On the dynamic nature of positional information

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Summary

Morphogenetic fields are among the most fundamental concepts of embryology. However, they are also among the most ill-defined, since they consist of dynamic regulatory processes whose exact nature remains elusive. In order to achieve a more rigorous definition of a developmental field, Lewis Wolpert introduced the concept of positional information illustrated by his French Flag model. Here we argue that Wolpert's positional information—a static coordinate system defining a field—lacks essential properties of the original field concept. We show how data-driven mathematical modeling approaches now enable us to study regulatory processes in a way that is qualitatively different from our previous level of understanding. As an example, we review our recent analysis of segmentation gene expression in the blastoderm embryo of the fruit fly *Drosophila melanogaster*. Based on this analysis, we propose a revised French Flag, which incorporates the dynamic, feedback-driven nature of pattern formation in the *Drosophila* blastoderm.

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Introduction

Over the last few decades, we have witnessed tremendous progress in the field of developmental genetics. A large number of genes involved in regulating developmental processes have been cloned and analyzed at the molecular level by genetic and, more recently, genomic approaches. However, despite this wealth of genetic information, we still lack an integrative view of how genes affect development, and hence phenotypes of tissues, organs and whole organisms. In other words, we lack an integrative view of a morphogenetic field.[1]

Morphogenetic fields were one of the most central concepts in embryology before they became eclipsed by the rise of developmental genetics and its focus on gene regulation.[2] According to C.H. Waddington, a morphogenetic field not only denotes a specific region of an embryo but, more importantly, incorporates all relevant processes, which through their interactions in time and space, regulate the development of a particular structure or the entire embryo itself.[3] In analogy to the field concept in physics, biological fields exhibit many regulatory powers. If cut in half, each half can regenerate a complete structure. If parts are removed, the remainder is able to compensate. If fields are brought into contact with each other, they fuse into a single field.[3]

In 1969, Lewis Wolpert introduced the concept of positional information—a biochemical coordinate system for developing tissues—in order to clarify and simplify the rather ill-defined notion of a biological field.[4] It was Wolpert's aim to provide experimentalists with specific molecular mechanisms for spatial pattern formation. Its continuing success demonstrates that his conceptual framework has proven extremely powerful and productive in guiding experiments and theoretical approaches to developmental biology.[5–8] One of its most notable achievements was the prediction and subsequent discovery of morphogens, chemical substances which form spatial gradients that affect development in a concentration-dependent manner.[9,10] The related concepts of positional information and morphogens will be reviewed in the first part of this paper.

Over the last few years, novel computational approaches—based on data-driven mathematical modeling—have been developed that allow us to keep track of a large number of simultaneous interacting regulatory processes in intact, wild-type developmental systems. In contrast to traditional genetic and molecular methods, the focus of these approaches lies explicitly on the complexity and dynamical aspects of development. We illustrate how such methods enable us to investigate the nature of regulatory processes constituting specific morphogenetic fields.

As an example, we review how one such mathematical modeling approach, the gene circuit method,[11–20] is applied to study pattern formation in the blastoderm morphogenetic field during early embryogenesis of the fruit fly *Drosophila melanogaster*. Our analysis focuses on the initial subdivision of the *Drosophila* embryo into distinct territories of gene expression, a process that has provided one of the most important sources of evidence for positional information.[5–7]

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We show that positional information in the blastoderm embryo is dynamic, feedback-driven and combinatorially complex, in contrast to the notion of a simple, static and instructive coordinate system. In the last and main part of this paper, we discuss the broad and important implications that this has for our understanding of positional information and morphogens, and hence of morphogenetic fields in general.

Modern morphogens and Wolpert's positional information

Before we can begin our discussion, we need to clarify what exactly is meant by 'morphogen' and 'positional information'. According to Lewis Wolpert, positional information is a mechanism by which cells have their position specified with regard to one or more points of reference in a developing tissue.[4] Position is determined by a cell parameter, called positional value, which changes monotonically with distance from a point of reference. Cells that have their position specified based on the same points of reference constitute a field. Reference points usually lie on the boundaries of the field. Unipolar systems have one positional value for each dimension of the system, while bipolar systems specify positional value by the ratio between two gradients with opposite polarity for each axis. Either way, positional information imposes a spatial coordinate system on a developing field, which uniquely determines the distance of each cell from the boundaries of the field.

Initial states of cells in a field are assumed to be equivalent. The cells then 'interpret' positional values by entering qualitatively different cell states depending on their positional values. This requires a threshold-dependent mechanism of interpretation, where cells with positional values above threshold will enter one state, while cells with values below threshold will enter another.[4,21] This type of mechanism relies crucially on very precise sensing of positional values by target cells. Although a theoretical study suggests that sufficiently accurate read-out of a gradient is possible,[22] it remains unclear whether such precision could be achieved in real developmental systems.

