Xenopus mesoderm induction: evidence for early size control and partial autonomy for pattern development by onset of gastrulation

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Summary

Experiments are described that examine the state of organisation of the presumptive mesoderm and ectoderm of the Xenopus embryo at stages up to the onset of gastrulation. It is shown that a process during blastula stages, establishing the normal proportions in which this cell population is partitioned to found the two outer 'germ layers', has a positive regulative property. An operation has been performed to excise the yolky endodermal core, at the beginning of gastrulation, leaving only the presumptive territories of mesoderm, neural tissue, epidermis and supra-blastoporal endoderm. This reveals that by this time a stable capacity exists within the induced tissue to express the craniocaudal sequence of the normal pattern, including the proper numbers of somite segments. The mediolateral organisation of such body patterns is however abnormal. The relevance of the observations to understanding mechanisms of axial pattern control is discussed.

Key words: size-regulation, induction, gastrulation, segment number, pattern formation.

Introduction

In early Xenopus development, ooplasmic localisations cause the process of cleavage to generate two cell types. One, derived from the yolky vegetal region, develops in isolation as endoderm, while tissue from the remainder, in similar circumstances, forms a structurally abnormal but wholly epidermal tissue (Smith et al. 1985). The mesoderm, giving rise to skeletal, muscular, excretory and mesenchymal tissues as the middle one of three cell layers after gastrulation, originates in an equatorial portion of the ectodermal territory because of specific inductive signals initiated from the endodermal one. The system has been studied by co-culturing of the normal signalling and response-competent regions, and more recently by use of isolated soluble proteins which are candidates for identity or close relationship with the endogenous signals (Nieuwkoop, 1985; Smith et al. 1985; Gurdon et al. 1985; Warner & Gurdon, 1987; Slack et al. 1987; Kimelman & Kirschner, 1987; Smith et al. 1988; reviewed in Smith, 1989). Since molecular understanding of signal and response mechanisms may be at hand, it is important to complement this with knowledge of the 'system properties' of mesoderm formation, considered as an episode of pattern formation (Wolpert, 1971; Meinhardt, 1982; Cooke, 1985a) within the animal cap region.

Quantitative proportions between the territories of the larval mesodermal structure have been reported to be regulated against natural variation in egg size, and hence mesoderm size (Cooke & Webber, 1985a). In this paper, I initially present data supporting this, and also showing that a similar constancy of proportion is kept between cell populations of mesoderm and ectoderm as a whole, in naturally different-sized embryos. These constancies could all in principle occur because eggs come each equipped with appropriate sized sources of inducers. In an older terminology, eggs may rely upon a balanced set of animal (ectodermal) and vegetal (endodermal) development tendencies at the outset. Alternatively, since establishment of the mesodermal territory is ultimately the stabilisation of a frontier between induced and remaining tissue in the wall of the blastula, the process of response to induction may have actively regulatory properties. A test for such properties is to equip very early embryos with experimentally enlarged or reduced initial territories of ectodermally prespecified tissue in relation to their undisturbed (vegetal) sources of induction, and to compare these with normally constructed siblings as to the proportions of tissue ultimately allocated to mesodermal and to ectodermal structure. I report here that such an experiment indeed reveals regulation of the position of the mesoectodermal boundary in relation to total tissue size, although the severe operating procedures also happen to destabilise the mechanisms that then allocate relative territory sizes for components within the mesoderm pattern.

How stable is the information for the extent of mesodermal territory, and for future organisation
within it, that exists within the tissue of the animal cap by onset of gastrulation at around stage 10 (Nieuwkoop & Faber, 1967)? Several recent studies have re-emphasised the largely cell-autonomous nature of the pattern of activities that generates force for shape-change during gastrulation (Keller et al. 1985; Keller & Daničkik, 1988; Cooke & Smith, 1989), suggesting that the sequence of such change is largely preorganised by the time of its onset. The sequence of cell movement is also thought to correlate in general with the craniocaudal sequence of axial pattern (e.g. Keller, 1976) and has been proposed to be a first expression of the positional variable in preinvoluted mesoderm that sets up at least the dorsal axial pattern (Cooke, 1985a,b; Cooke & Smith, 1989). But the extent of that autonomy of pattern, by the initiation of gastrulation, has never been tested directly. I describe here the major features of bodies that follow complete removal of yolky endoderm, probably including all initial sources of induction, at stages close to the start of gastrulation. They reveal great autonomy for craniocaudal aspects of pattern including segment number, despite great alteration in the geometry of the mesoderm at gastrulation. This has relevance to models for control of segmentation. By contrast, the results reveal that the full sequence of lateral and ventral differentiation in normal mesoderm requires conditions in the normal entire embryo which are not fulfilled in endodermless ones. ‘Correct’ allocation of cells to dorsal axial vs. latero-ventral modes of mesodermal differentiation appears to require normal geometry during or after gastrulation.

