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Biological notion of positional information/value in morphogenesis theory

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ABSTRACT The notions of positional information and positional value describe the role of cell position in cell development and pattern formation. Despite their frequent usage in literature, their definitions are blurry, and are interpreted differently by different researchers. Through reflection on previous definitions and usage, and analysis of related experiments, we propose three clear and verifiable criteria for positional information/value. Then we reviewed literature on molecular mechanisms of cell development and pattern formation, to search for a possible molecular basis of positional information/value, including those used in theoretical models. We conclude that although morphogen gradients and cell-to-cell contacts are involved in the pattern formation process, complete molecular explanations of positional information/value are still far from reality.

KEY WORDS: *positional information, positional value, pattern formation, morphogen, theoretical model*

Introduction

How an organism develops following a very precise plan starting from a single cell is among the most outstanding mysteries in biology. Although sharing the same genes, cells at different positions develop differently and concordantly, so as to produce the fine structures of different organs. The popular concept that cells "know" their positions within the organism, and translate the information of position into corresponding cell behaviors was summarized by Lewis Wolpert as positional information and positional value (Wolpert, 1969, 1989). Then these notions are extensively used with various meanings.

In some *Xenopus* transplantation experiments, cells that are transplanted to new positions adopt the fates of host cells, not donor cells (Grainger *et al*., 1988; Krneta-Stankic *et al*., 2010). These experiments prove that position does play a role in development. Nevertheless, there are also examples of complex structure formation without knowledge of position: (1) An early wing-bud progress zone of chick always induce a new set of ulna and radius, whether it is on an early wing bud or transplanted to a late wing bud with ulna and radius (Summerbell and Lewis, 1975). (2) Cellular automata model describes the configuration of many sites, where the evolution of each site only depends on its neighbors. This model can produce complicated patterns without knowledge of position (Wolfram, 1984). (Cellular automata are just toy models in developmental biology that lack feedback control toward specific large-scale outcomes. Therefore they are insufficient in explaining the regenerations.) (3) One or a few cells can self-organize into a simplified version of an organ *in vitro*, called an organoid (Gilmour *et al*., 2017). Thus the role of position in development is not self-evident.

Some information is used by experimentalists to determine the cell position. One choice is the morphogen concentration (Lander, 2013). For example, morphogens Shh and BMP form antiparallel gradients along the dorsal-ventral axis of embryo (Zagorski *et al*., 2017). Another choice is to encode position in expression levels of genes. Fibroblast cells from different sites have distinct gene expression patterns, especially for Hox family transcription factors, known as transcriptional signature (Rinn *et al*., 2006). The expression levels of four gap genes can locate a cell position with 1% accuracy along the anterior/posterior axis (Dubuis *et al*., 2013; Tkačik *et al*., 2015). Such information of position might not be related to development. For example, if the GFP gene is incorporated into another gene, whose expression level is position-related, then the expression level of GFP is also position-related.

Abbreviations used in this paper: AP, anterior-posterior axis of body/limb/wing; BM, the boundary model for development and regeneration; DV, dorsal-ventral body axis; ECM, extracellular matrix; PCM, the polar coordinate model; PD, proximal-distal axis of limb.

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Nevertheless, GFP does not interfere with the development. Such quantities are information of position, but not necessarily positional information. The goal of introducing positional information is not to find quantities that can locate the cell, but to understand how cells at different positions have different fates.

The definitions of positional information/value are theoretical and abstract, and the role of position in development is complicated. Thus we analyze studies of pattern formation and regeneration, and aim for clear and verifiable definitions of positional information/value (Section 2), and possible related molecular mechanisms (Section 3).

The notion of positional information/value is used in several theoretical models, such as the Polar Coordinate Model and the Boundary Model, for explaining and predicting phenomena in development and regeneration experiments (Bryant *et al*., 1981; French *et al*., 1976; Lewis, 1981; Meinhardt, 1983a; Mittenthal, 1981; Papageorgiou, 1984; Stocum, 1978). We introduce these models as good illustrations of positional information/value, and explore for possible molecular mechanisms (Section 4).

Notions of positional information and positional values

The concept of positional information initially introduced by Wolpert stated that (Wolpert, 1969) "The cells in a developing system may have their position specified with respect to one or more points in the system. This specification of position is positional information." Later the notion of positional value was introduced (Wolpert, 1989): "The strong version of positional information states that there is a cell parameter, positional value, which is related to position as in a coordinate system and which determines cell differentiation. A weaker version merely emphasises position as a key determinant in cell development and differentiation."

Wolpert's definitions are abstract and difficult to verify, thus the notions of positional information/value are interpreted differently by different authors, despite their frequent usage in descriptions of development and regeneration. The usage of positional information/value can be roughly classified into five categories:

(1) the current or future position of a cell, especially the position in the anterior-posterior (AP) axis of body or proximal-distal (PD) axis of leg (Tickle and Barker, 2013; Paré *et al*., 2014; Onimaru *et al*., 2015; Yokoyama *et al*., 2002;Nakamura *et al*., 2007; Bando *et al*., 2009; Moriyama *et al*., 2009);

(2) the expression levels of specific genes, which vary across cells at different positions (Dubuis *et al*., 2013; Rinn *et al*., 2006; Tkačik *et al*., 2015);

(3) the concentrations of morphogens (Gregor *et al*., 2007a; Lo *et al*., 2014).

