A Clock and Wavefront Model for Control of the Number of Repeated Structures during Animal Morphogenesis

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Most current models for morphogenesis of repeated patterns, such as vertebrate somites, cannot explain the observed degree of constancy for the number of somites in individuals of a given species. This precision requires a mechanism whereby the lengths of somites (i.e. number of cells per somite) must adjust to the overall size of individual embryos, and one which co-ordinates numbers of somites with position in the whole pattern of body parts.

A qualitative model is presented that does admit the observed precision. It is also compatible with experimental observations such as the sequential formation of somites from anterior to posterior in a regular time sequence, the timing of cellular change during development generally, and the increasing evidence for widespread existence of cellular biorhythms. The model involves an interacting "clock" and "wavefront". The clock is is a smooth cellular oscillator, for which cells throughout the embryo are assumed to be phase-linked. The wavefront is a front of rapid cell change moving slowly down the long axis of the embryo; cells enter a phase of rapid alteration in locomotory and/or adhesive properties at successively later times according to anterior–posterior body position. In the model, the smooth intracellular oscillator itself interacts with the possibility of the rapid primary change or its transmission within cells, thereby gating rhythmically the slow progress of the wavefront. Cells thus enter their rapid change of properties in a succession of separate populations, creating the pattern.

It is argued that the elements, a smooth oscillator, a slow wavefront and a rapid cellular change, have biological plausibility. The consequences of combining them were suggested by catastrophe theory. We stress the necessary relation between the present model and the more general concept of positional information (Wolpert, 1969, 1971). Prospective and ongoing experiments stimulated by the model are discussed, and emphasis is placed on how such conceptions of morphogenesis can help reveal homology between organisms having developments that are very different to a surface inspection.

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1. Introduction

(A) THE PROBLEM OF NUMBER CONTROL IN MORPHOGENESIS

Among the spatial patterns of cell activity occurring in early development is one type that poses particular problems for theories of morphogenesis. This is the occurrence, within the body patterns of some animals, of series of equivalent or homologous structures, their number (on the order of 10–50) being relatively invariant amongst individuals of a given species. The two most important cases are the segmentation of the arthropod embryo and vertebrate somite formation. The former segmentation is fundamental to the arthropod body pattern, since this pattern actually consists of the subsequent unique specialization of each of the originally demarcated segments, and the ordinal number of the segment undergoing each specialization is quite reliably the same in all genetically normal individuals. Development of other than the normal number of segments is rare (i.e. number is modally canalized—see Maynard Smith, 1960). There are senses, both morphogenetic (Lawrence, 1970) and genetic (Garcia-Bellido & Santamaria, 1972), in which the segments are repetitions of a developmental unit. Somite formation, by contrast, involves just two longitudinal tracts within one of the three basic cell layers of the embryo, and the coefficient of variation for number of somite blocks developed anterior to the tail region (up to some 30, dependent on species) is some 4% (Maynard Smith, 1960, and our own data on *Xenopus* larvae). The repeating pattern is so developed as to be in relatively or absolutely close register with other aspects of the body pattern, such as limb rudiments or internal organs. We shall refer to this as the co-ordination between, say, the somite pattern and the "whole body pattern". Thus we normally find a constant number of the repeated structures (spinal nerve roots, vertebrae), associated with somite formation, lying between pairs of distant markers in the anatomy such as the base of the skull and the rear of the pelvic girdle. Derivatives of somites having particular ordinal positions in the series therefore quite reliably pursue similar specializations in each individual (Straznicky & Szekely, 1967; Mark, 1975), though number of units formed in the remaining tail material is often more variable, and abnormal temperature regimes during development can bias the overall number (e.g. Lindsey, 1966).

In both the animal groups mentioned, development is known to be regulative in the classical embryologist's sense, although in arthropods this condition may only obtain at earliest stages, before cellularization of the embryo through cell boundary formation (Herth & Sander, 1973; Sander, 1975). In such embryos, if material is removed during early phases of development before cellular determination, a normally ordered and

proportioned whole-body pattern of differentiation is nevertheless achieved within the remaining material, This is accomplished without special cell migration or re-assortment, but rather by cells re-adjusting their trajectories of development according to their new relative positions in the whole material. Compensatory cell division does not at first restore the normal cell population, so fewer cells are available to form each part of the body pattern in the remaining embryonic tissue. In amphibian (vertebrate) development, following removal of nearly half the cells of the early embryo, such regulation causes development of normally formed bodies out of an initially smaller-than-normal cell population (Spemann, 1903; Schmidt, 1933). The question can now be posed as to whether, in this regulated development, the somites follow the strict rules of classical regulation, or some other rule. Is the number of somites in the pattern kept similar to normal in small embryos (necessarily by reducing cell number in each somite at its formation), or is somite size kept at a species-typical value in morphogenesis so that (again necessarily) only a number of somites proportional to the reduced body dimension can be formed?

In fact, numbers of somites are essentially normal in these circumstances, the distance in terms of cells between the separating fissures that define them being reduced accordingly (Cooke, 1975a,b). The comparable aspect of arthropod (insect) development has not to our knowledge been tested as explicitly. It now seems very likely, however (Sander, 1975, including symposium discussion), that the answer to the equivalent experimental question is the same; that number of segment units is kept constant by similar, or the same factors as regulate the whole body pattern, rather than that size of individual units is kept "species-normal". The implications of this phenomenon for theories of spatial organization in biology can now be discussed.

