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Positional information revisited

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Summary

Positional information has been suggested to play a central role in pattern formation during development. The strong version of positional information states that there is a cell parameter, positional value, which is related to position as in a coordinate system and which determines cell differentiation. A weaker version merely emphasises position as a key determinant in cell development and differentiation. There is evidence for boundaries and orthogonal axes playing an important role in positional systems. A positional signal is distinguished from an inductive interaction because the former specifies multiple states, confers polarity, and can act over a long range. A gradient in a diffusible morphogen is just one way of specifying position. There is now good evidence in several systems for substances which may be the morphogen for positional signalling. The product of the *bicoid* gene in early *Drosophila* development is the best prospect. Retinoic acid is unique in its ability to alter positional value and may also be a morphogen. The best evidence for positional value, a concept fundamental to positional information, remains a biological assay based on grafting. The idea of positional value uncouples differentiation and position, and allows considerable freedom for patterning. It is not clear whether positional value or differentiation involves a combinatorial mechanism.

Interpretation of positional information remains a central problem. There is good evidence that cells can respond differentially to less than a two-fold change in concentration of a chemical signal. It may be that interpretation involves listing the sites at which a particular class of cell differentiation will occur. The problem is made less severe when blocks of cells are specified together as in mechanisms based on an isomorphic prepattern. Isomorphic prepatterns could establish repeated structures which are equivalent and which are then made non-equivalent by positional information. This would enable local differences to develop. The combination of these two mechanisms may be widespread.

There is evidence that positional signals within a single animal and in related animals are conserved. It is not clear just how wide this conservation is, but it is at phylotypic stages, rather than in eggs, that similarity might be expected. It is nevertheless impressive that the polar coordinate model can be applied to regulation in systems as diverse as insects, vertebrates and protozoa. The molecular basis of positional signalling is just becoming accessible; the molecular basis of positional value is still awaited.

A brief personal history of positional information is provided in an appendix.

Key words: positional information, pattern formation, inductive interaction, retinoic acid, polar coordinate model.

Introduction

Pattern formation is a central problem in development (Wolpert, 1969). Positional information provides both a conceptual framework for thinking about pattern formation and also suggests possible mechanisms. The basic idea of positional information is that there is a cell parameter, positional value, which is related to a cell's position in the developing system. It is as if there is a coordinate system with respect to which the cells have their position specified. The cells then interpret their positional value by differentiating in a particular way. This differentiation may involve developing as a particular cell type or state, or it might involve changes in growth or motility (Wolpert, 1969, 1971, 1981, 1989*a*). (For a personal history, see Appendix).

Among the attractive features of positional information is that it provides a unifying concept for understanding the development and regulation of a variety of patterns. The only cell-to-cell interactions that are, in principle, required, are those necessary to specify position. Again, in principle, the same signals and positional values may be used to specify different patterns, the differences arising both from developmental history and from genetic constitution. Regeneration and regulation could be viewed in terms of changes in positional value and the specifying of new boundary regions: morphallaxis and epimorphosis could now be clearly distinguished. Perhaps the least attractive feature of positional information, if the basic idea is correct, is that it places a great burden on the process of interpretation.

The essential features of a coordinate system that is used to establish positional information are: boundaries with respect to which position is specified; a scalar, which gives a measure of distance from the boundary; and polarity, which specifies the direction in which position is measured from the boundary. Using such a framework forces one to construct specific and concrete models. For a one-dimensional system, all the necessary features can be provided by a monotonic decrease in the concentration of a chemical – a morphogen – which could be set up with a localized source or by reaction–diffusion. The concentration of morphogen at any point then provides a scalar measure of distance from the boundary, and the slope of the concentration gradient effectively provides the polarity.

Some confusion has arisen from assuming that a system of the kind just described can only be achieved by the diffusion of a chemical morphogen from a source (e.g. Davidson, 1989). That is not the case. A gradient in a chemical morphogen can be generated by quite other mechanisms, only some of which involve diffusion. Such mechanisms could, for example, be provided by a progress zone model in which the gradient is generated as a group of cells grow (Summerbell *et al.* 1973), or by cell-to-cell interactions (Babloyantz, 1979). Furthermore, the gradient of a chemical concentration is, itself, only a special case of the yet more general case. Position need not be recorded by the changes in concentration on a chemical. Positional value could be represented by a set of genes – additional genes being activated with increase in distance in a simple additive manner, such as 1, 12, 123...