(4) material or signal that leads to new structures/patterns (in the Boundary Model [Meinhardt, 1983a, 1983b, 2013]).

(5) an artificial parameter of cells, which describes the regeneration process (in the Polar Coordinate Model [Bryant *et al*., 1981; French *et al*., 1976]).

We first analyze these usage through the reflection on Wolpert's idea and related experiments. Then we propose our criteria of positional information/value: what is related to position and determines cell development?

Position is not the direct cause of differential development

The role of position in development has been verified by many experiments (Grainger *et al*., 1988; Krneta-Stankic *et al*., 2010). How does position participate in development? One possibility is that cells can directly sense their positions in a global coordinate system, which could determine cell fates. Another possibility is that cells sense their local environment, which is position-related.

Some experiments show that the role of position in development is complicated: (1) Local application of retinoic acid to regenerating amphibian limbs cut at the wrist can cause an extra radius and ulna to develop with a concentration of 2-4 μg/limb, an extra part of the humerus with 4-8 μg/limb, while 16 μg/limb gives a complete extra limb (Maden *et al*., 1985). (2) For chick embryos, when beads containing Noggin are placed in the webbing between digits 3 and 4, digit 3 anteriorly transforms into digit 2 (Dahn and Fallon, 2000). In these experiments, although cell positions are unperturbed, injected chemicals make cells develop as if they were at another position. This illustrates that changing the local environment through injecting proper chemicals is equivalent to changing the cell position. It is even possible that the effect of position change is cancelled out by injecting proper chemicals.

Therefore we conclude that a cell cannot directly measure its position, but just detect its position-related surrounding environment, such as the local concentration of chemicals, or the properties of neighboring cells. Although the detection range can be extended by specific structures, such as axon, long cytonemes or tunneling nanotubes (Dupont *et al*., 2018; González-Méndez *et al.,* 2019), cells are rather near-sighted in general. The causal chain is not position => cell fate, but position => local environment => cell fate. This causal chain means that position can only indirectly determine the cell fate through influencing the local environment. If we change the cell position, but force the local environment to be unperturbed, the cell fate should not change.

How are position-related factors produced?

If cells cannot directly measure the global position, how could cells at different positions have different fates? In other words, what is the source of asymmetry among cells?

(1) Due to fertilization, zygote itself is already asymmetric (Cowan and Hyman, 2004). This asymmetry can be passed to its descendants.

(2) Cell division may not be symmetric, especially in early development stages. For example, the cell division of sea urchin between 4-cell stage and 8-cell stage is asymmetric (Horstadius, 1939).

(3) Such differences within and among cells in (1) and (2) produce different environments.

(4) Different environments are interpreted differently by the same or different cells, which produce more differences in cells.

(5) Such differences in cells are further reflected in the environment, leading to a positive feedback loop: difference of cells <=> difference of environments.

(*) Alan Turing's reaction-diffusion model is also a possible source of asymmetry. In this model, slight asymmetry in chemical distribution resulting from stochastic fluctuations can be amplified by the system, resulting in stable and macroscopic asymmetric patterns (Turing, 1952).

Intracellular factors should not be included in positional information/value

So far, we have shown that in development, position-related factors are differences in environment (extracellular) and differences in cells (intracellular).

The positional information is introduced to describe the role of position in pattern formation, namely why cells at different positions have different fates. Here cell fate mainly means the specific gene expression pattern. If we also include intracellular factors, especially gene expression levels, in the definition of positional information, then positional information becomes a tautology of pattern, and loses its role in explaining pattern formation. In short, we cannot use "cells are different (in gene expression levels)" to explain "cells are different (in gene expression levels)".

Besides, the statement "cells could interpret the same positional information differently according to their characteristics" (Wolpert, 2016) also requires that intracellular factors are not positional information, otherwise different cells already have different positional information.

Therefore we propose a concise solution: exclude everything within a cell from its positional information. Now the positional information of a cell is independent of "what this cell is", and only reflects "where this cell is".

Definitions of positional information/value

As a conclusion of the above discussion, we propose three criteria for a factor to be the positional information of a given cell:
(1) Position-related: this factor is not the same at all different positions;
(2) Extracellular: this factor is outside of this cell;
(3) Direct cause: the change of this factor can directly affect the cell fate. It means that if this factor changes, while all other extracellular factors are forced to be unperturbed, the cell fate still changes.

Positional value is any quantified positional information

We can utilize these criteria to check whether some information is positional information or not, and search for possible molecular mechanisms of positional information. Under these criteria, positional information can be the concentration of a morphogen, or properties of neighboring cells that are related to cell-cell contact.