Each unit (somite or arthropod embryonic segment) of repeating patterns is qualitatively similar, at least at the cellular level, at its initial formation. There is apparently a regular spatial alternation between a few modes of cell behaviour, such that successive populations of cells become separated from similar populations forming immediately before and after them in spatial and temporal sequence down the body axis. The process is essentially the regular segmentation of material already there, rather than one of serial addition at one end by true growth (though in regeneration, in some organisms, the identical result can be produced by just such an alternative process). Turing and various later authors have developed theories whereby spatially periodic "prepatterns" might arise within embryonic tissue and then control such patterns (Turing, 1952; Gierer & Meinhardt, 1972; Wilby & Ede, in press). The concept of prepattern refers to a hypothetical spatial

distribution of some quite simple cellular variables, such as concentrations of morphogen substances, the distribution being isomorphic with the final pattern of cell activities to be seen. Cells would then respond by behaving according to local values of prepattern variables, in making simple "decisions" of a threshold character between alternative behaviours. So where patterns consist of controlled numbers of similar structures, the prepattern must consist of the same controlled number of spatial repetitions (e.g. peaks of morphogen concentration). Cellular response is conceived as having a rather all-or-nothing character, the problem of prepattern control thus being one of positioning periodically, in real space, threshold values for the variables that control the switch in cellular response. The fact that somites (and usually insect segments) form in regular series in time as well as space, presents no problem in itself, as some of the more recent prepattern models produce a series of peaks distributed along another, overall morphogen gradient (Gierer & Meinhardt, 1972).

Prepattern theories can now be modelled in terms of principles known to regulate biosynthesis in cells, and their performance simulated by computation. On the basis of this, when biologically plàusible degrees of control over the various interacting parameters are considered, cases such as somites seem very demanding because of the great regularity in size, or "wavelength" in the units, and the relative accuracy in control of a large number (Maynard Smith, 1960; Bard & Lauder, 1974). Furthermore, all current models for periodic prepatterns seem to us to share a particular rather deep property, whereby they can be challenged experimentally. They all postulate allosteric feedback interactions (positive or negative) between particular large and small molecules underlying morphogen synthesis, and then diffusion of such molecules for spatial interactions in time. For any actual such system therefore, with its particular molecular hardware, the "wavelength" generated between successive repetitions should be constant on a space or cell-number basis, and so the number of final pattern units should be closely dependent on the extent of the tissue in which morphogenesis is occurring. Variation in overall dimension at early stages, much greater than one normal pattern unit in extent, is nevertheless followed by development of normal numbers of somites. To achieve this, the actual parameters describing allosteric interaction and substance diffusion, and thus wavelength, would need to be modifiable according to some local correlate of overall body dimensions.

In an abnormally small whole amphibian embryo the width and depth of the somite-forming columns of cells has also been regulatively reduced, since these are features of the whole body pattern. At an imaginative stretch one could conceive of the local "diffusion" kinetics within such columns being appropriately altered, to give adjustment of the unit "wavelength"

of a Turing type prepatterning. Experiments in which the width and depth of the predifferentiated column is experimentally reduced, on one side only of a normal sized embryo (Cooke, 1975b), probably dispose of such an idea. The results of these early cell removal operations show that number (thus lengths) of somites formed is controlled only with reference to the embryo's long dimension, and not by the width or depth of tissue in which segmentation is occurring. This is also counterevidence to the suggestion (e.g. Waddington &.Deuchar, 1953) that the width/depth/length proportions of somites in a given species are due to local control mechanisms of some sort, acting as each block of cells is individualized from the remaining material.

The positional information gradient concept developed by Wolpert (1969, 1971) provides a satisfying and plausible formal explanation as to how pattern elements might each be adjusted in extent to changes in size of the whole (the process referred to above as regulation). In its present form it does this, however, only for patterns such as that of the basic body plan (mentioned earlier), where during one period of development cells are assigned a small number of unique determinations, e.g. as heart, fore and hind-limb rudiments, kidney, etc., according to relative position within the whole. Extending earlier gradient theories (e.g. Child, 1946), this model supposes that some quite simple early variable is spatially distributed in monotonically graded fashion across the entire developing system. Its local value can be perceived by cells, and determines the choice between the small available number of developmental pathways. This positional information (hence, p.i.) variable is preserved at particular "boundary" values in special organizing regions of the embryo, and its profile of change in space between such regions is mediated by cellular interactions.

Diffusion of a morphogen substance between local source and sink cells provides just one particular realization of this concept. Many others are possible (Wolpert, 1971; Goodwin & Cohen, 1969; Cohen, 1971). Their chief feature is that removal or internal transposition of cells is followed by restoration of all normal gradient values in their normal order, though the gradient in cell state, per cell may be more or less steep (after size alteration). This in turn mediates pattern regulation in later morphogenesis, since a normal range of values for the p.i. variable across the system results in a normal spatial distribution of the developmental pathways followed by the embryonic cells. A variety of mechanisms [such as that of a few, bistable, genetic switching systems (Kauffman, 1973, 1975)] could plausibly control cellular entry into one or other developmental pathway according to thresholds in values of such a variable, "read" directly by the cells and interpreted accordingly. It is when we consider the co-ordinated development

of the many-times-repeated aspects of body patterns that such direct inter-
pretation becomes implausible.

We know that numbers of somites are (a) usually in close register with the
whole body pattern and (b) approximately normal in abnormally small
bodies. Naively, a mechanism for achieving this would be to have the pre-
somitic cells, which are two longitudinal tracts down the body axis, responsive
to a particular regular succession of values of the p.i. variable or body
gradient, to which alone they react by performing the activity that defines
either the centres of somites or the fissures between them. But this requires
an implausible performance for any interpretative mechanisms working
within cells, all of which are in the same state of non-determination at the
time of laying down the pattern. Cells would be required to respond with a
particular programme of activity (say, de-adhesion from their neighbours, or
extra close adhesion) to each of some 15 to 30 discrete values of a variable, while
ignoring or reacting differently to all others in between. Furthermore, somites
are always regular in size within each embryo, and yet their number is not
absolutely invariant, relative to whole body pattern, within each species. We
observe complete body-patterns within which there happens to have developed
one more or one fewer somite than usual. This observation is incompatible with
a strict p.i./interpretation mechanism, since it implies that spatial regularity is
a deeper property of somites than their precise relation to other body parts.

There is striking evidence from haploid amphibian embryos (Hamilton,
1969), that the "wavelength" of the somitogenic process, which ultimately
determines somite number in the body, is controlled as a spatial extent of
cellular material rather than as a particular cell number. The more recent
results just discussed show that this "wavelength" in the repeating programme
of cell processes is itself adjusted, according to the overall dimensions within
which morphogenesis is occurring. The profile (i.e. steepness) of a gradient
in a variable, registering relative position in the body, is strongly implicated
in such control.

