsegmented paraxial mesoderm lie at right angles to the embryonic long axis and during segmentation they rotate through 90°, as can be seen in the last somite on the right-hand side of Fig. 2 A. During this movement the medial tip of the cells in the unsegmented mesoderm comes to lie at the anterior face of the

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Fig. 1. (A) Longitudinal section through somites of an Axolotl embryo, showing rosette arrangement of the cells. (B) Longitudinal section through an entire *Xenopus* embryo, stage 28; the section passes through the notochord and somites in the trunk. (C) Transverse section through a *Xenopus* embryo, stage 13; neural plate, notochord, paraxial mesoderm and premyocoel are visible. (D) Transverse section through a stage-23 *Xenopus* embryo behind the region of segmentation.
Fig. 2. (A) Longitudinal section of a stage-23 *Xenopus* embryo showing myotomal reorientation; the cells of the last somite on the right are not completely turned. (B) Transverse section through a somite of a *Xenopus* embryo at stage 23; the spindle-shaped cells of the myotome appear as small circles when cut at this angle; no myocoel is visible. (C) Enlargement of part of Fig. 1 (B); the turning of the myotomal cells in the plane of the notochord is clearly seen, while the dermatome remains unsegmented.
Somite formation in *Xenopus* somite and the coelomic tip moves posteriorly. When a block of mesodermal cells rotates in this way the premyocoel is automatically obliterated. Transverse sections of an embryo of the same age indicate the initial (Fig. 1D) and final (Fig. 2B) steps in reorientation. Transverse sections across the spindle-shaped somitic cells show them up as small circles arranged in a solid block with no myocoel at the centre (Fig. 2B). The dermatome does not become segmented but remains rather like a curtain draped over a stage while scene shifting takes place behind it. In a few sections it is possible to see the attachment of the dermatome to the posterior dorsal edge of the myotome.

![Diagram](image)

**Fig. 3.** Three-dimensional reconstruction of somite formation in *Xenopus*. The axial and paraxial structures are viewed from a ventro-lateral position on the left-hand side. Blocks of cells of the paraxial mesoderm are shown in the process of reorientation that accompanies somite formation.

It thus appears that segmentation in *Xenopus* embryos is very simple and involves only those parts of the somite which remain segmented when functional. The turning of premyotomal cells through 90° at once forms a somite and lines up the cells in their definitive positions. Figure 3 is a diagrammatic reconstruction of somite formation on the left side of the embryo. The myotomal mesoderm of the left side only is shown together with the notochord and neural tube. Anterior is to the left, and posterior to the right of the diagram.

At stage 28 (Fig. 2C) sections are clearer than at stage 23 since the cells are now less yolky. The reorientation of the cells to form a somite is the same and the cell length is the somite length.

(b) The regulation of somite formation

It is remarkable that segmentation proceeds in such an orderly fashion along the embryo, the same reorientation of mesoderm cells *en bloc* occurring as each somite is formed. This repetitious process is so regular that there is a very high
linear correlation between age and somite number ($r = 0.995$). The computation was based on data in Nieuwkoop & Faber (1956), which have been slightly simplified and are shown in Table 1. (The ultimate somite is delayed in its appearance and has not been included in the analysis.)

Table 1. Number of somites which have been formed by a certain age

(Nieuwkoop & Faber (1956): Normal table of *Xenopus laevis*, Daudin.)

<table>
<thead>
<tr>
<th>Age (h)</th>
<th>No. of somites</th>
</tr>
</thead>
<tbody>
<tr>
<td>...</td>
<td>19-7 20-7 21-7 22-5 24-0 24-7 26-5 27-5 29-5</td>
</tr>
<tr>
<td>...</td>
<td>3 5 6-5 8-5 9.5 12 15 16 17</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age (h)</th>
<th>No. of somites</th>
</tr>
</thead>
<tbody>
<tr>
<td>...</td>
<td>31-2 32-5 35-0 37-5 40-0 44.5 50-0 53-5 56-5</td>
</tr>
<tr>
<td>...</td>
<td>19 23 24-5 26-5 30 36 40 44 47</td>
</tr>
</tbody>
</table>

This computation, however, is not the important one. What is the embryo's 'counting' mechanism? An embryo might be able to count off its somites regularly if they were all the same size but they obviously are not. However there is evidence that the length of unsegmented mesoderm which is cut off might be the same in all somites. The width of a formed somite indicates the length of tissue before segmentation and rotation through 90°. Somite widths were therefore measured at the middle of each somite, which is located at the line of flexure lying lateral to the notochord. The number of myotomal cells in each somite was counted from horizontal sections passing through the notochord (as shown in Figs. 1B or 2A, C). Of the ninety-eight somites counted in ten embryos between stages 23 and 28, 20% were nine cells wide, 70% ten cells wide and 10% eleven cells wide. Mitoses are extremely rare in this tissue, and since there was no difference between anterior and posterior somites it is proposed that the embryo can count or measure ten cells worth of unsegmented mesoderm for reorientation.

