



HAL
open science

Positional information and patterning revisited

Lewis Wolpert

► **To cite this version:**

Lewis Wolpert. Positional information and patterning revisited. *Journal of Theoretical Biology*, 2010, 269 (1), pp.359. 10.1016/j.jtbi.2010.10.034 . hal-00653684

HAL Id: hal-00653684

<https://hal.science/hal-00653684>

Submitted on 20 Dec 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Author's Accepted Manuscript

Positional information and patterning revisited

Lewis Wolpert

PII: S0022-5193(10)00579-5
DOI: doi:10.1016/j.jtbi.2010.10.034
Reference: YJTBI6218

To appear in: *Journal of Theoretical Biology*

Received date: 13 October 2010

Accepted date: 26 October 2010



www.elsevier.com/locate/jtbi

Cite this article as: Lewis Wolpert, Positional information and patterning revisited, *Journal of Theoretical Biology*, doi:[10.1016/j.jtbi.2010.10.034](https://doi.org/10.1016/j.jtbi.2010.10.034)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting galley proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Positional Information and Patterning Revisited
 Lewis Wolpert, Cell and Developmental Biology, University College, London

It is 42 years since I published my article on positional information in this journal. In this 50th anniversary year it is pleasure to review the field. Positional information is a concept that relates to pattern formation, that is the development of spatial organisation in the embryo that results from cells differentiating at specific positions (Wolpert, 1969). The concept of positional information proposes that cells acquire positional identities as in a coordinate system, and then interpret their positions to give rise to spatial patterns. It had its origin over a hundred years ago when Hans Driesch was working on sea urchin development. He found that if he separated the cells of the embryo at the two cell stage each developed into a small but normal larva. This implies that patterning involves a mechanism for scaling – they are like a flag whose pattern is size invariant. He also claimed – mistakenly – that if any region of the early embryo were removed the embryo still developed normally. He therefore concluded that there was a co-ordinate system that specified the position of each cell so that it could develop in the correct manner. He also concluded that this was impossible and gave the coordinate system the mystical name *Entelechia*. Again he was wrong, as there has been progress in understanding how position in the embryo is specified, but major problems remain.

Positional information implies that cells have a graded set of values that varies continuously along an axis, and under the influence of the local positional information a cell obtains a positional value which they interpret their position by developing in particular ways. One of the main mechanisms for setting up positional information is based on gradients of morphogens which are diffusible molecules produced by the cells, whose concentration specifies position. This was first proposed by Crick (1970) because the lengths of the proposed gradients – such as those in the vertebrate limb and insect imaginal discs – are quite small, around 50 cells long (Wolpert, 1969). Early evidence for positional information came from studies on chick limb development with models for positional specification along both the proximo-distal (Summerbell, et.al. 1973) and antero-posterior axes. (Tickle, et.al 1975). There was also evidence for positional gradients in hydra (Wolpert, et.al. 1971) and in insect limbs (French et.al. 1976)

The Bicoid gradient in the early *Drosophila* embryo is the best understood system, but in this case a gradient is set up in a single cell and not in a multicellular system and so is basically different from gradients in multicellular embryos where the gradient is across cells. A special feature is that the protein gradient is present in the cytoplasm of a single cell and so it does not have to interact with cells or their membranes, and it enters directly into nuclei. Models of this gradient involving production, diffusion and degradation have been analysed, but none can account for all the observed characteristics of the gradient (Grimm et.al. 2010). It is suggested that better knowledge of Bicoid lifetime could help. The diffusion constant is similar to that of signal molecules Decapentaplegic and Wingless. Given the problems with this intracellular gradient one should not be surprised that the gradients that move through extracellular space raise many more problems (Gurdon, 2001).

The systems where it is thought positional information plays key role are the main body axes of embryos and appendages such as legs and wings. Some of the best evidence for positional information comes from regeneration experiments, and the patterning of the leg and antenna in *Drosophila* and the vertebrate limb.

Regeneration of amphibian limbs provides at present the best evidence for cells having positional values, and regeneration requires the specification of new positional values. There is good evidence for a continuous set of positional values along the limb from intercalation experiments (Pescitelli and Stocum 1981). Of major significance, in the newt limb a membrane molecule - Prod1 - is graded from one end of the limb to the other, the proximo-distal axis, and can be considered to be the molecular basis of positional information in the limb (Kumar et.al. 2007). Retinoic acid can proximalise the positional values along the limb, so that a whole limb will regenerate from a cut at the distal end; the treatment increases the concentration of Prod 1. This is excellent evidence for graded positional values.

Further evidence that cells have positional values comes from regeneration experiments that show intercalation of missing regions of invertebrate limbs (French et.al. 1976). There seems to be a set of positional values along the tibia of the cockroach leg since if a portion of the tibia is removed, the missing region will be replaced by intercalary regeneration. More striking, when a proximal cut tibia is grafted on to a more distal site making the tibia longer, intercalation makes the tibia even longer by intercalating the missing positional values. Another clear example of intercalation is from the salamander limb when a distal regeneration blastema is grafted in place of a proximal one. In insect limb intercalation the regenerated region comes mainly from the distal partner, whereas in salamanders it is the proximal partner that gives rise to the intercalated region. Intercalation also

occurs when ventral regions of the *Drosophila* leg imaginal disc are removed, as circumferential intercalation occurs..

