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Regulative feedback in pattern formation: towards a general relativistic theory of positional information

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Positional specification by morphogen gradients is traditionally viewed as a two-step process. A gradient is formed and then interpreted, providing a spatial metric independent of the target tissue, similar to the concept of space in classical mechanics. However, the formation and interpretation of gradients are coupled, dynamic processes. We introduce a conceptual framework for positional specification in which cellular activity feeds back on positional information encoded by gradients, analogous to the feedback between mass-energy distribution and the geometry of space-time in Einstein's general theory of relativity. We discuss how such general relativistic positional information (GRPI) can guide systems-level approaches to pattern formation.

Introduction

Ever since Hans Driesch's famous experiments on sea urchin embryos, it has been evident that developmental processes are capable of global regulation (Driesch, 1892). A small part of an embryo, such as a single totipotent cell, can regenerate a whole intact embryo. Driesch was so baffled by his results that he rejected a materialist explanation for this phenomenon and turned to vitalism instead (Driesch, 1914). The problem of embryonic regulation essentially reduces to the problem of regulative positional specification: how do cells adopt a state that is appropriate to their relative position within a developing embryo?

Classical embryology introduced the notion of a morphogenetic field to explain global regulatory capacities (reviewed by Gilbert et al., 1996). The morphogenetic field aims to capture the ability of the cells in a developing tissue to establish a pervasive influence that imparts information about the state of the whole tissue. Local interactions within the field then allow cells to access global information, and, in principle, to adopt states that lead to appropriate patterning of the tissue or embryo as a whole. However, owing to the lack of molecular evidence, the mechanistic basis of such fields has remained obscure.

Lewis Wolpert sought to address the issue of the missing mechanistic basis of embryonic regulation and developmental fields in 1968, when he proposed his French flag model to illustrate the concept of positional information (Fig. 1) (Wolpert, 1968). At the time, gene expression in development was largely considered to be a problem of temporal regulation based on the paradigm established by Jacob and Monod from their work on the *lac* operon of *Escherichia coli* (Jacob and Monod, 1961; Monod and Jacob, 1962). To shift focus back to the spatial aspects of pattern formation, Wolpert argued that cells must have some means of determining their relative position in a developing field (Wolpert, 1969). In contrast to the concept of the morphogenetic field, Wolpert suggested that this happens by a mechanism imposed on, instead of arising from within, the field. According to this view, embryonic fields are defined by their boundaries (Irvine and Rauskolb, 2001), and positional information provides a mechanism by which cells can measure their distance from these boundaries. Signalling from field boundaries specifies a positional value for each cell in the field. Interpretation of the positional value by a cell (that is, its adoption of a particular fate based on its positional value) is then thought to occur autonomously, according to each cell's particular developmental history.

This view implies that positional specification is a two-step process, where the establishment and interpretation of positional values are independent of each other (Fig. 1). In other words, positional specification is essentially a hierarchical, feed-forward process in which cells within a developmental field play a passive, 'interpretative' role. We refer to this as the 'classical' theory of positional information.

The most common way in which positional information is thought to be implemented is by morphogen gradients (Wolpert, 1968; Crick, 1970) (reviewed by Slack, 1987; Gurdon and Bourillot, 2001; Tabata and Takei, 2004; Ashe and Briscoe, 2006; Lander, 2007). The term 'morphogen' was introduced by Alan Turing (Turing, 1952) to denote any kind of form-giving or pattern-forming substance. In its more restricted, modern definition, a morphogen acts across several cell diameters to induce at least three different states of gene expression in its target cells in a concentration-dependent manner (Gurdon and Bourillot, 2001). Specific threshold concentrations in the gradient correspond exactly to the positions of boundaries of target gene expression, which in turn determine the developmental fate adopted by cells within the tissue (Fig. 1). In this way, the concept of classical positional information suggests that positional values correspond to a simple biochemical variable (morphogen concentration) that is measurable by responding cells. As the morphogen gradient is itself not influenced by the response of cells within the tissue, the specified positional values are independent of subsequent processes operating within the developmental field.

Classical positional information and the modern (restricted) definition of the morphogen concept fail to capture important aspects of positional specification. Some recent criticisms of these concepts focus on temporal aspects, i.e. the duration and timing, of morphogen signalling (Pagès and Kerridge, 2000), or on processes involved in the interpretation of the signal (Ashe and Briscoe, 2006; Jaeger and Reinitz, 2006; Lander, 2007). More generally, there are two main problems with the classical theory of positional information: it cannot account convincingly either for size regulation (the ability of the pattern of cell fates in a developing tissue to scale with the overall size of the tissue) or for the observed precision of patterning in the presence of perturbations or fluctuations (robustness). Wolpert's purely geometrical argument on size regulation in the French flag model (Wolpert, 1968; Wolpert, 1969)

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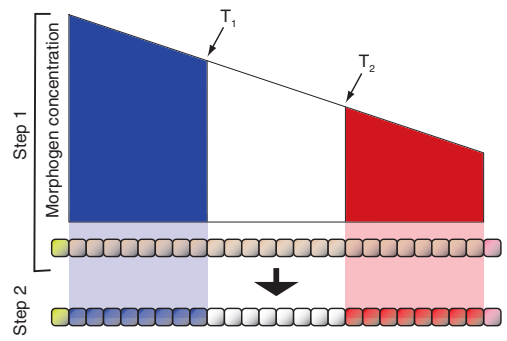