We next describe briefly the cellular anatomy of somitogenesis as it appears
in the amphibian *Xenopus*, to show how it lends itself to quantitative study
and to the pursuit of a theory of control. We then present the outline of
such a theory. This involves postulating a wavefront of sudden cell change,
passing along the pre-somite material and controlled in its time course by
the whole body p.i. gradient. It is then proposed that all the cells are also
coupled oscillators with respect to an unknown "clock" or limit cycle in the
embryo, periodically modulating the effect of the wavefront as the latter
progresses. The additional problem of co-ordination of somitogenesis in the
tail rudiment (Cooke, 1975a) where the process progresses for the first time
into truly growing tissue, will not be dealt with in detail.

(B) THE ANATOMY OF SOMITOGENESIS

Somitogenesis in the amphibian *Xenopus* is described at the light microscope level from wax embedded material, by Hamilton (1969). Her findings have been confirmed from glutaraldehyde-fixed and araldite-embedded embryos (Cooke, unpublished) and also extended to *Bombina*, another anuran amphibian. Figure 1 shows, schematically, how presomite mesoderm all along the body first migrates towards the midline to form the two longitudinal columns of spindle-shaped cells, one cell wide transversely and many cells deep dorso-ventrally. The actual width (i.e. degree of stretching of the long axes of the cells) and depth (number of ranks of cells in transverse section) both decrease smoothly in posterior regions, and the cells are also smaller there when the later somites are formed, presumably because more cell divisions have occurred since onset of egg cleavage. The process of somitogenesis itself then occurs in the successive isolation, by transverse fissures where cell de-adhesion occurs, of blocks of spindle-shaped cells. Each block is initially a regular number of cell-widths in extent, as counted along the animal's longitudinal axis, but then rotates through 90°, the medial edge moving forward. It thus becomes a single bundle of spindle-shaped cells, now a defined number of cells wide at any particular horizontal level in the embryo, and of course as many cells deep as the original presomite column. The process from visible onset of de-adhesion to completion of rotation does not normally occupy more than two successive blocks or presumptive blocks in the series at once. Each block is therefore half-rotated by the time the subsequent block is first defined by the next de-adhesion. In principle the positions of many subsequent fissures might already be determined in a "prepattern" sense, behind the latest visible morphogenesis at any one time, but there is no evidence that this is so.

The cells almost never undergo division (mitosis) once "blocked" and rotated. We know moreover, from histological sections, that each somite just after its formation shows a number of cells, in its width, comparable to that seen there after many subsequent somites have been formed. This number therefore remains a record of the local "wavelength" (in spindle-cell widths) of the somitogenic process as it occurred in each part of the body, even though stretching of the larva finally lengthens the spindle cells considerably (see Fig. 1). Somites actually form, within each embryo, with a clock-like regularity in time. This knowledge comes from experiments in which a large population of sibling *Xenopus* embryos, set to develop under constant conditions and synchronized at onset of somitogenesis, was sampled at regular intervals of laboratory time by fixing and dissecting batches of five embryos. Within no batch did number of somites formed vary by more
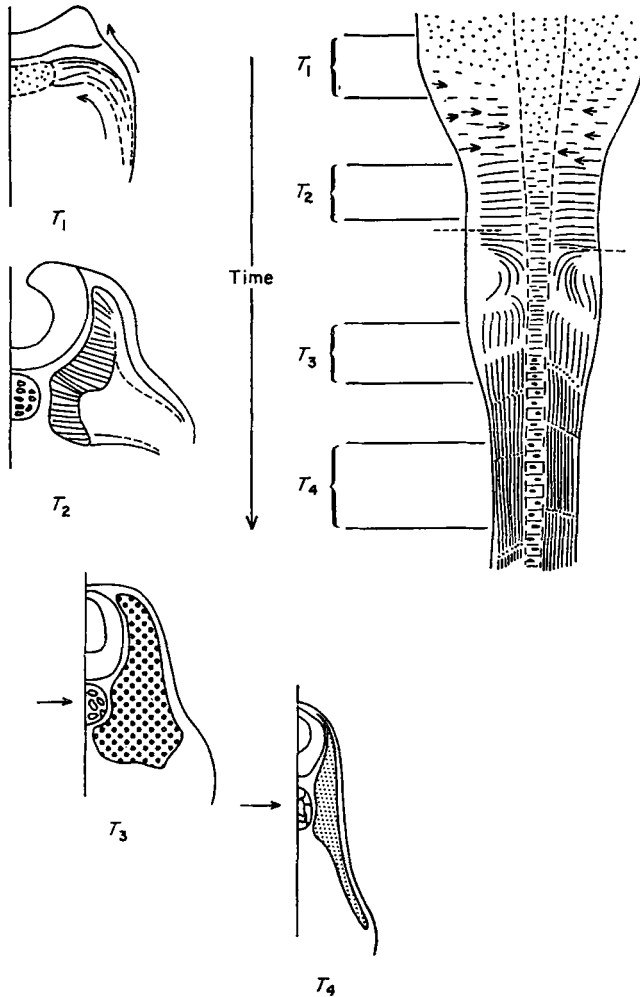
Fig. 1. Representation of the cellular anatomy of somite formation in *Xenopus*. Somite formation is represented in horizontal, longitudinal section through the notochord (mid-dorsal axial skeleton). Gradation from top to bottom represents advance in development with time at any one level of the head–tail axis. Each level passes stages T_1, T_2, T_3 and T_4, which are each shown in unilateral transverse section. Note initial demarkation of the mid-dorsal notochord cells from the mesoderm (see section 3) and then progressive migration towards the midline of the elongating pre-somite mesoderm cells to form into columns of spindle-shaped cells as at T_2, while the notochord starts differentiation. Formation into somite blocks, by de-adhesion then rotation of cell groups, is shown, completed by T_3. Stretching during continued notochord differentiation (vacuolation) increases greatly the lengths of formed somites, making their cells more slender as they differentiate (T_4). Numbers of cell-widths visible, at the notochord level marked in T.S., remains unchanged between T_3 and T_4, however.

than one, over formation of the first 30 somites! At any one temperature, this temporal regularity is accompanied by great repeatability in size of successive somites (say 9·5 cells' "wavelength" in anterior body regions, derived from averaging horizontal section cell counts for each somite within single embryos). Both features are reflected in the smoothness of an experimental curve such as that of Fig. 2, of cumulative cells in somites against time at 21°C. One of us (J.C.) found somites to form at one per 40 min anteriorly, slowing to, about one per hr posteriorly, but this may have been due to a cooling laboratory. The real plot for somites formed against developmental time may be linear (Murray Pearson—personal communication).