This last example forces one to ask what would *not* be regarded as a positional system. There is a weak sense in which the idea of positional information is used to refer to differences between cells in developing system. If each cell in a developing system has a unique specification this does not necessarily mean that they all have positional information in the sense of a coordinate system. Thus in the development of the nematode most cells have a unique specification and position by virtue of their lineage; but there are no boundaries and no scalars. In insects and vertebrates the dominant mechanism of pattern formation involves cell interactions rather than lineage (Wolpert, 1989*a*) but not all interactions involve positional information in the strong sense. For example, the development of the ommatidea in the *Drosophila* eye depends on cells' positions with respect to their neighbours (Tomlinson, 1988). However, this relationship is rather like the specification of position in folk-dancing or a rugby scrum rather than in a coordinate system. Positional information is about graded properties and it is in this strong sense, with its implications for a coordinate system, that it is considered here.

A surprising feature is that all positional fields are small, none being longer than about 1 mm in maximum linear dimension or about 50 cell diameters (Wolpert, 1969). In fact most are much smaller. The other characteristic feature is that the times required to specify position appear to be of the order of hours. It was these two features that lead Crick (1970) to propose a diffusible morphogen for setting up positional fields.

Axes and boundaries

The Cartesian nature of many developing systems is very striking in the sense that many systems seem to develop in relation to orthogonal axes. (One always has, however, to be careful to distinguish between our perception and description of the embryo, and the mechanisms used by the embryo).

In *Drosophila* the anteroposterior axis and the dorsoventral axis are at right angles and are controlled by quite different sets of genes; in Hydra regeneration the specification of a head along the main body axis is at right angles to the specification of the ring of tentacles; in sea urchins and many other embryos with radial cleavage, the first two cleavages are parallel with the animal–vegetal axis and the third at right angles to them; again in sea urchins, the development of the animal–vegetal and dorsoventral axes are at right angles as are the skeletal rods; in the developing vertebrate limb there seem to be different mechanisms for specification of the anteroposterior and proximodistal axes; the main body axis in vertebrates is specified by a mechanism different to that of the dorsoventral axis. Embryos like right angles.

Meinhardt (1984) has considered mechanisms whereby orthogonality of the axes may be specified and also how new boundary regions might develop within a primary positional field. In insects he invokes interactions at compartment boundaries.

In any coordinate system, the boundaries are fundamental and act as reference regions. They must provide both the polarity of the system and be linked to position. Some boundary regions are listed in Table 1. The key operational criterion for a boundary region is that other regions are specified in relation to it. Thus the polarising region of the chick limb bud can specify new structures in relation to it when it is grafted to different positions along the anteroposterior axis of the chick limb bud (Tickle *et al.* 1975). There is good evidence in the insect egg for two boundary regions with long-range influences on pattern: one anterior and one posterior. Sander (see 1984 review) demonstrated an activity that specifies both polarity and pattern at the posterior end of the *Euscelis* egg, and inferred the existence of an anterior activity. In *Drosophila*, both anterior and posterior organising centres have been demonstrated by cytoplasmic injections (Nüsslein-Volhard *et al.* 1987); cytoplasm from both regions can specify pattern and polarity when transplanted to ectopic sites, and, in addition, their effects are long range. Of particular importance for later development is the specification of a single line of cells at the end of each parasegment, which may act as a boundary region for the later development of the segments (Lawrence, 1981, 1987; Lawrence *et al.* 1987).

In regenerating or regulatory systems, the behaviour of the boundaries is of key importance (Wolpert, 1971).

In morphallaxis, new boundaries may be formed and new positional values specified in relation to it without necessarily any growth. By contrast, with epimorphosis, new positional values are generated by growth and the boundary values may play much less of a role.

Polarity

There is an intimate relationship between the gradient of positional information and polarity, the polarity being determined by the slope of the gradient (Lawrence, 1970). In terms of positional information, polarity determines the direction in which position is measured with respect to a boundary region. Polarity in other non-positional systems may have other meanings. Nevertheless the close relation between gradients and polarity is satisfying. In the chick limb bud, for example, the polarity of the hand can be reversed in relation to the polarizing region and the gradient it sets up (Tickle *et al.* 1975). In insects there is a direct marker of polarity provided by the direction in which epidermal structures, like hairs, point. This correlates well with the postulated gradients (Lawrence, 1973). Again, in regeneration in Hydra, polarity is tightly linked to gradients (Bode and Bode, 1984).

