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HYPOTHESIS

Positional information and reaction-diffusion: two big ideas in developmental biology combine

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ABSTRACT

One of the most fundamental questions in biology is that of biological pattern: how do the structures and shapes of organisms arise? Undoubtedly, the two most influential ideas in this area are those of Alan Turing's 'reaction-diffusion' and Lewis Wolpert's 'positional information'. Much has been written about these two concepts but some confusion still remains, in particular about the relationship between them. Here, we address this relationship and propose a scheme of three distinct ways in which these two ideas work together to shape biological form.

KEY WORDS: Reaction-diffusion, Biological pattern, Developmental biology, History of ideas

Introduction

The problem of patterning the embryo is almost synonymous with developmental biology itself. One can trace controversies about how embryonic pattern arises back to Aristotle, but in the late nineteenth and early twentieth centuries the problem was revisited by such greats as Boveri, Roux, Driesch, Spemann and Morgan. They recognised that cell fates in embryos are somehow spatially coordinated into 'patterns' and that some continuously varying properties or substances that form 'gradients' might achieve this coordination. They also recognised that information could be physically, or more likely chemically, transmitted from one part of an embryo to another (see Lawrence, 2001). However, vagueness as to what these chemicals might be and how they might work persisted. It was advances in biochemistry in the 1920s and 1930s that began to inspire increasingly concrete thinking about the physico-chemical nature of pattern formation. Then, in 1952, Alan Turing (see Box 1), the great mathematician, code-breaker and computer scientist, published *The Chemical Basis of Morphogenesis* (Turing, 1952). In the highly readable introductory part of this paper, he laid out in crystalline prose a perfectly formal statement of the problem of embryonic pattern formation, distinguishing it, for example, from 'mechanical morphogenesis'. He provided a conceptual solution – that of reaction-diffusion (RD) – but one that remained relatively obscure for a further 20 years. It took Lewis Wolpert (see Box 2), another non-biologist (who originally trained as a civil engineer), to again bring a hard edge to the problem in the late 1960s and early 1970s. In a series of eloquent theoretical review articles, Wolpert

distilled and synthesised much that was known and theorised regarding pattern formation, brilliantly focusing the term 'positional information' (PI) and providing, almost literally, a graphical icon for the field: 'The French Flag' (Wolpert, 1969, 1971).

The test of time has shown that, of the many theories regarding pattern formation, Turing's and Wolpert's remain pre-eminent. Wolpert's, being the conceptually simpler, is most often found in textbooks and courses, while Turing's is currently enjoying a resurgence. Wolpert, typically, proposed his concepts in a robust and contrarian way (the quotation below from his 1971 review is characteristic) and so it is often thought that the two ideas are mutually exclusive. In this Hypothesis article, we aim to overturn this impression and show that these two big ideas in developmental biology, despite indeed being conceptually distinct, are in fact wonderfully complementary and often collaborate to establish the complexity of developmental forms that we see. We first discuss the background behind Turing's and Wolpert's ideas, then introduce some real examples of each, before moving on to list the three main ways in which the two proposed patterning mechanisms might work together.

> "*This [positional information-based] view of pattern formation must be contrasted with those views which explicitly or implicitly claim that in order to make a pattern it is necessary to generate a spatial variation in something which resembles in some way the pattern… [Such a] view of pattern formation is characterised by the work of Turing (1952) [and others] and is the antithesis of positional information.*"
>
> Lewis Wolpert (1971)

Reaction-diffusion: a background to Turing's ideas

Turing took the boldest and baldest approach to addressing biological pattern formation by starting from first principles. He first distinguished chemical and mechanical morphogenesis. Chemical morphogenesis is what we now refer to as pattern formation. He left aside (as shall we) consideration of mechanical morphogenesis – that is, the movement and shape change of cells and tissues. He then postulated, long before modern developmental biology, that certain chemicals might have the property of instructing different cell fates based on their concentration. Although the term 'morphogen' later became associated with gradients of molecules imparting positional information, it was in fact Turing who gave us this term, in his own words: "*being intended to convey the idea of a form producer*" (Turing, 1952). It is worth pointing out that, in this original definition, a morphogen only needed to specify two different cell states (e.g. one novel and one default). Next, he tried to work out the simplest mathematical system of interacting morphogens that could spontaneously produce a pattern when starting from a uniform field of cells. His objective was to seek a simple set of equations that would drive a homogeneous distribution of chemicals into a pattern, but only when the chemicals were allowed to diffuse between the cells. Presumably through sheer mathematical intuition, he came up with

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DEVELOPMENT

Box 1. Alan Turing
Alan Turing (1912-1954), the British mathematician, is famous for a number of breakthroughs that altered the course of the twentieth century. In 1936 he published a paper that laid the foundation of computer science, providing the first formal concept of a computer algorithm. He later played a pivotal role in the Second World War, designing the machines that cracked the German military codes, enabling the Allies to defeat the Nazis in several crucial battles. His experience during the war with designing complex logic machinery also put him in prime position to turn his theoretical ideas of the 1930s into practical reality, and he was thus responsible for designing one of the first general-purpose digital computers in the late 1940s. This in turn led him towards another new field for which he was a founding member – artificial intelligence – the question of how to design machines that can think.

His contribution to mathematical biology is less well known, but was no less profound. He published just one paper (in 1952), but it triggered a whole new field of mathematical enquiry into pattern formation. Intriguingly, this interest in developmental biology was in fact also a logical extension of his previous research interests, as he increasingly wished to understand how nature's own 'thinking machine' – the human brain – was constructed during embryogenesis (Hodges, 1985).

an astonishingly elegant two-equation system that could do exactly this. His paper in fact describes six types of spatial pattern (some of which require three diffusing chemicals), including travelling waves and oscillations as well as stable periodic patterns, such as stripes or spots (reviewed by Kondo and Miura, 2010). For simplicity, here we use the terms 'RD' and 'Turing system' to mean only the stable stripe/spot version with a regular periodicity, because both he and we find these to be the most relevant to embryological development. This creation or discovery revealed for one last time before his suicide (as this was his last published paper) the genius of Turing's mathematical mind. Despite claiming that his system "*does not make any new hypotheses; it merely suggests that certain well-known physical laws are sufficient to account for many of the facts*", his discovery was so counterintuitive that it drew scepticism even from hard-core physicists. This is perhaps understandable: in all previously considered situations, diffusion destroys spatial patterns. Just think of a drop of ink in water – diffusion will cause the slow but sure dispersal of the ink molecules until all the water is faintly coloured. The original pattern – a spot – is destroyed; the final state has no spatial heterogeneities and thus no pattern. Diffusion would seem to be a quintessentially entropy-increasing, disorder-maximising process. The idea that diffusion itself could

Box 2. Lewis Wolpert
Lewis Wolpert (b. 1929) trained as an engineer and brought his engineering sensibilities to a series of papers on physical morphogenesis of the early sea urchin embryo in the 1960s. Toward the end of the 1960s he introduced the concept of positional information as an attempt to formalise and unify prior notions (dating as far back as Boveri in the nineteenth century) of 'gradients' and 'fields'. His own group's experimental work focused on patterning in *Hydra* and the chick limb and diversified to further theoretical problems, such as developmental precision and left-right handedness. His ability to throw questions into sharp relief by adopting and testing sometimes controversial stances has made him an inspirational figure with many of his protégés becoming prominent in developmental biology and UK science generally in their own right. Like Turing, Wolpert has made contributions well beyond developmental biology, most prominently in writing one of the first bestsellers to publicise depression – *Malignant Sadness: The Anatomy of Depression* – as more than just a low mood, as well as defending the ethics of pure science in contradistinction to the dangers of its technological application.

create a pattern – that it could drive the well-mixed ink back into a spot – was (and still is) very surprising.