Despite the above-mentioned regularity of size, somites do become smoothly and systematically smaller (fewer-celled) in posterior body regions after about somite 15. The decrease in slope, or parabolic shape of the curve of cells per time, as in Fig. 2, thus has one certain cause, and may have a second (slower formation of each somite) acting synergistically. Because
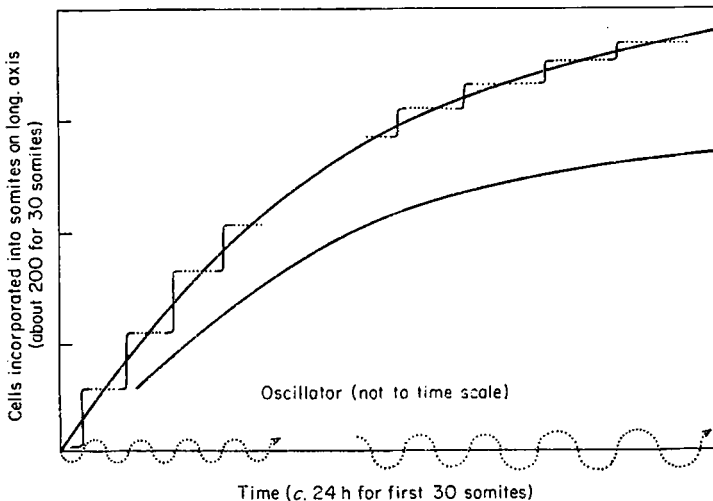

FIG. 2. The curves for rate of cell involvement in somite formation, experimentally determined and for a hypothetical small embryo. Cumulative numbers of spindle-cells, in horizontal sections, incorporated into somites (ordinate), are plotted against laboratory time (abscissa). Upper curve is experimentally produced from a control *Xenopus* population (see section 2), while the lower one is that expected for a regulated, experimentally size-reduced embryo. Hypothetical oscillator advances and retards recruitment of cells into new behaviour (oscillations not to time scale), so that the curve is actually manifested as a step function whose vertical components are the synchronous rotation of blocks of cells adhering together. The curve slope (cells per time) definitely decreases with time, because later somites are smaller and form either slightly less often (our data) or at the same regular rate as earlier ones. Result: the plot of cells per somite, per somite in the series is a near straight line.

cells in later formed somites are themselves smaller, histology shows that in terms of material between fissures, the wavelength decrease is even more accentuated than it is in terms of cell number. Examination of newly forming somites at widely differing body levels supports the following; that whereas width and depth of the somite column, and "wavelength", each decrease smoothly along the body axis, the proportions in these dimensions are by no means kept constant. In other words the small blocks of posterior regions are not simply scaled-down isomorphic versions of anterior ones. In fact the very first somites, where the wavelength is maximal, nevertheless have abnormally reduced widths and depths when formed, because the pre-somite column here fits in between head structures and is reduced. This is further presumptive evidence (see also section 1), that the deployment of fissures to establish the total number of somites is controlled with respect to the length of the whole body, and not by a local proportioning process.

We are ignorant of the biochemistry or even cell biology of the organized changes in adhesion and locomotory behaviour whereby this morphogenesis is brought about. Thus it is only appropriate at present for models of the pattern formation to be formal ones, that can be mapped onto whatever machinery is found to mediate cell behaviour. Anatomy of somite formation in the tailless amphibia is clearly secondarily simplified, in the evolutionary sense, but the more basic vertebrate version can be related to it easily enough. The essential problem of creating by cells' behaviour a set of discrete, self-adhesive populations separated by boundaries, is the same in the formation of more radially symmetrical "rosettes" as in salamanders, birds and mammals; the process is just harder to study quantitatively.

(C) THE MODEL

By the close of gastrulation, some hours before the earliest anterior somites are formed, the embryo is already a mosaic of regions, each determined as capable only of differentiating into particular parts of the whole body pattern (Holtfreter & Hamburger, 1955, for review). Gastrulae chopped in two in the future transverse plane at this stage, for instance, develop into quite well-formed anterior and posterior half-larvae, with no regulation and with numbers of somites that together add up to about the normal for whole bodies. Let us suppose that a longitudinal gradient of the whole body p.i., whatever its nature, is used at this early stage to determine two aspects of the future development. It sets cells to differentiate in qualitatively unique ways in different places, and also sets rates for the process of intracellular development, according to its local value, along the columns of cells that are determined as pre-somite material. There will thus be a rate gradient, or timing gradient along these columns, and we shall assume a fixed monotonic

(not necessarily linear) relation between rate of an intracellular evolution or development process, and local p.i. value experienced by a cell at the time of setting that rate.

Sufficiently complex molecular descriptions are not currently available for us to know the nature of the control of timing, direction and execution of the developmental programmes undergone by early embryonic cells. How many variables are involved in such control? Are many of the determining variables in the "metabolic" state-space of the cell (see, e.g., Goodwin, 1963), or are nearly all of them of macromolecular "switch-like" specificity? In view of this ignorance an adequately general description of the developing cell is as a multi-dimensional state space, or manifold. In this state space, the vectors must be such that a relatively small number of attractor domains exists, each corresponding to determination and then biochemical and hence cell-behavioural execution of one of the differentiation pathways open to cells of the species concerned. At the phenomenological level, cells in early embryos characteristically behave "smoothly" and stably with little overt change for considerable periods, interspersed with relatively rapid overt changes of behaviour and state of determination.

