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Development of segmentation in zebrafish

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Summary

Recent findings on the nature and origin of segmentation in zebrafish, *Brachydanio rerio*, are reviewed. Segmented peripheral tissues include the trunk and tail myotomes, that are derived from somitic mesoderm, and the pharyngeal arches that are derived from head mesoderm in addition to other sources. Two major regions of the central nervous system, the spinal cord and hindbrain, are also segmentally organized, as deduced from the distribution of identified neurones in both regions and by formation of neuromeres in the hindbrain that contain single sets of these neurones. Neural and mesodermal segments in the same body region can be related to one another by their patterns of motor innervation. This relationship is simple for the spinal/myotomal segments and complex for the hindbrain/pharyngeal arch segments. Development of the segments is also complex. Mesodermal and ectodermal progenitors have separate embryonic origins and indeterminate cell lineages, and the embryonic cells migrate extensively before reaching their definitive segmental positions. Results of heat-shock experiments suggest that development of myotomal and spinal segments are regulated coordinately in postgastrula embryos. Segmental patterning may be a relatively late feature of zebrafish embryonic development.

Key words: zebrafish, segmentation, metamerism, somite, neuromere, cell lineage.

Introduction

In certain invertebrates, the cellular and genetic events that eventually lead to segmentation begin very early during zygotic development. In leeches, after only a few cleavages, unique precursor cells are produced that embark on specific programs of divisions that sequentially generate founder cells (termed 'blast' cells) for successive segments (Weisblat & Shankland, 1985). Continued divisions of these blast cells give rise to the different kinds of tissues that form a single segment. In *Drosophila*, the first cells to arise during zygotic development express patterns of segmentation genes according to their positions in the blastoderm, and mutational analysis reveals that correct subsequent development depends critically on this initial expression (Nüsslein-Volhard & Wieschaus, 1980; for a review see Scott & O'Farrell, 1986). At the same stage, at least some of the blastoderm cells, those that will go on to develop epidermal derivatives of the adult, acquire segment-specific 'compartmental identities' that restrict the territories that their descendants will come to occupy (Garcia-Bellido *et al.* 1973).

In contrast, our understanding of the origin of segmentation of the vertebrate body is substantially less advanced. There is controversy about the extent to which different body structures are segmental in their organization (Northcutt & Gans, 1983; Jarvik, 1980). Even for structures such as somites that most researchers would agree are segmental, there is only the most general understanding of the cellular events and essentially no understanding of the genetic events that underlie the patterning. Here we describe recent work on segmentation and its origin in zebrafish, a simple vertebate amenable to both detailed developmental (e.g. Eisen *et al.* 1986; Kimmel & Warga, 1986) and genetic (Streisinger *et al.* 1981; Grunwald *et al.* 1988) study. In this creature, at least four series of segmented structures, two in the mesoderm and two in the central nervous system (CNS), can be identified by the second day of embryonic development

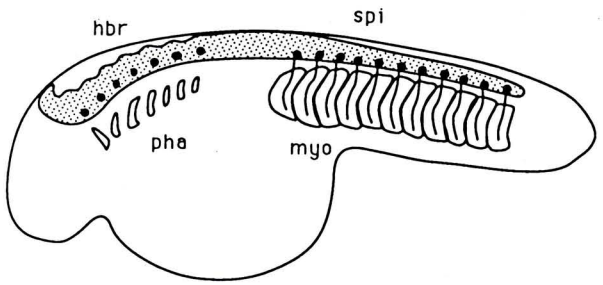

Fig. 1. Segmentation in the zebrafish embryo. By the definition that a segment means a set of structures iterated serially along the body axis (Stent, 1985), at least four segmental series of structures, illustrated schematically here in a left-side view of the embryo, can be identified by the end of the first day of embryonic development. Segmented structures in the periphery are the 7 pharyngeal arches (pha) of the head and 30 myotomes (myo) of the trunk and tail. Corresponding regions of the CNS, the hindbrain (hbr) and spinal cord (spi) are also segmented.

(Fig. 1). We summarize what we presently know, as well as what we would like to learn, about these separate patterns and their relationships to one another. In particular, we will show that the developmental processes that generate and assemble a 'body segment' (or 'metamere') in this vertebrate are complex, and they occur relatively late in development, as compared with the invertebrate species mentioned above. This may mean that in vertebrates, segments are patterned only secondarily, after initial processes that generate the different organ systems are well underway.

Mesodermal segments in the zebrafish embryo

As in other vertebrates, an early clear indication of segmentation in zebrafish is in trunk paraxial mesoderm, as somites form in a rostrocaudal sequence beginning shortly after epiboly is completed. The great majority of early somitic cells are myotomal and differentiate as axial skeletal muscle fibres. A number of hours after the trunk somites are present, mesodermal segmentation in the head arises in the form of the pharyngeal arches. These structures are eventually comprised of derivatives of all three germ layers, including the neural crest (Langille & Hall, 1988). When they can first be identified, and for at least 1–2 days thereafter, the pharyngeal arch segments are very much shorter in length along the rostrocaudal axis than the myotomal segments of the corresponding stage. The differences in both time of appearance and segment length suggest that the patterning of these two sets of mesodermal segments occurs separately and independently during development.

Segmental patterning of the central nervous system

Analysis of the distribution of embryonic neurones has provided evidence for segmental patterning of the major part of the length of the CNS; namely the spinal cord and the hindbrain. This is a new kind of evidence for segmentation and we treat it in some detail. As will be discussed further below, it is interesting that the neural segmentation is observed in the same body regions in which the mesoderm is segmented.

