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Towards an understanding of spatial patterning in development*

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Abstract

A characteristic feature of the way embryos develop is that, by and large, differentiated tissue types preserve topological order Therefore cells must have a means of sensing their (relative) positions in the early embryo. The present article describes two extreme possibilities for the means by which a cell can sense its position. One, positional cues can be provided by the mother by way of morphogenetic determinants laid down in the egg. Two, by means of intercellular interactions whose effect is to set up long-range patterns of external signals, embryonic cells can organise themselves spontaneously.

Key words: Development, pattern formation

1. Introduction

The desire to understand development, the process whereby a relatively unstructured entity, the fertilised egg, is transformed into a highly differentiated multicellular organism, dates from antiquity. Aristotle is said to have been the first to pose the issue in terms of two rival hypotheses, *preformation* and *epigenesis*, which are still valid today (though not in the same sense as that used by Aristotle; *see* Gardner[1]). Developmental biology has evoked a remarkable degree of interest in the last few years. Much of this interest stems from a feeling of anticipation, a feeling fuelled primarily by the successes of contemporary research in elucidating the molecular correlates of development. This has raised the hope that the answers to many long-standing problems may finally be at hand. There is even the expectation that the discovery of general laws of development is imminent. One law is tacitly accepted as valid by a large number of workers: development is under the control of, or even 'programmed' by, genes (Luria[2]; for a contrary view *see* Newman[3]).

The purpose of this essay is to present to a general readership, firstly the reasons why the ideas of preformation and epigenesis are still current; and secondly, in bare outline, how these concepts are analysed. I have avoided giving detailed references, so it is worth mentioning here that developmental biology is fortunate in possessing

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a rich and readily accessible literature. Pertinent to this article are the book by Bonner[4], who looks at the development of form from an evolutionary point of view, and a much older work by D'Arcy Thompson[5], who does so as a physicist or engineer might; a discussion of the problems of development within the overall context of our attempts at understanding the major problems of biology by Smith[6] and two recent books by Wilkins[7] and Slack[8] which constitute the best attempts yet to integrate what we have learnt about development into a conceptual whole. For those unfamiliar with selectionist arguments, Jacob[9] and Dawkins[10] provide an excellent introduction. Wilson's classic *The cell in development and heredity*[11] is a useful reminder that not all supposedly modern insights are new.

2. Background

To begin, I will try to highlight those properties of cells and cell groups which appear to be important for an understanding of pattern formation in animal development. A basic assumption is that rules or laws underlie the orderly behaviour of cell groups; only the discovery of such rules can justify the assumption. An alternative point of view might be, for instance, that development consists of the detailed unfolding of a genetic program, and that what we think of as rules governing multicellular behaviour are at best convenient mental constructs to aid developmental biologists. One can think about cells in two different ways, sometimes as 'test-particles' whose behaviour tells us something about the 'fields' which direct such behaviour, and at other times as the sources of the fields themselves. Eventually, so the hope goes, it should be possible to work out a self-consistent picture by combining the two views.

Whichever picture is adopted, the aim remains the same: an understanding of the arrangement of cellular phenotypes within the 3-dimensional space defined by the embryo. Here, by *phenotype* we mean, roughly speaking, what a cell 'looks like'. A better definition might be 'the spectrum of proteins made by a cell'. The problem, in other words, is that of space-dependent gene expression. This follows from the observation that during development, cells of the same genetic constitution make different proteins, that is, express different subjects of genes. Therefore, there must be factors extraneous to the genome which make them do so. In addition to possible differences in the past experiences of the cells, the factors must depend on (a) the external environment or (b) internal non-uniformities spontaneously arising as a consequence of mutual interactions [or, of course, on a combination of (a) and (b)]. There are circumstances in which isolated cells which share a common developmental history can exhibit different phenotypes even under identical environmental conditions. However, these phenotypes are necessarily arranged in random spatial patterns, and we will not discuss them. Except for assuming that the gross cellular phenotype is the signature of the underlying genotype, I will not touch upon problems relating to genetic structure and regulation of gene expression. Another important aspect which we will skip concerns the study of development from an evolutionary point of view, a very popular subject in the years immediately following Darwin. Haeckel and popular sentiment notwithstanding, the belief that the embryonic forms of organisms