Materials and methods

Operations were performed in $\text{NAM}^{-}$ strength NAM (Slack, 1984), pH adjusted to 7.0 with dilute HCl, on beds of 2% Noble Agar, using electrolytically sharpened tungsten needles and mounted loops of baby hair. For further details see Figs 1 and 3. After operations of both the types reported, ionic strength of the bathing solution was reduced to $\text{NAM}$ or less within one hour, and not returned to $\text{NAM}$ until the close of the gastrulation period.

Sectioning of embryos was always at stage 19 in the horizontal plane, except in the case of one endodermless set and its controls where the stage was 35 and the plane transverse. The complete set of 7 mm sections was taken, after double-wax embedding, and stained with Feulgen/Light Green/Orange G (Cooke, 1979). Estimates for the cell numbers in the clearly visible tissue layers and structures (see Figs 1D and 4A-J), and the proportions between them, were made by counting nuclei on regularly spaced subsets of sections and using appropriately derived correction factors in view of cell and nuclear size, section thickness and the proportion of sections sampled (Abercrombie, 1946; Cooke, 1979a, 1985a). Sampling density was a standard one in five sections for the larval (st. 35) form, and one in ten for the nearly spherical neurula form (one in five for the 3 grazing sections at ends of the series). Notochord, representing a relatively small cell number, was sampled more densely to reduce error, and the proportion of its contribution to total mesoderm was computed appropriately.

Additional larval bodies after endoderm removals, and their normal siblings, were fix-dissected under the binocular microscope, a few minutes after onset of fixation with 2% acetic acid in a solution of 1% $\text{K}_2\text{Cr}_2\text{O}_7$ in water (Cooke et al. 1987; Cooke & Smith, 1989).

Observations

Early size regulation under natural and experimental conditions

Table 1 shows the relative sizes of mesoderm and neuroectoderm (epidermis and derivatives plus rudiment of the central nervous system) in two stage-matched (st. 19) sets of neurulae with members of widely contrasting natural sizes. Set I was of synchronously obtained material from two different females characterised by the ovulation of unusually small and large eggs, respectively, while set II was of siblings collected from an unusual female ovulating eggs of two contrasting size-classes. All embryos had developed side by side, undisturbed at 22°C until fixation. It can be seen that both the overall allocation of ectodermally prespecified material to the mesoderm as defined by the end of gastrulation (Keller, 1976; Cooke, 1979b), and relative allocation to the notochord within the mesodermal pattern, are significantly controlled against overall size of the tissue field. Naturally smaller embryos may tend to have, relatively, somewhat large mesoderm and notochords. But this tendency if it exists is by no means as great as expected on the null hypothesis that the embryos of each set are a population in which whole mesodermes, and notochords, each tend towards one absolute size that is the average of those seen (see note to Table 1). Notochord extent is a sensitive index of the overall balance of pattern in Xenopus – in the sense of over-presentation, normality or under-representation of dorsal and anterior body structure – after various agencies that disturb this (Cooke, 1985a,b, 1986). External body form, which in turn correlates well with the relative extent of notochord internally, is also normally balanced in the naturally smallest and largest embryos seen. The probability thus is that positive natural regulation of the relative extents of whole germ layers and of mesoderm parts occurs in embryos of this type.

