Positional Information – a concept underpinning our understanding of developmental biology

Neil Vargesson

Author for correspondence:

Prof Neil Vargesson. School of Medicine, Medical Sciences and Nutrition. Institute of Medical Sciences. University of Aberdeen. Foresterhill. Aberdeen. AB25 2ZD.
Email: n.vargesson@abdn.ac.uk; nvargesson@gmail.com. Tel: 01224 437374.

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Abstract

It is now 50 years since Lewis Wolpert published the paper in which he set out the concept of Positional Information to explain how spatial patterns of cellular differentiation are generated. This concept has provided a universal model for pattern formation in embryonic development and regeneration and become part of the fabric of the field of developmental biology. Here I outline how Wolpert devised the concept of Positional Information and describe landmark studies from his lab investigating how Positional Information is specified in the developing chick limb.

Origins of the concept of Positional Information – sea urchins, *Hydra* and the 'French Flag Problem'

How a fertilised egg produces a fully patterned embryo and ultimately an independent organism, has fascinated generations of biologists for centuries. Over more than a century ago, some elegant experiments by the German embryologist Hans Driesch (reviewed in Driesch, 1908) on sea urchin embryos began to shed light on this. Driesch separated the two-cell stage embryo into single cells and found that each cell made a complete and perfect embryo but was half the normal size. Driesch speculated that the ability of the cells to regulate and each produce a whole embryo rather than an embryo with missing structures meant the cells must contain some special information to allow them to understand position. Yet, he didn't know what that information was. Driesch's work was one of the inspirations that helped lead Lewis Wolpert to formulate his concept of Positional Information.

Wolpert was originally a soil engineer in South Africa and became interested in cell division and cell biology while studying soil biology in London. He was introduced to sea urchin embryos via his PhD studies on the mechanics of cell division (Wolpert, 1960; Wolpert, 2018; see also Richardson, 2009; Tickle, 2002). After his PhD, Wolpert continued to work on sea urchins and spent several summers in Sweden with Trygve Gustafson investigating sea urchin development in detail (Gustafson and Wolpert, 1967). This work not only provided a complete account of gastrulation in an animal embryo but also defined it in terms of the repertoire of cellular activities. Meanwhile, in his own lab that he had set up at Kings College London in 1960, he was studying the movement of amoeba with two PhD students (Charles O'Neill and Chris Thompson and later Joan Morgan). Another PhD student David Gingell who joined later worked on membrane interactions between cells (Figure 1). It was at this time, that Wolpert started to focus on how the spatial organization of cellular differentiation is specified.

Wolpert chose *Hydra* as his model to investigate the specification of spatial organization as it is a simple organism which can regenerate when parts of it are removed. Work with several PhD students (Gerry Webster and Judy Hicklin) and his technician Amata Hornbruch demonstrated that a diffusible inhibitory gradient down the body produced by the head prevented the head forming in the wrong place (Webster and Wolpert, 1966; Hicklin et al., 1969; Wolpert et al., 1971) (Figure 1). A second more stable gradient was demonstrated to determine where the head forms (Wolpert et al., 1971). These experiments on *Hydra* taken together with the earlier work of Driesch led Wolpert to realise that the embryo was behaving like a flag, where the pattern is the same no matter what its size. This provided the foundation for his adoption of the "French flag problem" with its blue, white and red stripes as a model of pattern formation in developing tissues (Wolpert, 1968). The problem is how does a line of cells in which each cell has the potential to develop into blue, white and red actually form the French flag? Wolpert suggested that this could be accomplished if the cells are first informed of their position with respect to the boundaries or reference points at either end (Figure 2). To specify position, he suggested that a concentration gradient of some substance would be involved and that each cell would acquire a positional value according to the local concentration. In a second step, the positional value would then be

interpreted so that the cell differentiated into the appropriate colour. This model could account for the pattern being the same for 'flags' (tissues) of different sizes (Wolpert, 1968).

Wolpert first presented his French flag model at a meeting hosted by Conrad Waddington at Serbelloni on Lake Como in 1968 and it was well-received. However, a little later that year when he talked at Friday evening lecture at Woods Hole, the response was very negative (Wolpert, 2015; see also Richardson, 2009). The reasons were not clear, but it may have been because the American developmental biologist Gordon Child had previously championed the idea that gradients could be involved in embryonic development but had not been able to take it any further (Wolpert, 2015; Wolpert, 2018). However, Sydney Brenner, who had been at the lecture, encouraged Wolpert to write up his ideas and this resulted in the 1969 paper in the Journal of Theoretical Biology entitled 'Positional Information and the spatial pattern of cellular differentiation' (Wolpert, 1969).

It is worth highlighting some of the key points from this paper as these formed the basis for general principles. For example, Wolpert stressed the importance of polarity in providing reference points and how it would be important to identify them. He suggested that Positional Information could be specified in relation to such reference points in two main ways; one being by a concentration of a substance that varies according to distance from the reference points, as in the French flag model, the second by a mechanism for cell counting (Figure 2). He also highlighted the need for a mechanism for the differential response of cells, such as thresholds; an issue he later returned to with Julian Lewis and Jonathan Slack (Lewis et al., 1977).

Another important point in the 1969 paper was the universality of positional information in the sense that It applied to practically all multicellular organisms. Wolpert wrote "there is good reason for believing that there are a set of general and universal principles involved in the translation of genetic information into pattern and form". He illustrated this in the paper by showing how the concept of Positional Information could be applied to the early development of the sea urchin embryo, regeneration of *Hydra,* pattern formation in the insect epidermis and the development of the vertebrate limb. He suggested that it might also apply to early amphibian development, establishment of retinotectal connections, pattern of cell division in the crypts of the small intestine and regeneration of amphibian limbs and planarians.

Wolpert also elaborated on some implications of phylogenetic universality drawing again on the analogy of a patterning tissue with flags. He pointed out that if Positional Information is specified in the same way in the cells that make up the French flag and the British flag, then one can predict how a group of cells of the French flag will behave when transplanted into the British flag and vice versa. The cells will differentiate according to their position but will interpret that Positional Information according to their flag of origin ie: they will form the appropriate part of the flag from which they came (Figure 3). This point is further illustrated by the imaginal discs in *Drosophila* in which positional values are similar in each of the different discs. If the Hox gene *Antennapedia* which is normally expressed in regions that produce the legs of the *Drosophila* are expressed in the head region, the antenna develops into a leg. Moreover, clones of *Antennapedia* cells placed in the antenna imaginal disc can develop as leg cells but which part of the leg they form is dependent upon their position in

the imaginal disc; for example, if the clones are the tip of the disc, they can form leg claws (Postlethwait and Schneiderman, 1971; Wolpert, 2015; see also Wolpert et al., 2019). This indicates that Positional Information is the same between the imaginal discs of the antenna and leg, but because of Hox genes the cells interpret their positional values differently (Wolpert, 2015).