Although Turing systems are intrinsically non-intuitive, they are perhaps best thought of as a combination of local self-enhancement coupled with lateral inhibition. More specifically, we can consider Turing's original morphogens as short-range activators coupled with long-range inhibitors (Gierer and Meinhardt, 1972), each of which acts on itself as well as the other. Starting from a homogeneous spatial pattern, even small random molecular fluctuations can cause the activator to have slightly higher concentrations at certain positions (Fig. 1A). Because it has a positive effect on its own production, the activator levels will tend to rise further at that point (i.e. as the result of a positive-feedback loop). As the activator concentration rises it also boosts production of the inhibitor, which thus increases at the same position (Fig. 1A). However, the inhibitor is allowed to diffuse faster than the activator, and this has two important consequences for the system. The faster diffusion of inhibitor away from the peak results in lower repressive levels, such that the activator peak is able to stabilise itself. By contrast, in neighbouring regions the influx of inhibitor means that activator levels are repressed, preventing another peak from forming nearby (Fig. 1A). At a certain distance from the peak, the inhibitor is not strong enough to repress the formation of new peaks, so new ones can arise on either side (Fig. 1A). Because this distance is determined by the global parameters of the system (e.g. the diffusivity of the inhibitor), the resulting pattern is a spatially periodic distribution of concentrations (i.e. there is always the same distance between peaks; Fig. 1A), which in 2D usually produces spots or stripes. A crucial distinction from PI (discussed later) is that each peak does not form as a response to positional information unique to that point in the field of cells. It is a locally self-organising system, in which each peak is produced in exactly the same way as the others.

Turing's idea was almost ignored for two decades, in part due to the rigidity of the pattern's wavelength for a given set of parameter values, a rigidity that seems to be at odds with the ability of developmental patterns to adjust to natural variability or even dramatic experimental perturbation of embryo size (Harrison, 1987). Additionally, the early exploration of RD concluded that it was rather sensitive to noise or initial conditions and thus too unreliable for developmental patterning (Bard and Lauder, 1974), leading Conrad Waddington, perhaps the pioneer of British developmental biology in the 1950s and 1960s, to write that the Turing model was "*inherently chancy and likely to play a part only in the quasi-periodic dapplings and mottlings which often fill up relatively unimportant spaces*" (Waddington, 1956, as quoted in Harrison, 1987). However, in 1972 Gierer and Meinhardt revived the concept of RD by articulating and lucidly developing its terminology, implications and variations, and also by showing that regulation of pattern size was easily achievable (Gierer and Meinhardt, 1972). Many examples of biological patterning were subsequently explored, ranging from animal coat patterns (zebra, leopard and fish) to mollusc shell pigmentation patterns and even segmentation of insects, all of which seemed to display RD-like properties (Meinhardt, 1982). However, the proof that a Turing mechanism was the actual basis of these patterns was lacking, as the models were 'under-constrained' by the data (Oster, 1988), in effect because the technology of that era was not able to identify the relevant molecules. This deficit has only relatively recently begun to be corrected with clear examples of RD systems in which both an activator and an inhibitor with the necessary interactions have been identified (reviewed by Marcon and Sharpe, 2012; Economou and Green, 2014). Some of these molecular cases – for example, the patterning of palatal ridges (Economou et al., 2012) or digits (Raspopovic et al., 2014) during mouse embryogenesis – are discussed later in this article (Fig. 2).
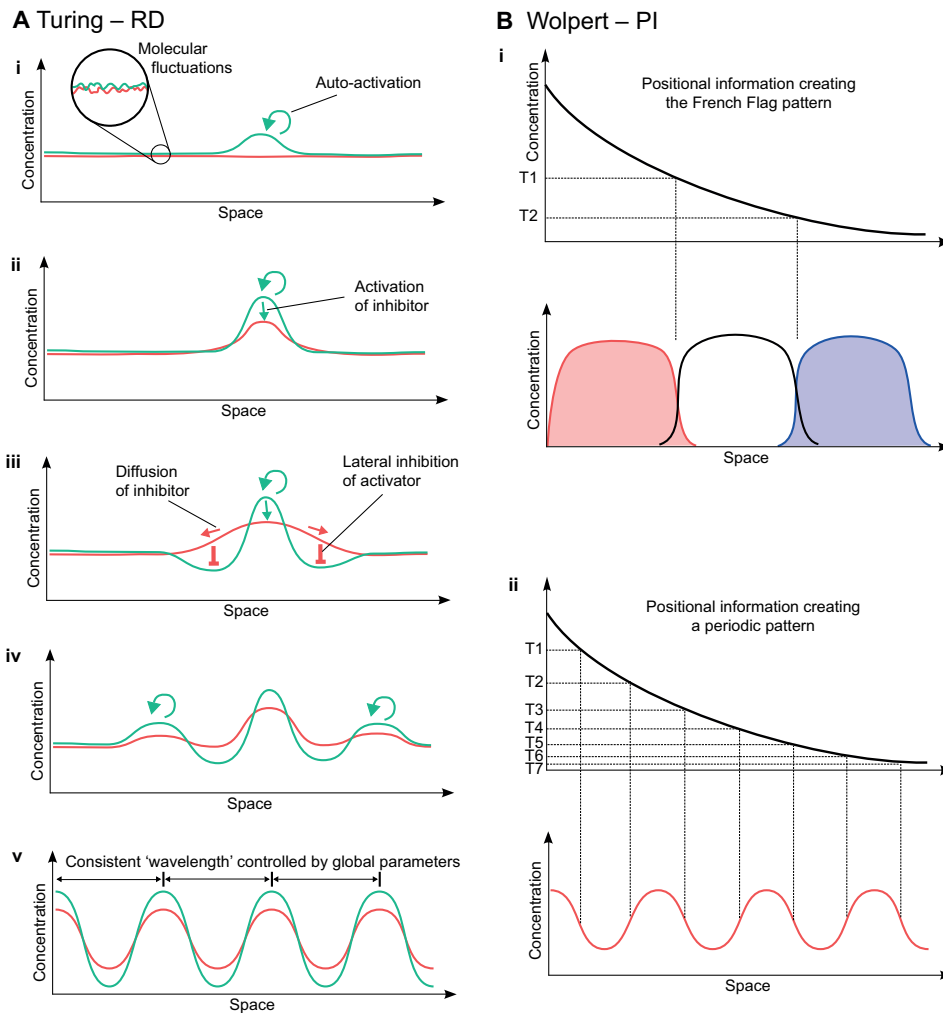
Fig. 1. The principles of reaction-diffusion (RD) and positional information (PI) systems. (A) There are two broad categories of Turing RD systems: the activator-inhibitor model and the substrate-depletion model. In the former case, the two molecular concentrations make periodic patterns that are in phase with each other. In the latter case, the patterns are out of phase with each other. Here, we illustrate the general self-organising nature of RD systems by reference to the activator-inhibitor model. Even an apparently homogeneous distribution of molecules across space will display molecular fluctuations. Some cells with a slightly higher level of activator will thus auto-enhance these levels, pushing up the concentration (i). Since the activator also enhances production of the inhibitor, levels of inhibitor will also rise at that point (ii). However, the inhibitor can diffuse faster than the activator, which has two consequences: first, at the position of the peak, inhibitor levels fail to accumulate sufficiently to repress the activator, whose positive feedback is able to stabilise its own high levels; second, the increase in inhibitor levels in neighbouring cells prevents levels of the activator from growing, thus creating a zone on either side of the first peak where no new peaks can form (iii). However, beyond these regions of 'lateral inhibition' new peaks can form (iv), so the whole system dynamically changes until a regular array of peaks and valleys is formed across the whole field of cells (v). (B) Wolpert's concept of PI describes a very different process. A prior asymmetry results in a graded monotonic distribution of a variable (usually the concentration of a morphogen), and cells use this distribution to make fate choices. A popular illustration of this concept is the French Flag Problem (i), in which the field of cells must be divided into three equal regions of different cell fates (represented by red, white and blue). It is increasingly believed that small networks of cross-regulating genes constitute the mechanism of morphogen interpretation. However, irrespective of the molecular mechanism, the effective calculation is to define threshold levels of morphogen (T1, T2) and to associate prespecified fates to the different concentration ranges between these thresholds. In principle, any pattern can be defined in this way, including a periodic pattern similar to those produced by RD (ii). However, in this case a large number of different positional values (T1 to T7) would have to be accurately defined, even though they subsequently map to just two fate choices.