Catastrophe theory has been used (Thom, 1973; Zeeman, 1974) in describing the possible intracellular and intercellular processes during development. The trajectory of development within a cell is modelled by the movement of a point within the manifold that represents the intracellular state space. Smooth motion along homeostatic surfaces within such manifolds may be interspersed with relatively sudden, unstable jumps to new such surfaces (i.e. catastrophes). Such a choice of description is by no means essential for understanding the present model but does lend itself well to thinking about ways in which developmental switches and programmes of activity may be ordered in space and time during morphogenesis. We are suggesting that the p.i. cellular variable itself sets the time, within individual somite cells, at which an instability will be reached followed by the catastrophe or by immediate competence to undergo the catastrophe if triggered, say by a catastrophe-associated signal from an anterior neighbour cell. According to preference, the catastrophe may be imagined as the switch-on or switch-off of a single, "executive" unit of genetic activity, or just the forceful entry of the cell state into a new attractor domain or configuration of metabolic-genetic activity (e.g. Kauffman, 1969). But let us assume that the sudden change in locomotory, and adhesive behaviour that occurs as cells participate in formation of a somite, is the expression of such a programmed discontinuity in their development.

Now a fixed relationship of p.i. (gradient) variable to cell development rate is proposed, and we know (at least on the positional information theoretical

paradigm) that after size reduction of an early embryo the gradient will be restored to a normal range of values. Thus, a wavefront in space of sudden cell behaviour change will pass down the body pattern in the somite column, and the rate of passage of this will be such as to traverse the entire column (relative to other markers in the head–tail axis) in unit time, whatever the size of the whole body pattern. This time period will be a characteristic of the species at any one temperature, because of (a) the fixed relation of intracellular development rate to p.i. value and (b) the normal gradient profile between boundaries of the regulated body pattern. Speed of the wavefront, in terms of cells per time, will be proportional to the length (cell number) of the embryo, for each part of the whole body pattern, provided that morphogenesis has regulated to wholeness after any early disturbance.

Such a wavefront might arise from any of the variety of underlying mechanisms. For instance, it might partake of the character both of a true wave, involving propagatory interactions between cells, and of a purely kinematic "wave" controlled, without ongoing cellular interaction, by a much earlier established timing gradient. The kinematic wave might be set up with respect only to onset of intrinsic competence for catastrophe, while a catastrophe-associated intercellular signal might mediate propagation of the actual rapid cell change down the body. But a gradient in the cells' intrinsic rates of development must limit the speed of the wavefront to account for regulation of the somite pattern. The smoothness of a purely kinematic wave might seem to depend upon extreme accuracy of control, over many hours of intracellular development rates in response to the early gradient. However, we might expect "thermal" fluctuations to be smoothed out, meanwhile, by intercellular diffusional communication for variables (substance concentrations) that decisively affect timing of the catastrophe. Indeed, the local reversal of developmental gradients of behaviour following grafting experiments (Waddington & Schmidt, 1933; Abercrombie, 1950) is evidence for such local interactions.

A smooth antero-posterior timing gradient (thus, wavefront) with respect to morphogenetic movements and differentiation in general, is a widespread empirical feature of early vertebrate and arthropod development. Moreover, in the case of experimental small *Xenopus* embryos, the process of formation of somites progresses from the head to the tail over the same period as in synchronously developing unoperated controls. So in a sense this element of the model is simply a surface description of a reality that itself requires an adequate explanation.

The morphogenesis we observe involves recruitment of cells, in discrete successive populations of regular size, into an activity which otherwise they might enter in smooth succession as a result of the wavefront. In fact,

the control problem is that of the repeatability of a number, so that the large unit time we propose as that occupied by transit of the wavefront must be partitioned by a regular series of much shorter time intervals, also characteristic of the species at a given temperature. We propose that the latter are provided by an oscillator, shared by all the pre-somite cells, with respect to which they are an entrained and closely phase-organized population, because of intercellular communication. Probably, this time period is that actually observed to count out the very regular morphogenesis of somites down the axis. But note that in principle both wavefront and "clock" might be earlier, very much faster processes than those observed in the final cell behaviour. By programming the latter behaviour, these hidden processes would then in effect be setting up a periodic pre-pattern, and visible somite formation would be a "secondary process" in the terms used by Zeeman (1974).

We conceive the oscillator as interacting with the wavefront by alternately promoting and then inhibiting its otherwise smooth passage down the body pattern. It could do this by affecting periodically either the onset of catastrophe in cells, or else those particular expressions of the rapid change which cause the new locomotory-adhesive behaviour. This can be visualized (Fig. 2) as converting the course of the wavefront into a step function in time, in terms of the spread of recruitment of cells into post-catastrophe behaviour. The observed distribution of somite centres or of the fissures of de-adhesion between them, would result because each population of cells had become self-adhesive and discrete, due to almost synchronous behavioural change, at a time significantly after the preceding such population, but significantly before the subsequent cells were competent to participate. Although the biochemistry of the control of mutual recognition, motility and adhesiveness in cells is little understood, it seems reasonable from the look of the process in various animals that this is how each somite is individualized.

The essential property of number preservation during morphogenesis follows from the species-typical, invariate values for both the transit time of the wavefront along the body pattern, and the period of the "clock" during this transit. The lengths of the cell populations recruited by clock and wavefront interaction, like the rate in cells per time of the wavefront itself, will be proportional to embryonic length.

The postulation of an endogenous oscillator of cell behaviour as controlling animal development is not as *ad hoc* as might seem. Such oscillations, with a period of minutes, are now known to be fundamental to slime mould morphogenesis (e.g. Robertson, 1972), where they were first discovered because the initially single amoebae aggregate across distances involving

vigorous motile behaviour. But the cells among which most early animal morphogenesis occurs are quite closely stacked in sheets, or else held in presumably contact-inhibited mesenchymal layers. Unpublished observations of time-lapse cinemicrography (J.C.) suggest in a preliminary way that when moving extra vigorously as in neurulation or bird gastrulation, such cells may propagate short-term periodicities of behaviour. Although most overt biological oscillators studied hitherto have had cycle times on the order either of fractions of a second (neuronal) or of many hours (circadian systems) we need not assume there is any *a priori* problem in postulating the intermediate, an hour long oscillator. Relaxation times of cyclical processes involving known levels of biosynthetic control machinery can accommodate intracellular oscillations of such periods (Goodwin, 1963). But known oscillators show independence from inhibition of macromolecular synthesis and from many metabolic inhibitors (Enright, 1971), so that universal models depending upon ion/membrane transport phenomena are becoming increasingly plausible (Njus, Sulzman & Hastings, 1974).