Some environmental factors are related to development, but are the same everywhere, such as temperature and oxygen concentration. These factors only satisfy criteria (2) (3), and can be named "non-positional information". One cell's own expression levels of specific genes only satisfy criteria (1) (3), and should be regarded as the characteristics of this cell, or the interpretations of its positional information. Nevertheless, gene expressions and positional information can affect each other. Cell position can be regarded as extracellular, since we can substitute one cell by another cell without changing its position. However, cell position is not the direct cause of cell fate. Thus position itself, which only satisfies criteria (1) (2), is not positional information. The above argument proves that none of the three criteria is redundant, in the sense that any two criteria do not imply the third one, and cannot describe positional information accurately.

In summary, each cell is a processor, which receives local information (positional and non-positional), and interprets such information based on its own characteristics, to determine its fate. Mathematically speaking, a cell corresponds to a function f, which receives positional information x and non-positional information c as input, and produces cell fate $f(x,c)$ as output. The function f depends on this cell's properties, especially gene expression levels. The cell fate $f(x,c)$ can further affect the positional information of neighboring cells.

Molecular foundations of positional information and positional value

In this section, we consider possible molecular mechanisms for positional information/value. To be related to positional information, a factor needs to be non-uniformly distributed, extracellular, and be the cause, not the result, of different cell fates. Under our definitions, position-related gene expression is not positional information, but an interpretation of positional information, and the interpretation mechanism of positional information within cells is not the main focus of this paper.

Known molecular mechanisms for positional information can be classified into two families: morphogen gradients and contacts between cells. The analysis of the experimental data shows the existence of mutual regulations between two families (Clevers and Nusse, 2012; Fernandez-L *et al*., 2009; Hayashi *et al*., 2015; Kumar *et al*., 2007; Lin *et al*., 2012; da Silva *et al*., 2002; Wu and Mlodzik, 2008). Wolpert, who first proposed morphogen gradient as positional information, even claimed that "diffusible gradients are out" (as the only explanation of positional information), and cell-cell interactions can fine-tune the crude positional information system set by morphogens (Kerszberg and Wolpert, 2009; Richardson, 2009).

Morphogen gradients as positional information

The hypothesis that morphogen gradients can influence positional information of cells and then pattern formation was formulated in the 1960's by Wolpert (Wolpert, 1969). By definition, a morphogen is a signaling molecule which induces different specific cellular responses, and governs patterns of tissue development, depending on its non-uniform concentration distribution.

Up to date, many molecules have been found that can play the role of morphogens in different processes and organisms (Table 1). Most morphogens are secreted proteins that form extracellular gradients, among which the most well-known examples are fibroblast growth factors (FGF), activins, bone morphogenetic proteins (BMP), Transforming growth factor-β (TGF-β), Wnt, Hedgehog (Hh), and Chordin proteins. Retinoic acid (RA) in animals and phytohormone auxin in plants are metabolite morphogens (Bryant and Gardiner, 1992; Brukhin and Morozova, 2011; Kim and Stocum, 1986; Ludolph *et al*., 1990; Paque and Weijers, 2016; Stocum and Thoms, 1984; Thoms and Stocum, 1984; Tuazon and Mullins, 2015; Uggla *et al*., 1996).

The formation of morphogen gradients can be regulated by numerous mechanisms (Wartlick *et al*., 2009). Gradients formed by free, hindered or facilitated diffusion (Müller *et al*., 2013), or by active transportation (Gregor *et al*., 2007b), can be further tuned by regulated secretion and internalization of morphogen and/or its receptor (Gore and Sampath, 2002; Gregor *et al*., 2007a; Kakugawa *et al*., 2015; Kunche *et al*., 2016; Lo *et al*., 2014). Furthermore, different feedback regulation loops can operate between morphogen gradients and their regulators (Ashe and Briscoe, 2006).

The differential interpretations of cells to morphogen gradients can be affected by many factors, such as morphogen concentrations, cofactors, duration of acting, already existing gene expression patterns and epigenetic modifications (Ashe and Briscoe, 2006; Bintu *et al*., 2016; Briscoe and Small, 2015; Chen and Dent, 2014; Christian, 2012; Sagner and Briscoe, 2017; Tabata and Takei, 2004; Watanabe *et al*., 2016). These factors form a huge gene regulatory

network. Thus it is extremely difficult to formulate the general rules for the interpretation of morphogen gradients by cells. For instance, RA can cause both gene activation and gene repression depending on several additional cofactors (Cunningham and Duester, 2015). In the *Drosophila* wing disc, only cells in the anterior part can respond to Hh, since they express the transcriptional effector Ci of the Hh pathway (Sagner and Briscoe, 2017). Silencing of pluripotency genes enables embryonic stem cell differentiation,

