CHAPTER SEVEN

Dynamic positional information: Patterning mechanism versus precision in gradient-driven systems

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Abstract

There is much talk about information in biology. In developmental biology, this takes the form of "positional information," especially in the context of morphogen-based pattern formation. Unfortunately, the concept of "information" is rarely defined in any precise manner. Here, we provide two alternative interpretations of "positional information," and examine the complementary meanings and uses of each concept. Positional information defined as Shannon information helps us understand decoding and error propagation in patterning systems. General relativistic positional information, in contrast, provides a metric to assess the output of pattern-forming mechanisms. Both interpretations provide powerful conceptual tools that do not compete, but are best used in combination to gain a proper mechanistic understanding of robust patterning.

While we feel information theory is indeed a valuable tool in providing fundamental insights into the nature of communication problems, it is certainly no panacea for the communication engineer, or a fortiori, *for anyone else.*

***Claude Shannon,* The Bandwagon, *1956*.**

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1. Introduction

The use of the term "information" is "strikingly prominent" in contemporary biology (Godfrey-Smith, 2007). Broadly speaking, it is motivated by the idea that biological activities—such as perception, cognition, and signaling—are best understood in terms of information processing and representation (Godfrey-Smith & Sterelny, 2007). In evolutionary biology, inheritance can be treated as the flow of information from parents to offspring (Godfrey-Smith & Sterelny, 2007). In cell and developmental biology, it has come to reflect the common notion that genes exert their causal effects by carrying information about their products and the phenotypic traits that result from their expression (Godfrey-Smith, 2007; Griffiths, 2001; Maynard Smith, 2000). This view originated with Erwin Schrödinger's book "What is Life?" (1944) in which he postulates a "codescript" underlying order in biology. It became firmly entrenched with the elaboration of the genetic code (Godfrey-Smith, 2007). Since then, it has taken on some very strong meanings, such as the view that genes are "made of information," that phenotypic traits are "encoded" by genes, or that regulatory processes can be viewed as the execution of a "genetic program" (Godfrey-Smith, 2007). Through the use of such computational metaphors, "information" has become a fundamental concept in biology.

But strictly speaking, "information" only applies to the genetic code, where DNA sequences can be said to encode RNA and protein products in a well-defined sense (e.g., Griffiths, 2001).[1] All other talk about information in biology uses the term vaguely, either denoting some kind of correlation between observables, or remaining entirely at the metaphorical level. This is one of the main reasons why the use of the concept has been heavily criticized. Sarkar (1996, p. 187), for example, observes that "there is no clear, technical notion of 'information' in molecular biology. It is little more than a metaphor that masquerades as a theoretical concept and (…) leads to a misleading picture of possible explanations in molecular biology." Similarly, Griffiths (2001) calls information "a metaphor in search of a theory," while Longo, Miquel, Sonnenschein, and Soto (2012) deny outright that information is a proper observable for biology, quoting Godfrey-Smith and Sterelny (2007), which state that "enthusiasm for

[1] Alternative splicing and RNA editing complicate the picture, but do not alter the fact that there is a strong and relatively straightforward correlation between DNA and RNA sequences.

information in biology has been a serious theoretical wrong turn" as it "fosters naive genetic determinism." We will encounter this particular problem again when we discuss information in the context of developmental biology.

At the heart of this conceptual confusion lies the fact that "information" can be defined and applied in two very different ways. In its weaker and more clear-cut sense, information measures contingent but non-accidental correlations between a signal and its source. This sense of the term originates with Claude Shannon's theory of information, which is concerned with the accuracy of signals transmitted through some kind of channel (Shannon, 1948). Shannon information is sometimes (rather confusingly) called causal information (Griffiths et al., 2015). This notion of information is counter-intuitive and has several important limitations. First of all, Shannon information is highest when the signal is random. It is not meant to capture the quantity of *meaningful* information contained in a signal. Because of this, Shannon information cannot convey false information. It cannot misrepresent, as happens, for example, with the transmission of a deceitful signal, or the misexpression of a gene during development (Godfrey-Smith, 2007). Moreover, Shannon information flows both ways: we can learn as much about the source from the signal as the other way around. However, information flow is asymmetric in most biological contexts: genes are inherited from parents to offspring only, development unravels genomic information over time, and inductive signaling conveys information from the inducer to the target tissue.

This is why "information" in biology is often used in a richer semantic or intentional sense of the term. In this sense, "information" is about the content or meaning of a signal. The richer semantic concept is required if we are to move from a mere description of correlations to a causal-mechanistic understanding of biology, for instance, if we want to explain *how* a particular tissue interprets an inductive signal, *how* a particular pattern is generated during development, or *how* a character trait has originated, been modified, and inherited across generations in evolution (Godfrey-Smith & Sterelny, 2007).[2] Only semantic information can distinguish between true and false signals (e.g., honest or dishonest advertising during mating rituals), or correct (wild-type) development and aberrations induced by genetic or

[2] Even a causal-mechanistic understanding of translation and transcription—the very processes that underlie the genetic code—requires a semantic notion of "information" since many aspects of these processes (e.g., which tRNA matches which particular amino acid) are evolutionarily contingent.

environmental perturbations. In return, semantic information poses some formidable conceptual problems of its own. How do we define "meaning" in a context without intentionality or a conscious interpreter? Meaning for whom? What is the signal about? How is its normativity, its proper function, defined and how did it come about? If we are to take semantic concepts seriously in biology—and not interpret them as merely metaphorical—then we need answers to these questions.

One way to interpret meaning in biology is through a teleosemantic (or teleofunctional) approach (reviewed in Godfrey-Smith & Sterelny, 2007). It provides a reductive explanation of semantic information as derived from evolution. Genes semantically specify their wild-type products and traits because their function has been adapted to this task by natural selection.[3] In this sense, adaptive evolution results in genes that truly represent the traits they are involved in generating. Meaning depends on an etiological account of function, which derives from evolution (Wright, 1973).

Unfortunately, there are many problems with this approach. It is overly adaptationist, assuming that all traits represented by genes have a proper function. Moreover, it cannot account for exaptations, and other contingent or opportunistic features of evolution. Finally, it ignores the fact that evolution at the genetic, gene network, morphological, and behavioral levels can often be quite dissociable (Needham, 1933; see also DiFrisco & Jaeger, 2019; Von Dassow & Munro, 1999). What is functionally conserved at one level may not be at another.

An alternative and complementary way to interpret meaning and function in biology is by examining how a particular process contributes to the overall self-maintaining dynamics of a living system. This is the systemic or organizational approach to function (Cummins, 1975; Mossio, Saborido, & Moreno, 2009). In this framework, the meaning or function of a process, trait, or signal is determined by its contribution to the continued and healthy survival of an organism.