Fig. 1A shows how an extensive group of blastomeres can be defined, in the 128-cell blastula, as not yet having stably recorded any inductive signals even though many of its descendants will normally be recruited into the mesoderm (Cooke & Webber, 1985b; Dale & Slack, 1987a). The descendants of the two animal octets from a typical pattern of 32-cell stage, when dissected at this later stage and cultured alone in saline, form no mesoderm but only ciliated epidermal tissue. If more vegetal material is included in such explants, evidence of mesoderm formation tends to be seen in the result (Fig. 1B). Accordingly, smaller and larger groups of blastomeres from within the stated uninduced territory have been reciprocally exchanged between sibling embryos as shown in Fig. 1C. Control operations in each set of siblings were constructed by replacing a moderate sized group of the blastomeres with a similar group from a donor. The pigmentation pattern across the
animal hemisphere is normally an indicator of presumptive dorsal/ventral polarity. In view of the possibility that dorsal/ventral differences may exist even at these early stages across ectodermal material, graft and surrounding pigment patterns were matched up as well as possible.

All cells from the graft and its surroundings will be allocated to ectoderm or to mesoderm, except for any isolated loose blastomeres which could become incorporated into the endoderm in a way most unlikely to affect the events of pattern formation within the animal cap. Normal cell contact appearance and cellular relationships, as apparent in fix-dissections (see Materials and Methods), are rapidly restored between newly joined regions within blastular animal caps. The vegetal zone of origin of inductive signals is undisturbed mechanically by the operations. The tissue proportions in which they were induced. Some feedback mechanism, operating to adjust or limit the proportional allocation which that partitioning had occurred. A highly significant trend is seen whereby mesoderm sizes are positively correlated with those of the fields of tissue in which they were induced. Some feedback mechanism, operating to adjust or limit the proportional allocation to mesoderm, is a dominant component in setting its size.

The control data points, clustered at a middle position on total size as would be expected, show the inherent variability in ectomesodermal partitioning in embryos after the operating procedures. This is little if at all greater than that in unoperated controls from the laboratory collection, as exemplified in Table 1, so we assume the operation per se to have little effect upon this particular aspect of the control of induction. Experimental data points span a twofold range of total mesoectodermal sizes, a variation greatly exceeding

For each allocation, within each embryo set, the null hypothesis was tested (chi²) that the smaller component (notochord, mesoderm) varies in absolute size around a mean that is the average of the sizes recorded, and independent of the size of the larger unit (whole mesoderm, total meso+ectoderm) of which it is part. Probability that the percentage allocations actually recorded could then occur is in each set less than 0·01.

Table 1. Partitioning between ectoderm and mesoderm, and allocation of mesoderm to notochord, in naturally large and small embryos at stage 19

<table>
<thead>
<tr>
<th>Set</th>
<th>S 1</th>
<th>S 2</th>
<th>S 3</th>
<th>S 4</th>
<th>S 5</th>
<th>L 1</th>
<th>L 2</th>
<th>L 3</th>
<th>L 4</th>
<th>L 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total mesoderm and ectodermal cell population x 10³</td>
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<td></td>
<td></td>
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<tr>
<td>Set I</td>
<td>22-4</td>
<td>21-8</td>
<td>25-1</td>
<td>23-4</td>
<td>22-7</td>
<td>54-6</td>
<td>52-7</td>
<td>49-7</td>
<td>56-0</td>
<td>26-1</td>
</tr>
<tr>
<td>Mesoderm allocation (% previous column)</td>
<td>49</td>
<td>42</td>
<td>43</td>
<td>39</td>
<td>46</td>
<td>42</td>
<td>35</td>
<td>36</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td>Notochord allocation (% mesoderm cells)</td>
<td>6-4</td>
<td>6-9</td>
<td>5-0</td>
<td>6-3</td>
<td>7-2</td>
<td>5-9</td>
<td>5-5</td>
<td>6-1</td>
<td>5-6</td>
<td>6-4</td>
</tr>
</tbody>
</table>

For each allocation, within each embryo set, the null hypothesis was tested (chi²) that the smaller component (notochord, mesoderm) varies in absolute size around a mean that is the average of the sizes recorded, and independent of the size of the larger unit (whole mesoderm, total meso+ectoderm) of which it is part. Probability that the percentage allocations actually recorded could then occur is in each set less than 0·01.
Fig. 1. The alteration of mesoectodermal extent. (A) The origin of mesoderm. A lateral view of a common pattern of *Xenopus* egg cleavage, 32-cell stage, is shown at left. A sectional view from camera lucida drawing of fixed specimens two cell cycles later is shown at right, with descendants of the two upper (animal pole) tiers from the 32-cell stage hatched. The limits of explants that include those cells, with or without some immediate neighbours, are indicated. The replica half-section has those regions of blastomeres stippled, from which cleavage products normally fated to become mesoderm are derived. These embrace material more vegetal than 'tier 2' derivatives but also include appreciable material from tier 1.