Regeneration of hydra provides evidence for positional gradients. In hydra the head organizer continuously produces the two signals that are transmitted to the body column, each of which is distributed in graded manner down the body column (Bode, 2009; Meinhardt, 2009). The head activation gradient determines how the cells behave along the body column, essentially providing positional information. It also provides the capacity to initiate head organizer formation. The head inhibition gradient prevents the head activation gradient from carrying out this latter function along most of the body column. By grafting a head to the basal end of a hydra and then determining how long it took for the inhibitor to prevent head regeneration when the host head was removed, together with computer simulation, suggested a diffusion coefficient of $2 \times 10^{-7} \text{ cm}^2/\text{sec}$ (Wolpert et al. 1972). This is remarkably similar to Crick's (1970) initial conjecture for setting up gradients. A somewhat different model suggests that positional information scheme may be realized by a set of hierarchically coupled pattern forming systems - a structure generates the precondition for a second structure but excludes this structure locally (Meinhardt, 1993). In this model, head, tentacle, and foot formation are under the control of separate activator-inhibitor systems. These systems are coupled via the source density. The head activator increases the source density and the activation takes place preferentially in regions of highest source density. The foot activation has the opposite behavior. Therefore, head and foot activation appear preferentially at opposite positions of the field. Since no direct inhibition between head and foot is involved, both structures can appear close to each other in experimental situations. Budding is regarded as a trigger of a second head activator maximum. Budding can occur only beyond a minimum distance from the head due to the head inhibitor and beyond a minimum distance from the foot since the source density would be too low there.

An important example providing evidence for positional information comes from the antenna and leg of *Drosophila*. If the Hox gene *Antennapedia* which is normally expressed in parasegments 4 and 5 of the *Drosophila* embryo, is expressed in the head region the antenna develops as a leg. Clones of *Antennapedia* cells in the antenna disc develop as leg cells, and the type of leg cells that develop depends on their position along the proximo-distal axis; if, for example they are at the tip they will develop as a claw. This strongly suggests that the positional values along the leg and antenna are the same, but because of the Hox genes the cells interpret their positional values differently. The downstream action of the Hox genes that control this process is not understood. The molecular basis of the positional values is also not known in this or any other system.

Setting up gradients

The central problems are how positional information is set up, how it is recorded, and then how it is interpreted by the cells. One simple example is where there is at one end a source of the morphogen, and at the other end a sink, both with fixed concentrations. This will result in a linear gradient being formed. If there is just a localized source and breakdown of the morphogen the diffusion will give rise to a gradient with an exponential form. Meinhardt (2009) has shown that primary pattern formation is possible if, and only if, a locally restricted self-enhancing reaction is coupled with an antagonistic reaction that acts on a longer range. A prototype of such a pattern-forming reaction consists of a short-ranging substance the activator, which promotes—directly or indirectly—its own production, and also regulates the synthesis of its rapidly diffusing antagonist, the inhibitor. But most models for setting up gradients do not consider this requirement.

Eldar et al. (2003) described how having a morphogen that stimulates its own degradation can provide a powerful way to build gradients that are highly robust to variation in the level of morphogen production at a source. They claim that exponential profiles cannot, at the same time, buffer fluctuations in morphogen production rate and define long-range gradients. To comply with both requirements, morphogens should decay rapidly close to their source but at a significantly slower rate over most of the field. Morphogens could enhance their own degradation, leading to a higher degradation rate close to their source through reciprocal interactions between the morphogen and its receptor. To generate steady-state gradients, morphogens must be degraded (Wartlick et al. 2009). For example Hedgehog (Hh) up-regulates its receptor Patched (Ptc) which in turn restricts the Hedgehog signalling range most likely by causing the internalization and degradation in regions of high Hh concentration. Thus, Hh enhances its own degradation.

A key feature of the signalling response in target cells is the control of receptor expression, which in turn alters the morphogen profile (Jaeger et al. 2008). In the case of Hh, signalling activity upregulates the expression of its receptor Patched (Ptc), which antagonises signal transduction by inhibiting the co-receptor Smoothed (Smo) and which restricts the movement of extracellular Hh

ligand across the tissue by sequestering it. Furthermore, the upregulation of Ptc changes the ratio of bound to unbound Ptc receptor, which alters morphogen read-out as bound Hh-Ptc complexes can titrate the repressive effect of unbound Ptc receptor. This feedback increases the amount of Hh that is bound, internalised and degraded resulting in a net sharpening and steepening of the gradient. In addition, this feedback is predicted to enhance robustness against fluctuations in Hh production, as an increase in morphogen production is counteracted by an increase in Ptc. Morphogens also can interact with one or more type of coreceptor, loosely defined as a cell surface morphogen binding protein that boosts the formation of morphogen-receptor complexes and/or enhances their signalling (Lander, 2007). In Hedgehog gradients there can be positive feedback, conversion of transcriptional inhibition to activation, bistability, and upregulation of receptor synthesis.

Morphogens diffusing in the extracellular space can be influenced by a variety of molecules such as HSPGs (heparan sulfate proteoglycan) which it is claimed can directly influence morphogen gradient formation at various levels, including morphogen movement, signalling, and trafficking (Yan and Lin, 2009). HSPGs can also interact with other molecules such as lipoprotein, which is required for morphogen movement and distribution. Lei and Song (2010) have established a mathematical model for morphogen gradient formation

In which the morphogens are synthesized in a local region, and they form a long distance gradient by binding to the chains of heparan sulfate proteoglycans and moving to target cells through the random walk of these chains.