(c) The effect of haploidy on somite development

Haploid embryos of *Xenopus* have been described briefly by Hamilton (1963). Their somites are formed in the same way as diploid somites but their dimensions are affected by volumetric considerations. Fankhauser (1945) pointed out that although haploid cells are half the volume of diploid cells, many haploid organs appear to compensate for reduced cell volume by an increase in the number of cells.

If one assumes that all the linear dimensions of haploid cells are reduced equally compared with diploid ones then in a haploid cell the linear dimensions should be $\sqrt[3]{0.5}$ times the corresponding diploid dimensions; that is, approximately 0.8 times. This difference is manifested in the length of haploid somites when compared with diploid somites since each somite is one cell long. The length of each post-otic somite was measured in two diploid embryos and three haploid embryos at stage 28. The average length for left and right of each pair
was plotted for each embryo (Fig. 4). Anterior somites are longer than posterior ones and at all levels haploid somites are about 0.8 times the length of diploid ones. However, the most posterior somites of haploid and diploid embryos were more nearly equal in length than was expected. These somites were in the process of turning and their length was not measured along the length of their constituent cells. Since the length of somites is the same as the length of the component cells, it might be possible for haploid somite cells to compensate by being the same length as diploid somite cells but much thinner. However, it appears that haploid somitic cells do not show such compensation.

Fig. 4. The lengths of individual somites are plotted against their position in the embryo. Anterior somites to the left, posterior to the right. A comparison is made between the lengths found in diploid embryos stage 27/28 and haploid embryos stage 27. •, Diploid; ○, Haploid.

Table 2. Distribution of somites according to the number of cells visible in them in a horizontal section through the notochord

<table>
<thead>
<tr>
<th>No. of somites</th>
<th>Mean cell count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diploid</td>
<td>8.65 ± 0.7</td>
</tr>
<tr>
<td>Haploid</td>
<td>11.3 ± 0.8</td>
</tr>
</tbody>
</table>

A further investigation was carried out to compare the widths of haploid and diploid somites in terms of cell numbers. Six haploid and four diploid embryos at stage 32 were sectioned horizontally at 8 μ and 10 μ respectively.
The distribution of cell numbers per somite is laid out in Table 2. Haploid somites contain more cells across their width than diploid somites do (11.3 ± 0.8 and 8.65 ± 0.7 respectively). However, when one takes into account the reduction by 0.8 times of the linear dimensions they have a mean width corresponding to 9.04 ± 0.75 diploid cells.

It thus appears that in width there has been compensation for smaller cell size in the haploid embryos, and it is concluded that in the embryo the somites are measured before reorientation, not in number of individual cells, but in the thickness of tissue into which a certain number of cells may fit.

COMPARISON OF SOMITE FORMATION IN XENOPUS AND IN OTHER VERTEBRATES

The initial stages in the development of mesodermal structures in Xenopus and other vertebrates are similar. The notochord is defined very soon after invagination, and is flanked by an unsegmented double layer of mesoderm. Balfour (1885) describes the process as seen in the Elasmobranchs as follows. 'The mesoblastic plates subsequently become divided for their whole length into two layers, between which a cavity is developed. The dorsal parts of the plates become divided by transverse partitions into somites, and these somites with their contained cavities are next separated from the more ventral parts of the plates. In the somites the cavities become eventually obliterated, and from their inner sides plates of tissue for the vertebral bodies are separated; while the outer parts, consisting of two sheets, containing the remains of the original cavity, form the muscle plates.' After a brief summary of early mesodermal development in the teleosts, amphibia, reptiles, birds and mammals, Balfour concludes: 'What was stated for the Elasmobranchii with reference to the general fate of the mesoblast holds approximately for all the other forms' (author's italics). However, in Xenopus, as soon as segmentation begins, differences from other vertebrates become apparent. These differences are represented diagrammatically in Fig. 5. In each diagram the mesodermal structures are shaded to indicate the shape and orientation of cells in the different areas. Each diagram is a composite of a Xenopus embryo on the left and another vertebrate on the right.

In both types of embryo before the neural folds appear it is very difficult to say just where the boundary between somitic and lateral plate mesoderm lies (Fig. 5A). During and immediately after neurulation the presomitic cells become spindle-shaped and almost entirely surround a cavity (Fig. 5B). This cavity is the forerunner of the myocoel in other vertebrates but, as we have seen, is obliterated in Xenopus when a somite is formed. In the others, a somite is formed by the cells pinching the myocoel off from the more lateral splanchnocoel (Fig. 5C, D).