Positional signalling and induction

By far the clearest demonstration of a positional signal in a developing system – clear in the sense that it can be directly visualised rather than being inferred from other properties – is in the insect egg. The gradient is in the protein coded for by the *bicoid* gene, which is a key gene in patterning along the anteroposterior axis (Driever and Nüsslein-Volhard, 1988*a*). Its discovery is particularly gratifying not only because of its importance, but because it has just the anticipated distribution of a morphogen which is made at a source and both diffuses and breaks down. There is also a ventral to dorsal gradient in the protein of the *dorsal* gene, but in this case its mRNA is uniformly distributed and the generation of the gradient requires a different mechanism (Steward *et al.* 1988).

Another example of a positional signal is the polarizing region in the chick limb for which there is good evidence for a graded signal – possibly retinoic acid – although the signal itself has not yet been unequivocally identified (Tickle *et al.* 1985).

The distance over which a positional signal has been shown to act seems to have decreased over the last two decades. As already pointed out, positional fields are small – usually only 20 to 30 cell diameters or less than 1 mm in maximum linear dimension. For intercalary regeneration it has been argued that the distances over which signals act is no more than a few cell diameters (Bryant *et al.* 1981) and this type of local interaction is said to characterise inductive interactions (Davidson, 1989).

The evidence that positional signals can act over greater distances, while limited, is persuasive. In Hydra there is very good evidence that an inhibitory signal can affect tissues about 1 mm distant (Bode and Bode, 1984). The polarizing region in the wing bud can propagate its signal across leg tissue about 100 μm thick (Honig, 1981) and the local application of retinoic acid can cause apical ridge extension about 100–200 μm away (Lee and Tickle, 1985). In amphibians the graft of an organiser seems to affect tissues several hundred microns away (Smith and Slack, 1983). Injections of cytoplasm into insect eggs seem to exert effects over distances of several hundred microns (Nüsslein-Volhard *et al.* 1987).

It would be a pity if one conflated all cell-to-cell interactions involving cell signalling. A positional signal and induction have distinct and different, and more important, useful meanings. Use of 'positional signal' should be confined to situations where the position of a group of cells is being specified, preferably where positional information is involved. A positional signal should be distinguished from induction in a number of ways (Table 2). Induction is defined as an interaction between two different tissues, one inducing, the other responding (Gurdon, 1987). This at once makes it different from positional signalling, for which two different tissues need not be involved. There are other differences. In general, induction specifies just one cell state such as muscle or neural tissue or cartilage, whereas a positional signal, which need not involve only one substance, specifies, by definition, multiple cell states. Related to this a positional signal is graded whereas induction is basically all-or–none. Along the same lines, induction is short range whereas a positional signal is usually specifying cell states over a greater distance. Again, a positional signal has 'polarity'

Table 1. *Boundary/reference regions*

Hydra	Head Foot	Bode & Bode (1984).
Sea urchin	Micromeres	Hörstadius (1973).
Insect	Egg ends Parasegment boundary	Nüsslein-Volhard *et al.* (1987). Lawrence *et al.* (1987).
Chick	Hensen's node Polarizing region limb	Hornburch, Summerbell & Wolpert (1979). Tickle, Summerbell & Wolpert (1975).
Amphibian	Organiser	Smith & Slack (1983).
Lepidopteran	Wing foci	Nijhout (1980).

Table 2.

Positional signal	Induction
Multiple states	Single state
Graded	All-or-none
Polarity	No polarity
Long range	Short range
Boundary region	Diffuse
Same tissue	Different tissue
Instructive	Instructive or permissive

whereas induction is not related to the polarity of either tissue. A positional signal is provided by, or linked to, a boundary region and as such is localised; inductive signals need not be. Finally, a positional signal is always instructive whereas an inductive signal may be either permissive or instructive. The distinctions made here are somewhat exaggerated to emphasise the differences.

It is thus far from clear to what extent the patterning of muscle in early amphibian development involves primarily induction or whether positional signalling is involved (Smith, 1989).

There is at least one situation where the distinction between induction and positional signal may be blurred; in those cases where induction might be thought of as transferring a set of positional values from one tissue to another (Wolpert, 1981). The classic example where this might be occurring is in primary embryonic induction, where it is claimed that if small pieces of gastrula ectoderm are placed at different positions along the axis of the endomesoderm of an exogastrula they differentiate neural structures according to their position. A similar explanation could explain homoiogenetic induction: induced tissues can themselves induce similar structures. It is thus of great interest that in *Xenopus laevis* the homeobox gene X1H box 1 is expressed at the same level in a narrow band of both neural and mesodermal tissues. This correlation is best explained by homeogenetic transfer of positional information (de Robertis *et al.* 1989).