Wolpert defines positional information as being entirely independent of its subsequent interpretation.[4] In this view, development is seen as a two-step process consisting of (1) pattern formation based on positional information, and (2) differentiation of cells specified by positional information according to the cell's genome and developmental history.[4,5,23] This implies that molecular mechanisms for pattern formation do not need to be specific to any developmental field, and may in fact be universal.[4,7] However, it also implies that processes in the target tissue must not alter the coordinate system established by positional information.

Positional information is a purely abstract concept that can be implemented by different physico-chemical mechanisms.[4] One such possible mechanism is based on biochemical oscillators with distinct periods, which show a monotonic increase or decrease in phase angle with distance from their point of origin.[4,24] Traditionally, most discussions of positional information have focused on another molecular mechanism based on morphogen gradients.[5–7,21] The term 'morphogen' was introduced by Alan Turing to denote a substance involved in morphogenesis by forming spatial patterns through chemical reaction and diffusion.[25] Later, 'morphogen' came to be used more specifically for substances forming concentration gradients in developing tissues.[9] To distinguish morphogen gradients from inductive processes (defined as local, all-or-none interactions between two neighboring tissues), the definition of 'morphogen' was restricted even further.[5,26] Thereby, a morphogen must not only be distributed as a gradient during development, it must also diffuse and act over long distances (compared to local inductive events), and must be directly and exclusively responsible for threshold-dependent induction of at least two different states of gene expression in its target cells. No additional morphogens or interactions within or among target cells should be required. We call this restricted definition of 'morphogen' the modern morphogen concept to distinguish it from Turing's more general, original definition.

Quite obviously, modern morphogens are ideally suited as carriers of positional information. By definition, they affect gene expression in target cells directly in a concentration-dependent manner and their concentration decreases monotonically with distance from their source. Estimates and measurements of diffusion constants in living tissue suggested that morphogen gradients can form within plausible time limits across a tissue diameter of less than about 100 cells,[9,27] which is true for most developmental fields.[4] It was the characterization of the Bicoid (Bcd) gradient in the *Drosophila* blastoderm embryo—the first morphogen gradient to be visualized directly[10,28]—that confirmed these theoretical considerations. Since then, many more morphogen gradients have been characterized in organisms ranging from slime molds to vertebrates.[26,27,29]

For most, if not all, of the morphogen gradients that we know today we do not have conclusive evidence on the molecular mechanisms involved in gradient interpretation.[26,30] In other words, we do not know if any candidate morphogen actually fulfills all the requirements of the modern morphogen concept. It has proven especially difficult to establish whether a morphogen is sufficient to induce different states in target cells, or whether other morphogens or regulatory interactions within the target tissue are required for its effect.[26,29,30]

As mentioned above, the Bcd gradient is one of the most important examples of a candidate morphogen implementing positional information.[5–7,29] It is involved in segment determination during early *Drosophila* embryogenesis, a process that will be outlined in the following section.

Drosophila segment determination

The insect body plan consists of serially repeated morphological structures called segments. In *Drosophila*, the boundaries of these segments are determined more or less simultaneously during the blastoderm stage of development, coinciding with cellularization of the syncytial embryo just before the onset of gastrulation.[31] Boundaries of morphological segments become visible much later—at the extended germ-band stage—preceded by transient parasegmental boundaries, which appear in the middle of each future segment during germ-band extension.[32]

The genetics of segment determination in the *Drosophila* blastoderm is very well understood. Screens saturating the entire genome of *Drosophila melanogaster* with mutations have led to the isolation of a complete—or almost complete—set of segmentation genes.[33,34] Based on their mutant phenotypes, these genes have been subdivided into maternal coordinate,[34] as well as zygotic gap, pair-rule and segment-polarity genes.[33] A majority of segmentation genes encodes transcription factors, which form a network of gene regulatory interactions. Analyses of genetic epistasis have revealed that the different classes of segmentation genes correspond to hierarchical regulatory layers in the segmentation gene network[35,36] (Fig. 1A). Thereby, the products of genes in higher layers (e.g. maternal coordinate genes) regulate genes in lower layers (e.g. gap genes), but not vice versa. In addition, there is cross-regulation among genes within the same hierarchical layer.

