REVIEW ARTICLE

Mechanisms of pattern formation in development and evolution

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SUMMARY

We present a classification of developmental mechanisms that have been shown experimentally to generate pattern and form in metazoan organisms. We propose that all such mechanisms can be organized into three basic categories and that two of these may act as composite mechanisms in two different ways. The simple categories are *cell autonomous* mechanisms in which cells enter into specific arrangements ('patterns') without interacting, *inductive* mechanisms in which cell communication leads to changes in pattern by reciprocal or hierarchical alteration of cell phenotypes ('states') and *morphogenetic* mechanisms in which pattern changes by means of cell interactions that do not change cell states. The latter two types of mechanism can be combined either *morphostatically*, in which case inductive mechanisms act first, followed by the morphogenetic mechanism, or *morphodynamically*, in which case both types of mechanisms interact continuously to modify each other's dynamics. We propose that this previously unexplored distinction in the operation of composite developmental mechanisms provides insight into the dynamics of many developmental processes. In particular, morphostatic and morphodynamic mechanisms respond to small changes in their genetic and microenvironmental components in dramatically different ways. We suggest that these differences in 'variational properties' lead to morphostatic and morphodynamic mechanisms being represented to different extents in early and late stages of development and to their contributing in distinct ways to morphological transitions in evolution.

Key words: Induction, Pattern formation, Morphodynamic development, Morphostatic development, Morphogenesis, Tooth, Brain, Limb, Evolution, Genetic networks

INTRODUCTION

Evolution of metazoan organisms has produced, over hundreds of millions of years, both phenotypic complexity and the developmental mechanisms by which such complexity is generated. During development a single cell becomes an organism composed of multiple cell types arranged in spatial distributions that can be both architecturally complex and functionally coherent. How this distribution of cellular phenotypes ('cell states') is attained through spatiotemporal regulation of gene interactions and cell behaviors is one of the main questions of developmental biology. To this end, considerable knowledge has been acquired during the last few decades about the genetic composition of multicellular organisms, how various genes and gene products interact, where are they expressed, and in which developmental processes are they involved.

Organismal development is enabled by *developmental mechanisms*. A developmental mechanism is understood in this paper as gene product interactions and changes in cellular behaviors (such as mitosis, apoptosis, secretion of molecular signals, cellular adhesion, differentiation, and so forth) that are required for and cause the formation of a particular arrangement of cell states in three-dimensional space (i.e., a 'pattern'; we reserve the word 'form' for the spatial arrangement of cells without considering their state). In formal terms the development of an organism can be described as transformation from one set of patterns to another set of patterns and here we aim to highlight the basic logic of the developmental mechanisms underlying these pattern transformations.

Causal explanations of pattern formation in an embryonic primordium require knowledge of all the genes, epigenetic determinants (that is, surrounding cell arrangements and other microenvironmental conditions in the embryo), and their interactions necessary for generating such a pattern from a previous pattern. In practice, causality can be inferred by testing how well a developmental mechanism predicts the 'variational' properties – the range of potential morphological outcomes.

It is common in theoretical discussions of development to distinguish two components of pattern formation (Wilkins, 2001). First, pattern formation through cell-cell signaling mechanisms (we will refer to these as *inductive mechanisms*) establishes cells with different states and different spatial relationships by signaling in two and three dimensions in

developing planar and solid tissues, respectively. Second, mechanisms that use cell behaviors other than signaling (we will refer to these as *morphogenetic mechanisms*) act on the previously established pattern to cause the formation of three-dimensional tissues and organs. As described in detail below, morphogenetic mechanisms change the spatial distribution of cells without changing cell states.

Morphogenetic and inductive mechanism act at all stages of development. Inductive mechanisms are generally implicated in developmental changes that produce new patterns. Because induction is a prerequisite for development to proceed no particular attention is normally paid to the order in which inductive and morphogenetic mechanisms function. We suggest, however, that the relative timing, including possible coincidence, of inductive and morphogenetic mechanisms can have major consequences for developmental dynamics and the range of potential morphological outcomes, and is therefore of central importance for the understanding of both development and morphological evolution. In fact the terms 'pattern', 'pattern formation' and 'morphogenesis' are often used in different and not always explicit ways. In this article, we define these terms in a way that does not make assumptions about how inductive and morphogenetic mechanisms interrelate in producing developmental change.

A key aspect of our treatment is the introduction (or rather appropriation) of the term 'morphodynamic' (distinguished from 'morphostatic') to characterize complex developmental mechanisms in which inductive and morphogenetic mechanisms interact with one another in a reciprocal fashion. The need for new concepts to bring order to the complexities of morphogenesis was anticipated by earlier investigators such as the cell biologist Paul Weiss, whose influential textbook of developmental biology was first published under the German title *Morphodynamik* (Weiss, 1926) and later published in English as *Principles of Development* (Weiss, 1939). The mathematician René Thom used the allied phrase "dynamics of forms" in his topological treatment of embryogenesis and human biology *Structural Stability and Morphogenesis* (Thom, 1975).

In the following sections we briefly review and classify the main types of developmental mechanisms for which there is experimental evidence. As noted, these can be characterized as basic mechanisms that employ only one or few cell behaviors. Although our main objective is to explore the ramifications of the heretofore overlooked morphostatic/morphodynamic distinction, we also emphasize that efforts to formulate useful computational models of developmental and evolutionary-developmental scenarios (Hunding et al., 1990; Mjolsness et al., 1991; Drasdo and Forgacs, 2000; von Dassow et al., 2000; Solé et al., 2000; Salazar-Ciudad et al., 2001a; Salazar-Ciudad et al., 2001b) will benefit from an accurate schematization of the full range of experimentally confirmed developmental mechanisms.