Fig. 1. The French flag model. Based on Wolpert (Wolpert, 1968). Positional specification by a morphogen gradient is implemented as a two-step process. Step 1: localised production of a morphogen at its source (green cell) and degradation at its sink (pink cell) leads to a linear gradient of decreasing morphogen concentration through the as yet undifferentiated tissue. Cells in the tissue sense whether they are exposed to morphogen concentrations below or above given thresholds (T_1, T_2). Step 2: cells become specified and later differentiate by turning on specific target genes (indicated by blue, white and red). Boundaries of target gene expression correspond exactly to the thresholds in the gradient. The arrow indicates the strictly feed-forward flow of information in this system. This 'classical' two-step view of positional specification naturally extends to more realistic non-linear gradients and systems where degradation is not restricted to a sub-population of cells (Slack, 1987).

depends crucially on the assumption of a linear gradient profile, and breaks down if more realistic exponential gradients are considered (Slack, 1987). Furthermore, classical positional information requires precise interpretation of the gradient, which renders it very sensitive to fluctuations in morphogen concentration [see appendix of Wolpert (Wolpert, 1989)].

Today, more than one hundred years after Driesch, we still lack any precise, mechanistic understanding of regulative phenomena and developmental robustness. In particular, the phenomenon of size regulation implies that developing embryos and tissues create their own adaptive spatial measuring system (or metric, see Box 1). In recent years, it has become increasingly clear that embryonic regulation and robustness require regulatory feedback between the dynamic metric systems implemented by morphogens, in the general sense used by Turing (Turing, 1952), and the target tissue they act upon. Data-driven computational modelling approaches, complementing the powerful tools of modern genetics, now allow such feedback to be studied quantitatively and in unprecedented detail. However, a suitable conceptual framework to guide such investigations is only slowly emerging, and many recent studies still rely (at least implicitly) on the classical conceptual framework of positional information.

Here, we introduce a revised and extended framework for positional specification within developing tissues that places a central emphasis on dynamics and regulative feedback. This is in contrast to the traditional concepts of positional information from which it is derived, as these are of a strictly feed-forward and static nature. Our proposed framework has interesting parallels with the concepts of physical space and time introduced by Einstein in his general theory of relativity (see Box 1). In the following sections, we present a number of examples that provide evidence in support of our revised conceptual framework, which we then describe in detail.

Regulative feedback and dynamic positional information

The classical concept of positional information has proven invaluable for guiding experimental research on pattern formation in developing fields. Although rarely acknowledged explicitly, Wolpert's ideas have inspired developmental biologists to search for and identify a number of candidate morphogen gradients and their respective regulatory targets (e.g. Green, 2002; Ephrussi and St Johnston, 2004; Tabata and Takei, 2004). However, a rapidly growing body of experimental evidence suggests that classical positional information is insufficient to account for the observed dynamics and regulative capabilities of gradient-based morphogenetic fields. Instead, the establishment of morphogen gradients turns out to be tightly coupled to their interpretation in a dynamic process, often involving multiple layers of regulatory feedback and interactions with the target tissue. In the following sections, we present a number of key examples that illustrate various levels of regulatory feedback involved in pattern specification. For more comprehensive reviews on feedback in signalling and patterning processes, see Freeman or Perrimon and McMahon (Freeman, 2000; Perrimon and McMahon, 1999).

Shifting gap domains in the *Drosophila* embryo

Regulative feedback can operate at many different levels. Our first example illustrates a 'semi-classical' case, where feedback occurs only between target genes and does not affect the concentration profile of the upstream morphogen Bicoid (Bcd; Fig. 2A, Fig. 3B). Bcd is a transcription factor that is encoded by a maternal gene. It is distributed as an exponential gradient emanating from the anterior pole of the early syncytial blastoderm embryo of *Drosophila melanogaster* (Driever and Nüsslein-Volhard, 1988). Its nuclear concentration profile remains constant throughout the period during which it initiates localised expression of its primary downstream targets, the zygotic gap genes, in broad overlapping domains (Tautz, 1988; Kraut and Levine, 1991a; Kraut and Levine, 1991b; Rivera-Pomar et al., 1995; Gregor et al., 2007a; Surkova et al., 2008). These domains are then stabilised and their boundaries sharpened by cross-repressive interactions between the gap genes (Jäckle et al., 1986; Eldon and Pirrotta, 1991; Kraut and Levine, 1991b; Clyde et al., 2003). Traditionally, it has been thought that boundary refinement through cross-regulation does not alter the position of gap gene expression domain boundaries, and, therefore, that the gap genes provide an excellent example of a 'French flag' encoded by a maternal gradient (Wolpert, 1989; Wolpert, 1996).

However, a quantitative network-level analysis of the gap gene system revealed that gap domain boundaries in the posterior part of the embryo undergo significant positional shifts towards the central region (Jaeger et al., 2004a; Jaeger et al., 2004b; Surkova et al., 2008). These shifts do not depend on concentration changes in maternal gradients, such as Bcd, and do not rely on gap protein diffusion. Instead, they are caused by asymmetries in gap gene cross-repression with posterior dominance occurring between gap genes that have overlapping expression domains (Fig. 2A). This means that each posterior neighbour represses its anterior neighbour more strongly than the other way around, leading to a cascade of asymmetric feedback. Because more anterior domains shift less than posterior ones, the entire gap gene expression pattern becomes compressed and sharpened towards the middle of the embryo, similar to the compaction of an accordion (Jaeger et al., 2004a; Jaeger and Reinitz, 2006).