Initial conditions for zygotic segmentation gene expression are given by spatial gradients of the maternal transcription factors Bcd (Fig. 1A), Hunchback (Hb) and Caudal (Cad).[37] Further maternal input is provided by the terminal maternal system,[38] which acts through the zygotic terminal gap genes *tailless* (*tll*) and *huckebein* (*hkb*) in the pole regions of the embryo.[39] Among the earliest targets of the above factors are the gap genes *hunchback* (*hb*), *Krüppel* (*Kr*), *knirps* (*kni*) and *giant* (*gt*) which are expressed in broad, overlapping spatial domains[40–44] (Fig. 1A,B). Gap gene products—in

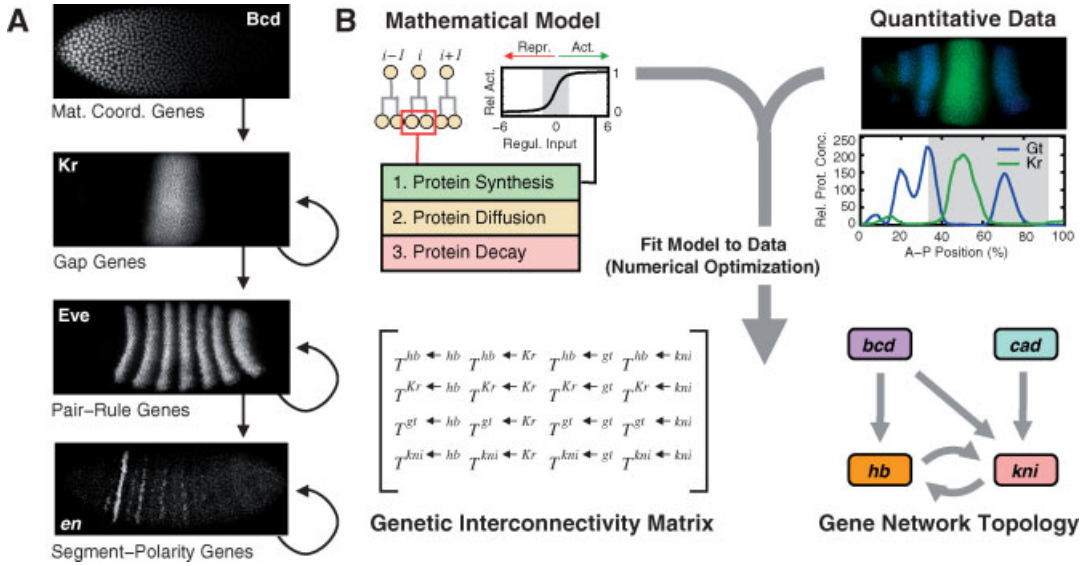

Figure 1. A: The segmentation gene network of *Drosophila melanogaster*. Maternal gradients of transcription factors such as Bicoid (Bcd), regulate expression of trunk gap genes *hunchback* (*hb*), *Krüppel* (*Kr*; shown), *knirps* (*kni*) and *giant* (*gt*) in one or two broad domains. Maternal coordinate and gap genes then provide regulatory inputs for pair-rule gene expression (e.g. *even-skipped* (*eve*)). Pair-rule genes in turn regulate the initial expression of segment-polarity genes in 14 narrow stripes (shown for *engrailed* (*en*) as newly forming pattern at blastoderm stage). Arrows indicate regulatory interactions within and between the hierarchical layers of the network. Anterior is to the left, dorsal is up in embryo pictures. Protein expression patterns are shown for Bcd, Kr and Eve; mRNA for *en*. **B:** The gene circuit method. Regulatory interactions are inferred from gene expression patterns by fitting gene circuit models to quantitative gene expression data (shown are protein expression profiles for Kr and Gt; gray background indicates the region of the embryo included in gap gene circuits). Gene circuit models consist of a one-dimensional row of nuclei (denoted by index *i*), which divide equally and synchronously at each mitosis. During interphase, protein synthesis, diffusion and decay occur within and between nuclei. Protein synthesis occurs according to a sigmoid regulation-expression function (the sum of individual regulatory inputs on the horizontal axis is plotted against relative activation of protein synthesis). Synthesis rapidly approaches zero or saturation outside the sensitive range (indicated by gray background). Arrows indicate increasing net repression (red) and activation (green). Regulatory interactions within a gene circuit are represented by the genetic interconnectivity matrix (shown here for interactions of *hb, Kr, gt* and *kni*), which contains regulatory information extracted from the data by the fitting procedure, and represents a functional network topology capable of reproducing the observed gene expression patterns.

combination with maternal factors—then regulate pair-rule genes, which are expressed in periodic patterns of seven stripes[45–51] (Fig. 1A). Pair-rule genes in turn establish the expression patterns of segment-polarity genes such as *engrailed* (*en*), whose 14 stripes of expression appear just before the onset of gastrulation[52,53] (Fig. 1A). These stripes of segment-polarity gene expression constitute a segmental prepattern in that they directly determine the positions of parasegmental boundaries later in development.[54–56]

In summary, it is the biological function of the segmentation gene network to establish a segmental prepattern of gene expression based on initial embryonic asymmetry and polarity provided by maternal gradients. During this process, we observe the increasingly more refined localization of expression domain boundaries of gap, pair-rule and segment-polarity genes. Here we focus on a computational analysis of how such boundaries are first established through the interpretation of maternal gradients by the gap gene system.

