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The Problem of Periodic Patterns in Embryos

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The problem of periodic patterns in embryos

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A segmented body-plan has developed at least twice during metazoan evolution: in the lineage including annelids and arthropods, where the segment is the unit of body structure, and in the ancestors of vertebrates, where a primary segmentation of the middle, mesodermal cell layer of the embryo imposes a spatially periodic character upon derivatives of other layers. The mechanism controlling the development of these periodic patterns has the property that the number of the serially homologous structures formed within each species is largely independent of the linear dimension, or scale, at which pattern formation occurs in individual cases. In this they contrast with other patterns of dispersed, homologous structures occurring in animal epidermis and dermis. The performance of various classes of model for the control of number in vertebrate somite formation are compared, in the light of experimentally and naturally observable properties of this aspect of pattern.

1. Introduction: general properties of segmental patterns

Early morphogenesis in animals of various types is accompanied by the formation of a set of fundamental units of structure, whose number (10–40) is relatively or absolutely invariant among normal individuals of each species. The units form in a series distributed regularly through the longitudinal axis of body pattern, each member of the series being founded by cellular events that are obviously homologous in a developmental sense. The term homology as applied to development may be understood by reference to its more familiar usage in comparative anatomical and evolutionary studies. Pieces of anatomy in two forms are diagnosed as homologous regardless of 'surface' appearance in the adults, if evidence suggests that they are expressions of sets of developmental processes that are derived from an ancestral set that occurred in a common evolutionary precursor form. Likewise, structures derived from different positions in the body plan are developmental homologues if one can follow their emergence from populations of cells that are obviously set up by serial, spatial repetition of some fundamental morphogenetic event in the early embryo. Such a mode of serial organization has almost certainly been achieved twice independently in evolution. Metameric segmentation of the mesodermal cell layer of the vertebrate gives rise to the somite series, which then imposes a segmental character on other organ systems. In the annelid–arthropod type of body, the fundamental unit of structure is the segment.

Details of the programmes of cellular activity whereby each segment is marked off from neighbours at its foundation vary even within the two groups, and it is unlikely that they are important to an understanding of the overall organization controlling numbers and positions of these structures in the body. Indeed, for the insect segment pattern there is evidence that what appears to us as the salient 'boundary' between units is of no particular significance morphogenetically (Wright & Lawrence 1981). Rather, there is simply the repetition of a smooth sequence, or cycle, of cell states, corresponding in a one–one manner with the number of repeats

of the segmental structure. Details of somitogenesis in one particular vertebrate only will be mentioned later, since they have rendered quantitative studies possible.

Development is of course characterized by many other regular or quasi-regular spatial periodicities, usually on a more local scale. Examples are the distribution of follicles, scale- or feather-germ rudiments in vertebrate dermis, or the patterns of microchaetes, sensillae, etc., in the arthropod epidermis. In such 'spacing' patterns however, one cannot point to any particular elements, in one specimen and another, and say that they are 'the' identical ones. Numbers have a certain indefiniteness even among normal individuals (see Lawrence 1970).

The impression of a different basis for these two types of periodic pattern is strengthened when the (conceptually) simple experiment is performed of requiring the morphogenesis to occur, from its beginnings, within an abnormally sized field. They then behave differently, as shown in figures 1 and 2. Feather germs appear to be determined and spaced within available tissue because centres form at random that will each give rise to one rudiment, but which effectively capture a set area of tissue by inhibiting development of any other centres within it. Control is essentially local. If, due to drug inhibition of early growth, a chick limb is abnormally short at the time of feather determination (McLachlan 1980), the number of units is correspondingly reduced. The remarkably low variability of flight feather germ number among normal individuals of a bird species is thus due to the exactness with which growth is controlled in relation to the onset of cell determination (see, for example, Summerbell & Wolpert 1973). If a vertebrate embryo is caused to develop with an abnormally small body pattern (Cooke 1975), then provided that regulation has ensured that a whole body (i.e. with head and tail) is being produced, the cell numbers devoted to each somite at its foundation are reduced in such a way that the numbers of somites, and their positions with respect to other body parts such as limb buds and the endodermal gut mass, are approximately or absolutely normal. An unknown, effectively global system, which regulates the completeness and proportions of the body as a whole, is acting also to normalize the numbers of repeated body elements at the expense of their individual sizes. Enough experimental work has been done on the arthropod (insect) segmental pattern to reveal that it too has this fundamental response to the challenge of abnormal scale, provided that manipulation of the latter is done at the very early stages of embryogenesis required for regulative behaviour in these systems.

The skeletal pattern of vertebrate limbs has a quasi-periodic character, especially in its antero-posterior aspect. On comparative anatomical and evolutionary grounds we might expect it to have a basis akin to that of spacing patterns for dermal structures, and not like segmentation. Yet at least in tetrapods, where the numbers of elements are reasonably small, normal morphogenesis gives an invariate pattern. I do not consider that we yet know enough about the mechanism of limb pattern formation to say whether its correctness at foundation actually requires control of tissue size, making it a very well canalized spacing pattern (see Cooke 1981*a*; MacWilliams & Papageorgiou 1978; Newman & Frisch 1979), or whether it has more the character of the scale-independent mechanism whereby the overall body plan is established. Growth control is certainly closely involved in the response of pattern to experimental disturbance in limb morphogenesis (Cooke & Summerbell 1980) but not in that of the primary body axis (Cooke 1975, 1979*a*, 1981*a*, *b*).

We have now defined, by exclusion, the class of patterns that are to be considered in the rest of this article, namely metameric segmentation patterns of whole bodies. What other salient properties of them, of relevance in thinking about their underlying mechanisms, emerge from natural observation as opposed to experimental probing?