Morphogen

The word morphogen was coined by Turing (1952). Its original meaning was in relation to pattern formation; the distribution of the morphogen reflected the resulting overt pattern as in the isomorphous prepattern mechanism described above. The essential features of this meaning should be retained but extended to include a concentration gradient that specifies position. An inductive signal is not a morphogen as it does not specify pattern.

What then are the criteria for identifying a morphogen? (Some aspects are considered by Slack and Isaacs (1989) but they include induction). The substance must be distributed in a pattern that generates a pattern; changing the distribution must alter the pattern in the expected manner; blocking the interaction of the putative morphogen with the cells should prevent pattern formation. At present no morphogen meets all these requirements. The most promising candidates are the *bicoid* protein in early insect development; retinoic acid in limb development (Thaller and Eichele, 1987); and possibly the peptide involved in Hydra regeneration (Schaller and Bodenmuller, 1981) and DIF in the slime mould (Williams, 1988). Of all these the *bicoid* protein is the most impressive since it can be measured directly, and altering its concentration profile alters early patterns. While it is unwise to be too enthusiastic about a particular morphogen, as the history of embryology is littered with false trails, retinoic acid is unique, so far, in its ability to alter positional value, both in development and regeneration (Brockes, 1989).

Positional value

One of the most important concepts related to positional information is that positional value is a cell parameter. The most direct evidence for the existence of a cell parameter that correlates with position comes from experiments of the kind initially carried out by Bohn. He showed that intercalary regeneration in the cockroach leg could be interpreted in terms of a graded set of positional values and the intercalation of intermediate values when non-contiguous regions were placed next to each other. This type of model has been formalised to account for a very wide range of experiments by the polar coordinate model (French *et al.* 1976; Bryant *et al.* 1981). The importance of the model – the details need not concern us here – is that cells have a biological property, positional value, that determines their regulative and regenerative behaviour when grafted to different positions. There are two key features to this behaviour. First, when cells with disparate positional values are juxtaposed, intercalation occurs to smooth out those disparities (cf. Winfree, 1984). Second, and more important, the positional values of the cells are independent of the structures that they form. This second point is essential, for it dissociates position from differentiation. In principle, any structure – bristle, sex comb and so on – could be formed at any position. This uncoupling is fundamental to the concept of positional information and is particularly clearly seen in genetic mosaics in insects (Bryant, 1974). These mosaics not only illustrate uncoupling but also show the identity of positional values in different organs in the same animal.

Molecular genetics has enabled the activity of a wide variety of genes to be mapped during early insect development (Ingham, 1988). The question is whether any of these can be regarded as providing the cells with a positional value. In general the answer seems to be in the negative, though genes like *hunchback* might loosely be thought of as providing blocks of positional value along the main axis. It is not clear to what extent the expression of the homeotic genes can be thought of in terms of positional value.

It is also far from clear whether the specification of cell state or cell differentiation is combinatorial or not. In a combinatorial system the number of signals required, or genes activated, to specify a cell would be

small in relation to the total number of specified states. For *Drosophila* at least, while more than one gene is used to specify cell states in early development, the number of genes seems to be similar to the number of states. On this criterion specification would not involve a combinatorial mechanism. Further support for this view comes from the ability of single genes, such as *myo-D*, to cause transfected cells to differentiate into muscle (Davis *et al.* 1987).

While the molecular basis of positional value remains unknown, the report of a position-specific antigen in the developing chick limb is of great interest. This antigen does not correspond to any particular structure but is confined to a position along the anteroposterior axis of the limb (Ohsugi *et al.* 1988).

Non-equivalence

Closely related to positional value is the concept of non-equivalence. Non-equivalence is the property possessed by cells of the same differentiation class that makes them different from one another (Lewis and Wolpert, 1976). It is fundamental to evolution for it enables structures that contain the same overt differentiated cell types, like digits, to develop differently. Non-equivalence is a natural consequence of positional information because even cells that differentiate in the same way have an intrinsic difference related to their positional value. The epidermal cells of insects are clearly non-equivalent as is shown by the intercalation experiments. While positional information demands non-equivalence, the presence of non-equivalence does not, however, imply positional information.

It is worth first noting that in vertebrates, at least some cells are equivalent and others non-equivalent. Thus the connective tissue cells of limbs are non-equivalent but it seems that both for limbs and the head, muscle cells are equivalent. There is also evidence, in the head at least, that endothelial cells, initially at least, are equivalent (Wolpert, 1988*a*).