The gene circuit method

Gene circuit models[11] are computational tools that enable us to extract regulatory information from (wild-type) quantitative gene expression data through the following four steps. (1) We formulate a mathematical model incorporating basic assumptions about mechanisms of gene regulation. (2) We collect quantitative gene expression data. (3) We make the model reproduce observed expression patterns as closely as possible by fitting the model to the data. (4) We analyze the regulatory information within each resulting gene circuit by tracking individual regulatory contributions to each gene across space and time.[13,16,17,20]

The basic objects of the model are dividing blastoderm nuclei arranged in a row along the anteroposterior (A-P) embryonic axis (Fig. 1B). Each nucleus contains transcription factors whose levels of concentration are governed by regulated protein synthesis, protein decay and diffusion between neighboring nuclei[11,13] (Fig. 1B). We use coarse-grained kinetic equations that approximate the exact biochemistry of transcription and translation with a sigmoid regulation-expression function[11] (Fig. 1B). Regulatory interactions are determined by parameters that constitute a genetic interconnectivity matrix (Fig. 1B). Each effect of a specific transcription factor on a target gene is described by a single element of this matrix. Negative parameter values represent repression, positive values activation.

We find estimates for each parameter value—and thus for the topology of the gene network—by fitting the model to quantitative segmentation gene expression data[57-62] (Fig. 1B). This is achieved by numerically calculating expression patterns from the model, and then evaluating the sum of squared differences between model output and expression data for each gene, nucleus and time class for which we have data. This sum is then minimized by changing parameter values and either retaining or discarding the new set of parameters depending on criteria determined by a suitable numerical optimization method.[15,19,63] The result of this process is a gene circuit—defined as a specific set of parameter values—which have been extracted from the data by the fitting procedure.

Gap gene circuits

Our recent analysis of the gap gene network illustrates how gene circuits can be used to gain new insights into a system that has been studied extensively using traditional experimental approaches.[16,17,20] This analysis has yielded a much more dynamic picture of the gap gene system than previously thought. It demonstrated that there are two distinct phases of gap gene regulation. First, early gap domain boundaries are established by maternal factors.[20] Although timing of expression and boundary positions of these early gap domains vary considerably between embryos, averaged boundary positions remain constant over time at this stage.[20,64] Second, the onset of gap–gap cross-regulation leads to sharpening, refinement and systematic shifts in the positions of gap domain boundaries[16,17] (Fig. 2A). Thereby, activation by maternal factors is only a prerequisite for zygotic repressive boundary control, which counteracts broad activating inputs in a spatially specific manner.[17] Both phases of regulation are required for the correct positioning of gap domains. However, the viability of maternal *hb* mutants,[65–67] together with the increasing precision of segmentation gene expression over time.[68,69] suggest that—to some extent—the second phase is able to suppress variation in the first.

Our study confirmed earlier results indicating that the basic pattern of gap gene expression in staggered 'alternating cushions' of mutually exclusive expression domains (Hb/Kni, and Kr/Gt; Fig. 2A) is due to strong mutual repression between these genes,[43,70,71] while Tll represses gap gene expression at the posterior pole[42,43,72] (Fig. 2B). In addition, there is a second layer of repressive interactions between those gap genes that show significant overlaps between their expression domains[40,42,43,72–74] (Fig. 2A). Our analysis revealed that these interactions show a clear A-P asymmetry focused on the central *Kr* domain[16,17] (Fig. 2A,C). Posterior of that domain, these interactions constitute a spatial cascade of asymmetric repressive feedback loops with posterior dominance. Thereby, the absence of repression by their anterior neighbors allows gap genes to be activated in the posterior part of that neighbor's domain, where they initiate repression in turn (see, for example, *kni* and *gt* in Fig. 2A). This is made possible by the concomitant anterior shift of each gap gene's complementary repressor (e.g. Kr in the case of *gt*). Such repressive invasion of the anterior neighbor's territory causes systematic anterior shifts in the regions of active gap gene