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

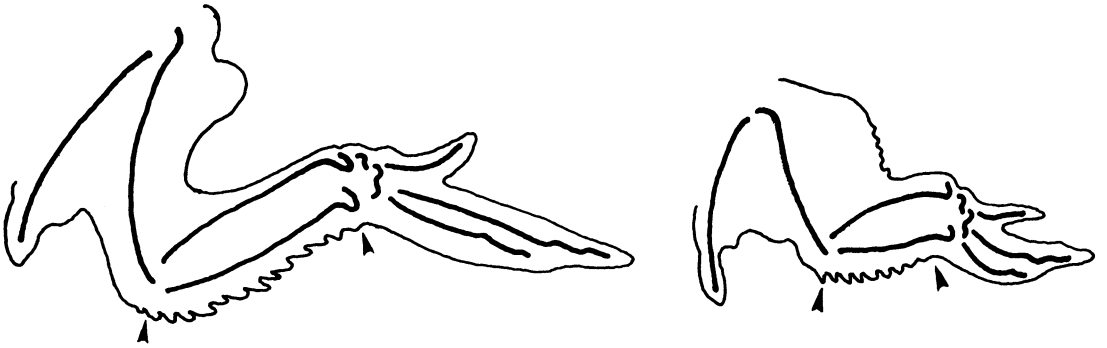

FIGURE 1. A spacing pattern with number dependent on tissue size. Soft tissue and skeletal outlines of two developmentally synchronous chick wings are shown at around 10 days of development. In the limb at right, inhibition of growth with the analogue 6-aminonicotinamide has decreased the tissue field size at stages of feather germ initiation, though allowing 'non-repetitive', or large-scale pattern proportions to be normal. The result, as counted between the arrowed positions of wrist and elbow joints, is a decrease in the number of essentially normal-sized flight-feather germs that is founded (McLachlan 1980).

1. Number control can be dramatic. Advanced arthropods have characteristically a particular, modally canalized (Maynard Smith 1960) number of segments typical of whole taxonomic groups. In annelid worms, control is usually not so tight, but the specialized Hirudinea (leeches), with modal canalization for a number of segments in excess of 30, may represent the most extreme known achievement of the control system. In vertebrates, when numbers of somites appearing between, say, skull and pelvic girdle articulations are considered, control appears less strict. Mean numbers up to around 25 are highly conserved across taxonomic groups of reptiles, birds or mammals, as well as within species. But the canalization of individuals around the mean is less than modal, as individuals with deviations of one or even two units in vertebral number are not rare, and in some species individuals exist in two frequent, adjacent number classes as well as the more deviant ones. Appearance may, however, be deceptive. In the arthropod body, coordination of segment number with other features is automatic in that segments *are* the anatomy. In vertebrate development there is evidence that the longitudinal columns of tissue that become segmented as somites are in fact sliding with respect to the remaining tissue during the stretching movements of axial morphogenesis and differentiation, rather as if a slide rule made uniformly of indiarubber were subjected to more powerful stretching forces in one of its components than in the other. Subsequent demarcation of particular somites as those that 'coordinate' with particular body markers (like the limb skeletons or girdles) may be in part an epigenetic process induced by their contiguity with those developing structures. There is thus more than one factor involved in the reliability of spacing achieved for vertebrate somites, and the accuracy with which wavelength is adapted to the length of the somite-forming column may be comparable with that attained in the arthropod segmentation mechanism. The spare segments of the tail region, however, do bring the total complement up to numbers where we could not expect, and never observe, modal canalization within species (Fowler 1970).

2. The production of particular numbers of units as such appears to be controlled, in both vertebrates and invertebrates, by separate mechanisms acting earlier in development than do the mechanisms that then assign a particular specialized character to each segment's products. This shows up most dramatically in insect development, where segment number itself is modally canalized. Only surgical alteration of the geometry of the earliest embryo, induction of bipolarity, or a very restricted set of gene mutations acting again during earliest development will

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

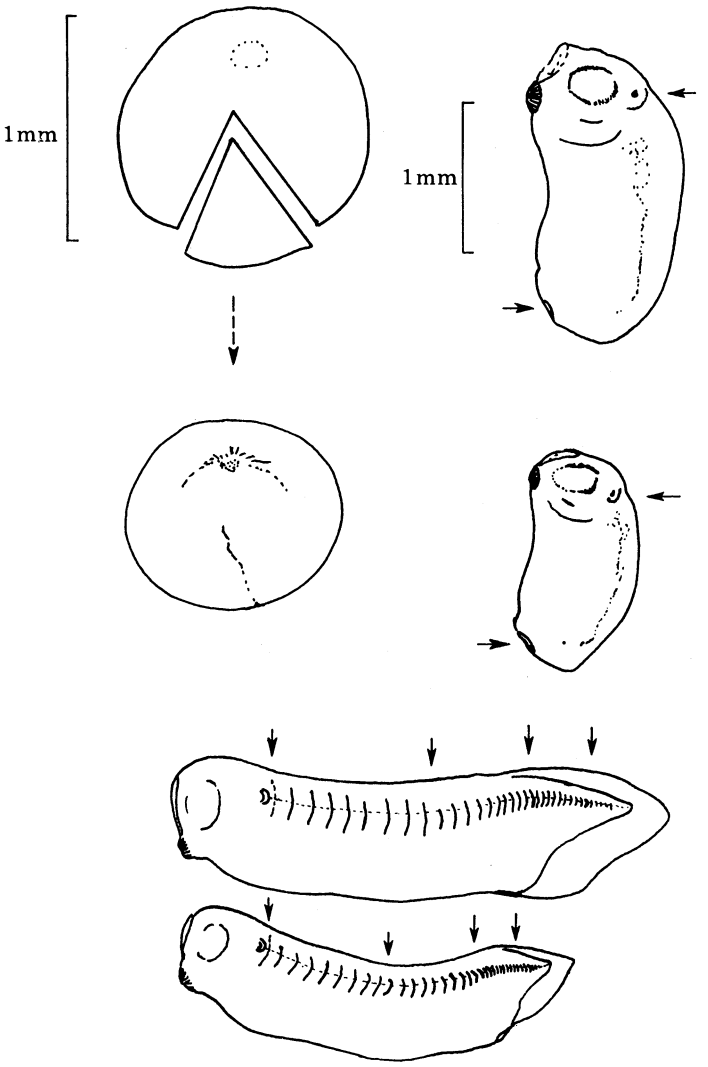

FIGURE 2. Segment number is highly conserved against variation in individual embryo size. The upper diagrams show excision of a sector of the amphibian blastula, opposite the site of the presumptive dorsal lip, giving an artificially few-celled embryo in which morphallactic regulation ensues to produce a harmoniously proportioned, small whole-body pattern. Heavy arrows show pattern markers (ear vesicle and proctodaeum) between which segment number can be counted accurately. Numbers of segments finally founded in normal and small larvae, and their positions in the body, are highly regulated even over numbers near 30. Fewer cells are incorporated into the head–tail dimension of each segment in experimental embryos, in order that this can occur (Cooke 1975).