Previously, much of the evidence for non-equivalence has relied on biological experiments such as grafting. Homeobox genes have now opened up a new approach, and, in the mouse for example, there are striking differences in expression from presomite stages onwards along the anteroposterior axis that do not correspond to any obvious morphological boundaries (Holland and Hogan, 1988). As Holland and Hogan point out these patterns of expression are consistent with, but of course do not in any way prove, a role for homeobox genes in establishing domains of positional value in the mammalian embryo. It is thus of great interest that Ruiz i. Altaba and Melton (1989) have found that a *Xenopus* homeobox (Xhox 3) gene is expressed in a graded manner along the anteroposterior axis of the embryo and that different fates along this axis correspond with the level of the homeobox gene.

Interpretation

The weakness of a positional model has always been the process of interpretation. There are two main problems. First it has always seemed very difficult to imagine how, for example, a monotonic concentration gradient in a single morphogen could specify the necessary number of positional values. Cells, it is thought, would not be able to distinguish reliably between small changes in concentration of a chemical. The second problem is that even if cells acquired a discrete set of positional values, it is far from clear how these would be used to specify a particular pattern, particularly if two-, rather than one-, dimensional patterns are considered (Wolpert, 1985).

If each cell has a discrete and remembered positional value then the specification of that positional value is partly a problem of thresholds. This is not necessarily so if there is an isomorphism between positional information and the way it is expressed – that is, if the continuous gradient in positional information is expressed directly as a continuous gradient in some cellular property such as the number of adhesive sites or the rate of a particular chemical reaction (Wolpert and Stein, 1984). In this case, the responses could be continuous and graded as in adhesive properties (Zackson and Steinberg, 1988) or enzyme distribution (Sweetser *et al.* 1988). However, the problem of thresholds does occur if these properties are retained even when the signal is withdrawn, and some mechanism is then required to maintain the state of the cell, particularly through cell multiplication.

There are several models for the interpretation, or at least the transformation, of a continuous change in chemical concentration into a set of discrete states. One class of model relies on a threshold property arising from a positive feed-back loop (Lewis *et al.* 1977; Meinhardt, 1982). Another could be due to the phosphorylation of a protein (Goldbeter and Wolpert, unpublished). Both give sharp transitions under the influence of an increasing concentration of a morphogen.

How small a change in concentration can cells detect? Studies on chemotaxis (McRobbie, 1986) suggest that in that in some situations cells can detect changes in concentration of about 1 % this might involve temporal rather than spatial differences. More direct evidence comes from the effect of putative morphogens on development and from gene dosage studies (Table 3). Consider first retinoic acid. Local application of retinoic acid to chick wing buds can specify the development of a digit **2**, **3** or **4**, the corresponding concentration being 0.9 nM, 2.5 nM and 25 nM (Tickle *et al.* 1985). Again, local application of retinoic acid to regenerating amphibian limbs cut at the wrist can cause an extra radius and ulna to develop with a concentration of 2–4 mg/limb, an extra part of the humerus with 4–8 mg/limb while 16 mg/limb gives a complete extra limb (Maden *et al.* 1985). These cells are able to respond in rather a dramatic way with changes in concentration as low as a two-fold difference. A similar conclusion might be drawn from studies on the role of lin-14 in the development of the nematode, where mutations that eliminate or elevate the activity of the gene change cell fate

Table 3. *Concentration and interpretation*

System	Character	Concentration change
Insect	Head/thorax boundary	*bicoid* protein × 1.1 (Driever & Nüsslein-Volhard, 1988*b*).
Chick limb	Digit character	Retinoic acid × 2 (Tickle, Lee & Eichele, 1985)
Amphibian limb regeneration	Level of duplication	Retinoic acid × 2 (Maden, Keeble & Cox, 1985).
Amphibian mesoderm	Cell differentiation	MIF × 2 (Smith, Yaqoob & Symes, 1988)
Nematode	Cell fate	Lin-12 × 2 (?) (Ambros & Horvitz, 1987).

(Ambros and Horvitz, 1987). Again there is evidence that the anchor cell produces a graded signal that can stimulate adjacent cells to differentiate into two different types depending on their distance from the anchor cell. In addition the cell nearest the anchor cell appears to inhibit its neighbours (Sternberg, 1988). This means that cells can detect differences in concentration that vary over one cell diameter. A final, and crucial, example comes from changing the concentration of the protein of the *bicoid* gene in early *Drosophila* development. A 10% change in concentration alters the position of the head/thorax boundary 15% (Driever and Nüsslein-Volhard, 1988*b*). Taken together these results suggest that quite modest changes in concentration can lead to different cell behaviours. The problem remains but seems somewhat less formidable.