In addition to the lack of molecular evidence for RD, the 1980s and 1990s brought a discovery that seemed to make RD models unnecessary. This was the surprising result in *Drosophila* that the periodic segments of the larva, an apparently archetypal periodic pattern for which RD might have been expected to be involved, are built up not by a single global periodic patterning process but rather by a stepwise subdivision of the embryo by a multiplicity of overlapping gradients (Fig. 2A). Thus, each segment or stripe of gene expression is controlled and positioned independently of the others (Akam, 1989a,b; Lawrence, 1992). This contributed to the notion that RD was overly complex

and provided a clear example of the main alternative idea to Turing's: PI.

Wolpert and the concept of positional information

The problem that Wolpert sought to address in the late 1960s and early 1970s was distinct from Turing's. Instead of asking how a periodic pattern could arise from nothing, he asked how a more complex pattern could be determined from simple prior asymmetries in the tissue, and how the scale of this pattern could be coordinated over a whole tissue space. In other words he was not seeking a self-organising mechanism that could 'break symmetry' by itself, but rather was defining a system
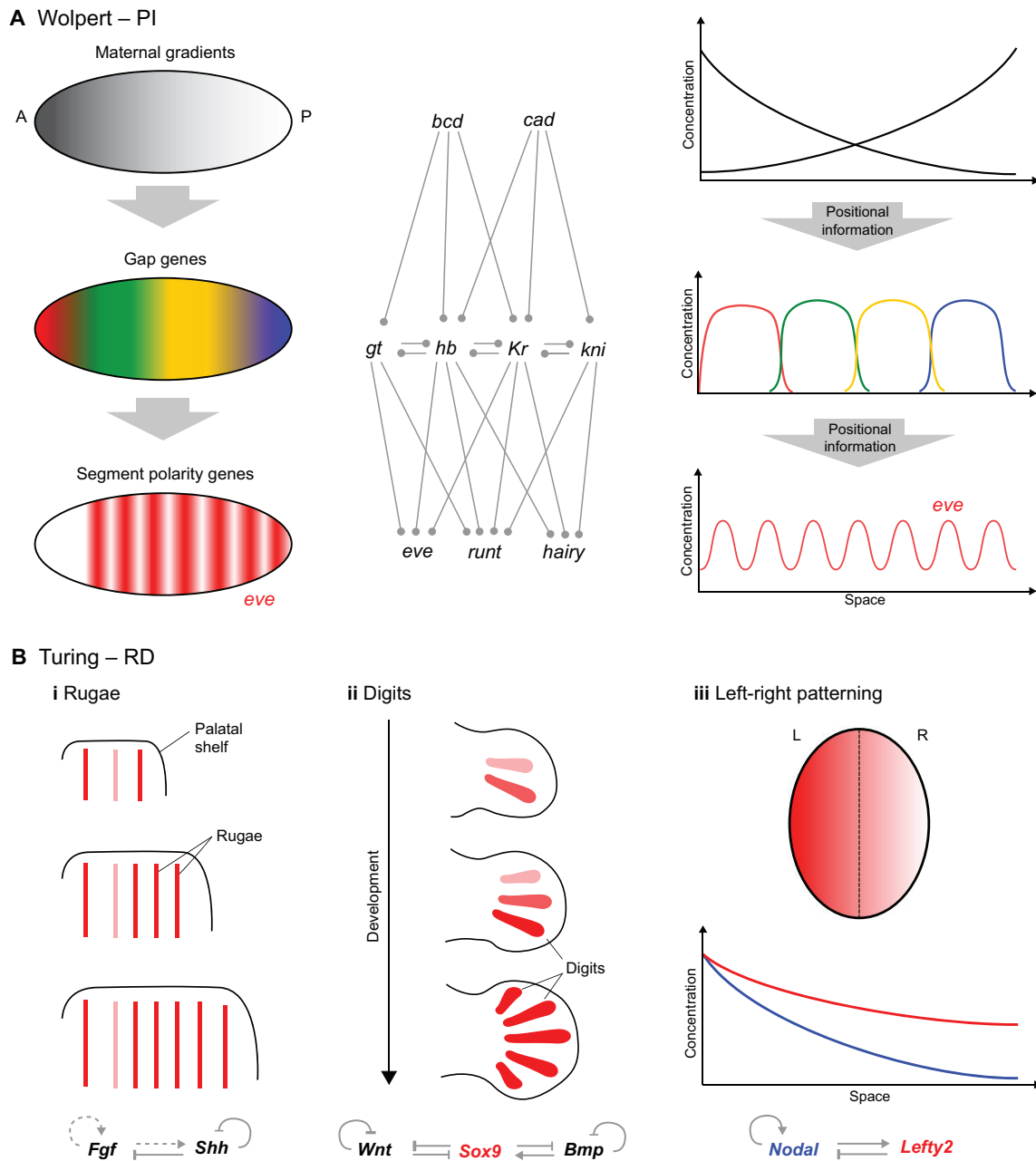
Fig. 2. Examples of real patterning systems. (A) Molecular patterning in the early *Drosophila* embryo is considered a convincing example of PI. It is generally represented as a one-dimensional system along the anterior-posterior (A-P) axis. Initial asymmetries result in broad monotonic gradients of morphogens across the field, which directly regulate the gap genes. Differences in morphogen concentrations at each position of the field provide distinct inputs to the gap gene network, which, through various cross-regulatory interactions, convert these smooth spatial differences into more discrete molecular patterns (the various coloured domains). This more complex molecular pattern of gap genes then provides the positional information for the next level of gene regulation – the segment polarity genes, which are each expressed as a series of seven stripes. The diagram is intentionally schematic in order to illustrate the concept and therefore not all relevant genes have been included. (B) Three examples of RD systems from mouse embryos are shown. In the patterning of rugae (i), the palatal shelf grows in size and a series of expression stripes develops, which determine where each ruga will form. The spacing of these stripes is self controlled by the activator-inhibitor pair of Fgf and Shh. In the limb buds (ii), the positions of future digits are created as a Turing pattern driven by a feedback loop between Wnt and Bmp signalling and the transcription factor Sox9. This is equivalent to a substrate-depletion Turing model, rather than an activator-inhibitor model. The distinction between the left (L) and right (R) side of the body is driven (or at least enhanced) by an RD system comprising Nodal and Lefty2 (iii), which creates broad gradients of morphogen concentration across the field.