In what follows, we will focus on the specific problem of "positional information" in developmental biology and how it is used to study pattern formation by morphogen gradients (Wolpert, 1968, 1969, 1989, 1994, 1996; see, Briscoe & Small, 2015 and Green & Sharpe, 2015, for recent reviews and contextualization). We provide a short historical overview on definitions and applications of the term, and examine how they correspond

[3] This mirrors the approach which claims to use teleonomy—purposive function evolved by natural selection—to explain teleological aspects of organisms (Mayr, 1965; Pittendrigh, 1958).

to either one of the two basic meanings of information introduced above. We discuss the use of Shannon information theory to study error propagation and precision in gradient-based pattern formation. We compare this to more semantic, causal-mechanistic, approaches to the same problems, and show how the two perspectives can complement each other to yield a deeper understanding of robust developmental patterning. Finally, we discuss how discussions about function inform our view of the evolution of positional information, and provide a number of suggestions for future research directions in the field.

2. Positional information and developmental biology

Information talk first appears in the field of developmental biology through the invention of the "genetic program" metaphor, which historically originated in two convergent ways (reviewed in Peluffo, 2015). On the one hand, Jacob and Monod (1961) noted that the logic of transcriptional regulation—in bacteria, and presumably also in other organisms—provides the basis for a "co-ordinated program of protein synthesis and the means to control its execution" (Peluffo, 2015, p. 686).[4] On the other hand, Mayr (1961) stipulated that such a genetic program shaped by natural selection can explain the apparent goal-orientedness and function of the organismic traits and behaviors it determines. In this sense, informational metaphors were seen as a solution to the problem of teleology in biology (see our discussion of the teleosemantic approach in the Introduction). Primarily, however, the program metaphor represents the idea that the genome encodes a complete set of instructions for the construction of an organism under the influence of a given environment (see DiFrisco & Jaeger, 2019, for a detailed criticism of this idea).

A few years later, Apter and Wolpert (1965) review some less well-known efforts to apply information theory directly to the development of an organism. They compare a naïve approach, which literally considers development as a communication channel between embryo and adult, and a more sophisticated, program-based approach inspired by the arguments of Jacob, Monod, and Mayr. The paper focuses on the problem of preformationism: is it realistic, or even possible, that the egg contains a sufficient amount of information or instructions to determine the adult

[4] This idea closely resembles Schrödinger's (1944) "hereditary code script," but Jacob and Monod do not seem to have been aware of Schrödinger's book and do not cite it in their publication (Peluffo, 2015).

phenotype? This question is easy to answer for the naïve approach. Several authors in the late 1950s and early 1960s had attempted to estimate the (Shannon) information content of successive developmental stages, from egg to the full-grown organism. Although afflicted by much uncertainty and serious controversies over what to measure, they conclude that the information content of the adult must be orders of magnitude higher than that of the egg. Therefore, development cannot be treated directly with information theory, as Shannon information can never increase between source and signal (Apter & Wolpert, 1965). Much later, Developmental System Theory (DST) returns to this argument, and bases its criticism of informational approaches to development on this fundamental point (see, for example, Griffiths & Gray, 2001; Griffiths & Stotz, 2018; Oyama, 1985).

The case of the genetic program is more difficult to judge. Apter and Wolpert (1965) note that much of the information increase from egg to adult is due to the increasing complexity of spatial organization. Earlier authors (especially Monod) had neglected this rather obvious problem when comparing bacteria to elephants. This raises two important questions. First, how much of this information is redundant, meaning that it does not require an explicit representation in the egg? And second, how do we measure aspects of complexity, which go beyond the capabilities of Shannon's information theory? Apter and Wolpert (1965) focus on the first of these questions asking how a "program for development" could implement increases in spatial complexity.

As a consequence of this discussion, Wolpert (1969) develops the notion of "positional information" through a simple conceptual model of gradient-based patterning, the French Flag (Wolpert, 1968), which is intended to illustrate how spatial information can be "encoded" in a developmental program (Fig. 1A). The gradient-based French Flag model consists of a one-dimensional tissue, made up of a row of cells. At one end of the tissue, there is a source of diffusible morphogen; at the other end, there is a sink, where the morphogen molecule gets degraded. If there is no morphogen production or degradation in between source and sink, diffusion will generate a linear concentration gradient of the morphogen across the tissue (Fig. 1A). This gradient is then read out by its target cells. If above or below specific thresholds of morphogen concentration, a target cell will activate alternative sets of genes that determine its future fate. To distinguish it from a general inductive signal, the morphogen gradient must span at least two such thresholds, leading to three (or more) distinct territories of gene expression in the target tissue, colored blue, white, and red in case of the French

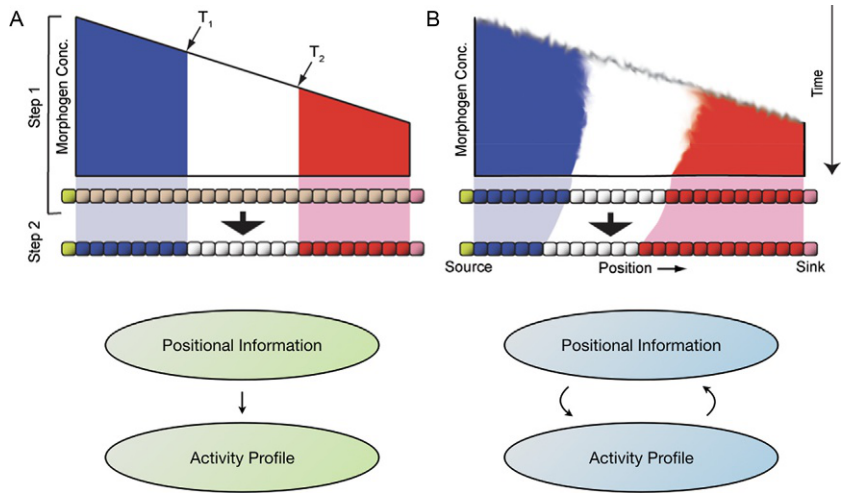

Fig. 1 The French Flag Model and Positional Information. (A) Wolpert's original gradient-based French Flag Model (1968). A morphogen diffuses from its source (green) across a tissue toward its sink (pink), where it is degraded. Concentration thresholds in the resulting spatial gradient (T1 and T2) are read out by cells in the tissue, leading to the establishment of different territories of target gene expression (blue, white, and red). This model treats development as a two-step process: first, positional information is imposed on the target tissue by the morphogen gradient (step 1). Later, this information is interpreted by cells in the tissue leading to distinct pathways of differentiation (step 2). Concentration thresholds in the gradient correspond exactly to borders of expression territories. Downstream activity profiles are determined by the morphogen in a feed-forward manner. (B) A revised French Flag, incorporating target domain shifts and increasing precision over time. Both depend on feedback regulation involving target genes. In this revised model, which is now explicitly dynamic, there is no longer a precise correspondence between concentration thresholds in the gradient and the final position of target domain boundaries. Positional information now explicitly depends on feedback regulation from downstream activity. *Simplified from Jaeger, J., Irons, D., & Monk, N. (2008). Regulative feedback in pattern formation: Towards a general relativistic theory of positional information. Development, 135, 3175–3183 and Jaeger, J. (2011). The gap gene network. Cellular and Molecular Life Science, 68, 243–274.*

Flag (Fig. 1A). The pattern scales across variable tissue sizes as long as the strength of the source and the sink are held constant. In this model, morphogen concentration is said to "encode positional information," which can be decoded by the target cells to reduce their uncertainty about where in the tissue they are located (Wolpert, 1969). In this sense, "positional information" provides a static and feed-forward coordinate system, which is imposed onto the tissue by the signal encoded in the spatial distribution of the morphogen gradient.