The second problem is that even with a positional field in which each cell is uniquely specified, it is necessary to interpret positional values so as to generate a pattern. A formal solution is that each cell contains a complete specification of the behaviour of every cell in every position. There must be a complete list of all the cells that will differentiate as type A, and another list of those that form type B and so on. For systems like the vertebrate skeleton it seems unlikely that there is a positional specification for every cell that develops into, for example, cartilage. This could partly be resolved if contiguous cartilage cells were specified as a group, and a prepattern mechanism (see below) can provide just such a mechanism. Even so positional fields are small and what seems inelegant or unlikely to us might look quite different to the cell. Moreover there is some evidence that there may be a listing of the type postulated for sensory bristles in *Drosophila*. There are 11 sensory bristles on the thorax of the fly which can be removed, often in pairs, by a series of mutations in the *achaete–scute* complex (Ghysen and Dambly-Chaudiere, 1988). Molecular analysis has shown that the phenotype of most scute alleles can be correlated with their location on the chromosome. These different sites on the chromosome might be thought of as being activated at appropriate positional values. The sites would correspond to sites that were specifically activated in those cells at particular positional values and the sites would then correspond to the list of positions at which interpretation of the bristle phenotype occurred. There is evidence, in addition, for lateral inhibition increasing the precision of the bristle patterning.

The assumption in the previous example – indeed in the very concept of differentiation *via* interpretation – is that states of cell differentiation are, as it were, specified as a single event. While it is not an absolute requirement, the implication is of a single switch and is in strong contrast to any combinatorial mechanism of specification. It is far from clear whether or not the specification of cell differentiation is combinatorial (see above).

Spacing patterns and prepatterns

A weakness of the original positional information model was, perhaps, that it tried to do too much. It was recognised, quite early, that repeated or metameric patterns like somites or stripes might be generated by a different mechanism (Wolpert, 1971). While the actual mechanism for segment formation or stripe generation is not known, it is not unreasonable to consider that in some cases, at least, they could be generated by an isomorphic prepattern (MacWilliams, 1978).

An isomorphic prepattern, unlike positional information, provides a pattern of cell activities which reflects the overt pattern that will develop. For example, the digits in the hand could result from a wave-like distribution in a chemical morphogen with the peaks and troughs specifying the digits. A number of models, mainly based on Turing's (1952) original ideas of a reaction–diffusion mechanism, have shown how such prepatterns could be generated (Nagorcka, 1989). All these mechanisms generate wave-like patterns that could generate repeated structures. A crucial feature of all these is that the peaks and troughs are all the same. One could not alter one structure without affecting all and this greatly limits the classes of patterns to which they can give rise. But, by combining them with positional information, which will make the waves non-equivalent, a very large variety of patterns can be generated (Fig. 1).

The idea of wave-like prepatterns is more attractive than the evidence in its favour. The evidence is, as yet, just not there. A key case is the segmental pattern in early *Drosophila* development for which wave-like patterns could provide the mechanism, but the basis of

segmentation with its beautiful stripes remains, so far, unknown. It is of great interest that the early periodic expression of a pair-rule gene is established by different regulatory elements that do not respond to periodic spatial cues (Goto *et al.* 1989).

In one case, at least, there is good evidence that positional information alone is not sufficient to specify the pattern, and that is the chick limb (Wolpert and Stein, 1984). While models based on positional information can account for a wide variety of experimental results, there are at least two telling experiments that show its inadequacy. The positional information model suggests that, for pattern along the anteroposterior axis of the developing limb, position is specified by a signal – possibly a gradient in a morphogen like retinoic acid – produced at the posterior margin by the polarising region. However, if the mesoderm of the limb bud is disaggregated into simple cells, reaggregated, and enclosed in normal limb ectoderm, limb-like structures, including digits, can develop (Patou, 1973). This strongly suggests that some sort of self-organising prepattern mechanism exists.

The second result comes from a consideration of the specification of the humerus. The predictions of the positional model concerning the development of the humerus when a polarising region is placed at different positions along the anteroposterior axis do not hold. The expected duplication and eliminations do not occur. The results might be better explained if the humerus were specified by a wave-like prepattern. For example, it would only be possible to get an additional humerus if there were substantial widening of the limb to allow the development of another peak (Wolpert and Hornbruch, 1987).

In more general terms the suggestion is that the solution to the French Flag problem (Wolpert, 1969) may involve first specifying three regions by a prepattern and then making them different with a positional signal. A possible interaction between specification of a repeated structure and positional information is in relation to size regulation in somite formation (Cooke, 1988).