TABLE 1

MORPHOGEN GRADIENTS AND THEIR EFFECTS IN DIFFERENT TISSUES AND ORGANISMS

Morphogen	Effect on Positional value	Tissue	Organism	Stage	Gradient
Metabolites					
Retinoic Acid	Proximal	Limb (R)	*Ambystoma mexicanum*	Larval	-
			Notophtalmus viridescens	Adult	-
	Posterior	Limb (R)	*Ambystoma mexicanum*	Larval	AP, max in P
			Notophtalmus viridescens	Adult	-
		Wing (D)	*Gallus domesticus*	Embryo	AP, max in P
		Hindbrain	*Dario rerio*	Somitogenesis	AP, max in P
			Mus musculus	Somitogenesis	AP, max in P
	Ventral	Limb (R)	*Ambystoma mexicanum*	Larval	-
	-	Embryo	*Mus musculus*	Somitogenesis	AP, two tailed, max in trunk mesoderm, low in hindbrain and caudal zone
		Embryo	*Danio rerio*	Gastrula, Somitogenesis	AP, two tailed
Auxin		Embryo	*Arabidopsis thaliana*	Embryo	Steep gradient
		Root/shoot lateral meristems	*Arabidopsis thaliana*	Adult	Steep gradient
Extracellular proteins					
Spätzle	Ventral	Embryo	*Drosophila melanogaster*	Embryo	DV, max in V
Wingless		Wing disc	*Drosophila melanogaster*		Secreted from DV boundary (center of disc)
Dpp	Dorsal	Embryo	*Drosophila melanogaster*	Embryo	Dorsal Midline -> Lateral, Max in Dorsal Midline
	Dorsal	Wing disc (D)	*Drosophila melanogaster*	Larval	Medial (slightly A)-> Lateral (A and P sides)
Gbb			Drosophila melanogaster		
Screw			Drosophila melanogaster		
Bmp2		Embryo	*Paracentrotus lividus*	Blastula	DV, max in D
			Xenopus laevis		
Bmp4		Embryo	*Paracentrotus lividus*	Blastula	DV, max in D
			Xenopus laevis		
		Embryo	*Danio rerio*	Gastrula	
Squint			Danio rerio		
Cyclops			Danio rerio		
Southpaw			Danio rerio		
Gdf6a		Retina	*Danio rerio*		
Shh	Posterior	Wing (D)	*Gallus domesticus*	Embryo	AP, max in P
		Limb (D)	*Mus musculus*	Embryo	AP, max in P
	Dorsal	Neural Tube	*Mus musculus*	Embryo	DV, max in V
Nodal			Mus musculus		
Fgf8	Rostral	Neocortex	*Mus musculus*	Early neurogenesis -postnatal	-
Wnt11	Dorsal	Embryo	*Xenopus laevis*	Early embryo	Extracellular signal
Xnr family (1,2,4,5,6)	Mesoderm	Embryo	*Xenopus laevis*	Blastula	DV
XTC-MIF	Dorsal	Embryo	*Xenopus laevis*	Blastula - Mid-gastrula	
Intracellular proteins					
Bicoid	Anterior	Embryo	*Drosophila melanogaster*	Blastoderm	AP, max in A, mRNA
Caudal	Posterior	Embryo	*Drosophila melanogaster*	Blastoderm	AP, max in P
Dorsal	Ventral	Embryo	*Drosophila melanogaster*	Embryo	DV bell shaped, max in V
Hunchback	Anterior	Embryo	*Drosophila melanogaster*	Embryo	AP, max in A
Nanos	Posterior	Embryo	*Drosophila melanogaster*	Embryo	AP, max in P, mRNA

(D)= developing, (R)= regenerating. Table built from (Agius *et al.*, 2008; Benkovics and Timmermans, 2014; Boualem *et al.*, 2008; Briscoe and Small, 2015; Chen and Schier, 2001; Chitwood *et al.*, 2009; Cho *et al.*, 1991; Cunningham and Duester, 2015; Dessaud *et al.*, 2008; Ferguson and Anderson, 1992; Foe and Alberts, 1983; Fukuchi-Shimogori and Grove, 2001; Gritli-Linde *et al.*, 2001; Gurdon *et al.*, 1994; Hamaratoglu *et al.*, 2014; Kessel, 1992; Kim and Stocum, 1986; Ludolph *et al.*, 1990; Maden, 1982, 1983b; Marshall *et al.*, 1992; Matsuda *et al.*, 2016; McDowell *et al.*, 1997; Mlodzik and Gehring, 1987; Moussian and Roth, 2005; Niswander *et al.*, 1993; Onimaru *et al.*, 2015; Plouhinec *et al.*, 2013; Ramel and Hill, 2012; Restrepo *et al.*, 2014; Scadding and Maden, 1994; Smith *et al.*, 1991; Stocum and Thoms, 1984; Tao *et al.*, 2005; Thaller and Eichele, 1987; Thoms and Stocum, 1984; Tickle and Barker, 2013; Tickle *et al.*, 1982; Towers *et al.*, 2012; Tuazon and Mullins, 2015; Uggla *et al.*, 1996; Wharton *et al.*, 1993; Zeng *et al.*, 2001).