Primary motoneurones are present segmentally in the spinal cord (Westerfield *et al.* 1986; Myers *et al.* 1986). These neurones are prominent cells, and probably the first neurones to differentiate in the ventral part of the CNS (Hanneman & Westerfield, 1988; for a review see Kimmel & Westerfield, 1988). Primary motoneurones are present in clusters in the spinal cord, a single cluster being present on each side of each spinal segment (Fig. 2). A spinal segment corresponds in length to a myotomal segment, and the spinal and myotomal segments are in close

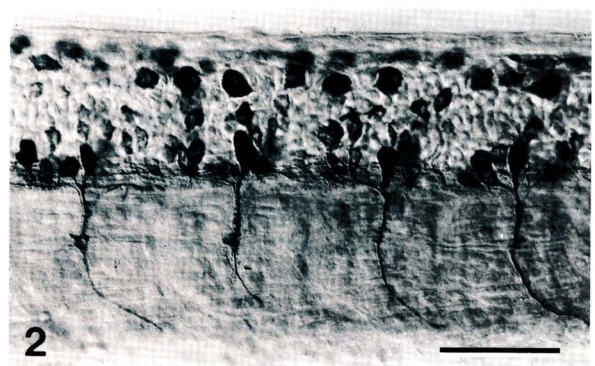

Fig. 2. Primary motoneurones are segmentally arranged in the zebrafish spinal cord. Embryonic neurones in a parasagittal section through an embryo at 24 h (h: hours postfertilization at 28·5 °C), were labelled using the neurone-specific monoclonal antibody zn-1 (described in Trevarow, 1988). Spinal segments 8 through 11 were photographed, using Nomarski interference contrast optics to show also unlabelled cells. The spinal cord is in the upper part of the figure and myotomes are in the lower part. Rostral is to the left and dorsal is to the top. In each segment, a cluster of motoneurones project axons through a single ventral root that courses ventrally through the middle of each myotome (Myers *et al.* 1986) (myotomal borders are faintly visible). The caudalmost cell in each cluster, named CaP, is darkly labelled. Its axon projects to the ventral part of myotome, curving caudally as it does so. Two other primary motoneurons, MiP and RoP (Eisen *et al.* 1986), are present in each cluster. In addition to some labelled interneurones, a row of distinctive neurones are labelled in a dorsal row. These are sensory Rohon-Beard cells, present in variable numbers in each segment. The preparation was made by R. BreMiller. Bar, 50 μm.

register along the entire length of the trunk and tail (unlike amphibians; see Westerfield & Eisen, 1985). Three kinds of primary motoneurones have been distinguished on the basis of their positions within the clusters, and on the projection of their axons within the periphery; the three innervate nonoverlapping territories of somitic axial muscle (Westerfield *et al.* 1986). A single motoneurone of each type is present in each cluster; thus primary motoneurones are uniquely identifiable cells. The three primary motoneurones in the same cluster grow their axons in a strict order, relative to one another, but there is no strict temporal relationship between outgrowth in adjacent segments, or in opposite sides of the same segment (Eisen *et al.* 1986; Myers *et al.* 1986).

We do not know if other types of spinal neurones are segmentally distributed. Spinal interneurones that arise earliest in development may be present segmentally, as suggested from the appearance of antibody-labelled preparations (unpublished observations). Rohon-Beard neurones, dorsally located sensory cells, are present in variable numbers in each segment and do not show any segmental patterning in their peripheral arbours (Myers *et al.* unpublished data). Likewise, secondary motoneurones, which arise several hours after the primary ones (Myers *et al.* 1986), are not arranged in an obviously segmental manner, but appear to occupy a rather continuous column along the ventral cord. Segmental patterning in the spinal cord might thus involve only a subset of its neurones.

The hindbrain is also segmented. There are about nine segments, three each in its 'rostral', 'middle' and 'caudal' regions (Hanneman *et al.* 1988). The rostral seven of these segments are well known; they contain sets of interneurones, termed reticulospinal neurones (Fig. 3), and early in development the segments form prominent swellings along the surface of the neural tube that are termed neuromeres (Fig. 4). The length of a hindbrain segment, at stages when the neuromeres are most prominent, is about the same as a spinal segment (and also a myotome).

Small and specific groups of reticulospinal neurones develop in the centres of each neuromere (Mendelson *et al.* 1986*a*,*b*; Hanneman *et al.* 1988). These first reticulospinal neurones to differentiate eventually develop into prominent and distinctive cells that, like the spinal primary motoneurones, can be identified individually (Kimmel *et al.* 1982; Metcalfe *et al.* 1986). Specific reticulospinal neurones occupy characteristic locations in specific hindbrain segments, and generally a cell in one segment shares certain morphological features with particular cells at equivalent positions in adjacent segments. By these shared features, the neurones were classified into a number of families, and it was proposed that each