alter segment number or the nature of the positional information for pattern control that is set up within the segments (Herth & Sander 1973; Nüsslein-Volhardt & Wieschaus 1980). Presence of a variety of normal gene products is, however, necessary, up to late developmental stages, to assure normal consignment of their special characters to the segments in correct sequence (see, for example, Lewis 1978). In vertebrates the evidence is (as usual) less direct. It resides in the fact that when we consider first the overall number of vertebrae represented between skull and pelvis, and then the subdivision of this number into sets having been ascribed the character of neck, thoracic or lumbar segments, the latter processes are seen to have been less accurately canalized than is the overall number. Individuals vary much more with respect to how many of

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

their vertebrae have thoracic compared with lumbar character than they do with respect to the total of vertebrae preceding the junction of spine with pelvic girdle. The message of such observations is that the development of metameric patterns is in no sense accomplished by repetition of a programme of variations upon some unit of developmental activity until the series ends, marked by some final variant. Rather, an archetypal spatially repetitive pattern of units is marked out, and then a subsequent set of boundaries between zones of various developmental tendencies is erected, the latter contriving to be (approximately or absolutely) in register with particular positions in the series of units.

3. Great regularity in spacing of the units at the time of their foundation, that is, in the 'wavelength' of the repetitive morphogenetic process in tissue, is a more fundamental feature than number itself in these patterns. After their initial demarcation, units can grow and differentiate quite differently, since tissue within each acquires non-equivalence (Lewis & Wolpert 1976) or position value (Wolpert 1971) that distinguishes it from tissue at other locations. Therefore, to know the spatio-temporal pattern of segmentation itself we must observe the actual process, and this has been done more intensively in vertebrates, particularly amphibians (Hamilton 1969; Elsdale *et al.* 1976; Pearson & Elsdale 1979), than in anthropods. Segmentation events range from appearing almost synchronous, to occurring in a prolonged, rhythmical and coherent sequence down the embryo from head to tail, with little overlap between episodes of cellular activity whereby cell populations of successive segments are marked off. Equal lengths of tissue in the head-tail dimension are characteristically allotted to each segment for much of the pattern, but if there is a deviation from this in posterior regions it is itself of a simple, regular form. Thus in amniote vertebrates the tissue length marked off between successive fissures, after about the first 20, declines in such a way that when cumulative cell number or tissue segmented is plotted against somite number formed (or time, since fissure formation remains rhythmic), a parabolic or asymptotic shape results (Cooke & Zeeman 1975). Initiation of visible events for formation of all segments in a body may occur within a time that is short relative to that required to complete any one of them (e.g. some insect blastoderms), or else be stretched out so that serial formation is obvious (all vertebrates). The impression of a coherent, head–tail wave of activity in the embryo is always apparent, however, even if time-lapse photography needs to be used.

4. In keeping with the deep spatio-temporal regularity that segmentation exhibits is the observation that where final segment number in these patterns is not invariate within species, this is never because 'missing' or interpolated boundaries interrupt the regular sequence in some individuals. It is, rather, an expression of the limited exactness with which 'wavelength' has been controlled in relation to overall size of the developing tissue, or to the positions of pattern markers other than somites in the body. Mutations that delete particular segment boundaries are not known, and even those 'segmentation' mutants that have recently been discovered have the character of dissecting or disrupting the process as such, rather than affecting particular numbered segments in a series. A segment of particular unique character is never missed out due to genetic mutations in *Drosophila*, but rather re-assigned with a segment character associated with adjacent positions in the series. All this suggests that there are no genes 'for' segment *number* N or $N+1$, but only perhaps genes 'for' a particular segment character which, because of the reliability or coordination of development, becomes associated with segment N or $N+1$.

5. In animals with less than modal canalization of segment number, especially in the posterior

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

body regions, environmental conditions during the period of segment morphogenesis can bias the number attained (for review, see, for example, Fowler 1970). Among vertebrates, some forms seem much more susceptible to such perturbations than others. It would appear that in the larvae of boreal amphibians, adapted to accomplish normal development under varied and fluctuating ambient temperatures in small bodies of water, mean and variance of segment number are remarkably buffered against this variable. Marine fish larvae and bird embryos, evolved (for different reasons) under very reliable temperature ranges, are by contrast highly susceptible to experimental decanalization of vertebral number (Lindsey & Harrington 1972; Lindsey & Moodie 1967).

The foregoing observations are not pedantic, or not merely so. They are important in my view because they make it almost impossible for us to entertain one particular theory for the control of these patterns. The idea of positional information (Wolpert 1969, 1971), presented elsewhere in this symposium, offers the clearest currently available framework for thinking about control of what we might call *unique* or non-repeating features of the patterns that are determined within embryonic tissue. Position and proportions of the head structures, and limb, kidney and heart territories, as well as the non-equivalence for schedules of growth and differentiation at various axial levels, form examples of these aspects of pattern in the vertebrate gastrular mesoderm where the somite boundaries also become determined. The idea is that a simply graded profile in some cellular variable or signal (concentration of a morphogen would be one hypothesis) is set up along the tissue, local levels of this signal being used to direct the pathways of cell activity and commitment at each position. A system of interpretative machinery is required within cells, implicitly involving the control of gene activity directly, to 'read' the continuous positional variable and produce an appropriately distributed set of cell states. Extreme dedicatees of this idea (and as is frequent with scientific ideas, its author does not extend his allegience to it as far as do some of his followers) would assume that somites or segments are positioned by an extreme form of direct gradient interpretation. Thus a special, regularly distributed subset of values of the positional variable is assumed to code for the special cell behaviour that demarcates boundaries between the repeated structures, the overall range of values for the variable and their spatial profile being agreed to be normal in all regulated (i.e. whole) bodies regardless of individual size. Such a mechanism, with its formally straightforward way of ensuring numbers and positions, might appeal to the human engineer designing some self-constructing automaton. It would be ruled out, I suspect, for cells or embryos, because of inherent accuracy limitations in the kinds of processes that are available to them. The signal gradient must have a mathematically simple shape to be plausible, so that the extreme regularity and systematism in the positioning of newly founded segment boundaries would require, on this model, a regular systematic subset of signal values used to generate a particular cell behaviour at regular locations. There is no selective requirement for constant somite or segment size; indeed they go on to become unique sizes for adaptive reasons. Why, therefore, should the boundary positions not be freed from this constraint at the outset? The model would also predict precise registration of somite boundaries with the other features of the body pattern, and modal number canalization (not the case in vertebrates), besides the occurrence of genetic mutations or developmental mistakes, or both, whereby *particular* boundaries fail to form (corresponding to non-function of interpretive machinery), which are not observed. As we shall see, a smooth body-gradient of position value is almost certainly involved in segment number control and coordination with the body plan, but not in the foregoing direct and demanding way.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