(B) Specification at the stage of operation (shown in A). Explants that include just the hatched blastomere material form only wrinkled epidermis when cultured in simple salines (left). Inclusion of a little more vegetal material often causes development of slightly elongated, mesenchyme-filled explants (right) in which cement gland induction (arrows) occurs. (C) The operation to alter size of the territory competent to induction. Embryos are shown about one cleavage cycle after the reciprocal grafting of larger and smaller groups of blastomeres at 128-cell stage. The actual grafts are now shown hatched, but both are from within the hatched region of A. Embryo shape is maintained by snugly fitting agar wells as indicated. Individuals not healing to surface continuity within 40 minutes, or showing any clone of cleavage-retarded cells, were rejected. (D) Transverse sections through operated siblings with smaller (left) and larger (right) ectomesodermal populations, at late neural stage. The mass of endoderm is unchanged by operation, but there has usually been relatively more elongation, giving a smaller cross section in siblings with larger mesoderms presumably by the greater force of convergent extension movement. Note relative thinness and stretching of mesoderm and epidermis of embryo with size-reduced animal cap territory. Left, 32,000 mesoderm and ectoderm cells, 46% mesoderm. Right, 54,000 mesoderm and ectoderm cells, 42% mesoderm. np = neural plate; nc = notochord; p = paraxial mesoderm; lp = lateral plate mesoderm; ep = epidermis; end = endoderm. Scale bar = 1 mm approx.
what would occur in any one batch of normal sibling embryos.

The situation is probably different as regards the balance of the differentiations that constitute pattern within mesoderm. The only measure of this in the present embryos is relative cell number allocated to the notochord, a small structure already distinctive and scorable in the neurula. As noted above, notochord proportion correlates well with overall balance of mesodermal pattern and is well regulated among naturally different-sized ectomesodermal fields in *Xenopus*. The operating procedure involved in constructing the present embryos appears to destabilise pattern formation as judged by this criterion (data not shown). Among both the large and small mesoderms, relatively normal and abnormally small notochords are found apparently at random, and only the largest of them constitute what would be normal allocations for their mesoderms (5.5–7%, see Table 1). In addition, many of the experimental mesoderms have less than normal of the convergent extension that precedes somite formation, and a small narrow neural plate. In the large mesoderm of Fig. 1D, for instance, which shows relatively normal cross-sectional appearance, a slender notochord and abundant ventrolateral tissue is nevertheless evident. I return in the discussion to the question of the 'regulation' of intramesodermal pattern after blastular microsurgery in *Xenopus*.

**Development without endoderm from onset of gastrulation**

Fig. 3 shows the operation whereby the entire yolky endodermal core is removed from embryos, at some time during a period extending 20 minutes either side of the first appearance of the stage 10 indented dorsal blastoporal lip. This operation is clearly easier to perform accurately once the lip appears, but in all cases results in a 'shell' of tissue comprising presumptive epidermis and nervous system (ectoderm), some large proportion of the presumptive mesoderm, and the superficial cell layer immediately above the lip position in the dorsal sector known as the suprablastoporal endoderm (presumptive archenteron roof). This latter structure, despite its endodermal nature, is almost certainly specified by induction, whereas the remaining yolky mass is descended from the egg region that is the initial source of induction. Preliminary observations showed that a very complete and normally ordered dorsal axial pattern is obtained, despite the abnormally complete convergence and thus elongation that is mechanically allowed by absence of the yolky endoderm (see Fig. 3B–E). A series of 35 such operations was therefore performed exactly at onset of stage 10 (3 separate egg batches). These were fixed for comparison with control siblings as regards epidermal, neural and mesoderm cell numbers, at stage 19 or at stage 35 larval bodies, or were explored by fix-dissection at the latter stage.