Since normal patterning of several early embryos occurs over quite different sizes as in the Driesch experiment, there is a scaling effect which most models of morphogens do not take into account. There are several proposed mechanisms such as two diffusible molecules that emanate from opposing poles and define the activation profile through their ratio. A model based on expansion-repression by Ben-Zvi and Barkai (2010) suggests that scaling emerges as a natural consequence of a feedback topology. Patterning is defined by a single morphogen, whose profile is shaped by a diffusible molecule, the “expander”. The expander functions, directly or indirectly, to facilitate the spread of the morphogen by enhancing its diffusion or protecting it from degradation. In turn, expander production is repressed by the morphogen. Mathematical analysis shows that scaling is achieved provided that the expander

is stable and diffusible. Another model by Ben-Zvi et al. (2008) provides a quantitative explanation for the capacity of the *Xenopus* embryo to scale pattern with size. Three key features of the Bone Morphogenetic Protein (BMP) patterning network underlie their mechanism. First, patterning is governed by a shuttling-based mechanism, where the BMP ligands are effectively transported by a common BMP inhibitor (Chordin) to the ventral-most part of the embryo, establishing a sharp, power-law decaying activation profile. Second, the presence of two BMP ligands, which differ in their affinity to the inhibitor Chordin, allows for a range of possible steady-state profiles, depending on the relative abundance of the two ligands. Thirdly, the negative auto-repression of the BMP ligand, Admp, is used for sensing embryo size, and effectively tunes the pattern with size. These three features are claimed to lead to a robust and sharp gradient that is properly scaled with embryo size.

Morphogen profiles can be optimized to buffer against noise. Saunders and Howard's (2009) analysis suggests that if external noise dominates, algebraic profiles will be favored; if internal and external fluctuations are of a similar magnitude then exponential profiles will be preferred, and if internal noise is the dominant source of error they expect close to linear morphogen profiles.

There is evidence that simple diffusion in the extracellular space may not be the only mechanism for setting up a gradient. In the developing fly wing morphogens such as Dpp (Decapentaplegic) and Wg (Wingless) are believed to form gradients of concentration providing positional information. Dynamin-dependent endocytosis was required for spreading of Dpp, but not Wg. The cellular mechanisms of spreading are thus different and the spreading of Dpp requires endocytic, intracellular trafficking (Kicheva, A. et al. 2007). Dpp morphogen gradient formation and function require interaction of Pent, a secreted Dpp antagonist, with glypicans (Vuilleumier et al. 2010). Glypicans bind and enrich extracellular Dpp at the cell surface of the wing disc epithelium, and glypican-bound ligand is then passed either to receptors for endocytosis and signalling or to glypicans of neighboring cells for further lateral movement, facilitated transport activity. Pent is critically involved in keeping these two activities in balance.

The study of morphogen gradients has focused on secreted molecules, partially because these molecules can move extracellularly over large distances. However, molecular gradients along a cellular tissue can arise without requiring any dynamics in the extracellular space (Ibañes and Belmonte, 2008). On the one hand, transport from cell to cell through gap junctions can occur and cells could be the transport vehicle of the molecule. These kinds of transport might enable the formation of gradients of non-secreted molecules. Molecular gradients can also be formed by the

dilution of the molecular content on cells that continuously divide and become displaced away from a source

A quite different mechanism for specifying position is based on time. The progress zone model for proximo-distal specification of position in the developing limb in the suggests that cells in the progress zone under the apical ridge are dividing and measure how long they remain there – the more proximal cells spend the least time (Summerbell et.al. 1973). This model is controversial (Towers and Tickle,2009). Another important example is specification of position along the antero-posterior axis of the mesoderm in vertebrates. There are oscillations related to somite formation, and the cells could measure the time at which the somites are formed (Gaunt,2000; Aulehla and Pourquié. 2010) and this could define their position and activate Hox genes.

Evidence for gradients.

In addition to the systems referred to at the beginning, there are a number of others which provide important evidence for positional information in development that include the antero-posterior pattern of the vertebrate limb, the dorso-ventral patterning of the vertebrate neural tube, and the axes in *Xenopus*.

The antero-posterior pattern of the chick wing is best seen as the three digits, called 2,3, and 4 as they are different and of which 4 is the most posterior digit. More posterior to digit 4 is a region called the polarizing region which can pattern the digits. If another polarizing region is grafted to the anterior of the early limb bud the pattern of digits is then 432234. Evidence that the signal is graded is shown by grafting just a small fragment of a polarising region anteriorly, as then the pattern is 43223, and an even smaller piece will result in just another digit 2 developing. This suggests that a high level of the signal specifies digit 4, while lower levels specify digit 3 and the yet lower level digit 2. The polarizing region produces Sonic Hedgehog (Shh) and different doses of it give similar results. Also removing the grafted Shh signal give the expected result – short periods of signalling induce just digit 2 while longer periods give 432234. Just how Shh acts remains unknown (Tickle,2006; Towers and Tickle,2009) but the evidence for a graded positional signal is strong. Meinhardt (1983) has postulated that positional information for secondary embryonic fields, like limbs, is generated by a cooperative interaction between two pairs of differently determined cell types. Positional information is thus generated at the boundaries between cells of different determination. The latter are assumed to result from the primary pattern formation in the embryo.

There is good evidence that sonic Hedgehog secreted by the notochord provides a graded signal for the specification of the cells in the ventral neural tube (Dessaud et.al 2010). The model is more about the interpretation of the gradient and is described below. While the regulation of body axis specification in the common ancestor of bilaterians remains controversial, BMP signalling appears to be an ancient program for patterning the secondary, or dorso-ventral, body axis, but any such program for the primary, or antero-posterior, body axis is debated (Niehrs,2010). Posterior Wnt/beta-catenin signalling could be such a mechanism and it evolutionarily predates the cnidarian-bilaterian split. A Cartesian coordinate system of positional information may thus be set up by gradients of perpendicular Wnt and BMP signalling and is conserved in bilaterians, and orchestrates body axis patterning and contributes to both the relative invariance and diversity of body forms. In vertebrates, the BMP and Wnt gradients are both dependent on yet another signalling gradient, namely that of Nodal.

