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A note on segmentation and the scale of pattern formation in insects and in vertebrates

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Little or nothing was said, by workers on vertebrate somite formation at this symposium, about theories for the overall control of segment size, and thus the number of segments in the pattern. The current mood may well be that further modelling of the machinery of segmental patterning should await knowledge of the spatial patterns of synthesis of all relevant gene products, because understanding of the dynamics of segmentation in insects is only just beginning to emerge even though such knowledge for *Drosophila* genes is well advanced. What can be said, nevertheless, about the 'system properties' of the segmentation process in the two groups?

It is now clear that within the long-germband-type insect blastoderm, the spatial scale with which the repeating pattern underlying segmentation is set up can be adapted to an abnormal expansion of the scale on which the plan for the body as a whole is being determined. This has always seemed likely simply from inspection of partial, but expanded, larval body plans resulting from ligature or centrifugation of eggs, or from maternal-effect mutations that partially reverse the polarity of pattern formation (e.g. Sander, this Symposium; Schubiger & Newman, 1982; Nüsslein Volhard, 1977; Mohler & Wieschaus, 1986). In such cases, segmental patterns that embrace more tissue or cells per segment than normal can be seen from the earliest stages. Expansion of the 'wavelength' of the repeats of domains of activity for genes such as pair-rule or segment-polarity genes can now be seen directly in *Drosophila* blastoderms where experimental genetic manipulation has locally expanded the scale for body patterning as a whole, as in dosage manipulation of the 'bicoid' gene (Fröhnhofer & Nüsslein Volhard, 1987) or in cases of double abdomen.

The normal scale at which the *Drosophila* machinery works happens to be such that the most restricted of the stripes of gene activity are only one blastoderm cell wide at their initiation, so that artificial expansion is the only experimental challenge that the system can meet. Such expansion does, however, imply that the mechanism producing a periodic pattern takes its reference directly from set positions within a previous system that registers relative distances between the ends of the plan as a whole (Wolpert, 1969), rather than being dominated by an intrinsic 'chemical wavelength' within the tissue. This is in accord with a prominent current model for organization of long-germband insect body plan (Meinhardt, 1986 and this Symposium). It is not what we should expect if reaction and diffusion mechanisms of the type proposed by Turing (1952), or arrangements with similar descriptions but involving local mechanical variables like elastic tension and cell adhesiveness, lay at the heart of segmentation. On such theories, where control is due solely to local organization, segment size would depend only on the values of parameters intrinsic to each species and time of development (diffusion constants of morphogens, rates of enzyme-catalysed processes, adhesivity or deformability of cells). It would not therefore be susceptible to feedback modification according to the distance between other landmarks in the emerging pattern.

Although much new information, some of it molecular, is already emerging about vertebrate segmentation, it is fair to say that the present symposium has left it still unclear whether that segmentation plays as fundamental an ontogenetic role, in vertebrates, as it does in animals of the annelid/arthropod type. Segmental organization of the axial locomotory organ is of obvious adaptive significance in chordates and, in vertebrates, the final character of the derivatives of segments varies with position of origin in the body. But is division of the embryonic material into segmental units part of the *mechanism* of its regionalization into a body plan? We shall undoubtedly know the answer at the next major symposium on vertebrate segmentation but, in the meantime, we can at least inquire whether the mechanism that sets up segments indeed responds to the

scale of the pattern forming in individual embryos at early stages. Only in this way could somite *number* be regulated towards constancy in the body plan of each vertebrate species, since embryo tissue size at the time of pattern formation seems quite variable even under natural conditions.

Such regulation for somite number against embryo size had been assumed by experimental embryologists and was positively stated to occur after a series of surgical experiments creating amphibian embryos of abnormally small size or with asymmetrically scaled columns of paraxial mesoderm (Cooke, 1975, 1977). Such experiments are not so clear of interpretation as was thought at the time, however. Both our ideas about when segment primordia become set aside and our understanding of the fate map, and thus of what prospective material was removed to give small gastrulae, are somewhat modified from those then in force. A clearer test is perhaps the one whose essential results are illustrated in Fig. 1. The appearance in horizontal sections through recently segmented and segmenting somites at around position 15 in the series is shown for embryos from synchronously fertilized sibling *Xenopus* eggs that varied about twofold in diameter. *Xenopus* females are occasionally found to ovulate two size-contrasting classes of eggs at once. The yolk-laden embryo is for some time a nongrowing system. Cell number control is dominated by the relation of genome numbers to cytoplasmic volume up to mid-blastula stage (Newport & Kirschner, 1982) and then, perhaps, by the achievement of particular nucleo-cytoplasmic volume ratios for various cell types. Whatever the mechanism, total mesodermal cell numbers at stages of anterior somite formation are strongly a function of original egg size. In such embryos, the formation of segments (and, particularly, their determination at an unknown prior time) is being carried out within different-sized populations of similar-sized cells, as well as within tissues of different absolute spatial extents. Overt segmentation tends to run slightly ahead in very small embryos in terms of real time, but fixation times have been arranged such that closely corresponding somites within the trunk region are in the act of segmentation in the different individuals of each set. It is apparent that there is indeed an adaptation of the scale at which the events of segmentation occur to the overall scale of body pattern (five matched sets from three different egg batches investigated). When such embryos are compared, the cell numbers that separate successive fissures in the craniocaudal dimension vary in a way that is closely in accord with that expected from the ratio of total cell numbers in their mesodermal cell sheets, i.e. a linear dimension ratio of approx. 0·75 for a two-dimensional cell number ratio of approx. 0·5.

