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Mechanisms of vertebrate segmentation

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That all higher organisms possess some segmental organization during their development is presumably no coincidence. Segmentation appears to be an important developmental strategy with which to build and diversify different body regions. It can be studied at many levels, ranging from molecule and genome, through cells and their interactions, to morphogenesis and whole body pattern; and there is no compelling reason for thinking that the study of any one level will prove more revealing than that of any other. The study of vertebrate segmentation in fact provides a particularly good example of this philosophy. The application of classical and molecular genetic analysis to *Drosophila* development (reviewed by Akam, 1987) has yielded a large amount of information about the genes that play a role in the development of segmental patterns in this organism. Springing directly from this analysis comes the hope that similar methods applied to vertebrate development, exemplified by the discovery of the homeobox in the vertebrate genome, will be just as successful. But will the identification of vertebrate segmentation genes complete our understanding of the segmentation process? We believe not, and our chief purpose in this review is to identify and discuss the variety of different levels at which one can approach the major events involved.

The cell biology of somite development

The very recognition of the vertebrates as a distinct animal phylum rests upon the most conspicuous form of vertebrate segmentation, the vertebral column. Vertebrae develop from the somites, which form by the sequential epithelialization of two mesenchymal rods of mesoderm, the segmental plates. In higher vertebrates the segmental plates are laid down during the process of gastrulation, appearing on each side of the midline neural epithelium as the primitive streak regresses along the anterior–posterior (A–P, craniocaudal) axis of the embryo (Figs 1A, 2). At the primitive streak stage, the major contribution to the somitic mesoderm appears to be from the epiblast adjacent to the anterior part of the streak; mesoderm cells emerging from this part of the streak also contribute to the notochord (Spratt, 1955; Rosenquist, 1966; Nicolet, 1971; Tam & Beddington, 1987). As development proceeds, neurulation takes place in the midline, and the segmental plates come to flank the neural tube and notochord (Figs 1A, 2). In the chick embryo, somites form from the anterior end of each plate at an approximate rate of one pair every 100 min. During segmentation the length of each plate remains relatively constant, as more cells are added both by mitosis within the plate and by recruitment of cells at its posterior end. Even before overt segmentation, a metameric arrangement of segmental plate cells is visible with scanning electron microscopy (Meier, 1979), although the significance of these 'somitomeres' is unclear (see below).

Each somite is first constructed as an epithelial sphere, whose radially arranged cells line a small central lumen. The lumen contains a cluster of cells which retain a mesenchymal arrangement (Fig. 1B). Several hours later, the cells in the ventromedial part of the somite lose their epithelial arrangement to form, along with the luminal cells, the mesenchymal **sclerotome**. The sclerotome, together with the notochord, gives rise to the vertebral column. The dorsolateral cells of the somite retain their epithelial arrangement, producing the **dermomyotome** (Fig. 1A,C). This later subdivides further: those cells situated immediately beneath the ectoderm, comprising the **dermatome** (Figs 1C, 3), eventually disaggregate and give rise to the dermis of the trunk and to some muscle cells (Christ *et al.* 1986); the cells that appear between the dermatome and the sclerotome remain closely packed as the **myotome** (Figs 3, 4), forming the axial skeletal muscle (Christ *et al.* 1978).

With the exception of certain amphibia such as *Xenopus* (Hamilton, 1969), the essential elements of cell behaviour during the formation and subsequent development of the somite are similar in all ver-

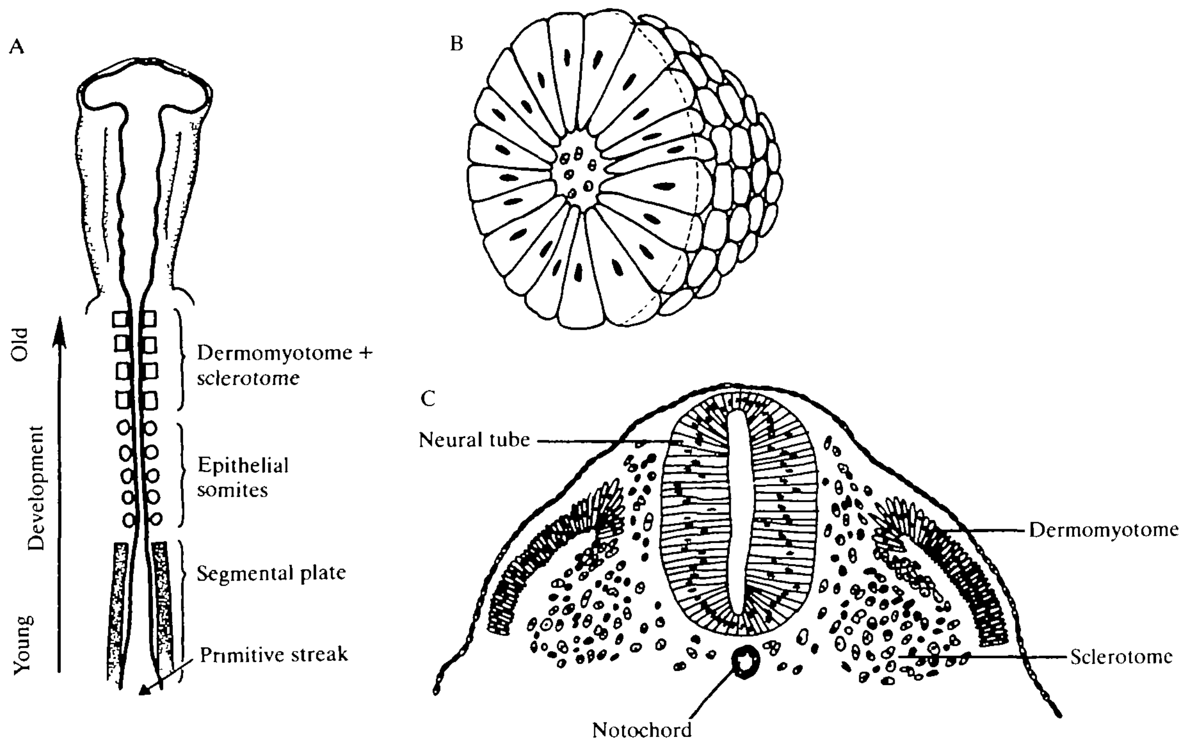


Fig. 1. Stages of somite formation in the chick embryo. (A) Diagram of the main stages of somite formation. Somitogenic cells arise towards the posterior end of the embryo (bottom in the diagram) and remain in the segmental plates until they segment into epithelial somites, in anteroposterior sequence. One pair of somites forms every 1·5 h. About 7 h (5-somite-pairs worth) after its formation, each somite differentiates into dermomyotome and sclerotome portions. (B) Diagram of half of a newly formed epithelial somite. The somite is an epithelial sphere, a single cell in radius, but containing a few mesenchymal cells within its lumen. The spherical somite is enveloped by a basal lamina (not shown). (C) Diagram of a transverse section through a 2- to 3-day chick embryo showing a pair of somites after its differentiation into dermomyotome and sclerotome.

tebrate classes (see Borisov, 1971). Each somite appears when a group of cells undergoes a change in organization from mesenchyme (the segmental plate) to a rosette, simultaneously displaying the cell–cell associations typical of an epithelium (Trelstad *et al.* 1967; Revel *et al.* 1973). Cells in the segmental plate bear gap junctions and tight junctions, but the latter do not form the large interconnected networks typical of a mature epithelium.