TABLE 2

MEMBRANE/EXTRACELLULAR COMPONENTS OF SIGNALING PATHWAYS REGULATING CELL FATES AND ORGAN DEVELOPMENT/REGENERATION

Protein	Regulates (at the cellular level)	Regulates (at the organ/tissue level)	Organ	Organism
Ds/Fat signaling				
Ds and Fat	proliferation	leg regeneration, leg intercalary regeneration, leg length and diameter	Leg (R)	*Gryllus bimaculatus*
	orientated division	organ shape	Eye or wing	*Drosophila melanogaster*
	orientated division	leg length and shape	Leg	*Drosophila melanogaster*
	polarity	hair polarity, vein development, wing size	Wing	*Drosophila melanogaster*
Fj	proliferation	organ development	Eye, leg or wing	*Drosophila melanogaster*
Fz/Stan/Vang-PCP				
Fz	-	bristle orientation	Wing	*Drosophila melanogaster*
Notch				
Notch1	-	somitogenesis	Somites	*Mus musculus*
	-	organ regeneration	Head and tentacle	*Hydra*

Ds = Dachsous, Fz = Frizzled, R= regenerating. Table built from (Adler *et al*., 1998; Baena-Lopez and García-Bellido, 2006; Baena-López *et al*., 2005; Bando *et al*., 2009, 2011; Brodsky and Steller, 1996; Conlon *et al*., 1995; Gee *et al*., 2011; Grusche *et al*., 2011; Gubb and Garcia-Bellido, 1982; Hayashi *et al*., 2014; Li *et al*., 2009; Morin-Kensicki *et al*., 2006; Münder *et al*., 2013).

which influences further interpretation of morphogen gradients (Chen and Dent, 2014; Sagner and Briscoe, 2017).

Our current knowledge of morphogens and their role in pattern formation is still limited. For each well studied organism in developmental biology (*Xenopus*, mouse, chicken), only a limited amount of morphogens and corresponding mechanisms are known, which cannot explain the large number of tissues, each of which requires a different morphogen configuration. Either there are more unknown morphogens, or there are more unknown mechanisms of known morphogens. Besides, the formation and differential interpretations of morphogen gradients already require difference among cells, which implies different positional information. Thus the fundamental role of morphogens as a basis for positional information in a complicated living body is still questionable. This also meets Wolpert's reflection on his own ideas (Kerszberg and Wolpert, 2009; Richardson, 2009).

Cell-cell contacts as positional information

Cell-cell contact is a local mechanism between neighboring cells. It is achieved either by direct contact between cell surfaces of adjacent cells, involving membrane proteins and oligosaccharide residues (Morozova *et al*., 2006; Suprasert *et al*., 1999; Zablackis *et al*., 1996), or by indirect interaction through extracellular matrix (ECM). The spatially inhomogeneous cell-cell contacts induce appropriate and localized cellular responses, which ultimately result in the formation of proper patterns. It has been shown that cell-cell contact could affect cell size, shape, polarity, and cell behavior, such as differentiation, migration, proliferation, division orientation, and survival (Baena-López *et al*., 2005; Bando *et al*., 2009; Brodsky and Steller, 1996; Goodrich and Strutt, 2011; Yang *et al*., 2002). Such effects have been verified in diverse tissues and organisms (Table 2).

ECM is an extracellular macromolecular network, which is related to cell adhesion, differentiation and cell-cell communication (Abedin and King, 2010; Theocharis *et al*., 2016). Cells could affect local ECM, and further induce responses from neighboring cells. In axolotl, grafted ECM could induce or inhibit limb regeneration, depending on the position of ECM source. The effective ingredient of ECM on regeneration might be heparan sulfate (Phan *et al*., 2015).

Among cellular properties linked to cell-cell contacts, cell adhesiveness appears to be position dependent, notably in regenerating amphibian blastemal limbs and chick wing buds (Nardi and Stocum, 1984; Yajima *et al*., 1999). For example, cell surface protein Prod1, which plays a role in cell adhesion in the newt limb (Kumar *et al*., 2007; Nomura *et al*., 2016), is more expressed in newt limb proximal blastemas compared to distal blastemas (da Silva *et al*., 2002; Maden, 2002). The proximalization effect of retinoic acid is achieved through increasing the concentration of Prod1 (Kumar *et al*., 2007).

Cell-cell contacts often trigger intracellular signaling cascades, which lead to the expression of specific target genes. Currently, only a few signaling pathways have been reported to link cell-cell contacts to cell fate determination. Besides, the majority of a signaling pathway lies within the cell, which does not count as positional information.

The Hippo signaling pathway plays an important role in regulating organ size and regeneration (Bando *et al*., 2009; Bando *et al*., 2018; Hayashi *et al*., 2015). Two components of this pathway, Four-jointed (Fj) and Dachsous (Ds), are in opposing gradients in different *Drosophila* tissues, including eye, wing imaginal disc, pupal wing and scutellum (Bosveld *et al*., 2012; Goodrich and Strutt, 2011; Yang *et al*., 2002).

Another example is the Notch signaling pathway. It is triggered by an interaction between the Notch receptor and its ligands in the Delta/Serrate/Lag2 (DSL) family, which are all extracellular parts of transmembrane proteins (Bray, 2016). Notch signaling pathway plays an important role in fine-grained patterning processes such as the formation of checkerboard-like differentiation patterns and sharp boundaries between developing tissues (Shaya and Sprinzak, 2011).