2. Plausible theories for number control, and some experimental results

Reaction–diffusion-based models for production of repeating patterns in tissue (pre-patterns), of 'activation' peaks or high concentration for some morphogen molecule, have a long record as proposals for the control mechanism of repetitive aspects of biological pattern (Turing 1952; Gierer & Meinhardt 1972; Murray 1981). As candidates for control of metamerism, they hover on the edge of plausibility in the absence of experimental challenge, and then fail decisively, in my view, to meet that challenge. Briefly, periodic pre-patterns emerge when one plots, with suitable adjustment of parameters, the performance of equations that model the spatial behaviour of hypothetical interlinked biosynthetic pathways for production of substances within a tissue. Short-range autocatalytic activation is involved, followed by longer-range inhibition of earlier progress towards an activated state because of a more diffusible, inhibiting feedback signal. The degree of control over larger numbers of pattern units, and the spatial regularity of the pattern, pose *a priori* problems for such a system in metameric segmentation (Bard & Lauder 1974). Maynard Smith (1960) pointed out that control of a large number could be achieved by hierarchical operation of successive Turing systems superimposed, each with only a plausible degree of accuracy (e.g. modal canalization of five units). Vertebrate somite number gives no experimental evidence of being laid down by such hierarchical sequential subdivisions of territory (Cooke 1975), while only one early-acting mutation has so far given a hint that some such hierarchical organization may underlie segmentation in the *Drosophila* blastoderm (Nüsslein Volhardt & Wieschaus 1980), and this is not diagnostic for pre-pattern mechanisms.

A decisive limitation of reaction–diffusion pre-patterns is that numbers of peaks or singularities generated are sharp functions of the spatial extents of tissues in which they operate, unless the reaction and diffusion parameters can have their values adjusted within tissue by appropriate feedback from the overall size. Only then can the wavelength of the local processes be adapted to the available cell number to control the number of units founded. It is precisely this experimentally verified property of segmentation patterns that appears to rule out prepatterns, since one cannot imagine an appropriately *systematic* feedback from the overall, longitudinal dimension of the system onto local processes of the Turing sort. One can imagine a *trend*, e.g. for smaller overall size of embryos, by increasing diffusional loss to the medium, effectively to decrease the diffusion term for inhibition and thus shorten wavelength (though there is no evidence for increased diffusion from small embryos). It is, however, the systematic adjustment of somite length to body length that in my view rules out this class of models for the pattern. They remain real candidates for other periodicities, however, e.g. integumentary stripe patterns and, just possibly, vertebrate limb skeletal pattern. In the latter, element number may be controlled and constant in the grown animal because of initial control of timing and tissue size when any pre-pattern was being generated, followed by growth of the determined territories. We would like to grow up a frog from a size-reduced embryo to see if it had abnormally few stripes, patches or spots, but have not yet been able to do so.

A further set of ideas about somites derives from Waddington (e.g. Waddington & Deucher 1953). It is based on the proposal that undifferentiated tissues in embryos are essentially like liquids with very high surface tension, or at least show many of the properties of liquids on a very long timescale. Steinberg and his associates are prominent modern champions of this view (Steinberg 1970), though it is to be wondered how far the structure and social behaviour in cultured aggregates of cells resembles that in even undifferentiated embryonic tissues *in vivo*.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The idea about somite formation is somehow easier to test (and disprove) than to describe. It is proposed that the process whereby a column of pre-somite tissue breaks up into actual somites resembles, say, the breaking of a jet of water into drops. The number of drops produced is largely a function of surface forces, so that a finer jet will give rise to smaller drops. In other words, somites tend to have constant shape or constant proportions at their formation. A regulated, miniature embryo body will possess appropriately thinner and shallower (as well as shorter) pre-somite columns, because of appropriate regulation in the medio-lateral dimension of pattern formation by positional information or its equivalent. The local physics of the segmentation process will ensure that the head–tail distance between fissures is appropriately reduced, leading essentially to the preservation of somite number.

In many vertebrate forms, even inspection of normal morphology at the time of segmentation suggests that the regular placement of fissures is made with respect to position in the overall head–tail sequence of cells alone. The somite column varies in width and depth at this time, having special configurations in certain regions of the embryo anatomy that appear not to affect the longitudinal 'wavelength' of somitogenesis. In the progressively shorter, posterior amphibian somites particularly, width–length–depth constancy is not observed. A simple experiment disposes of the idea. By removing many mesoderm cells from one side of the gastrula, embryos can be made with very asymmetric numbers of cells, and thus thickness and depth of the somitogenic columns, on either side of the dorsal midline. Such embryos form numbers of somites that are both normal and symmetrical, on either side (Cooke 1977). We are brought back to the idea that the system that signals cell position within the body plan as a whole is somehow integrating the wavelength of somitogenesis with body size.