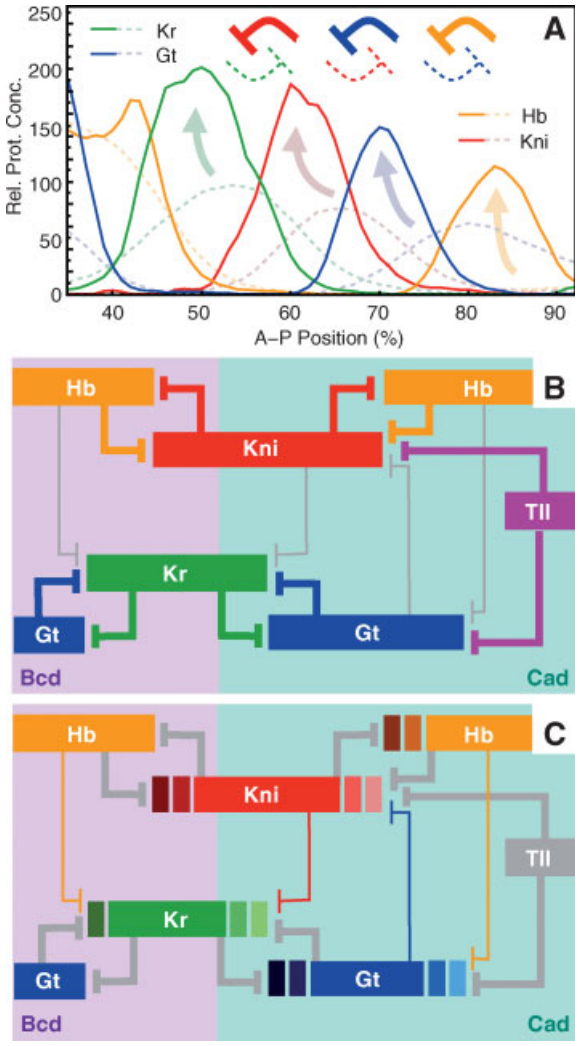

Figure 2. Summary of our dynamic analysis of the gap gene network. **A:** Gap gene expression patterns at early (dashed lines) and late (solid lines) blastoderm stage (taken from the FlyEx database: http://urchin.spbcas.ru/FlyEx). The horizontal axis represents A-P position (where 0% is the anterior pole), the vertical axis relative protein concentration. Arrows indicate sharpening, refinement and shifts of gap domain boundaries. T-bars illustrate that asymmetric repressive feedback with posterior dominance between overlapping gap genes is responsible for the boundary shifts. **B:** 'Alternating cushions' mechanism for maintenance and refinement of gap domain boundaries during cycle 14A. **C:** Asymmetric repression leading to anterior shifts of gap domain boundaries posterior of the central *Kr* domain. Protein expression domains of *hb* (orange), *kni* (red), *Kr* (green), *gt* (blue) and *tll* (magenta) are shown schematically. Anterior is to the left. Shifts in domain boundaries are indicated by bars of increasing color intensity toward the direction of the shift. Background color represents main maternal activating inputs by Bcd (purple) and Cad (turquoise). T-bar connectors represent repressive gap–gap interactions.

expression with reference to their respective protein domains, which in turn leads to the observed anterior shifts of the protein domains themselves.[16]

The shift mechanism described above does not depend on diffusion of gap proteins between neighboring nuclei and relies exclusively on gap–gap cross-repression.[16] It therefore alters positional information provided by diffusible maternal factors through a mechanism that is based entirely on zygotic regulatory feedback within each blastoderm nucleus. This is inconsistent with the role of Bcd as a classic morphogen.[10,28]

Dynamic positional information

Wolpert's positional information and the modern morphogen concept both require a clear distinction between the establishment and the interpretation of positional information. They implement a static coordinate system, which is imposed on a purely passive target tissue, presumably at a particular moment in developmental time after the morphogen gradient has reached steady state. It is possible that such a mechanism underlies the positioning of gap domain boundaries which do not shift over time—such as the posterior boundary of the anterior *hb* domain.[7,16,41,75] In contrast, our analysis strongly suggests that this conceptual framework is inadequate to explain the dynamic, feedback-driven positioning of expression domain boundaries for *Kr, kni* and *gt* in the posterior region of the embryo.

The first complication is that there is no single morphogen gradient in the *Drosophila* blastoderm. Maternal gradients of Bcd and Hb synergize through an unknown molecular mechanism in determining boundary positions of target gene expression domains.[12,28,76] This is not simply equivalent to a bipolar gradient system as described by Wolpert.[4] Although Bcd and Hb gradients are established with regard to distinct, anterior and posterior points of reference,[10,41,77–79] they form gradients of equal anterior polarity and thus cannot convey positional information through the ratio of their respective concentrations. Moreover, Cad does not contribute at all, since its graded distribution depends on Bcd and, furthermore, does not appear to convey any positional information to its target genes in any case.[20,80,81]

More importantly, positional information in the blastoderm embryo is fundamentally not static. There is no single moment in time that defines *the* coordinate system determining domain boundaries of segmentation gene expression. Instead, positional information is in constant and rapid flux. For a large majority of gap domain boundaries, concentration levels of maternal gradients are not directly correlated to boundary positions of target gene expression domains. Thus, positional information in the *Drosophila* blastoderm cannot be reduced to a single, constant positional value represented by maternal morphogen concentration, but becomes increasingly dynamic and combinatorial over time. While the positions of early gap domain boundaries are determined by maternal factors

alone,[20] these boundaries then become sharpened and shifted by zygotic gap–gap cross-regulation.[16,17] This happens in a manner independent of maternal gradients.