Fig. 4 shows a series of histological appearances in transverse section, and the appearance of the whole body stripped of skin in endodermless and control developments compared. A normal, if contracted, pharyngeal architecture usually exists, presumably derived from the suprablastoporal endoderm with appropriate anteroventral mesoderm. Heart is always absent, and throughout the abnormally long thin trunk region the pattern is completed ventrally by at most a slender tract of lateral plate type mesoderm. Some axes are essentially entirely of notochord and somite in section behind the head region. Tail is of rather normal gross morphology but may show incoherent segmentation. Pronephros is well-formed, and may almost complete the mesoderm mid-ventrally in the absence of heart, lateral plate and liver rudiment. The central nervous system appears massive in the more ventral parts of its cross-section, even though, perhaps as a mechanical effect of the difficulty of rolling to a tube within the small body cross-section, its roof is often thin and stretched. A frequent abnormal feature of pattern is the absence of territories anterior to the eyes, and the fusion of their paired fields to give a large synophthalamic mass of retina.

Experimentals from three sets of operations were fix-dissected, along with synchronous controls, when segmentation had reached the stage with around 20 segmented somites in controls (st. 26/27). Table 2 shows that in addition to the frequently clear organisation of somites (see Fig. 3D), the number segmented, per time, follows the same course in experimental as in control embryos despite the considerably more con-
Fig. 3. The autonomous state of organisation for axial pattern from the onset of gastrulation. (A) Sectional view of a beginning gastrula, showing how the entire endodermal core (End) and the small amount of already involuted mesoderm (Inv) are cleanly removed with tungsten needles, the remaining shell of tissue allowed to contract for 10 minutes and then the outer continuity restored with a plate of gastrula animal pole ectoderm (below). (B) Sketches of the normal (above) and operated (below) larval appearance. Greater axial extension has occurred, and the forebrain structure is absent or reduced in the operated case, but a high degree of structure is nevertheless apparent. cg = cement gland; oc = optic cup; ev = ear vesicle. (C) Section showing brain structures normally associated with mesodermal pattern at the anteriormost notochord level, but in an endodermless body as in B. These are now bent around the notochord tip. oc = brain region showing retinal pigmentation and associated hypophyseal anatomy adjoining notochord (nc). The other brain region is probably midbrain. (D) Horizontal section at mid-axial level of endodermless body showing massive notochord and perfect somites. (E) The tail, the last region to undergo morphogenesis, in another endodermless specimen. Notochord (nc), tailbud somite (s) and spinal cord are all formed grossly normally, although there is sometimes a loss of coherence in segmentation accompanied by failure of tail expansion in the absence of post-anal endoderm. Scale bar for C, D, E = 100 µm approx.

Table 3 shows cell number estimates for whole germ layers and their major identifiable territories, notochord, epidermis and neural plate/tube, in examples of endodermless development and their synchronous control siblings at stage 19. The data make it clear that the pattern deficits – as opposed to geometrical changes – in the experimentally altered development are linked with altered allocation of available material to particular
Regulation and organisation of Xenopus mesoderm

Table 2. Number of somites segmented with time in endodermless embryos and synchronous control siblings

<table>
<thead>
<tr>
<th>Hours after st. 10 operation at 20°C</th>
<th>17</th>
<th>23</th>
<th>29</th>
</tr>
</thead>
<tbody>
<tr>
<td>Set 1</td>
<td>Exp.</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7-9</td>
<td>8-4</td>
<td>20-6</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Set 2</td>
<td>Exp.</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6-9</td>
<td>7-1</td>
<td>19-4</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Set 3</td>
<td>Exp.</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5-8</td>
<td>6-2</td>
<td>19-4</td>
</tr>
</tbody>
</table>

Four experiments and four control siblings from each set were fix-dissected at each of the three times (number of hours after stage 10) shown at the heads of columns. Entries refer to the mean numbers of somites segmented (8 sides) per group.

Discussion

Early size regulation

There is little evidence that the process of mesoderm induction in itself alters the early mitotic schedule (Symes & Smith, 1988) or that regional specificity within the mesoderm alters cell cycles on a large scale before neurula stages (Cooke, 1979a,b) when the cycle slows progressively in dorsal axial mesoderm. The data on mesoderm/ectodermal partitioning of Table 1 and Fig. 2 must therefore represent a rather constant, but not large, under-estimate of the proportion of the original animal cap cell descendants respecified by the spread of induction. There is no reason to believe the estimate will itself be of altered accuracy when pattern is formed to a different scale in different individuals. It therefore seems that around 50% of the cleavage products of the region competent to respond to induction are in fact recruited into the primary mesoderm, in a way that is substantially adjusted to tissue size. This adjustment operates both in undisturbed embryos and in those where size of the field of competent tissue is manipulated in relation to a constant sized origin of inducers at the 128-cell stage. Because of this, and in view of in situ lineage-labelling data from 32- and 64-cell territories. The differences between control and experimental allocations cannot be solely due to a variable inclusion of preinvoluting mesoderm at operation. Some of the isolated ectomesoderms possess total cell numbers in the normal range for these structures in whole siblings. Dorsal axial mesoderm pattern, and the nervous system that is thought to be induced by ‘dorsal’ mesoderm are allocated expanded amounts of tissue, at the expense of ventral territories and of epidermis, respectively. Overall allocation as between mesoderm and ectoderm is often remarkably near normal (cp. Table 1 and Fig. 2), confirming that essentially all presumptive mesoderm has been included and suggesting that its induction is in an advanced state of completion by the onset of activity in the first-involuting cells.