Once the somite has formed, the epithelial cells are polarized, with the Golgi zone located near the apical (luminal) cytoplasm and tight junctions close to the luminal border (Revel *et al.* 1973; Lipton & Jacobson, 1974). Actin and α-actinin are also concentrated at the apical zone (Ostrovsky *et al.* 1983; Lash *et al.* 1985). The basal cell surface rests on a basal lamina that covers the somite, separating it from several adjacent tissues. The basal lamina contains collagen, laminin, fibronectin and cytotactin (Bellairs, 1979; Thiery *et al.* 1982; Rickmann *et al.* 1985; Crossin *et al.* 1986; Duband *et al.* 1987: Tan *et al.* 1987).

Several recent studies have concentrated on the mechanisms that might underlie the mesenchymal–epithelial transitions characteristic of somite development. Using an assay for cell adhesion (Curtis, 1969), Bellairs *et al.* (1978) showed that disaggregated somite cells are more adhesive than those of the segmental plate. Similarly, Cheney & Lash (1984) found that cell–cell adhesion increases at the anterior end of the segmental plate, immediately before somite formation. These findings suggest that the epithelialization of the segmental plate is accompanied by an increase in cell–cell adhesion.

Several molecular mechanisms have been identified that could mediate cell–cell adhesion during somite formation in the chick embryo. For example, the calcium-dependent adhesion molecule N-cadherin is expressed at low levels in posterior regions of the segmental plate, but increases in the more anterior regions. When segmentation occurs, the molecule becomes concentrated at the apical part of the newly epithelial cell surface (Hatta *et al.* 1987; Duband *et al.* 1987). The subsequent disaggregation of the ventromedial portion of the somite to form the mesenchymal sclerotome is preceded by a loss of N-cadherin immunoreactivity in precisely this region

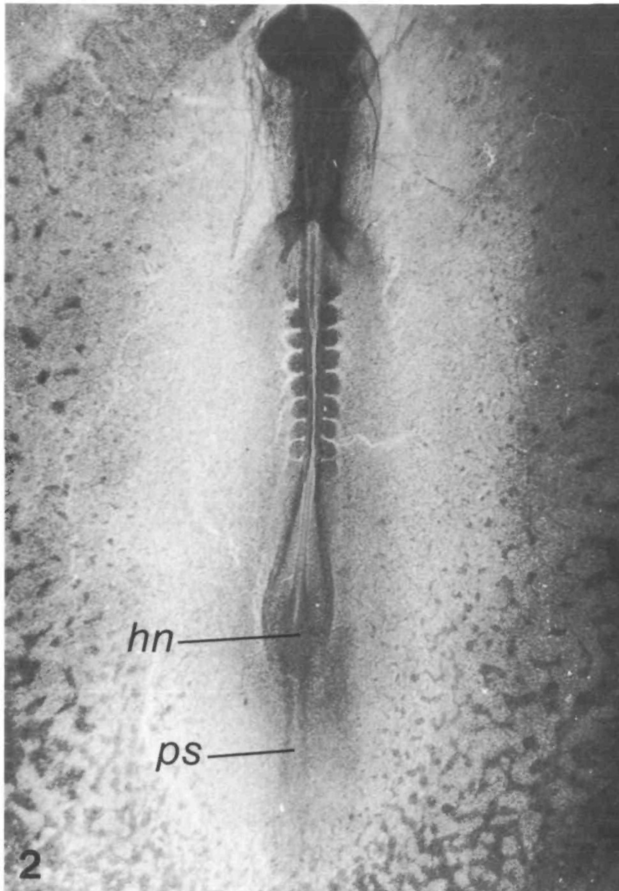


Fig. 2. Whole mount of 8-somite chick embryo. Compare with Fig. 1A. The neural tube opposite the segmental plate is still open, and the notochord can be seen between the elevated neural folds at this level. More posteriorly, Hensen's node (*hn*) is clearly visible, as is the primitive streak (*ps*).

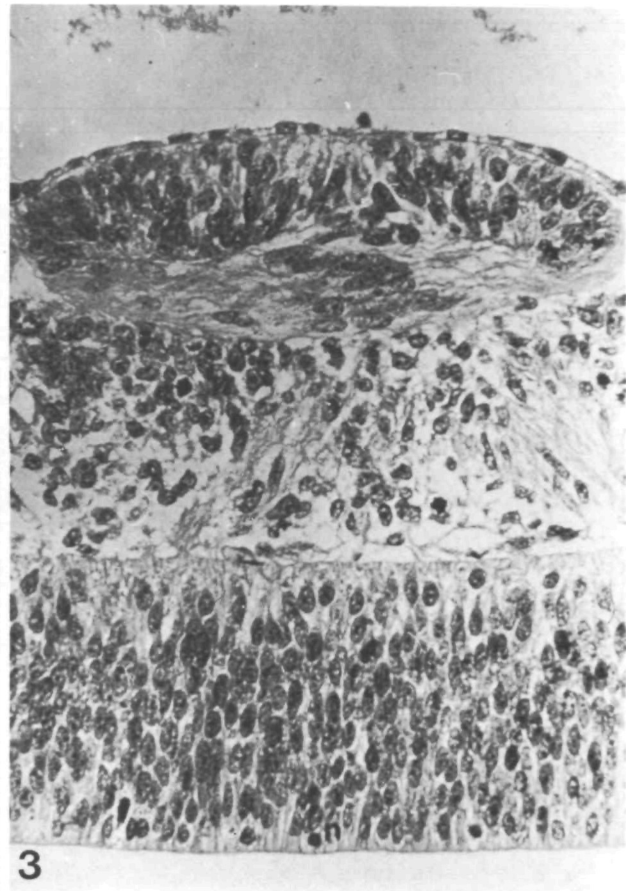


Fig. 3. Somite in a human embryo. Coronal section through one segment of a human embryo of approximately 9 weeks of gestation, stained with haematoxylin and eosin. Motor axons can be seen leaving the neural tube (towards the bottom of the photograph), traversing the anterior (right in the picture) half of the sclerotome towards the myotome. Note that the length of the myotome corresponds to that of one myoblast, each of which stretches over the whole width of the segment. The dermatome portion of the somite is still epithelial and can be seen just under the ectoderm, at the top of the photograph. It may be relevant to observe that the dermatome appears to display a change in cell morphology and orientation at a position corresponding to the middle of the segment.

of the epithelium; and Fab fragments of monoclonal anti-N-cadherin antibody cause disaggregation of chick somites *in vitro* (Duband *et al.* 1987). N-cadherin appears to be closely related to the adhesion molecule A-CAM (Volk & Geiger, 1986), which is localized to adherens junctions between epithelial cells. Given the epithelial nature of the newly formed somite, it is reasonable to expect that the components of epithelial cell–cell associations, such as N-cadherin, will appear at some stage during somitogenesis.