Wolpert (1969) argues that positional information is a "mechanism" according to which cells in a developing tissue have their position specified in relation to one or more reference points in the system (the source and the sink at the boundaries of the tissue). Cells that have their positional information specified with respect to the same points constitute a field. This reduces the classic embryological concept of the morphogenetic field to a "mechanism" represented by the genetic program in a spatial patterning context. Importantly, specification occurs before, and is disconnected from, subsequent cellular and morphological differentiation. Spatial pattern formation is seen as an essentially two-step feed-forward process. This allows Wolpert (1969) to speculate that positional information may be universal between different lineages of organisms, a view he later saw confirmed by the discovery of conserved Hox gene clusters and their expression across animal phyla (Wolpert, 1994, 1996).

The discovery of molecular gradients involved in patterning a wide variety of developmental systems led to the widespread adoption of the term "positional information" far beyond its originally intended scope. While some of its use can be precisely defined in the context of gradient-based patterning (see, for example, Briscoe & Small, 2015), most of it remains vague and metaphorical, indicating a general sense that "cells know where they are in a developing embryo." This has led to controversies and discussions analogous to those concerning the use of "information" in biology in general. Here, we will focus on two recent attempts to make the term precise: on the one hand, work on patterning precision that interprets "positional information" in terms of Shannon's theory; and on the other hand, work on the mechanisms of patterning dynamics that provides an alternative interpretation of "positional information," not as a mechanism, but as a metric for embryonic patterning. This metric is an epiphenomenon of the underlying patterning dynamics.

3. Precision in patterning: Positional information as Shannon information

Positional information, as originally defined by Wolpert in 1969, proposes that spatial asymmetry in a signal can be used by cells to determine their relative position in a tissue. Concentration thresholds in the gradient determine boundaries between distinct territories of gene expression (see Fig. 1A). 20 years later, Wolpert's model received a great boost in popularity with the discovery that a spatial gradient of the Bicoid (Bcd) protein

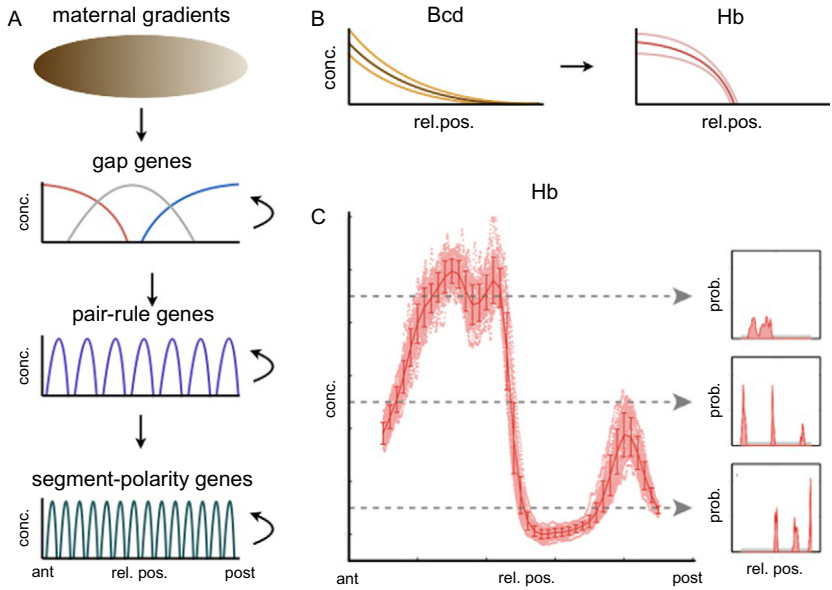

Fig. 2 Error propagation through the segmentation gene network in *Drosophila*. (A) The blastoderm-stage embryo of *Drosophila melanogaster* is patterned through hierarchical interactions among regulatory layers of the segmentation gene network. Regulatory input is provided by maternal morphogen gradients such as the Bicoid (Bcd) protein gradient (shown in brown, projected onto a schematic embryo). Maternal gradients regulate expression of the zygotic gap genes. Maternal and gap genes together then generate the periodic 7-stripe patterns of pair-rule genes which, in turn, activate the segment-polarity genes, whose 14-stripe patterns form a molecular prepattern for the process of morphological segmentation, which occurs later in development. Curved arrows indicate cross-regulation among members of each hierarchical layer in the network. (B) Bcd (brown) activates early expression of the gap gene Hunchback (Hb, red) in a concentration-dependent manner. Fluctuations in Bcd and Hb concentrations are indicated by bright lines. (C) Positional information as Shannon information: the main graph shows measured Hb expression data (dots), with the average activity profile (red line) and error bars indicating standard deviations. Dashed arrows indicate specific concentration levels of Hb. Small graphs show the probability (prob.) of being at a certain position, given a specific concentration of Hb. From these distributions, the mutual information between concentration and relative position can be calculated. In this way, positional information can be shown to correspond to Shannon information (see text). (C) Is highly simplified from Dubuis, Samanta, and Gregor (2013)). Unless indicated otherwise, graphs in all panels show relative protein concentration levels (conc.) plotted against relative position (rel. pos.) along the anteroposterior axis: anterior (ant) is to the left, posterior (post) to the right.

determines position in a concentration-dependent manner in the early blastoderm embryo of the vinegar fly *Drosophila melanogaster* (Fig. 2A) (Driever & Nüsslein-Volhard, 1988a, 1988b; Struhl, Struhl, & Macdonald, 1989). Bcd is a prototypical example of what Turing (1952)

had called a morphogen (a form-giving substance; see also Jaeger & Reinitz, 2006). This experimental work established a causal link between morphogen gradient distribution and the resulting fate map of the embryo. But it did not yet provide a detailed quantitative characterization or mechanistic explanation of the intermediary developmental processes. These processes are governed by hierarchical regulatory interactions between the layered components of the segmentation network: gap, pair-rule, and segment-polarity genes (Fig. 2A) (Akam, 1987; Ingham, 1988).