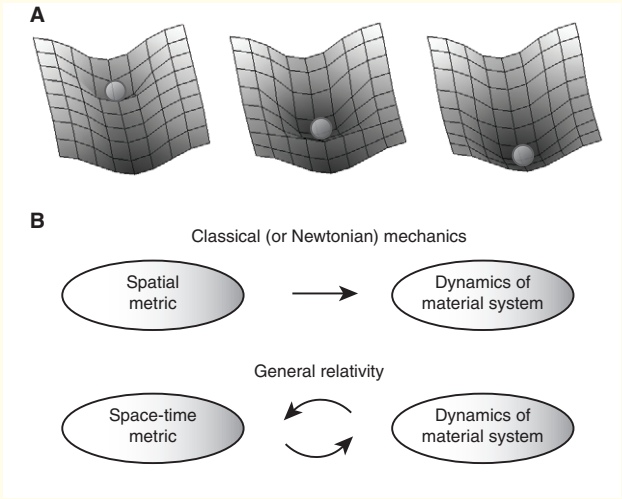
The shift of domain boundaries as a result of target gene cross-regulation implies that there is no one-to-one correspondence between concentration thresholds in the maternal gradient and the positions of

Box 1. Metrics, and dynamic feedback in general relativity

The dynamics of material systems depend explicitly on the relative spatial locations of their components. Distances between components are determined by a function called a metric, which encodes the geometry of the space; in a 'flat' Cartesian space, the separation of two points is given by Pythagoras' theorem, whereas curved spaces (such as the surface of a sphere) require different metrics. Classical mechanics presumes the existence of an inert spatial metric that acts as a passive 'arena'. Although the forces acting on bodies depend on their spatial separation, the metric is itself unaffected by material systems. A principal motivation for the development of general relativity was Einstein's dissatisfaction with this immutability of the metric:

"*… it is contrary to the mode of thinking in science to conceive of a thing (the space-time continuum) which acts itself, but which cannot be acted upon*" (Einstein, 1967).

General relativity denies this immutability, providing a description of gravity radically different to that of classical physics. Rather than generating a gravitational field in a fixed spatial geometry, matter generates a dynamic space-time metric. In turn, the geometry specified by this metric determines the dynamics of the material systems within it (illustrated schematically in A by a ball rolling down, and at the same time altering the slope of, a 'valley' in a two-dimensional space). The transition from an immutable to a dynamically responsive metric inextricably links the dynamics of material systems and the metric (see B).



target domain boundaries over time. Thus, the static nuclear Bcd gradient does not impose positional information on its target tissue. Rather, Bcd provides only an initial bias towards the expression of certain target genes, whereas positional information in the *Drosophila* blastoderm is encoded dynamically by the positions of expression boundaries of zygotic downstream factors. As these boundaries constantly shift, positional information needs to be seen as a dynamic process rather than a static metric. Moreover, it does not simply correspond to the concentration of Bcd (or any other morphogen), but rather consists of changing combinations of maternal and zygotic transcription factors expressed in a given nucleus over time. This example shows that even 'semi-classical' positional information cannot be simply equated to a specific chemical entity, such as a morphogen gradient, but is combinatorial and dynamic in nature (Jaeger and Reinitz, 2006).

Hedgehog, Wingless and Decapentaplegic in the *Drosophila* wing imaginal disc

More generally, the dynamic response of cells to a morphogen can provide feedback onto the shape of the morphogen gradient itself (Fig. 3C). In the *Drosophila* wing imaginal disc, the secreted proteins Hedgehog (Hh), Wingless (Wg) and Decapentaplegic (Dpp) form spatial gradients over several cell diameters to regulate global aspects of wing development, including size, shape and vein positioning (reviewed by Crozatier et al., 2004; Tabata and Takei, 2004). They act as morphogens by inducing the expression of target genes in a concentration-dependent fashion. Wg and Dpp emanate from, and form gradients centred on, the dorsoventral (DV) and anteroposterior (AP) compartment boundaries of the disc, respectively; *hh* is expressed in all posterior cells and the Hh protein forms a gradient in the neighbouring anterior compartment. In each case, a key feature of the signalling response in target cells is the control of receptor expression, which in turn alters the morphogen profile (Fig. 2B).

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 which restricts the movement of extracellular Hh ligand across the tissue by sequestering it (Fig. 2B) (Chen and Struhl, 1996). 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 (Casali and Struhl, 2004). This feedback increases the amount of Hh that is bound, internalised and degraded close to the AP boundary, resulting in a net sharpening and steepening of the gradient (Eldar et al., 2003). 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 receptor levels (Eldar et al., 2003). An analogous negative-feedback loop has been found in vertebrate embryos (Goodrich et al., 1996; Marigo et al., 1996; Marigo and Tabin, 1996).

By contrast, Wg signalling activity downregulates its receptor, Frizzled2 (Fz2), leading to reduced levels of receptor close to the Wg source at the DV boundary of the wing disc (Fig. 2B) (Cadigan et al., 1998). High Fz2 levels have been shown to increase Wg protein stability away from the source (Fig. 2B) (Cadigan et al., 1998). Conversely, Fz2 has also been shown to co-operate with a second receptor, Arrow, to internalise and degrade Wg (Piddini et al., 2005), suggesting that the interaction between Wg and Fz2 acts to differentially regulate Wg stability across the disc in a complex manner. As with Hh, feedback regulation of receptor levels is predicted to sharpen the morphogen gradient and to increase the robustness of the signalling system against fluctuations in the morphogen source (Eldar et al., 2003).