Table 3. Partitioning between ectoderm and mesoderm, and allocation of ectoderm to nervous system and mesoderm to notochord, in endodermless embryos and normal siblings at stage 19

<table>
<thead>
<tr>
<th>Total mesoderm and ectoderm cell population x 10⁶</th>
<th>Mesoderm allocation (%)</th>
<th>Notochord allocation within mesoderm (%)</th>
<th>Neural allocation within ectoderm (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Set I Exp. 1</td>
<td>34-7</td>
<td>33</td>
<td>9-1</td>
</tr>
<tr>
<td>2</td>
<td>39-2</td>
<td>39</td>
<td>8-5</td>
</tr>
<tr>
<td>3</td>
<td>32-0</td>
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</tr>
<tr>
<td>4</td>
<td>41-7 Mean 39-6</td>
<td>39 Mean 38</td>
<td>8-2 Mean 8-4</td>
</tr>
<tr>
<td>C 1</td>
<td>39-5</td>
<td>44</td>
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</tr>
<tr>
<td>2</td>
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<td>42-7</td>
<td>37</td>
<td>5-7</td>
</tr>
<tr>
<td>4</td>
<td>41-0 Mean 42-0</td>
<td>41 Mean 40</td>
<td>6-3 Mean 6-0</td>
</tr>
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<td>Set II Exp. 1</td>
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<td>42</td>
<td>8-7</td>
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<td>36-5</td>
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<td>39-9 Mean 41-1</td>
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<td>3</td>
<td>43-6 Mean 45-0</td>
<td>41 Mean 41</td>
<td>6-9 Mean 6-3</td>
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<td>Set III Exp. 1</td>
<td>33-7</td>
<td>31</td>
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<td>3</td>
<td>42-6 Mean 40-6</td>
<td>40 Mean 40</td>
<td>6-0 Mean 6-4</td>
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</tbody>
</table>

Augmentation of notochord and of neural tube proportions was not mainly due to selective loss of ventrolateral mesoderm and epidermis at operations. Thus all but one experimental notochord and two experimental neural systems contained more cells than any of their control counterparts.
stages (Dale & Slack, 1987a; Cooke & Webber, 1985b), it is most unlikely that mesodermal lineages are founded only as a ring, one or two cells wide, directly contacting endodermal cells. Release of inducer appears possible from the first cleavages, and competence to respond, from the 64-cell stage (Jones & Woodland, 1987), but the \textit{in situ} process appears to take place by diffusion or active propagation of signals across responsive tissue over much of the blastular stage. Cooke \textit{et al.} (1987) have reported the transmission of induction from competent newly induced cells to further uninduced ones.