resemble the adult forms of their ancestors has no basis in fact (if anything the opposite is true in the case of the primates, as a comparison of baby and adult gorilla skulls with that of an adult human shows). More recent, and more interesting from our point of view, is the work of Waddington[12], who argued that developmental systems exhibited an intrinsic stability as a by-product of natural selection for phenotypic constancy (ideally, for *the* phenotype which corresponded to a maximum of fitness). Waddington based his ideas on two generally held beliefs: (a) For every species there is such a thing called the 'wild type'; and different wild type individuals are remarkably like one another in spite of varying genetic constitutions, and (b) When the wild type is mutated, its phenotypic variation goes up, as testified by the usage of words like *penetrance* and *expressivity*. Except in rare cases, and these usually concern inbred strains used in laboratory experiments, one does not speak of *the* wild type today. However, the fact remains that one observes developmental stability in so-called wild-type strains, and instability in mutant strains.

To the extent that we do not believe that the details of development are rigidly specified from fertilization onwards, it becomes important to understand the basis for the stability of complex genetic and epigenetic systems. There is an argument that any sufficiently large system with a reasonable degree of connectivity is expected to be unstable in the absence of specially designed constraints. Two studies have something interesting to say about the problem of stability. Starting from the hypothesis that genes function as bistable switches and as controlling elements for other genes, Kauffman has shown that the potentially astronomical number of distinct states accessible to almost any genotype collapses into a manageably small number of stable cycles. This results from assuming that even if randomly connected, the number of inputs to a gene is small, ideally just two. Kauffman also compares the number of such cycles with the number of differentiated cell types to which a given genotype can give rise. Newman and Rice have used a different approach in attempting to account for the stability of epigenetic systems. They show that stability is a natural consequence of the observation that the rates of most enzyme-catalysed reactions are monotonically increasing functions of substrate concentrations. As a result, steady states tend to be sinks (stable equilibria) in metabolic space. Beyond the fact that because living systems maintain and increase their degree of internal order, their entropy must keep decreasing (*see* Lwoff[13]), thermodynamic theories of biological pattern are of doubtful value. At best, thermodynamics can help us to specify the gross constraints within which a system has to function, and is therefore of little help in choosing between detailed rival models.

3. Development as differential gene expression

For our purposes, 'development' implies (a) a progressive restriction in the actual or potential phenotype of a cell and, more generally, (b) a non-equivalence among different cell types even when this is not reflected at the level of the visible phenotype.

To take the more familiar feature first: in their gross morphology, ultrastructure, reactions to stimuli and patterns of protein synthesis, cells start showing differences

as development proceeds (differentiated cells are so unlike one another that at times it helps to turn the superorganism concept the other way round and think of a multicellular organism as an extremely complex social network of specialized individuals). The point is that these differences reflect differences in gene expression and not in genotype, though one cannot rule out at present the possibility that during normal development a small subset of genes gets differentially mutated in different cell types. Spemann showed long ago that any of the early cleavage nuclei could support the development of an entire newt larva if compelled to do so. The implications of Spemann's finding have been strengthened and generalised by the following observations: (i) the amount of nuclear DNA in an animal cell is the same from one tissue to another but varies from species to species; (ii) genes which are known to be expressed in only specialised cell types are present in the same number of copies in all cell types[14]; (iii) nuclear transplantation in amphibia, insects and mammals suggests that a differentiated nucleus has the complete spectrum of differentiation potencies contained within it; and (iv) under certain circumstances fully differentiated cells and tissues can regenerate to give rise to other cell types, with or without passing through an undifferentiated intermediate stage, and at times even in the absence of cell division (regeneration resembles normal development in certain respects).