The embryonic differentiation wave model is a mechanochemical model based on cytoskeletal functioning. The activity of cytoskeleton, especially the organelle cell state splitter, influences the nucleus through changing gene expression levels. Such influence is then transmitted to other cells through cell-cell mechanical contacts (Gordon, 1999; Gordon and Brodland, 1987; Gordon and Gordon, 2016a, 2016b; Lu *et al.,* 2012). This is an example of mechanical factors in pattern formation (Bellas and Chen, 2014; Chanet and

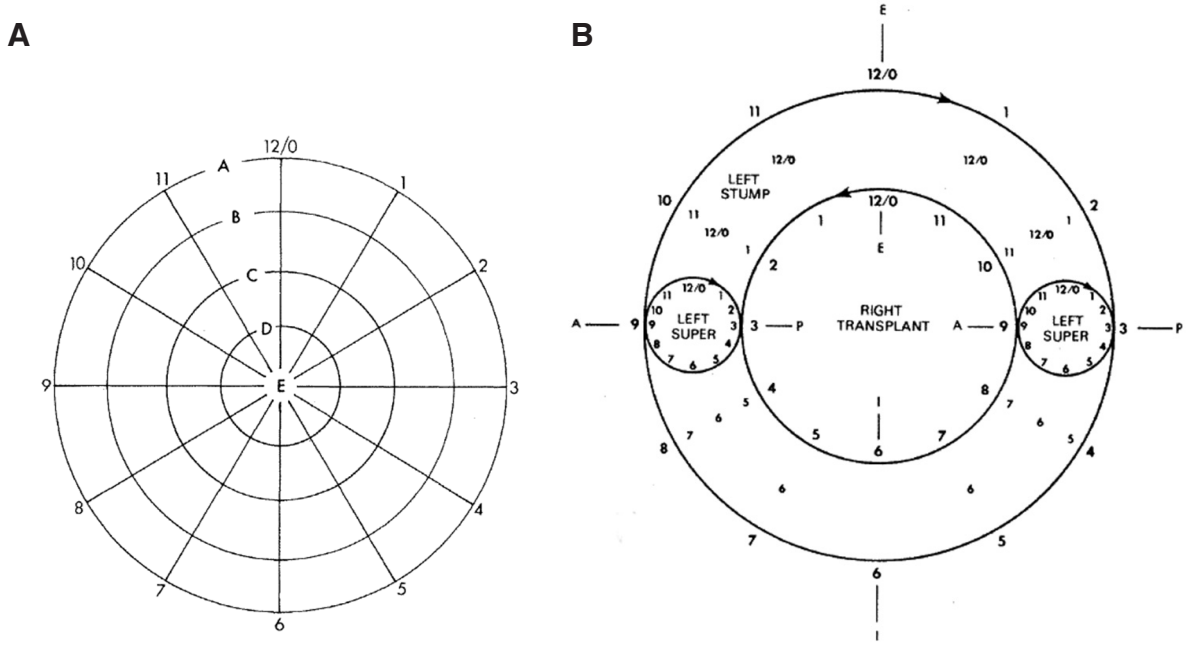

Fig. 1. The polar coordinate model. *The model is illustrated in* **(A)** *that the coordinate of each cell has a radial component (A-E) and a circumferential component (0-12). In* **(B)***, the model explains the supernumerary legs in the contralateral leg grafting experiment. Cells regenerate between inner circle (graft) and outer circle (stump), and acquire positional values according to the shortest intercalation rule. At each of two anterior/posterior positions, cells have two shortest routes, which form a full circle. Each full circle follows the complete circle rule, and forms a supernumerary left leg. A, P, I and E stand for anterior, posterior, internal and external. Super is the newly formed supernumerary leg. Figures extracted from (French* et al.*, 1976).*

Martin, 2014).

There is a theory for pattern formation, in which cell behaviors and cell-cell contacts are encoded by the combinations of specific cell surface markers (Bessonov *et al.*, 2019; Minarsky *et al.*, 2018; Morozova and Penner, 2015; Morozova and Shubin, 2013; Wang *et al.*, 2020). The role of cell-cell contact in pattern formation is still far from clear, especially on molecular level. Besides, part of the cell-cell contact mechanism is intracellular, which should not be included in positional information.

Theoretical models built on positional information and positional value notions

The notions of positional information/value are used in several theoretical models for explaining and predicting regeneration experiments (Bryant *et al.*, 1981; French *et al.*, 1976; Lewis, 1981; Meinhardt, 1983a; Mittenthal, 1981; Papageorgiou, 1984; Stocum, 1978). These models are good illustrations of positional information/value, although they have different usage of related notions. We need to emphasize that such models only cover a small fraction of all pattern formation phenomena.

The Polar Coordinate Model (PCM)

The Polar Coordinate Model (PCM) uses the notion of positional values to formulate common rules of regeneration, which could explain several outcomes of regeneration experiments of amphibian limbs, cockroach legs, and *Drosophila* imaginal discs (Bryant *et al.*, 1981; French *et al.*, 1976). The PCM proposes that each cell contains specific positional information, which is represented in a polar coordinate system. These values have radial (A-E) and circumferential (0-12, where 0 coincides with 12) components, defining the cell's position on the proximal-distal axis and on the section of the organ (limb, imaginal disc) respectively (Fig. 1A).

