Control of somite number in normal and \textit{amputated} mutant mouse embryos: an experimental and a theoretical analysis

By O. P. FLINT,\textsuperscript{1} D. A. EDE,\textsuperscript{1} O. K. WILBY\textsuperscript{1} AND J. PROCTOR\textsuperscript{2}

From the Departments of Zoology and Mathematics, University of Glasgow

**SUMMARY**

A regulation is shown for size and number of serially repeated axial structures, the somites, in a mammalian embryo. The mammalian embryo is normally inaccessible to operation at post-implantation stages. This problem is resolved by the quantitative analysis of somite size, number and development in a recessive mutant of the mouse, \textit{amputated}, whose axial length is greatly reduced. The effect of the gene simulates an experiment ablating part of the embryonic tissue available for somitic segmentation. Regulation occurs at the time when the somite is first formed, by control of the quantity of cells included in each new somite. A model is devised for the control of somitic segmentation which explains most of the features observed and which can be simulated on a computer.

**INTRODUCTION**

Among the problems of morphogenesis, the generation of serially repeated structures is an especially challenging one and the most striking examples of such structures in vertebrates are the somites - the blocks of mesodermal cells which arise in sequence along each side of the embryonic axis from the posterior boundary of the head (otic capsule) to the tail. Hamilton (1969) studied their development in the toad \textit{Xenopus} and showed, using haploid embryos in which cell size is reduced, that the quantity of material included in each somite is a measured length of presomitic mesoderm rather than a specified number of cells. Further studies by Cooke (1975) showed that \textit{Xenopus} which have been reduced in size by removal of some vegetal material at the blastula stage show a capacity for regulation of somite number; that is they produce the normal number of somites from a greatly reduced number of cells.

This raises the question of whether the capacity for such regulation exists also in the embryogenesis of higher vertebrates or whether, like the capacity for

\textsuperscript{1} Authors' address: Department of Zoology, University of Glasgow, Developmental Biology Building, 124 Observatory Road, Glasgow G12 9LU, U.K.

\textsuperscript{2} Author's address: Department of Mathematics, University of Glasgow, Glasgow G12 9QQ, U.K.
limb regeneration in post-embryonic stages, it is limited to certain amphibians. Studies we are making on a new mutant gene in the mouse have given us the opportunity of investigating this aspect of somitogenesis in the mouse and also analysing the relation of somitogenesis to some other embryonic processes. The mutant, amputated, is a single recessive gene (Meredith, 1965; Flint, 1976), one of whose effects is to reduce the embryonic length thus simulating the experimental lesions of Cooke (1975). In interpreting our results we have had to take into account one basic difference between the amphibians and higher vertebrates; in amphibians somitogenesis does not begin until all the presumptive mesoderm has been invaginated at the blastopore lip, but in higher vertebrates it begins anteriorly while presumptive mesoderm is still being invaginated at the primitive streak, and more somites are initiated in sequence as the node at the anterior end of the streak moves backwards. In amphibians, therefore, the embryonic axis is present as a measurable length of mesoderm before the beginning of somitogenesis and regulation of somite size may occur by direct relation to it as Cooke (1975) suggests, but if regulation does occur in higher vertebrates it must be governed by some different relationship since in them the length of axial mesoderm is being continuously extended as somite initiation takes place.

METHODS

The mice used were from a CBA/101 hybrid control stock and a mutant stock derived from this by introducing into it the recessive lethal gene amputated (Flint, 1976). To obtain mutant embryos, known heterozygote males were mated to possible female heterozygotes. Embryos were dissected out into Tyrode solution. Measurements were made while they were still living, following the scheme shown in Fig. 1. Length of each embryo was measured along its axis (i.e. neural tube) from the most anterior surface of the mesencephalon (arrowed) to the posterior neuropore. The antero-posterior length of each somite was also measured midway between the dorsal and the ventral surface. Length of presomitic paraxial mesoderm was also measured. The number of embryos used to measure each variable can be seen in Table 1.

Statistical analyses of the results consisted of regression analysis, analysis of variance and covariance. These are routine techniques and descriptions can be found in Snedecor & Cochran (1967) and Sokal & Rohlf (1969). We have accepted probabilities (P) of 0·05 and less as an indication of significance.

RESULTS

A description of the embryos. Mutant homozygotes are shorter than their wild-type littermates (Fig. 1, 4, Table 3). This includes all stages we have measured up to 26 somites when, in the normal embryo the tail begins visible outgrowth (Grüneberg, 1956). The tail fails to grow in the mutant but at later
Table 1. The numbers of embryos used for the measurements described in the text. Except where indicated these are separate groups of embryos.