For readers who enjoy conceptualizing visually, though maybe not for others, the development of the embryo can be graphed in three axes as in Fig. 3. In Fig. 3(a), real space and real (developmental) time are horizontal, and the state-space or manifold representing intercellular states is collapsed onto one, vertical axis. The progress of the whole embryo is then represented by a folded surface (see also Thom, 1973; Zeeman, 1974), in a space where the analogue of the overall vector driving development in both its stable and catastrophic phases would be the force due to gravity. Only a sector of the space axis some three somites long is shown, so that the fold edge marking the catastrophic instability in intracellular development recedes in time through, say, 3 hr within this sector of tissue. Considered as a wavefront in real space, the sudden change or competence to undergo it spreads through the tissue in 3 hr. We then express the limit cycle of the oscillator as shown, in real time and in some dimensions of the intracellular state space, assuming that cells are so closely phase linked as to be effectively locally synchronous on a developmental time scale. The oscillator interacts with the homeostatic surface that includes the catastrophe fold in each cell, so that as the cells move in smooth succession towards this fold (according to spatial gradient of developmental rates organized by p.i.), their entry into instability and fast change is rhythmically gated in time. Alternatively the catastrophe fold is visualized as parallel to the real space axis as in Fig. 3(b). Then a head–tail row of cells has to be represented as points moving through time on the upper and lower surfaces, obliquely to the fold edge. On the catastrophe fold, the undulation in time ensures that cells arriving at the unstable fold edge undergo rapid change in regular groups.
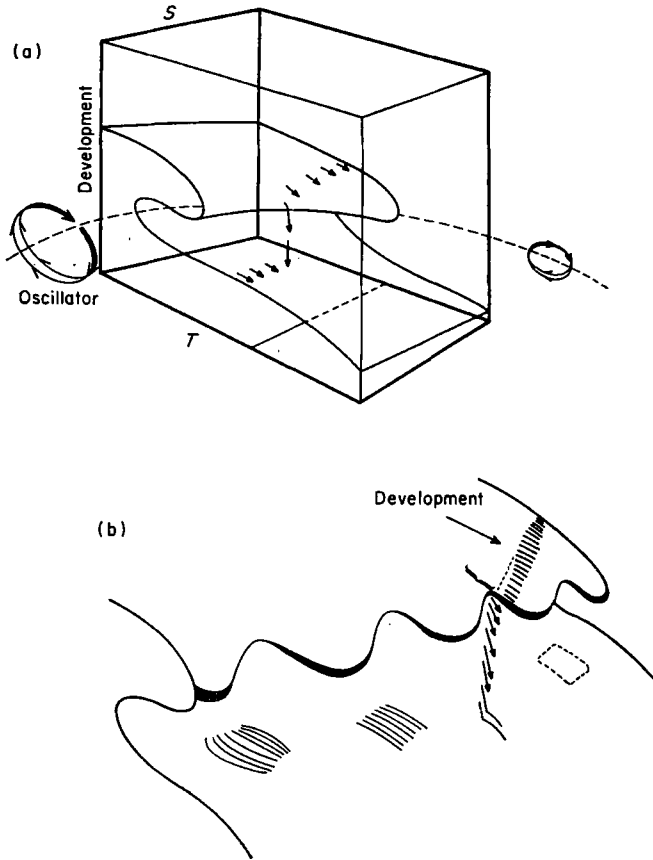
FIG. 3. Topological representations of the model for control of somite number. (a) A section of the embryonic axis a few somites long graphed in real space (S) in head–tail axis, real developmental time (T) (i.e. onset of somite formation at each level) and a dimension representing intracellular development (vertical, with gravity as analogue of the vectorial nature of development). The fold in the descending surface, representing onset of fast unstable cell change involved in somitogenesis, is oblique to the time and space axes. Thus of any longitudinal string of somite-forming cells, some will not yet have changed, a group will be changing and the rest will be in a new era of slow development (differentiation) following change. The hypothetical oscillator that controls the grouping of these cells is represented as a point describing a limit cycle, in real time and in some of the intracellular biochemical dimensions (vertical). The dashed line shows that it involves oscillation in the position of the instability or fold-edge. (b) The same surface is shown, but with real space and time represented by the rippled shape of the fold-edge and by drawing a string of cells travelling through their development (i.e. time) at an angle to the axis of the fold-edge, so that all must meet it. Cells thus undergo change in a succession of discrete, synchronized groups in time and space. A formed and differentiating, a just-formed, and a forming block are shown, while still on the upper surface are the presumptive cell-group of the subsequent somite (dotted lines and bracket).

At this point it is tempting to digress, and note that immediately before the first somites are formed, the pre-somite columns themselves have been demarcated in the mesoderm by a longitudinal pair of fissures of cell de-adhesion, separating them off from the mid-dorsal strip of the cells that then differentiate as the unsegmented notochord. Both the formation of these fissures and the first stages of notochord differentiation progress very quickly indeed down the long axis, so that notochord demarcation is nearly syn-chronous over at least the anterior parts of the body pattern. So mesodermal cells are capable of creating fissures of de-adhesion at this time, and if a clock is utilized in somite formation we should expect it already to be operating in cells at slightly earlier stages. Why does the notochord column not segment? We can say that the wavefront of cell change is many times faster-moving in this case, as compared with somitogenesis, either because the p.i. gradient is of very shallow profile in the midline or because the relation between p.i. and the timing of cell change is different during noto-chord formation. One can imagine on many grounds, especially if genuine propagation of catastrophe events were involved, that a fast progressing wave might not be susceptible to periodic interruption, by a given cycle of events in the cells, that would convert a more slowly travelling one into a step-function.