that would depend on earlier heterogeneities or polarities across the tissue, and use these to create more complex patterns downstream. This led him to propose that differences in morphogen concentration across space could be gradual enough such that many different positions could be reliably defined based simply on their differences in concentration (Wolpert, 1969, 1971).

This was not the only difference between Wolpert's approach and the Turing models. In depictions of RD, stripes or spots of morphogen directly produce stripes or spots of cell types in the resulting tissue, implying that a close correspondence exists between the shape of a morphogen distribution and the shape of the resulting pattern. Wolpert's idea explicitly rejected this direct coupling of the

DEVELOPMENT

resulting pattern to the prior morphogen distribution (a coupling referred to as 'pre-pattern') by introducing an interpretation step. Specifically, Wolpert proposed that an interpretation step allows a smooth, monotonic morphogen concentration gradient to give rise to any arbitrary pattern (Fig. 1B), be it periodic, such as with spots and stripes, or non-periodic, like the sequence of red, white and blue in the French flag (hence his invocation of the 'French Flag Problem' as a paradigm task for cellular patterning mechanisms). The development of *Drosophila* segments is a perfect illustration of this (Fig. 2A): each stripe is defined independently by its unique position in a succession of local concentration gradients of the gap genes (which are themselves initially set up by Bicoid and Hunchback proteins), rather than as one iteration of a self-organised periodic pattern. The interpretation step also allows that the exact same pair of orthogonal morphogens could provoke cells to form an American Flag pattern in one species and a French Flag pattern in another. In effect, the morphogen concentrations could act as positional coordinates along an axis – hence the term 'positional information'. Cells would respond to (i.e. interpret) the local concentration of morphogen and make whichever 'colour' or fate choice was appropriate for that position. This is illustrated by heterotypic (interspecies) grafts, such as the classic grafting of frog mouth skin into a newt mouth to obtain frog mouthparts, which were performed in Hans Spemann's group in the 1930s (Spemann and Schotté, 1932).

Wolpert was not the first person to consider that spatial fields of chemicals would be involved in organising patterns of cell differentiation. The notion that gradients establish developmental pattern had a long history going back to the nineteenth century, and has been brilliantly summarised by Lawrence (2001). Lawrence himself articulated one of the most detailed ideas around the same time as Wolpert (Lawrence, 1970), and even Francis Crick weighed in on the subject (Crick, 1970). However, Wolpert's name stuck to the gradients-plus-thresholds idea, perhaps because he focused on the consequences of this idea more explicitly and more extensively than others in the field.

This type of patterning mechanism has two important consequences. The first is that the PI system operates in two mechanistically distinguishable steps, although in practice they can overlap in time. The system that produces the positional information (most commonly a morphogen gradient) is distinct from the system that interprets the positional information (some fate-choice mechanism within the cells, such as a gene regulatory circuit). Second, it allows for the possibility that overt biological pattern can vary evolutionarily while the underlying morphogen coordinate system is preserved and reused in multiple species and during the development of multiple tissues. As with Turing's RD concept, the idea of PI significantly preceded its molecular proof, but such proof was forthcoming in the discovery of the Bicoid gradient in *Drosophila* (Fig. 2A) and the demonstration that in *Xenopus* a TGFβ superfamily protein growth factor could trigger different cell fates separated by concentration thresholds (reviewed by Green and Smith, 1991; Lawrence, 1988; Wolpert, 1989). Another compelling example of the PI concept was the discovery that Hox genes encode positional information along the anterior-posterior axis of all animals (Akam, 1989b). In other words, the encoding of positional values has remained the same, while the interpretation of this information has evolved to create many different body plans.

For a variety of reasons, Wolpert's ideas quickly eclipsed Turing's. First, the limitations of Turing's initial formulation of RD and Waddington's devastating critique of it continued to resonate. Second, as mentioned above, the concept that diffusion can create organised

spatial heterogeneities is not, and never was, intuitive, and the fact that it involved differential equations put off many biologists (and still does to this day). By contrast, PI is wonderfully intuitive – even simple – coming across as a practical engineer's solution (no coincidence, given Wolpert's training as an engineer) rather than an esoteric mathematician's. Third, even Turing himself acknowledged that "*most of an organism, most of the time, is developing from one pattern into another, rather than from homogeneity into a pattern*" (Turing, 1952). Thus, self-organisation seemed to be a rarity, rather than a central phenomenon in development. Fourth, in pointing out the relative inflexibility of a pre-pattern mechanism compared with the versatility of a generalisable, reusable chemical coordinate system, Wolpert correctly emphasized a distinction between the mechanisms but in language that often suggested mutual exclusivity (Wolpert, 1971). Lastly and most importantly, some classic experimental observations appeared to favour Wolpert over Turing. In particular, grafting experiments in the chick wing bud published in the same year as Wolpert's ideas suggested that the three digits were each specified by their own distinct concentration of a hypothetical morphogen secreted on the posterior side of the bud (Saunders and Gasseling, 1968). Apparently, the entire digital arrangement, including its periodic pattern, could be explained by cells simply interpreting their coordinates in a morphogen landscape. This powerful story was the first blow to the popularity of RD in the early 1970s, and a second blow – the discovery that *Drosophila* segments are patterned independently of each other (as discussed above; Fig. 2A) – almost killed it off. By the time of the commentary by Akam entitled *Making Stripes Inelegantly* (Akam, 1989a), few self-respecting developmental biologists could allow themselves to be distracted by the phenomenological allure of Turing's idea.