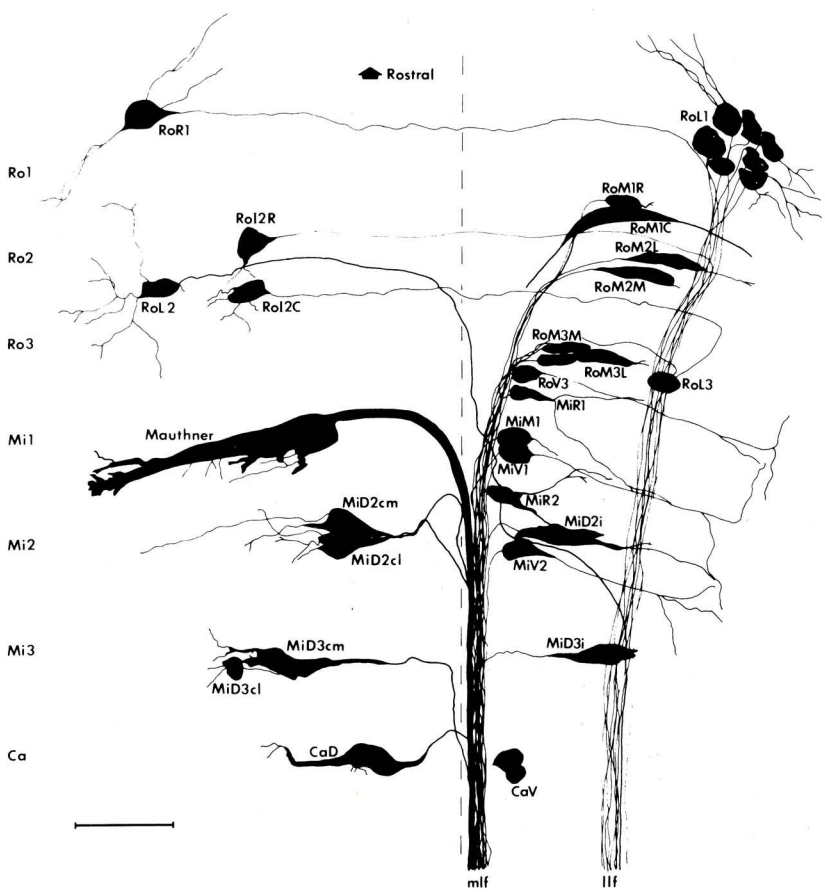

Fig. 3. Hindbrain reticulospinal neurones are segmentally distributed. A dorsal view (rostral to the top), of the 5-day-old larval hindbrain, revealing a ladder-like pattern of cells. The cells at each rung of the ladder (labelled Rol-Ca on the left side of the drawing) develop within a single embryonic neuromere (cf. Fig. 4). Neurones that have crossing projections to the spinal cord are shown on the left and those with ipsilateral projections shown on the right. In the animals, all of them are present bilaterally; most of the types (19/27) represent single identified cells on each side of the midline; others (e.g. RoL1 neurones) are present in clusters. Except for the Mauthner cell, the cells are named according to their positions and axonal projections. (From Metcalfe *et al.* 1986.) Bar, 25 μm.

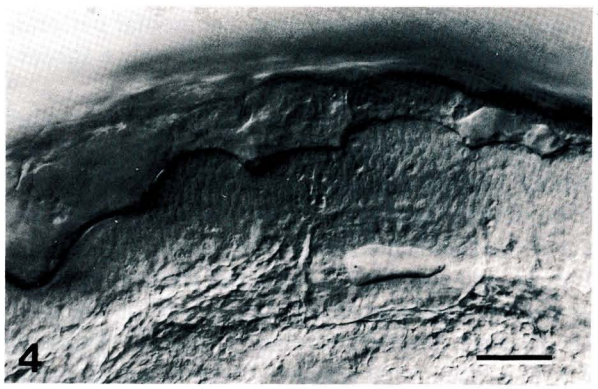

Fig. 4. Neuromeres are present in the hindbrain of the zebrafish embryo. A live 18 h embryo was photographed from the left side (dorsal to the top), using Nomarski optics. The otic vesicle is visible beside the brain. (From Hanneman *et al.* 1988.) Bar, 50 μm.

family represented a set of segmental homologues (Metcalfe *et al.* 1986).

For example, the axon of the Mauthner neurone (the best known of the reticulospinal cells) crosses the midline within its own segment and then descends to the spinal cord by a particular route. Equivalent pathways are taken by only two other reticulospinal cells (named MiD2cm and MiD3cm; see Fig. 3). One of these neurones is present in each of the next two more caudal segments. The three neurones (i.e. the set including the Mauthner neurone) share other specific morphological (Kimmel *et al.* 1982) and developmental characteristics, including when they are born and grow axons (Mendelson, 1986*a*,*b*). Thus, in spite of some differences (that can be considered to be segment-specific) being present among these three identified cells, it seems reasonable to imagine that they share important determinants of development. In the case of other families of reticulospinal neurones, the iterated cells are nearly exact copies of one another, as is also observed of the spinal primary motoneurones (Myers *et al.* 1986).

After the first neurones begin to differentiate in each hindbrain segment others are added in the same segmental pattern, including other reticulospinal neurones (Mendelson, 1986*b*) and neurones of other types, as demonstrated by staining them with monoclonal antibodies (Trevarrow, 1988). Radial glial fibres also develop segmentally; rows of these fibres form partition-like structures between the central region of each neuromere and its rostral and caudal borders (Trevarrow, 1988). Study of this later period of development is hampered by the fact that a substantial compression of the hindbrain occurs longitudinally along the neuraxis (Hanneman *et al.* 1988), which tends to obscure the segments and the segmental relationships among the neurones they contain. For example, seven motor nuclei, representing cranial nerves IV through VII (and including two nuclei each for nerves V–VII) are located in the region of the hindbrain in 5-day-old larvae that develops from the first seven hindbrain neuromeres of the embryo (Kimmel *et al.* 1985). One possibility is that a single one of these motor nuclei arises within each neuromere, yet because of the compression, such a strict relationship cannot be deduced by examining the larval brain directly; developmental studies of the segmental origins of cranial motoneurones need to be carried out.

The caudal region of the hindbrain is not as well characterized. Motor nuclei of two more cranial nerves, IX and X are present there. The region is one of transition between the brain and spinal cord; two types of interneurones (T-interneurones and ic interneurones) located in the caudal hindbrain segments are also found in rostral spinal segments. These interneurones both occur not once, but about twice per segment (see Kimmel *et al.* 1985; Trevarrow, 1988). As in the spinal cord, neuromeres are not prominent.

Patterns of motor innervation

The segments of the CNS and the mesoderm can be related to one another by considering the patterns of motor innervation. For the spinal myotomal segments of the trunk and tail this relationship is simple and direct: the single set of primary motoneurones present in each segment in the spinal cord projects through a single ventral root (Fig. 2) and their synapses are restricted to the immediately overlying myotome (Westerfield *et al.* 1986).