There is evidence for gradients in the early *Xenopus* embryo and Smith et al. (2008) have concluded that morphogens in the *Xenopus* embryos traverse responding tissue through the extracellular space, and not by transcytosis or via cytonemes. BMP4 is predominately ventrally expressed in the gastrula and neurula and acts as a morphogen, patterning the dorso-ventral (DV) mesoderm and neuroectoderm in a concentration-dependent manner (Niehrs,2010). The Spemann organizer and its derivatives secrete BMP antagonists, such as Chordin, Noggin and Follistatin, which are expressed in the dorsal midline. A BMP4 activity gradient is established through the diffusion of BMP antagonists and the action of the Chordin protease Tollid, which regulate gradient polarity and shape by establishing and maintaining a dorsal BMP sink. Perpendicular to the *Xenopus* DV BMP gradient is an Wnt/ β -catenin gradient which is thought to pattern the embryo along its antero-posterior (A-P) axis. Multiple Wnts are expressed in the gastrulating embryo and probably cooperate in establishing the gradient. The Wnt signalling gradient is high posteriorly and low anteriorly, owing to the anterior expression of Wnt antagonists.

Evers et.al. (2008) proposed that positional information provided by D–V and A–P growth factor gradients in the developing amphibian embryo is integrated at the level of Smad1/5/8. Chordin, a secreted BMP antagonist, is expressed in the dorsal region of the vertebrate embryo during the

gastrula stage, while on the opposite side at the ventral pole BMPs are expressed at high levels. Wnt signals are strong in the posterior region and weaken anteriorly. Cells at different positions within these two Cartesian axes may read these morphogen gradients and determine the embryonic body plan that specifies the place at which the various organs will be subsequently formed. An elaborate system of extracellular protein–protein interactions has been suggested to regulate these gradients. Kiecker and Niehrs (2001) found that dose-dependent Wnt signalling is both necessary and sufficient for AP patterning of the neuraxis and Wnt/ β -catenin signalling occurs in a direct and long-range fashion within the ectoderm.

Plouhinec and De Robertis (2009) consider the short time frame for the establishment of a D-V gradient in a 1.2 mm *Xenopus* embryonic field of about 10,000 cells, less than 4 h at 22°C from blastula to mid-gastrula, and ask whether the BMP gradient reached a steady state or is the gradient interpreted still in the transient phase? This could affect which molecular process is important in the formation of the gradient. The Chordin–BMP biochemical pathway is self-adjusting due to a combination of intracellular transcriptional regulation and extracellular biochemical events that drive the flow of BMPs. It determines dorsal-ventral positional information (Zakin and De Robertis, 2010). High concentrations of activin, a member, like BMP, of the TGF-beta superfamily, induced the formation of notochord and muscle in *Xenopus*, whereas lower levels resulted in the formation of ventral and lateral cell types such as mesenchyme and mesothelium (Smith et.al.1991, Smith 2009). Increasing time of exposure to a single concentration of activin had a similar dose-dependent effect, with more dorsal structures forming with increased duration.). Many important unanswered questions concerning the *Xenopus* gastrula patterning gradients remain, particularly their quantitative nature. Of particular importance is the timing of the proposed gradients as they may not be active at the same time and some may be active at different times.

Quantitative analysis of gradients is important but difficult, but we do need to know how the concentration varies along the axis. There are as yet preliminary data but no detailed quantitative studies. Members of the nodal family are thought to form a morphogen gradient in the developing zebrafish embryo and to be essential for pattern formation. Mesoderm and endoderm are believed to develop due to high levels of nodal signalling, while cells experiencing the lowest concentrations of nodal signalling become ectoderm. Harvey and Smith(2009) used two different approaches to visualise the intensity of nodal signalling within individual cells. Nodal causes phosphorylation Smad2 and it interacts with Smad4 and together they enter the nucleus. They used bimolecular fluorescence complementation to visualise the formation of a complex between Smad2 and Smad4. They found that the cells at the margin experience the highest levels of nodal signalling and cells positioned away from the margin and towards the animal pole experiencing lower levels.

It has been argued that morphogen gradients in living zebrafish embryos are established and maintained by two essential factors: fast, free diffusion of single molecules away from the source through extracellular space, and a sink function of the receiving cells, regulated by receptor-mediated endocytosis Yu et al. (2009). Evidence was provided by examining single molecules of Fgf8 in living tissue by fluorescence correlation spectroscopy. Their results support a simple mechanism, involving a localized source, Brownian diffusion through the extracellular space and a sink in the target tissue generated by receptor-mediated endocytosis, to form and maintain a morphogen gradient.

Two studies used quantitative imaging to measure the distribution of the dorsal protein along the dorso-ventral axis of the early *Drosophila* embryo (Bothma et.al. 2010). One claimed that the region of graded nuclear Dorsal in the ventral region does not extend to the dorsal half of the embryo, and that the expression boundaries of key genes in this region are not determined by Dorsal, whereas another reported a smoothly changing nuclear concentration gradient that spans nearly the entire circumference of the embryo.