Another major conclusion in the 1969 paper based on analysis of the data available at the time was that Positional Information is specified in fields of about 50 cells over about 10 hours, which is the approximate size of many tissues in early development undergoing patterning, for example *Hydra*, sea urchin gastrula, chick limb bud (Table 1). Francis Crick subsequently published a paper suggesting that this could be accomplished by diffusion (Crick, 1970). In more recent work with Michel Kerzberg, Wolpert has argued however that diffusion may not be sufficiently reliable to specify Positional Information (Kerzberg and Wolpert, 1998; Kerzberg and Wolpert, 2007; see also Richardson, 2009).

Positional Information to explain chick limb morphogenesis
Following a move to The Middlesex Hospital Medical School located in the Windeyer Building in 1966, Wolpert switched to the developing chicken limb as a model system to investigate how pattern formation comes about through Positional Information. This was because he thought that this would be more relevant to medical students than regeneration in *Hydra.* Wolpert's lab was joined by several talented PhD students and postdocs who made important discoveries (see below) that supported the concept of Positional Information. This work established the chick limb as a leading model for studying Positional Information (see also Davey et., 2018). A PhD student Dennis Summerbell joined Wolpert's lab in 1969 (Figure 4) and established in the lab the tools and assays to study chick limb development, most of which had been previously devised by John Saunders (Saunders, 1948; Saunders and Gasseling, 1968). He was soon joined by Julian Lewis, a postdoc who focussed on more theoretical aspects of the work and proposed the principle of non-equivalence (Lewis and Wolpert, 1976; see later). Later Cheryll Tickle, and PhD students Geoff Shellswell, Nigel Holder, John McLachlan and Jim Smith also joined the group working on chick limb development (Figure 4). Jonathan Slack carried out postdoctoral studies on amphibian limb regeneration and also on theoretical aspects of Wolpert's model.

The Progress Zone model for proximo-distal limb outgrowth
Wolpert's ideas (1969) had implied that Positional Information would be specified in a co-ordinate system but he started his work on the chick limb by focussing on how position is specified along the proximo-distal axis of the developing chick wing ie: how do cells "know" if they are to make the proximal bones like the humerus or more distal bones like the digits? It had already been shown by John Saunders that the apical ectodermal ridge, the thickened rim of ectoderm at the tip of the chick wing bud, was required for bud outgrowth and that the parts of the wing are laid down in sequence from proximal to distal (Saunders, 1948). In his 1969 paper, Wolpert wrote "In terms of the French Flag analogy, the development of the pattern of the limb is not the growth of a small French Flag but the laying down first of the blue region, then the white and finally the red, as the region increase in length". He suggested that the specification of structures along the proximo-distal axis depended on distance from the body wall (Wolpert 1969). However, a series of transplantation experiments by Dennis Summerbell in which undifferentiated distal tips from early wing

Table 1

Linear size of positional fields in terms of cell numbers

System	Approximate Size
Axial length of *Hydra littoralis* ectoderm	60
Early amphibian gastrula – animal pole to dorsal lip	30
Early sea urchin gastrula – animal pole to vegetal pole	30
Early starfish gastrula – animal pole to vegetal pole	50
Larval insect segment – epidermal cells from front edge to back	50-100
Diameter of retina at stage 29	30
Mesenchyme of chick limb from trunk to apical ridge at stage 24	<100
Width of amphibian medullary plate	40
Imaginal disc of leg of Drosophila before determination occurs	<100

buds were grafted onto proximal stumps of late limb buds and vice versa, undifferentiated distal tips from late wing buds grafted onto proximal stumps of early wing buds, resulted in duplications of structures along the proximo-distal axis or loss of structures respectively. This indicated that structures along the proximo-distal axis do not develop according to distance from the body wall but instead that the limb bud tip behaves autonomously (Summerbell et al., 1973). To explain these findings Lewis Wolpert, Julian Lewis and Dennis Summerbell proposed that the region of undifferentiated mesenchyme directly beneath the apical ridge at the tip of the bud acts as a progress zone and that the time spent in the progress zone specifies positional values – the longer cells remain in the progress zone, the more distal the positional value (Summerbell et al., 1973; see also Summerbell and Lewis, 1975; Figure 5). They proposed that the role of the apical ectodermal ridge is to maintain the progress zone. The timing mechanism might be linked to the cell cycle and it was calculated by Julian Lewis that each of the elements of the limb are specified by one cell cycle each. Thus, all elements of the chick wing could be patterned within 7 cell cycles (Lewis, 1975). So, presumptive limb elements can each be specified in a similar number of cells, and then develop their individual characteristics (ie: a humerus versus a carpal bone) through intrinisic growth programmes (see also Lewis and Wolpert, 1976). A few years later work in the lab showed that irradiating early wing buds caused the loss of proximal structures but distal structures were relatively unaffected (Wolpert et al., 1979); a result predicted by the progress zone model. The cells that survive after irradiation spend longer in the progress zone in order to replace the cells that are lost and thus produce mainly distal structures. This was proposed as a potential explanation for thalidomide-induced phocomelia (Wolpert et al., 1979).

Nearly 30 years later, the progress model for proximo-distal pattern formation in the limb was challenged by a study from Cliff Tabin's lab who suggested that the cells in the early wing bud were pre-specified and that the pre-specified structures then expanded in a proximo-distal sequence to lay down the parts of the wing as the bud elongates (Dudley 2002). This prespecification model also provided an alternative explanation for the loss of proximal structures in irradiated wing buds because there would be less time for proximal pre-specified cell populations to replenish lost cells and undergo the necessary expansion to form an element (Galloway et al 2009). The most recent work suggests that the proximal structures may be pre-specified but that positional values for structures distal to the elbow are specified by a progress zone mechanism (Saiz-Lopez et al., 2015; Towers et al., 2012). Current thinking of the mechanism underpinning thalidomide-induced phocomelia is that thalidomide targets the blood vessels resulting in localised cell death (reviewed in Vargesson, 2015; Vargesson, 2019).