In the head pharyngeal region, there does not appear to be a simple 1:1 relationship between the series of hindbrain neuromeres and the pharyngeal arches, although the pattern of innervation is not completely known. Motor nuclei present in some of the hindbrain segments, including the first segment and two located more caudally, do not supply the pharyngeal arches at all, but project to extrinsic eye muscles (Kimmel *et al.* 1985). If relationships that have been well shown for other teleosts hold for the zebrafish, then two motor nuclei (of nerve V) supply the first, or mandibular, pharyngeal arch and two motor nuclei (of nerve VII) supply the second or hyoid arch.

The pattern of innervation of the pharyngeal arches that form the definitive gills is particularly interesting. As described for adults of other teleosts (Kanwal & Caprio, 1987; Morita & Finger, 1987), in the zebrafish embryo cranial nerve IX seems to innervate a single pharyngeal arch (the rostral-most gill arch) while

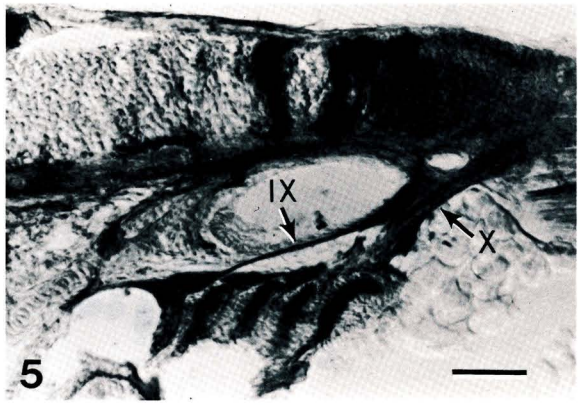

Fig. 5. Innervation of the gill arches (near the bottom of the photograph) derives from the caudal hindbrain (top). Nerve IX (the glossopharyngeal) innervates the first gill arch and nerve X (the vagus) innervates the remaining ones. Sagittal section (dorsal upwards and rostral to the left) from a 48 h embryo stained with the monoclonal antibody zn-5 that labels a variety of structures in this section (see Trevarrow, 1988), and photographed with Nomarski optics. The otic capsule is in the centre of the photograph (compare with Fig. 4). Hindbrain segment length is indicated by the staining of two vertical bands of cells in the upper centre; these are hindbrain commissural cells that flank a single neuromere (Trevarrow, 1988). Each pharyngeal arch is also lined by stained cells. Note that pharyngeal segment length is somewhat less than hindbrain segment length at this stage. Original data of B. Trevarrow. Bar, 50 μm.

nerve X innervates a series of four arches (those behind the first one) (Fig. 5). Both nerves stem from the caudal hindbrain, a region that could include only three segments (Hanneman *et al.* 1988). However, Kanwal & Caprio (1987) have described the distribution of these nuclei in the adult catfish as being present in an 'overlapping segmental pattern'. Their analysis (see also Morita & Finger, 1987) revealed a rostrocaudal somatotopic ordering of motoneurones innervating the gills within the vagal lobe; a region derived from the caudal hindbrain of the embryo. One possibility suggested from their study is that we have incorrectly assigned three segments to the embryonic caudal hindbrain and, in fact, more are present, compressed into less space than the segments that neighbour them rostrally and caudally. Another possibility, that seems more likely at present, is that the 'segmental' pattern of innervation of the gills is not one in which a single arch is innervated by motoneurones derived from a single neural segment of the embryo.

Cell lineage and morphogenesis of segments

As mentioned in the Introduction, particular stereotyped cell lineage relationships are present among cells that form individual body segments in invertebrates. Therefore, it is of intererest to examine the lineages of segmentally related cells in zebrafish. Use of lineage-tracer dye techniques revealed that early cleavages generate clonal families of cells that typically develop a great variety of cell fates in a highly indeterminate fashion (Kimmel & Warga, 1987*a*). In contrast, cells present later, at gastrula stage, regularly generate clones restricted to a single kind of tissue (Kimmel & Warga, 1986).

At the onset of gastrulation, the progenitors of the mesodermal and neural segmented tissues mostly lie in separate fields (Fig. 6A). This is unlike the case in segmented invertebrates, where the cells whose progeny contribute different structures within the same metamere lie in fairly close register with one another even at early developmental stages. Rather, in zebrafish the segments are assembled after rather extensive cell morphogenesis, occurring during epiboly and subsequently. In particular, as shown in Fig. 6B,C, there is considerable shearing of the relative positions of ectodermal and mesodermal cells during the convergence and extension movements of gastrulation (Keller *et al.* 1985).

Not only do the mesodermal and ectodermal cells move separately relative to one another, but also, cells lying near one another within one of the fields at the beginning of gastrulation tend to move far apart. For example, Fig. 7 shows a single clonal group of cells in the early gastrula that eventually generated muscle fibres distributed over 11 segments. Such scattering precludes fate mapping the positions of progenitors of individual myotomes during gastrulation, and strongly suggests that the cells have not acquired any specification of segmental identity throughout at least the gastrula period.