The Hedgehog signalling pathway has been highly conserved during evolution, and plays a central role in the patterning of a range of tissues in both vertebrates and invertebrates. Many of the proteins responsible for regulating Hedgehog signalling and transport are themselves targets of Hedgehog signalling, leading to multiple levels of feedback within the system. According to Irons et al. (2010) regulation of Hedgehog transport and stability by glypicans, as well as multiple overlapping feedbacks in the Hedgehog response network, can combine to enhance the robustness of positional specification against variability in Hedgehog levels. A feature of this process is the precision and robustness of the resulting patterns of cell fate in the face of fluctuations in protein levels and natural variability in the size and genetics of individuals (Jaeger et.al. 2008). An additional group of factors, which both regulate and are regulated by Hedgehog signalling, are Heparan sulfate proteoglycans (HSPGs), such as Dally and Dally-like (Dlp). Dally and Dally-like are both up-regulated in response to Hedgehog signaling in the insect imaginal wing disc, leading to a stripe of high expression in anterior

cells near the anterior–posterior border. This stripe overlaps with the Hedgehog signalling region and both proteins have been shown to influence the signalling pathway, in response to both high and low levels of Hedgehog. They suggest that regulative feedback and transport could play a central role in ensuring robustness and size regulation in morphogen responses, in a range of developmental processes. In both *Drosophila* and *Xenopus* embryos, a combination of experimental and theoretical work has shown that the Bone Morphogenetic Protein (BMP) gradient—which patterns the dorso-ventral body axis—is robust against changes in the levels of important signalling factors and scales according to embryo size (Ben-Zvi et al., 2008). The models predict that this striking robustness depends on a combination of facilitated BMP transport and local regulation of BMPs in response to signalling. In the model of Irons et al. (2010) Hh diffuses in two forms, one normal, the other bound to HSPG. These results confirm the robustness of Hedgehog signalling to changes in the Hh production rate during *Drosophila* wing development. Their results predict that the regulation of Hedgehog transport and stability by glypicans, as well as multiple overlapping feedbacks in the Hedgehog response network, can combine to enhance the robustness of positional specification against variability in Hedgehog levels. However their model does not deal with real diffusion in the intercellular space, or where the glypicans are.

In the *Drosophila* wing imaginal disc Decapentaplegic (Dpp) functions as a long-range morphogen to control patterning and growth. Dpp protein is secreted at the antero-posterior compartment boundary and is probably the positional signal for patterning both the anterior and posterior compartments along the antero-posterior axis. Dpp appears to form a concentration gradient across the wing disc that provides a long-range signal controlling the localized expression of the genes for the transcription factors Spalt, Omb and Brinker in the wing disc. There is a connection between setting up this and how cells respond to it (Vuilleumier et al. 2010). Both processes are linked by pentagone (pent), a transcriptional target of BMP signalling encoding a secreted negative regulator of the pathway. Absence of pent in the wing disc causes a severe contraction of the BMP activity gradient resulting in patterning and growth defects. Pent interacts with the glypican Dally to control Dpp distribution and provide evidence that proper establishment of the BMP morphogen gradient requires the inbuilt feedback loop embodied by Pent. Further complexity is provided by Dally-like which is a glypican-type heparan sulfate proteoglycan containing a protein core and attached glycosaminoglycan chains. In *Drosophila* wing discs, Dally-like represses short-range Wingless signalling, but activates long-range Wingless signalling (Yan et al., 2009).

There is however, no good evidence for the quantitative aspects of any of the gradients just described, or details how they are set up. While polarized Wnt and BMP signalling in body axis formation is a conserved feature throughout animal evolution, the mechanisms by which this polarity and the gradients are set up are diverse, ranging from the sperm entry point in amphibian embryos determining the DV axis, to gravity in chick embryos determining the AP axis, and to cytoplasmic determinants in the *Drosophila* egg determining the AP and DV axes (Niehrs, 2010).

Gradients of auxin are required for tissue patterning within the embryo and the root of plants (Benkova et al. 2009). Graded auxin distribution seems to be crucial for cell specification within the root meristem, and cells seem to react flexibly to changes in the auxin level by modulation of their developmental fate. Computer studies have shown that this is a plausible mechanism for the generation of the auxin distribution patterns in the root of plants. Mironava, et al. (2010) have demonstrated that the reflected flow mechanism that relies on the presence of positive and negative regulations between auxin and expression of its carriers provides not only for self-organization of the observed auxin distribution in the root, but also can explain much of the positional information in root patterning.

Interpreting Position

The function of a gradient is to specify position and how this is recorded and appropriate genes turned on and off is a central problem. Ashe and Briscoe (2006) reviewed how morphogens act as graded positional cues that control cell fate specification in many developing tissues. The way in which a signalling gradient regulates differential gene expression in a concentration-dependent manner raises several mechanistic issues, such as how responding cells perceive and interpret the concentration-dependent information provided by a morphogen to generate precise patterns of gene expression and cell differentiation in developing tissues. The mechanisms of gene regulation by morphogen signalling must provide a means to translate small differences in signal strength into threshold responses in which all-or-none changes in gene expression allow the selection of discrete cell identities in the developing tissue.

Given a gradient in positional values how is this interpreted so that cells at specific positions behave in a special manner? The most detailed model of how gradients can be interpreted, comes from how Shh signalling from the notochord assigns the positional identities of distinct neuronal subtype progenitors throughout the ventral neural tube (Dessaud et al. 2010). Assays of intracellular signal transduction and gene expression indicate that the duration as well as level of signalling is critical for morphogen interpretation. Progenitors of the ventral neuronal subtypes are established sequentially, with progressively more ventral identities requiring correspondingly higher levels and longer periods of Shh signalling. Moreover, cells remain sensitive to changes in Shh signalling for an extended time, reverting to antecedent identities if signalling levels fall below a threshold. Thus, the duration of signalling is important not only for the assignment but also for the refinement and maintenance of positional identity. Together the data suggest a dynamic model for ventral neural tube patterning in which positional information corresponds to the time integral of Shh signalling. This suggests an alternative to conventional models of morphogen action that rely solely on the level of signalling. The dynamic nature of Shh mediated pattern formation is emphasized by the requirement for continued Shh signalling to maintain the more ventral progenitor identities even after the gene expression profiles that define these progenitor domains have been induced.