Fibronectin has also been implicated in experiments by Lash and his colleagues. Adding cellular fibronectin to cultures of chymotrypsin-disaggregated segmental plate cells stimulates cell aggregation (Lash *et al.* 1984). More recently, Lash *et al.* (1987) have shown that aggregation can be produced simply by the addition of the peptide GRGDS. Since this sequence has been identified as the cellular recognition site of fibronectin (Pierschbacher & Ruoslahti, 1984; Yamada & Kennedy, 1984), Lash *et al.* (1987) have suggested that the peptide might act as a specific trigger for somite formation *in vivo*. Certainly fibronectin is present at the appropriate stage, being localized to those regions of the segmental plate which lie adjacent to ectoderm and endoderm. It is necessary, however, to postulate additional mechanisms that localize somite formation at the anterior end of the segmental plate *in vivo*. One possibility would be an increased expression of the receptor for the peptide in this region. This has been assessed using the monoclonal antibodies CSAT and JG22, which

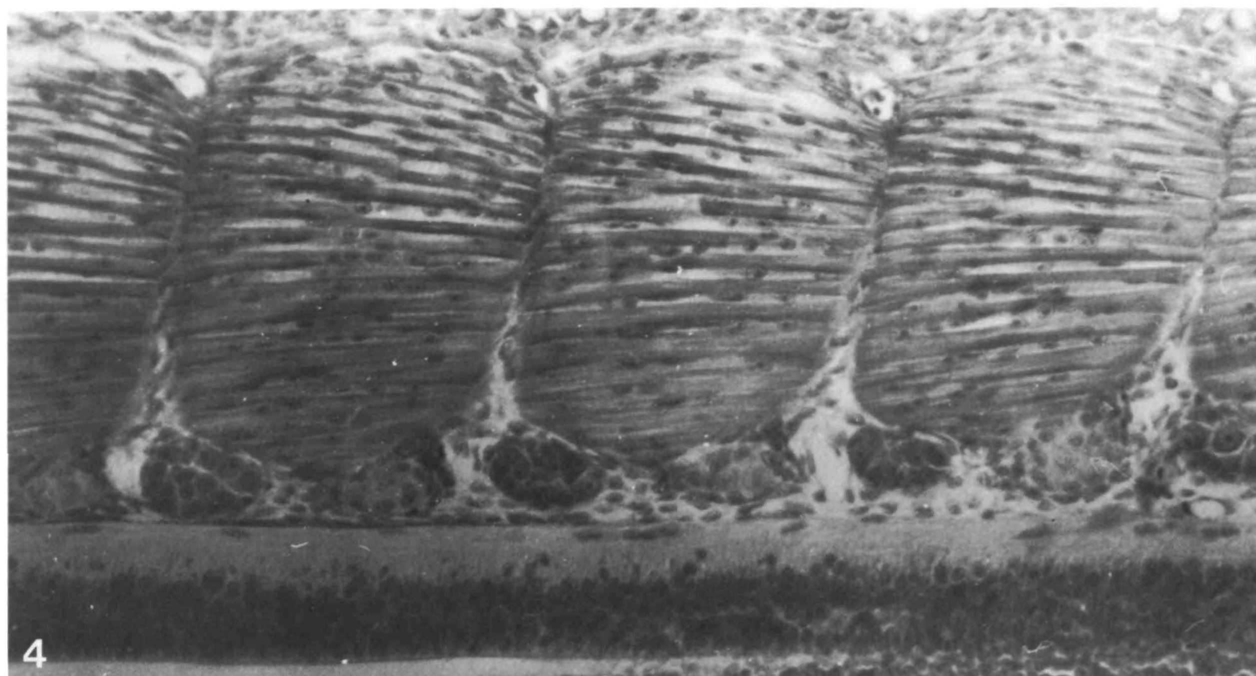


Fig. 4. Segmentation in the salmon trout. Unlike the chick and human embryo (cf. Fig. 3), the segments in this fish, as well as in some amphibians, consist mainly of myotome. Each sclerotome is a small group of cells between the neural tube (visible at the bottom of the section) and the tall myotome. Nevertheless, the dorsal root ganglia are restricted to the anterior half of each of these small sclerotomes. Longitudinal section. Note that, as in other embryos (cf. Fig. 3), each myotome is one stretched myoblast cell in length.

recognize a 140 000 M_r cell surface receptor complex involved in adhesion to fibronectin and related to the integrin receptor family (Horwitz *et al.* 1985; Ruoslahti & Pierschbacher, 1987). CSAT and JG22 staining surrounds the segmental plate, but is present only at low levels within it and does not vary along its length (Krotoski *et al.* 1986).

Finally, the cell adhesion molecule N-CAM has been implicated in the experiments of Duband *et al.* (1987). As determined immunohistochemically, N-CAM is expressed at low levels in the posterior segmental plate, but increases at the anterior end. The cells of the epithelial somite are immunoreactive for N-CAM over their entire surface. Immunoreactivity does not decline in the sclerotome during the early stages of somite disaggregation, and monoclonal or monovalent polyclonal N-CAM antibodies do not dissociate explanted epithelial somites. It appears, therefore, that N-CAM expression does not correlate as tightly with the mesenchymal–epithelial transitions of somite formation as does N-cadherin expression.

It is probably naïve to suppose that any one molecular system plays the dominant role in somite formation; one might imagine, for example, the sequential operation of separate recognition and adhesion systems as the somite cells aggregate into an epithelium. It will not be surprising, therefore, if other molecules, such as desmosome constituents, turn out to be involved in the process. As a further example, the cells of the epithelial somite also express a membrane-bound receptor for complex sulphated polysaccharides, which is found subsequently at the apical part of the dermatome (Cook *et al.* 1988). This raises the possibility that carbohydrate-binding activities play a role during somite formation. The earliest interactions between segmental plate cells might be expected to involve molecules, such as glycoconjugates, which make up the outermost part of the cell periphery; these interactions could then be consolidated by other adhesion systems.

Half segments

The study of the interactions between the developing peripheral nervous system and the somite-derived sclerotomes has shown that each sclerotome is divided into anterior and posterior parts. When motor axons emerge from the developing neural tube in chick embryos, they traverse exclusively the anterior halves of the adjacent sclerotomes. Reversal of a short length of segmental plate about the A–P axis, before axon outgrowth, results in axon growth through the posterior (original anterior) parts of the reversed segments (Keynes & Stern, 1984). Neural crest cells also migrate through the anterior rather

than posterior sclerotome (Bronner-Fraser, 1986; Rickmann *et al.* 1986; Teillet *et al.* 1987; Loring & Erickson, 1987).

Recent grafting experiments in chick embryos (Stern & Keynes, 1987) have shown that the anterior and posterior populations of sclerotome cells maintain their distinct segmental positions because they are unable to mix with one another. For example, when the anterior half of an epithelial somite is grafted in place of one whole somite, the resulting sclerotome cells retain their anterior character, mixing only with the anterior sclerotome cells of the next-posterior segment. The 'compound' sclerotome so produced has an unusually large anterior portion and contains a correspondingly large spinal nerve (Fig. 5). Where the grafted anterior sclerotome cells confront sclerotome of posterior character, at the posterior edge of the next-anterior segment, a boundary develops. Grafting quail half-somites into chick embryos confirms that the interactions between sclerotome cells can be described by a simple rule: *cells from* **like** *sclerotome halves mix with one another, while cells from* **unlike** *halves do not; when unlike cells interact, a boundary develops between them* (Stern & Keynes, 1987).

The A–P sclerotomal subdivision exists in all vertebrate classes (see Keynes & Stern, 1984), and we discuss below its implications for somite formation and neural development. It may also be important for the further development of the sclerotomes into the segmented vertebral column. Remak (1855), who first observed the subdivision of the sclerotome, suggested that it allows a 'resegmentation' ('Neugliederung') whereby, on each side of the embryo, the anterior half of one sclerotome merges with the posterior half of the next-anterior sclerotome to form one vertebra. This would cause a phase shift of the vertebrae relative to the myotome-derived axial muscles, allowing the muscle segments to span, and thereby move, the vertebrae.