<table>
<thead>
<tr>
<th>Measurements</th>
<th>8.5 days (+/+)</th>
<th>9.5 days (+/+, am/am)</th>
<th>9.5 days (+/+, am/am)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axis length</td>
<td>16</td>
<td>—</td>
<td>32</td>
<td>36</td>
</tr>
<tr>
<td>Most recently formed somite</td>
<td>16</td>
<td>—</td>
<td>32</td>
<td>36</td>
</tr>
<tr>
<td>Ant/post lengths of somites 5, 7, 9, 11</td>
<td>—</td>
<td>—</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>Length of each somite in 18-somite embryos</td>
<td>—</td>
<td>—</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Total cell number in most recently formed somite (from serial sections)</td>
<td>—</td>
<td>—</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Somite number</td>
<td>—</td>
<td>80</td>
<td>79</td>
<td>30</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>377</td>
</tr>
</tbody>
</table>

Comparison of somite size in mutant and normal embryos. At all levels of the body axis mutant somites are smaller than those in normal embryos (Fig. 2). This is confirmed by analysis of variance (P < 0.001). At some levels of the body axis mutant somites are closer in size to normal somites than elsewhere. But even where mutant and normal somites are closest in size, at the level of somites 3, 4 and 5, the difference is statistically significant (P < 0.001). There is a significant overall change in somite size with level in the body axis (normal and amputated, P < 0.001). Anterior somites are larger than posterior somites.

There is no difference between the sizes of somites in normal or in mutant embryos at the time of segmentation, whether they are destined to be anterior or posterior. Direct measurement of living embryos shows that over all the stages measured there is no change in the size of the most recently formed somite (Table 3, Fig. 4). But at all times this somite is 50% smaller in amputated than in normal embryos. The cell number included in this most recent somite is 13-2.
correspondingly reduced in the mutant (amputated, $134 \pm 22.96$; normal, $277 \pm 6.81$ cells; $P < 0.001$).

Since anterior somites are larger than posterior somites it follows that somites grow. This can be seen in Fig. 3 where the change in size of somites 5, 7, 9 and 11 is plotted against developmental age (somite number) in mutant and normal. Each particular mutant somite remains consistently smaller than its normal counterpart through all the stages we have measured. This is confirmed by statistical analysis (Table 2).

How therefore do somites grow? Is it by growth of the cells themselves as appears to be the case in *Xenopus* (Hamilton, 1969) or is it by an increase in cell number? An analysis of cell density in the somite dermatome of every somite in the axis of a pair of amputated and normal littermates shows that somites grow mainly by increase in cell number. Cell density does not change from somite to somite (17-somite normal embryo, average dermatome cell density: $11.32 \pm 2.99$ cells per $10^6 \mu m^2$ and no significant difference between somites, $P > 0.75$; 13-somite amputated littermate, c.d.: $12.66 \pm 3.19$ cells per $10^6 \mu m^2$ and $P > 0.75$; 7–8 sections counted per somite). But, as has already been shown, anterior somites are much larger than posterior somites (Fig. 2). Clearly, only if the cell density were to decrease with increasing somite size could we say that cell growth played a major role in the growth of the dermatome.

**Regulation of somite size and number.** We have counted somite number in 189 embryos at 9.5 days of development. There is no difference between amputated and normal littermates (amputated $= 19.04 \pm 4.26$; normal $= 18.90 \pm 3.51$, $P > 0.50$). Nor is there any difference between these normal litter mates (+/+, +/am) and wild-type embryos (+/+) from the control stock (+/+ $= 18.31 \pm 4.68$, $P > 0.05$) thus excluding any heterozygous effect. In spite of a reduction in axial length in the mutant, it produces the same number of somites as in the normal embryo. A regulation for somite size and number has taken place. We can deduce from the evidence already presented how and at what stage this regulation is effected.

From the earliest stage of their formation there is a difference of size between mutant and normal somites. This difference is maintained, without deviation, through all the stages we have observed (Fig. 3, Table 2). Regulation for size must therefore have occurred at the time of somite formation. It is clear that the regulation is effected by controlling the number of cells included in each somite because normal somites at the time of their formation are about twice the size of amputated somites (Fig. 4) and (see above) contain a proportionately greater number of cells.