A major mechanism that has been extensively investigated exploits differences in the affinity of the transcriptional effector for binding to sites with different DNA sequences. At a minimum, to meet the definition of a morphogen, a graded signal must be able to direct the generation of at least two distinct cell types at different concentrations. Theoretical analysis has raised the possibility that graded signals can achieve up to 30 thresholds (Lewis et al., 1977); however, empirical evidence has typically identified between three and seven distinct thresholds, but many more may be involved. Positive-feedback loops in responding genes can also play a role in gradient interpretation and provide a mechanism for the generation of all or none responses at threshold levels of signalling. Permanent activation of a particular gene can be achieved by a positive nonlinear feedback combined with a competition between genes responsible for alternative pathways (Meinhardt, 2009). Details on the precision and refinement of gradient interpretation are still vague in most cases. Coupled with this is the issue of how interpretation can remain accurate when gradients are scaled to accommodate the variability in tissue sizes during development. It is possible that the regulatory circuits also provide an explanation for the hysteretic, or persistent, feature of the response of cells to a gradient (Lewis et al., 1977). Since the level of a gradient may change over time it has been proposed that there is a 'ratchet effect' (Gurdon et al., 1995) which results in cells retaining gene expression profiles characteristic of the highest concentration of signal to which they have been exposed.

Interpreting the local level of a gradient can involve thresholds. Thresholds are a central but somewhat neglected aspect of cellular processes in development. An analysis has been made of the conditions in which different thresholds can be generated in the covalent modification of a number of target proteins when the concentration of an effector is continuously increased. It is assumed that the effector, which could represent a morphogen, activates, for example, kinases that phosphorylate the proteins. Thresholds are found when the modifying enzymes are saturated by their protein substrates in conditions of zero-order ultrasensitivity (Goldbeter and Wolpert, 1990). The study of different morphogens has shown that the graded signal that is interpreted may depend on the specific morphogen gradient. Thus, the graded signal can be the steady-state amount of morphogen around a cell, which might be measured by the number of bound receptors or, alternatively, by the ratio of bound to unbound receptors (Gurdon and Bourillot, 2001).

Contrary to the view that the profile of Hedgehog determines a pattern of gene activity, Nahmad and Stathopoulos (2010) have proposed a different model. They investigated temporal effects on gradient formation and the eventual patterns of certain target genes. Their study suggests that Hh-dependent patterning in the *Drosophila* wing disc depends on temporal changes of the morphogen profile but, unlike the classical morphogen model, it does not primarily depend on concentration thresholds defined by the distribution of the gradient; instead, patterning is controlled directly by the architecture associated with the Hh gene network, particularly by the feedback that results from Hh-dependent *ptc* upregulation and Ptc-dependent ligand sequestration.

In *Drosophila*, the morphogen Wingless produced in the wing's prospective distal region activates target genes in a dose-dependent fashion to organize the proximodistal pattern. Piddini and Vincent (2009) showed that, in parallel, Wingless triggers two nonautonomous inhibitory programs. Cells flanking the source of Wingless produce a negative signal that inhibits Wingless signalling in nearby cells. Additionally in response to Wingless, all prospective wing cells produce an unidentified signal that dampens target gene expression in surrounding cells. Thus, cells influence each other's response to Wingless through at least two modes of lateral inhibition.

Saunders and Howard (2009B) suggest that some morphogen gradients may be interpreted before reaching steady-state. Theoretical analysis suggests that such pre-steady-state readout can provide reliable positioning of gene boundaries and it may even be preferable to steady-state measurement if there are large variations between embryos in the morphogen production rate. Furthermore, time-varying morphogen concentrations offer the additional advantage that they can define the expression of multiple genes at similar spatial positions but at different times. The spatiotemporal dynamics of cellular responses to morphogens can depend on the changes of the morphogen gradient itself, the dynamics of its signal transduction, the downstream interactions between target genes, or a combination of all three (Kutejova, et al. 2009). It is a challenge to determine which of these steps introduce nonlinearity and/or are rate-limiting for a response in each case, and how these mechanisms achieve the accuracy and robustness that characterizes embryonic development. This will require quantitative analysis and measurements of the morphogen concentration, signalling activity, and target gene activation in real time.

Conclusions

Lander et al. (2009) point out that after hardly a decade of intensive study, we find ourselves awash in a sea of diverse and intriguing mechanisms for conferring one or another type of robustness on morphogen-mediated patterning. Mechanisms that operate at the level of gradient formation include self-enhanced morphogen degradation, serial transcytosis, presteady state patterning, and competition between morphogens for binding to inhibitors. Mechanisms that operate at the level of morphogen detection and interpretation include morphogenetic apoptosis, cell rearrangement, integration of signals from multiple morphogens, and various types of local cell-to-cell signalling.

There is still very limited information on how gradients are actually set up and particularly quantitative measurement of the gradient. While there are numerous papers analysing gradient formation and mathematically modeling, there are major problems with all of them. Firstly, they all have the morphogen diffusing in the extracellular space and while it is recognised that external factors can influence the gradient there is no attempt to deal with the variability of these external factors and the complex pathways between cells. Secondly there is not a single case in embryos where a reliable gradient has been detected or identified with specific values of its concentration. For these reasons we have argued that extracellular diffusion is not reliable enough to specify positional information and other mechanisms must be involved (Kerszberg and Wolpert, 2007). One possibility is that diffusible mechanisms set up a crude gradient which is then transformed into a more reliable form. Another is based on cell to cell interactions. Lawrence et al. (2008) have proposed a model for establishing planar cell polarity in the abdominal epidermis of *Drosophila* based on cell-cell interactions. One protein, Frizzled is graded and while it is not yet known how the direction of polarity is set across a tissue, but it is now clear that key interactions that determine and maintain the polarity of individual cells take place at the epidermal cell junctions. An asymmetry between the two sides of a junction is established by interactions between proteins in the adjacent cell membranes. The model of Le Garrec et al. (2006) posits that a weak Frizzled activity gradient is read by asymmetric molecular complexes built at cell interfaces around the cadherin Flamingo. It is probable that these last two mechanisms have the potential, if suitably extended, to provide positional information. It still remains to be known just what roles positional information may play in the development of the embryo. The whole field has some way to go.