Well-nigh another 20 years had to pass before the methodological advances required to start addressing these issues became available. Gregor, Wieschaus, McGregor, Bialek, and Tank (2007) provide the first detailed experimental characterization of the temporal dynamics, reproducibility, and precision of the Bcd gradient. The authors estimated the precision limit of positional information encoded by the Bcd gradient in the presence of diffusion-induced noise (Gregor, Tank, Wieschaus, & Bialek, 2007). The detected amount of spatial precision in the gradient was remarkably high, very close to the predicted theoretical limit. Even more unexpected, however, was the precision with which Bcd positions the expression domain boundary of one of its targets, the gap gene *hunchback* (*hb*) (Fig. 2B). In fact, the measured levels of precision for the *hb* domain boundary are not compatible with purely feed-forward regulation by Bcd alone. In the absence of other candidate maternal inputs, Gregor, Tank, et al. (2007) invoked the possibility that nuclei in the blastoderm embryo must be able to perform spatial averaging when measuring Bcd concentrations. This mechanism reappears in later theoretical work (Hillenbrand, Gerland, & Tkačik, 2016; Sokolowski & Tkačik, 2015), but is currently not supported by any experimental evidence.

Be that as it may, these results do suggest a surprisingly tight level of control very early in the patterning process. This is further corroborated by quantitative measurements of maternally deposited *bcd* mRNA in *Drosophila* wild-type and mutant embryos, which reveal that fluctuations in the levels of mRNA are as small as those measured for Bcd protein, and that the amount of mRNA is directly proportional to *bcd* gene dosage in the mother (Petkova, Little, Liu, & Gregor, 2014; see also Liu, Morrison, & Gregor, 2013). This raises the possibility that developmental precision and reproducibility in the *Drosophila* embryo is controlled, or at least initiated, by the mother.

One central aspect of the quantitative work discussed so far is the attempt to precisely measure the amount of positional information encoded in an

observable gene expression pattern. How is this achieved? Dubuis, Samanta, et al. (2013) introduce a quantitative framework, which is further developed in Tkačik, Dubuis, Petkova, and Gregor (2015). As a first step, Dubuis, Tkačik, Wieschaus, Gregor, and Bialek (2013) generated a dataset, in which the concentrations of all four trunk gap genes—*hb*, *Krüppel* (*Kr*), *knirps* (*kni*), and *giant* (*gt*)—were measured simultaneously by immunofluorescent staining in the same embryo. By a careful dissection of experimental versus biological "errors," this allowed the authors to quantify relative expression levels as well as (co-)variation of expression patterns. To capture the positional information present in each pattern, Dubuis, Samanta, et al. (2013) assess entropy (uncertainty) reduction in each blastoderm nucleus by quantifying the mutual information between morphogen concentration and spatial position (Fig. 2C).

Mutual information is one of the central quantities in information theory (Shannon, 1948). Shannon proposed his theory as a means to quantify communication through a noisy transmission channel. As an input, a message is encoded by a transmitter. This message is then sent down the channel and decoded by the receiver, providing the read-out or output of the system. The channel is noisy: information only decreases, but never increases during transmission. In his theory, Shannon introduced the concept of entropy as a measure of uncertainty in a random variable. He defined mutual information as a measure of the statistical dependence between two random variables, in the case of the transmission channel, its input and output. More specifically, mutual information measures the reduction in the uncertainty (or entropy) of the output given a noisy measurement (or transmission) of the input.

Mutual information is extremely useful and broadly applicable. This has several reasons: first, it accounts for both linear and non-linear correlations between variables. Second, it is independent of the units of measurement, always being measured in bits (roughly, the number of yes/no questions that would need to be answered to account for the measured reduction in uncertainty/entropy). Finally, mutual information is symmetric. It does not depend on the direction of the flow of information. We can predict the state of the output, given a certain input, but we can also estimate the state of the input, given a certain output.

By formally equating positional information to mutual information between morphogen concentration and spatial position, Dubuis and colleagues enable the straightforward application of Shannon's theory to pattern formation (Fig. 2C). We can now measure the positional information contained in any observed morphogen distribution. Individual gap gene

profiles, for example, contain approximately two bits of information (*hb*: 2.25 bits; *Kr*: 1.95 bits; *kni*: 1.75 bits; *gt*: 1.84bits; Dubuis, Samanta, et al., 2013). Interestingly, this is almost twice the amount of information that would be carried if gap genes were simple on/off switches: graded concentration profiles obviously matter. Together, all four gap genes encode 4.27 bits of information, evenly distributed along the anteroposterior axis of the embryo, which is sufficient to convey a unique identity to each nucleus based on its particular spatial position (Dubuis, Samanta, et al., 2013; Petkova, Tkačik, Bialek, Wieschaus, & Gregor, 2019).

Unfortunately, there is something not quite right with this simple picture of tight maternal control with subsequent feed-forward transmission of positional information. When the amplitude of the Bcd protein gradient is perturbed experimentally, the resulting changes in downstream gene expression are corrected, or canalized, toward the wild-type state (Liu et al., 2013). Generally, the precision of gap and pair-rule expression increases and becomes more independent of maternal inputs over time (Dubuis, Tkačik, et al., 2013; Petkova et al., 2019; Surkova et al., 2008). This requires cross-regulatory interactions downstream of the maternal gradients (Manu et al., 2009a), indicating that regulatory feedback among target genes is essential for the control of patterning robustness, reproducibility and ultimately precision in the *Drosophila* blastoderm.

One way to tackle this issue is to treat the gap gene network as an optimal decoder of positional information, as first suggested in Liu et al. (2013). A later study tests this suggestion explicitly by "predicting" the position of stripe positions for the pair-rule gene *even-skipped* (*eve*) under the assumption that the positional information provided by maternal gradients is optimally decoded through the gap genes (Petkova et al., 2019). The authors show that the terminal maternal system is affecting the precision of gap genes expressed in the central region of the embryo (where terminal genes are not expressed). This leads to the claim that downstream regulatory interactions integrate different maternal inputs across the embryo, which may explain why precision at late stages is higher than predicted from feed-forward regulation by Bcd alone.

Theoretical studies provide further support that integration of multiple gradients can lead to higher precision. Applied to the opposing gradients of Bcd and the posterior gradient of Caudal (Cad) protein in the Drosophila embryo, this work found that positional information, measured in terms of maximum likelihood, was highest in the middle of the embryo and reduced toward the poles (Morishita & Iwasa, 2009, 2011).

All of this leaves us with intriguing evidence that information decoding may be optimal, and with the fact that patterning is astonishingly precise already early on in the *Drosophila* blastoderm, but without any convincing mechanistic explanation of how any of this could be achieved. The focus of all the work described so far is on feed-forward error propagation from maternal gradients to their downstream targets. This (perhaps somewhat excessive) focus may explain the necessity to invoke implausible mechanistic assumptions such as spatial averaging to account for the observed levels of precision. But are gap genes really just integrating inputs from different maternal systems, or do they actively contribute to the control of patterning precision through cross-regulatory feedback over time? The evidence we have described so far simply cannot answer that.

As we have mentioned above, Shannon-style positional information is symmetrical, and therefore agnostic concerning the flow of information in the system. Another problem is that we do not even have a proper null model to tell us what levels of precision (or what kind of error propagation behavior in general) we should expect from a heavily feedback-driven regulatory system such as the segmentation gene network. Shannon's information theory is ill-suited for this context.