The greatest hope of understanding the regulation of positions and numbers for regular segmental patterns in animal embryos seems to be offered by what has been termed the 'escapement' class of models (Pearson & Elsdale 1979). An escapement, in human (-clock) technology, is a device whereby the total quantity of some reservoir of potential energy such as a raised weight or a coiled spring, rather than being released in a rapid continuous process, is released as a precisely regular series of smaller events, which thus measure out time. The standardized events are used as the unit of time and counted. In the technological case the mechanism itself usually ensures also that the energy release occupies a very long time; it is a brake as well. But this need not be so. Biological escapement models for segmental patterns assume that the equivalent of the kinetic motor of the process is a prolonged smooth unfolding or developmental progression, that occupies about the same length of physiological time (= real time at a particular temperature) to run its course from its anterior beginnings to its posterior conclusion in the body plan of all complete embryos of any one species. The progression takes the form of a continuous wave, with respect to rapid change of some type among cells, i.e. in behaviour or surface properties, or both. The wave passes down the body and takes the long, species-invariant time to traverse the entire pattern regardless of the actual length of tissue or number of cells involved. The whole embryo may be involved, even though the wave is only used to control pattern periodicity in particular structures in the vertebrate. It is important to understand that the wave passage is slow, despite rapid change by individual cells at each locality, because it is the expression of a pronounced timing gradient whereby cells at each position in the body plan have been developing autonomously at different rates, which are graded systematically and smoothly along the tissue. The cellular development that has been graded in this way is one whereby, having undergone no manifest change for some time which varies with the preset rate, cells reach a point where sudden overt novelty occurs in their properties. In other words

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

the 'wave' is not a wave at all in the sense that physical scientists would reserve for the term, but rather a *kinematic wavefront* (Zeeman 1974), having been set up by prior arrangement rather than being propagated by present intercellular communication. Any true propagating wave of cellular activity would have a particular rate of passage per cell, e.g. the waves of cell movement in slime mould aggregation (Robertson 1972) or in gastrulation of vertebrate blastoderm embryos (Robertson 1979). It would therefore pass down the body in developmental times proportional to the length of tissue available within individuals. What we require, what is understandable in terms of a kinematic wavefront, and what is indeed observed upon inspection of experimentally small embryos and their normal siblings, is a species-invariant passage time for the sequential recruitment of all available tissue into segmented somites: larger embryo-faster wavefront; smaller embryo-slower wavefront.

The 'escapement' must then consist of a regular repetitive process that partitions this smooth wavelike sequence into a controlled number of 'steps', of constant short duration, thereby causing the apportionment of regular-sized groups of cells into membership of successive somites or segments. We in fact observe, in normal and small sibling amphibian embryos that completed gastrulation synchronously, that formation of anterior somites and then formation of somites at particular posterior positions in the pattern (i.e. similarly *numbered* somites) also occur synchronously. Thus wavefront passage time *and* the cycle-time of the escapement are species-determined entities, regardless of scale, and interact to give a constant dimensionless number (laid out as a spatial series of entities, the somites (Cooke & Zeeman 1975)).

Restricting our modelling activities now to the vertebrate case because there is no requirement for homology of mechanisms as between phyla, we can ask what might underlie the species-invariant passage time for a wavefront, and what might be the nature of a species-invariant 'escapement' periodicity. So far, the model has after all been only a formalized redescription of the empirical surface, so to speak, of somite segmentation watched in any vertebrate embryo. The wavefront mechanism takes us into the mysterious territory of fundamental pattern formation in the antero-posterior axis and the idea of positional information (see § 1). This will involve an understanding, beyond our present one, of what is occurring in cells during the movements of gastrulation. We observe that cells are given an intrinsic rate of development that varies as a smooth function of the order in which they are situated in the head–tail sequence of the mesodermal mantle, the fastest-evolving cells lying at the head end and the slowest being produced at the back. This could occur in two ways. Positional information (Wolpert 1969, 1971), regulating the general proportions of pattern by giving a full and proportioned gradient profile of signal regardless of cell number, may be set up along the already invaginated mesoderm. The rate of cells' development could then be set, as part of the 'non-equivalence' among cells induced by their response to values in this gradient. Just as in the 'French flag problem' of Wolpert, a complete gradient implies a whole pattern, so in all regulated neurula stage embryos the absolute range of rates of development would be the same and distributed smoothly between front and back ends of the body pattern. The problem here is that we know experimentally that the mesoderm has attained stable non-equivalence, both for eventual type of differentiation and for timing of episodes of rapid cell change, quite soon after passage through the blastoporal lip or its equivalent at each level. There is not much time for a gradient system, as we think of it, to have operated. Furthermore the geometries and packings of the mesodermal population before and after passage through the blastopore are so different (though we do not yet understand fully *how* different) that it is difficult to conceive how any fine-grained positional system for use in the later mesoderm could be established before gastrulation. Alternatively, we might

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

suppose that non-equivalence for the body pattern and for developmental rate in axial mesoderm is generated by a timing mechanism connected with the process of gastrulation itself. Gastrulation is a deeply conservative process throughout vertebrates, despite superficially different appearances among embryos of various types and at different stages in the head–tail sequence of mesoderm formation. It is simply the rather sudden recruitment of successive cells into a new behaviour and contact–adhesive situation as they invaginate and migrate forward to give the head–tail sequence of the definitive mesoderm. In other words, if the entire episode of gastrulation occupies about the same time in big and little embryos of a species, which it appears to do, this might be because regulation has already assured the existence, in pre-invaginated or presumptive mesoderm, of cells with a complete normal range of developmental rates, as well as pattern-forming tendencies. The times at which cells (in the head–tail sequence) change behaviour so as to invaginate and migrate forward will be the first expression of this, and the times of sudden change, which we require for the wavefront of somite formation, will be a subsequent expression. Gastrulation and axis formation are seen as comprising a series of wavefronts of cell activity in tissue, organized by an overall gradation of developmental rate (Cooke 1979*b*.)

Explantation and grafting experiments on embryonic material from stages of axis formation reveal that, by whatever mechanism, cells are able to have preset the rates at which they proceed towards some well marked episodes of change, and to maintain autonomy for this on a quite local scale (Deuchar & Burgess 1967; Christ *et al.* 1972; Menkes & Sandor 1977).