Elegant though this idea is, there is actually no direct evidence for resegmentation. Ever since Remak's day, accounts of the development of the vertebral column have been based entirely upon painstaking descriptions of serial sections, and (for obvious technical reasons) the fate of each half-sclerotome has not been traced accurately. Verbout (1976, 1985) has pointed out that before the segmentation of the vertebral column becomes visible, the sclerotome cells first form an apparently unsegmented aggregate around the notochord. The extent to which sclerotome cells from neighbouring segments mix within this aggregate is unknown, however, and the nonmiscibility of A and P cells de-

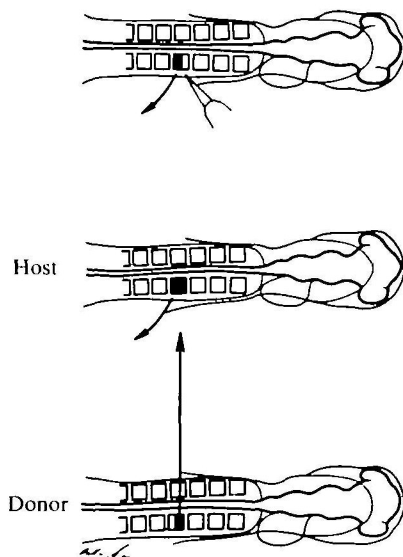
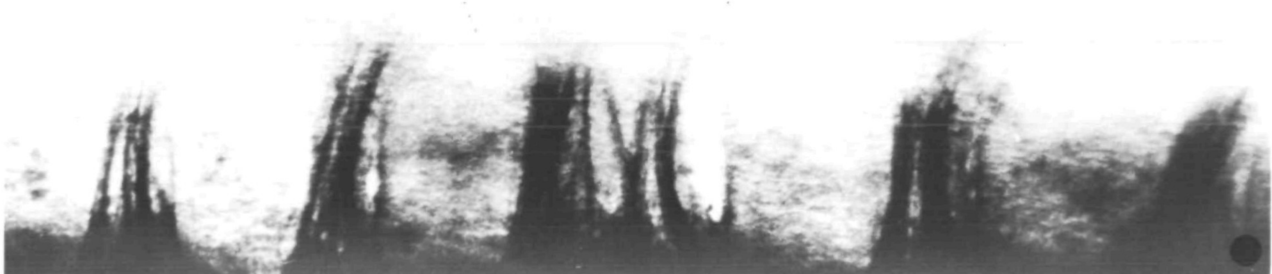


Fig. 5. Construction of double-anterior compound somites. (A) Two different microsurgical techniques that can be used: the posterior half of one somite may be removed; alternatively, a whole somite may be removed from a host embryo and the anterior half of a somite from a donor embryo grafted in its place. (B) Result of the operation: a larger than normal spinal nerve can be seen traversing the enlarged anterior portion (right in the picture) of the double-anterior somite. (Figures from Stern & Keynes, 1987.)



scribed above could apply only to other, more lateral, regions of sclerotome.

Using the chick/quail chimaera grafting technique (Le Douarin, 1969), and grafting quail half-somites into host chick embryos, both anterior and posterior halves are seen to contribute to the bone, cartilage and disc tissue of the vertebral column (Stern & Keynes, 1987). Although this result could be explained by invoking differences in the migratory behaviour of quail and chick cells in the chimaeras, it would appear that the two sclerotome halves have identical fates in terms of cell differentiation. More detailed lineage studies are needed, however, to assess their fates in terms of final position within the vertebral column. Such information has become all the more important, now that molecular markers are being identified which are restricted to particular perinotochordal regions along the A–P axis. For example, the transforming growth factor, TGF-β type 1, is first expressed in both sclerotome halves, but is later localized in the developing vertebral body rather than intervertebral disc (Heine *et al.* 1987).

Models of somite formation

If we now have a reasonably clear picture of the major features of cell behaviour during the development of individual somites, we are much more ignorant about the mechanisms that generate and control the metameric pattern overall. This is reflected in the number of different models that have been proposed to account for somite formation, whose strengths and weaknesses we outline here.

(A) *Induction models*

Many models for somite formation have sought to explain segmentation in terms of inductive interactions between the presumptive somitic mesoderm and neighbouring embryonic tissues (see Bellairs, 1980, for review). In essence, the neighbouring tissue is regarded as being primarily (if not overtly) segmented, and it imparts this property to the mesoderm by some form of interaction. As an example, Hensen's node might induce segmentation as it regresses along the embryonic axis; or the neural tube or notochord might confer the segmental information. These models are unsatisfactory in many respects, not least because the principal issue, namely how the primary metameric pattern is established, is not addressed, but is passed on to another tissue instead. No single tissue has in fact been shown to induce somite formation. Somites can appear in the absence of Hensen's node, notochord, neural epithelium or 'somite centres' (e.g. Bellairs, 1963; Stern & Bellairs, 1984*a*), and after removal of the endoderm (Bellairs & Veini, 1980). There is no good evidence, then, that inductive interactions are responsible for initiating the metameric pattern. It is clear from other experiments, though, that some of the neighbouring tissues are required for the maintenance of the somites. For example, somites rapidly lose their structural integrity when separated from the neural tube and notochord (e.g. Teillet & Le Douarin, 1983; Stern & Bellairs, 1984*a*). The precise nature of these more 'trophic' interactions between somite mesoderm and surrounding tissues remains unexplored.

(B) *Prepattern models*

Curt Stern introduced the term 'prepattern' as a 'descriptive term for any kind of spatial differentness in development' (Stern, 1954). An important problem arises when applying this concept to somite formation: like the induction models described above, prepattern models do not explain how the periodicity is actually created. While induction models displace the patterning influence in space, these models displace it in time. For the sake of completeness, however, we shall discuss two ideas of this kind.

Meier and his colleagues (Meier, 1979; Jacobson & Meier, 1984, 1986) have suggested that the segmental plate of the chick and other vertebrate embryos already displays a metameric arrangement of cells, visible by scanning electron microscopy. Each repeat-unit is called a 'somitomere' and the number of somitomeres is said to correspond to the number of presumptive somites generated by the segmental plate when isolated.

Alongside the lack of explanatory power, there is a further difficulty for the concept of a somitomeric prepattern. In order to maintain a fixed arrangement of somitomeres, it is essential that there be little or no cell movement within the segmental plate, or at least that any movement be restricted to a single somitomere. This is not the case, however. Considerable cell movement has been observed in time-lapse films of the chick segmental plate (Stern & Keynes, 1986; unpublished observations). In the mouse, the same conclusion has been reached by analysis of the distribution of cells derived from isotopic grafts of radiolabelled caudal mesoderm (Tam & Beddington, 1987).

Bellairs (1980, 1986) has suggested that the pattern of somites is derived from a prepattern of somitogenic 'clusters', located somewhere near the posterior end of the segmental plate, to which new cells are added by cell division and by recruitment from the primitive streak mesoderm. Each somite is related directly to one founder cluster, and regardless of the size of the embryo, the size of each somite depends upon the number of cells available for recruitment by each

cluster. A critical test for this model might be to isolate the transitional region between the primitive streak and the posterior portions of the segmental plate; a reduced number of somites should form from it, corresponding to the number of presumptive somites within it.

(C) *Positional information models*

Applying ideas developed from the study of arthropod segmentation to vertebrate embryos, Meinhardt (1982, 1986) has suggested that the metameric pattern is produced by confrontations between groups of cells with differing identities. An A–P gradient of 'positional information' is used to generate these states, by reaction–diffusion mechanisms; each presumptive somite cell first oscillates between the states before attaining its final, stable identity.