References

- Ashe, H.L., Briscoe, J, 2006. The interpretation of morphogen gradients. *Development*. 133:385-94.
- Aulehla, A., Pourquié, O., 2010. Signalling Gradients during Paraxial Mesoderm Development. *Cold Spring Harb. Perspect. Biol.* 2 a000869
- Barkai, N., Shilo, B-Z., 2009. Robust Generation and Decoding of Morphogen Gradients. *Cold Spring Harb Perspect Biol.*; 1(5): a001990
- Benková, E., Ivanchenko, M.g., Friml, J., Shishkova, S., Dubrovsky, J.G., 2009. A morphogenetic trigger: is there an emerging concept in plant developmental biology? *Trends Plant Sci.* 14:189-93.s
- Ben-Zvi, D., Shilo, B-Z., Fainsod, A., Barkai, N., 2008. Scaling of the BMP activation gradient in *Xenopus* embryos. *Nature* 453, 1205-1211

- Ben-Zvi D, Barkai N., 2010. Scaling of morphogen gradients by an expansion-repression integral feedback control. *Proc Natl Acad Sci U S A.* 107:6924-9.
- Bode,H.R., 2009. Axial Patterning in Hydra. *Cold Spring Harb Perspect Biol.* 1(1): a000463.
- Bothma,J.P., Levine,M., Boettiger,A., 2010. Morphogen Gradients: Limits to Signalling or Limits to Measurement? *Current Biology* 20, R232-R234
- Crick, F. H. C., 1970. Diffusion in embryogenesis, *Nature*, 225, 420
- Dessaud,D., Ribes,V., Balaskas,N., Yang, L.L., Pierani,A., Kicheva,A., Novitch,B.G., Briscoe,J., Sasai,N., 2010. Dynamic Assignment and Maintenance of Positional Identity in the Ventral Neural Tube by the Morphogen Sonic Hedgehog. *PLoS Biol.* 8: e1000382.
- Eldar,A., Rosin,D., Shilo,B-Z., Barkai,N., 2003. Self-Enhanced Ligand Degradation Underlies Robustness of Morphogen Gradients. *Developmental cell*,635-
- Evers.E., Fuentealba,L.C., and De Robertis.E.M.' 2008. Integrating positional information at the level of Smad1/5/8. *Current Opinion in Genetics & Development* 18, 304-310
- French, V., Bryant, P.J., Bryant, S.V., 1976. Pattern regulation in epimorphic fields. *Science.* 193, 969-81.
- Gaunt, S.J. 2000 Evolutionary shifts of vertebrate structures and Hox expression up and down the axial series of segments: a consideration of possible mechanisms. *Int. Jnl. Dev. Biol.* 44:109-17.
- Goldbetter, A., Wolpert, L., 1990. Covalent modification of proteins as a threshold mechanism in development. *Jnl.Theoretical Biol.*142:243-250.
- Gurdon, J. B., Mitchell, A., Mahony, D., 1995. Direct and continuous assessment by cells of their position in a morphogen gradient. *Nature* 376,520 -521.
- Gurdon,J.B., Bourillot, P.Y., 2001, Morphogen gradient interpretation. *Nature* 413: 797–803
- Harvey,S.A., SmithJ.C., 2009. Visualisation and Quantification of Morphogen Gradient Formation in the Zebrafish. *Plos Biol* 7
- Ibañes,M., Belmonte, J.C.I., 2008. Theoretical and experimental approaches to understand morphogen gradients. *Mol Syst Biol.* 2008; 4: 176.
- Irons,D.J., WojcinskiA., Glise,B., Monk.N.A., 2010. Robustness of positional specification by the Hedgehog morphogen gradient. *Developmental Biology* 342, 180-193.
- Jaeger,J., Irons,D., Monk,N., 2008. Regulative feedback in pattern formation: towards a general relativistic theory of positional information. *Development* 135, 3175-3183.
- Kerszberg,M., Wolpert,L., 2007. Specifying Positional Information in the Embryo: Looking Beyond Morphogens. *Cell* 130; 205-9.
- Kicheva,A., Pantazis,P., Bollenbach,T., Kalaidzidis,Y., Bittig,T., Jülicher,F., González-Gaitán M., 2007. Kinetics of morphogen gradient formation. *Science* 315, 521-5.
- Kiecker,C., Niehrs,C., 2001. A morphogen gradient of Wnt/ β -catenin signalling regulates anteroposterior neural patterning in *Xenopus* *Development* 128, 4189-4201
- Kumar, A., Gates, P.B., Brockes J.P., 2007. Positional identity of adult stem cells in salamander limb regeneration. *C R Biol.* 330, 485-90.