Feedback also plays an important role in the formation and interpretation of the Dpp gradient. *dpp* expression is localised to cells at the AP boundary of the wing disc, from where it establishes protein gradients in both the A and P compartments. The response of cells to these concentration gradients, mediated through the receptor Thickveins (Tkv), patterns the surrounding wing disc tissue (reviewed by Affolter and Basler, 2007). Tkv is downregulated by Dpp signalling activity, although indirectly and in co-operation with Hh, affecting both the read-out and the shape of the Dpp gradient (Fig. 2B) (Lecuit and Cohen, 1998; Funakoshi et al., 2001; del Alamo Rodriguez et al., 2004). As in the case of Ptc and Hh, Tkv inhibits Dpp movement through the tissue, and increased levels of Tkv far from the source sensitise target cells to the Dpp signal (Fig. 2B) (Lecuit and Cohen, 1998).

Wg and Dpp also play a role in cell proliferation, providing evidence for an additional layer of feedback between the shape of the gradient and the size of the target tissue (Rogulja and Irvine, 2005; Baena-Lopez and García-Bellido, 2006). Moreover, the levels of the Dpp receptor Tkv have been shown to be important for size regulation in the wing and haltere (Crickmore and Mann, 2006). Overexpression of *tkv* leads to wing discs of reduced size, whereas decreasing Tkv levels in the (much smaller) haltere disc increases its size.

These examples show that the shapes of three key morphogen gradients in the wing disc are modulated by receptor feedback in responsive cells. An important consequence is that the morphogen-induced signalling activity in each cell is sensitive to the response of all the cells in the responsive tissue. Receptor feedback thus provides a concrete mechanism for encoding, in the morphogen gradient itself, information about the dynamic response of the tissue as a whole.

Sonic Hedgehog in the vertebrate neural tube

An excellent example of how complex regulative feedback occurring at multiple levels is integrated to lead to a coherent spatial and temporal response in the target tissue is provided by the patterning of the vertebrate neural tube by Sonic Hedgehog (Shh) (reviewed by Ingham and Placzek, 2006; Dessaud et al., 2008). Shh, a vertebrate homologue of *Drosophila* Hh, is secreted from the notochord and the ventral neural tube, and diffuses to form a ventral-to-dorsal gradient that is required for specifying discrete progenitor domains from which different neural subtypes derive (Briscoe et al., 2000). The mechanisms underlying this process show striking similarities to both the gap gene network and the wing disc gradients discussed above.

As with Bcd and the gap gene network, cross-repressive interactions amongst target genes play an integral part in interpreting the Shh gradient, affecting the final positions of the different progenitor domains (Fig. 2C) (Briscoe et al., 2000; Vallstedt et al., 2001; Pachikara et al., 2007). Primarily, cross-repressive interactions exist between two main classes of transcription factors, one of which is activated (or de-repressed) at low levels of Shh signalling and the other at high levels. In addition, repressive interactions among genes of each target class further refine the borders between progenitor domains, leading to substantial shifts in domain boundaries over time. Overall, the Shh gradient provides a bias for localised target gene expression, which is then refined by cross-regulatory interactions between downstream targets that lead to dynamic shifts in their respective expression domains. For instance, a recent study by Dessaud et al. (Dessaud et al., 2007) demonstrated that the expression domain of the *Olig2* gene first expands and then contracts once levels of the transcription factor Nkx2.2 build up in the ventral-most part of the neural tube (Fig. 2C). In addition, the final pattern of cellular responses in this system depends on both the strength and duration of exposure to the Shh morphogen, exemplified by the finding that *Olig2* expression requires merely a brief exposure to Shh signalling, whereas *Nkx2.2* becomes activated only after sustained exposure (Dessaud et al., 2007).

As in the *Drosophila* wing disc, feedback between cellular response and the morphogen gradient is important in this system. In fact, such feedback is required for the temporal integration of the signalling response described above. Target cells become desensitised to the Shh signal over time as a consequence of Ptc1 upregulation (Marigo and Tabin, 1996; Dessaud et al., 2007), which leads to increased levels of unbound receptor that inhibit signalling