**Possible mechanisms for the regulation of somite size.** The amputated embryo reaches the same developmental stage (i.e. somite number) as the normal embryo at approximately the same time after fertilization. Development in the amputated embryo therefore keeps pace with the normal embryo; organs emerge
Fig. 1. Normal (a) and amputated (b) embryo littermates, unfixed at 9.5 days of gestation. PM and S indicate, respectively, the lengths we measured when estimating the size of the presomitic paraxial mesoderm and the somites.
at the same rate. But the *amputated* embryo is smaller than the normal embryo and in order that the same proportion of the shorter embryonic axis should emerge at the same time the rate of axis formation in the mutant must be retarded. Streak regression must therefore be retarded. We have both *in vivo* and *in vitro* evidence to show that *amputated* cells are less motile than normal cells (Flint, 1976; Flint & Ede, 1978). We believe that it is because of this reduced motility that streak regression, a morphogenetic movement involving individual cells (Nicolet, 1965; Spratt, 1957), is retarded in the mutant.

Somite size may regulate for embryo size because the genetic programme in each cell may precisely define every future parameter of the embryo: axis length, somite number, somite size, available presomitic paraxial mesoderm length, etc. The grounds we have for rejecting such an hypothesis of completely
Table 2. Equations describing the growth of somites 5, 7, 9 and 11 in normal and amputated embryos. The graphs of these equations are drawn in Fig. 3

The statistical analysis asks whether there is a significant increase in somite length (Y) with somite number (X) and whether there are significant deviations of the points from a linear regression (would a curvilinear regression make a better fit?). In addition we have by analysis of covariance compared normal and amputated regressions for each pair of somites to see whether there is any difference in somite growth rate (slope) or somite size at any stage (elevation). There is nowhere any difference in growth rate but amputated somites are consistently smaller than normal somites.

Table 3. Regression equations calculated for the graphs shown in Fig. 4

A similar statistical analysis to that described under Table 2 is carried out. In this case, Y is the variable parameter described in the column headed Y, and X is always somite number (developmental stage).
mosaic development are those of the complexity of genetic programme required and that such mosaic development is not characteristic of amniote development. Given that this is the case, somite size is most probably regulated at the time of somite formation by control mechanisms acting on the presomitic paraxial mesenchyme, and these mechanisms are either directly or indirectly related to the size of the embryo.

While axis length increases throughout the somite stages we have examined, the size of the most recently formed somite remains constant (Fig. 4, Table 3). Therefore there can be no direct relationship between axis length and somite size. The quantity of available paraxial mesenchyme on the other hand exactly parallels most recently formed somite size in that it too remains constant throughout the somite stages we have observed (Fig. 4, Table 3), and this suggests a more direct relationship. There is also a comparable difference in both the size of mutant and normal most recently formed somites, and available paraxial mesoderm lengths (somite length: normal = 133.82 ± 32.57 μm, amputated = 74.32 ± 15.55 μm; parax. mesoderm length: normal = 1112.93 ± 122.33 μm, amputated = 550.69 ± 86.56 μm). The ratio of normal to amputated in both cases is approximately 2:1.

If these correlations are not entirely coincidental it is also interesting that the length of presomitic paraxial mesenchyme in mutant and normal is of the same
order as that proposed by Crick (1970) for a morphogenetic field whose properties might be determined by a simple diffusion gradient. The average length of the whole embryo at 9.5 days, however, would not permit such a mechanism (normal = 6.14 ± 0.78 mm, amputated = 4.15 ± 0.92 mm).

A model for the control of somite size through node regression. The argument: It is possible to explain the generation of many types of developmental pattern,
Fig. 5. Results of a computer simulation based on the model discussed in the text, indicating the effect of reduced node regression rate on somite size. Graph (a) is the simulation for the normal embryo. Three stages (a, b and c) in the development of the standing morphogen wave are shown. Directly underneath P is a representation of the graph as a linear strip of cells. The black dotted cells are those engaged in the continuous destruction of morphogen (the troughs) while in between cells are actively synthesizing (stable peaks). Two clear bands are indicated where the next trough will appear. Beneath P, I shows how we think this pattern of morphogen synthesis affects the appearance of somites. There is a delay between the formation of the trough and the full formation of the fissure. Therefore though there is a set of zones within the paraxial mesoderm corresponding to the troughs (B, C and D), there will be a fairly long strip of unsegmented paraxial mesoderm (PM) between the node and the first fully-formed fissure (A). We have observed in time-lapse films zones of increased cellular activity within the PM corresponding to the troughs and to the later formed fissures. S is the most recently formed somite. The effect of reducing node regression rate in the computer simulation on the form of the standing wave and consequently somite size is shown in (b).