The second, less obvious, feature of development is that in many cases apparently identical groups of cells behave differently when probed appropriately. For instance, (a) the hundreds of thousands of axons which make up a nerve bundle may be capable of individually innervating only a very restricted set of target sites; (b) patches of skin removed from different regions within the same segment of an insect cuticle elicit qualitatively different responses when they are transplanted elsewhere; (c) the growing cells in an insect imaginal disc become progressively restricted to occupying sub-territories—'compartments'—within the disc. Apart from the case of compartments in the fruit fly *Drosophila*, we cannot say at present whether the examples of cellular non-equivalence listed above reflect differential gene expression, albeit in a manner not immediately obvious (*e.g.*, differences in minor cell-surface constituents), or whether they mirror differences in stably maintained levels of the same gene product(s).

We must note that the rule of genomic constancy in development is not always obeyed. The classical case is that of the nematode *Ascaris* in which only those cells which are destined to enter the germ line carry the complete diploid number of chromosomes. Dimunition of chromosome number in presumptive somatic cell nuclei is also found in some insects. One of the two X chromosomes is inactivated at random in every cell of a female mammal. As a complementary class, one also knows of selective gene amplification during development. Ciliates provide an exception to the rule that stable changes in phenotype must reflect changes in gene expression: surgical alterations in their surface morphology can be transmitted faithfully over hundreds of generations.

3. Mosaicism *vs* regulation

As stated in the beginning, Aristotle is generally credited with the first clear statement

of the problem: does the essence of development lie in the growth of a preformed miniature embryo, or in a progressive elaboration of structure within an unstructured whole (epigenesis)? Since the sperm and egg are themselves highly differentiated cells and are necessary for the next round of development, development is a cyclic process. Today the issue is posed as one of mosaic or regulative tendencies. Mosaicism sees the embryo as a patchwork of limited, non-interacting and, in extreme form, distinct potentialities already present at the earliest stages. According to the regulative viewpoint the dominant factor in development is the ability of embryos to spontaneously generate order by virtue of interactions between cells or cell groups. The operational distinction is that local damage to a mosaic embryo shows up as a missing part or parts in the adult; a regulative embryo can partially, or even fully, compensate for such damage. In practice, the distinction is blurred, because (a) most embryos are both regulative and mosaic depending on how early or late the test is made, and (b) the egg, the presumed seat of all preformed or mosaic tendencies, is itself the final product of a series of epigenetic stages ("the chicken is only the egg's way of making another egg"). Nonetheless, there is an interesting question to be asked, and that is whether there are cases of regional differences within the egg that have a casual role to play in the development of regional differences within the embryo. To put it differently: is development better posed as an initial value problem or as a boundary-value problem? We will first examine the case for mosaicism.

5. Evidence in favour of mosaicism

Cytoplasmic determinants, which by definition are localised extranuclear factors capable of selectively influencing gene expression, constitute explicit proof of mosaicism. They have been shown to be present in a large number of orders (the mammals are an exception). There is evidence for determinants being either freely diffusible in the cytoplasm or bound to cell organelles or membranes. One way in which these determinants could affect pattern is for them to directly influence precusors of spatial organisation; another way would be for them to disrupt the temporal coordination of developmental events. I list a few examples of cytoplasmic determinants.

5.1. *The polar plasm in insects*

In holometabolous insects, zygote nuclei end up in the neighbourhood of the posterior pole of the egg and give rise to future germ cells. The cytoplasm at the posterior pole appears different from that elsewhere (even at the level of the light microscope) and is called the pole plasm. In some insects only future germ-line nuclei retain their full complement of chromosomes, and constriction and centrifugation experiments show that this is because the pole plasm protects those nuclei which enter it. More direct evidence for a determining role comes from transplantation of pole plasm in *Drosophila*. Basically, the observation is that when the pole plasm is transplanted into ectopic regions of the cleavage embryo, the nuclei which migrate there can develop into germ cells. Conversely, nuclei from other regions develop into germ cells if transplanted into the posterior pole. The precise nature of the germ cell determinant(s) is unknown.