Morphogen gradients and antero-posterior patterning of the limb

The next question Wolpert addressed was how position is specified along the antero-posterior axis of the limb so that for example the different digits form in their proper places. In his 1969 paper, Wolpert interpreted the results obtained by Saunders and Gasseling with grafts of a small region of the posterior-distal mesenchyme of the developing chick wing bud (which they later coined as the Zone of Polarising Activity (ZPA) or polarising region) in terms of his ideas about Positional Information and polarity potential. When this region is transplanted to the anterior margin of a second wing bud, it caused mirror image duplications of the digits (Saunders and Gasseling, 1968). Wolpert proposed that the ZPA is

a reference point and that graded polarity potential with the high point at this reference point specifies Positional Information. Cells nearest to the ZPA would have high polarity potential and those far away would have a lower polarity potential. This would give rise to the pattern of digits in the normal chick wing and the duplicated patterns of digits following ZPA grafts (Wolpert, 1969). A series of experiments by Cheryll Tickle and Dennis Summerbell in which they grafted a ZPA to different positions in a host wing bud showed that the character of a digit that developed in response to the graft depends on distance from the polarizing region as predicted (Tickle et al., 1975). Furthermore, they showed that the wing bud widened following a ZPA graft to the anterior margin. It was then discovered that ZPA grafts stimulate cell proliferation, thus growth is needed to allow the limb bud to widen to allow a complete pattern of duplicated digits to develop (Cooke and Summerbell, 1980). Jim Smith later demonstrated that when a wing bud was irradiated after a ZPA graft, the most anterior digits are lost from the duplicated digit patterns (Smith and Wolpert 1981). Further work began to investigate how antero-posterior and proximo-distal positional values interact in the 3D coordinate system proposed to pattern the limb (Summerbell, 1974; Summerbell and Lewis, 1975).

The experiments above focussed on the pattern of cartilage differentiation in the developing chick wing but was the muscle pattern specified in the same way? Geoff Shellswell showed, for example, that following a polarizing region graft that the pattern of wing muscles is also duplicated (Shellswell and Wolpert, 1977). Wolpert's ideas put forward in the 1969 paper envisaged that Positional Information would determine "the nature of molecular differentiation" of a cell as transplantation experiments by Searls in the 1960's had suggested that cells in the chick wing differentiate into either muscle or cartilage according to their position (Janners and Searls, 1970; Searls, 1967). Subsequently however it became clear that the myogenic cells of the muscles of the limb are actually a separate cell population which migrate into the limb bud from the somites and differentiate in the limb bud (Christ et al., 1977; Chevallier et al., 1977) and that the connective tissues differentiate from the lateral plate mesoderm. Thus, it is the connective tissues whose differentiation is determined by Positional Information in the developing limb and not myogenic differentiation. Furthermore, cells that differentiate into the same connective cell type, for example, cartilage, can have different positional values. This is the principle of non-equivalence which proposes that differences in positional values can be interpreted so that for example each cartilage element has its own intrinsic growth programme (Lewis and Wolpert 1976; see also, Wolpert, 1989).

Another prediction of Wolpert's French flag model as applied to antero-posterior pattern formation in the chick wing was that the response to the ZPA morphogen would be dose dependent. This was tested by Jim Smith. He treated the ZPA with different doses of gamma-irradiation to reduce the number of cells in the graft and found that only additional anterior digits were specified (Smith et al., 1978). Later, Cheryll Tickle found that grafting just a few ZPA cells induced only an additional anterior digit and higher numbers were needed to induce more posterior additional digits (Tickle, 1981). Furthermore, in line with the conclusion of Wolpert's 1969 paper about the length of time required to specify Positional Information, Smith found that ZPA grafts need to be in place for 16 hours to produce a duplicated anterior digit and 20 hours to also induce duplications of more posterior digits (Smith, 1980). Thus, the ZPA controls the pattern and identity of the digits of the chick wing in a dose responsive

manner. The more polarizing region cells grafted or the longer cells are exposed to the ZPA the more posterior the digit identity.

Additional work in the Wolpert Lab also demonstrated that mouse ZPA grafts into chick wing buds could cause chick digit duplications, demonstrating that the mouse limb also used a ZPA signalling-like mechanism to specify digits and further demonstrating Wolpert's view that Positional Information is a universal concept operating in multiple species (Tickle et al., 1976).

A challenging issue that arose was whether Positional Information was specified by a long range signal from the polarizing region as predicted by Wolpert's French flag model or whether local interactions occurred as predicted by an intercalation mechanism put forward to account for regeneration in imaginal discs of *Drosophila* and regeneration in amphibian and cockroach limbs (French et al., 1976; Javois and Iten, 1981; Javois and Iten 1982; see also Wolpert, 2015). Work by Lawrence Honig, a postdoc in the lab, found that the signal from the ZPA can act over a long range in the chick wing, where an anterior wing digit could be induced in cells that were separated by a 200um wide piece of leg tissue from a ZPA graft (Honig, 1981). Amata Hornbruch also carried out experiments where multiple ZPA grafts were placed in donor limb buds (Wolpert and Hornbruch, 1981; Figure 4E). The results further supported the idea of long-range signals from the ZPA to establish digit identity (Wolpert and Hornbruch, 1981). Instead of seeing a single mirror-image set of duplicated digits (which intercalation theory hypothesised would happen as cells would intercalate to make the correct positional values for a single set of duplicated digits), a range of different digit identities, and in some cases multiple digit duplicate structures was seen according to the positions and distances apart of the ZPA grafts (Figure 4E).

A crucial issue was of course to identify the molecular nature of the ZPA morphogen. Experiments treating ZPA grafts with a range of biochemical inhibitors showed that RNA synthesis inhibition reduced the ability of ZPA grafts to induce digit duplications (Honig et al., 1981). Then Juliet Lee, an undergraduate summer student working together with Tickle found unexpectedly that local application of retinoic acid could mimic the effects of grafts of the polarizing region (Tickle et al., 1982). Retinoic acid was the first defined chemical discovered which could alter positional values in the developing chick wing. We now know that the ZPA signal is actually Sonic hedgehog (Shh) which is produced by the ZPA (Riddle et al., 1993). Retinoic acid specifies ZPA position (reviewed in Davey et al., 2018; Tabin and Wolpert, 2007) while Shh specifies the different digits of the chick wing at different concentrations and/or with different lengths of exposure (Yang et al., 1997). It has more recently emerged that although the morphogen gradient model can explain the digit patterns of the chick wing, it can not simply be applied to limbs with more than 3 digits. In the mouse limb, for example, while the pattern of the three anterior digits could be specified by different Shh concentrations, the two posterior digits come from the ZPA itself and their identities must be specified instead by the length of exposure to Shh (Harfe et al., 2004; reviewed in Tickle and Towers, 2017).