The problem is that the information-based approach has been expanded from its initial application to a well-defined problem—the feed-forward regulation of early *hb* expression by its only known activator Bcd (e.g., Gregor, Tank, et al., 2007; Gregor, Wieschaus, et al., 2007)—to an overall paradigm for studying patterning in the complex regulatory context of the gap and pair-rule networks (e.g., Liu et al., 2013; Petkova et al., 2019). By doing so, has this approach transgressed its proper limits? To answer this question, we need to take a closer look at the mechanisms of gene regulation underlying pattern formation in the *Drosophila* blastoderm.

4. Patterning precision versus patterning mechanism

Measurements of expression co-variation are not only useful for measuring positional information, but also enable us to make inferences about other aspects of stability in pattern formation. Of particular interest in this regard is the claim that biological systems must be in a state of criticality to be both robust and adaptable (see Mora & Bialek, 2010). As Kauffman (1993) put it, biological systems must be poised at "the edge of chaos"—stable against small perturbations yet close to a bifurcation boundary where the behavior of the system can change drastically and abruptly.

Critical systems exhibit a number of characteristic signatures. Krotov, Dubuis, Gregor, and Bialek (2014) assume that domain boundaries can be modeled by a simple two-gene network. From these simple models, they derive four predicted signatures of criticality, which they identify in the gap gene expression data from Dubuis, Tkačik, et al. (2013). This leads them to conclude that the gap gene system is in a state of criticality all along the anteroposterior axis of the embryo (Krotov et al., 2014). Unfortunately, this work is based on a number of simplifying assumptions which call this conclusion into question. Their models only consider the interactions of overlapping gap genes (and do not even specify these in any detail), ignoring the strong repression between genes with mutually exclusive spatial domains (Jaeger, 2011). Furthermore, the analysis focuses exclusively on local patterning at boundary interfaces. Finally, the authors assume that gap gene patterns are at steady-state, which is not the case (see below). Consequently, their analysis fails to consider the transient expression dynamics of gap genes, and neglects much of the regulatory complexity of the system.

Although this work may be seen as a step toward mechanistic investigation of patterning precision, it also highlights the need for dynamic models with the relevant level of detail that have been rigorously validated against experimental evidence. Only such models will yield solid mechanistic insight. Luckily, the *Drosophila* blastoderm provides a unique opportunity for such a detailed, data-driven modeling approach (see Jaeger, 2009, 2018; Jaeger, Manu, & Reinitz, 2012). Over the past two decades, quantitative data sets of segmentation gene expression have been generated (see Ashyraliyev et al., 2009; Surkova et al., 2008, among others), that enable us to fit detailed models of gap gene regulation (Ashyraliyev et al., 2009; Crombach, Wotton, Cicin-Sain, Ashyraliyev, & Jaeger, 2012; Jaeger, Blagov, et al., 2004; Jaeger, Surkova, et al., 2004; Manu et al., 2009a, 2009b; Perkins, Jaeger, Reinitz, & Glass, 2006; Verd et al., 2018; Verd, Crombach, & Jaeger, 2017; Verd, Monk, & Jaeger, 2019).[5] The solutions resulting from these fits provide a detailed representation of the complex regulatory mechanisms governing the dynamics of gap gene expression. These mechanisms are entirely consistent with the extensive experimental evidence that is available in this system (reviewed in Jaeger, 2011).

[5] This data-driven modeling approach has also been extended to non-model species of flies (Diptera), such as the moth midge *Clogmia albipunctata* (Crombach, Garcia-Solache, & Jaeger, 2014), and the scuttle fly *Megaselia abdita* (Crombach, Wotton, Jiménez-Guri, & Jaeger, 2016).

What have we learned from these mechanistic models? The first important point to note concerns the dynamics of gap gene expression. While boundaries of gap gene expression domains remain stationary over time in the anterior, they shift toward the anterior in the posterior region of the embryo, resulting in an accordion-like narrowing and sharpening of the shifting domains (Fig. 3A) (Jaeger, Blagov, et al., 2004; Jaeger, Surkova, et al., 2004; Surkova et al., 2008). These two qualitatively different types of expression dynamics are governed by different regulatory mechanisms. Stable anterior domain boundaries result from different nuclei converging toward different attractor states in a multi-stable dynamic regime (Fig. 3A) (Manu et al., 2009b; Verd et al., 2017). For example, a nucleus may converge toward a state of high *hb* expression, or toward a state of high *Kr* expression, depending on the amount of maternal morphogen it has been exposed to. In contrast, shifting domain boundaries in the posterior result from an underlying damped oscillator mechanism (Fig. 3A) (Verd et al., 2018). Each nucleus in this region of the embryo goes through part of a stereotypical succession of gap gene expression whose temporal sequence (from *Kr–kni* to *gt–hb*) is imposed by the oscillator. Different nuclei start at different positions in this sequence—they are phase-shifted with regard to each other—depending on their maternal inputs. This leads to an apparent anterior movement of posterior domains—so-called kinematic shifts[6]—although no gap protein is actually being transported across the tissue.

A second important point, which affects our understanding of the stability of the system, is that the gap gene network never reaches steady state. Gap gene dynamics remain transient throughout the blastoderm stage (Verd, Crombach, & Jaeger, 2014; Verd et al., 2017). Even though far from steady state, gap gene regulation shows canalysed behavior in the posterior region of the embryo, as first demonstrated by Manu et al. (2009a, 2009b). Canalization means that system trajectories converge toward each other long before the system approaches its attractors. In the case of the damped oscillator, it happens because of strong repressive feedback between complementary gap genes *hb/kni* and *Kr/gt* (Verd et al., 2019). This may provide a possible mechanism for the observed increase in precision of gap gene expression over time.

[6] A Mexican wave in a soccer stadium is a good example of such a kinematic phenomenon. It originates from a temporal sequence of arm movements by each spectator, and travels around the stadium without any people actually changing position.