5.2. *The anterior determinant in Smittia and Chironomus*

In the chironomid midge *Smittia* an extensive series of experiments have led to the following observations: (i) local perturbations at the anterior pole of the early embryo can convert the normal segmented body pattern, made up of head, thorax and abdomen, into a pair of mirror-imaged half abdomens; (ii) among these perturbations are UV irradiation and RNase treatment; and (iii) the effect of UV can be reversed by subsequent irradiation with light of higher wavelength. These findings are consistent with the existence of a morphogenetic factor, an RNA-protein complex, in the anterior part of the egg. The presence of the factor in an active form appears to break an intrinsic anterior–posterior symmetry of the egg. Equally interesting, but less precise, are centrifugation experiments done with very early embryos of *Smittia* and *Chironomus*: depending on the angle of centrifugation and the g-value, one can get a range of phenotypes from normal embryos to double heads, double abdomens, and completely inverted embryos. Centrifugation of amphibian eggs has been known since the days of Driesch and Morgan to produce the same sorts of effects.

5.3. *The grey crescent in Xenopus*

In the frog *Xenopus*, the grey crescent is a cortical pattern which forms as a consequence of the extensive migration of pigment following fertilization. It marks out the future dorsal side of the embryo. About 12 cleavages after fertilization, this is also the site at which gastulation begins. Curtis showed in a series of experiments that grafts of grey crescent material (taken from 1- to 8-celled embryos) were able to induce a secondary point of gastrulation and, ultimately, two embryonic axes. A fertilised egg from which the grey crescent was removed went through normal mitosis and cleavage but failed to gastrulate. All this was taken to mean that the cortical area containing the grey crescent contained morphogenetic information for initiating gastrulation and a secondary axis. Subsequent investigations by Gerhart and colleagues have shown that matters are not so simple: the effect of the grey crescent can be overidden by orientating the egg abnormally, implying that internal cytoplasmic gradients can influence the direction of the dorsal-ventral axis.

5.4. *Maternal effect mutations*

If cytoplasmic determinants exist, and if they are present in the unfertilized egg, their production and distribution must be under the control of the maternal genome. Therefore, there ought to be mutations affecting embryonic (and for that matter adult) pattern, with the mutant phenotype being restricted to the *progeny* of mutant females. The classic case is that of the snail *Limnea* whose spiral shell reflects the handedness of its early cleavages. In nature, the spiral is normally a right-handed helix, and occassionally left-handed deviants are found. When self-fertilised, broods from the same animal are identical with respect to handedness, but either kind of parent can produce either sort of brood. The effect is due to a single gene in the mother, with the allele for right-handedness dominant to the one for left handedness; the phenotype of the offspring is determined by the genotype of the mother.

An extensive study of maternal mutations and their effect on embryonic development comes from the fruit fly *Drosophila*. A whole set of *grandchildless* mutants is known in which females lay eggs whose germ-cell determination is defective. Some of these could be due to a defect in the pole plasm, but interestingly the earliest such mutant identified (in *D. subobscura*) turned out to have delayed nuclear migration into the posterior region as the cause of embryonic sterility. There are also maternal effect mutants in Drosophila which affect embryonic body pattern in a rather specific fashion; for instance, *bicaudal*. The most extreme phenotype in this case is that of two mirror-imaged half embryos consisting of caudal segments alone. When one examines the range of *bicaudal* phenotypes, the observations are consistent with the following model. Normal segmental determination is under the control of a monotonic morphogenetic gradient, and mutant forms result from the gradient turning back on itself to a greater or lesser degree. *Bicaudal* illustrates an important link between morphogenesis, evolution and developmental genetics; the point has to do with the symmetry of living forms. The evolutionary transition from one set of forms to another almost invariably involves a higher order of symmetry going over into a lower one. As a result, mutants which cause gross disruptions to body pattern are often suggestive of atavism in that they display a higher form of symmetry than the wild type.

6. Evidence in favour of regulation

A famous painting by Mansur shows a zebra with twenty stripes on its back. The antecedents of this particular zebra are unknown, but let us assume that it developed from a fertilized egg which developed as a single entity. Suppose now that the developing embryo had split into two, and that Mansur's zebra was one of a pair of identical twins. Would it then have ten strips? If the answer is no, and if a zebra always has twenty evenly spaced stripes irrespective of the overall size of the embryo from which it develops, we have a typical example of regulative development. The problem posed by regulation is one of constancy of biological form irrespective of size. More precisely, in certain classes of organisms the fate of a cell at position x in the embryo depends, not on x as such, but on the ratio x/L where L is the size, or other appropriate measure, of the embryo's linear dimension. This dependence on x/L is also referred to by the term scale invariance, because it indicates that certain spatial properties of the developing embryo are independent of the scale of measurement used.