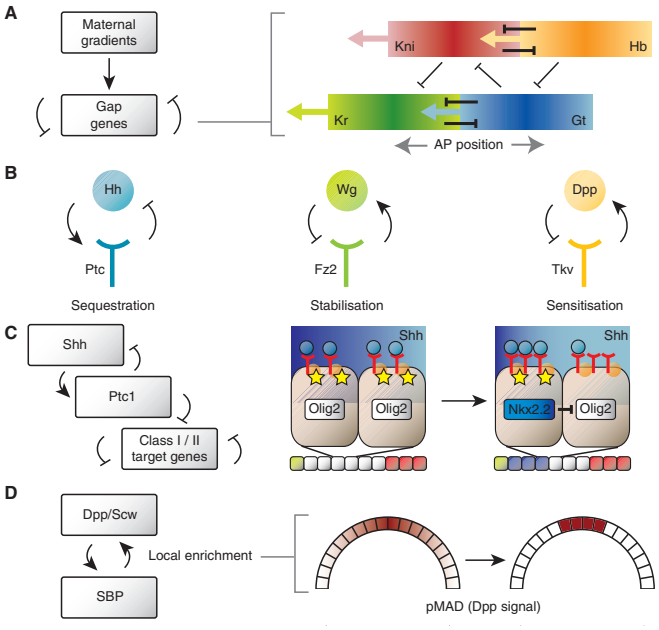


Fig. 2. Examples of regulatory feedback involved in positional specification by morphogen gradients. (**A**) The gap gene system of *Drosophila melanogaster.* Maternal morphogen gradients (such as Bcd) bias target nuclei towards the expression of specific gap genes according to their position along the anteroposterior (AP) axis of the embryo. The positions of the central and posterior domains of *Krüppel* (*Kr*), *knirps* (*kni*), *giant* (*gt*) and *hunchback* (*hb*) are shown diagrammatically, indicating the two pairs of staggered, mutually complementary domains along the AP axis (anterior, left). Cross-repressive feedback between complementary gap genes stabilises and sharpens these patterns (thick T-bars). A second layer of cross-repression with posterior dominance (thin T-bars) leads to anterior shifts in expression domain boundaries (indicated by coloured arrows). (**B**) Feedback between signalling ligands (morphogens) and their receptors or downstream pathways in the *Drosophila* wing disc. (Left) Hedgehog (Hh) signalling upregulates Patched (Ptc) receptor expression, which inhibits Hh movement by sequestering it extracellularly. (Centre) Wingless (Wg) signalling downregulates expression of its receptor Frizzled2 (Fz2) close to the Wg source. High levels of Fz2 away from the source stabilise the Wg protein. (Right) Decapentaplegic (Dpp) signalling downregulates expression of its receptor Thickveins (Tkv) close to its source. Tkv in turn sensitises cells away from the source to low levels of Dpp signalling. (**C**) Sonic Hedgehog (Shh) in the vertebrate neural tube (NT) is regulated by multiple levels of feedback. Shh (blue circles) up-regulates expression of its receptor Ptc1 (red), which inhibits signalling by repressing the co-receptor Smoothened (Smo, orange circles). This desensitises cells in the region of the gradient where ligand is limiting (in the dorsal NT, right) and alters the shape of the gradient (indicated by different blue shading). Yellow stars indicate signalling events. Brief Shh signalling activity induces expression of the target gene *Olig2* (white). Maintained levels of Shh induce *Nkx2.2* (blue), which in turn represses *Olig2* (T-bar). Red indicates dorsal (Class I) Shh target genes, such as Irx3 and Pax6. (**D**) Dorsoventral patterning in the *Drosophila* embryo. Dpp/Scw heterodimers diffuse dorsally in a complex with Twisted Gastrulation (Tsg) and Short Gastrulation (Sog). They are captured and enriched at the dorsal midline by a surface bound ligand binding protein (SBP), whose expression is upregulated by Dpp signalling. This leads to the sharpening and narrowing of the dorsal stripe of Dpp activity (measured as the concentration of phosphorylated MAD, pMAD) through bistability in the cellular response.

activity by repressing Smo (Fig. 2C). Such temporal modulation of the Shh signal is by no means unique to the neural tube. For example, during mouse limb and zebrafish muscle development, the level and duration of Shh exposure is essential for determining cellular response (Wolff et al., 2003; Ahn and Joyner, 2004). In addition, the Ptc1 upregulation is likely to limit the spread of Shh in a manner analogous to the Hh-Ptc interaction in the wing imaginal disc. Shh upregulates an additional antagonist of its own signal, Hip1 (Chuang and McMahon, 1999; Goodrich et al., 1999). Hip1 binds Shh at the cell surface, and can therefore limit the movement of Shh across the tissue. Finally, these different forms of feedback can also regulate cell proliferation, with the neural tube becoming overgrown in *Ptc1 Hhip1* double mutants (Jeong and McMahon, 2005).

In summary, regulatory feedback in this system occurs at four different levels. First, upregulation of Ptc affects the shape of the gradient itself. Second, upregulation of Ptc leads to the desensitisation of cells experiencing low levels of, and a short exposure to, the Shh signal. Third, regulatory feedback between target genes leads to temporal shifts in boundary positions. Lastly, morphogen signalling affects cell proliferation. All of these feedback interactions are crucial for determining the correct size and position of each neuronal progenitor domain. If any of these forms of feedback fail, the range and strength of Shh signalling is significantly expanded (Jeong and McMahon, 2005; Dessaud et al., 2007). This implies that the establishment and the interpretation of the Shh gradient rely on a complex interplay between signal strength, signal duration, cell proliferation, and interactions involving Shh receptors and target genes.

Dorsoventral patterning in *Drosophila* and vertebrates

In the above examples, morphogens are produced in a 'signalling centre' that acts as a boundary; the morphogens then establish gradients that pattern the surrounding tissue. However, it is also possible for positional information to be specified by broadly expressed proteins that are then localised through feedback-regulated transport. One such example is involved in DV patterning during early *Drosophila* embryogenesis (reviewed by O'Connor et al., 2006). It illustrates that positional specification that is driven by feedback is a very general phenomenon and that it is not limited to gradient-based fields.