A REPERTORY OF BASIC DEVELOPMENTAL MECHANISMS

Cell autonomous mechanisms

Cell autonomous developmental mechanisms all involve one cellular behavior: mitosis. Thus, cells do not interact mechanically or by signaling. See Fig. 1.

Division of a heterogeneous egg

With few exceptions (mammalian and some turbellarian clades) different parts of the egg contain different protein or mRNA gene products. Non-uniformities in the egg may result from asymmetric assembly of materials from follicle or nurse cells during the course of oogenesis, or non-uniformities inherent to all cells (Gilbert, 2000; Muller, 2001). In some cases, as in *Drosophila* (Riechman and Ephrussi, 2001), the oocyte is patterned by inductive interactions with the cells in the gonads.
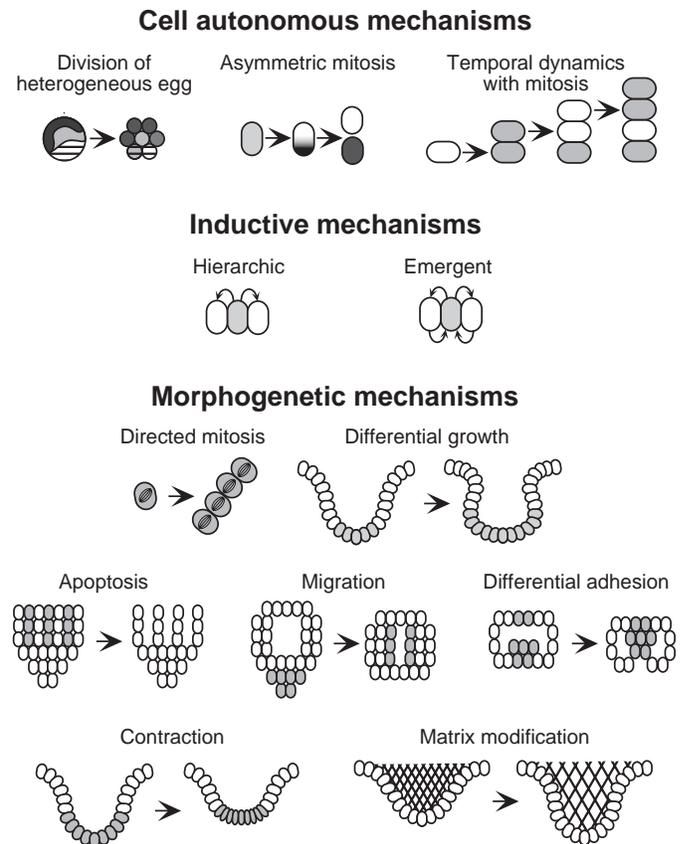

Fig. 1. Schematic examples of the basic developmental mechanisms. *Division of an heterogeneous egg*: different parts of the egg bind different molecules (indicated by different shading) resulting in different blastomere cells. *Asymmetric mitosis:* molecules are differentially transported into different parts of a cell resulting in different daughter cells. *Internal temporal dynamics coupled to mitosis*: cells that have oscillating levels of molecules before their division can produce spatial patterns. *Hierarchic induction*: inducing cell (gray) affects neighboring cells but the induced cells (white) do not affect the production of the inducing signal. *Emergent induction*: inducing cell affects neighboring cells, which in turn signal back affecting the production of the inducing signal. *Directed mitosis*: consistently oriented mitotic spindles may direct tissue growth. *Differential growth*: cells dividing at a higher rate (gray) can alter tissue shape. *Apoptosis*: transformation of an established pattern into another can result from apoptosis affecting specific cells (gray). *Migration*: cells can migrate to a new location. *Adhesion*: a change in pattern can result if a set of cells have differential adhesion properties (strong adhesion among gray cells). *Contraction*: differential contraction of cells can cause buckling of a tissue. *Matrix swelling, deposition, and loss*: matrix swelling can cause budding.

Asymmetric mitosis

Nearly all cells exhibit some kind of internal polarity causing gene products or mRNAs to be distributed into different parts of a cell and become incorporated into different daughter cells. The difference with the previous mechanism is that here gene products or mRNAs are asymmetrically transported to the future daughter cells while the mother cell is dividing, whereas in the previous case no transport occurs during cleavage. A non-random pattern results from asymmetric mitosis if cells take invariable positions after division. Asymmetric mitosis is found in the early cleavage divisions of many groups such as nematodes (Bowerman and Shelton, 1999), mollusks (Collier, 1997), ctenophores (Freeman, 1976) and annelids (Bissen, 1999), but also in later processes such as the formation of the central nervous system of *Drosophila* (Doe and Bowerman, 2001). In some cases, cell signaling may also determine which daughter cell will receive which set of factors (Doe and Bowerman, 2001).

Internal temporal dynamics coupled to mitosis

Temporally cyclical expression of genes can produce a pattern if oscillation becomes decoupled from cell division. Cyclical gene expression can result from closed chains of molecular events that trigger each other in a sequential fashion ('dominoes') or by genetic networks with inherent oscillatory dynamics ('clocks') (Murray and Kirschner, 1989). If, when cells divide, one of the daughter cell stops or resets its temporal dynamics, then cells can acquire different states depending on the time of their mitosis. As in the case of asymmetric mitosis, an invariable positioning of cells is required in order to generate non-random patterns. This mechanism has been proposed for the segmentation of hirudean leeches, oligochaetes (Weisblat et al., 1994), short germ-band insects (Newman, 1993; Salazar-Ciudad et al., 2001b), the somitogenesis of vertebrates (Newman, 1993) and in the formation of morphological structures, such as the limb and the tail, involving 'progress zone' growth (Duboule, 1995). Experimental evidence for this mechanism is still limited, but in vertebrates it has been shown that expression of genes involved in somitogenesis exhibit oscillatory behavior (Maroto and Pourquié, 2001).