The Wolpert Lab – later work on the chick limb and pattern formation
All the work outlined above resulted in the establishment of a conceptual framework upon which the subsequent discoveries of the molecular basis of development could be built. For

example, Wolpert and Tickle collaborated with Denis Duboule and his PhD student Juan Carlos Izpisua- Belmonte to investigate the way in which Hox gene expression is controlled in the developing chick wing (Izpisua-Belmonte et al., 1991; Izpisua-Belmonte et al., 1992). In an extraordinary period of discovery in the early 1990's, work by many labs identified the secreted morphogens involved in specifying Positional Information in the chick wing that Wolpert's ideas had predicted. These extracellular signalling molecules include FGFs, Shh, Wnts, BMPs (Francis et al., 1994; Laufer et al., 1994; Niswander et al., 1994; reviewed in Tabin and Wolpert, 2007).

Wolpert himself continued to investigate Positional Information in chick limb development as well as discuss, support and argue for the concept at every opportunity he could (Figure 6; Figure 7). Keiichi Akita, a post-doctoral researcher, studied the role of the limb ectoderm in specifying dorso-ventral pattern, putting forward a novel model (Akita, 1996) while Neil Vargesson, a PhD student, also worked with Jon Clarke and Cheryll Tickle to produce detailed fate maps of the developing chick wing by marking small groups of cells with DiI and comparing cell fate with gene expression pattern changes (Vargesson et al., 1997).

Among the last experiments carried out in Wolpert's lab (see Table 2 for a list of Wolpert lab key personnel) was an investigation by PhD students Adrian Hardy, Michael K. Richardson and colleagues on gene expression associated with self-organization of a limb prepattern (Hardy et al., 1995). The existence of a self-organizing prepattern had been shown many years previously by Patou in her experiments with cells disaggregated from chick limb buds. When the cells were reaggregated and stuffed into an ectodermal jacket, the recombinant buds developed into limb-like structures with recognizable digits (Patou, 1973). Experiments by Amata Hornbruch in the 1980's had also shown that the ZPA only patterns the limb distal to the humerus indicating that the humerus is specified early before the ZPA is established, perhaps through a wave-like prepattern mechanism (Wolpert and Hornbruch, 1987). Wolpert, initially working with Wilfred Stein, suggested that "the solution to the French Flag problem may involve first specifying three regions by a prepattern and then making them different with a positional signal" (Wolpert 1989, see also Wolpert and Stein 1984). This integration of a periodic prepattern by a Turing-type mechanism (Turing, 1952) and Positional Information which may occur not only in the limb but also in many other developing systems has been reviewed by Green and Sharpe (2015).

Conclusion

The concept of Positional Information and pattern formation as first described by Lewis Wolpert (Wolpert, 1969) to explain how cells become different from one another and produce different structures changed the field of developmental biology and still underpins much of current developmental biology thinking today. It is a unifying and universal concept that provided a major stimulus to the field suggesting a new way of thinking about development and provoking discussion and setting challenges.

The first concrete example of the way in which a concentration gradient of a morphogen can specify a pattern is the development of *Drosophila* blastoderm where the bicoid transcription factor protein provides an antero-posterior gradient (Driever and Nusslein-Volhard, 1988a; Driever and Nusslein-Volhard, 1988b). Another early example of a morphogen gradient was discovered again in the *Drosophila* blastoderm where the dorsal

transcription factor protein forms a dorso-ventral nuclear gradient (Roth et al., 1989; Rushlow et al., 1989; Steward, 1989). Subsequently gradients of secreted extracellular morphogens were found to pattern the embryonic dorso-ventral axis, the antero-posterior axis of the segments and the imaginal discs in *Drosophila*. In vertebrates, gradients of secreted morphogens pattern not only the digits of the limb but also, for example, the germ layers (Chen and Schier, 2001) and the dorso-ventral axis of the neural tube (reviewed in Placzek and Briscoe, 2018; Cohen et al., 2013). Furthermore, as predicted by the concept of positional information, the same morphogen molecule can be used over and over in development and in different animals without dictating a specific cell fate. For example, hedgehog proteins are involved not only in providing Positional Information in the development of the body segments in *Drosophila* but also in the chick wing and neural tube. Yet, questions remain about the mechanisms establishing these gradients of secreted morphogens and their precision (for further detail see Jaeger and Martinez-Arias, 2009; Wolpert, 2011; Wolpert, 2015; see also Wolpert et al., 2019).

Another outstanding issue relates to the molecular basis for positional values and how they are interpreted. Wolpert has suggested that the best candidate for a molecule that encodes positional value is Prod 1 a membrane bound protein involved in newt limb regeneration (Kumar et al., 2007; Wolpert, 2015). It is however well-established that Hox genes are among the target genes expressed in response to extracellular morphogens and they can interpret positional information by controlling regional identity in both arthropods and vertebrates. In a similar way to the Hox genes that control segment identity in *Drosophila*, there are transcription factor genes in flowering plants that control organ identity e.g petals versus carpals (Coen and Meyerowitz, 1991; Krizek and Fletcher, 2005; see also Meyerowitz, 1994; Wolpert et al., 2019).

The impact of the 1969 landmark paper (Wolpert, 1969) is highlighted by being cited over 2700 times according to Google Scholar and almost 2000 times according to Web of Science and Scopus. This number of citations is almost certainly an underestimate of its actual influence as the concept is now found in all text books of developmental biology (eg: Slack, 1983; Wolpert et al., 2019). Indeed, the paper has influenced and been cited by authors in a broad range of subjects not just in developmental biology but also mathematical modelling, computer simulations and engineering (Figure 8). The paper's influence, even 50 years since its publication, means it is still cited today, indeed just in 2019, over 25 papers have cited it ranging in topics from regeneration studies to muscle patterning and stem cell fate (Figure 9).