Maternal gradients and gap domain boundaries together go on to determine the positions of pair-rule stripes, which show dynamic shifts similar to those observed for gap domain boundaries.[62] It is reasonable to assume that such gap and pair-rule shifts affect the positioning of segment-polarity stripes, and hence the positioning of parasegmental domain boundaries.[56]

In summary, positional information in the *Drosophila* blastoderm can be said to consist of dynamically changing combinations of maternal and zygotic protein concentrations, depending not only on maternal morphogens but also on shifting positions of segmentation domain boundaries due to zygotic downstream gene regulatory interactions. This implies an active, rather than a passive, mode of gradient interpretation and blurs the distinction between establishment and interpretation of positional information.

The return of the Turing morphogen

Based on the above, it is apparent that the modern morphogen concept is too restrictive to be usefully applied to segment determination in the *Drosophila* blastoderm. Results presented here, and the fact that spatial precision of segmentation domain boundary positions increases throughout the blastoderm stage[68,69] are certainly not interpretable within the framework of the modern morphogen concept. Even worse, it can be misleading to assume that morphogens must be responsible for threshold-dependent determination of multiple downstream gene expression boundaries. We suspect that this may have affected interpretation of mutant expression data, which led to controversial claims about Hb and Kr being morphogens (in the strict, modern sense), since they were thought to determine other gap domain boundaries by activation thresholds.[72,82–84] In these studies, alternative explanations based on gap–gap cross-repression were neglected although they are equally or more consistent with experimental evidence.[17]

Therefore, we suggest a return to a morphogen concept that is closer to Turing's original definition. It includes any chemical substance whose inhomogeneous distribution in a developing tissue affects differential states of downstream gene expression in a concentration-dependent manner. This definition consciously excludes any details on the regulatory mechanism responsible for the effect on target gene expression. It may involve activation or repression of target genes, and may occur in combination with other morphogens or gene regulatory interactions within target cells. In fact, this definition includes downstream factors, such as gap, pair-rule and segment-polarity genes, which are expressed in spatial patterns more complex than gradients. In this view, positional information is no longer seen as a coordinate system encoded by a single morphogen, but rather as a complex, dynamic process involving varying combinations of morphogen concentrations.

Evocators and the active role of target tissue

Our criticism of Wolpert's positional information not only emphasizes the context-dependence—instead of universality—of morphogen action, but also the active—rather than passive—role of target tissue in determining positional information. Very similar arguments have been made by other authors.[8] Positional information was received unenthusiastically by C. H. Waddington when Wolpert first presented it in 1968, since Waddington thought that it depended too much on the precise read-out of morphogen concentration.[5] In fact, our redefinitions of morphogen and positional information are closely related to Waddington's conceptual framework.[3,85] The requirement of specific downstream regulatory interactions for proper interpretation of positional information is equivalent to Waddington's tissue competence. Similarly, the context- and target-dependent effect of maternal morphogens is reminiscent of Waddington's concept of the evocator. Here, the emphasis lies on the target tissue, which is seen as an unstable system with the potential to enter several distinct developmental pathways upon reception of the signal conveyed by the evocator. The nature of these pathways is itself dynamic and determined by the current state of competence of the cells in the target tissue. The evocator merely acts as a trigger. Therefore, different evocators can induce very similar dynamic responses. In light of this, it is interesting to note that nematoceran flies and mosquitoes have no Bcd gradient, but show gap gene expression patterns that are very similar to those observed in *Drosophila*.[86,87]

Active interpretation of positional information was also suggested by theoretical studies of morphogen gradients.[88,89] Based on these studies, Hans Meinhardt proposed mechanisms of gradient interpretation that relieve dependence on thresholds by an active, self-organizing role of interactions among target genes. Subsequently, Meinhardt used more specific models of the gap gene system to illustrate how gap–gap cross-regulatory interactions can sharpen and stabilize boundaries of gap domains after they have been set up by maternal gradients.[90,91]

Finally, the importance of gene regulatory interactions within the target tissue for specification of positional information in the *Drosophila* blastoderm has now been confirmed by an experimental study. Molecular analyses of regulatory elements for *hb*, *kni* and the head gap gene *orthodenticle* (*otd*) suggested that concentration-dependent activation by Bcd depends on the number, arrangement and affinity of Bcd transcription factor binding sites in the respective regulatory regions.[44,75,92] However, a systematic analysis of all currently known Bcd-responsive regulatory elements failed to find any correlation between the number and affinity of predicted

Bcd-binding sites and the boundary positions of corresponding reporter gene expression patterns.[93] Instead, boundary positions depend on the presence of additional predicted binding sites for Hb and Kr. This indicates that Hb and Kr—both regulated by Bcd themselves[94–97]—are crucial for mediating Bcd's regulatory effect on its target genes.