The revival of Turing's idea

Recently, however, there has been a strong revival of Turing's idea, as many specific molecular hypotheses have been made. Initially, the focus was on periodic patterns that are non-identical from one individual to the next; for example, the distribution of the thousands of hair follicles or feather buds in the skin of mammals or birds. Various proposals for the diffusible activator and inhibitor have been made in each case: Wnt as the activator and dickkopf (Dkk) as the repressor (Sick et al., 2006), ectodysplasin receptor (Edar) as the activator and bone morphogenetic proteins (Bmps) as inhibitors (Mou et al., 2006), Bmp7 and Bmp2 as antagonistic regulators (Michon et al., 2008), and also a more complex proposal involving a combination of fibroblast growth factor 4 (Fgf4) and sonic hedgehog (Shh) as the activators and Bmp2 and Bmp4 as the inhibitors (Jung et al., 1998).

In contrast to these patterns, which contain thousands of repeated elements, two recent proposals have been made for periodic structures that show a reliably stereotypical pattern across all individuals within a species. These show that, far from being a mechanism only for superficial structures and decoration (Waddington's 'mottlings and dapplings' mentioned above), RD is certainly involved in patterning highly determined and anatomically crucial patterns as well. The first concerns the hard palates of mammals, which have a series of parallel ridges (four in humans, eight in mice) that help masticate food. It is now believed that these physical ridges, known as rugae, are initially patterned by a molecular Turing mechanism (Fig. 2B) in which an Fgf signal acts as a diffusible activator and Shh as the inhibitor (Economou et al., 2012). The second case represents one of the longest-debated questions of Turing patterning in the field – the patterning of digits during limb development. That this was a Turing RD system was first proposed back in 1979 (Newman and Frisch, 1979), but because strong

evidence of the molecular basis was lacking at that time, the proposal was generally resisted by the community. The widely accepted alternative was that digit patterning was based on PI, specifically a gradient of Shh signalling emanating from the posterior region of the limb bud. However, a recent series of manipulative experiments coupled with computer modelling has provided strong evidence that this is indeed a Turing RD system based on Wnt and Bmp (Raspopovic et al., 2014). The model is slightly more complex than the popular activator-inhibitor mechanism, as it is a version of the substrate-depletion model (Gierer and Meinhardt, 1972) but involves three molecules instead of two (Fig. 2B).

Finally, studies have also proposed that the processes of germ layer specification (Müller et al., 2012; Schier, 2009) and left-right patterning (Shiratori and Hamada, 2006) in the early vertebrate embryo are based on Turing systems, but with an interesting difference – the resulting pattern is not periodic. It is believed that the protein Nodal acts as the activator and Lefty2 as the repressor (Fig. 2B), and their interactions result in a broad molecular gradient that allows cells to distinguish which side of the embryo they are on. The key feature that indicates a Turing system is that the gradient is self-organising through the dynamics of the activator-inhibitor pair. Unlike the PI concept, which explains how a prior asymmetry is converted into a specific pattern (Fig. 1B), the Nodal-Lefty network requires no prior signalling centre on one side of the embryo. It spontaneously forms the gradient from an initial maternal bias (in the case of germ layer specification) or from cilia-driven directional fluid flow (in the case of left-right patterning) through local auto-activation and long-range inhibition. The final pattern is a single gradient rather than a periodicity because the effective wavelength is significantly larger than the size of the tissue.

Further details of proposed real RD systems will not be discussed here and the reader is directed to recent reviews on Turing RD systems (Kondo and Miura, 2010; Marcon and Sharpe, 2012; Meinhardt, 2012; Roth, 2011). Instead, we use the last case discussed, that of left-right patterning, as the ideal starting point for discussing how RD and PI may interact and collaborate with each other.

The striking feature of the pattern formed by Nodal and Lefty2 is that it is a single smooth gradient across the spatial domain. This indeed looks just like a morphogen gradient from a PI system, and is therefore likely to act as one. However, it also highlights the probable dual nature of this particular case – the gradient is created by Turing dynamics (rather than by a polarised signalling centre), but is nevertheless potentially capable of providing positional information to the cells of the field, as every cell could experience a unique concentration of the signals.

Combining distinct concepts: bringing RD and PI together

As highlighted thus far, Turing's RD and Wolpert's PI have often been considered as two opposing ideas and as alternative mechanisms for a given pattern. It was even possible for Lionel Harrison to write in 1987 that 'Turingians' and 'Wolpertians' constituted two (British) 'tribes' (Harrison, 1987). Here, we would like to suggest that 'is it RD or PI?' is the wrong question. Despite maintaining that they are clearly distinct mechanisms (as described above), we question whether they should be seen as alternative explanations for a given patterning event. On the contrary, we argue that in many cases they are likely to work together, with each providing their distinct benefits to the system: Turing providing the benefits of symmetry-breaking and self-organised regularity, and PI being a flexible way to specify regional differences and to tinker with pattern formation during evolution. Indeed, in many cases it

might be impossible to reliably build a pattern using only one approach or the other. We believe that there are three distinct modes (Fig. 3) by which the two systems can collaborate to build relevant biological patterns as follows.

Mode 1: RD acting upstream of PI

Wolpert's concept depends on a spatial gradient to provide positional information, but it does not specify how that gradient is created. The commonly discussed scenarios start with a prior asymmetry, such as morphogen molecules secreted from cells on one side of the tissue, which decay and diffuse across the field to create the gradient. However, any mechanism that creates a gradient is in principle sufficient to provide positional information. For example, the classical progress zone model in limb development posited a gradient of 'cellular ages', rather than a gradient of chemical concentrations, and yet it was explicitly a PI model (Summerbell et al., 1973). Turing systems provide a mechanism by which a gradient can be created, since each spot or stripe within the periodic pattern is not a step function but a peak in morphogen concentration surrounded by a morphogen gradient. Each subregion of the Turing pattern that goes from 'peak' to 'valley' is a spatial monotonic gradient, and different cells within this subregion could perceive different positional values (Fig. 3A). Thus, the size of a group of cells fated to become, for example, a hair follicle, depends on the interpretation of a threshold level of a morphogen even though the spacing of such maxima may have resulted from RD. Furthermore, both theoretically and experimentally, it has been proposed that sometimes a Turing pattern is created in a field that is not big enough for multiple stripes. The case of left-right patterning described above provides a perfect example: the Nodal and Lefty2 molecules constitute a Turing system that only has enough space to make half a wavelength – from one peak to one valley – which gives rise to a perfect gradient (Fig. 2B) that could, theoretically, be subsequently interpreted into multiple positional values. In this case, these would be manifested as either left or right side-specific genes being expressed. Examples of downstream interpretation have also been proposed for cases in which multiple stripes or spots are made. Hair follicles and feather buds differ from pigment spots with respect to patterning because they have an internal structure of concentrically organised morphogenesis and differentiation. Although this could be set up in two steps, with RD establishing the location of the centres and the centres subsequently releasing a second set of morphogens, it is equally possible that the initial RD patterns can be interpreted according to thresholds for different concentrations of the primary morphogen pair.

We must emphasize here why Mode 1 is not a pure PI system. The primary reason is because this mode does not require an upstream (earlier) asymmetry – it does not require a polarised signalling centre. It is thus very different from classic gradients, such as the polarised expression of Bicoid in the early *Drosophila* embryo, whose asymmetry is provided by the mother, or of Shh in the posterior region of the limb bud, whose localisation depends on prior molecular asymmetries established in the main body of the embryo. Instead, in a Mode 1 mechanism, the RD part describes the spontaneous self-organising manner in which the gradient has formed, and the PI part describes the way in which cells subsequently interpret this gradient to choose different fates.

Mode 2: RD acting in parallel with PI

The second possible mode involves cells integrating information from both systems simultaneously but independently (Fig. 3B).
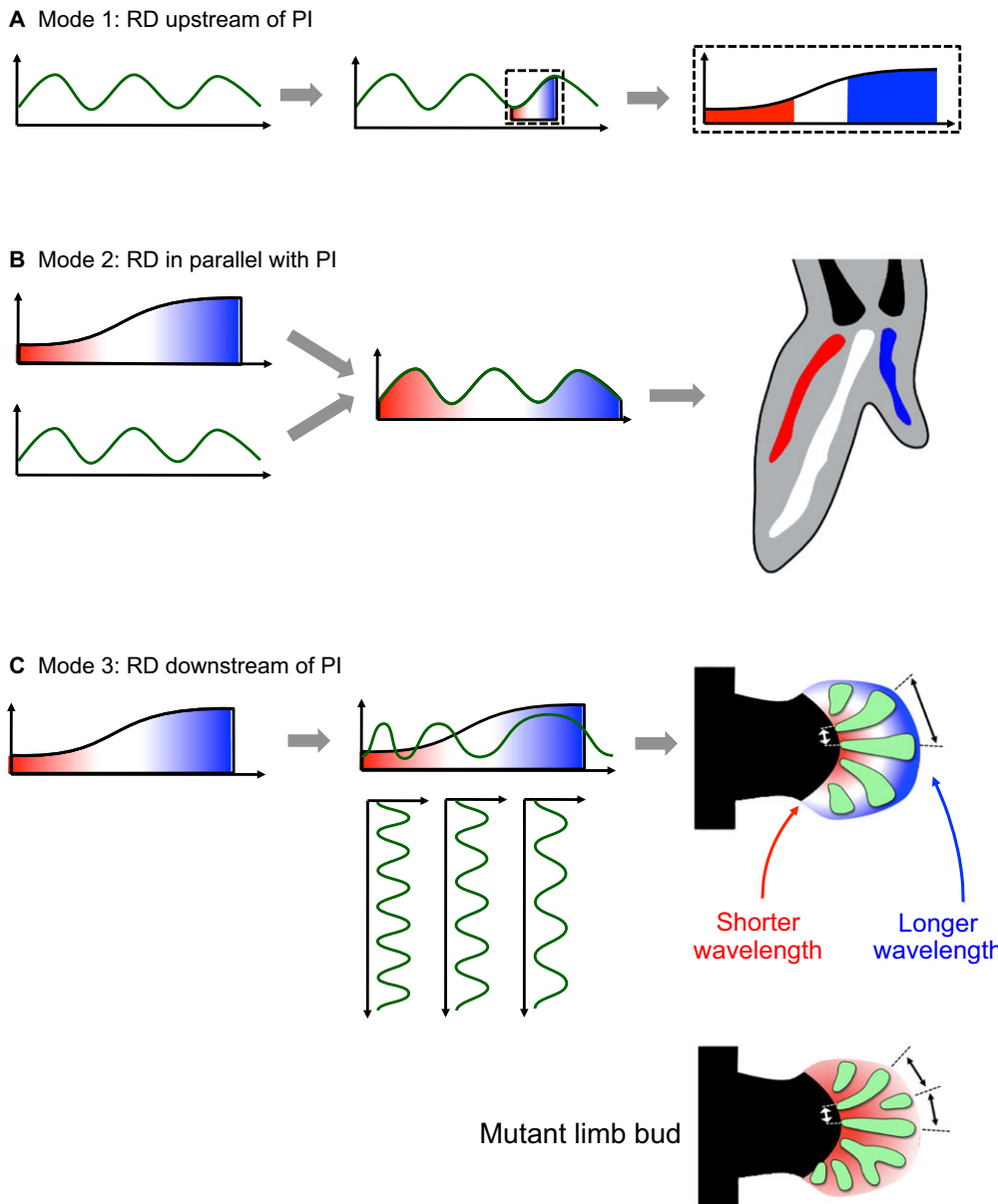
A Mode 1: RD upstream of PI

B Mode 2: RD in parallel with PI

C Mode 3: RD downstream of PI

Shorter wavelength

Longer wavelength

Mutant limb bud

Fig. 3. Modes of RD-PI interaction. The RD pattern is represented in green in all cases, while the interpretation of PI is indicated with the red, white and blue of the French flag. (A) In Mode 1 (RD acting upstream of PI), a morphogen Turing pair spontaneously makes a periodic pattern. Each subregion corresponding to a local monotonic gradient (e.g. dashed box) can then serve as positional information for subsequent interpretation by cells. (B) In Mode 2 (RD-PI acting in parallel), which is seen, for example, in the developing limb bud, an RD system produces a periodic pattern along the anterior-posterior axis, which will distinguish digit from interdigit. Independently of this periodic pattern, a PI gradient is created, which allows the initially equivalent digits (peaks of the green RD pattern) to adopt different identities (probably through controlling growth rates, rather than directly controlling cell fates). (C) In Mode 3 (PI acting upstream of RD), a periodic RD pattern develops but under the influence of a PI gradient, such that the wavelength varies along the length of the PI gradient. In this example, the red positions create a shorter wavelength, while the blue regions create longer wavelength. This mode of interaction appears to be important in the mouse limb bud, as the periodically patterned digits 'splay out' distally. Mutants of distal Hox genes and *Gli3* support this theory, as they show supernumerary digital structures that are short and distally restricted and exhibit branching of digits; both of these features suggest that the wavelength is not controlled sufficiently along the proximodistal axis in these mutants, resulting in abnormal digital morphologies.

This type of dialogue is exemplified by current models of digit specification in the vertebrate limb. The limb bud develops a periodic gene expression pattern prefiguring the digits, for which evidence points overwhelmingly to a Turing mechanism (Raspopovic et al., 2014; Sheth et al., 2012). An important feature of the early pattern is that the digits appear very regular in width and length – the intrinsic periodicity of a Turing system is unable to directly impart differences between the five digits (Fig. 2B). However, it is also clear that the different digits do gradually vary in size, especially in birds, and these differences, which lead to what we call 'digit identity', are probably driven by a longer range gradient of Shh that is superimposed upon, but independent of, the periodic patterning. It thus appears that two different types of cellular decision are driven by the two different patterning mechanisms: (1) the choice of whether to become a digit cell or an interdigit cell is driven exclusively by the Turing system, with no input from the Shh gradient, whereas (2) the slightly later decision of whether to become a big digit 3 or a small digit 1 must be largely driven by a PI-based system, such as the

Shh gradient, with no direct input from the Turing system. It is still not understood how Shh controls such a digit identity choice, but it is plausible that it operates directly through the differential control of proliferation rates (such that the cells in digit 3 proliferate faster than the cells in digit 1) and perhaps also more indirectly on other differences between the digits (e.g. by influencing the shape of the handplate, which indirectly impacts on each digit).

The particular view elaborated above is still just a hypothesis to illustrate our concept of Mode 2. But it indeed appears plausible that the creation of a complete hand requires each cell to correctly make both decisions independently – which cell type to become (digit/interdigit) versus how fast to grow (digit identity) – but with the information for each choice coming separately from two different patterning mechanisms. Whether other structures that display periodicity with variations, such as teeth, also employ the two mechanisms in parallel remains to be seen, although there is evidence in at least the case of teeth for both RD and long-range gradient patterning (Maini, 1997; Depew et al., 2002).

Mode 3: RD acting downstream of PI

There is a trivial sense in which any patterning mechanism could be downstream of PI. A field of cells can be subdivided into smaller regions by PI, and each subregion can then perform a new independent patterning process, which could be an RD pattern. However, we also hypothesize that RD can be downstream of PI in a more specific, dependent manner, which is our third proposed mode (Fig. 3C). Although most simple Turing patterns are very regular and periodic across the whole spatial domain, some biological cases of Turing patterns show evidence of higher level control. Stripes may need to have a longer wavelength in one region of the tissue and a shorter wavelength in another region. Again, the limb provides a beautiful example of this spatially controlled wavelength modulation. Specifically, the periodic pattern of the digit gene expression stripes develops not as a series of truly parallel stripes but instead as a radial arrangement with digits fanning out to fill the width of the paddle-shaped distal limb. This fanning out violates the constant wavelength rule for RD systems because the wavelength at the proximal (wrist) end of the digits is shorter than that at the distal (fingertip) end. The finding that failure to modulate the wavelength leads to branching (bifurcation) of the stripes (Sheth et al., 2012) provides evidence that the periodic patterning is normally subject to a type of positional signal that varies along the proximodistal axis of the limb. In other words, the RD patterning process seems to be controlled by, and thus is downstream of, a PI system. It has been proposed that a known distal-to-proximal gradient of Fgf signalling modulates not only limb outgrowth but also the wavelength of digit periodicity. This Fgf gradient is, at least in a rather specific sense, providing positional information. Although the cell fate (digit versus interdigit) is not directly determined by PI, the wavelength of the Turing system at any point in the limb bud tissue is determined by its position along the proximodistal axis, and this in turn (indirectly) does control individual cell fates.

Although experimental evidence has been provided for the role of Fgf in determining the pattern wavelength of digits, we again emphasize that our primary goal here is to provide a concrete hypothesis of Mode 3 patterning, rather than a definitive account of digit patterning. A clear idea of how widespread this mode is across the field of developmental biology must await the results of future studies. Additionally, we acknowledge that the general idea of spatially non-uniform parameters creating non-uniform periodicities has been discussed before (Maini, 1997; Meinhardt, 2012), but our specific goal here is to position this idea in the context of the other two modes discussed above – indeed, to provide a conceptual framework for the three different ways that RD and PI can interact.

Perspectives

Our goal here has been to discuss possible ways in which RD and PI concepts can work together, and to provide a clear framework that compares and contrasts three hypothetical modes of interaction. In particular, we propose that PI mechanisms involving long-range gradients can operate in rather specific manners downstream of, parallel to, or upstream of RD systems. In other words, robust self-organising RD mechanisms can underlie PI gradient establishment (e.g. during left-right patterning by Nodal-Lefty), be superimposed on a PI gradient to provide 'periods' with different identities (e.g. to specify digit identity), or be themselves modulated by wavelength-modulating PI gradients (e.g. in the case of digit splaying). The concrete examples given are relatively recent and thus the ideas presented here should be taken as a conceptual framework and

a hypothesis, the full confirmation of which will depend on further discoveries over the coming years.

Additionally, it should be noted that Modes 1 and 3 could theoretically occur simultaneously, constituting a feedback loop between RD and PI. In other words, morphogen levels from a Turing RD system could specify different positions, directly altering the spatial distribution of a molecule that in turn controls the Turing wavelength. This combined feedback loop could be seen as complementary to Mode 2 (in which both processes occur, but independently – they do not directly alter each other). However, we prefer not to add this as a fourth mode. Our intention is that our list contains the most basic 'building blocks' of the possible RD-PI interactions, and more complex mechanisms can be considered by combining them in various ways.

In conclusion, our framework argues that, contrary to the historical separation of PI from RD in most developmental biologists' minds, these two processes, although very different from each other, are nevertheless probably in dialogue in many cases, collaborating to produce a variety of different patterns with different types of behaviours. Of course, other patterning mechanisms have been identified in the field; for example, the clock-and-wave mechanism that is thought to be important in the periodic patterning of somites. However, even this type of mechanism has internal submodules that look much more like PI processes and RD processes than was once thought (e.g. Sen et al., 2010). Many layers of complexity in underlying transcriptional and feedback networks might be involved, and so the next task is to establish the extent to which these basic principles capture the properties of the patterning mechanism or affect aspects of it such as timing, robustness and evolvability. In the meantime, the collaboration between RD and PI mechanisms provides a useful conceptual synthesis. We hope that in the future PI-based and RD-based patterning will be taught and written about together as a magnificent pair of insights.

References
Akam, M. (1989a). Drosophila development: making stripes inelegantly. *Nature* **341**, 282-283.

Akam, M. (1989b). Hox and HOM: homologous gene clusters in insects and vertebrates. *Cell* **57**, 347-349.

Bard, J. and Lauder, I. (1974). How well does Turing's theory of morphogenesis work? *J. Theor. Biol.* **45**, 501-531.

Crick, F. (1970). Diffusion in embryogenesis. *Nature* **225**, 420-422.

Depew, M. J., Lufkin, T. and Rubenstein, J. L. R. (2002). Specification of jaw subdivisions by Dlx genes. *Science* **298**, 381-385.

Economou, A. D. and Green, J. B. (2014). Modelling from the experimental developmental biologists viewpoint. *Semin. Cell Dev. Biol.* **35**, 58-65.

Economou, A. D., Ohazama, A., Porntaveetus, T., Sharpe, P. T., Kondo, S., Basson, M. A., Gritli-Linde, A., Cobourne, M. T. and Green, J. B. A. (2012). Periodic stripe formation by a Turing mechanism operating at growth zones in the mammalian palate. *Nat. Genet.* **44**, 348-351.

Gierer, A. and Meinhardt, H. (1972). A theory of biological pattern formation. *Kybernetik* **12**, 30-39.

Green, J. B. A. and Smith, J. C. (1991). Growth factors as morphogens: do gradients and thresholds establish body plan? *Trends Genet.* **7**, 245-250.

Harrison, L. G. (1987). What is the status of reaction-diffusion theory thirty-four years after Turing? *J. Theor. Biol.* **125**, 369-384.

DEVELOPMENT

Hodges, A. (1985). *Alan Turing: The Enigma of Intelligence*. London: HarperCollins/Counterpoint.

Jung, H.-S., Francis-West, P. H., Widelitz, R. B., Jiang, T.-X., Ting-Berreth, S., Tickle, C., Wolpert, L. and Chuong, C.-M. (1998). Local inhibitory action of BMPs and their relationships with activators in feather formation: implications for periodic patterning. *Dev. Biol.* **196**, 11-23.

Kondo, S. and Miura, T. (2010). Reaction-diffusion model as a framework for understanding biological pattern formation. *Science* **329**, 1616-1620.

Lawrence, P. A. (1970). How do cells know where they are? *Adv. Sci.* **132**, 121-128.

Lawrence, P. A. (1988). Background to bicoid. *Cell* **54**, 1-2.

Lawrence, P. A. (1992). *The Making of a Fly: The Genetics of Animal Design*. Chichester: Wiley-Blackwell.

Lawrence, P. A. (2001). Morphogens: how big is the big picture? *Nat. Cell Biol.* **3**, E151-E154.

Maini, P. K. (1997). Bones, feathers, teeth and coat markings: a unified model. *Sci. Prog.* **80**, 217-229.

Marcon, L. and Sharpe, J. (2012). Turing patterns in development: what about the horse part? *Curr. Opin. Genet. Dev.* **22**, 578-584.

Meinhardt, H. (1982). *Models of Biological Pattern formation*. London: Academic Press.

Meinhardt, H. (2012). Turing's theory of morphogenesis of 1952 and the subsequent discovery of the crucial role of local self-enhancement and long-range inhibition. *Interface Focus* **2**, 407-416.

Michon, F., Forest, L., Collomb, E., Demongeot, J. and Dhouailly, D. (2008). BMP2 and BMP7 play antagonistic roles in feather induction. *Development* **135**, 2797-2805.

Mou, C., Jackson, B., Schneider, P., Overbeek, P. A. and Headon, D. J. (2006). Generation of the primary hair follicle pattern. *Proc. Natl. Acad. Sci. USA* **103**, 9075-9080.

Müller, P., Rogers, K. W., Jordan, B. M., Lee, J. S., Robson, D., Ramanathan, S. and Schier, A. F. (2012). Differential diffusivity of Nodal and Lefty underlies a reaction-diffusion patterning system. *Science* **336**, 721-724.

Newman, S. A. and Frisch, H. L. (1979). Dynamics of skeletal pattern formation in developing chick limb. *Science* **205**, 662-668.

Oster, G. F. (1988). Lateral inhibition models of developmental processes. *Math. Biosci.* **90**, 265-286.

Raspopovic, J., Marcon, L., Russo, L. and Sharpe, J. (2014). Digit patterning is controlled by a Bmp-Sox9-Wnt Turing network modulated by morphogen gradients. *Science* **345**, 566-570.

Roth, S. (2011). Mathematics and biology: a Kantian view on the history of pattern formation theory. *Dev. Genes Evol.* **221**, 255-279.

Saunders, J. and Gasseling, M. (1968). Ectodermal-mesenchymal interactions in the origin of limb symmetry. In *Epithelial Mesenchymal Interactions* (ed. R. Fleischmajer and R. E. Billingham), pp. 78-97. Baltimore: William and Wilkins.

Schier, A. F. (2009). Nodal morphogens. *Cold Spring Harb. Perspect. Biol.* **1**, a003459.

Sen, S., Ghosh, P., Riaz, S. S. and Ray, D. S. (2010). Spatial periodicity induced by a chemical wave train. *Phys. Rev. E Stat. Nonlin. Soft Matter Phys.* **81**, 017101.

Sheth, R., Marcon, L., Bastida, M. F., Junco, M., Quintana, L., Dahn, R., Kmita, M., Sharpe, J. and Ros, M. A. (2012). Hox genes regulate digit patterning by controlling the wavelength of a Turing-type mechanism. *Science* **338**, 1476-1480.

Shiratori, H. and Hamada, H. (2006). The left-right axis in the mouse: from origin to morphology. *Development* **133**, 2095-2104.

Sick, S., Reinker, S., Timmer, J. and Schlake, T. (2006). WNT and DKK determine hair follicle spacing through a reaction-diffusion mechanism. *Science* **314**, 1447-1450.

Spemann, H. and Schotté, O. (1932). Über xenoplastische Transplantation als Mittel zur Analyse der embryonalen Induktion. *Naturwissenschaften* **20**, 463-467.

Summerbell, D., Lewis, J. H. and Wolpert, L. (1973). Positional information in chick limb morphogenesis. *Nature* **244**, 492-496.

Turing, A. M. (1952). The chemical basis of morphogenesis. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **237**, 37-72.

Waddington, C. H. (1956). *Principles of Embryology*. London: George Allen & Unwin.

Wolpert, L. (1969). Positional information and the spatial pattern of cellular differentiation. *J. Theor. Biol.* **25**, 1-47.

Wolpert, L. (1971). Positional information and pattern formation. *Curr. Top. Dev. Biol.* **6**, 183-224.

Wolpert, L. (1989). Positional information revisited. *Development* **107**, 3-12.

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