Inductive mechanisms

Cells can affect each other by secreting diffusible molecules, by means of membrane-bound molecules or by chemical coupling through gap junctions. A large number of mechanisms which use only these developmental functions are capable of pattern formation. In inductive mechanisms tissue pattern changes as a direct consequence of changes in cell state. This, in turn, is due to the processing or interpretation of signals sent by other cells. In certain cases, inductive pattern formation assumes a simple form, that is, one cell or tissue type will change the state of another cell or tissue type from what it would have been without the interaction, with no morphological consequence following directly from this. In other cases a morphological consequence accompanies, or follows closely upon, the change in state of the induced target cells. Since our aim here is to show how such *composite* inductive-morphogenetic mechanisms comprise highly divergent categories of developmental mechanisms, we will focus initially on the simple case without immediate morphological consequences.

Examples of simple inductive mechanism are mesendoderm induction in amphibians by maternal factors produced by the Nieuwkoop center (Harland and Gerhart, 1997), and the short-range signaling hierarchy in the echinoid blastula, in which the oral-aboral axis is established by signaling from the micromere tier to the macromeres, which, in turn, signal the mesomeres (Davidson et al., 2002). Other examples include generation of the gradient patterns of gap gene products in the *Drosophila* syncytial blastula induced by the patterns of maternal gene products, and the subsequent induction of striped patterns of pair-rule gene products, based on these gap patterns (Rivera-Pomar and Jackle, 1996).

Many basic inductive mechanisms appear to be based on *hierarchic* genetic networks (Salazar-Ciudad et al., 2000). In such networks a territory (or a single cell) may signal another, and this second may respond to such signaling by sending a signal back. This back-signal, however, does not affect the signaling rate or capacity of the first territory. Inductive mechanisms can also be based on *emergent* genetic networks in which cells or territories send signals in a way that is affected by neighboring cells' responses to such signals (Salazar-Ciudad et al., 2000). Emergent genetic networks, which comprise reaction-diffusion mechanisms (Turing, 1952; Meinhardt and Gierer, 2000; Salazar-Ciudad et al., 2001a) but also include other mechanisms in which cells affect one another in reciprocal ways, such as those used in the Notch-Delta signaling system (for details, see Salazar-Ciudad et al., 2000), have been suggested to underlie limb skeletal patterning (Newman and Frisch, 1979; Miura and Shiota, 2000a; Miura and Shiota, 2000b), pigment patterning in the butterfly wing (Nijhout, 2001), feather bud spacing in avian skin (Jiang et al., 1999; Prum and Williamson, 2002) and fish colour patterns (Kondo and Asai, 1995). Theoretical studies have indicated that hierarchical and emergent mechanisms together exhaust the possibilities for simple inductive mechanisms (Salazar-Ciudad et al., 2000), and have explored their variational properties (Salazar-Ciudad et al., 2001a).

Morphogenetic mechanisms

A number of patterning mechanisms use cellular behaviors other than signaling (although signaling may have been active at a prior stage). These mechanisms alter pattern by affecting form. This can be defined as a mechanism that changes the relative arrangement of cells over space without affecting their states.

Directed mitosis

Intracellular or extracellular signals can affect the direction of the mitotic spindle. Once the mitotic spindle assumes a set direction, new cells are forced to be positioned at specific places. The central nervous system of *Drosophila*, for example, forms by the dorsally directed budding of presumptive neuroblasts from the ectoderm (Broadus and Spana, 1999). This produces two cordons of neuroblasts that extend longitudinally in the ventral part of the embryo. Asymmetric mitosis and inductive signals are involved in determining which cells will become neuroblasts, but their localization is ultimately determined by the control of mitotic spindle orientation. External inductive signals have been shown to

direct the mitotic spindle in the first divisions of *C. elegans* (Goldstein, 2000) and in the leech (Bissen, 1999). In ctenophores the form of the whole blastula is attained through precise regulation of the orientation of the mitotic spindle (Freeman, 1976).

Differential growth

A change in a pattern can be produced if, in a previously existing pattern, cells with different states divide at different rates. The new pattern depends on the previous pattern, the relative rates and directions of mitosis and on other epigenetic factors such as the adhesion between cells and the influences of surrounding matrices. One such example is the establishment, maintenance, and waning of the growth plate during the formation of long bones in vertebrates (Sandell and Adler, 1999).

Apoptosis

A pattern can be transformed into another if some of the cells undergo apoptosis. Apoptosis can be strictly dependent on a cell's lineage, or triggered by interaction, or abrogation of interaction, with surrounding cells (Meier et al., 2000). Although apoptosis, in the first instance, is a cell autonomous function, the patterning consequences depend on the existence and arrangement of surrounding cells. The associated *developmental mechanism* is thus morphogenetic rather than cell autonomous. A wide range of developmental processes are dependent on apoptosis, including the outflow tract and valves of the heart (Poelman et al., 2000), development of neural circuitry in the brain (Kuan et al., 2000), and freeing up of the digits during vertebrate limb development (Chen and Zhao, 1998). In particular, it has been shown that the final shape of the interdigital membranes depends on the amount of apoptosis in such membranes (Gañan at al., 1998).

Migration

Cells can rearrange their relative positions without changing their states simply by migrating. Migration can be directionally random, random but speeded up by an ambient chemical signal ('chemokinesis'), or have a preferred direction in relation to a chemical gradient ('chemotaxis') or an insoluble substrate gradient ('haptotaxis'). While mesencephalic neural crest cell migration in the mouse appears to be controlled in part by a chemotactic response to members of the FGF family of growth factors (Kubota and Ito, 2000), migration of trunk neural crest cells in the chicken appears to depend on more random dispersal mechanisms (Erickson, 1988). The migration of premuscle cells into the developing vertebrate limb is regulated by both chemokinetic and chemotactic responses to hepatocyte growth factor (Lee et al., 1999). Regardless of the migratory mechanism, specificity of outcome will also, in general, be controlled by the adhesive environment of the destination sites (Lallier et al., 1994).