The 1969 paper concludes with an insightful statement that still rings true today: 'The provision of a universal co-ordinate system to which the cell's genome can respond is probably the most effective way of exploiting the fact that each cell has a full complement of genetic information, and it also enables a tremendous variety of patterns to be formed'. Thus, Positional Information underpins pattern formation in tissues in vertebrates and in invertebrates and it is how the cells interpret the information that leads to tissue and patterning differences. This is the challenge for the next 50 years of Positional Information, to identify the precise molecular controls that underpin Positional Information and the long-term stable memory of positional value in developing and regenerating tissues.

Table 2

Wolpert Lab Staff (Family Tree):

1960's Kings College London – Sea Urchin, *Hydra,* Cell adhesion

Charles O'Neill, Chris Thompson
Joan Morgan, Gerry Webster
David Gingell, Stuart Clarkson

1960's The Middlesex Hospital Medical School – *Hydra*, Slime moulds, *Xenopus*, chick limb development and somitogenesis

Judy Hicklin, Amata Hornbruch
David Garrod, Paul Farnsworth
Christine Slack, Anne Warner
Dennis Summerbell, Stephanie Webber

1970's The Middlesex Hospital Medical School – *Hydra*, amphibian limb regeneration, chick limb development, chondrogenesis

Amata Hornbruch , Richard Wakeford
Pat Clissold, Anne Crawley, Anthony Smith, Jonathan Slack, John Gayley
Dennis Summerbell, Julian Lewis, Lulwah Al Gaith, Margaret Goodman, Moira Cioffi, Cheryll Tickle, Muriel Sampford, Geoff Shellswell , Nigel Holder, John McLachlan, Jim Smith, Margaret Bateman, Lawrence Honig, Betsy Gregg, Christine Harrison,
Paul Rooney, Chris Cottrill, Charlie Archer, Jacquie Morris

1980's The Middlesex Hospital Medical School – chick limb development and somitogenesis, pigmentation patterns, handedness

Amata Hornbruch, Julian Schofield, David Wilson, Euan Taylor, Esther Bell, Philippa Francis-West, Sally Weale
Michael K Richardson
Christine Hoyle

1990's The Middlesex Hospital Medical School and University College London – chick limb development, feather patterns

Adrian Hardy, Ronald Nittenberg, Keiichi Akita, Han-Sung Jung, Neil Vargesson

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Figure Legends

Figure 1 – The Wolpert Lab circa 1960s
The Wolpert Lab first studied sea urchins, *Hydra*, and cell movements in Amoeba. A. David
Gingell (who worked on Amoeba); B. Amata Hornbruch and Judy Hicklin (who worked on
Hydra); C. Lewis Wolpert (with Judy Hicklin in background). It was Wolpert's work on sea
urchin gastrulation that led him to coin the phrase '*gastrulation is the most important time
of your life*' which he mentioned over dinner at a scientific meeting in the 1980s (Wolpert,
2015). His comment was added to by Jonathan Slack in 1983 (J. Slack, personal
communication) to create the now famous quote '*it is not birth, marriage or death, but
gastrulation which is truly the most important time in your life*'(Slack, 1983). Thanks to
Amata Hornbruch for supplying the images.

Figure 2 – The French flag model of pattern formation
Populations or lines of cells (up to about 50 – see Table 1) start out equal but each cell has
the potential to develop as blue, white or red. To do this, the line of cells is exposed to a
concentration gradient of some substance which gives each cell a unique positional value
defined by the concentration at that point. Each cell then interprets the positional value it
has acquired and differentiates into blue, white or red according to a predetermined genetic
program thus forming the French flag pattern.

Figure reproduced with permission and figure legend modified from Wolpert et al., 2019
'Principles of Development, 6th Edition'. pp29. Oxford, UK. Oxford University Press.

**Figure 3 – The Universal positional field concept: cells interpret their position according to
their developmental history and genetic make-up.**
Two populations of cells can use the same positional information to produce different
patterns due to differences in genome. For example, the white and grey rectangles
represent two independent positional fields that will give rise to slightly different flags – the
French flag on the middle left panel and the US flag on the middle right panel. Transplants
of a small piece of tissue from one flag to the other flag at an early stage of development
results in the graft developing according to its new position and positional information but
with its original pattern (from its genome). Lower panel: Imaginal discs of Drosophila
similarly use the same Positional Information to produce differently patterned appendages.
Mutations in the Antennapedia gene can result in in transformations into leg structures.
This indicates the cells share the same positional information but it is interpreted depending
on position.

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'Principles of Development, 6th Edition'.pp 450. Oxford, UK. Oxford University Press.

Figure 4 - The Wolpert Lab circa 1970s
A.Group picture of Wolpert Lab in December 1976 taken on the roof of the Windeyer
Building, Middlesex Hospital Medical School; John McLachlan, Julian Lewis, Anne Crawley,
Margaret Goodman, Nigel Holder, Geoff Shellswell, Jim Smith, (Margaret Bateman hiding
behind), Muriel Sampford, Lulwah Al-Ghaith, Cheryll Tickle, Lewis Wolpert, Julia Hunt. B. The

4-handed Dennis Summerbell from 1973 underpinning the sense of fun in the lab as well as hard work. C. Lewis Wolpert. D. Amata Hornbruch. E. Cartilage stain of a 10 day old wildtype chicken forelimb; Cartilage stain of a 10 day old chicken forelimb following two ZPA grafts – one at somite 16 and one at somite 18 resulting in 3 sets of digits (performed by Amata Hornbruch; Wolpert and Hornbruch, 1981); F. Nigel Holder; G. Julian Lewis; H. Jim Smith; I. Cheryll Tickle and Dennis Summerbell. Credit and thanks to Amata Hornbruch for providing the images used.

Panel A: Reproduced with permission from Tickle, C. Int J Dev Biol. 2002; 46: 863-867.