There are various candidates for biological escapement mechanisms. The simplest but least orthodox is that proposed in the 'clock and wavefront' model for number control (Cooke & Zeeman 1975). It is postulated that all cells of the pre-segmented column of tissue are phase-entrained with respect to a smooth biochemical limit cycle or oscillator, of the general type found to underlie widespread periodicities on timescales much longer (Winfree 1970) and shorter (Winfree 1972) than that, on the order of 100 min, characterizing morphogenesis of each segment. Intercellular communication entraining the oscillator in the head–tail dimension is assumed to be rapid relative to the progress of the developmental wavefront just discussed, so that the latter will advance along tissue that is undergoing a rotation through the phases of the oscillator that is regular and close in periodicity to that occurring within individual cells themselves. We now suppose that the oscillator interacts with other properties of the cell, to give a coherent phase-domain wherein *expression* of the rapid change marking the wavefront passage is permitted in a cell, should the right time have come, alternating with a phase domain in which such expression is suppressed. Passage of the wavefront, which is really the sequential recruitment of cells into the activities of somite formation, will thus be gated or punctuated rhythmically as it progresses, giving rise to successive populations of cells each of which is associated together because they began a rapid period of change in motile–adhesive properties nearly synchronously, but at a time differing from populations headwards and tailwards of them. This does indeed correspond to the anatomy, and to what little is known of the cell biology, of somite formation (Hamilton 1969; Bellairs *et al.* 1980). The cell population of each segment corresponds to wavefront passage through one oscillator cycle, having been released almost synchronously into new behaviour at transition from inhibiting to permissive phase of the latter. The model is presented in visual form in figure 3.

Other escapement candidates have been proposed by Mitchison (see appendix to Cooke & Zeeman 1975) and by Flint *et al.* (1978). The former, very ingenious proposal uses the idea of a

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

wavefront of altered communication (physiological coupling) among cells, accompanying the developmental wavefront and involving intermittent breakage of coupling within the column of cells to mark off each successive somite's population. Rhythmical alteration between coupling and uncoupling in mesoderm during somite formation is indeed observed in amphibians (Blackshaw & Warner 1976), though its status as cause or consequence of somite patterning is unclear. The model of Flint *et al.* (1978) is designed to explain the process in embryos that gastrulate by node regression, but as mentioned above I cannot see any differences of consequence between gastrulation of different vertebrate forms, and to the extent that it works in one it would work for all, as must any plausible model. The oscillator escapement within individual cells is replaced by a limit cycle that involves first biosynthesis and then a switch to degradation of a morphogen, initiated within tissue upon its recruitment into the definitive

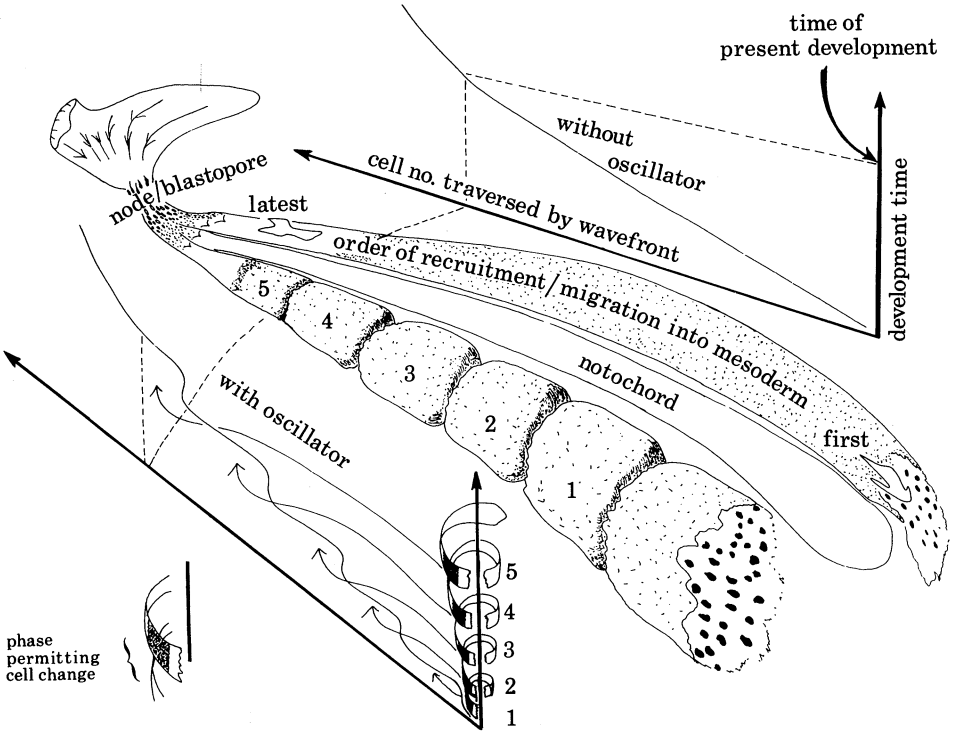

FIGURE 3. The clock and wavefront escapement model for control of segment number. A generalized instance of vertebrate gastrulation is depicted with cells of the blastoderm or deep neurectoderm changing their behaviour in sequence and passing in to take up their positions in the head–tail (early–late) sequence of the axial mesoderm. It is proposed that the positional signal system (a gradient or a 'progress zone') that regulates pattern in the antero-posterior dimension is also used to set the *rates* of mesoderm cell development in a smoothly graded manner, leading to a succession of *wavefronts* of cell change at subsequent times, passing from head to tail. The change denoting participation in somitogenesis is organized as such a wavefront, graphed on the upper (left) side of the body in the axes of developmental time and cumulative tissue traversed within the long dimension of the paraxial mesoderm. Since a complete pattern is correlated with a complete range of developmental rates, total *transit time* for the wavefront will be similar in regulated embryos of all sizes in a species. On the lower (right) side of the body it is shown how wavefront progress could be gated and turned into a series of regular steps by interaction with a regular cellular *oscillator*, phase-linked and entrained in all the prospective somite cells. Only one part of the oscillator cycle would allow expression of the rapid behavioural change by a cell, even though that cell had become competent to undergo change because or its axial position (i.e. the wavefront had just passed it). Regular sized, successive populations of the cells would then undergo change almost synchronously among themselves, individualizing a series of somites whose number would be the quotient of wavefront transit time divided by the oscillator period; this would be nearly constant for a given species.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

mesoderm at the node. This leads to production of a series of concentration peaks and troughs whose scale (governing the size of somite cell populations) is a function of the rate of node regression or tissue recruitment. It has already been mentioned that the latter rate is itself a function of the overall size or cell number in individual body patterns of a species. We do not understand the mechanism of this, but it is another expression of the series of wavefronts, each of constant passage time, characterizing a complete body pattern. The equations governing the potential alteration between morphogen synthesis and destruction within cells are reminiscent of Turing equations, but with tissue recruitment at one edge of the cell sheet partly replacing the effects of diffusional space in the production of standing waves of concentration. I should intuitively have expected this model escapement to resemble reaction–diffusion pre-patterning in its scale invariance of wavelength, but upon simulation it apparently shows the possibility of the requisite wavelength adaption.