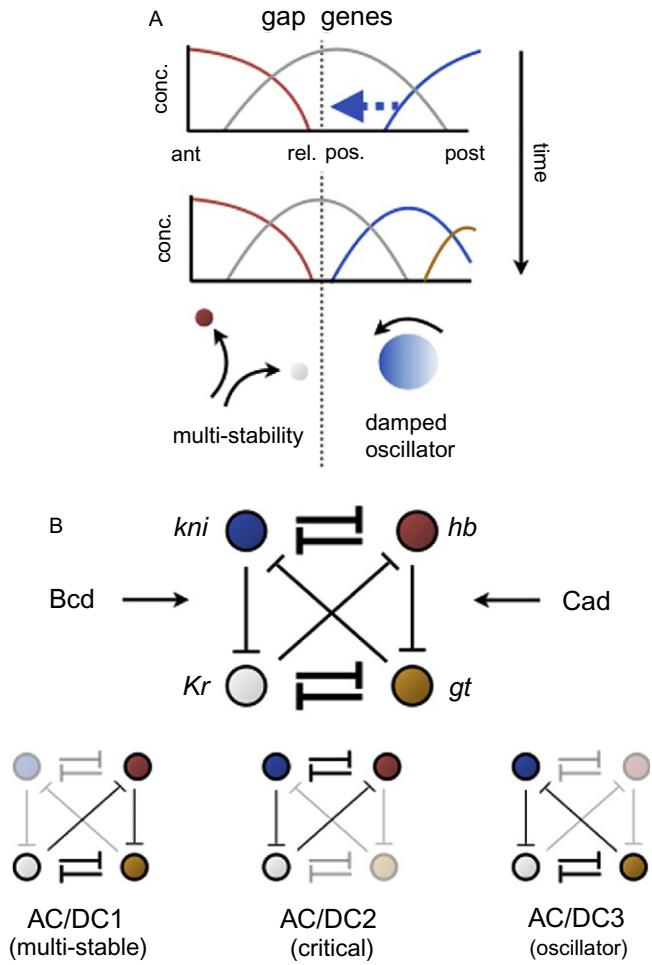

Fig. 3 Mechanisms for gap gene regulation. (A) The dynamics of gap gene expression differ between the anterior (ant) and posterior (post) halves of the *Drosophila* embryo. In the anterior, domain boundaries remain stationary while in the posterior, they shift toward the anterior over time (indicated by dashed blue arrow). Stable boundaries in the anterior are positioned by a multi-stable dynamical regime that causes different nuclei to converge toward different stable attractor states (represented as red and white circles indicating high expression of the red and white gene, respectively). Shifting boundaries in the posterior are governed by a damped oscillator mechanism, which causes nuclei to cycle through a stereotypical succession of gene expression states (only shown for white and blue here). Nuclei are phase-shifted with respect to each other, depending on their position in the embryo. This leads to the kinematic shifts of the domain boundaries in this region. (B) Schematic regulatory topology of the gap gene network (including external activating inputs by maternal gradients of Bicoid, Bcd, in the anterior, and Caudal, Cad, in the posterior). T-bars indicate repressive cross-regulatory

Our third and last point is that the gap gene system exhibits modular dynamics, even though its regulatory structure (or topology) shows no modularity at all (Fig. 3B) (Verd et al., 2019). This is because different subsets of gap genes are expressed and active in different regions of the embryo, which allows us to identify three individual subcircuits, or dynamical modules: AC/DC1 consists of *hb*, *Kr*, and *gt* and contributes to patterning through its multi-stable regime in the anterior region of the embryo; AC/DC2 consists of *hb*, *Kr*, and *kni*, and is in a critical state throughout the central region; AC/DC3 consists of *Kr*, *kni*, and *gt* and implements the damped oscillator in the posterior. Interestingly, AC/DC2 straddles the bifurcation boundary between stationary and shifting domains in the middle of the embryo (Fig. 3B). This suggests that the gap gene system is critical in the central region only, while exhibiting stable dynamics further anterior and posterior, contradicting the claim by Krotov et al. (2014) that the network is critical throughout. What kind of consequences this may have for patterning precision remains an open question.

5. General relativistic positional information (GRPI)

In contrast to statistical work based on information theory, our detailed analysis of the dynamics driven by the gap gene system provides a realistic and accurate causal-mechanistic explanation of *how* gap genes pattern the fly blastoderm. This mechanistic aspect of patterning is not in contradiction but complementary to questions of precision and error propagation. However, only the latter can be handled within the framework of Shannon's information theory. To confuse the two aspects amounts to a

interactions between gap genes *hunchback* (*hb*), *Krüppel* (*Kr*), *knirps* (*kni*), and *giant* (*gt*). Line thickness indicates relative interaction strength. The gap gene network can be subdivided into three dynamical modules: AC/DC1 is in a multi-stable regime, active in the anterior; AC/DC2 is critical, exhibiting both multi-stability and mono-stability with spiraling trajectories in the central region of the embryo; AC/DC3 is in a mono-stable regime with spiraling trajectories, active in the posterior. All subcircuits share the same network topology, but consist of distinct subsets of gap genes, with overlap between the modules. *Simplified from Verd, B., Clark, E., Wotton, K. R., Janssens, H., Jiménez-Guri, E., Crombach, A., & Jaeger, J. (2018). A damped oscillator imposes temporal order on posterior gap gene expression in Drosophila. PLoS Biology, 16, e2003174; Verd, B., Monk, N., & Jaeger, J. (2019). Modularity, criticality, and evolvability of a developmental gene regulatory network. eLife, 8, e42832. Graphs in (A) show relative protein concentration levels (conc.) plotted against relative position (rel. pos.) along the anteroposterior axis: anterior (ant) is to the left, posterior (post) to the right. See text for details.*

category error, muddling statistical with causal-mechanistic explanation (see also Calcott, Pocheville, & Griffiths, 2018). Causal-mechanistic explanation of patterning calls for a radically different, semantic, interpretation of "positional information."

The dynamic conceptual framework of "general relativistic positional information" (GRPI) (Jaeger, Irons, & Monk, 2008; Jaeger & Reinitz, 2006) provides such a semantic interpretation[7] (Fig. 1B). It treats positional specification in strictly processual terms: there is no particular point in time at which a cell becomes specified in terms of its position within the tissue. Instead, specification is seen as a dynamic process that ends with stable determination of cell fate. There is no invariable coordinate system as in the French Flag anymore. More importantly, there is no one-to-one correspondence between morphogen concentration and cell fate. Instead, the interpretation of the signal depends on the state of the target cell, dynamically changing over time (Jaeger et al., 2008). This is very close to Waddington's interpretation of morphogens as "evocators," exerting their effect dependent on the "competence" of the target cells (Jaeger & Reinitz, 2006; Waddington, 1940, 1956). Here, the role of the receiving cell is one of active, dynamic, and context-dependent interpretation. The cell needs to be ready (or "competent") for the signal to evoke an appropriate response. Therefore, the relevant content of the signal becomes fundamentally semantic and context-dependent. Moreover, the shape of the morphogen gradient itself is often altered in the process (Jaeger et al., 2008).

Instead of a statically imposed coordinate system, we now have a dialectic interaction between gradient and target tissue (Fig. 1B) that establishes a dynamic positional metric, which is not only actively interpreted but also actively altered by the receiving cells. Conceptually, this is analogous to the difference between absolute space and time in Newtonian versus dynamically malleable spacetime in Einstein's theory of general relativity (Jaeger et al., 2008). Just as spacetime geometry emerges from the interplay between massive objects with their environment, so does GRPI emerge from reciprocal interactions between gradient and tissue. In this sense, it is not a "mechanism" in the sense of Wolpert (1968, 1969), but a mere reflection, an epiphenomenon, of the underlying regulatory dynamics.

[7] Since its original publication, there has been some debate on whether GRPI should be seen as a type of "information" at all. In what follows, we will argue that it should indeed, as it can be smoothly integrated into a systemic or organizational account of function and semantic information.