Similarly, prospective neural cells scatter after gastrulation begins, suggesting that they have not been committed to occupy particular segmental locations either. The fate map positions of hindbrain and rostral spinal cord are completely overlapped in the dorsal half of the early gastrula (Fig. 6A); cells sort from a common early field into these two distinct regions of the CNS. A feature that is not shared with the mesoderm is that during CNS morphogenesis, dispersed clonal patterns of neural cells arise that have a regular appearance; including cells distributed bilaterally and periodically along the neuraxis (Fig. 8). Sometimes the neurones in such clones can be identified as bilateral or segmental homologues (e.g. Kimmel & Warga, 1986; 1988). In other cases, the cells differentiate as different neuronal types (work in progress). Moreover, quantitative analysis showed that whereas clonally related cells are distributed periodically along the neuraxis, the period

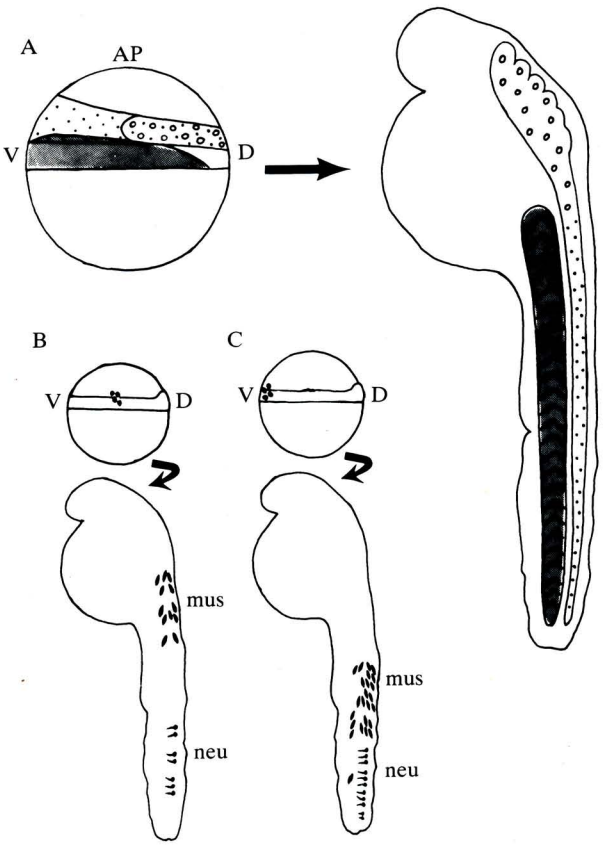

Fig. 6. (A) Progenitors of myotomes (shaded) and neural segments (stippled for the spinal cord and open circles for the hindbrain) occupy largely separate positions at the onset of gastrulation, at 50 % epiboly. Somitic (and also probably pharyngeal arch) mesoderm derives from cells present in a crescent-shaped zone largely in the ventral half of the blastoderm, and near its margin where it borders the yolk. Hindbrain and spinal cord progenitors map to a band of cells that completely encircles the blastoderm, including its dorsal half (i.e. opposite to most of the prospective muscle cells) and a little distance away from the blastoderm margin. The fate map was obtained by labelling single cells at late blastula stages, scoring the positions of the clones relative to the embryonic shield (that marks the dorsal side) in the early gastrula, and scoring fates of the labelled cells after they had differentiated in the embryo at 24–30 h. (B,C) The effect of shearing in the cell movements that generate these tissues. They show the positions of myotomal muscle cells (*mus*) and spinal neural cells (*neu*) present in single clones derived from injection of a lateral (B) and ventral (C) cell present within the zone where somite and spinal cord overlap in the fate map. Notice that in both cases, even though the progenitors of both tissues started from the same position, the labelled muscle cells come to lie in segments rostral to the labelled neural cells. *AP*, animal pole; *D*, dorsal; *V*, ventral (from Kimmel & Warga, 1987*a*; and in preparation).

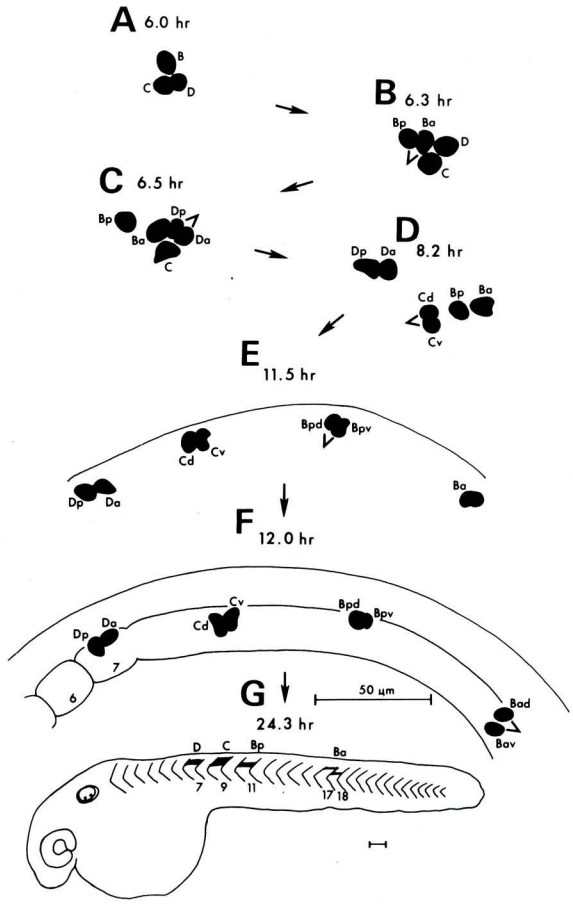

Fig. 7. Progenitors of somitic muscle cells disperse along the embryonic axis. The movements and divisions of individual cells, which were present in a single clone that was labelled earlier with lineage tracer, were followed in a live embryo during gastrulation and somite formation. The designations of the cells code their lineage relationships (e.g. cell B divides (v) at 6·3 h (B) to generate cells B.a and B.p). The cells invert their positions and move apart, with most of the movement occurring between 8 and 12 h, prior to segmentation of the somites they come to occupy. Note that a division at 12 h (F) generates a cell pair, B.av and B.ad, that separate and develop into postmitotic muscle fibres in two successive myotomes; 17 and 18 (G). In other clones analysed similarly, sibling cells that generated muscle fibres were observed to separate into dorsal and ventral portions of the same or different myotomes and, in segments of the tail of the embryo, into myotomes on the left and right sides. Thus there are no indications of segmental restrictions being present through the terminal divisions of muscle cells (from Kimmel & Warga, 1987*b*). The 50 μm calibration bar in (F) applies to (A–F). G is at lower magnification: bar, 50 μm.

length was not the same in different embryos and usually was not simply related to segment length (Kimmel & Warga, 1986).

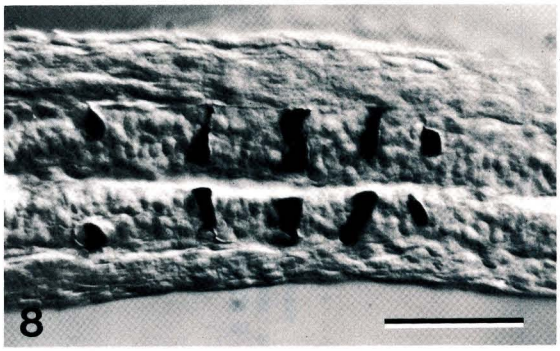

Fig. 8. Clonally related cells in the spinal cord are often distributed in a regular pattern along the neuraxis, and opposite to one another across the midline. The clone was descended from a blastula cell injected with horseradish peroxidase, which was subsequently developed histochemically in this horizontal section through the tail (from Kimmel & Warga, 1986). Bar, 50 μm; approximately the same as a segment length.

Cell lineage may not specify segmental patterning

Cell lineage analyses have revealed that in zebrafish the mesodermal and neural segmented structures arise from separate populations of progenitor cells whose lineages are indeterminate. There are no indications that cell lineage might specify segmental identity; in particular, the proposal that in *Xenopus* motoneurones are related by early lineage to the muscle fibres they innervate (Moody & Jacobson, 1983) is not supported by the studies in zebrafish. We suggest that the cellular events that control segmentation in zebrafish operate rather late, only after the cell rearrangements of the gastrula stage are accomplished. We note that in both the neural anlagen and the paraxial mesoderm, extensive cell migrations cease before there are any visible signs of segmentation. This cessation occurs in a rostrocaudal wave; e.g. in Fig. 7 it can be seen that caudally located cells continue to move away from the rostral ones after the latter have stopped migrating. The terminal cell divisions that generate the early muscle fibres (Kimmel & Warga, 1986*b*; S. Pike, unpublished observations) and neurones (Mendelson, 1986*a*; Myers *et al.* 1986) also occur at roughly the time that cell movements stop at a given location along the axis. It may be that cells receive instructions about their segmental relationships only after they have settled into, or near to, their definitive positions.

Heat shock produces parallel disturbances in myotomal and spinal segments

As discovered in frogs (Elsdale *et al.* 1976) and then observed in other vertebrates (Veini & Bellairs, 1985; Armstrong & Graveson, 1988) and invertebrates (Mee & French, 1986), somite-forming cells at a particular stage in their development become abuptly and transiently sensitive to a brief heat shock. Patterning of one or a few somites is disturbed; not those that were observed to be forming when the heat shock was delivered, but those that only begin to form some hours later. It has been proposed (see Elsdale & Davidson, 1986) that the period of heat-shock sensitivity marks the time when the cells are actually initiating segmentation, even though the somite furrows do not appear until later.

We compared the responses of the myotomes and spinal segments to heat shock as an approach towards unravelling relationships between these two segmented systems in zebrafish. As described above, myotomal and spinal segments become closely associated, structurally and functionally, although their early origins are separate.

Heat shock applied to a postgastrula embryo affected somitic development in essentially the same way as in frogs (Elsdale *et al.* 1976; Cooke, 1978); it produces a short zone of pattern disturbance along the file of myotomes, with normal-looking myotomes present both rostral and caudal to the affected region (Fig. 9). The borders of the affected myotomes are misplaced. The muscle fibres in these segments are sometimes too short, or too long, and sometimes not correctly aligned.

Also as in frogs, the time that cells are sensitive to heat shock is significantly ahead of when they form visible somites. In zebrafish, a wave of somite formation moves rostrocaudally along the axis at a roughly linear rate of 2·0 somites added per hour (Hanneman & Westerfield, 1988). The period of heat-shock sensitivity also moves in a rostrocaudal wave, and at the same rate – about 2 somites per hour (Fig. 10). The sensitive period precedes somite furrowing by 2–2·5 h; four or five normal somites form after the heat shock, before the first abnormal somite forms.

Spinal cord segmentation was also sensitive to heat shock (Fig. 11). There were several different effects; changes in the positions of the motor axons within the myotome, and changes in the positions and morphology of the motoneuronal cell bodies within the spinal cord. These changes occurred only where the myotome pattern was disrupted; not outside of that region. They were regularly, but not invariably, observed (see legend to Fig. 11).

Thus both mesodermal and neural segmentation is disturbed by heat shock. That mesodermal and neural pattern disturbances are present at the same position along the axis seems unlikely to be purely coincidental; rather, the finding suggests that the development of the myotomal and spinal segments has become

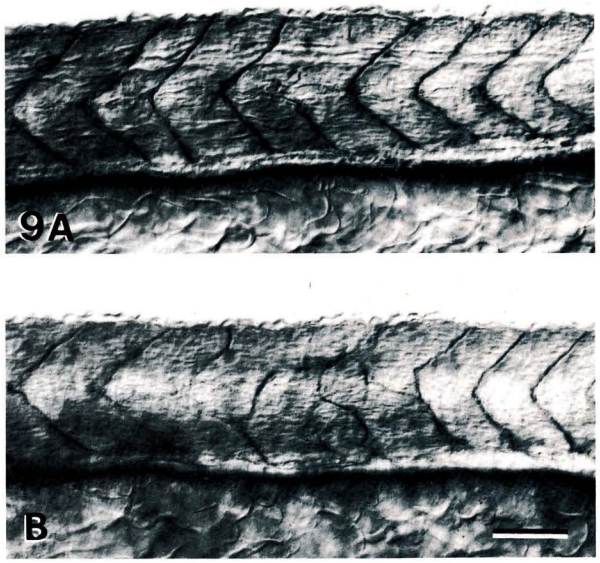

Fig. 9. Abnormal myotomes develop after heat shock. The myotome borders (transverse myosepta) are the most-revealing structures with respect to the disruption. These borders are rich in fibronectin in 1-day-old embryos (Frost & Westerfield, 1986), and here, in whole mounts at 28 h, they are labelled with an antifibronectin antibody. For these experiments and those described in Figs 10 & 11, the heat shocks were at 39–41 °C for 20 min, beginning here at 12 h (hours post-fertilization at 28·5 °C). (A) Heat shock produces disturbances in myotomes 11–14, including an incomplete segment border between two adjacent myotomes that results in their partial fusion. (B) Another, more severe, disturbance in the region of myotomes 10–13, in which the segment borders both wander and terminate abnormally. The muscle fibres were severely disorganized. Original data of D. Sepich. Bar, 50 μm.

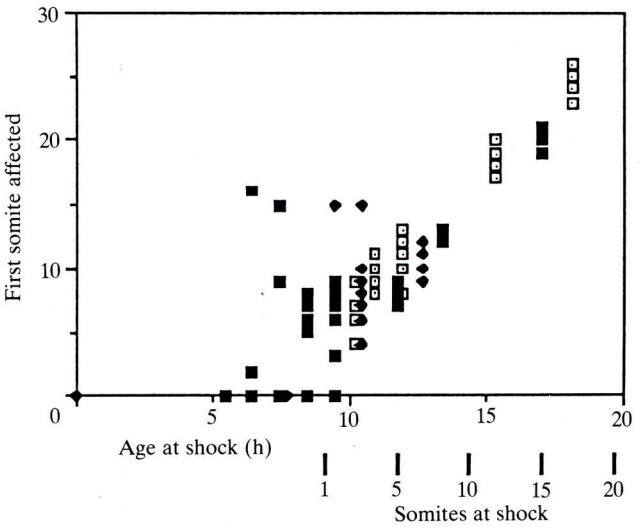

Fig. 10. A wave of sensitivity to heat shock progresses rostrocaudally in the postgastrula embryo. Three separate clutches of embryos, developing from timed fertilizations (Streisinger, 1981) were used, and are represented by the three different symbols. Single heat shocks were given, beginning at the time indicated on the horizontal axis. The position of the most rostral disturbed myotome was noted in each embryo, and each collection of points at given time shows the range of these disturbances in sets of 10–20 embryos. Original data of D. Sepich. The number of somites already formed at the time of the heat shock is also shown, from data of Hanneman & Westerfield (1988).

coordinate by postgastrula stages. Heat shock might affect patterning in both tissues directly or, alternatively, heat shock might affect one of them only indirectly, propagated from a pattern disturbance in the other tissue by cell interactions. Evidence from grafting experiments in chick embryos suggests that the segmental pattern of motoneuronal ventral roots depends on the mesoderm; motor axons appear to grow preferentially through the anterior portions of the somites in this species (review; Keynes & Stern, 1987). Thus in zebrafish, heat shock may directly effect mesodermal segmentation, and only secondarily affect neural segmentation, including the positions of the motoneurones within the spinal cord.

Nature of zebrafish metameres

We have seen that the assembly during embryogenesis of the prospective ectodermal and mesodermal cells into the segments is a complex process, involving considerable cell rearrangements. As revealed by the heat-shock experiments, some time after gastrula stage the development of spinal and myotomal segments becomes coordinated, perhaps by interactions occurring between cells of these two lineages. Presumably as a consequence of this coordinate development the two sets of segments then continue to be in close register along the body, with a simple functional relationship (in terms of the pattern of motor innervation) between them. In view of these interrelations, the concept of a metamere as a meaningful unit of organization in the trunk and tail of the body is arguably an appropriate interpretation of the data. Learning how the mesodermal and ectodermal cells might interact provides an exciting prospect for future work. In *Xenopus*, numerous close contacts are present between the cells of the neural tube and paraxial mesoderm before the time that somites form (Nordlander *et al.* 1981), which could provide for such interaction.

We presently know considerably less about the early developmental relationships of the segmented structures of head pharynx region. For example, important information is lacking about the early nature and fate of paraxial mesoderm in the zebrafish

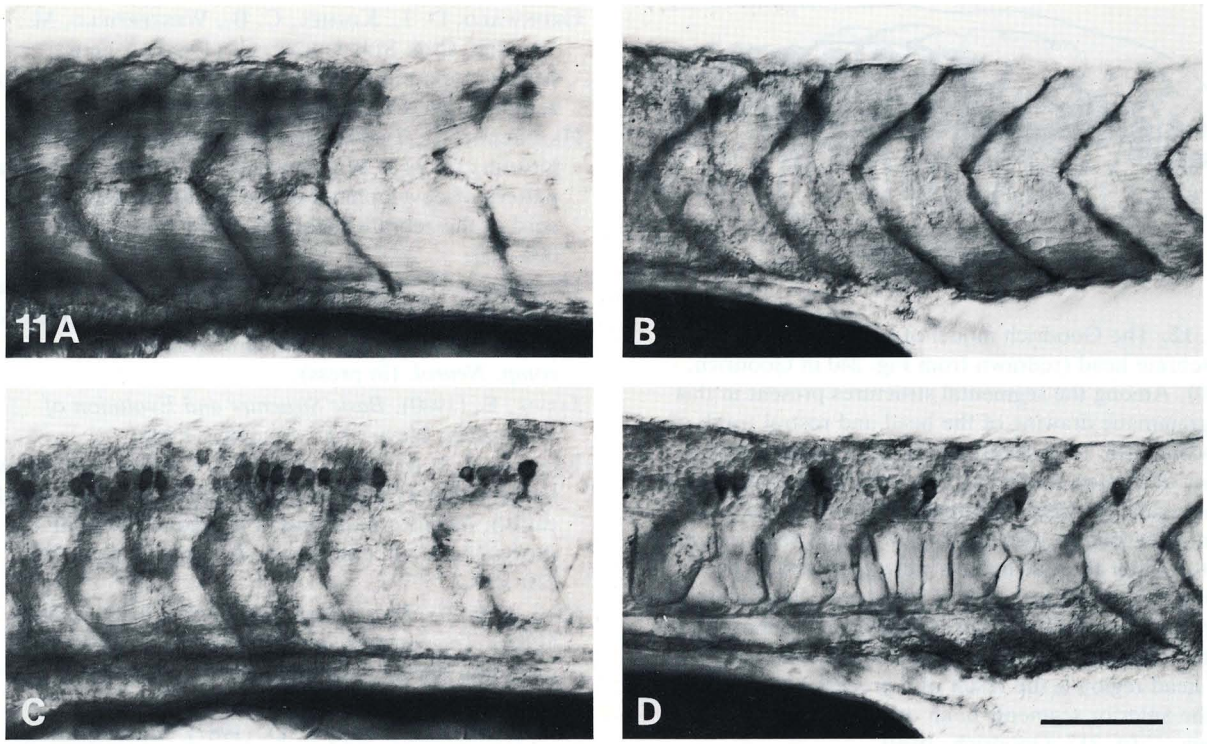

Fig. 11. Heat shock disturbs the positions of spinal primary motoneurones. The heat shock was given at 12·5 h, timed to produce a mesodermal disturbance at about the level of the 10th myotome (A,C). More caudally, at about myotome 15 (B,D), the pattern appears normal. For each comparison, the upper micrographs (A,B) show the positions of the myotomal transverse myosepta (labelled with antifibronectin antibody as in Fig. 9), and the lower micrographs (C,D) are of the same fields at a deeper plane of focus to show the zn-1 labelled spinal neurones (as in Fig. 2). At the time of the heat shock, the progenitors of the primary motoneurones in trunk segments are undergoing their last round of cell division (Myers *et al.* 1986). The animals were fixed after an additional 14–18 h, to let the young neurones differentiate. Outside of the disturbed region the labelled primary motoneurones are arranged segmentally (D), but the pattern appears disorganized in (C). Although not shown here, the neurones projected axons into the periphery in a pattern that is also abnormal. For quantification, the rostrocaudal distribution of the cell bodies of CaP motoneurones (that stain darkly with the zn-1 antibody; see Fig. 2 & Myers *et al.* 1986) was measured in twelve heat-shocked embryos that had a zone of abnormal somites. In seven of these embryos, CaP motoneurones innervating the disrupted myotomes were specifically dislocated, positioned either too close together or too far apart from one another in the spinal cord (by 30 % or more of the usual segmental spacing). In three of the twelve experimental embryos (and in three control embryos) no CaP dislocations were observed, and in two experimental embryos the overall pattern was too variable to detect any specific changes in the region where the somite pattern was abnormal. Original data of D. Sepich. Bar, 50 μm.

head. In other vertebrates, including the medaka (Martindale *et al.* 1987), head mesoderm is reported to be organized into segmental units termed somitomeres, but these structures have not been described in zebrafish. According to a commonly cited model, illustrated in Fig. 12, metameres in the caudal part of the vertebrate head include a number of components; neuromeres of the CNS, ganglia and nerves of the peripheral nervous system, somites and gill arches. But we have seen that the patterns of motor innervation, relating neuromeres and the periphery, are complex. Furthermore, in zebrafish, the pharyngeal arch segments come into approximate positional register with hindbrain neuromeres in a complicated fashion in the very late embryo; the arches grow in length while the hindbrain segments compress. Axial compression also moves the first somites into the head region so that they lie dorsally to some gill arches in the manner shown in Fig. 12; this juxtaposition is secondarily derived. Thus the model of head metamerism shown in Fig. 12 is, at best, strained. One must determine the earlier developmental relationships between CNS and peripheral segmented tissues of the head before a meaningful understanding of head segmentation can be achieved.

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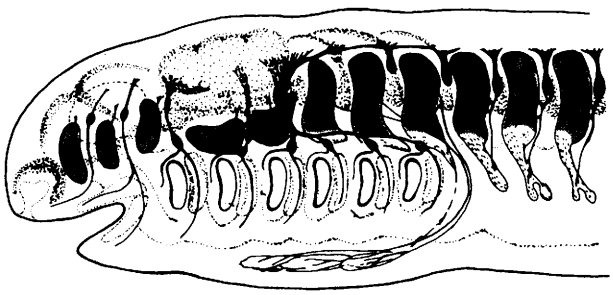

Fig. 12. The Goodrich model of metamerism of the vertebrate head (redrawn from Fig. 240 in Goodrich, 1930). Among the segmental structures present in this diagrammatic drawing of the head and rostral trunk of a selachian fish, brain neuromeres lie in register with a series of somites (including proposed head somites) and pharyngeal gill arches. Peripheral nerves, spinal and cranial ganglia are also shown (for details see Goodrich, 1930). All of these structures were proposed to be included in single metameres. Moreover, head metameres were proposed to be serially homologous to those of the rest of the body: "It has therefore been concluded that the head region is the result of a process of cephalisation of the anterior segments in an originally uniform series..." (p. 217, Goodrich, 1930).

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