at least in principle, by appeal to the concept of 'positional information' (Wolpert, 1969), but it is much less plausible to do so in the case of any sort of periodic pattern and Wilby & Ede (1975, 1976) have proposed a wave-form gradient model to account for the development of such patterns in the cartilage skeleton of the limb-bud. Here there is no regulation, but we show that this model may be extended to situations where it does occur, and in particular, that it is capable of generating the patterns of somitogenesis we have described above, including the modifications which occur in the mutant mouse.

Our work suggests the involvement of the unsegmented paraxial presomitic mesenchyme, specifically its length, in the control of somite size at segmentation. The model we propose and which we have simulated successfully on the computer (see Fig. 5) assumes firstly that two bands of paraxial mesoderm are created on either side of the notochord by the passage of the regressing node through the primary mesenchyme (Bellairs, 1963; Nicolet, 1970; Lipton & Jacobson, 1974a, b), and secondly that as each new group of cells is recruited
Analysis of somitogenesis in the mouse

into the presomitic mesenchyme band the cells start synthesis of a morphogen. The morphogen is created faster than it can diffuse away. The first recruited anterior cells will therefore initially accumulate most morphogen. At a certain threshold level irreversible destruction of the morphogen is initiated and the resultant local instability creates a trough of morphogen with a peak immediately behind it just below the threshold level. The peak is stable because diffusion into the morphogen destruction region never allows the adjacent peak cells to reach threshold level. However, further away from the first trough of morphogen destruction morphogen levels can rise in the paraxial mesoderm to threshold level and accordingly a series of troughs and peaks are created in the unsegmented mesenchyme in front of the node. Each trough of morphogen at a later stage initiates fissure formation, defining the somites. The distance between peaks depends upon the rate of node regression so that the faster the node regresses the greater the distance between peaks, and the larger the somites. We have already argued that node regression in amputated must be retarded and our model therefore can explain the smaller somites.

The troughs of morphogen defining where fissures are about to appear in the mesenchyme give a certain mosaic character to the unsegmented paraxial mesenchyme which is confirmed by experiment. For example, when Lanot (1971) reversed strips of chick paraxial mesenchyme in situ he found that segmentation did not begin at the new anterior end but at the old anterior, i.e. the new posterior end. We have also seen in time-lapse films (unpublished results) of cultured chick material bands of increased cellular activity in the paraxial mesenchyme which correspond to the fissures which will appear later at the site of each band.

The computer simulation: a formal description. The basic model has four stages of cellular activity: (1) initiation of synthesis of a morphogen, producing a (2) rise in morphogen concentration to a critical threshold level, at which there is (3) an irreversible switch from synthesis to destruction of the morphogen, linked to cell determination and differentiation and leading to (4) a fall in morphogen concentration and the establishment of a sink. Since there is free diffusion between cells at all stages the sink developed in Stage 4 maintains the morphogen concentration in neighbouring cells below the threshold level and so allows a periodic pattern to develop. In the original limb cartilage model (Wilby & Ede, 1975) the pattern develops across a static tissue from an initiation region located at one edge and there is no capacity for regulation since all the relevant interactions are either within cells or between neighbouring cells so that there is no reasonable way in which these can be modified at long range. But with a simple change in the pattern initiation process, making it subject to an external control, a sufficient capacity for regulation is introduced. This external control is, as we have proposed, the passage of the node.

A computer simulation programme based upon this model, with the appropriate modifications for somitogenesis, has been run on the IBM 375-series
computer at the Edinburgh Computer Centre. Because of restrictions imposed by the complexity of the programme we have been forced to limit the simulation to a single string of cells. This considerably reduces the flexibility of the system, which nevertheless still illustrates the way in which somites are generated in this model. The string of cells, representing the presomatic mesoderm, is continuously lengthened by addition of cells at one end as the node regresses, and shortened by subtraction of cells at the other as they become incorporated in each new somite.