The term *positional information* was coined by L. Wolpert in 1969 as a possible explanatory framework for regulative embryos. Positional information stands for that function of position which causes otherwise identical cells in a regulative embryo to follow different developmental pathways. Some biologists are of the opinion that Wolpert was merely rewording old ideas, but this is incorrect (quite apart from the fact that reformulations can be immensely useful). What he proposed was a view of development which one might call programmatic or algorithmic. In essence, he suggested that for a certain class of systems, position (more accurately, relative position) *per se* was a developmental variable, and that at some stage cells assessed their

positions in an embryo and used this assessment in developing further. The manner in which positional information was used would depend on the genotype of a cell and on its previous developmental history. However, nothing precluded the basis for specifying position from being universal, constituting as it were an epigenetic code.

As is true of all theories in biology, it is important to realise that a programmatic view of development is justified only in so far as it is supported by experiment. For example, one can write down a single mathematical formula for working out the period of a pendulum, but no one would claim that the pendulum uses the formula to compute its period; all it does is to follow dynamical laws which automatically ensure that the period has a definite value. Similarly, and in contrast to the positional information point of view, the course of development could well be an automatic consequence of the chemical reactions governing cell division and cell–cell interactions; it may just so happen that we find it convenient to describe it in terms of positional information. To repeat, the concept of positional information provides for a certain way of looking at development. At the heart of the concept is the notion of position as a developmental variable. Further, if only relative positions are of interest, there is the possibility that the system for specifying position might be universal.

The origins of positional information theory can be traced back to the earliest days of developmental biology, and in particular to Driesch's celebrated experiment (1891) of separating the blastomeres of a 2- 4- or (with certain restrictions) the 8-cell stage of the sea urchin embryo. When he did this, in every case the isolated blastomere could give rise to a perfectly formed larva, of diminished size but normal proportions. The phenomenon, which he described as 'harmonious equipotential development', and we call 'regulation', is widespread among the various phyla; human identical twins are a familiar example.

The pattern that displays regulation in blastomere separation experiments can be thought of as monotonic, of something varying in a steady and gradual fashion from one extremity to the other. One can also think of periodic patterns, for instance, the 8 bands on the back of an armadillo. The 8-banded armadillo is almost always born as a member of identical quadruplets. Depending on the degree of separation of the early blastomeres, one can in rare cases find twins, or just a single individual, arising from a fertilised egg. These are larger than the quadruplets, but always have the same number of evenly spaced bands—8—on the back.

The important point brought out by regulative development is that a part of an organism can have the developmental potentialities of the whole. Or, as first stated by Drisech, the future fate of a cell in an embryo depends on its relative position. There are other systems which bring out the implications of regulation more directly, and they involve the reconstitution of a whole individual from a part of it in the absence of any compensatory growth. The coelenterate *Hydra* provides a familiar example. Another is the slime mould *Dictyostelium* which goes through a cigar-shaped embryonic or slug stage. Cells in approximately the anterior fifth of the slug differentiate into one type and the cells in the posterior four-fifths, into another. This

embryo can be repeatedly fragmented perpendicular to its length, and the dimunitive masses differentiate to give rise to the same two cell types as the parent, and more importantly, can do so in the same proportions. Crucially, the distribution of future cell types within the diminished embryos also follows an anterior-posterior pattern.