(D) TESTS OF THE MODEL

Just as the model has two elements, two types of experiment are suggested by it. They attempt to alter the number and size distribution of somites in ways consistent with having disturbed a gradient profile and a clock, respectively. After early operations transposing sectors of presomite material between disparate levels of the body axis, we might expect regions containing abnormal numbers and sizes of somites. These would be understandable as regions where abnormal slope or steepness of the p.i. gradient, and thus rate of passage of the wavefront of cell change, had obtained at the time of somite formation. They would be the equivalent in somitogenesis of the miniature patterns of cuticle types seen in the regions of grafts in insect epidermis (Stumpf, 1968), where graft/host interaction mediating gradient regulation is incomplete at the time that the gradient sets the pattern (Lawrence, 1970). Such experiments on amphibian gastrulae have been started and will be reported elsewhere, but the work presents difficulty, histologically and in interpretation of results. Abnormally long and short somites are seen, however, in regions of experimental larvae which show by their anatomy that host–graft interaction, rather than self-differentiation of graft and host material, have occurred.

Most classical grafting experiments studying amphibian morphogenesis have been performed either too early or too late to be of interest in the present context. In the first case, the whole-body p.i. gradient regulates its profile completely, as judged by normal morphogenesis. After late transpositions, pre-somitic tissue presumably merely self-differentiates with regard to somite number and to time, and the sequence of morphogenesis can also "cross" gaps made late in the pre-somite column, to proceed with normal timing posterior to them (Deuchar & Burgess, 1967). Such results suggest that the timing gradient is determined early on in cells by p.i. and furthermore that cells do not require immediate signalling from neighbours for passage of the wavefront of cell change, even though signalling might normally be involved in ensuring continuity and smoothness of the process. We stress that any empirical demonstration, that somite size can be varied in a way related to steepness of a hypothetical gradient after grafting operations, is not distinctive of the model presented here as opposed to a direct positional information/interpretation model (see section 3). Such demonstration would merely reinforce the belief that somites cannot be controlled by a repeating prepattern of the Turing class.

The much more distinctive, second experimental approach is that of attempting to unlink an oscillator from its normal tight co-ordination with the timing gradient, so producing abnormal somite numbers and sizes, possibly in otherwise normal animals. Somite number is highly constant across a wide range of developmental temperatures in *Xenopus*, even though development of the pattern may take $2\frac{1}{2}$ times as long at 17°C as at 26°C, and similarly, concentrations of respiratory inhibitors that slow development somewhat, leave the somite number normal if overall development is normal. Thus we must assume that the oscillator is really quite deeply embedded in the intracellular developmental process, maybe itself measuring out or setting the longer term rates of other aspects of development, including the catastrophe underlying the wavefront. But we cannot cling to such a saving clause through too many empirical failures to unlink normal somite number from normal development, lest we be accused of ascribing the harmony of morphogenesis to the harmonious revolutions of the heavenly spheres.

A small list of substances is becoming known to affect the free-running frequencies of a wide variety of circadian and other biological oscillators (Enright, 1971; Pittendrigh, Caldarola & Cosbey, 1973). Chief among them is heavy water (D_2O) which slows the frequencies of many systems at concentrations tolerable for development.

Development of embryos in heavy water media until they have formed about their first 20 somites does indeed alter the number of somites found

between the base of the skull and the hind limb-bud, to a small but significant degree. The situation is complicated, however, by the effects of the D_2O upon the stretching processes in the embryos and also, apparently, on the cell-division schedule in the pre-somite cells (Cooke, unpublished data).

A further approach to be pursued consists of attempts to perturb the hypothetical "clock" by very short stimuli of a shock nature. The disturbances in final somite pattern that follow such perturbations, at the population level, might be found to correspond with the phenomenology ("temporal topology") that has been established and reviewed by Winfree (1975) as distinctive of perturbed biological oscillators in a variety of systems. These oscillators appear to be describable as limit cycles in a space that defines phase and amplitude around a singular state.

A critical-sized perturbation delivered at critical phase, rather than phase-resetting the oscillator as other similar perturbations do, brings the system into the neighbourhood of the singular state, following which amplitude is reduced or obliterated over many cycle-times, or else the system recovers with random phase. These types of disturbance should each be distinctively noticeable on appropriate local examination of the somites, if the appropriate mode of short-term perturbation can be found.

(E) THE DISTRIBUTION OF SOMITE SIZES IN THE NORMAL EMBRYO

Characteristically, in the early embryos or larvae of a variety of vertebrate types, somites are equally sized in the anterior and middle parts of the body-pattern, and then form a smooth gradation of decreasing sized populations of cells posteriorly into the tail. Because of the simple anatomy and lack of mitosis in somite muscle cells described in *Xenopus*, the numbers of cells cut off in the longitudinal dimension, between all the intersomitic fissures for about the first 30 somites, can be recorded in horizontal histological sections. Using these in conjunction with the curve for rate of somite formation per somite (see section 2), derived from the same population of embryos, two curves can be plotted. These are for total cells in the long axis incorporated into somites, against time (see experimental curve of Fig. 2), and against number of somites formed. The decreasing rate of wave front progression posteriorly is expressed as fewer-celled somites there; especially so if possibly slower tempo of the clock (see earlier) is taken into account. Ignoring cell boundaries and considering only space or distance across cell surface, as mentioned before (section 2) we have another component of slowing of the wavefront, not incorporated in such curves, due to the smaller cells when somites are formed posteriorly. None of the curves reaches zero slope, but somites were still being made in the tail at the time of preparation for histology in these embryos.