In *Drosophila*, *dpp* is initially expressed uniformly in the dorsal-most 40% of the embryo. Subsequently, Dpp protein is shuttled to the dorsal midline of the embryo, establishing a steep concentration gradient that specifies the extraembryonic amnioserosa and dorsal ectoderm (Fig. 2D) (Dorfman and Shilo, 2001). This gradient is achieved by dorsal diffusion of Dpp and its paralogue Screw (Scw) in complex with the Twisted Gastrulation (Tsg) and Short Gastrulation (Sog) proteins, the latter of which is expressed in (and diffuses from) the ventral domain (Holley et al., 1995; Marques et al., 1997; Ross et al., 2001; Shimmi et al., 2005). This process gives rise first to a shallow dorsal gradient, which subsequently matures into a narrow distribution of Dpp with very steep boundaries around the dorsal midline. This refinement involves positive feedback between the complexed ligand heterodimers and an, as yet, unidentified surface bound ligand-binding protein (SBP), which increases ligand concentration locally (Fig. 2D) (Wang and Ferguson, 2005). An analogous refinement is involved in the positioning of cross-veins during *Drosophila* wing development by Dpp and its paralogue Glass bottom boat (Gbb), with the secreted Crossveinless2 protein identified as the upregulated SBP (Serpe et al., 2008).

Modelling results confirm that positive feedback can account for the observed sharpening of the gradient as it creates a spatially bi-stable response, where the fate of each cell is determined by both the strength and duration of Dpp/Scw signalling (Umulis et al., 2006). Moreover, these studies suggest that this system is robust to changes in the gene dosage of *scw* and *sog*, and of the receptor *tkv*, and that it exhibits scale invariance across changes in embryo size of up to 40% (Shimmi et al., 2005; Umulis et al., 2006).

A slightly different type of regulative feedback leads to size regulation during DV patterning in *Xenopus laevis* embryos (Ben-Zvi et al., 2008). Analogous to *Drosophila*, the vertebrate BMP ligand homologues of Dpp and Scw are shuttled to the ventral pole of the embryo by chordin, a vertebrate homologue of Sog, which is released by the organiser tissue at the dorsal blastopore lip. In addition, vertebrates have a BMP ligand called anti-dorsalising morphogenetic protein (ADMP), which is co-expressed with chordin in the organiser. ADMP is negatively regulated by BMP signalling. Because of this, the system reaches steady state when ADMP accumulates at sufficient levels to repress its own expression dorsally. Modelling studies of this feedback mechanism have shown that it can lead to a gradient of BMP signalling, the range of which scales perfectly with embryo size and thus explains size regulation in isolated dorsal halves of *Xenopus* embryos, which develop into small but complete tadpole larvae (Ben-Zvi et al., 2008).

General relativistic positional information

In Wolpert's original conception, the field of positional information produced by a localised source of morphogen is read and interpreted by responding cells without the information in the field being changed significantly. In this sense, it resembles the logical structure of classical or Newtonian mechanics in physics, where the relative positions of bodies are determined with reference to the static geometry of space that is itself unaffected by any objects or processes that are referred to it. This view depends on making a well-defined distinction between an imposed field (the morphogen gradient specified by the boundaries of the developing tissue) and an interpretation system (which resides within the responding cells; Fig. 1) (Wolpert, 1969), and implies that there is a unidirectional transfer of information from the field to the responding cells (Fig. 3D). However, the specific examples that we have described show that positional specification by a wide range of different morphogens depends on regulative feedback from responding cells (Fig. 2, Fig. 3D). Such feedback from the cellular response system, which we believe to be of central importance in positional specification, does not fit into the traditional framework of positional information.

The field of positional information specifies a spatial metric (see Box 1) that is used by responsive cells to determine their relative position in the developmental field. In the examples discussed above, the form of this metric is dynamic and is determined in part by feedback from responding cells. Interestingly, the transition from classical positional information, in which the form of the metric is independent of the response system, to this new framework has parallels with the change in the status of the space-time metric in the transition from classical mechanics to general relativity (compare B in Box 1 with Fig. 3D). In classical mechanics, the spatial metric specifies a passive 'arena' in which physical processes take place; although the form of the metric affects the dynamics of physical processes, the metric is independent of objects and processes within it. Therefore, the logical structure is strictly feed-forward, just as in classical positional information. By contrast, in general relativity, the geometry of space-time (encoded by the metric) is dynamic and

depends on feedback from the mass-energy distribution within it. For this reason, we refer to our revised concept as general relativistic positional information (GRPI).

GRPI extends the 'classical' model of positional specification by explicitly incorporating dynamics and regulative feedback. This feedback can occur at multiple levels, such as between morphogens and cell surface receptors, and/or amongst downstream target genes. Moreover, where morphogens play a role in cell proliferation, there is an additional layer of feedback, as the resulting change in tissue size alters the relative shape of the gradient. One of the primary features of GRPI, and a common consequence of such feedback mechanisms, is that the activity profile of the target tissue (expression of downstream targets), the positional specification system, the tissue geometry and the morphogen profile are dynamic and inter-linked. Therefore, any mechanism that leads to a dynamic response within the target tissue, for example, where target gene response is determined by both the strength and duration of signalling, can also be incorporated within GRPI.

An important aspect of GRPI is that there is no longer any simple correspondence between positional value and any specific biochemical variable, such as the concentration of a morphogen. In other words, GRPI is not a 'thing' but a dynamic state of the system that implements a kind of biological space and time within developing fields. It can consist of rapidly changing combinations of factors, such as signalling and transcription factor levels, and can incorporate non-genetic elements, such as ionic potentials and mechanical stress. This implies that it is no longer sufficient to measure single biochemical variables of a system as proxies for positional value, or to study regulatory interactions between isolated genes. Instead, our notion of GRPI requires characterisation of the dynamic state of an entire developing system in order to understand how position is specified.