Differential adhesion

Cell adhesion is the defining property of multicellular organisms. It is an indispensable requirement for cell shape, differentiation and migration. A large, but limited number of pattern changes can be produced in tissues by constituent cells expressing different adhesion molecules or the same molecules at different levels. Hence, differential adhesion can cause subpopulations of cells to sort out into distinct groups. In a solid epithelioid tissue compartments may have straight or curved boundaries, or engulf or be engulfed by each other, depending on the magnitude of the adhesive differences (Steinberg, 1996). If adhesion is expressed nonuniformly on the surfaces of individual polarized cells, interior spaces or lumens can form in solid tissues (Newman and Tomasek, 1996). In planar epithelia, polar expression of adhesion along with differential adhesion of subpopulations can produce invaginations, evagination, placodes and the formation of cysts (Newman, 1998). Convergent extension, a reshaping of tissue masses during gastrulation which involves cell intercalation (Keller et al., 2000) can also be accounted for by energy minimization in populations of anisotropic cells (Zajac et al., 2000), particularly those that exhibit 'planar cell polarity' (Mlodzik, 2002). In well-studied cases some of these processes also involve mitosis or cell contraction, but this is not strictly required. Differential adhesion and cell polarity or anisotropy are in principle sufficient to achieve these morphological outcomes. Altered adhesion is also the final step in the set of transformations known as epithelial-mesenchymal and mesenchymal-epithelial conversions. An example of the first occurs during development of the neural crest (Le Douarin and Kalcheim, 1999) and the second occurs during the formation of the kidney tubules (Davies and Bard, 1998).

Contraction

Individual cell contraction mediated by actin-myosin complexes can have morphogenetic effects on neighboring cells and the tissue as a whole. Contraction of tissues during development is thought to trigger shape change and determine the character of the morphological outcomes (Beloussov, 1998). Contraction is propagated in epithelial tissues by direct physical attachment and in mesenchymal tissues by the extracellular matrix. In a planar epithelium contraction can also lead to buckling, and thus invagination or evagination (Newman, 1998). A recent study considered the role of myocardial contraction in trabeculation in the developing heart (Taber and Zahalak, 2001).

Matrix swelling, deposition and loss

The cells of mesenchymal and connective tissues are surrounded and separated by semi-solid or solid extracellular matrices. Changes in pattern may be accomplished by increased hydration or swelling of a preexisting matrix, increase in the amount of matrix separating the cells, or matrix degradation. During development of the avian eye, the primary corneal stroma swells in anticipation of its invasion by mesenchymal cells from the periphery (Hay, 1980). This swelling has been found to be controlled by tissue-specific, developmentally regulated proteolysis of collagen IX (Fitch et al., 1998). Vertebrate limb chondrogenesis is an example of a developmental process in which cellular rearrangement occurs as a result of matrix deposition. Here there is dispersal of newly differentiated chondrocytes within compact precartilage mesenchymal condensations and consequent flattening of more peripheral mesenchyme into a perichondrion (Hall and Miyake, 2000). Developmentally regulated matrix degradation, particularly of basement membrane components, has the capacity to alter cell positional relationships. Such changes are important in triggering new developmental events, for example

during sea urchin gastrulation (Vafa et al., 1996) and mammary gland morphogenesis (Werb at al., 1996).

VARIATIONAL PROPERTIES OF THE BASIC DEVELOPMENTAL MECHANISMS

The three categories of basic developmental mechanisms described above each have their characteristic *variational properties,* that is, capability of generating novel morphological outcomes if an element of the mechanism is changed. Autonomous mechanisms are implicated mainly in early development and are incapable of producing many pattern variations. This is because the extent to which internal cellular spatial asymmetries can be used to found distinct lineages is limited. Similarly, the coupling of internal temporal dynamics to mitosis is restricted in the number and arrangement of cell types that can be generated by constraints on the intrinsic length of the cell cycle relative to that of any internal cell state clock (Newman, 1993; Salazar-Ciudad et al., 2001b).

The variational properties of inductive mechanisms are discussed in previous work (Salazar-Ciudad et al., 2000; Salazar-Ciudad et al., 2001a). In essence, for the same amount of molecular variation inductive mechanisms that contain a self-organizing component ('emergent') typically produce more, and more complex, patterns than those that are organized in a hierarchic fashion. In contrast to emergent mechanisms, in which similarly constructed networks can generate very different patterns, hierarchic mechanisms based on similar gene networks tend to generate patterns that are similar to one another. Furthermore, complex patterns are difficult to attain by hierarchic networks: in general, a hierarchic network capable of producing a particular complex pattern would have to contain many more genes and many more connections among them than an emergent network capable of producing that pattern. These characteristics entail a more complex relationship between phenotype and genotype in emergent mechanisms than in hierarchic ones.

The variational properties of morphogenetic mechanisms have also been widely discussed (Newman and Müller, 2000; Beloussov, 1998; Alberch, 1982; Oster and Alberch, 1981). Morphogenetic mechanisms have a strong dependence on the epigenetic context and changes in their molecular components or microenvironments can have dramatic phenotypic effects. Morphogenetic mechanisms, furthermore, often involve mechanical interactions between cells and extracellular matrix. This implies that their outcomes depend on such aspects of the developing system as the material (e.g., viscoelastic, cohesive) properties of cells and extracellular matrix or their spatial distribution (Newman and Müller, 2000). In particular, tissues and extracellular matrices may respond very differently to stresses depending on their form and the relative orientation of the stresses to which they are subjected (Beloussov, 1998).

COMBINING INDUCTIVE AND MORPHOGENETIC MECHANISMS

Apart from the earliest stages of development when, among very few cells, autonomous mechanisms are frequent,

development is a *composite* of temporally and spatially ordered inductive and morphogenetic mechanisms. Below we discuss how the degree and sequence by which inductive and morphogenetic mechanisms are combined has dramatic implications for the variational properties, and evolution of development. Inductive and morphogenetic mechanisms can be combined into composite developmental mechanisms in two different ways. Usually it is assumed that inductive mechanisms act first to establish groups of cells with equivalent states of gene expression, for example, expressing the same transcription factors. Then this set of cells, which we will refer to as a 'gene expression territory' or, for brevity, 'genetic territory', employs one or more morphogenetic mechanisms. [Here we purposely avoid terms already in use such as 'morphogenetic field' (Sander, 1994) or 'equivalence group' (Stent, 1985) which, unlike gene expression territory, presuppose some notion of prospective cell fate.] We call this class of composite mechanisms, in which a pattern of genetic territories is first established and the resulting tissue undergoes a consequent change in form, *morphostatic* (Fig. 2).

Morphodynamic mechanisms, in contrast, make use of inductive and morphogenetic mechanisms simultaneously (Fig. 2). Thus, the forms of genetic territories are changing (via morphogenetic mechanisms) at the same time as some of the genetic territories are participating in inductive interactions by sending and receiving molecular signals. This results in continual change in the set of cells (and their spatial distribution) receiving a given concentration of a signal. The forms of these receiving territories depend on the form of the sending territories, as well as the form of the territories expressing the relevant receptor and the distances and relative orientations of both types of territories (Fig. 3).
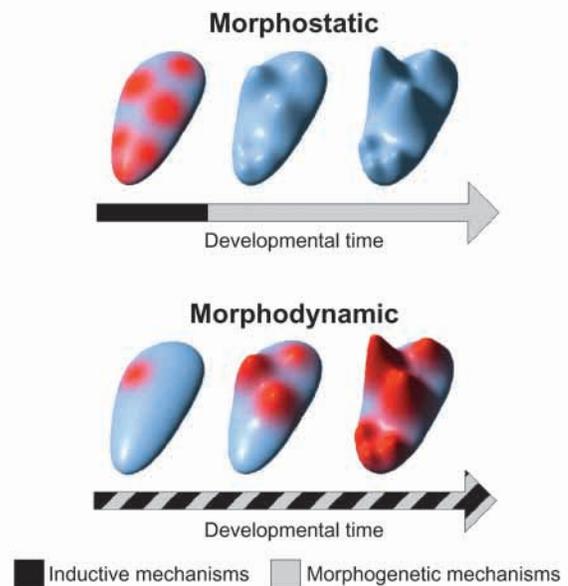

Fig. 2. Combining signaling and morphogenesis. Inductive (signaling) and morphogenetic mechanism can be combined to generate *morphostatic* mechanisms where induction (in red) temporally precedes growth, producing the final forms. *Morphodynamic* mechanisms, in contrast, integrate inductive and morphogenetic mechanisms and can often be difficult to separate as induction and final development of the shape are concurrent.
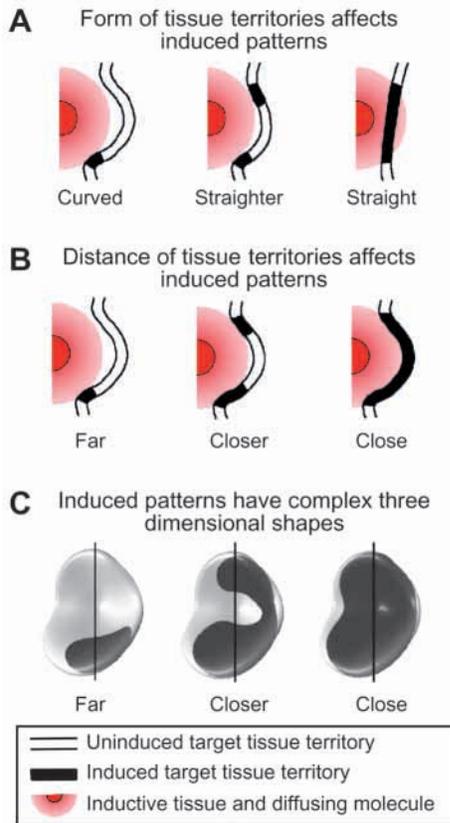
A Form of tissue territories affects induced patterns

Curved   Straighter   Straight

B Distance of tissue territories affects induced patterns

Far   Closer   Close

C Induced patterns have complex three dimensional shapes

Far   Closer   Close

☐ Uninduced target tissue territory
■ Induced target tissue territory
● Inductive tissue and diffusing molecule

Fig. 3. Morphodynamic interactions can result in complex patterns. (A) Forms of interacting territories can affect induced patterns. In this example, curvature of the target tissue affects whether one small, two small or one large territory is induced (black). (B) Distance of interacting territories can also affect the number and size of induced territories. Note that beyond a certain distance between territories, no pattern changes will occur. (C) Actual spatial patterns of induced territories can be complex with large changes produced by small changes in interacting territories. The actual patterns may also be difficult to infer from histological sections (e.g., vertical line in C represents location of corresponding sections in B).

The logic of these two types of composite mechanism is completely different. In morphodynamic mechanisms the functioning of the morphogenetic mechanisms and the inductive mechanisms is causally interdependent, so that changes in a genetic component of the morphogenetic mechanism (or in microenvironmental determinants of the developing form) can affect the locations and forms of the territories sending and receiving signals and thus produce large changes in the final pattern. In morphostatic mechanisms such interdependence does not exist; genetic changes in the morphogenetic mechanism or environmentally driven form changes would, in general, have only limited effect on the final form. The extent to which morphogenetic mechanisms act synchronously with inductive mechanisms will determine whether a composite developmental mechanism is morphostatic or morphodynamic. While gradations between the two categories exist, many developmental outcomes are produced by mechanisms that clearly fit one or the other

description, and the distinction is useful in the analysis of both morphological development and evolution.

Implications at the cellular level

How cells respond internally to received signals in order to coordinate their behaviors and produce the coherent pattern transformations discussed above is a current area of interest in developmental biology. It is therefore significant that morphodynamic and morphostatic mechanisms have different implications for the internal logic used by cells to produce patterns. During development cells are constantly sending and receiving molecular signals. The network of transcription factors and transduction molecules within a cell integrates the cell's previous history with received signals and then alters cell behaviors. The transduction of received molecular signals elicit, in target cells, the production of signaling, structural or catabolic molecules (Montross et al., 2000; Carnac at al., 1996), apoptosis (Su et al., 2001; Barlow et al., 1999; Ferrari et al., 1998), mitosis (Hu et al., 2001; Cecchi et al., 2000; Salser and Kenyon, 1996) expression or repression of cellular receptors (Panchision et al., 2001; McPherson et al., 2000) and/or changes in the contractility or adhesivity of cells (Wacker et al., 2000; Lincecum, 1998; Packer et al., 1997; Jones et al., 1992).

In morphostatic mechanisms once a cell has attained a new cell state through signaling (a state that depends on the received signal and on the cell's previous developmental history) it follows an autonomous, temporal program of behavioral changes that is specified, mainly, by the transcriptional factors it now expresses. Since the spatial configuration of signals can be established by emergent as well as hierarchical inductive mechanisms, the hallmark of the morphostatic mechanism is not the absence of reciprocal cell interactions in generating this configuration, but rather the causal separation between setting up the signals and the cell behavioral response to such signals.

The positional information metaphor (Wolpert, 1969; Wolpert, 1989), in which cells acquire their fates as a result of exposure to different concentrations of a signaling molecule, is one example of a morphostatic mechanism. Different developmental outcomes arise not from differences in the mechanisms by which genetic territories attain their forms, but in the different interpretation of this positional information. The nature of this interpretation is unspecified, but, as Wolpert explicitly proposes morphogenetic mechanisms act after and subordinately to inductive mechanisms (Wolpert, 1989), it is clear that interpretation implies the following of some sort of autonomous genetic program. The spatiotemporal coordination of cell behaviors required in development is assumed to be the outcome of the autonomous use, by each cell, of its own genetic program specified though an inductive signaling environment, in this case, the local concentration of a chemical gradient.

Morphodynamic mechanisms do not require a precise interpretation of signal concentrations or a temporal genetic program for every cell. Instead complex patterns arise through the collective spatiotemporal co-ordination of cell behaviors in the course of simultaneous cell signaling and form changes. Cells cannot be said to follow a program, but are rather moved along a developmental trajectory by continual interaction with a changing molecular-geometric microenvironment. At each moment the cell computes the behavioral changes it will

undergo based on the network of transcriptional factors and signal transduction molecules it expresses and signals it receives. The cell's responses at any moment may be relatively simple (although in the long run they may have complex consequences). In morphodynamic mechanisms it is not only what happens inside responding cells that is significant. The 'intermediate phenotype' at each moment is also causally determinative: that is, the shapes of, and relative distances and orientations among inducing and induced territories. Thus when an inductive interaction takes place between two territories it is not only important to know how this signal is interpreted by the receptive cells but also what are the forms of the inductive and receiving territories, and how are they changing in three-dimensional space as a result of the action of the morphogenetic mechanisms. Several experimental examples illustrate these points.

Developmental evidence

Brain development

The developing vertebrate brain is subdivided into territories expressing specific adhesion molecules and transcriptional factors (Rubenstein et al., 1998). Specific signaling molecules expressed in the territory boundaries are involved in patterning the brain. Pax6 is a transcription factor known to affect the expression of adhesion molecules and in the mouse brain, during stages E9.0-E12.5 (Stoykova et al., 2000), this protein is expressed at territory boundaries where the neuroepithelium is folding (Grindley et al., 1997). *Pax6* mutants exhibit morphological abnormalities originating at these stages, involving partial failure of such folding, enlargement of the boundary between two of the prosomeric segments of the diencephalon, and changes in the relative sizes of the prosomeres (Grindley et al., 1997; Warren and Price, 1997). This abnormal folding both results from and changes the relative spatial position of the territory boundaries and thus of the genes expressed in them. It is this reciprocity between changing shape and changing patterns of gene expression that marks this process as morphodynamic (Grindley et al., 1997).

The diffusible signaling molecules Shh and Wnt7b are expressed in regions of the developing brain altered by the *Pax6* mutation (Epstein et al., 1999; Grindley et al., 1997; Warren and Price, 1997). Both affect proliferation and Wnt7b also affects adhesion (Brault et al., 2001). By virtue of the effects of Wnt7b and Shh on proliferation and adhesion, the territories affected by these factors undergo continual alteration in form. But in certain cases the territories affected by Shh and Wnt7b are also territories that express the factors. The consequence is that the *Pax6* mutant exhibits nontrivial changes in the spatial patterns of expression of signaling genes coordinated with, and inextricable from, the morphological effect represented by misfolding. The three-dimensional context (i.e., form) within which morphogenetic mechanisms are deployed at one stage in the *Pax6* mutant and, presumably, the normal brain, thus have a causal role in determining patterning in later stages. Unlike developmental outcomes of morphostatic mechanisms, which can be schematized as two-step processes in which the establishment of a new cell pattern leads subsequently to a new form, during brain development changes in form and pattern reciprocally bring one another about in a morphodynamic fashion.

Mammalian tooth development

Multiple lines of evidence indicate that tooth development employs morphodynamic mechanisms. Mammalian cheek teeth, in particular, possess complex morphologies consisting of different arrangements and shapes of cusps. Tooth crowns consist of overlying enamel, produced by inner enamel epithelium, and underlying dentine, produced by dental mesenchyme. During development, before the formation of enamel and dentine, tooth shapes are formed by unequal growth and folding of the inner enamel epithelial-mesenchymal interface (Butler, 1956; Jernvall and Thesleff, 2000). The formation of cusps begins from their tips and is mediated by epithelial signaling centers, the enamel knots. Cells of the enamel knots are non-proliferative although they express signaling molecules, such as FGFs and Shh (Jernvall and Thesleff, 2000) that stimulate proliferation and survival of the areas surrounding the enamel knots. The formation of enamel knots and cusps is roughly sequential and takes place simultaneously with signaling linked to enamel knot formation (Jernvall et al., 1998) and cusp growth (Jernvall et al., 1994; Kettunen et al., 2000). Thus, the relative locations of knots are changing while they are sending signals.

Teeth most probably develop in a morphodynamic fashion since induction and morphogenesis take place at the same time and interdependently. Furthermore, to date no molecular prepatterns manifesting the final tooth cusp patterns, or unique genes or combinatorial code for individual cusps has been reported (Jernvall and Thesleff, 2000). These kinds of evidence would be indicative of morphostatic mechanisms and would also suggest that individual cusps would be relatively free to vary in size independently of one another. However, the variational properties of cusps within a tooth show that the presence and size of later forming cusps depend on the position and size of earlier developing cusps (Jernvall, 2000). This again suggests that formation of new enamel knots and molecular signaling depend on, and is reciprocally linked with, the preceding morphology, which is consistent with morphodynamic mechanisms.

Reciprocity of molecular patterning and morphogenesis is also implicated in *Tabby* mouse mutants by affecting the size and overall degree of enamel knot signaling (Pispa et al., 1999), resulting not only in smaller teeth but also in globally altered shapes. An empirically derived morphodynamic mechanism for tooth formation has been recently tested using mathematical modeling (Salazar-Ciudad and Jernvall, 2002). This morphodynamic model, while only containing essential components of known molecular interactions and their effects on growth, was able to predict both the course of tooth shape development and dynamics of gene expression patterns. Furthermore, simple changes in model parameters are able to reproduce well known evolutionary changes in tooth shapes, suggesting that morphodynamic mechanisms may promote evolutionary versatility (Salazar-Ciudad and Jernvall, 2002). The intricate manner by which developing tooth shape alters the diffusion and local concentration of molecular signals in this morphodynamic models suggests that predicting phenotypic effects of molecular manipulations may be very difficult without mathematical approaches and knowledge about developing morphology.

The preceding examples should not be taken to imply that

all developmental processes employ morphodynamic mechanisms. In the developing vertebrate limb, for example, the pattern of skeletal elements is specified by inductive mechanisms well before the occurrence of precartilage mesenchymal condensation (Wolpert and Hornbruch, 1990; Dudley et al., 2002), the latter being the first morphological change distinguishing skeletal tissue from adjacent nonskeletal tissue and the result of a morphogenetic mechanism (Newman and Tomasek, 1996). Although inductive and morphogenetic mechanisms acting earlier and later set the shape of the limb bud and refine the shapes of individual elements, the developmental mechanism that generates the basic skeletal pattern from a homogeneous distribution of mesenchymal cells is morphostatic.

EVOLUTIONARY IMPLICATIONS

The different modes of functioning of morphodynamic and morphostatic mechanisms produce dramatically different ranges of potential morphological outcomes. The forms of territories that morphostatic mechanisms are capable of producing are confined to those generated separately by inductive and morphogenetic mechanisms or by morphogenetic transformation of territories formed by inductive mechanisms. Additionally, morphodynamic mechanisms can be expected to produce all the forms of territories resulting from all possible spatial interactions of all the possible forms produced by morphostatic and inductive mechanisms (Fig. 3). The reason for this is that whereas release of signals can take place, in either case, from territories of given shape and size, both the dependence on distance of diffusion from such territories and the three-dimensional forms of territories can cause extensive variation in the spatial pattern of the cells receiving these signals.

It is important to note that the difference between morphodynamic and morphostatic composite mechanisms relates to how basic inductive and morphogenetic mechanisms are combined (Fig. 2). Indeed, a morphodynamic and morphostatic mechanism can involve the same basic inductive and morphogenetic mechanisms and thus the same genetic information. From what we have said in the previous paragraph it follows that morphodynamic mechanisms can produce additional forms without additional genetic information.

Because morphodynamic mechanisms can use the spatial epigenetic information (i.e., the form and relative orientations of the territories sending and receiving signals) present in the emerging phenotype at each point in development to alter later development, such mechanisms exhibit dependency on the 'intermediate phenotype'. This property permits developing systems that

employ morphodynamic mechanisms to generate more phenotypic variation for less molecular variation than morphostatic mechanisms. In addition to the intermediate phenotype of the forming pattern, patterns in the rest of the embryo may also influence morphodynamic mechanisms. Thus morphodynamic mechanisms acting in the context of more complex phenotypes may facilitate morphological innovation. This can be exhibited ontogenetically, where a wide variety of forms (for example teeth, or convolutions of the neocortex) can be generated by the use of the same set of mechanisms in slightly different developmental contexts, or phylogenetically, where small genetic changes can lead to significant evolutionary changes.

The integrated nature of signaling and morphogenetic aspects of development causes morphodynamic mechanisms to prescribe a more complex relationship between genotype and phenotype. The dependency of developmental outcome on the intermediate phenotype in such mechanisms makes it possible for small molecular changes to give rise to relatively large
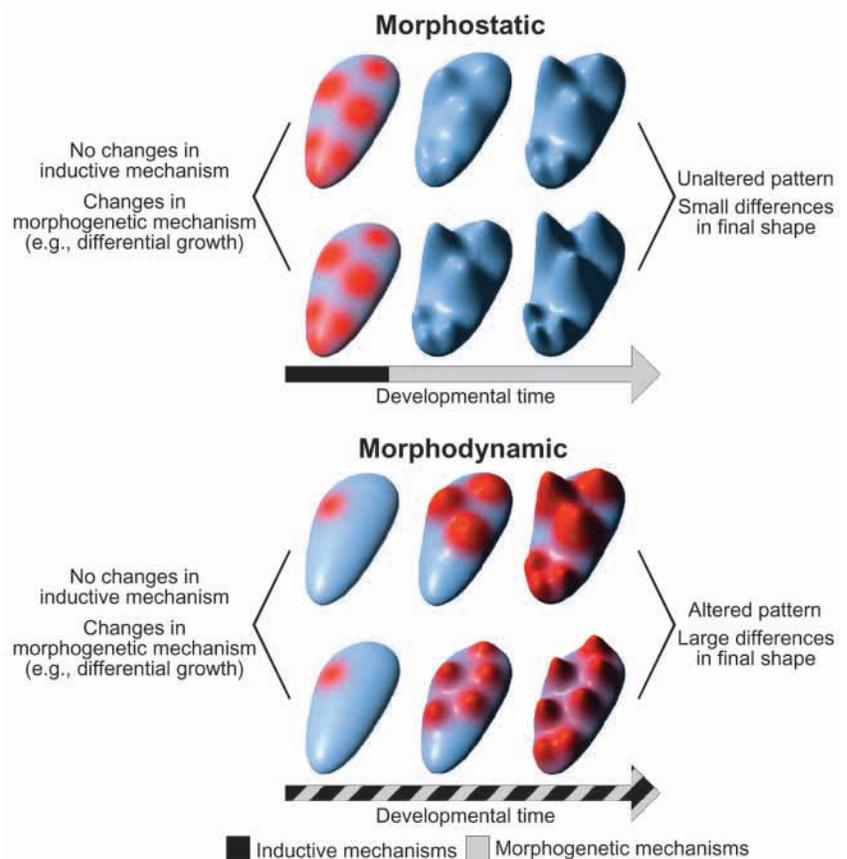

Fig. 4. A schematic illustration of how morphostatic and morphodynamic mechanisms have different variational properties. A simple change in tissue growth does not affect induction (red) and the resulting pattern in a morphostatic system because only growth of initially induced territories is affected, resulting in slightly blunter or sharper features. In morphodynamic mechanisms small changes in growth can alter induction of new territories (Fig. 3), resulting not only in blunter or sharper features, but completely altered patterns. Morphostatic mechanisms would require large changes in induction of territories in order to produce comparable change, particularly in the case of positional information systems where each new territory would require a unique signal or signal concentration. In general, morphodynamic mechanisms can be hypothesized to produce more disparate morphological outcomes than morphostatic mechanisms.

phenotypic effects in some cases and no effects in others (Figs 3, 4). While a typical morphodynamic mechanism will not necessarily be more prolific in generating patterns than a typical morphostatic mechanism, the range (i.e., disparity) of different patterns potentially produced by a given morphodynamic mechanism will usually be wider (Fig. 4). Conversely, in many cases genetic changes would have no phenotypic effects in morphodynamic mechanisms (Fig. 3B). One reason for this is that patterns produced by morphodynamic mechanisms will often vary in a 'discontinuous' fashion with small genetic changes, and the intermediate patterns would not be possible (Fig. 3) (see Salazar-Ciudad and Jernvall, 2002). In this sense morphodynamic mechanisms are both protean and developmentally constrained. In contrast, for morphostatic mechanisms most genetic changes will have small phenotypic effects (Fig. 4), and patterns intermediate between any two distinct ones would often be found.

We suggest, therefore, that compared with morphostatic mechanisms, morphodynamic mechanisms are more often involved in the generation of morphological innovations during evolution because the range of forms they can attain for the same amount of molecular variation is larger. For most patterns, the genetically most parsimonious mechanisms are morphodynamic. In morphodynamic mechanisms, small changes in a gene product can result in highly non-linear effects that can produce new morphological structures.

Earlier work has suggested that over the course of evolution a developmental pattern produced by an emergent morphostatic mechanism may persist, while the mechanism by which the pattern is generated evolves into a hierarchical one (Newman, 1993; Salazar-Ciudad et al., 2001a; Salazar-Ciudad et al., 2001b). In an analogous fashion, progressive partial substitution during evolution of morphodynamic mechanisms by morphostatic mechanisms producing the same pattern can be expected. This is because, compared to morphodynamic mechanisms morphostatic mechanisms can produce more finely-tuned phenotypic variations. In other words, more continuous phenotypic variation can be produced. In addition a simpler relationship between phenotype and genotype allows them to produce such changes relatively rapidly. These two properties are probably adaptive for patterns under strong stabilizing selection. Such substitution of morphodynamic by morphostatic mechanisms would likely require many generations and may, in general, not go to completion since in many cases it may be evolutionarily adaptive to produce the same pattern with two different mechanisms [especially if they have different variational properties (Nowak et al., 1997)].

Generally, morphological innovations have been proposed to appear more often in later development because they are less likely to disrupt global developmental processes at those stages (Riedl, 1978). This suggests, in turn, that morphodynamic mechanisms would be found more often in later development where, in addition, already existing complex intermediate phenotypes would allow them to produce variation, and thus respond to selective pressures more easily. Conversely, at earlier developmental stages, which would have had more evolutionary time to change, morphostatic mechanisms may have become superimposed on, and in some cases, substituted for, morphodynamic mechanisms.

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