Changes in morphogen concentration with time (Δ[M]i) and the resultant patterns of cell differentiation are found by the stepwise application of the following simple algorithmic equations:

(i) For 1 < i < n

\[ Δ[M]_i = \sum_{i=n-1}^{i=2} \left( (M)_i \times (1.0 - 2d) + d \times (M)_{i-1} + f_i(S) \times f_i(D) \times DK \times t \right). \]

(ii) For the terminal cells i = 1 and i = n the diffusion terms are \( (M)_i \times (1.0 - d) + d \times (M)_{i-1} \) and \( (M)_n \times (1.0 - d) + d \times (M)_{n-1} \). t = Time step, \( (M)_i \) = morphogen concentration in the ith cell, \( d \) = rate of cell to cell diffusion, \( S \) = rate of morphogen synthesis, \( D \) = rate of morphogen destruction, \( DK \) = rate of spontaneous decay, \( f_i \) = state function.

For a cell in the ‘synthetic state’ where \( (M)_i \) has never exceeded the threshold \( T \), the value of \( f_i(S) = S \) and \( f_i(D) = 1.0 \). Where \( T \) has been reached, the cell is in the destructive state and \( f_i(S) = 0.0 \) and \( f_i(D) = D \). To achieve gradient stability and minimize error accumulation a small time step is used \(- t = 0.1 \).

Cells are added stepwise to the model system, one at a time; the node regression rate \( R \) is therefore the reciprocal of the elapsed time between additions. To date, 112 simulations have been run in which the following ranges of variables have been used: \( S = 0.01 \) to \( 0.05 \); \( D = 0.7 \) to \( 0.999 \); \( d = 0.1 \) to \( 0.33 \); \( DK = 0.996 \) to \( 1.0 \); \( R = 0.25 \) to \( 1.0 \).

Not all, but 36% of the simulations showed the direct dependence of somite length on node regression rate shown in Fig. 5a, b, indicating that the selection of appropriate parameters cannot be arbitrary. In 28% the relation between node regression rate and somite length was inverted and in the remaining 36% an unstable or irregular pattern was produced, ranging from the inclusion of simple errors, e.g. a single 1/8/2 in a 2/10/2 string, to complex forms such as 2/11/1/7/1/7/2 and 2/5/1/2/1/3/2/2, which represent imbalances between various components of the simulations. The imbalances may be paralleled in reality in various mouse mutants such as pudgy (Grünberg, 1961) and Crooked-tail (Matter, 1957) in which somites are of variable size and intersomitic fissures are irregular and ill-defined.

A serious restriction of the simulation, though not of the model, is that its one-dimensional quantized character limits fissure width to two alternatives (one cell and two cells) despite a range of somite lengths from 2 to 17 cells, so
that the regulative ability of the model is limited to two basic states—1-cell-wide fissure/short somite and 2-cell-wide fissure/larger somite. Consequently, if all variables except node regression rate are kept constant a reduction of somite size is not produced until node regression rate is reduced by half. Though this is, in fact, the magnitude of change in our mutant and operated embryos, the biological situation will obviously be more complex and if our computer simulation had been 3-dimensional, with the greatly increased cell interactions which that would give, regulation would be continuous as it appears to be in the real embryo.

**Control of somite number.** As to a mechanism for achieving the normal species-specific number of somites, for which some sort of global control must be postulated, we have presented no suggestions. The number does follow, given appropriate modification of the rate of node regression with varying axis length. A control of node regression rate is required; we believe this may involve a modification of cell behaviour, such as would certainly account for the reduced node regression rate observed in *amputated*. A possible global control does emerge from the clock and wave front model proposed by Cooke & Zeeman (1976) for somite formation in *Xenopus*. This model requires that axis length be laid down prior to somite formation, but in the mouse, as in the chick, the primitive streak is still regressing and the embryonic axis still forming while the first somites are being produced. A modification of the Cooke–Zeeman model may be conceived which would fit the experimental results reported here, but more general and experimentally unsupported assumptions are required of the castrophe theory type of model than for the one which we present.

The wave-form gradient can therefore successfully model somitogenesis in its essential aspects, notably the definition of somite boundaries and the development of intersomitic fissures, including the capacity for regulation in adjusting somite number to overall axial length. More data is required to put real values to the model variables, and it is, of course, one of the chief functions of such a model to stimulate the necessary investigations. Our observations on mouse embryos have shown that node regression plays a key role in controlling somite size and number, and our simulations have suggested how this may come about, but of course the problem of how node regression rate itself is first specified and then regulated remains; in this connexion a detailed analysis of node regression and factors affecting it is clearly called for.

We wish to thank the SRC and ARC for financial support.

**REFERENCES**


O. P. FLINT AND OTHERS


(Received 31 October 1977, revised 19 December 1977)