One possible response to the discovery of dynamic feedback is to state that any split of the positional specification system into two distinct conceptual components, a metric-generator and a response system, is artificial and should be avoided. Rather, developing fields have to be understood in terms of the regulative dynamics of the entire spatially distributed system. Although this approach is consistent, we believe that there is value in maintaining a conceptual framework that preserves the notions of metric-generating and response systems while incorporating a two-way interaction between them. One major advantage of such a framework is that it puts an explicit emphasis on the spatial aspects of development, which, as stated above, was Wolpert's original (and still very much valid) motivation for introducing the concept of positional information (Wolpert, 1969).

In the case of gradients established by the movement of morphogens away from a localised source or boundary, these sources can often be considered as 'organisers' for a tissue. The GRPI formalism preserves the valuable concept of organisers, while elevating the status of the remainder of the tissue from passive 'organisee' to that of an active participant in the process of organisation. Indeed, organisers can themselves be dynamic emergent features of the organisation process, residing in a particular (relative) position in a tissue, rather than in a fixed population of cells (Joubin and Stern, 1999).

A similar conceptual split is often useful in analysing the dynamics of physical systems. Several different conceptual approaches to understanding the nature and consequences of the feedback between matter and space-time can be adopted (Friedman, 1983; Monk, 1997). A formalism that emphasises the dynamic interaction between a 'quasi-static' space-time metric and material bodies can yield valuable

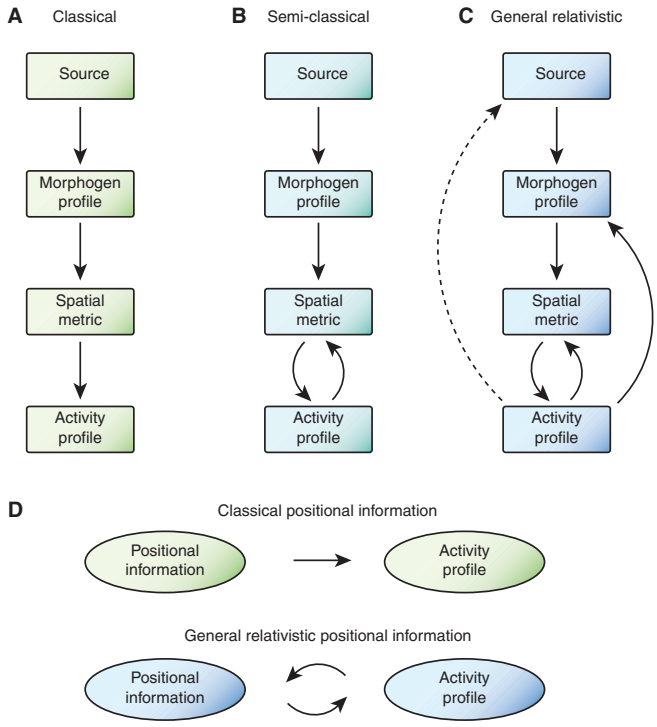


Fig. 3. Logical structure of conceptual frameworks for positional specification. (**A**) Classical: strictly feed-forward flow of information from the morphogen gradient, which specifies a static spatial metric that is imposed on the target tissue. The activity profile of the target cells has no influence on the metric. An example is the French flag model. (**B**) Semi-classical: feedback between target genes alters the metric over time without affecting the morphogen gradient itself. An example is the *Drosophila* gap gene system. (**C**) General relativistic: multiple levels of feedback exist between the cellular response (activity profile) and the metric, the morphogen profile and potentially also the morphogen source. See Fig. 2B-D for examples. (**D**) Logical structure of classical versus general relativistic positional information (GRPI). Compare with B in Box 1 for conceptual parallels to classical versus relativistic physics.

conceptual insight into the consequences of feedback. For example, in studying the dynamics of the solar system, the dominant contributor to the overall gravitational field is the sun, and the motion of the planets can be approximated by considering them as small 'test particles' that are affected by the field generated by the sun without themselves affecting this field. This view provides a framework in which the specific effects of feedback onto the field (the metric) can be studied in detail as perturbations to the strictly feed-forward dynamics. An early triumph of general relativity was that it provided an explanation for the anomalous precession of the perihelion of Mercury's orbit in terms of a small perturbation to its classically predicted orbit caused by feedback onto the space-time metric (Einstein, 1916).

Despite the strong conceptual parallels between Einstein's theory of general relativity and GRPI, there are some notable differences as well. First, although gravitational fields are governed by known general laws, relatively little is understood about the laws that govern morphogenetic fields. In fact, it seems unlikely that any such general laws exist. Moreover, there is conservation of mass-energy in physics, whereas it is highly improbable that any such conservation applies to the activities of cells, as they are open

thermodynamic systems. This suggests that there is no finite, well-defined set of rules that govern developmental processes. It also implies that while physicists can deduce specific cases from general laws, biologists will have to study as many particular examples of developmental systems as they can in order to learn what generalisations can usefully be made about them.

Conclusions

The data we review demonstrate that positional specification is a dynamic process that is driven by feedback. This basic insight is not new. In fact, it formed the foundation for the fundamental concept of classical embryology, the morphogenetic field, a concept that has been eclipsed by the reduction of embryology to molecular genetics (reviewed by Gilbert et al., 1996) (see also Jaeger and Reinitz, 2006). Classical positional information was an attempt to redefine the developmental field concept based on specific molecular mechanisms (morphogen gradients), while retaining a focus on regulative spatial patterning (Wolpert, 1968; Wolpert, 1969; Wolpert, 1989; Wolpert, 1996). The concept of positional information is still useful for making explicit the spatial nature of positional specification. However, Wolpert's definition of a field loses much of the explanatory power of the original morphogenetic field concept through its neglect of regulative feedback (Jaeger and Reinitz, 2006).