It cannot be claimed that in the few years since its publication, experimental evidence distinctively in favour of one or other version of the idea of a biological 'escapement' mechanism for number control has been obtained. Work with short temperature shocks in amphibian development, during the period of somite formation, has, however, built up a picture of a distinctly two-component process of pattern control: a wavefront of hidden cell change that sweeps down the body a set distance ahead of the visible morphogenesis of somites, and a second process with which this interacts (Elsdale *et al.* 1976; Pearson & Elsdale 1979). The second process has responses to and recovery kinetics from high temperature that differ from those of the wavefront, allowing experimental unlinking of the normally coordinated processes (Cooke & Elsdale 1980). The closely controlled yet not species-invariate numbers of somites, and the data on buffering against environmental variation in species from different habitats, can also be taken as circumstantial evidence in favour of a two-component process for number control.

3. The problem of developmental homology

I hope that contributions to a meeting such as this, and many of their audience, would agree that a goal of research in the field that we are discussing is to reach an understanding whereby 'surface' appearances and behaviours that seem very different, as between different animal forms, can be seen as manifestations of rather a few underlying mechanisms that are widely used in achieving certain organizations in biology. As an extreme example, the mesodermal component of each of the large, exact number of segments in the leech is founded as a left–right pair of single cells in the embryo (Fernandez & Stent 1980), each cell on each side being one member of a strict lineage derived by a series of asymmetrical divisions of one of the early cleavage products (teloblasts). In an advanced insect such as a dipteran, the invariant segment number is produced by rather exact geographical subdivision of the preformed cell layer of the blastoderm into a series of populations thereafter characterized by non-intermixing of cells between them. Yet with due apology to taxonomists whose mode of viewing the animal kingdom is more updated and adventurous than my own, I profess to a faith that these two apparent ways of counting, when erecting segment patterns, are manifestations of one underlying process that was 'discovered' by organisms ancestral to the annelid–arthropod–mollusc group of proterostomatous animals. The notion of homology overrides, at least in my mind, clues (or red herrings?) that might be offered by the anatomical peculiarities of embryos in particular forms. In certain relatively primitive insect and crustacean embryos, for example, while the first few segments of the body complement are demarcated within a blastoderm, a fixed subsequent

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

number is added from a restricted growth zone at the rear end. This formally resembles the addition of segments in worms like the leech, but here the process of segmentation marks off substantial clusters of cells, so that we can be sceptical of the notion that, in those other forms, cell lineage actually holds the key to the mechanism of counting.

Looked at from any standpoint other than an anthropocentric one, chordates must class as a morphologically very homogenous subphylum, yet the appearances of their gastrulation and axial morphogenesis strike the beginner as highly diverse. Since in all probability they have possessed no segmented common ancestor, one must accept that the organization of segmentation and its number control in proterostomes and in chordates might be quite different, from historical contingency alone. But to propose that vertebrate somites might be formed and regulated by different mechanisms in different groups would be like suggesting that some profound and successful analysis of segment number control in lobster development was irrelevant to a potential understanding of the phenomenon in wasps.

Problems of evolutionary homology between forms and of developmental homology between structures within forms can be seen as very closely interrelated. A fascinating case is that of somite formation at different antero-posterior levels of the body plan in an embryo such as the amphibian. Here, the relation between growth and the laying down of pattern alters, apparently profoundly, during morphogenesis of the sequence, while the pattern units are obviously homologous throughout in the developmental sense of the term. Two modes of pattern control have been identified in vertebrate development. The first, operative during the cleavage and gastrula stages before the onset of real growth, takes account of overall dimension or cell number available in adjusting the scale of pattern parts, including, as we have seen, the size of somites at foundation. It then corresponds to the concept of the 'harmonious equipotential system' (Driesch 1929) or of positional information, even though the response of somite size to a positional gradient could only be indirect (see § 1). The second control mode may be an adaptation of the first to circumstances where true growth and early differentiation have already begun even though pattern parts still remain to be specified. Cells of new potentiality are only supplied from a small pool of labile tissue at the extending tip of the system (Summerbell *et al.* 1973; see also Cooke 1975), while elsewhere the already-determined tissue proceeds to *express* pattern by differentiation with or without further growth. It is thus hard to see how such a later system might take account of the total scale of the embryo in the size of the pattern elements that it continues to lay down; short of feedback from circulating 'size' hormones, the processes controlling pattern in the 'progress zone' must have an essentially local character. While early, relatively anterior, repetitions of the somite demarkation process (up to say 10 or 15) occur under the first control mode mentioned above, more posterior ones are made under conditions approaching the second, which operates in the growing posterior axis. It is therefore unsurprising, though somehow amusing to discover (see figure 4) that in *Xenopus* embryos that have begun somite formation as artificially small, few-celled neurulae and adapted their anterior somite sizes appropriately, this fact is progressively forgotten in later axial morphogenesis. Somites numbered from around 20 onwards in the body pattern are founded on a species-typical scale, being indistinguishable in cell number in initially small embryos and in their normal siblings.