Due to removal or implantation of tissues, cells with non-adjacent values confront. In such cases, new cells are generated, and their positional values should fill the gap (intercalation). The intercalation occurs under two rules (French *et al.*, 1976). The first rule, shortest intercalation rule, states that intercalation occurs with the shortest route. For example, when cells with values 1A and 5A confront, the intercalation generates cells as 1A (2A, 3A, 4A) 5A, not 1A (0A=12A, 11A, 10A, 9A, 8A, 7A, 6A) 5A. The second rule, complete circle rule, states that when one radial level is full, it will produce cells with more distal positional values (distal transformation). For example, when cells with values 1B-12B are all present (complete circle), cells at C level will be generated.

The PCM could explain the occurrence, orientation and handedness of supernumerary limbs developing after contralateral (on the opposite side of the animal) grafts in cockroaches and amphibians, and certain types of 180° ipsilateral (on the same side of the animal) rotations in amphibians (French *et al.*, 1976). For example, in contralateral graft, the grafted limb and the original stump have different positional values, therefore during healing, new cells are generated, and their positional values are determined by the shortest intercalation rule. Such intercalation will produce two more full circles of circumferential values, and the complete circle rule leads to two supernumerary limbs, whose handednesses are both opposite to the grafted limb (Fig. 1B).

Later, the rules of the PCM were questioned and modified ((Bryant *et al.*, 1981; Stocum, 1978, 1981). The modified versions of the PCM can well explain formation of supernumerary limb elements after 180° ipsilateral chick limb bud rotation (Javois and Iten, 1986), retina regeneration in *Xenopus* (McDonald, 1977), post-