What might be the basis of position-dependent variation in cell fates? This too is a question dating from the days of classical embryology, and it has been known since then that there are broadly two kinds of explanation possible. One is to say, as we have seen, that the oocyte is a mosaic of qualitatively different cytoplasmic or cortical constituents, and that the fate of cell in an early embryo depends on what portion of the maternal cytoplasm it inherits. Clearly this hypothesis will not do for a regulative system, because it predicts that separated fragments of an embryo must develop, like Jarāsandha*, into complementary (perhaps mirror-imaged) structures. In the case of *Dictyostelium* the hypothesis cannot even be entertained because there is nothing equivalent to an oocyte: the amoeboid cells which come to make up the embryo are spatially separated mitotic products of a single cell and are believed to have the same cytoplasmic constitution because they are (in many laboratory experiments) genetically identical and raised in the same environment. This leads us to the other possible explanation for regulative development. The explanation is that spatial differences in regulative systems arise from a spontaneous breakdown of homogeneity, a breakdown postulated to arise as a natural consequence of intercellular interactions. (The process can be imagined to be analagous to the magnetisation which appears in a piece of iron when it is cooled below a critical temperature). Once this is accepted as a possible explanation, the problem becomes one of determining the means by which cells communicate with each other so as to lead the appearance of patterns independent of total size.

At this stage we can list what needs to be done to demonstrate the usefulness of *positional information* as a concept. Experimentally, it has to be proven that there is some measurable property of a developing system which scales with size. The property has to be in the nature of something recognisable in the system well before the onset of overt cytodifferentiation (since, by assumption, the pattern of differentiation regulates anyway). Secondly, though this is not essential, it would be extremely convincing if one had hints of 'universality'. Theoretically, a necessary exercise would be to work out testable models for cell–cell interactions which gave rise to regulative patterning. The progress made so far in carrying through this program has been limited.

The best evidence for universality comes from the homeotic mutations of *Drosophila melanogaster*. These are mutations in which one body segment of the adult is replaced by another in whole or in part. *Antennapedia* is a mutation in which part of the antenna is replaced by a leg. The interesting feature of this mutation is that precisely that part of antenna develops into leg as would be appropriate to the location of expression of the mutation. If the extremity of the antenna is transformed,

* Jarāsandha, a figure from mythology, was born in two distinct pieces, a left half and a right half Subsequently these were joined to give rise to a normal body

it gives rise to distal-most leg structure; if proximal regions of the antenna are transformed, they give rise to proximal leg structures, and so on. The implication is that whatever signals in development mean 'proximal' or 'distal' or 'middle', they are the same in antenna and leg. These experiments have now been generalised in enough cases for us to think in terms of a basic similarity of signals involved in position sensing in the developing body parts of the fly. Another striking instance of common signals comes from studies of early limb development. The zone of polarizing activity (ZPA), region of tissue at the posterior flank of the developing limb, can be transplanted to the anterior margin. When this is done the skeletal structure of the adult limb shows mirror-imaged pairs of posterior elements. These results hold good even in cross-species ZPA transplants amongst amphibia, birds, reptiles and mammals.

Experimental verification of universality in position-sensing is bound to be difficult. Is there any property of a developing system which is manifestly scale-invariant and is detectable well ahead in time of irreversible differentiation? The one clear candidate is tip regeneration in the embryonic slug stage of *Dictyostelium*[15]. The anterior-most boundary of the slug has a button-shaped mass of cells called the tip, and if the front half (or some other fraction) of a slug is cut off and removed, the anterior surface of the back half regenerates a new tip. The time for tip regeneration depends only on the relative position of the cut; in a slug of total length L, a cut made at a distance x from the front causes a new tip to generate after a time which depends only on the ratio x/L, and not on x or L separately. Whether tip regeneration times are casually related to the spatial pattern of cell differentiation, and if so how, is still a matter of conjecture.