Figure 5 – Development of the vertebrate limb using Positional Information
The limb grows out from the flank under the control of the apical ectodermal ridge and the zone of polarising activity. The main cartilage structures of the limb are laid down in a proximal to distal sequence as demonstrated by Saunders (1948). Wolpert was impressed by experiments where pieces of mesenchyme from the early limb bud were removed, and found the limb reformed nearly normally (see Wolpert, 1969). This indicated to Wolpert that early limb bud cells have positional values and their behaviour depends on their position. His lab proposed the Progress Zone model in 1973 to explain how positional value and skeletal element differences come about (Summerbell et al., 1973). They proposed that the longer the cells remain in the 'Progress Zone' directly beneath the apical ridge the more distal the resulting structure would be. As cells leave the 'Progress Zone' their identity is the determined. Thus, if cells measure the time they spend in the 'Progress Zone' this could specify their position along the proximo-distal axis. Red labelled cells fall out the zone early and as the limb continues to grow become proximal. Whereas green cells remain in the zone throughout development and become distal. Other models to explain proximo-distal limb outgrowth using molecular signals have also been proposed and which have been influenced by the concept of Positional Information, for example, the 'progenitor model' (Dudley et al., 2002), the 'Two-signal model' (Zeller et al., 2009) and the 'Signal-Progress Zone model' (Saiz-Lopez et al., 2015) (see also Wolpert et al., 2019, chapter 10).

Figure modified and reproduced with permission from Wolpert et al., 2019 'Principles of Development, 6th Edition'. pp461. Oxford, UK. Oxford University Press.

Figure 6 – Leaving the Middlesex Hospital and Windeyer Building, 1996
Before Wolpert's retirement the Middlesex Hospital became part of University College London (UCL) and as a result the Wolpert lab eventually left the Windeyer Building in 1996 and moved to the Medawar Building on the UCL main campus – many former students and postdocs and staff came to a leaving lab party in June 1996. A. Lewis Wolpert and Cheryll Tickle; B. Dennis Summerbell and Cheryll Tickle; C. Nigel Holder, Geoff Shellswell and John McLachlan; D. Neil Vargesson and Lewis Wolpert (Jonathan Slack on the far left; Michael K Richardson in the doorway); E. Lewis opening a bottle of wine; F. Maureen Maloney who was Lewis's personal assistant from 1966 until he retired.

Figure 7 – The 1996 British Society of Developmental Biology Meeting in Bath, UK.
The British Society of Developmental Biology (BSDB) dedicated its Autumn 1996 Meeting to Lewis Wolpert to celebrate 30 years of Positional Information. A. Meeting Poster (kindly

obtained and supplied by Jonathan Slack); B. Official 1996 Bath Meeting T-shirt owned by and modelled by Jonathan Slack. Together with many fantastic talks held in honour of Lewis, including talks from former lab staff as well as collaborators and colleagues from around the world, was a Cabaret based on his contributions (and quotes) to the field of developmental biology; C. Cheryll Tickle hosting the Cabaret as the fortune teller 'Morphogen (Mystic) Meg'. D. Cliff Tabin (wearing a specially made hedgehog costume) and Denis Duboule (with electric bow tie); E. Joy Richman, Julian Lewis, Brigid Hogan, Jonathan Bard, Vernon French performing in the Cabaret; F. Quote from the Editor (Vernon French) of the 1997 BSDB Spring Newsletter reviewing the 1996 BSDB Autumn Meeting. Credit and thanks to Jonathan Slack for images A and B.

Figure 8 – Subjects of papers that have cited Wolperts 1969 *Journal of Theoretical Biology* paper
Many different and varied subjects/topics have cited the 1969 paper – not unexpectedly developmental biology is the biggest source of citations but subjects such as mathematics, plant sciences, evolutionary biology, physics and engineering have consistently cited the paper. Source: Web of Science.

Figure 9 - Year by Year citation record for Wolperts 1969 *Journal of Theoretical Biology* paper
The 1969 paper has been cited over 2000 times and what is very interesting is it is cited as perhaps more today than when it first came out in 1969. Source: Web of Science.

Table 1 – Linear size of positional fields in terms of cell numbers
Wolpert calculated that Positional Information only needed to move across 50 cell diameters (which he called a positional field) in order to establish positional value to pattern embryonic tissues and could be complete in 10 hours. Remarkably 50 cell diameters is the approximate size of many early forming embryonic tissues in vertebrates and invertebrates.

Reproduced with permission from Wolpert L. *J Theor Biol*. 1969; 25:1-47.

Table 2 - List of key personnel in Wolpert Lab
Over Lewis Wolpert's career he supervised over 30 PhD students (including Dennis Summerbell, Nigel Holder and Jim Smith) with his last students as a full-time supervisor being Adrian Hardy (who graduated in 1996), Han Sung Jung (1997) and Neil Vargesson (1998). Wolpert also supervised many postdoctoral fellows (who included Julian Lewis, Jonathan Slack, Cheryll Tickle and Anne Warner). The Wolpert lab in the Windeyer Institute was a vibrant, exciting, collaborative and focused environment for research, learning, and questioning. The lab was also an inspirational place to be, encouraging scientific critique, independent thinking balanced with a sense of fun and enjoyment (as well as enjoying the aroma of methyl salicylate) (Figure 4). Indeed, Lewis encouraged students and postdocs to come up with their own ideas and do the experiments and to publish them sometimes independently of him and with his support! Many of the former students and postdocs from the Wolpert Lab went on to their own very successful research careers and many are world leaders in their respective fields and have further created a legacy as new generations of