During the subsequent differentiation of the somite of other vertebrates the myotome cells alter their orientation, becoming elongated antero-posteriorly.
As the cells elongate further they interdigitate with their partners across the myocoel, then, when they fuse, myotubes can be formed which stretch from one end of the somite to the other (Przybylski & Blumberg, 1966; Riabova, 1966). The myocoel thus finally disappears.

Fig. 5. A comparison of somite formation in *Xenopus* and other vertebrates. In all cases the pictures are a composite of *Xenopus* on the left and another vertebrate on the right. (A) Transverse section of late gastrula/early neurula. (B) Transverse section of early tail-bud stage, posterior to segmentation. (C) Transverse section through somites of tail-bud stage. (D) Longitudinal section through segmented and unsegmented regions of an embryo.

In *Xenopus*, on the other hand, muscle bands develop and traverse the entire length of the somite within the somitic cells as they were originally laid down. In other vertebrates the dermatome is an important constituent of the somites. These mesoderm cells, which initially are part of the outer sheet of unsegmented mesoderm, become segmented with the rest of the somite but lose their segmentation when they spread out under the epidermis and become the
dermis. *Xenopus* dermatome does not become fully segmented but remains a more or less intact sheet lying under the dorsal epidermis. Further migration is necessary.

There are of course many similarities in the formation of somites between *Xenopus* and other vertebrates. Somites are laid down one behind the other and fairly regularly. Romanoff (1960) gives some previously unpublished data on the rate of somite formation at different temperatures. His graphs show a uniform rate of formation for the first thirty pairs of somites provided that the temperature is between 37.5 and 41.5 °C. The last ten somites appear to arise more slowly. Perhaps chicks and other rosette-formers also have a way of counting blocks of cells from paraxial mesoderm which might explain the regularity which is observed.

### CONCLUSION

The presumptive somitic material of the early neurula and the fully differentiated axial musculature and dermis of *Xenopus* appear to be very similar to those of other vertebrates. Yet the intervening stages of morphogenesis are strikingly different. These differences manifest themselves in greater simplicity and economy of cell movements in *Xenopus*.

The finding that *Xenopus* somites have constant dimensions in two planes (length and width at the notochord) makes it easier to evaluate the attempts at alteration of somite size of Waddington & Deuchar (1953), in which extra presomitic mesoderm was added dorsally to *Triturus alpestris* neurulae. They found that the width and height were much more affected than the length. Although the role of the notochord and neural tube (Kitchin, 1949; Muchmore, 1951; Spratt, 1957; Fraser, 1960) and of transmissible substances (Deuchar & Burgess, 1967) in the regulation of somite formation in vertebrates is still obscure, it is hoped that by using *Xenopus* it will be possible to quantify experimental results and arrive at a greater understanding of the processes underlying somite formation.

### SUMMARY

1. The processes leading up to and including segmentation of the paraxial mesoderm into somites are described, starting from stage 12½.

2. The cells of the paraxial mesoderm lie at right angles to the long axis of the embryo before a somite is formed; afterwards they lie parallel to the long axis. Segmentation is accomplished by rotation of a block of cells through 90° about a vertical axis. Each somite is one cell long and about ten cells wide.

3. The myocoel is obliterated during reorientation and the dermatome does not become segmented.

4. Haploid embryos cannot compensate for reduced cell length since somites are constitutionally one cell long. They do compensate for small cell width by an increase in the number of cells across a somite.
5. It is suggested that the regular addition of somites during embryogenesis of vertebrates is in some way a reflection of a basic counting mechanism.

Résumé

La formation des somites chez Xenopus

1. On décrit les processus conduisant à la segmentation du mésoderme paraxial en somites, et la segmentation elle-même, à partir du stade 12½.

2. Les cellules du mésoderme paraxial sont situées à angle droit par rapport à l'axe longitudinal de l'embryon, avant qu'un somite ne se forme; ensuite, elles se disposent parallèlement à cet axe; la segmentation est réalisée par la rotation d'un bloc de cellules, sur 90°, autour d'un axe vertical. Chaque somite possède la longueur d'une cellule, et la largeur d'environ dix cellules.

3. Le myocoèle est oblitéré pendant la réorientation et le dermatome ne se segmente pas.

4. Les embryons haploïdes ne peuvent pas compenser la réduction de longueur des cellules, étant donné que les somites ont la longueur d'une seule cellule. Ils régulent la largeur des somites par accroissement du nombre de cellules dans l'épaisseur d'un somite.

5. On suggère que l'adjonction régulière de somites au cours de l'embryogenèse des Vertébrés reflète en quelque manière un mécanisme de numération de base.

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Références


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