Gurdon (1989) has recently proposed that mesoderm extent, as monitored by formation of the muscle pheno-
Infected plants soon infect their immediate neighbours in the lawn or forest (the competent field of ectodermal cells). A transmissable disease first infects one edge of a tissue, rapidly transmitted inhibition allows regulation of positional information against overall size variation. The present results (Fig. 2) show that for mesoderm as a territory in the whole embryo, some more positive principle that sets proportion in play. How might the spatial extent of induction be controlled in the required way? It should be noted that there may to some extent be an initial ‘pepper and salt’ distribution of mesodermised cells in the blastocoel, with the final boundary established or refined by a local cell sorting phenomenon or indeed a co-operative ‘community’ effect (Gurdon, 1988). The extent to which such a mechanism operates in Xenopus, or in other vertebrates where the geometry of mesoderm formation is different, is for future investigation. But the proportioning mechanism probably operates fundamentally by position-dependent specification. There is much indirect evidence that a feedback inhibitor of the response to inducer, as well as inducers or ‘activators’ themselves, are present in the natural system (Smith, 1987; Cooke et al. 1987; Cooke et al. 1989), and that appreciable levels of inducing signal actually reach regions of competent tissue beyond those where a mesoderm response is normally seen. These are the arrangements expected for a reaction–diffusion based ‘morphogen’ gradient system, in which the action of rapidly transmitted inhibition allows regulation of positional information against overall size variation (Gierer & Meinhardt, 1972; Meinhardt, 1982). The control principle proposed is best illustrated by analogy. Suppose a transmissable disease first infects one edge of a lawn or forest (the competent field of ectodermal cells). Infected plants soon infect their immediate neighbours via a local transmission process, and so on. But, at the same time, diseased plants release an agent that can confer resistance, and which diffuses rapidly within the community as a whole (e.g. because air- or insect-borne). Further transitions to the diseased state will stop at a frontier, whose position could reflect the proportion of the population, rather than the absolute number, that are infected.

Fig. 4. The larval body after the operation of Fig. 3, in relation to control development. (A–D) Transverse sections through the normal larval body at (grazing) forebrain/eye; hindbrain/ear vesicle, heart and pronephric levels, respectively. (E–H) Sectional levels, equivalent to those shown above them, through a sibling larval body after endoderm excision at stage 10 onset. In relation to the normal pattern, note in E the fused retinal area wrapped around truncated forebrain field, in F the visibly expanded sections of notochord and hindbrain and loss of ventral structures, in G the continuation of previous features and thin expanded neural roof, and complete absence of heart, and in H the restricted strip of mesoderm more lateroventral than the expanded pronephros. (I, J) Mid-trunk sectional levels of the same normal and endodermless siblings. Note notochord size and greatly diminished allocation to ventral mesoderm in J. (K, L) Normal and endodermless sibling whole larvae fixed in K2Cr2O7/Acetic acid and stripped of skin. Note absence of gut and truncated anterior neuraxis (with synophthalmia) in L, but the great normality of somite segmentation pattern (cp. Fig. 3D). ev = ear vesicle, fb = forebrain, hb = hindbrain, nc = notochord, s = somite, g = gill mesenchyme (N.B. neural crest and mesoderm-derived), h = heart, pn = pronephros, lpl = lateroventral mesodermal types.

Scale bar for A–J = 100 μm approx.

type in cultured combinates, is largely a function of proximity to source of inducer and the limited spread of signal, i.e. of local mechanisms. The present results show that for mesoderm as a territory in the whole embryo, some more positive principle that sets proportion in play. How might the spatial extent of induction be controlled in the required way? It should be noted that there may to some extent be an initial ‘pepper and salt’ distribution of mesodermised cells in the blastocoel, with the final boundary established or refined by a local cell sorting phenomenon or indeed a co-operative ‘community’ effect (Gurdon, 1988). The extent to which such a mechanism operates in Xenopus, or in other vertebrates where the geometry of mesoderm formation is different, is for future investigation. But the proportioning mechanism probably operates fundamentally by position-dependent specification. There is much indirect evidence that a feedback inhibitor of the response to inducer, as well as inducers or ‘activators’ themselves, are present in the natural system (Smith, 1987; Cooke et al. 1987; Cooke et al. 1989), and that appreciable levels of inducing signal actually reach regions of competent tissue beyond those where a mesoderm response is normally seen. These are the arrangements expected for a reaction–diffusion based ‘morphogen’ gradient system, in which the action of rapidly transmitted inhibition allows regulation of positional information against overall size variation (Gierer & Meinhardt, 1972; Meinhardt, 1982). The control principle proposed is best illustrated by analogy. Suppose a transmissable disease first infects one edge of a lawn or forest (the competent field of ectodermal cells). Infected plants soon infect their immediate neighbours via a local transmission process, and so on. But, at the same time, diseased plants release an agent that can confer resistance, and which diffuses rapidly within the community as a whole (e.g. because air- or insect-borne). Further transitions to the diseased state will stop at a frontier, whose position could reflect the proportion of the population, rather than the absolute number, that are infected.