PhD students and postdocs appreciate and work to determine the molecular basis of Positional Information.
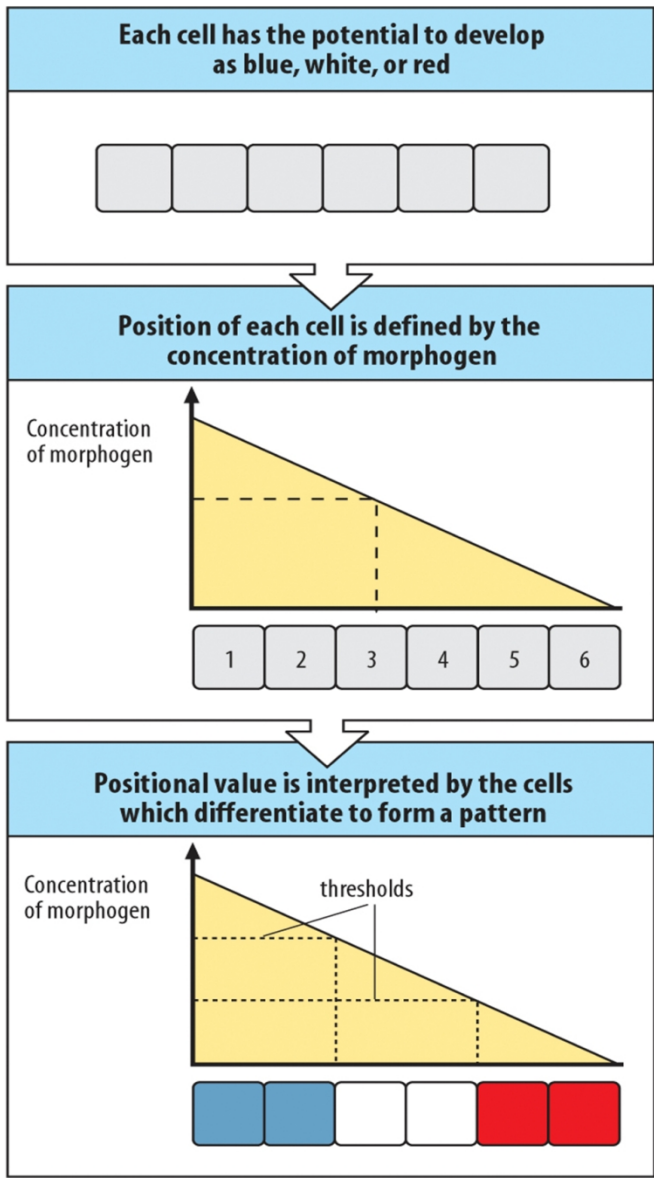
Figure 1