It is confirmed, then, that the more anterior and early-developing portion of the vertebrate segmental pattern has scale adaptation capacities similar to that in the long-germband insect blastoderm, whether or not its genetic basis is similarly organized. In amniote vertebrates, where the embryo as a whole is allocated from a much larger, growing, population of cells making up a blastodisc, such scaling capacities may be used differently, or seldom used at all, but they are unlikely to be absent. In accessory axial patterns developing far from the host pattern after grafting of Hensen's node in the bird embryo, sets of very small somites are seen. Since these develop synchronously with the anterior 'host' somite segments, they are unlikely to represent precocious second 'tail' patterns, and are probably partial, but small, second body patterns.

But what of later-forming, posterior, portions of the vertebrate pattern, obviously continuous with that which precedes them, but controlled more locally in relation to growth among a pool of tissue in the extending 'tailbud'? The posterior majority of the final segment numbers forms, in most vertebrates, during an extended period when processes of true growth are taking over in providing the still undifferentiated tissue at the posterior of the body. Davidson has described the situation with respect to the later segmentation of anuran amphibians at this Symposium. There are obvious parallels between typical vertebrate morphogenesis and one particular version of insect development, believed primitive, that differs from the 'Drosophila' version whose molecular description is most advanced. In this, the 'short-germband' version, a posterior majority of the segmental complement becomes visibly marked out (and, we can presume, set up) over some time in an anteroposterior sequence, in a relatively restricted space at the back of the extending embryo. We might expect most of the cellular mechanisms of segment morphogenesis and any genetic compartmentation involved in segment maintenance to remain the same throughout the sequence of segmentation in each type of embryo. But the factors determining the spatial scale of the patterning must become autonomous and local in nature, as the development progressively moves away from the time when the whole embryo was a 'field' in which potentially global intercellular signalling was available to control morphogenesis. It is hard to imagine how a particular piece of tissue, the tailbud, which was a very small element in the initial body plan, could 'remember' during the course of its own growth and morphogenesis that it was founded within a smaller- or a larger-scaled example of that plan. Some further observations, on *Xenopus* larvae that began somite