As regards a theoretical understanding of regulative development, the question is whether there are models for pattern formation which are reasonable in terms of the assumptions they make *and* exhibit regulation. As of today, the short answer is no. This answer has to be qualified. The models for pattern formation which have been examined in most detail are those involving chemicals (morphogens), and those of a qualitatively different sort, for example, ones based on cellular electrical potentials and ion transport, have not been studied in sufficient depth. The way in which one sets up a chemical theory of morphogens is to start out by assuming that there is a controlling chemical (or set of chemicals) whose level within the tissue is an indicator of specific patterns of metabolic activity characteristic of the differentiated state. One then postulates plausible biochemical reactions for the synthesis and degradation of the chemical and also models transport of the chemical from one cell to another (the simplest form of transport would be ordinary diffusion). In effect, the developing tissue mass is reduced to a reaction–diffusion system. By specifying initial levels of the morphogen(s) at different locations together with appropriate boundary conditions (the extreme possibilities being that the morphogen either leaks out freely or that it remains confined within the system), the system can be followed through time. Reasonable schemes can lead to a spontaneous origin of form of pattern. Namely, if one waits long enough, morphogens reach different steady-state levels in different regions. Both periodic and monotonic (gradient) distributions can result. The interesting thing is that this happens even when the system is completely homogenous (except

for the boundaries) and initial conditions specify a constant level of morphogen everywhere. Therefore, the problem of self-organisation of biological pattern is in principle solvable. Unfortunately, the very features that make for a solution ensure that the pattern which results cannot regulate. The reason is easy to see: all such models have a built-in scale of size. Typically, if the morphogen is produced (or destroyed) at a rate K and diffuses from cell to cell with a diffusion coefficient D, the scale of size is $\sqrt{D/K}$. Therefore, if the system as a whole has a linear dimension L, the number of pattern elements it can contain is $L/\sqrt{D/K}$ and so is *not* independent of L.

7. Problems for the future

(1) Mechanical forces play a very important role in the development of form and shape, but how they might be organised and coordinated is only beginning to be investigated[16]. The phenomenon of exogastrulation[17] which can be induced in amphibia by inverting dorsal blastoporal tissue prior to normal invagination suggests that there may be significant local autonomy (as opposed to global coordination) in the expression of forces leading to tissue movement and specific contacts.

(2) The spectacular success of 'wet' biochemistry and molecular biology, and a philosophical attitude favouring the steady state ('homeostasis') has tended to obscure subtle but important features of living systems, in particular their temporal organization. Cyclic AMP oscillations in *D. discoideum* constitute the best known example of a role in development for periodic signalling between cells. Evidence for Ca^{2+} oscillations, with periods in the range suitable for subserving developmental functions, is coming from a number of systems. The regulation of temporal patterns in intercellular communication needs to be explored.

(3) While it is accepted that somatic development does not entail any irreversible genetic changes, the existence and potential importance of imprinting, or sex-of-parent-dependent reversible modification of DNA, is being increasingly appreciated[18]. Reversible modifications offer, firstly, an obvious means of generating variant phenotypes within the same heritable genotype and, secondly, of mediating the influence of the environment on development. Canalization, a phrase coined by Waddington to describe the stability of development, can be thought of as a form of 'genetic learning' by means of which advantageous phenotypes start by being inducible by the environment through physiological adaptation and, over the course of generations of selection, end by becoming constitutive. The standard explanation of canalization is that it involves selection at modifier loci, just the sort of loci expected to regulate imprinting. Explicit models for genotype–environment interactions would make possible a better understanding of developmental canalization and its role in evolution.

(4) The functioning of genetic networks has to be examined keeping evolution in mind. Kauffman's elegant studies on randomly connected Boolean networks show

that given certain assumptions, a system made up of elements, each receiving two inputs and delivering one of two outputs, can exhibit stable patterns of cyclic behaviour; each cycle is identified with a differentiated cell type[19]. The system begins to exhibit undesirable features as the number of inputs is increased beyond two, and this is almost certainly because connections in the model are made at random. In addition, the integrity of individual cycles does not appear to be maintained as networks increase in complexity. It would be interesting to study these networks as they evolve starting from a small number of elements. As the number increases, newer elements will have to be integrated with the pre-existing ones. This might necessitate 'mutating' a newly added element so that its pattern of connections is almost, but not exactly, the same as that of a pre-existing element. The starting point of the exercise could be to construct a simple network with a plausible pattern of connectivities which, on the basis of identifying an *ON* element with an actively transcribing gene, sets out to mimic at least earliest features of embryonic pattern formation. Cellular automation models or biomorphs might offer a practical route to building such an embryo[10].

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