- Kutejova,E., Briscoe,J., Ki,A., 2009. Temporal dynamics of patterning by morphogen gradients. *Current Opinion in Genetics & Development*. 9,315-322
- Lander,A.D., 2007. Morpheus Unbound: Reimagining the Morphogen Gradient *Cell* 128, 245-256
- Lander,A.D., Lo,W-C., Nie,Q., Wan,F.Y.M., 2009. The Measure of Success: Constraints, Objectives, and Tradeoffs in Morphogen-mediated Patterning. *Cold Spring Harb Perspect Biol*. 1(1): a002022
- Lawrence, P.A., Struhl, G., Casal, J., 2008. Planar polarity: one or two pathways. *Nat. Rev. Genet*. 8: 555–363.
- Le Garrec, J.F., Lopez, P., Kerszberg, M., 2006, Establishment and maintenance of planar epithelial cell polarity by asymmetric cadherin bridges: a computer model. *Dev Dyn*. 235:235-46.
- Lei,J., Song,Y., 2010. Mathematical Model of the Formation of Morphogen Gradients Through Membrane-Associated Non-receptors. *Bulletin of Mathematical Biology* 72: 805–829
- Lewis, J., Slack, J. M., and Wolpert, L., 1977. Thresholds in development. *J. Theor. Biol*. 65,579 -59
- Meinhardt,H. 1983. A boundary model for pattern formation in vertebrate limbs. *J. Embryol. exp. Morph*. 76, 115-137
- Meinhardt,H. 1993 A Model for Pattern Formation of Hypostome, Tentacles, and Foot in Hydra: How to Form Structures Close to Each Other, How to Form Them at a Distance. *Developmental Biology* 157, 321-333
- Meinhardt,H., 2009. Models for the Generation and Interpretation of Gradients *Cold Spring Harb Perspect Biol*. 2009 ; 1(4): a001362.
- Mironova,V., Omelyanchuk,N.A., Yosiphon,G., Fadeev,S.I., Kolchanov,N.A. , Mjolsness,E., and Likhoshvai, V.A., 2010. A plausible mechanism for auxin patterning along the developing root. *BMC Systems Biology*, 4:98
- Nahmad M., Stathopoulos, A., 2010. Establishing positional information through gradient dynamics: A lesson from the Hedgehog signalling pathway. *Fly (Austin)*.4, 1-5.
- Niehrs,C., 2010. On growth and form: a Cartesian coordinate system of Wnt and BMP signalling specifies bilaterian body axes. *Development* 137, 845-859
- Pescitelli, Jr.M.J. and Stocum,D.L. 1981. Nonsegmental organization of positional information in regenerating *Ambystoma* limbs. *Developmental Biology*. 82, 69-85
- Piddini,E., Vincent,J.P., 2009. Interpretation of the Wingless Gradient Requires Signalling-Induced Self-Inhibition *Cell* 136, 296–307
- Plouhinec,J-L., and De Robertis, E. M. 2009. Systems Biology of the Self-regulating Morphogenetic Gradient of the *Xenopus* Gastrula. *Cold Spring Harb Perspect Biol*.; 1,a001701.
- Saunders,T., Howard,M., 2009A. Morphogen profiles can be optimized to buffer against noise. *Physical Review E* 80, 041902
- Saunders,T., Howard,M., 2009B. When it pays to rush: interpreting morphogen gradients prior to steady-state. *Phys. Biol*. 6, 046020.

- Smith, J.C., Hagemann, A., Saka, Y., Williams, P.H., 2008. Understanding how morphogens work. *Philos Trans R Soc Lond B Biol Sci.* 363, 1387–1392.
- Smith, J.C., 2009. Forming and Interpreting Gradients in the Early *Xenopus* Embryo. *Cold Spring Harb Perspect Biol.*; 1(1): a002477.
- Summerbell, D., Lewis, JH & Wolpert, L. 1973 Positional information in chick limb morphogenesis. *Nature*, 244, 492-6
- Tickle, C., Summerbell, D. & Wolpert, L. 1975 Positional signalling and specification of digits in chick limb morphogenesis. *Nature*, 254, 199-202
- Tickle, C., 2006. Developmental cell biology: Making digit patterns in the vertebrate limb. *Nature Reviews Molecular Cell Biology* 7, 45-53
- Towers M., Tickle, C., 2009. Growing models of vertebrate limb development. *Development*. 136:179-90.
- Vuilleumier, R., Springhorn, A., Patterson, L., Koidl, S., Hammerschmidt, M., Affolter, M., Pyrowolakis, G., 2010. Control of Dpp morphogen signalling by a secreted feedback regulator *Nature Cell Biology* 12, 611 – 617
- Wolpert, L., 1969. Positional information and the spatial pattern of cellular differentiation. *Jnl. Theoretical Biology*. 25, 1-47
- Wolpert, L., Hicklin, J. & Hornbruch, A. 1971 Positional information and pattern regulation in regeneration of hydra. *Symp. Soc. Exp. Biol.*, 25, 391-415
- Wolpert, L., Clarke, M.R.B., Hornbruch, A., 1972. Positional Signalling along Hydra. *Nature* 239, 101-105
- Yan, D., Lin, X., 2009. Shaping Morphogen Gradients by Proteoglycans. *Cold Spring Harb Perspect Biol.* 1(3): a002493.
- Yan, D., Wu, Y., Feng, Y., Lin, S-E., Lin, X., 2009. The Core Protein of Glypican Dally-Like Determines Its Biphasic Activity in Wingless Morphogen Signalling *Developmental Cell* 17, 470–481
- Yu, S.R., Burkhardt, M., Nowak, M., Ries, J., Petrasek, Z., Scholpp, S., Schwill, P., Brand, M., 2009. Fgf8 morphogen gradient forms by a source-sink mechanism with freely diffusing molecules. *Nature* 461, 533-536.
- Zakin, L., De Robertis, E.M., 2010. Extracellular regulation of BMP signalling. *Curr Biol*. 20, R89-92