There is a further reason for wishing to invoke a prepattern mechanism for repeated patterns. From an evolutionary point of view it is easy to see how such patterns could arise with a reaction–diffusion mechanism and a single threshold, whereas a positional model requires multiple thresholds which would be almost impossible to provide *ab initio*.

The combination of a spacing pattern with positional information could provide the basis for a variety of different patterns. The pattern of vertebrae might be thought of in these terms. Similarly feather patterning in birds may involve a spacing pattern to place the feathers in a regular pattern and positional information to specify both the nature of the individual feathers and whether feathers will form. Mammalian teeth may represent yet another system with a repeated pattern whose individual members then diverge. It may even turn out that all segmental structures where the segments are different are generated by a combination of the two mechanisms.

Morphogenesis

Change in form during development is brought about by cellular forces such as localised contractions and changes in adhesion. Recent studies have placed great emphasis on the pattern of expression of cell adhesion molecules (Edelman, 1986). The view adopted here is that all such changes are an expression of a prior patterning process. Changes in form occur as a result of an earlier patterning event. Many of the changes may be thought of in terms of interpretation of positional information. This is by no means exclusive and other patterning mechanisms and induction itself could be responsible.

This view should be contrasted with the idea that mechanical forces could, themselves, generate spatial patterns as in the case of cartilage condensation in limb

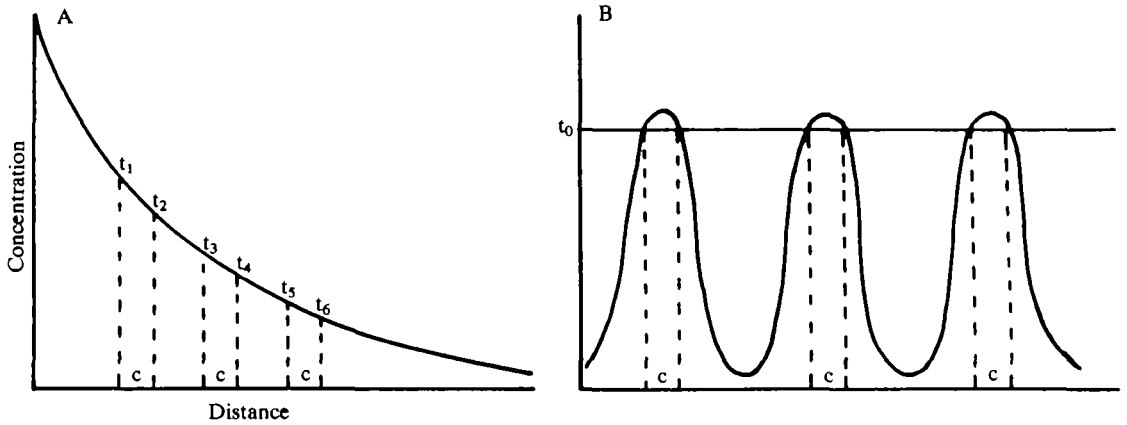

Fig. 1. Diagrams to illustrate a positional information mechanism (A) and a prepattern mechanism (B) to specify three regions of, say, cartilage. The positional information mechanism requires six thresholds, t_1–t_6, whereas the prepattern mechanism just one, t_1. The positional information mechanism could be combined with the prepattern to make the three regions non-equivalent.

development or the development of feather follicles (Oster *et al.* 1985). For the limb, at least, this model has severe defects (Wolpert, 1989*b*).

Universality

One of the attractive features of positional information was that it offered the possibility of universality. That is, in principle, the same coordinate system and the same signals could be used again and again. This ambitious expectation has not been quite fulfilled. There is, however, good evidence that within the same animal – the imaginal discs of insects, the forelimb and hindlimb of vertebrates – the same signals are used. In addition, developing and regenerating amphibian limbs use similar signals (Muneoka and Bryant, 1982) and so do the limbs of amniotes (Tickle *et al.* 1976; Fallon and Crosby, 1977). Nevertheless there is only limited evidence for the conservation of positional signals across phyla. However, the discovery of the homeobox has revealed a highly conserved region of many genes in different phyla possibly involved in patterning. Also there are an increasing number of reports in which similar proteins, such as EGF-like molecules and receptors seem to be involved in patterning. This is very encouraging. Perhaps we will find that there is a common language, just different dialects.