Still, GRPI is a type of semantic information, since it conveys a "meaning" to the cell, namely, about its time- and context-dependent relative position in a developing embryo. Its role within the organism is to establish the proportions and relative positions of different parts and organs. This is a central contribution to the continued survival of the organism: without it, a living system could not mature to its adult stage and reproduce. Therefore, it has a clear systemic or organizational function (see Cummins, 1975; Mossio et al., 2009). Note again that this interpretation is fundamentally different to the information-theoretic analysis of error propagation, which asks how precise a pattern can be. Here, we ask instead, *how* the specific pattern of an individual organism comes to be in the first place. The first question makes no sense in the absence of the second. But only answering both questions together yields a complete understanding of how development proceeds. Yet, surprisingly, there are only very few studies that have tried to integrate the two approaches.

6. Mechanisms for patterning precision

One of the few studies that integrates patterning mechanisms and precision in the way suggested above examines morphogen-based patterning in the vertebrate neural tube (Zagorski et al., 2017). Different populations of neurons develop at different dorso-ventral positions in this growing tissue (Fig. 4A). Cell specification depends on two antagonistic morphogen gradients: one of Sonic Hedgehog (Shh) emanating from the floor plate and ventral neural tube, and another one of bone morphogenetic protein (BMP) emanating from the dorsal end of the tissue (Fig. 4A). As the neural tube grows, cells experience changing concentrations of both morphogens. As in the case of the gap gene network, cross-repressive interactions among target genes alter the response of the receiving cells over time. At this level of abstraction, patterning in the fly blastoderm embryo and the vertebrate neural tube follow very similar regulatory principles (see Briscoe & Small, 2015).

Zagorski et al. (2017) ask a very simple question: what kind of regulatory network provides the most accurate fit to the observed target gene expression patterns given an antagonistic arrangement of contrapolar morphogen gradients of Shh and BMP? What is special about this study is that the authors fit their model to both expression patterns and a decoding map based on a maximum-likelihood analysis of the system (based on the methodology of Tkačik et al., 2015). This type of analysis predicts error propagation patterns based on the assumption that the system achieves optimal decoding of the

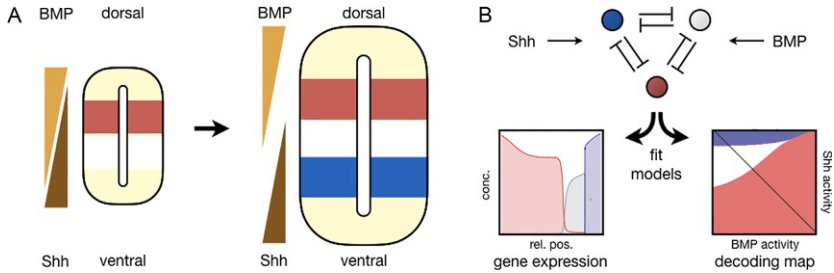

Fig. 4 Patterning and optimal decoding in the vertebrate neural tube. (A) The vertebrate neural tube is patterned by two antagonistic morphogen gradients: Sonic Hedgehog (Shh) and bone morphogenetic protein (BMP). These gradients induce different states of target gene expression (shown in red, white, and blue). As the tissue grows over time, cells experience changing concentrations of morphogens. Cross-regulatory feedback between target genes further modifies the boundary positions of expression territories. (B) Models of the response network implement various combinations of target gene interactions drawn from the fully connected topology shown here. These models are fit to both spatio-temporal gene expression data and a decoding map derived from maximum likelihood under an optimal decoding assumption. This forces fitting solutions to reproduce both the dynamics and fluctuations of gene expression correctly. The best-fitting solution across all models is achieved with a fully connected network topology. See text for details. *Panel (A) simplified from Briscoe, J. & Small, S. (2015). Morphogen rules: Design principles of gradient-mediated embryo patterning. Development, 142, 3996–4009.; Panel (B) simplified from Zagorski, M., Tabata, Y., Brandenberg, N., Lutolf, M. P., Tkačik, G., Bollenbach, T., Briscoe, J., & Kicheva, A. (2017). Decoding of position in the developing neural tube from antiparallel morphogen gradients. Science, 356, 1379–1383.*

positional information provided by the upstream morphogenetic gradients. Dynamical models, implementing various topologies of target gene networks, were fit to both gene expression data and the predicted decoding map (Fig. 4B). This approach evaluates which network topology gets closest to optimal decoding while faithfully reproducing the observed patterns of gene expression.

The network topology that emerges from this dual-aspect fitting procedure is a fully connected network with reciprocal repressive interactions between all target genes (Fig. 4B) (Zagorski et al., 2017). As long as we cannot constrain the fit with more detailed evidence on individual regulatory interactions, it may not be surprising that this is the case. One reason is methodological: the more interactions in a model, the more free parameters there are, and hence degrees of freedom for the fit. Another reason is dynamic: feedback regulation generally allows for tighter and more fine-grained control. Lastly, we cannot entirely exclude the possibility that there may be a missed alternative topology that would fit the data and

decoding map better, since the search of parameter space is not exhaustive, and the convergence to the best solution never guaranteed for such complex model-fitting procedures.

But despite all these caveats, there is progress here. First of all, this study shows, for the first time, that it is *possible* to achieve optimal decoding in a morphogen-driven patterning system, while closely reproducing the observed dynamics of gene expression. And second, it introduces an integrative methodology of mechanistic modeling combined with error-propagation analysis that should be used much more widely in the study of developmental regulation, not only in the context of morphogen-based pattern formation.

Indeed, such an integrative approach will be necessary to reconcile causal-mechanistic and information-based studies of the segmentation gene system of *Drosophila*. As it stands, our knowledge of these two complementary aspects of gene regulation in the fly blastoderm, are based on incompatible assumptions.

How patterning occurs in the gap gene system has been studied with data-driven models of the network that fit the dynamics of the observed (averaged) patterns of gene expression very closely (Ashyraliyev et al., 2009; Jaeger, Blagov, et al., 2004; Jaeger, Surkova, et al., 2004; Manu et al., 2009a, 2009b; Verd et al., 2018, 2017, 2019). These models are fully compatible with the available experimental evidence on gap gene regulation (reviewed in Jaeger, 2011), but do not reproduce the observed patterns of expression fluctuations in the system very accurately. In contrast, our knowledge of error propagation and optimal decoding in the system is based on extremely precise, quantitative measurements of expression (co-)variation, in the absence of realistic mechanistic models of the underlying regulatory dynamics (Dubuis, Samanta, et al., 2013; Dubuis, Tkačik, et al., 2013; Krotov et al., 2014; Liu et al., 2013; Petkova et al., 2019). These measurements indicate that decoding must be near optimal in the gap gene system, but we do not know *how* this is achieved at the level of regulatory mechanisms. As already mentioned, these two approaches do not compete, but represent two different sides of the same coin. The sobering truth is that, as long as they remain in contradiction, we cannot claim to truly understand all important aspects of patterning in this most carefully studied model system for pattern formation.