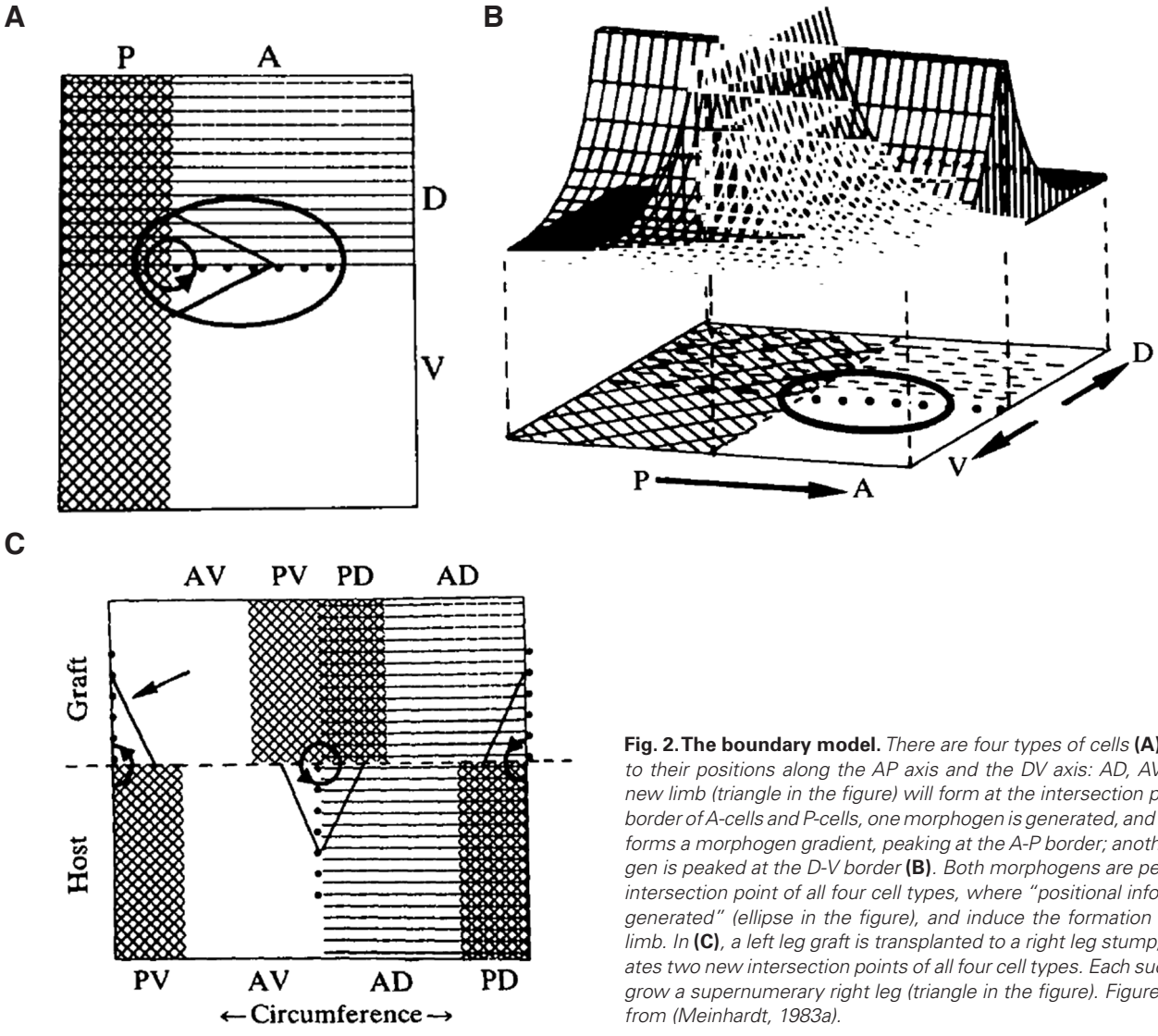

Fig. 2. The boundary model. *There are four types of cells* **(A)**, *according to their positions along the AP axis and the DV axis: AD, AV, PD, PV. A new limb (triangle in the figure) will form at the intersection point. At the border of A-cells and P-cells, one morphogen is generated, and its diffusion forms a morphogen gradient, peaking at the A-P border; another morphogen is peaked at the D-V border* **(B)**. *Both morphogens are peaked at the intersection point of all four cell types, where "positional information (is) generated" (ellipse in the figure), and induce the formation of the new limb. In* **(C)**, *a left leg graft is transplanted to a right leg stump, which creates two new intersection points of all four cell types. Each such point will grow a supernumerary right leg (triangle in the figure). Figures extracted from (Meinhardt, 1983a).*

regeneration (regeneration of missing structures of the embryo at the adult stage) of the ctenophore Mnemiopsis leidyi (Henry and Martindale, 2000), and describe regeneration in theoretically all epimorphic fields (Lewis, 1981, Papageorgiou, 1984). However, these models cannot explain the formation of some types of supernumerary limbs in axolotl (Maden and Mustafa, 1982; Tank, 1981) and newt (Papageorgiou and Holder, 1983).

The Boundary Model

The Boundary Model (BM) explains the grafting experiments of vertebrate limbs using the notion of positional information, associated with morphogen concentrations. In the BM, it is assumed that the production of a morphogen requires two or more cell types. For example, one cell type produces an inactive morphogen, which is activated by another cell type. Then the distribution of this morphogen will be peaked at the boundary of different types of cells. When there are three or four types of cells involved, the intersection point of boundaries will be the locally unique peak of the morphogen (Fig. 2 A,B). This intersection point, where "positional information (is) generated", will become the center of (re)generation (Meinhardt, 1983a, 1983b, 2013). In the BM, positional information represents material or signal that leads to new structures/patterns.

The BM assumes there are four cell types (AV, PV, PD, AD) surrounding a limb. After contralateral grafting, there are two more intersection points of all four cell types, which will create two supernumerary limbs (Fig. 2C). The orientation and handedness can be predicted from the properties of the intersection point. The BM avoids a problem in the PCM: how do cells know which the shortest route is (Meinhardt, 1983a)? The BM can explain some experiments where the PCM fails, such as supernumerary limbs observed after limb rotations with different angles, and the regeneration of double posterior limbs in axolotl (Campbell and Tomlinson, 1995; Maden, 1983a; Meinhardt, 1983a; Nacu *et al*., 2016; Stocum, 1978; Vincent and Lawrence, 1994).

Molecular implementations of positional information and positional value in theoretical models

The radial component of positional values in the PCM describes the position of a cell along the PD axis of the limb. One possibility is the Ds/Fat system in cell-cell contact mechanisms. For each

cricket leg segment, Ds and Fat form opposite gradients, and the steepness of the Ds/Fat gradient controls growth along the PD axis of the regenerating leg (Bando *et al*., 2009; Bando *et al*., 2018). Such repetition of Ds/Fat gradient in each leg segment is consistent with predictions of the PCM, which postulates that positional values are repeated in each leg segment (French *et al*., 1976). However, it is still a problem why the morphogen concentrations are kept after transplantation and regeneration.

The circumferential component of positional values in the PCM describes the position of a cell along a circle. No morphogen gradient or cell-cell contact mechanism has been shown to act along a circle. Besides, one morphogen is not enough to encode the circumferential position. A morphogen cannot be continuously distributed along a circle, while satisfying that different places have different concentrations.

In the BM, new structure formation depends on morphogen gradients, which are formed at the boundary of several different cell types. There is no molecular evidence for the generation of a morphogen at the boundary. Besides, the boundary is difficult to determine, since "what cells are of different types" is unclear. The formation of different cell types requires another mechanism, which is unknown, especially on molecular level.

Discussion and Conclusion

Through the analysis of previous definitions and related experiments, we propose three criteria for positional information/value, which are clear and verifiable. In brief, the positional information of one cell is the position-specific information of any surrounding materials (chemicals and cells) which could affect the fate of this cell. Positional value is any quantified positional information.

Analysis of current knowledge in the field allows us to conclude that though morphogen gradients and cell-cell contacts are involved in positional information, there is still a long distance from a complete molecular structure of positional information in pattern formation. Some molecules are related to positional information in the theoretical models PCM and BM. However, these molecules are not enough to explain everything.

Besides positional information by Wolpert, there is another idea of pattern formation: reaction-diffusion model by Turing (Turing, 1952). Reaction-diffusion model can explain the formation of stripes, spots (Kondo and Miura, 2010), digits (Raspopovic *et al*., 2014) and somitogenesis (Cotterell *et al*., 2015). Reaction-diffusion model, like cellular automata, is locally self-inducing, and does not require non-uniform distribution of chemicals, thus it is often considered as an opposing idea to positional information. However, there have been discussions of connecting reaction-diffusion with positional information (Green and Sharpe, 2015).

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