The Drunken French Flag

The theoretical principles of positional information and morphogen gradients have been famously illustrated by a simple cartoon, the French Flag model[4,21] (Fig. 3A). This model consists of a tissue represented by a linear row of N cells. We denote cells by C_i $(i = 1 \ldots N)$. There is a source of a morphogen (with concentration M) in cell C_0 at the left-hand boundary, and a sink where the morphogen is degraded in cell C_N at the right-hand boundary of the tissue. Assuming that the morphogen diffuses from source to sink and is not degraded in cells in between, M will form a linear gradient across the tissue at steady state.[98]

Cells in the tissue are initially in identical states S_0, but can be induced to enter three mutually exclusive states S_1, S_2, S_3, which are represented by the colors blue, white and red, respectively. We now assume two thresholds T_1 and T_2 in M, such that M induces different cell states in target cells as follows: cells enter S_1, if $M > T_1$, S_2 if $T_1 > M > T_2$, and S_3, if $T_2 > M$. This results in the establishment of a tricolor pattern resembling the French flag (Fig. 3A). Positions of boundaries between different colors correspond exactly to the positions of thresholds T_k in the graded distribution of M.

Based on a simple geometrical argument, it can be shown that this simple model is capable of maintaining relative positions of thresholds and boundaries as the tissue is expanded or contracted as long as M is held constant in C_0 and C_N.[21] Note that this mechanism of size regulation will not work if a more realistic localized source, dispersed sink model is assumed where M is degraded at equal rate in all cells of the tissue.[27] This leads to an exponential gradient of M at steady state[98] as observed for Bcd in the blastoderm embryo.[10,62,68] In this more realistic model, a second posterior gradient plus a mechanism of interpretation based on ratios of concentrations of both gradients are needed for size regulation.

To summarize our argument so far, we suggest a revised French Flag model. The diagram shown in Fig. 3B now features an explicit representation of time. Similar to the traditional French Flag model,[21] initial positions of boundaries at time t_0 are determined by morphogen M in a concentration-dependent manner. Note that it may be impossible to determine time t_0 precisely due to variability in the time of initial activation of target gene expression.[20,64] Additional morphogens may be involved (not shown). Domain boundaries shift and become refined in a manner dependent on regulatory interactions in the target tissue. These boundaries do not have to be set by precise interpretation of thresholds, since initial errors in boundary position can be corrected at later stages. In this model, there is no direct correspondence between boundary positions and thresholds in M. Moreover, it has now become impossible to predict dynamics and regulatory behavior of the system based on simple, generalized geometrical arguments. In contrast to Wolpert's argument