7. Conclusions

Here, we have argued that information-based and causal-mechanistic approaches to pattern formation seek complementary types of explanations,

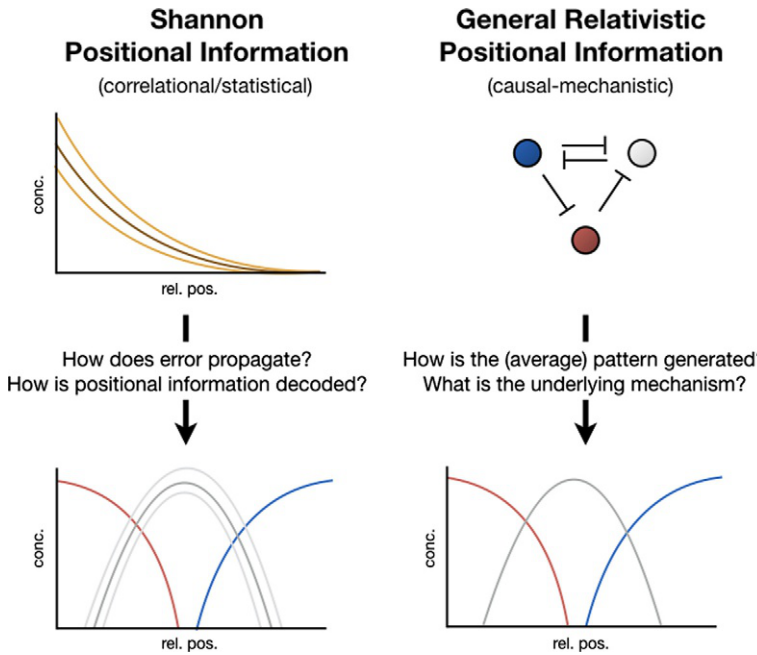

Fig. 5 Two different kinds of explanation. Shannon positional information and General Relativistic Positional Information (GRPI) are two alternative ways in which the concept of information can be made precise in the context of pattern formation. The represent two entirely different kinds of explanation, two different types of perspectives, on the same underlying pattern-forming system. See text for details.

which are of a fundamentally different nature (Fig. 5). Shannon's information theory provides powerful tools to investigate precision, error propagation and decoding of positional information in patterning systems. If we interpret positional information *sensu* Shannon, however, it no longer represents an instructive program or mechanism of pattern formation as argued by Wolpert (1968, 1969). Information-based explanations are correlational (statistical), while mechanistic explanations must be framed in causal terms. The latter require detailed experimental evidence on the relevant interactions among components of the underlying regulatory network, as well as a detailed and accurate dynamic model of the system to show how these interactions synergize to generate the observed patterning output (see DiFrisco & Jaeger, 2019).

In contrast, information-based explanations are independent of mechanistic detail (Tkačik et al., 2015). On the one hand, this can be advantageous if we are interested in a broad theory of patterning precision, which aims at

predicting rather than explaining features of regulation. For instance, an information-theoretic framework enables us to test the hypothesis that transmission of information is close to optimal decoding in different classes of regulatory networks, and asks what types of target gene expression patterns indicate such optimal decoding (Hillenbrand et al., 2016; Tkačik, Callan, & Bialek, 2008; Tkačik et al., 2015; Tkačik, Walczak, & Bialek, 2009, 2012; Sokolowski & Tkačik, 2015; Walczak, Tkačik, & Bialek, 2010; reviewed in Tkačik and Walczak (2011). This is certainly a fascinating question to pursue. Preliminary evidence suggests that optimal decoding may indeed apply both in the *Drosophila* blastoderm and the vertebrate neural tube (Dubuis, Samanta, et al., 2013; Gregor, Tank, et al., 2007; Petkova et al., 2019; Zagorski et al., 2017).

However, it remains to be seen whether this insight can be further generalized. In particular, it is not at all clear whether the essential underlying assumption of optimal decoding holds in a broader developmental and evolutionary context. Model organisms are often chosen because of their short generation times and fast development. As a consequence, these creatures often show genetically hardwired modes of development, while slower developers are likely to rely more heavily on a combination of genetic and cellular-physical mechanisms of morphogenesis (see, for example, Love, 2018; Newman, 2008, 2012; Newman & Bhat, 2009; Salazar-Ciudad, 2010). This may introduce a bias toward model systems exhibiting high levels of decoding optimality. It will be challenging but worthwhile to gain the evidence on patterning in slow-developing non-model organisms required to resolve this issue.

On the other hand, the lack of specificity of information-based approaches prevents us from learning anything particular about the mechanisms underlying any given developmental system. This is a serious limitation, because details *do* matter in biology. Evidence from the few developmental systems for which we have suitable experimental and modeling-based data indicate that biological pattern formation is heavily feedback-driven (e.g., Briscoe & Small, 2015; Jaeger, 2018; Jaeger et al., 2008). Unfortunately, we do not yet know how the flow of positional information is regulated in feedback-heavy systems. Shannon's original theory is not applicable in the presence of regulatory feedback, and efforts to extend it have not gone beyond very simple examples of genetic auto-regulation (Tkačik et al., 2012). An integrative approach, combining information-based as well as causal-mechanistic analysis and modeling with detailed experimental evidence will be required to transcend this fundamental limitation.

There is one last philosophical point to make: our argument reveals that information-based and causal-mechanistic approaches provide different perspectives on the same underlying reality. Both perspectives deal with the same physico-chemical processes, the same regulatory systems composed of material entities and the dynamic interactions between them. Depending on our interpretation, these interactions result in an overall flow of information, or an overall mechanistic flow of cause-and-effect. Therefore, information theory provides us with an alternative angle to the problem of pattern formation. But information is not a thing. It is not a substance. Genes cannot be "made of information" and the "genetic program" remains a metaphor (Godfrey-Smith, 2007).

Instead, information is a conceptual tool, to be employed with a clear understanding of its meaning. Unfortunately, there is much loose talk about information in biology, which leads to confusion and unnecessary arguments. As Shannon (1956) himself noted, "information is no panacea." It must be used in clearly specified ways. We have shown that there are two alternative ways of precisely defining "positional information" in the context of pattern formation. Shannon information helps us to understand statistical patterns of error propagation and decoding in pattern-forming systems. General relativistic positional information (GRPI), in contrast, is a semantic metric for cells to "read" and "interpret" their relative position in a growing embryo. Both of these conceptual tools are complementary. They are most practical and powerful when used together with a clear understanding of their domains and limits of application.

Acknowledgments

This contribution is dedicated to all researchers out there who use the term positional information without having the faintest clue what it may mean. An early draft of this argument was presented in the context of the workshop "Causal Foundations of Biological Information," organized by Paul E. Griffiths and the recently deceased Karola Stotz, at the Konrad Lorenz Institute for Evolution and Cognition Research in Klosterneuburg. A late but heartfelt thank you to both for the invitation to talk and the discussions at the workshop. J.J. thanks Murillo Pagnotta for our fascinating and inspiring exchanges about information in biology.

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