What factors could underlie such a trajectory of the wavefront? We suggest two alternative sorts of explanation. Firstly, we deduced from the coherent head to tail sequence of somite formation that the relation between the initial p.i. value and the local timing of the catastrophic change in cells was monotonic, but it need not be linear. Figure 4, in which the wavefront of the catastrophe in real space is drawn passing into a graded surface
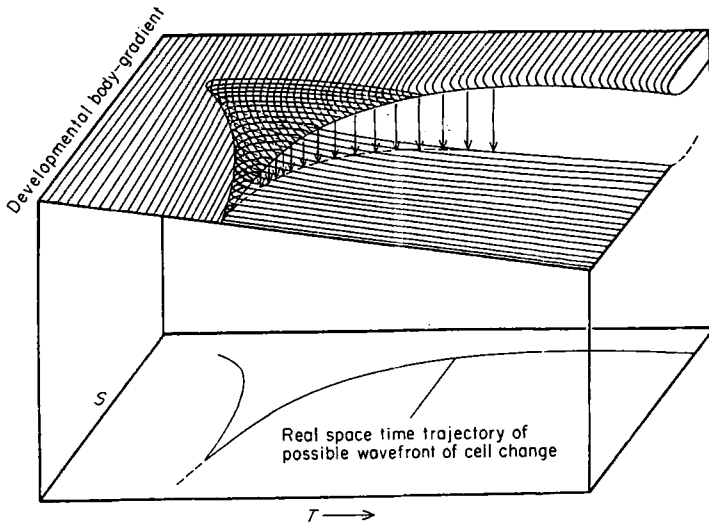

FIG. 4. A cusp-catastrophe unfolding into a graded surface. This shows a possible way in which the dynamics or shape of the surface in the state space within cells may condition the trajectory in real space (S) and time (T) for morphogenetic changes occurring as a wavefront in a linked sheet of cells. In the present instance, such a concept may help in understanding the size distribution of original somite masses in the embryonic body, though it has more general significance (see text section 5, and Zeeman, 1974).

(i.e. the length of the embryo as a p.i. organized gradient of decreasing development rate), itself suggests how the wavefront trajectory might describe a branch of a cusp. Projected down onto the space/time plane only, this cusp branch eventually comes parallel to the time axis; that is, a stable frontier forms in space between changed and never-to-be-changed cells (see Zeeman, 1974). Now since somites form to the tail-tip, our frontier here is virtual, as it were beyond the growing tail-tip. While the range of gradient values actually found within the embryo never prohibits the spread of somite morphogenesis, there could exist in posterior regions an increasingly non-linear relation between p.i. value and the successively later onset of competence for the rapid cell change, reflected in slower progress of the wavefront

and smaller somites. This might occur even if the p.i. gradient itself were better expressed as a linear change in real space, the non-linearity coming in the relationship of catastrophe timing within the cell to p.i. value, whereby the wavefront in real time could describe a section of one branch of a cusp as shown in Fig. 4.

Alternatively, we can have no meaningful *a priori* expectation that the profile of p.i. in the body axis is in any sense linear, rather than convexly non-linear which would also produce the observed distribution of somite sizes. A "steeper" progression of gradient values in tail regions would lead to a slower wavefront progression, thus shorter somite populations. The mode of maintenance of positional cues in growing tissue is controversial (Summerbell, Lewis & Wolpert, 1973; Wolpert, 1975, see symposium discussion) but certain experiments on amphibian tail rudiments suggest that after about the level of somite 15, there is no longer any longitudinal cellular interaction in maintaining the position gradient, but rather a special terminal posterior zone of tissue where cells of new values are generated (Cooke, 1975a). In this case any "shape" of gradient is plausible as there are no longer any stability (diffusion) problems.

The locus of such non-linearity (i.e. is it in real space, the gradient, or in the intracellular machinery of response to the gradient?), may never become meaningfully definable, biochemically, but possibilities like those outlined above enable us to conceive how varied types of patterns of cell determination may be controlled synchronously within the embryo, so as to remain spatially co-ordinated as is observed. The replicability of the timing of somite formation, in embryos beginning development synchronously, is evidence with which the overall timing of cells' activities can be controlled. The known "accuracy" of biological clocks in other systems is very great (Winfree, 1975; Enright, 1971), such that interaction of wavefront and clock could quite plausibly produce a co-efficient of variation in somite number, within species, as low as that observed.

2. Conclusion

Although abstract, we do not feel that these hypothetical ways of thinking about development are vacuous. They promote searches for the entities (in this case "clock" and "wavefront") that they postulate, at least at the phenomenological level. If validated at this level, then even without a molecular analysis, they allow us to proceed with considering how form might be re-created reliably in each ontogeny, as well as changed during evolution. For instance, although our model (Zeeman) and experiments (Cooke) refer in the first instance to Amphibia, a similar model would appear

equally valid for vertebrates as different, superficially, as birds or mammals in their early development. In such ways we might see more clearly the homology between the superficially very different early morphogenesis of distantly related organisms, which is only hinted at on comparative anatomical criteria.

APPENDIX A

A Threshold Model

We have seen that it is generally difficult for models based on gradients, thresholds, etc., to allow the degree of number control observed for somites and arthropod segments. However, one type of threshold model that would behave appropriately has been suggested by Graeme Mitchison, of the MRC Laboratory of Molecular Biology, Hills Road, Cambridge. By analogy with particular positional information models for *Hydra* morphogenesis (Wolpert, 1971, for review), imagine two gradients, in substances P and I, running in homopolar manner from head to tail of the embryo. Assume linearity and assign an arbitrary normal range of values (1 to 0) to each gradient. Suppose that P (non-diffusible) is fixed within cells throughout somite formation, while I can diffuse rapidly to equilibrate in concentration across local groups of cells when they are coupled (but separated from the source/sink boundary regions). At first all cells become uncoupled, before the "wave" of cell change is initiated in its passage down the embryo. Passage of the wavefront then involves successive recoupling of cells. As a group of coupled cells grows in size by recruitment of those posterior to it, the joint "coupled" value of I will fall, so that eventually the difference $(I-P)$ will rise to some threshold value within the tailward cells of a coupled group. If the response to this threshold is failure to couple, of the cells where it obtains, with those behind them then a population of cells will become functionally isolated as a unit. If boundary values of the body gradient P and I and for the $(I-P)$ threshold are fixed, unit number will regulate over different lengths. This model still postulates a wavefront, but not an endogenous oscillator whose temperature coefficients must co-ordinate with that of the wavefront transit in poikilothermic animals.

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