Review

Positional information and patterning revisited

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A B S T R A C T

The concept of positional information proposes that cells acquire positional values as in a coordinate system, which they interpret by developing in particular ways to give rise to spatial patterns. 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. Central problems are how positional information is set up, how it is recorded, and then how it is interpreted by the cells. A number of models have been proposed for the setting up of positional gradients, and most are based on diffusion of a morphogen and its interactions with extracellular molecules. It is argued that diffusion may not be reliable mechanism. There are also mechanisms based on timing. There is no good evidence for the quantitative aspects of any of the gradients and details how they are set up. The way in which a signalling gradient regulates differential gene expression in a concentration-dependent manner also raises several mechanistic issues.

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1. Introduction

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 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 coordinate 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

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(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 and Bourillot, 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 Prod1. This is an 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 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. 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 $2 \times 10^{-7} \, cm^2/s$ (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 to 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 very 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 developed leg cells 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 controls this process is not understood. The molecular basis of the positional values is also not known in this or any other system.

2. 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, and this results in the formation of a linear gradient. 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. However 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) upregulates 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 Smoothened (Smo) and this 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 can also interact with one or more type of co-receptor, 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 heparan sulfate proteoglycans (HSPGs), which are claimed to 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) 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 and Barkai (2010) 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. Third, 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 are expected to be 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 developing fly wing morphogens such as Decapentaplegic (Dpp) and Wingless (Wg), they are believed to form gradients of concentration providing positional information. Dynamin-dependent endocytosis was required for spreading Dpp, but not Wg. The cellular mechanisms of spreading are thus different and the spreading of Dpp requires endocytic, intracellular trafficking (Kicheva 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 suggests that cells in the progress zone under the apical ridge divide 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.

3. Evidence for gradients

In addition to the systems referred 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 anterio-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 4i 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 polarizing 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 yet lower level digit 2. The polarizing region produces Sonic Hedgehog (Shh), and its different doses give similar results. Also removing the grafted Shh signal gives 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 the cells of different determinations. The latter are assumed to result from the primary pattern formation in the embryo.

There is a 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 an evidence for gradients in the early Xenopus embryo and Smith et al. (2008) 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, Tolloid, which regulates gradient polarity and shape by establishing and maintaining a dorsal BMP sink. Perpendicular to the Xenopus DV BMP gradient is a 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 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 selfadjusting 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., 2008; 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 are essential for pattern formation. Mesoderm and endoderm are believed to develop due to the 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 the cells positioned away from the margin and towards the animal pole experience 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. The 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 proteins responsible for regulating the Hedgehog signalling and transport are themselves the 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 signalling 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 and 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 of 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 provides 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 (Benková et al., 2009). Graded auxin distribution seems to be crucial for cell specification within the root meristem, and the 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. Mironova et al. (2010) demonstrated that the reflected flow mechanism, which relies on the presence of positive and negative regulations between auxin and expression of its carriers, not only provides self-organisation of the observed auxin distribution in the root but can also explain much of the positional information in root patterning.

4. 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. In addition 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 that graded signal is 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 (Goldbetter and Wolpert, 1990). The study of different morphogens has shown that the interpreted graded signal 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) 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 proximo-distal 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. In addition 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.

The need for quantitative precision is challenged by fluctuations in the environmental conditions and by variations in the genetic makeup of the developing embryos. Self-enhanced morphogen degradation and pre-steady-state decoding provide general means for buffering the morphogen profile against fluctuations in morphogen production rate (Barkai and Shilo, 2009). Saunders and Howard (2009b) suggested 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 characterize embryonic development. This will require quantitative analysis and measurements of the morphogen concentration, signalling activity and target gene activation in real time.

5. 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 the other type of robustness on morphogen-mediated patterning. Mechanisms that operate at the level of gradient formation include self-enhanced morphogen degradation, serial transcytosis, pre-steady-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 mathematical modeling, there are major problems with all of them. First, 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. Second 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) 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.

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