Here, we attempt, in a similar spirit to Wolpert's original efforts, to reconcile the old phenomenological concepts of classic embryology with positional information. The main purpose of our effort is to shift our focus back to the intrinsically dynamic and regulative nature of positional specification, while maintaining Wolpert's mechanistic rigour.

The advantages of regulatory feedback are obvious. Since its early days, positional information has been criticised for its lack of robustness and its heavy reliance on the precise interpretation of minute differences in morphogen concentration [see appendix of Wolpert (Wolpert, 1989)]. Feedback-based systems, by contrast, allow for increased stability against expression noise, mutation or fluctuations in the environment. This is substantiated by the fact that mathematical models incorporating three of the previously described feedback interactions, Hh-Ptc, Wg-Fz2 and Dpp/Scw-SBP, show significant robustness to changes in the levels of signalling factors (Eldar et al., 2003; Shimmi et al., 2005; Umulis et al., 2006).

A key challenge that must be met by conceptual frameworks for positional specification is to provide a mechanism for size regulation (e.g. Gregor et al., 2005; Lott et al., 2007; Gregor et al., 2008). Classical positional information is severely limited in this regard, as a morphogen gradient only encodes information about the boundaries of the developmental field. By incorporating local feedback from dynamic cell states onto the morphogen gradient itself, GRPI provides an explicit mechanism for achieving locally encoded global regulation (e.g. Ben-Zvi et al., 2008). This was one of the main strengths of the original concept of the morphogenetic field, which was lost in the transition to Wolpert's fields, whose definition relies entirely on their boundaries without considering processes within the field (Jaeger and Reinitz, 2006).

Note that feedback and long-range signalling need not be entirely chemical, as the mechanical properties of tissues can play a central role in these processes (Forgacs and Newman, 2005). For example, it has been proposed that local mechanical feedback in response to tissue compression or stress can lead to global growth regulation under the control of the Dpp gradient in the wing imaginal disc (Shraiman, 2005; Hufnagel et al., 2007; Aegerter-Wilmsen et al., 2007). Once the size of the disk exceeds a certain limit, cells on the margin no longer receive enough morphogen to proliferate and therefore constrain the space available to those cells still dividing in the centre, halting growth throughout the disk. The morphogen gradient is both affected by and affects growth patterns in the tissue. Such interaction between patterning and tissue growth appears to be a very widespread phenomenon in developmental systems.

It is important to stress again that positional specification relies not on a static 'thing' (such as a chemical gradient), but on a complex process involving the target tissue. This implies that downstream factors and their interactions need to be considered when analyzing positional specification by morphogen gradients. The following example illustrates how ignoring such interactions can lead to results that are inconclusive and difficult to interpret.

A recent, quantitative study in *Drosophila* concluded that precision in the positioning of an expression domain boundary of the gap gene *hunchback* (*hb*) is due exclusively to high precision in the Bcd gradient (Gregor et al., 2007b). The authors measured the transcriptional response of *hb* with respect to Bcd concentration without considering the known gap-gap cross-repressive interactions. Sensitivity analysis of this interaction showed that the time required to obtain the measured *hb* precision exceeds the age of the embryo at the relevant developmental stage. This led the authors to propose that precision was based on spatial integration of signal interpretation across neighbouring nuclei (Gregor et al., 2007b). It remains unclear how such spatial integration could be achieved. In addition, closer scrutiny of the fluctuation levels in Bcd and *hb* reveals that positioning of the target gene expression boundary is still more precise than fluctuation levels in the gradient (Reinitz, 2007), and that the spatial distributions of positional errors in gradient and downstream expression boundaries become increasingly uncorrelated over time (Holloway et al., 2006). In light of the above, it remains plausible that regulatory feedback among target genes contributes to the observed levels of precision, and that no spatial integration of signal is required. It seems that this possibility was not even considered by Gregor et al. (Gregor et al., 2007b) because we are used to equating positional information with concentration levels of morphogen gradients. By contrast, the authors of a recent study of the Dpp gradient in *Drosophila* wing discs, although focussing exclusively on direct, instructive interactions of the gradient with its target genes as well, suggested that the lack of achievable precision by morphogen signalling alone indicates a role for downstream regulation in the patterning process (Bollenbach et al., 2008).

The above example illustrates that classical positional information still influences current experimental design and can lead to complications in the interpretation of experimental evidence. GRPI is intended to clarify these issues, as it shifts the emphasis away from the notion of morphogen gradients as simple biochemical co-ordinate systems, on to a dynamic metric that allows cells to measure their relative position within a developing field that itself changes in response to the activity of those cells. The underlying biochemical mechanisms are likely to be diverse and change rapidly over time, involving a range of regulatory feedbacks on multiple levels. Mechanisms of this type are the focus of the emerging paradigm of systems biology, which shifts the emphasis of experimental approaches away from individual biochemical findings to dynamic regulatory principles that integrate biochemical processes. The study of such principles requires researchers to keep track of many simultaneous interactions. This is impossible without the help of computational models and their analysis using the methods and concepts of dynamical systems theory (Strogatz, 2001). Most

developmental biologists today are not yet familiar with these methods and concepts. GRPI illustrates why we need to understand the dynamic behaviour of complex systems to understand positional specification, and provides a guiding metaphor that will be useful in focussing integrative studies of the complex feedback systems that underlie regulative spatial patterning in development.

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