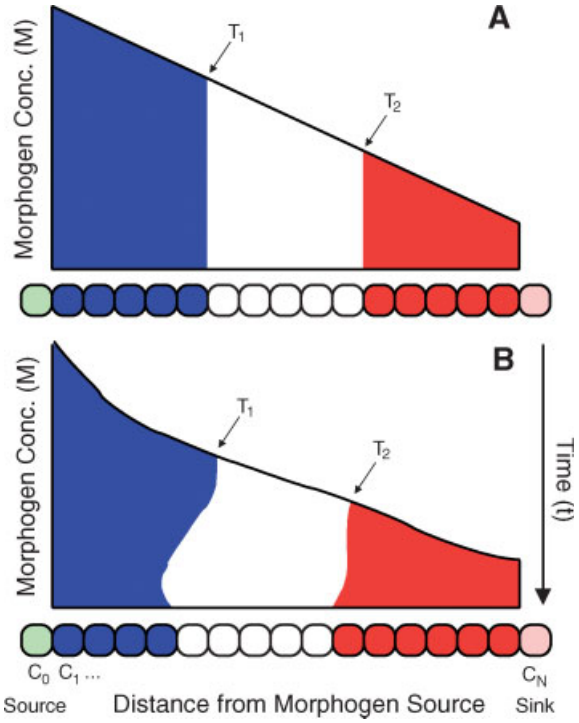

Figure 3. The Drunken French Flag. **A:** Wolpert's original French Flag model.[21] The model consists of a row of cells in a developmental field $C_0 \ldots C_N$ (bottom). Positional information is specified relative to field boundaries which coincide with the source (C_0, light green) and the sink (C_N, pink) of a morphogen. Morphogen concentration M (at steady-state) is plotted against distance from its source. Initially equivalent target cells enter distinct, mutually exclusive states based on thresholds (T_1 and T_2) in the concentration of the morphogen, establishing different territories of target gene expression (shown in blue, white and red). There is direct correspondence between thresholds T_1, T_2 and boundary positions in the target tissue. Although shown here for a cellularized tissue, the French Flag model can be, and has been applied to the syncytial *Drosophila* blastoderm.[7] **B:** The Drunken French Flag represents active and time-variable interpretation of positional information by the target tissue. Time t is shown schematically as running downwards along the vertical axis. An irregular (noisy) gradient emphasizes independence of the model with regard to gradient shape. Lopsided and curved boundaries represent temporal shifts of boundaries under context-dependent downstream control. There is no more direct correspondence between thresholds T_1, T_2 and the shifting position of domain boundaries in the target tissue.

on size regulation,[4,21] the present argument about context-dependent interpretation of positional information is not affected by the assumption of any specific profile of *M* as long as the gradient's profile remains monotonic. The Drunken French Flag model works just as well—or as badly—if an exponential instead of a linear gradient of *M* is used, and if irregularities—due to molecular noise[68]—are present (Fig. 3B). In summary, our version of the French Flag emphasizes the complexity and the unique character of each developmental field, rather than suggesting a universal regulatory mechanism of pattern formation.

Morphogenetic fields

One of the main motivations for formulating the theory of positional information was to lend more conceptual rigor to the biological field concept.[4,5,7,21] Wolpert has suggested that his definition of a developmental field is equivalent to—but both simpler and more specific than—the somewhat vague definition of the morphogenetic field.[2–4] We argue, however, that Wolpert's fields have lost important features of the original field concept. The latter relies on the complex, interacting processes occurring within the field to define its regulatory and developmental capabilities. In contrast, Wolpert's field concept considers processes occurring within the field as irrelevant for its definition. Instead, it relies on the idea of a common coordinate system, and is therefore defined by purely spatial rather than regulatory relationships.

The vagueness of the original field definition is mainly due to a lack of data on the specific molecular mechanisms and dynamical principles, which could give more precise meaning to those mysterious 'interacting developmental processes' that constitute a field. Data-driven models of developmental processes—such as gap gene circuits—are now able to provide some of that missing specificity. They suggest regulatory mechanisms for spatial pattern formation that are testable and consistent with experimental evidence.[17] However, they go beyond what is attainable by traditional experimental approaches. Mathematical models allow us to keep track of the many simultaneous regulatory processes and feedbacks occurring in a field, and to cope with the complexity of intact, wild-type developmental systems. It is difficult to imagine how we could have unraveled the nested regulatory feedback loops that cause dynamic shifts in gap domain boundaries without the help of computational modeling.[16,17] This is an important methodological advance, since it enables us to link the dynamical properties of an intact morphogenetic field to specific regulatory mechanisms in a way that is difficult to achieve by traditional experimental means.

Conclusions

One major problem with concepts such as fields, morphogens and positional information is that they can be, and have been, used in many different, often inconsistent ways. In particular, our use of positional information to describe a complex, dynamic process is so different from its original definition that the question arises whether it should not be replaced by a new term. We are not sure that this is justified at this point. The basic meaning of positional information is that it provides cells with a measure of where they are within a field. This is still true for dynamic positional information in the blastoderm embryo, where boundaries of gene expression domains reflect the subsequent morphological subdivision of the embryo into segments. These shifting boundaries can thus be said to convey positional information[14] although they are not equivalent to a simple coordinate system.

The argument that we have presented is hardly new. Positional information and morphogenetic fields have been contrasted and criticized many times based on evidence from the existing experimental literature[2,4,7,8,99] In contrast to these earlier studies, we have argued that data-driven computational models can provide evidence that is qualitatively different from that available in the literature, since they enable us to study the specific dynamic principles of whole morphogenetic fields. We believe that knowledge of such specific regulatory mechanisms is an essential prerequisite for uncovering potential general principles of development.

Here we have restricted ourselves to one specific example of a morphogenetic field. For two main reasons, we are confident, however, that our conclusions are relevant to a wider range of developmental processes. First, the dynamic interpretation of maternal gradients in the syncytial blastoderm embryo occurs autonomously within each nucleus.[16] Therefore, equivalent mechanisms could easily occur in cellularized embryonic tissues. Second, we have demonstrated how one of the most important examples of a developmental process thought to be governed by strictly instructive and hierarchical developmental signals in fact relies on regulative feedback. This suggests that static metaphors—such as that of an embryonic coordinate system—are of limited use and that the fundamentally dynamic nature of all developmental phenomena should be reflected in the concepts, and methods, used for the study of embryogenesis.

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