Figure 2

antennal segments

antenna

arista

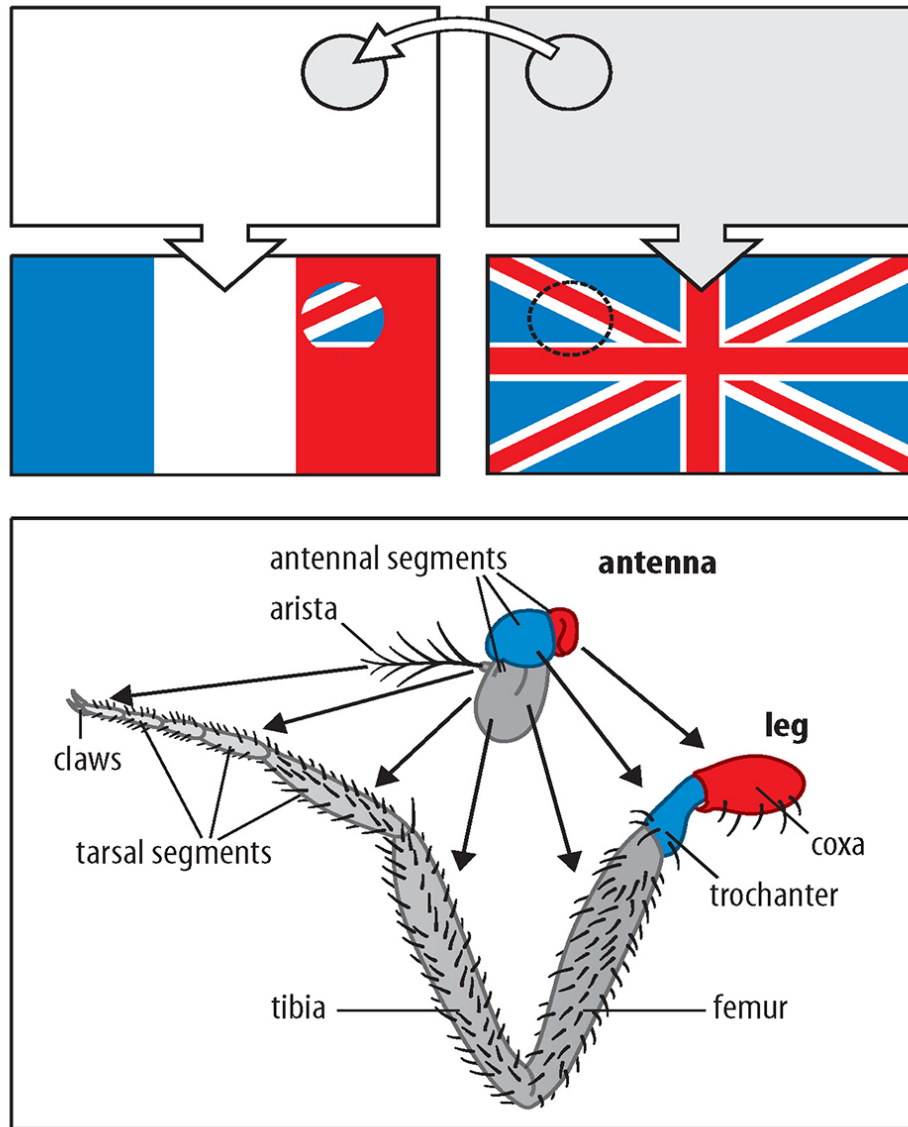claws

leg

tarsal segments

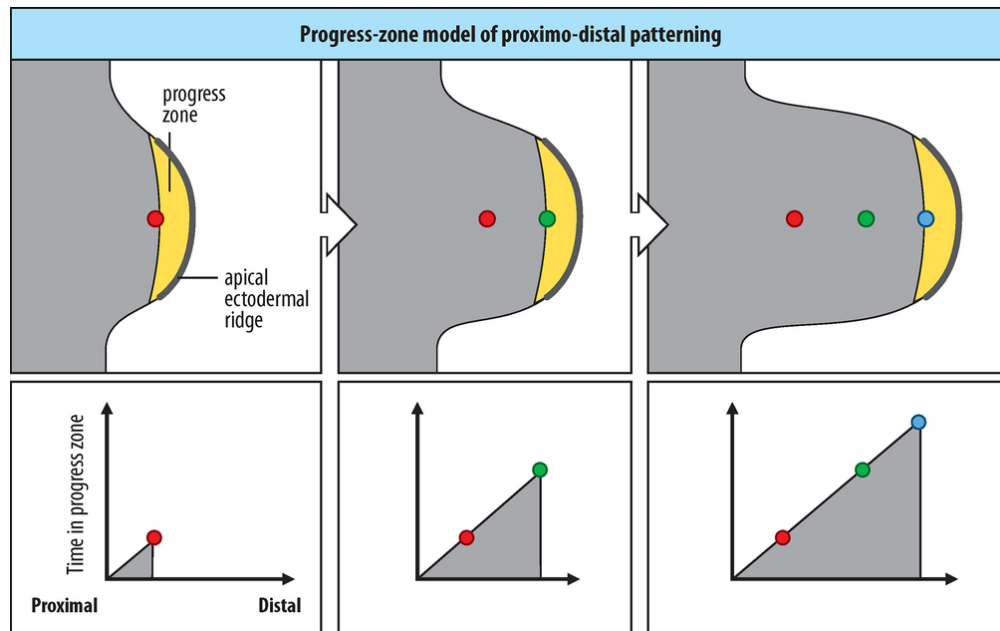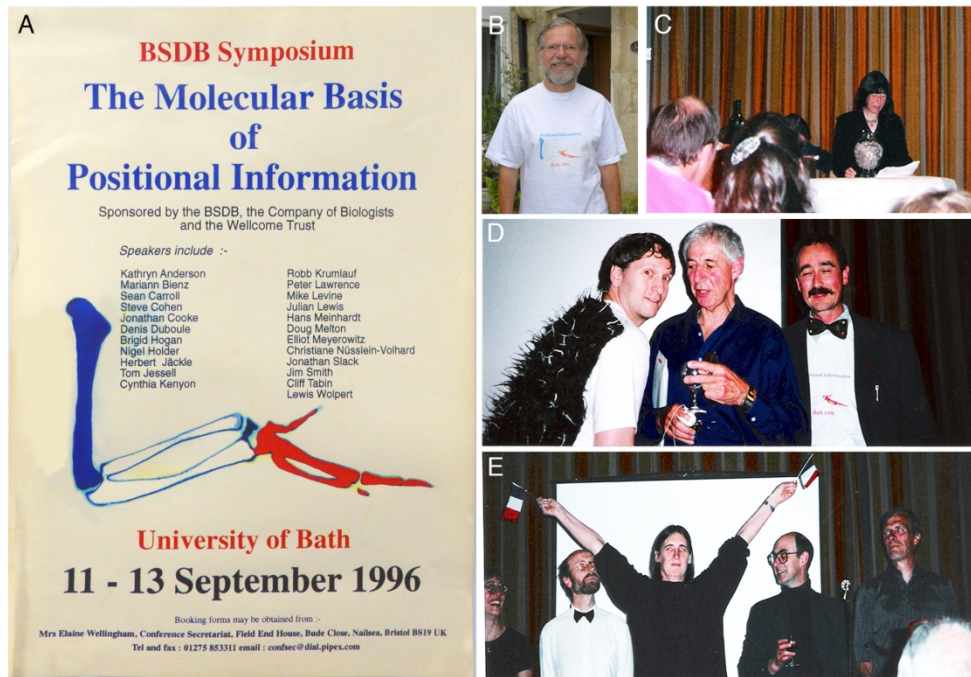coxa

trochanter

tibia

femur
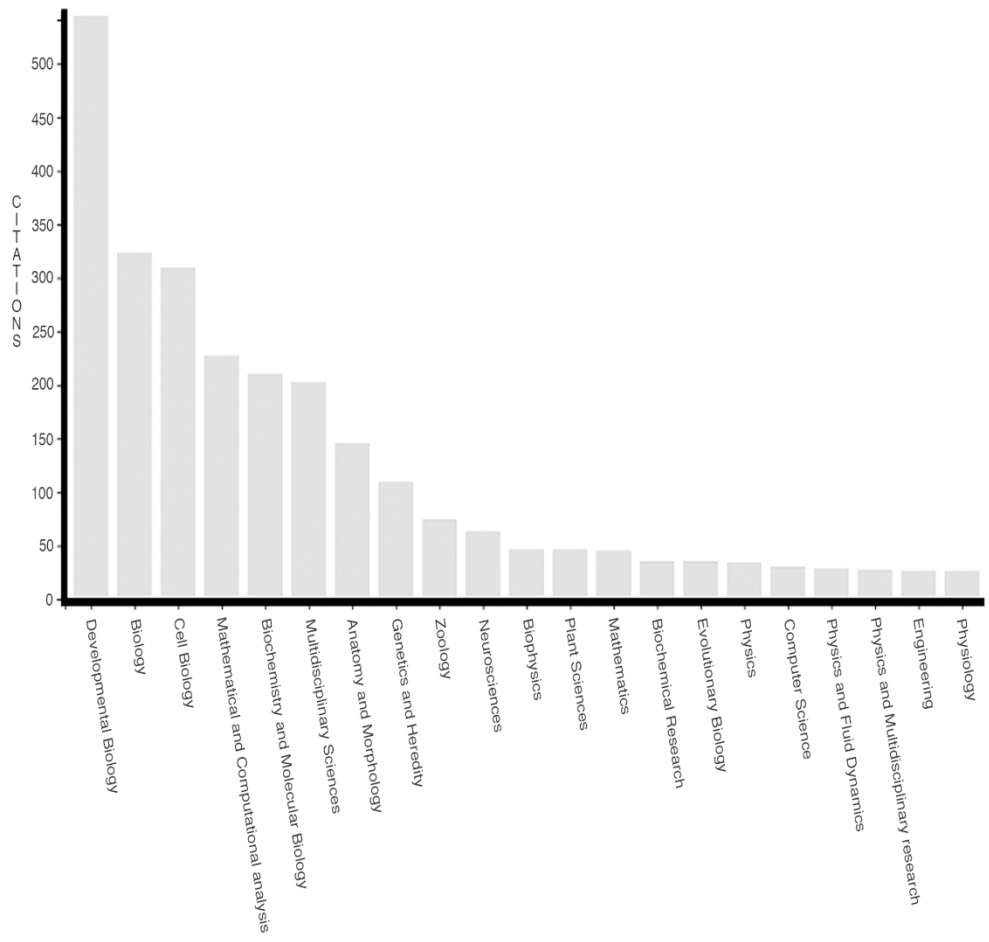
Figure 3

Figure 4

Figure 5

85x53mm (300 x 300 DPI)

Figure 6

F (Ed. Many of my generation - undergraduates in the late 1960s/early 1970s - perhaps particularly appreciate Lewis. We are 'in' Development directly because of his ideas of Positional Information and his enthusiasm, and we have enjoyed the insight and the arguments over the years. As he would say (and did say in Bath), "terrific - how right I was!")

Figure 7

Figure 8

Figure 9