A simple possibility would be that each somite is subdivided into nonmixing anterior and posterior halves, with somite borders forming as a result of interactions between cells of the differing (i.e. A and P) states. Ambiguity arises, however, with respect to the placing of the borders, which must be at *alternate* A–P confrontations. To resolve this problem, Meinhardt suggests that somites might instead be subdivided threefold, with regions designated S(egment boundary), A and P: the borders would then arise, unambiguously, at each P–S confrontation. Another solution (Meinhardt, 1986) is to suppose that the primary metameric pattern is generated by a system with a two-segment (rather than single-segment) periodicity, being allocated in a manner reminiscent of the pair-rule genes in *Drosophila*. Consecutive somites could alternate between two states, say O (odd) and E (even), each one somite in length, with borders determined by consecutive O–E confrontations.

The fact that each sclerotome is divided into A and P parts (see above) is at least consistent with models based on segmental subdivision, and confrontations between A and P states do generate sclerotome borders (Stern & Keynes, 1987). Moreover, an alternation of A and P states should produce a border in approximately the middle of each sclerotome, and such a border (the fissure of von Ebner) does indeed exist (see Keynes & Stern, 1984).

We have argued previously (Stern & Keynes, 1987) that the A–P sclerotome subdivision and immiscibility may serve to *maintain* the boundaries between consecutive sclerotomes; in the absence of some such restraining mechanism, the newly mesenchymal cells of the sclerotome would be expected to mix freely with one another, destroying the periodic pattern of vertebrae and associated segmental nerves and blood vessels. It is less clear, however, whether the initial *development* of somites as epithelial spheres could be based upon similar mechanisms. A subdivision of the epithelium into A and P parts could be envisaged, perhaps, with each part one cell in length; but since the epithelium comprises only two cells along the A–P axis, or is reduced to one (e.g. *Xenopus*, Hamilton, 1969), a further subdivision (e.g. to three states) is harder to imagine. Border formation based upon O–E confrontation, and maintained by A–P confrontation, is perhaps more attractive, because it could represent a way of distinguishing between intersegmental (O–E *and* A–P) and intrasegmental (A–P only) borders for later resegmentation.

One prediction of Meinhardt's model (Meinhardt, 1986) is that the oscillation period should coincide precisely with the time taken for one somite to form, being approximately 100 min in the chick embryo. With a cell cycle duration in the chick mesoderm of about 10 h, each cell cycle would simultaneously comprise about 6 oscillations through the putative states. It is perhaps difficult to envisage the molecular basis of an oscillator with such a period, although repetitive calcium transients with a period shorter than the cell cycle have been described in fertilized sea urchin eggs (Poenie *et al.* 1985) and hormone-stimulated hepatocytes (Woods *et al.* 1986).

(D) *The 'clock and wavefront' model*

Cooke & Zeeman (1975) proposed that the periodic arrangement of somite blocks in amphibian embryos could be produced by the combined action within presumptive somite cells of two main events: an intracellular oscillator, or clock, and the passage of a single 'kinematic wave' of somitogenic cell determination travelling along the A–P axis of the embryo. The somitogenic cells oscillate synchronously with respect to some (necessarily ill-defined) biochemical state, being able to express the properties required for overt segmentation during only part of this oscillation cycle. If the wave passes them in the 'off' part of the cycle they accumulate as a group, having undergone the determination change in close synchrony, but being unable to segment. When the 'on' part of the cycle reappears the group proceeds to segmentation. The model therefore proposes that an interplay between the clock and the wave gates the somitogenic cells into groups, producing a punctuated pattern.

An important feature of the model is its prediction that the total number of somites will be unaffected by variations in embryo size; both the time taken for the wave to propagate along the A–P axis and the oscillation period are constant for each species, regardless of the actual length of the embryo. If the embryonic length is reduced (Cooke, 1975), the wave is postulated to move at a slower rate than in normal embryos, resulting in a normal number of somites. By

suggesting that the clock is coupled to a standing gradient, rather than a propagating event, Slack (1983) has since produced a slight modification to the model, allowing it to explain the control of somite number somewhat more elegantly: it is easier to envisage how an embryo might adjust the slope of a gradient rather than the propagation rate of a wave.

Whether the metameric pattern is under a global control of the kind predicted by this model, or whether instead it represents the sum of a series of cell-autonomous events, is an important unresolved issue. The regulation of somite number may prove to be the exception rather than the rule during vertebrate development. It has been demonstrated in *Xenopus* (Cooke, 1975) and in a mouse mutation (Flint *et al.* 1978); but treatment of mouse embryos with Mitomycin-C, which kills up to 80 % of the cells of the embryo, greatly alters the resulting number of somites (Snow & Tam, 1979; Gregg & Snow, 1983; Snow & Gregg, 1986); and the same is true for a variety of species reared at abnormal temperatures (e.g. bony fish – Orska, 1962; amphibians – Lindsey, 1966; reptiles – Fox, 1948; birds – Lindsey & Moodie, 1967; mammals – Lecyk, 1965; reviewed by Fowler, 1970).

To date, all the evidence advanced in support of the model comes from one class of experiment, examining the effects of heat shock. Exposure of *Xenopus* embryos for a brief period to an abnormally high temperature (37·5 °C), at an appropriate time during development, produces single localized anomalies of the somite pattern. These anomalies have been explained by supposing that heat shock alters the action of the wave at a single critical point in its passage, resulting in somite abnormalities a short while later. The delay between the time of the heat shock and the appearance of the anomalous segments reflects the time interval between the commitment of a group of cells to segment and the expression of that commitment (Pearson & Elsdale, 1979; Elsdale & Davidson, 1986).

Recent heat-shock experiments in chick embryos (Primmett *et al.* 1988*a*) are not consistent with this interpretation, however. As in *Xenopus*, a single heat shock applied to 2-day chick embryos can generate discrete somite and vertebral anomalies; but these anomalies appear at *repeated* positions (up to four) along the body axis, with a reliable and constant repeat interval of 6–7 somites between them. Because a pair of somites forms every 100 min, this interval corresponds remarkably closely to the cell cycle time (9–10 h) in the segmental plate mesoderm (Primmett *et al.* 1988*b*). This observation supports the idea that there is an oscillatory event, perhaps linked to the cell cycle, which plays a role in gating those cells that will segment together and is susceptible to heat shock. It is not compatible, though, with the notion of a *single* event corresponding to a determinative phenomenon, which is susceptible to heat shock.

(E) *A cell-cycle model for segmentation*

The segmental plate of the chick embryo contains some 12 presumptive somites. This implies that the third and fourth anomalies observed following heat shock (Primmett *et al.* 1988*a*) are probably associated with cells that have not entered the plate at the time of shock. One should also bear in mind that the creation of an intersomite boundary simultaneously defines the posterior half of the somite actually forming and the anterior half of the next somite to form. In other words, somite formation proceeds in 'parasegmental' rather than segmental steps (cf. *Drosophila*; Martínez-Arias & Lawrence, 1985).

We have recently proposed a simple model for the control of segmentation, which allocates populations of presomitic cells to individual parasegments (Primmett *et al.* 1988*b*). The model proposes the following.

(1) The time interval between the segmentation of consecutive parasegmental precursor populations (100 min) is equal to 1/6 or 1/7 of the cell division cycle.

(2) There is some degree of cell division cycle synchrony between those cells destined to segment together.

(3) A short time before segmentation, those cells destined to form part of the same parasegment increase their adhesion to one another, regardless of their position within the segmental plate.

(4) This increase in cell adhesion always takes place at the same time point of the same cell cycle, a fixed number of cell cycles after that cell population becomes committed as somitic. The segmenting cells can sort out from cells that are not yet competent to form a parasegment, that is, from those destined to form other parasegments.

(5) 1/6–1/7 of the cell cycle duration after formation of a parasegment, the cells become epithelial, forming the somite.

This model suggests that heat shock and other disturbances (Primmett *et al.* 1988*a*,*b*) transiently arrest the clock at some critically sensitive phase of the cell division cycle, so altering the size of the group of cells that become adhesive at the time of segmentation. The repetitive nature of the anomalies is accounted for, as the sensitive phase recurs during the lineage history of the segmenting cells. The model proposes that cells destined to form the same parasegment possess a certain amount of cell division synchrony. This has, in fact, been found to be the case: a high proportion of the cells at the anterior and posterior ends of the segmental plate are in the M-

phase of the cell cycle (Stern & Bellairs, 1984*b*; Primmett *et al.* 1988*b*).

To account for the rhythm of somite formation, a degree of hysteresis must be postulated. Hysteresis could be achieved, for example, if there were two special time points around the time of the mitotic division occurring nearest the time of somite formation (that nearest the cranial end of the segmental plate). The first of these might occur towards the end of the G_2 phase immediately preceding somite formation, and the second would be close to the start of the next G_1 phase. The time interval between the two points should be 1·5 h. The synchrony between those cells destined to segment together is not perfect, however, and some cells will arrive at the G_2 point earlier than others. These 'pioneer' cells might produce a signal, to which any cell situated between the two special points of the same cell cycle would respond by increasing its adhesion to its similarly responding neighbours. This mechanism punctuates the pattern, the size of each parasegment being determined by the number of cells that respond together at the critical time period.

This model differs from those discussed above in that the metameric pattern is generated by a process that does not rely on global signals, is linked to the cell division cycle and is independent of the position of the cells within the embryo. It is also important to note that it requires no separate oscillator with a period shorter than that of the cell division cycle.

Segmentation, cell lineage and cell determination

For a full understanding of the process of vertebrate segmentation, it is important to know when cells become committed to particular fates. We also need an accurate description of the lineage of the cells involved, which is not yet available. These matters are discussed in detail elsewhere (Stern *et al.* 1988), and we will draw only the main conclusions here.

Since an isolated chick segmental plate can form somites (e.g. Packard & Jacobson, 1976), a mesoderm cell is determined as somitogenic at the *latest* by the time of its entry into the segmental plate, which is equivalent to about 12 somites (2 cell cycles) before overt segmentation. The *earliest* time at which determination could occur is less certain, and may vary for different vertebrate classes. In the developing zebrafish, Kimmel & Warga (1986) have shown that individual gastrula cells produce clones that are confined to single tissues, such as the somitic mesoderm. The descendants of pregastrula cells, however, are not so restricted, suggesting that heritable restrictions in cell fate first arise during gastrulation in this species. Cell lineage experiments in the chick, however, suggest that there exists a population of cells at the posterior end of each segmental plate which gives rise both to somite tissue and to other mesodermal derivatives, including blood, vascular endothelium, mesonephros and the lining of the coelom (Stern *et al.* 1988).

Experiments using allophenic mice (Gearhart & Mintz, 1972) have shown that somites are not derived from a single founder cell. It would be interesting to know whether somites (or parts of somites) represent lineage 'compartments' comparable to those of the epidermis of the fly, in which the cells constituting the compartment comprise *all* the surviving progeny of the founder cell group. In the case of the zebrafish, an early gastrula cell can give rise to progeny in more than one somite-derived myotome along one side of the embryo (Kimmel & Warga, 1987). If, then, a myotome is a compartment, its founder cells must be generated at a stage later than early gastrulation. The results of single-cell lineage experiments mentioned above (Stern *et al.* 1988) are not consistent with the idea that chick segments are compartments. After injection of a single segmental plate cell with a fluorescent marker, the labelled descendants that participate in somite formation (about 30–40 cells after two days) are confined to a region one segment in length, and the clones never cross more than one A–P boundary. However, while some of these clones are in register with a segment, others are aligned with a parasegment, indicating that there is overlap between the lineages of segments and parasegments.

The fates of cells destined to form dermomyotome and sclerotome in the chick embryo appear to diverge around the time of somite formation (Gallera, 1966; Jacob *et al.* 1974). Various arguments (discussed in greater detail elsewhere: Stern & Keynes, 1986; Stern *et al.* 1988) lead us to believe that the distinction between A- and P-sclerotome cells is also established at about the same time, during the process of epithelial somite formation. Those cells that remain at the anterior end of the segmental plate, adjacent to a somite boundary for some time before epithelialization, become anterior; those that separate from the remainder of the segmental plate during epithelialization become posterior.

It is also important to know when the *regional* properties of the somite derivatives (skeletal elements, dermis and muscle) become determined during segmentation. The myoblasts derived from the dermomyotome probably become committed to form particular muscles, with appropriate motor innervation, only when they reach their destinations, such as the limb (Christ *et al.* 1977; Chevallier *et al.* 1977; Keynes *et al.* 1987). The skeletal and dermal somite derivatives, on the other hand, may be specified regionally at an earlier stage. For example, after

transplantation of thoracic segmental plate to the cervical region in the chick embryo, the graft gives rise to thoracic vertebrae and ribs (Kieny *et al.* 1972), and to a thoracic plumage pattern (Mauger, 1972).

Segmentation and neural development

In higher vertebrates, the subdivision of the sclerotomes into anterior and posterior halves is responsible for generating the segmental arrangement of the peripheral nervous system. Both motor and sensory roots (Keynes & Stern, 1984) and sympathetic ganglia (Lallier & Bronner-Fraser, 1986) develop in phase with the anterior half-sclerotomes (Fig. 3). While the A–P subdivision also exists in fishes and amphibia (see Keynes & Stern, 1984), it is less certain that it determines peripheral nerve segmentation in all lower vertebrate species. In salmon trout embryos, for example, the segmental dorsal root ganglia develop in the anterior half-sclerotome (Fig. 4); but in another teleost, the zebrafish, the primary motor axons pioneer pathways on each myotome from a midsegmental ventral root (Eisen *et al.* 1986). In *Xenopus* embryos, motor axons (Kullberg *et al.* 1977) and primary sensory axons (Taylor & Roberts, 1983) grow out from an intersegmental position. It seems likely that in some lower vertebrates the earliest peripheral nerves grow out at a stage when few sclerotome cells intervene between the spinal cord and the myotomes. Under these conditions, while segmentation in the mesoderm is still likely to be the overriding influence causing segmentation of the spinal nerves (Lehmann, 1927; Detwiler, 1934), myotome cells or intersegmental extracellular matrix may be more important determinants of axon position than sclerotome cells.

The preference of axons and crest cells for anterior rather than posterior sclerotome provides an attractively simple system for the molecular analysis of nerve cell guidance; differences must exist between A- and P-sclerotome cells, which can be detected by growing nerve cells (Keynes & Stern, 1984). Immunohistochemical studies using antibodies to laminin and fibronectin, both of which are known to influence axon and crest cell growth *in vitro*, have failed to reveal any differential distribution of these molecules within the sclerotome (Rickmann *et al.* 1985; Krotoski *et al.* 1986; Duband *et al.* 1987). The same holds for the adhesion molecules N-CAM and N-cadherin (Duband *et al.* 1987). To date, the following differences between A- and P-sclerotome have been identified: the localization of binding sites for peanut lectin (PNA) to P cells (Stern *et al.* 1986); the localization to A cells of the (probably identical) glycoproteins cytotactin (Tan *et al.* 1987) and tenascin (Mackie *et al.* 1988), and of butyrylcholinesterase activity (Layer *et al.* 1988); and the localization to P cells of a cytotactin-binding proteoglycan (Tan *et al.* 1987).

The differences detected with PNA appear to be directly related to qualitative changes in the surface glycoprotein structure of A and P cells. *In vitro*, axons grow more extensively on A cells than on P cells (Stern *et al.* 1986; Tosney, 1987), and preliminary experiments with affinity-purified PNA receptors indicate that inhibition of crest and axonal migration by these receptors may be responsible for the observed preference for A cells (J. Davies, G. M. W. Cook, R. J. Keynes & C. D. Stern, unpublished observations). The properties of the cytotactin-binding proteoglycan described by Tan *et al.* (1987) may also be relevant here: the molecule becomes concentrated in the posterior half-sclerotome and, *in vitro*, provides a poor substrate for crest migration. It is possible, then, that the proteoglycan is inhibitory for crest migration *in vivo*, but since this molecule is evenly distributed within the sclerotome during the initial phase of neural crest migration, it cannot be solely responsible for the segmented pattern of crest migration.

During the first stages of somite formation in chick embryos, cytotactin is localized to the basal lamina surrounding the epithelial somite (Crossin *et al.* 1986). By the 30 somite stage, however, it also becomes detectable in the anterior halves of the newly-formed sclerotomes (Tan *et al.* 1987), correlating with the simultaneous appearance here of neural crest cells. Tenascin has a similar distribution in quail embryos (Mackie *et al.* 1988). Since cytotactin appears in the sclerotome after ablation of the neural crest, and both cytotactin and tenascin are produced by cultured somite cells, these authors suggest that the source of cytotactin/tenascin is sclerotome rather than neural crest. Tan *et al.* (1987) and Mackie *et al.* (1988) also find that neural crest cells round up when cultured on cytotactin/tenascin. It is not entirely clear, at present, how to reconcile this apparently poor adhesion of crest cells to cytotactin/tenascin *in vitro* with the evident preference of the crest for A- rather than P-sclerotome *in vivo*. One possibility is that cytotactin/tenascin is important for preventing presumptive dorsal root ganglion cells from migrating far from the neural tube. Since dorsal root ganglia do not form in the occipital sclerotomes (Lim *et al.* 1987), it will be interesting to determine the distribution of cytotactin/tenascin at these segmental levels. Another possibility is that stronger repulsion by P cells is the more critical event in directing crest cells through the sclerotome.

Segmentation in the central nervous system

To understand the development of the vertebrate central nervous system (CNS), it is important to identify the mechanisms that provide the CNS with regional variations in connectivity and cell arrangement. There are, for example, obvious anatomical differences between the adult forebrain and spinal cord, and more subtle regional differences along the length of the spinal cord. Could early segmental subdivisions be instrumental in achieving regional specification of the CNS?

Morphological segments in the developing neural tube ('neuromeres') have, in fact, been recognized for many years, following their original description by von Baer in 1828 (see Vaage, 1969, for review). In the cranial region, there is a striking positional correspondence between the anterior neuromeres, which later give rise to the fore-, mid- and hindbrain, and the adjacent somites (in lower vertebrates; Goodrich, 1918), somitomeres (in higher vertebrates; Jacobson & Meier, 1984), and branchial arches. In chick embryos, the hindbrain segments ('rhombomeres') are particularly conspicuous, and are separated by boundaries of low cell density (Lumsden & Keynes, in preparation).

Despite the many descriptions of neuromeres their significance remains unclear. It is tempting to suggest that they might represent units of cells with related lineal origins, perhaps analogous to invertebrate compartments, with distinct end-products in terms of defined CNS regions; the lineage analysis necessary for this to be confirmed, or otherwise, has yet to be undertaken. There have also been remarkably few attempts to relate the overt neuromeric pattern to any underlying pattern of development of individual neurones within the neural tube. There may, for example, turn out to be a tight correspondence between particular cranial nerve nuclei and their origins from particular rhombomeres, as was suggested by Streeter (1908) and later denied by Neal (1918). Recent observations (Lumsden & Keynes, in preparation) do suggest such a relationship (Fig. 6). In the larval zebrafish hindbrain, serially repeated clusters of reticulospinal neurones have been described (Metcalfe *et al.* 1986), which may also reflect segmental development within the CNS. It is interesting that the number of neuronal clusters (seven) in the zebrafish hindbrain exactly matches the number of rhombomeres in the chick. Segmentally arranged neurones have also been described in the spinal cord of *Amphioxus* (Bone, 1960) and several vertebrates (Huber, 1936; Whiting, 1948; Anderson *et al.* 1964; Myers, 1985). Finally, the recent description of a monoclonal antibody with specificity for neurones in the telencephalon, but not other CNS regions (Mori

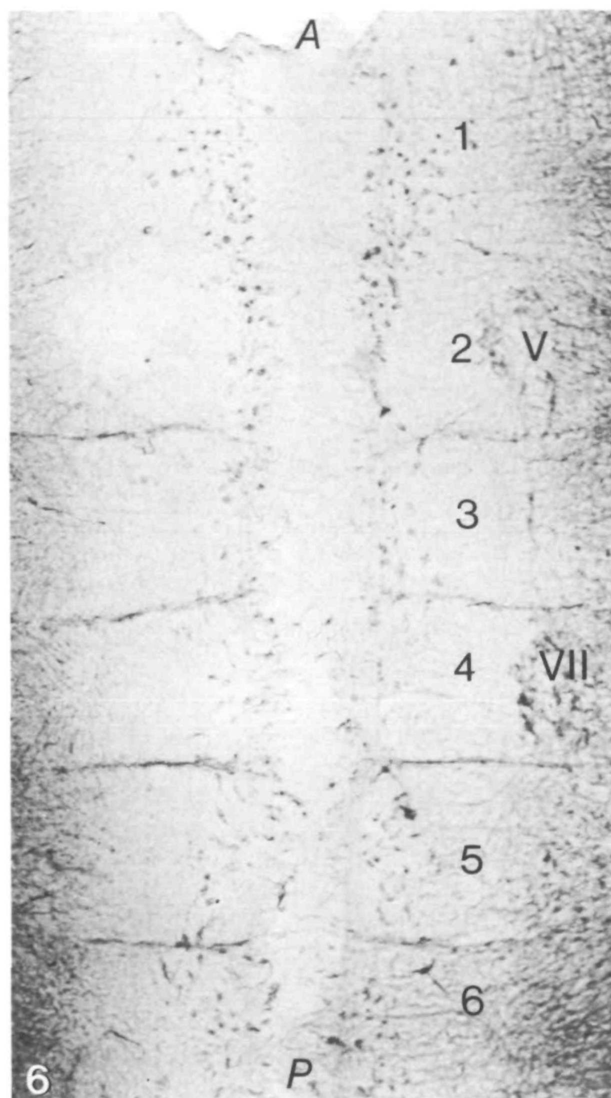


Fig. 6. Rhombomeres in a chick embryo at stage 16. Whole mount of hindbrain, viewed from pial aspect, stained with anti-68×10^3 M_r neurofilament antibody (gift of Dr P. Hollenbeck), and visualized by indirect immunoperoxidase. The rhombomeres are labelled '1' through '6'. The spinal roots of cranial nerves V (trigeminal) and VII (facial) lie in the middle of rhombomeres 2 and 4, respectively (they were avulsed completely on the other side, hence not visible there). Axons appear constrained to the inter-rhombomere boundaries. *A*, anterior. *P*, posterior.

et al. 1987), raises the possibility that position-specific antigens may exist in the developing CNS. If more such antigens are identified, it will be important to assess their boundaries of expression in relation to the neuromeric boundaries.

Patterns of expression of vertebrate homeobox genes

The advent of molecular genetics has stimulated

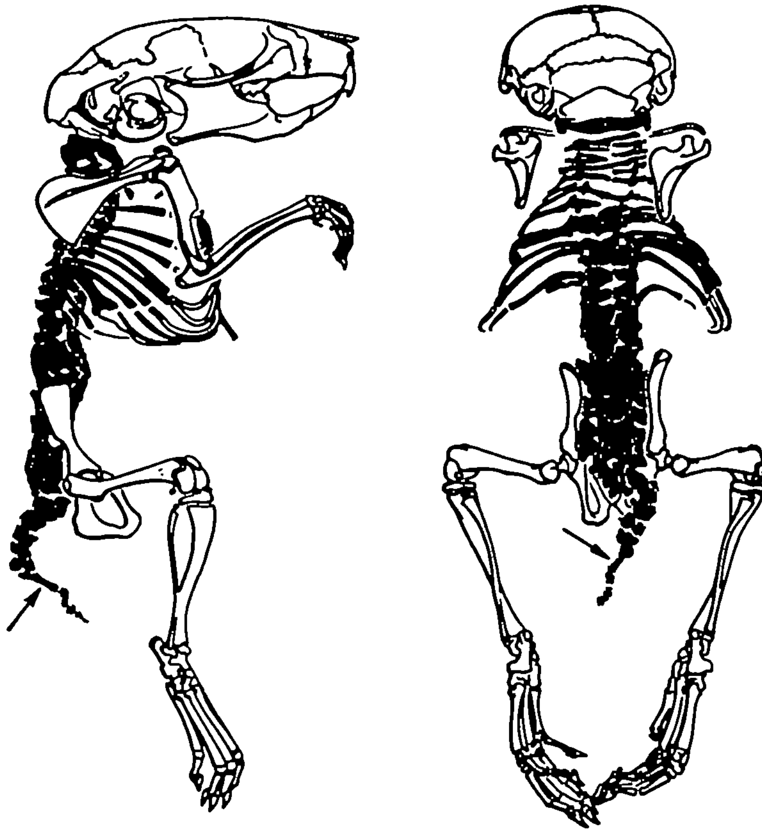


Fig. 7. Skeleton of 23-day-old female mouse, homozygous for the mutation '*pudgy*' (*pu*). Grüneberg describes the phenotype most graphically: 'The whole axial skeleton is in a state of chaos. It is astonishing that an animal can live and occasionally even breed in such a frame.' The arrow shows the single normal vertebra. (Reproduced from Grüneberg, 1963, with kind permission of Cambridge University Press.)

considerable interest in the development of body pattern in *Drosophila* (for review, see Akam, 1987). With the discovery of the homeobox in the vertebrate genome (McGinnis *et al.* 1984), the hope has arisen that the same approaches will open the way to an understanding of pattern formation in vertebrate development. There are now many published descriptions of the patterns of expression of homeobox genes in vertebrate embryos, and there has been much speculation about the role of these genes in specifying different regions of the body plan. What is clear, though, is that unlike *Drosophila* the patterns described do not correlate in any obvious way with the process of segmentation. Rather, expression is found along broad regions of the embryonic body, and is often particularly marked in the CNS (see Stern & Keynes, 1988, for review). To take one example (Holland & Hogan, 1988), at 12·5 days of development the mouse homeobox gene *Hox 2.1* is expressed in a domain extending from the hindbrain along the length of the spinal cord; the anterior boundary of expression lies around the level of the first to the third somite, below the otic vesicle. One day later, expression is localized to the occipital and cervical cord; the gene is also expressed in several other embryonic tissues, such as the mesoderm of stomach, lung and kidney, but not in the somites.

Other homeobox genes have different, equally distinctive and overlapping patterns of regional expression. It is intriguing that, of the genes studied so far, none is expressed anterior to the developing hindbrain during early development. Since the anterior boundaries of CNS expression lie within the hindbrain for most of the genes, there may be some connection between the appearance of these boundaries and the development of the rhombomeres. However, with the possible exception of *Hox 1.5* (Gaunt *et al.* 1986), the boundaries of gene expression and rhombomeres do not appear to coincide precisely. If functional parallels with *Drosophila* can be drawn, then, the vertebrate patterns correspond more closely to those of the 'gap' or 'selector/homeotic' genes than the 'pair-rule' or 'segment polarity' genes (Nüsslein-Volhard & Wieschaus, 1980). We might, therefore, expect the vertebrate homeobox genes to be concerned more with the specification of broad body domains than with the development of segmental body patterns.

Vertebrate genes concerned with segmentation

Hans Grüneberg was the first to recognize the importance of studying mouse mutants as a means of understanding developmental patterning processes in vertebrates (Grüneberg, 1943, 1963). In his book 'The Pathology of Development' (Grüneberg, 1963)

he attempted to classify the various skeletal mutants according to the presumed primary site of action of each mutant gene, and included a specific category of mutation causing disorders of segmentation. A useful up-dated account of these mutants, together with others described more recently, has been provided by Johnson (1986).

Several mutants placed in the segmentation class are worth noting. In mice homozygous for '*pudgy*' (Grüneberg, 1961) and '*rib fusions*' (Theiler & Stevens, 1960), the axial skeleton shows extensive fusions between adjacent vertebrae and ribs (Fig. 7), and the sclerotome boundaries are incomplete or irregular. Vertebral and rib fusions are also a feature of the mutations such as '*loop-tail*' (Strong & Hollander, 1949), '*fused*' (Theiler & Gluecksohn-Waelsch, 1956), '*rachiterata*' (Theiler *et al.* 1974), '*crooked tail*' (Matter, 1957), '*malformed vertebrae*' (Theiler *et al.* 1975) and '*rib-vertebrae*' (Theiler & Varnum, 1985), in which irregularities of the pattern of epithelial somites are found during early development. In mutants such as '*undulated*' (Grüneberg, 1954) and '*tail-kinks*' (Grüneberg, 1955), the earliest visible defect lies in the sclerotome, whose subdivision into A and P halves is abnormal.

There is every reason to hope that progress will soon be rapid in characterizing these mutants in more detail at the cell and molecular level. Consistent with the multiplicity of separate loci, one could envisage many different mechanisms causing these phenotypes, ranging from defects in cell–cell adhesion to disturbances in the allocation of cells as A- or P-half-sclerotome. Whether any of these genes will turn out to be analogous to segmentation genes in the fly, such as those of the segment-polarity class, remains an interesting and open question.

Conclusions

We have described in some detail the process of vertebrate segmentation where it is best understood, namely in the somitic mesoderm. Segmentation may also be an important feature of the development of the vertebrate head, CNS, and pro- and mesonephric systems. It seems likely, however, that during the course of vertebrate evolution some body regions (such as the tetrapod head and limb) may have evolved more complex tissue patterns by the fusion of adjacent segmental regions. The apparent lack, in vertebrates, of segmentation mutants analogous to those found in *Drosophila* could suggest that the mechanisms of vertebrate segmentation are fundamentally different to those in the fly; and if segmentation evolved independently in the arthropod and vertebrate lines, perhaps this is not surprising. The similarities, such as the operation of homeobox genes and the subdivision of segments into A and P parts are striking, nevertheless, and may hint at deeper parallels to come. For the moment, the molecular genetic approach to the study of segmentation is much better advanced in *Drosophila*, while more is known about the cell biology in vertebrates. It is to be hoped that those working in the respective fields will have much to learn from each other in the forseeable future.

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