The state of organisation at onset of gastrulation

The observations of Figs 3 and 4 show that, while induction may be a prolonged process during pregastrular stages, an advanced and stable state has been reached at the very onset of gastrulation. This state contains the preconditions that control many aspects of mesoderm organisation, though it is not meaningful to say that the pattern is determined within the tissue at this stage. The sequence of neural induction, probably reflecting craniocaudal sequence of specificities in axial mesoderm, is normal. The absence of preoptic area and fusion of eye fields could reflect omission of a small but crucial group of the first-involuting, presumptively anterior mesoderm at the time of separating the tissues, since such omission cannot be compensated for by regulation (Cooke, 1985c). Alternatively the normal separation of the optic field into two areas may require the broadening of the neural plate, which is somehow dependent on the outer germ layers being stretched.
around a massive, relatively incompressible endodermal core. The most striking aspect of endodermless development from stage 10 is the normal segmentation sequence and number, preserved despite the quite altered geometry of gastrulation, and changed shape, cell number and packing of the almost fused columns of paraxial mesoderm. This simple but singular fact argues strongly against model mechanisms for segmentation proposing that somite number is controlled as some indirect consequence of the local mechanical conditions and shape in the segmenting tissue. Instead, without speaking directly to the molecular nature of the machinery involved, it argues for the concept that some physiological variable having to do with rates of development, and possibly with periodicities, is already so organised within the pregastrular mesoderm cell population that the proper number of discrete anatomical units are allocated and express their segmentation behaviour in the correctly timed and arranged sequence. It must increase the belief that, however it is set up, segment number is a profound and not an incidental aspect of regionality in the vertebrate body (Cooke & Zeeman, 1976; Primm et al. 1989).

The absence of heart, and shift of allocation away from 'lateroventral' in favour of dorsal axial structure after stage 10 endoderm excision, reflect a lesser state of preorganisation with respect to mediolateral pattern as compared with dorsal craniocaudal sequence at onset of gastrulation in Xenopus. Jacobson & Sater (1988) have drawn attention to possible differences in timing, as between subclasses of amphibians, for requirements for endoderm in the induction of heart mesoderm. One possible meaning of the present results is that in Xenopus an inductive signal from endoderm, of a regionally specific or else a general 'permissive' kind, is required later than stage 10 for stabilisation of heart formation.

Another possible explanation, embracing both the absence of heart and the expanded notochord and paraxial mesodermal territories, is in terms of pattern formation by gradients of morphogen signals within the plane of the mesoderm itself during and after gastrulation. Such interactions have an 'asymmetrical' character whereby dorsal tissue emits signals which will dorsalisate ventral tissue, but not vice versa (Slack & Smith, 1983; Dale & Slack, 1987b). Following the endoderm excision operation, tissue presumably repacks as gastrulation proceeds, as the cross-sectional cellular distance between dorsal and ventral meridians becomes much less than would be allowed in the normal gastrula/neurula, and the tissue has in any case an inbuilt mechanism leading to dorsal convergence. In these circumstances a simple 'diffusion' mediated, mediolateral gradation in a signal from a dorsal origin might be expected to 'flood out', giving a partial, dorsally overbalanced sequence of signal intensities which will not permit the specification of fully ventral structure. The expanded allocation to nervous system, in the cross-section of the ectodermal layer, would follow from the wider tract of mesoderm now active in the next major inductive episode, neural induction. The results of Table 2, for notochord and for nervous system allocations taken together, make clear that the precise allocation between notochord and somite territories, and that between the whole dorsal axial and more lateral mesodermal domains, is not stabilised until well into gastrulation (see Dale & Slack, 1987b; Smith & Slack, 1983; Cooke, 1983). Heart is relatively 'dorsally' derived from the early cleavage fate map, so that its omission from these patterns may not seem consistent with the above hypothesis. It is nevertheless worth noting that XTC-MIF, an inducer of distinctively dorsal mesoderm, can directly or indirectly prevent heart specification in the embryo when injected into the blastocoel of blastulae (Cooke & Smith, 1989).

An operation drastically changing geometry and cell packing in earliest mesoderm can allow striking normality of expression of craniocaudal pattern while substantially disturbing mediolateral pattern. This should strengthen belief that the vertebrate mesodermal body plan is built up at later stages by interacting but different mechanisms of organisation that primarily relate to these two anatomical dimensions, even though many early manipulations on whole embryos produce only co-ordinated effects upon them.

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References


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