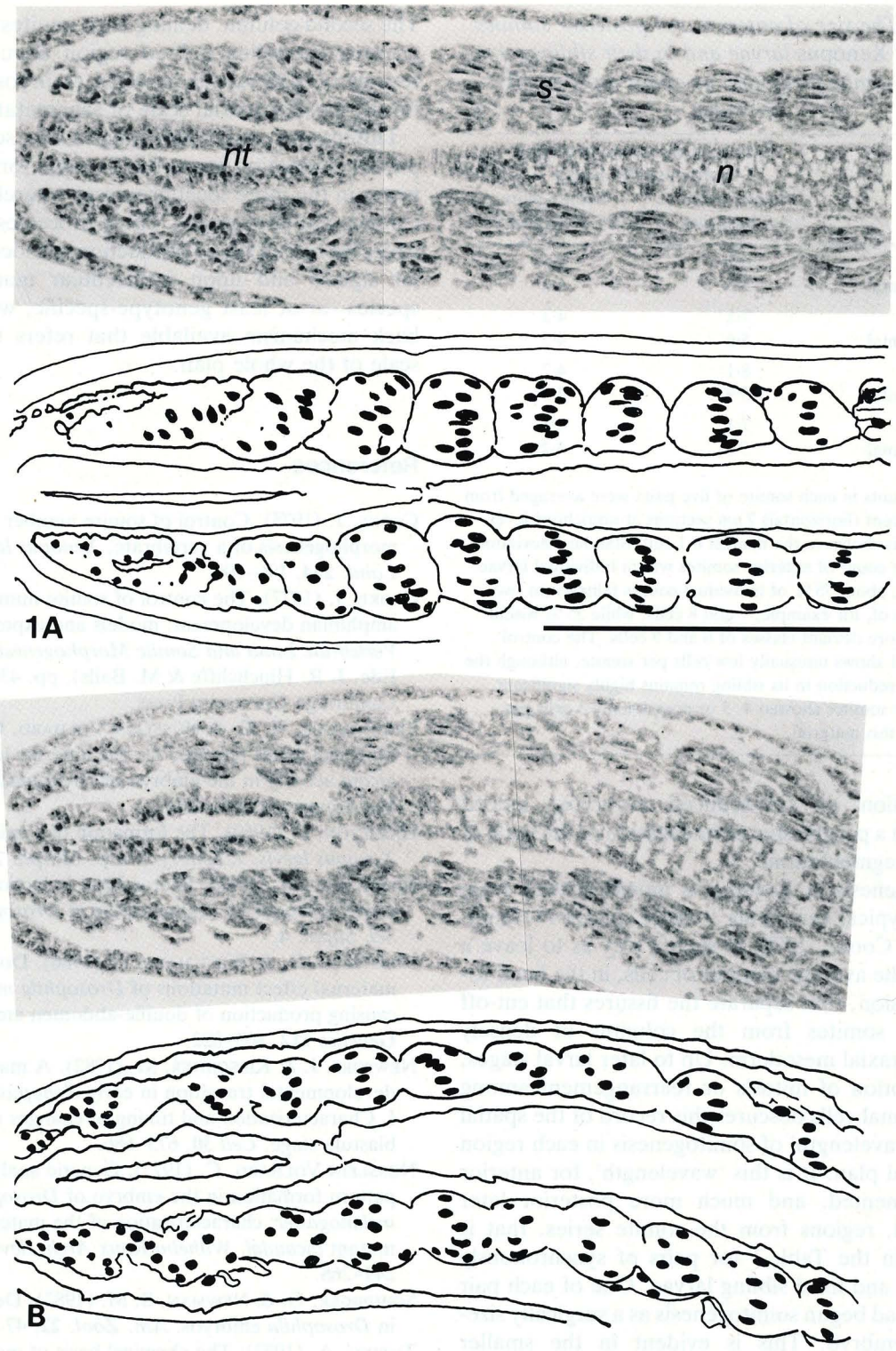

Fig. 1. Horizontal 7 μm sections at notochord/neural tube level through the region of segmentation in sibling *Xenopus* embryos at young tailbud stage. The mesoderm of the embryo in A contains nearly twice the number of cells of that in B (ratio 1:0·52) and equivalent, posterior trunk somites are in course of segmentation. *Camera lucida* outlines of each section are included with the positions of nuclei (Feulgen-stained in the original) marked in. Somites in *Xenopus* rotate through 90° at their formation, so that the spindle-shaped cells between notochord and skin represent the original craniocaudal interfissure cell number. It is readily seen that fewer cells are involved between successive segmentation events in the craniocaudal axis in the fewer-celled embryo and that each segment is smaller in this as well as in the cross-sectional plane (ratio in nuclei transected per somite 1:0·63). Thus the process of segmentation measures neither particular numbers of cells (see also Hamilton, 1969) nor amounts of tissue. *n*, Notochord; *s*, somite segment; *nt*, neural tube. Bar, approx. 0·5 mm.

Table 1. *The size of anterior and posterior somites in normal* Xenopus *larvae and in their siblings that began somite formation as abnormally small neurulae*

Sibling pairs	Mean cells per somite in original anteroposterior length: Somite pairs 3–7 inclusive	Somite pairs 26–30 inclusive
1. Control	6·2	4·4
Experimental	5·3	4·8
2. Control	7·2	4·3
Experimental	5·6	4·5
3. Control	8·1	4·2
Experimental	5·9	4·3
4. Control	7·1	4·1
Experimental	5·6	4·2

Nuclear counts in each somite of five pairs were averaged from four nonadjacent (horizontal) 7 μm sections at notochord level. The average is shown to the nearest 0·1 cell. Standard deviation in the nuclear count of anterior somites within individual larvae is around 0·8, about 75 % of individual counts falling into two modal classes of, for example, 7 and 8 cells, while 25 % would occupy the more deviant classes of 6 and 9 cells. The control larva of pair 1 shows unusually few cells per somite, although the effect of size reduction in its sibling remains highly significant. The posterior somites showed 4, 5 or occasionally 3 cells per section in all this material.

determination as size-reduced embryos, indeed reveal such a progressive normalization of the scale of posterior segmentation.

Somitogenesis in *Xenopus* is modified away from the archetypical vertebrate 'rosette' pattern (Hamilton, 1969; Cooke, 1977) in such a way as to leave a record of the average number of cells, in the head-to-tail dimension, that separate the fissures that cut-off successive somites from the columns of densely packed paraxial mesoderm. Up to later larval stages, no resumption of mitosis or rearrangement among the myotomal cells obscures this record of the spatial scale or 'wavelength' of somitogenesis in each region of the axial plan. It is this 'wavelength', for anterior early segmented, and much more posterior later segmented, regions from the somite series, that is recorded in the Table 1 for pairs of synchronously developed and fixed sibling larvae. One of each pair of larvae had begun somitogenesis as a surgically size-reduced embryo. This is evident in the smaller cellular scale of segmentation in the first column, that refers to somites presumed to be determined at gastrula to neurula stages, depending on one's preferred theory for the pattern-forming mechanism. The second column, dealing with somites set up in the growing extending tailbud region about 24 h later, reveals no differences between embryos in the uniformly smaller spatial scale of segmentation.

Differences among individuals in the scale at which pattern formation starts out become forgotten, as it were, in the more local processes whereby pattern is extended at later stages. These processes, dependent upon the rate of tissue production, on local intercellular signals and upon intracellular machinery, are species- or at least genotype-specific, with no feedback mechanism available that refers them to the scale of the whole plan.

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