In looking for universal mechanisms and signals, it is likely that the systems in which they are least likely to be found are eggs and very early development. Yolky eggs, for example, provide highly specialized systems compared to the phylotypic stages of development, such as the germ band stage in insect development and the head process/somite stage in vertebrates (Wolpert, 1989*b*).

A further feature of universality is that many of the features of regulation and regeneration described by the polar coordinate model apply to organisms as diverse as insects and amphibia (Bryant *et al.* 1981) and even to protozoa (Frankel, 1984).

Conclusions

Patterning by positional information provides a relatively simple mechanism for making a wide variety of patterns. Alas, compared to 21 years ago, that simplicity now seems more like simple-mindedness. Things seem, at this stage, much more complicated, particularly since molecular genetics has transformed our understanding of early insect development (Ingham, 1989). While there is evidence for some aspects of a positional information mechanism, there are also other interactions. Boundaries, positional signals, positional value and interpretation are still useful concepts that perhaps have to be considered in relation to more complex systems that may involve prepatterns and induction and other cell-to-cell interactions. Nevertheless, for some processes, such as those involved in regeneration, the polar coordinate model still provides a powerful framework for systems as diverse as protozoa, insects and vertebrate limbs. It would be a great pity at this stage to conflate positional signals, positional values, induction and morphogens. Each still has a useful meaning. Morphogens are only just beginning to be identified and we may have to be even more patient for a molecular understanding of positional value.

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Appendix

The idea of positional information came to me in early 1968. It was a very exciting few days when everything became clear and obvious. Naturally it has not remained so. But at that time all the problems we had been struggling with could at once be explained if cells had their position specified as in a coordinate system and then used this information to determine how they would differentiate.

The origin of the idea came from several sources. The most important was the recognition that the problem of pattern formation and regulation was being grossly neglected. Pattern formation was not part of the embryologist's conceptual equipment at the time, though some workers like Waddington and Rose did recognise its importance. My work on sea-urchin morphogenesis with Trygve Gustafson had introduced me not only to development, but to the founders of the Swedish gradient theory, both Runnstrom and Hörstadius. From the beginning I had great difficulty with gradient theory and how it could explain patterning and size regulation. Gradients at that time were, in the tradition of Child (1941), meant to reflect metabolic rates and I could not understand how they worked or gave rise to pattern. Rate advantage did not make much sense. When marriage ended trips to Sweden I chose Hydra as a simple regulating system to work on pattern formation.

I like to think that I invented the term 'pattern formation'. I had great difficulty finding a suitable name and even consulted a classicist to see if another word would do. For pattern, as normally used in English, is not quite the right word, the essential connotation being template. Pattern formation does, now, seem to have just the right meaning.

With Hydra came the French Flag Problem (Wolpert, 1968). This focused in a very simple way on a basic patterning system that required a solution. Both the sea urchin and Hydra were like a French flag, and with Gerry Webster, Michael Apter and Mary Williams we developed all sorts of quite simple and ingenious models. Michael Apter's solution – he was a psychologist with an interest in computers – was to number the cells in a line from both ends. It was then easy to specify the red, white and blue regions. I initially thought it completely artificial and unrealistic, and dismissed it.

Another motivation was a desire for universality and how to get from the genes to pattern. I was convinced that there had to be universal mechanisms – so many of the phenomena seemed so similar. Also I needed a mechanism which could make use of the fact that all cells had the same genetic information. Positional information thus came from the French flag via Apter's numbering and universality. I think Stern's genetic mosaic studies on insects was important for it suggested a simple interpretation.

I first presented the idea at one of Waddington's 'Theoretical Biology' meetings at the Villa Serbelloni. I travelled from Milan in a taxi with Brian Goodwin who was excited by the idea and, with Cohen, soon developed the phase-shift model (Goodwin and Cohen, 1969). Waddington, by contrast, was unenthusiastic, since he recognised that interpretation carried too large a burden.

In the summer of that year I was at Woods Hole. I presented the new ideas at a Friday evening discourse, which had a very large audience. The reception was very hostile. They did not like being told that for the limb, for example, they had completely missed the problem: it was not in epithelial mesenchymal interactions, but in patterning of cartilage and muscle that the real problem lay. Only Sydney Brenner was encouraging though Howard Schneiderman was at least interested.

Several aspects were not new. Driesch had spoken of position and coordinate systems at the end of the last century, but thought it was impossible. More important, Stumpf in a lovely experiment, had said very much the same thing and Peter Lawrence's ideas on gradients were along very similar lines (Wolpert, 1986).

Gradients had become very unfashionable, and Crick's (1970) support and interest were, I think, crucial to making positional information more or less respectable.

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