What does this mean in terms of the idea that a wavefront of cell change, governed by a body position variable, is gated by a rhythmic escapement mechanism to produce segments? We conclude that the rate of passage of the early portion of the wavefront has been adapted to overall cell number at that stage because position values in cells have been regulated by an overall communication system to give a complete range in the tissue available. Ultimately, however,

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

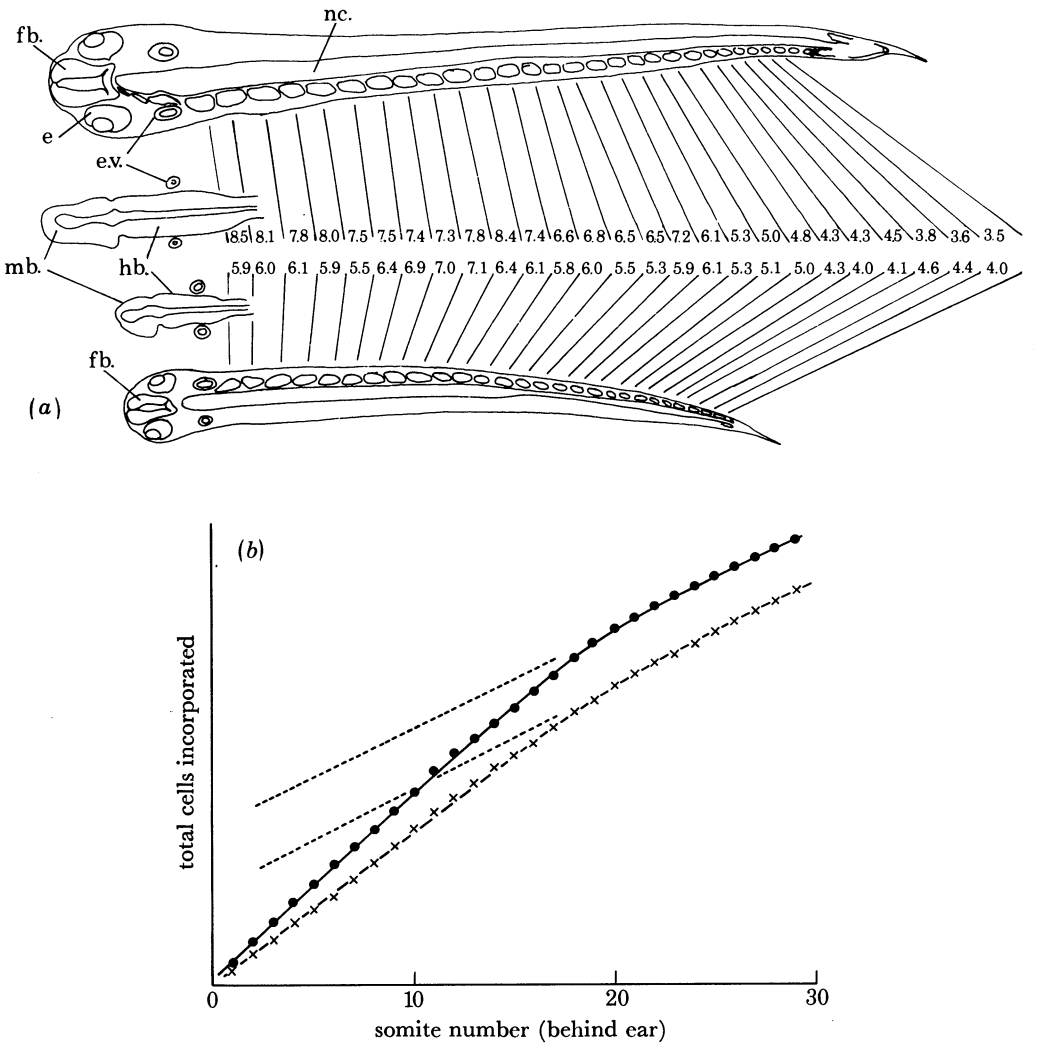

FIGURE 4. Adaptation of somite size to overall body size is lost in later-forming parts of the axial pattern.

(*a*) Somite numbers, positions and dimensions compared in a typical larva and in its sibling which began somitogenesis as a neurula with artificially low cell number. The cell number in the original length (right and left somites pooled and averaged over four horizontal sections along the notochord) is significantly reduced in anterior somites of the experimental embryo, but approximates progressively to that normal for the axial positions concerned as the pattern extends posteriorly (see also figure 2). The brain is also shown at an additional, more dorsal sectional level to confirm the reduction of its scale in harmony with the small body in which it was founded. Abbreviations: e, eye; e.v., ear vesicle; fb., forebrain (diencephalon); mb., midbrain; hb., hindbrain; nc., notochord. Positions of equivalent somites are related by lines leading towards the columns of numbers that give the cellular dimensions. The first somite extending behind the ear vesicle in the larva is normally considered as no. 3 in the definitive series. For the way in which somitogenesis in amphibians of the type used here forms a lasting record of the cell number in the 'wavelength' of somite formation, see Hamilton (1969) and Cooke & Zeeman (1975).

(*b*) Curves for cumulative cells incorporated into somites in the antero-posterior dimension (ordinate) against number of somites formed, equivalent to developmental time (abscissa), for a pair of larvae similar to those shown in (*a*). The stage (*Xenopus*) is 35, near the posterior completion of somitogenesis. The small scatter of the data points reveals the accuracy or spatial rhythmicity of the process (●, control; ×, experimental). While the curves diverge regularly up to around the 12-somite stage, there is a transition whereby from around somite 18 onwards the dimensions in which pattern is laid down are indistinguishable in the two embryos, as revealed in the parallel plots at later stages. This picture is typical, though the abnormally flat curve does not show up in the body as an abnormal distribution of somite lengths and positions, probably because the ends of the longitudinally disposed cells can 'slide' in relation to the notochord to adapt the pattern to stretching all along the body.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

the wavefront for late parts of the pattern is moving through tissue that has been recruited from the dividing pool in the posterior growth zone (Cooke 1979*b*). Here the progression of tissue position values per amount of tissue produced (hence, the speed of the wavefront of cell change) must be some function of growth rate in, or time of residence in, the pool of labile tissue at the tip. These are species-determined, not individual size-determined, factors. We need assume no different character for an escapement process in different regions, but only for part of the mechanism generating the wavefront. The gradual transition between one control mode and another may well occur at different positions in the body pattern in different kinds of embryo within the vertebrate (or for that matter the annelid–arthropod) series.

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PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES