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Dynamical patterning modules: physico-genetic determinants of morphological development and evolution

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Abstract

The shapes and forms of multicellular organisms arise by the generation of new cell states and types and changes in the numbers and rearrangements of the various kinds of cells. While morphogenesis and pattern formation in all animal species are widely recognized to be mediated by the gene products of an evolutionarily conserved 'developmental-genetic toolkit', the link between these molecular players and the physics underlying these processes has been generally ignored. This paper introduces the concept of 'dynamical patterning modules' (DPMs), units consisting of one or more products of the 'toolkit' genes that mobilize physical processes characteristic of chemically and mechanically excitable meso- to macroscopic systems such as cell aggregates: cohesion, viscoelasticity, diffusion, spatiotemporal heterogeneity based on lateral inhibition and multistable and oscillatory dynamics. We suggest that ancient toolkit gene products, most predating the emergence of multicellularity, assumed novel morphogenetic functions due to change in the scale and context inherent to multicellularity. We show that DPMs, acting individually and in concert with each other, constitute a 'pattern language' capable of generating all metazoan body plans and organ forms. The physical dimension of developmental causation implies that multicellular forms during the explosive radiation of animal body plans in the middle Cambrian, approximately 530 million years ago, could have explored an extensive morphospace without concomitant genotypic change or selection for adaptation. The morphologically plastic body plans and organ forms generated by DPMs, and their ontogenetic trajectories, would subsequently have been stabilized and consolidated by natural selection and genetic drift. This perspective also solves the apparent 'molecular homology-analogy paradox', whereby widely divergent modern animal types utilize the same molecular toolkit during development by proposing, in contrast to the Neo-Darwinian principle, that phenotypic disparity early in evolution occurred in advance of, rather than closely tracked, genotypic change.

1. Introduction

The body plans of essentially all the modern types of animals—sponges, molluscs, arthropods, echinoderms, chordates, and so on, emerged within a period of 20 million years or less (Rokas *et al* 2005) about 550–530 million years ago (Conway 2006). The last common ancestors of the first multicellular metazoan forms and their unicellular 'cousins' such as choanoflagellates thrived in the Ediacaran period (approximately 635 Mya) and presumably possessed a non-robust and developmentally transient sheet-like multicellularity (King *et al* 2008). The morphologies that are inferred from fossil evidence of the subsequent Cambrian period are, however, of a much more complex and diverse type. This implies that Cambrian metazoan organisms explored morphospace with a fluency that was astonishing given the evolutionary time within which it took place. Not only that, this exploration was exhaustive: it now seems likely that no entirely novel body plans have arisen since that period (Passamaneck and Halanych 2004).

This is not what would have been predicted by the standard model for evolutionary change—the Neo-Darwinian synthesis. In that model, where a fairly straightforward relationship between genotypic and phenotypic change is assumed, the engine of evolution is change (under natural selection) in the populational frequency of genes with small effects on the phenotype. Morphological evolution should therefore be gradual, rather than abrupt, as seen in the early metazoa.

The discrepancies of these findings from the predictions of the standard model, when probed deeper, are even more serious. Since evolutionary change supposedly tracks genetic change, it would be expected that the genes that mediate developmental morphogenesis and pattern formation would have changed dramatically during origination of the disparate metazoan body plans. But this is also not the case. The genes of the 'developmental-genetic toolkit' are highly conserved among all metazoan phyla. In fact the proteins specified by these genes have changed so little during the more than half-billion years since the common ancestor of chordates and arthropods was extant, that their coding sequences will often function in development when swapped between the embryos of mice and fruit flies (see, e.g., Gehring (2002)).

These observations present the following puzzle: how did large-scale morphological evolution take place so rapidly without much change in the genes specifying the proteins that mediate development? There are two possible answers: (i) unusually intense selection on regions of DNA that do not encode proteins (e.g., the regulatory regions of the genes) led to extremely rapid, but still incremental, diversification of form during a narrow period of time at the Precambrian–Cambrian boundary; and (ii) non-genetic/epigenetic determinants were responsible for generating many different organismal forms during that period, with genetic change occurring after this rapid episode of diversification. Scenario (i) underlies the analysis of many articles in what may be called the 'Neo-Neo-Darwinian' mode, and is well summarized in a recent book by Carroll (2005). Scenario (ii), which we favor, proposes that early multicellular forms were subject to the action at the mesoscopic scale of physical processes characteristic of viscoelastic and chemically excitable matter, and thus assumed the three-dimensional structures and patterns generic to these materials (Newman *et al* 2006). We will describe this latter view in what follows. In doing so, we will present evidence in support of the idea that molecular functionalities that evolved to serve unicellular life inescapably took on new morphogenetic roles in the new physical environment and larger spatial scale entailed by the transition to multicellularity.

2. Definition of dynamical patterning modules

By dynamical patterning modules (abbreviated DPM) we mean a set of molecules produced in a cluster of cells, along with one or more physical effects mobilized by these molecules so as to generate an aspect or alteration in the cluster's form or pattern. Roughly speaking, 'form' comprises shape, size and topology, and 'pattern' comprises specific arrangement of cell types.

The molecules of DPMs, usually proteins, but in some cases polysaccharides, or a combination of both, mediate such effects as cell–cell adhesion and phase separation of differentially adhesive cell populations, oscillation in cells' biochemical state, short-range laterally acting inhibitory effects, generation of structural anisotropy across individual cells, diffusion across cell masses and alteration of the rheologic properties of the intercellular microenvironment. These molecules mostly evolved in single-celled organisms prior to the evolution of the metazoa, and only took on their DPM-associated roles with the change of spatial scale that was a consequence of multicellularity. Indeed, one of the DPM categories, cell–cell adhesion, which is the necessary condition for multicellularity, is based on cell surface proteins that must have had a different function in unicellular antecedents.

The choanoflagellates are a group of organisms that are genetically related to the metazoa (Wainright *et al* 1993, King and Carroll 2001, Snell *et al* 2001, Lang *et al* 2002) and are their closest living nonmetazoan ancestors (Philippe *et al* 2004). Depending on laboratory culture conditions, choanoflagellates are unicellular, or form small colonies. The ancestors of choanoflagellates are considered to have evolved before the *eumetazoa*, organisms with more complex body plans ranging from animals like corals and hydra to the most complex ones like the mammals. They also appear to have arisen before the most primitive metazoa, the placozoa (Miller and Ball 2005) and sponges. Many of the DPM molecular components are found in the choanoflagellates. A number of them are first seen in sponges or cnidarians (hydroids, corals, jellyfish), the simplest eumetazoa (Müller and Müller 2003). Because DPMs, by definition, are molecules plus the physical processes they mobilize, their molecular constituents are a functionally specific subset of the developmental-genetic toolkit (Newman and Bhat 2008).

An assumption in what follows, on which there is general agreement, is that molecular mechanisms for switching gene expression patterns between alternative states, in many cases mediated by toolkit genes that specify transcription factors, had evolved before the emergence of multicellularity (Wilkins 2002, Davidson 2006). Therefore cell differentiation per se, though an essential process in both embryonic development and the origination of developmental systems (see Forgacs and Newman (2005) for a review), does not enter into our description of the DPMs other than in its capacity to be deployed by mechanisms of pattern formation (Salazar-Ciudad *et al* 2003).

In each of the subsections below, we elaborate on some of the important DPMs. We focus on the roles of the associated toolkit molecules in unicellular organisms, the new physical processes they come to embody with changes in spatial scale, and the novel biological functions they perform under these new conditions. We also deal with some specific instances when two or more DPMs combine spatiotemporally to mobilize mesoscale[1] physical phenomena

[1] Although the term has different referents in different subfields, here we use it to refer to condensed materials on a scale $\sim 10^{-4} - 10^{-3}$ m; i.e., larger than a typical biological cell but smaller or equal to a functional unit of a developed organ.

(e.g., biochemical oscillation, reaction-diffusion patterning instabilities) that are novel in a biological context. Each DPM is given a three-letter abbreviation that is also used in the table. Because our objective here is to provide an account of DPM-mediated morphogenesis and pattern formation from a physics-oriented perspective, we provide only a basic description of downstream signaling effects of the DPMs. Additional molecular details can be found in Newman and Bhat (2008).

3. Mobilization of physical effects in dynamical patterning modules

3.1. Cell–cell adhesion and differential adhesion

Cell adhesion, which in modern metazoa is mediated by several different classes of integral membrane proteins, is a necessary and sufficient condition for establishment of at least a primitive form of multicellularity. Homologs of cadherins, a ubiquitous class of transmembrane proteins that mediate cell adhesion via their Ca^{2+} binding domains, as well as of adhesion-mediating C-type lectins, are encoded by the choanoflagellate genome (King *et al* 2003, 2008). Although many present-day choanoflagellates are colonial, such proteins, which can be rather numerous in extant forms (Abedin and King 2008), may have evolved in free-living cells prior to the origin of metazoa to serve purposes other than cell–cell adhesion, e.g., defense against pathogens and recognition and capture of prey (King *et al* 2003). Because protein–protein association is a property that can be modulated by microenvironmental factors, formerly non-adhesive cells could have acquired a tendency to aggregate as a result of changes in external conditions, such as the ionic content of the oceans (Kazmierczak and Degens 1986).

Although single-celled organisms may have adhered to surfaces in occupying particular environmental niches, adhesion mediated by homophilic proteins is by definition irrelevant to unicellular life. Thus, when pre-existing cell surface molecules took on the new role of mediating the formation of cell clusters, the first set of DPMs emerged (those we designate ADH; see table 1) and with them, the first multicellular organisms. This example clearly illustrates the character of DPMs as one or more cellular molecules mobilizing a physical force or effect so as to bring about a change in a multicellular form or pattern.

In a cell aggregate mediated by reversible bonds between cell attachment molecules, individual cells can move around in a random fashion and exchange positions with their neighbors. Cells in aggregates, of course, do not undergo Brownian motion like molecules in liquids. But individual cell movements, though driven by complex intracellular machinery, do not generally have preferred directions, and bonds between cells are weak. Cell aggregates thus behave formally like liquids (Steinberg and Poole 1982), exhibiting the formal equivalents of viscosity and surface tension (Forgacs *et al* 1998). Furthermore, if subpopulations within a multicellular aggregate possess sufficiently different numbers of cadherins on their cell surface, they would sort out into islands of more cohesive cells within lakes composed of their less cohesive neighbors (Steinberg and Takeichi 1994). Eventually, by random cell movement, the islands coalesce and an interface is established across which cells will not intermix (Steinberg 1998). The physical basis of this sorting out of cell populations is equivalent to phase separation of two immiscible liquids, such as oil and water (Forgacs and Newman 2005). The result is the formation of multilayered structures in which the more cohesive tissue (the one with stronger bonds between its cells) will always be partly or fully engulfed by the less cohesive one. The thermodynamic principle of minimization of free energy thus ensures a reliable organized morphological outcome despite the fact that there is no specific 'genetic program' for this behavior (Steinberg 1998).

Ancient cadherins or lectins, then, acting in an environment that permitted them to mobilize the physical force of adhesion, became not only the mediators of colony formation, but of the automatic development of embryo-like structures consisting of distinct cell layers or 'compartments', where no interchange or mixing of cells occurs across the common boundary (Crick and Lawrence 1975, Garcia-Bellido 1975), and which have a reproducible spatial relationship to one another. We use the three-letter abbreviation DAD for the DPM that mobilizes differential adhesion to induce tissue multilayering (see table 1).

Aggregates of isotropic, mobile cells mediated by cell adhesion molecules are 'solid',[2] with a spherical geometry due to the minimization of surface tension. Even when differential adhesion comes into play and the aggregates become multilayered the default topology remains solid, though the aggregate geometry may no longer be spherical.

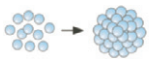
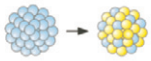
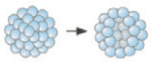
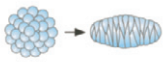
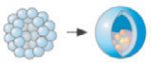
If the ratio of high-cadherin-expressing to low-expressing cells is not stringently regulated (as was likely the case in the most ancient metazoans), the resulting organisms would have many different, poorly defined, morphologies. A cell's biochemical state, including its level of expression of cadherins and other cell surface molecules, however, is determined by gene regulatory networks (GRNs), dynamical systems capable of switching between alternative stationary or oscillatory states of gene expression or biosynthesis, depending on the cell's microenvironment (Davidson 2006). In the following subsection we describe an ancient pathway that in a multicellular context, can stabilize the coexistence and relative numbers of cells occupying different biochemical states. Coupled to differential adhesion, this would have enabled the emergence of ancient metazoan cell clusters with reliable proportions in different compartments. Beyond this, numerous mechanisms of spatial pattern formation, even those unrelated to modulation of adhesion, would have been facilitated.

3.2. Lateral inhibition and cell fate choice

The Notch pathway is a highly conserved signal transduction system that mediates cell fate decisions in embryonic and

[2] We use the term 'solid' here not in contrast to 'liquid' (see above for the application of this term to tissue behavior), but in the topological sense: lacking internal cavities.

Table 1. Key dynamical patterning modules, their respective molecular constituents and physical principles, roles in evolution and development, and schematic representations.

DPM	Molecules	Physics	Evo-devo role	Effect
ADH	Cadherins	Adhesion	Multicellularity	
LAT	Notch	Lateral inhibition	Coexistence of alternative cell states	
DAD	Cadherins	Differential adhesion	Phase separation, tissue multilayering	
POL_a	Wnt	Cell surface anisotropy	Topological change, interior cavities	
POL_p	Wnt	Cell shape anisotropy	Tissue elongation	
ECM	Chitin, collagen	Stiffness, dispersal	Tissue solidification, elasticity, EMT	
OSC	Wnt + Notch	Chemical oscillation	Segmentation, periodic patterning	
MOR	TGF-β/BMP FGF, Hh	Diffusion	Pattern formation	
TUR	MOR + Wnt + Notch	Dissipative structure	Segmentation, periodic patterning	

adult tissue. The integral membrane protein receptor Notch interacts with one of its transmembrane ligands Delta, Serrate (also known as Jagged) and Lag2 (the DSL proteins) (Ehebauer *et al* 2006). Juxtacrine interaction between the ligands and Notch receptor causes a cleavage of the latter's intracellular domain, resulting in the nuclear transport of the intracellular cleaved portion. This fragment then interacts with a class of transcriptional repressor proteins, turning them into transcriptional activators. Based on the receptor involved and the cell's developmental state, the pathway forces the cell to choose one of its potential fates, whatever those may be.

Cells interacting with one another through Notch–DSL juxtacrine signaling are generally biochemically equivalent prior to the start of the signal transduction. Typically, the cells of a progenitor population will all express both Notch and a DSL and be in the same metastable state of gene expression. As the interaction proceeds, the population undergoes a bifurcation in its state with one or a small contiguous group of cells increasing their DSL levels and the cells surrounding them become Notch-activated and are thereby prevented from assuming the same fate as the central group. This state is a stable steady state and the radial directional nature of state transformation has been referred to as 'lateral inhibition' (Simpson 1997). Because of the inherent symmetry of the initial state, achieving the preferred directionality that is often required for biological functionality is nontrivial and requires accessory mechanisms (Zhu and Dhar 2006).

The Notch signaling pathway can also operate in a cell-autonomous fashion; that is, the receptor and ligand are on the same cell, rather adjacent ones (Sakamoto *et al* 2002). This suggests that the ancestral role of the Notch pathway may have been to mediate the switching of individual cells between one state and another (e.g., sporulation) due to environmentally induced association between their Notch and DSL proteins. It is plausible, therefore, that the transition to multicellularity would turn a cell autonomous mechanism into a juxtacrine one, and a single-cell-state switching mechanism into one mediating lateral inhibition. It is also possible that Notch–DSL signaling reinforced an inherent propensity of interacting identical cells to force one another into distinct dynamical states (Kaneko and Yomo 1999, Furusawa and Kaneko 2002).

Just as the ADH and DAD DPMs mobilized adhesive features of single cells analogous to those of molecules to generate cell aggregate properties analogous to those of liquids, the Notch-related DPM (which we designate LAT; see table 1) mobilizes *reactive* properties of single cells analogous to those of molecules to generate systems analogous to chemical reactions. The result of the 'reaction' of cells via Notch–DSL signaling is a mixture of 'products', cells that are different from the starting mixture. Another consequence of this interaction is to exert control over the relative numbers of cells of different states in an aggregate. In general, the patterns that form will be fine-grained since the spatial scale of juxtacrine signaling is small. Since the Notch pathway genes are ubiquitous in the sponge and eumetazoan proteome but are absent in choanoflagellates, it is reasonable to infer that this DPM arose as a means of creating bifurcations in cell states that would stabilize the ADH- and DAD-based sorting out of cells. The alternative cell states would, for example, exhibit high and low levels of cadherin expression.

3.3. Induction of apical-basal and planar cell polarity

Employing the above set of DPMs, multicellular aggregates can transform their morphology from sheet-like configurations

with non-robust morphology into relatively stable solid spheroids. However, cells can become polarized along their apical-basal (A/B) axis (Karner *et al* 2006b), or oriented in a plane orthogonal to this axis (planar cell polarity (PCP); Mlodzik 2002, Karner *et al* 2006a) by exposure to Wnts, a family of secreted factors that interact with cell surface receptors of the Frizzled family. These individual cell behaviors, acting in a multicellular context, permit aggregates to overcome the morphological defaults of solidity and sphericality.

Although secreted Wnts are not present in the choanoflagellates (Ryan and Baxevanis 2007), the Wnt pathway has deep roots in cellular evolution. The MO25 family of proteins which mediates a key step in Wnt's A/B polarity-inducing activity in multicellular animals such as nematodes, insects and vertebrates, has a homolog in the fission yeast *Schizosaccharomyces pombe*, where it is essential for polar growth and regulation of cell division (Mendoza *et al* 2005); in its absence the actin cytoskeleton becomes depolarized and the otherwise cylindrical cells adopt a round morphology (Mendoza *et al* 2005). The ability of the Wnt pathway to create an apicobasal axis in otherwise spherical or amorphous cells predates even the divergence of choanoflagellates from fungi. Even in the extant bauplans, this ability remains an individual cell-specific property and in fact can be triggered independently of Wnt in isolated animal cells by activation of Lkb-1, a downstream effector of the pathway (Karner *et al* 2006b).

The cell polarizing effect of Wnt signaling can give rise to novel morphological outcomes when it occurs in the context of multicellular aggregates. If the polarity is manifested, for instance, in demarcation of a 'cadherin-rich' and a 'cadherin-free' portion of the cell membrane, the cells, instead of forming a solid aggregate, will orient themselves such that their adhesive portions bind to each other while their less adhesive regions enclose a free space or lumen. This topological change is brought about by the physical principle of minimization of free energy (Forgacs and Newman 2005).

The identification of what appear to be small, hollow, cell clusters in Chinese fossil beds of the Precambrian has suggested that interior cavities or lumens were among the earliest innovations of metazoan evolution (Chen *et al* 2004, Hagadorn *et al* 2006). Since Wnts, which mediate A/B polarization, are particularly prevalent in cnidarians, the most ancient of the eumetazoa (Miller *et al* 2005, Lee *et al* 2006), it is highly plausible that the advent of the Wnt-associated DPM enabling A/B polarization helped drive the morphological transition between the Ediacaran-early Cambrian biota and those of the mid-Cambrian explosion. We designate this DPM, which mobilizes the physical consequences of the A/B polarized expression of adhesion to create lumens, POL_a (table 1).

Planar cell polarity (PCP), in which cells are polarized orthogonal to the A/B axis is brought about as a consequence of activation of the Wnt pathway receptor Frizzled, although like A/B polarity it can also potentially be activated independently of ligand (Karner *et al* 2006a). The morphological consequences of planar polarity in a multicellular context can be dramatic. Elongated cells with anisotropic adhesive properties are predicted to spontaneously align and intercalate among one another, leading to narrowing of the tissue mass in one direction accompanied by elongation in the orthogonal direction (Zajac *et al* 2003). These cellular rearrangements and tissue reshaping effects are involved in tissue elongation processes throughout the metazoa, including 'convergent extension', which establishes the elongated body axis during gastrulation in the amphibian embryo (Keller 2002). We term the DPM that mobilizes the effects of elongation in the plane orthogonal to the cell's A/B axis POL_p (see table 1).

The Wnt signal transduction pathways, then, impart polarity to a cell's surface properties and shape. While this may have been an important mechanism for some unicellular functions like cell division, in a multicellular context it enabled spherical and solid cell aggregates to acquire internal lumens, tissue axes and elongated shapes.

Physically, these two polarity-associated morphogenetic phenomena have analogies in the behavior of certain polymers. Lumens form in tissues in which cells exhibit A/B polarity for reasons similar to the familiar ones by which amphipathic polymers form liposomes in aqueous media: one end of the cell (the adhesive portion) or molecule (the hydrophobic portion) is energetically favored to associate with the corresponding portions of other cells or molecules, leaving the other, physically distinct portion (the cell's nonadhesive region or the molecule's charge-polarized region) free to interact with the medium. The deviation from tissue fragment sphericality resulting from the anisotropic shape (due to PCP) of the component cells has its own physical analogy in the formation of ellipsoidal nanoparticles, rather than spherical ones, when the molecular subunits are certain main-chain liquid crystalline polymers (Yang *et al* 2005). The analogy in this case may be less exact, however. Tissues undergoing convergent extension elongate orthogonally to the long axis of the cellular subunits as a result of the unique intercalary behavior of the structurally polarized cells; it is not clear what the relationship is between the microarrangement of liquid crystal molecules and the axes of the ellipsoidal nanoparticles (Yang *et al* 2005).

3.4. Biochemical oscillations

It is well known that an appropriate balance of positive and negative feedbacks in any dynamical circuit, including gene regulatory networks, can lead the system to exhibit temporal oscillations in its characteristic state variables (e.g., molecular concentrations; Goldbeter 1996). Biochemical oscillation can take place within the confines of a single cell (Reinke and Gatfield 2006), but when coordinated across cell boundaries by juxtacrine and short-range paracrine signaling it has the potential to drive morphogenetic change. A variety of chemical mechanisms can give rise to oscillations, the most familiar being the coupled positive and negative feedback dynamics that produces limit cycles (Strogatz 1994). In living tissues, where the cells perform as 'reactors' that are much more elaborate than the reactions of non-living chemical systems (see below), other types of oscillatory dynamics are

possible, such as negative feedback coupled with time lags in the production of key components (Lewis (2003), Monk (2003), see below) (OSC, table 1).

The 'segmentation clock' of vertebrate embryos is one example of the operation of biochemical oscillation in a morphogenetic process. Somitogenesis is the process by which distinct blocks of tissue, precursive to the vertebrae and associated muscles, form in a progressive spatiotemporal order along the central axis of vertebrate embryos. The expression of genes of three signaling pathways (Notch, Wnt and FGF) oscillates with a time period which corresponds to the rate of somitogenesis (Pourquié 2003). The mechanism of oscillation in all three cases is believed to depend on negative feedback with time delay in the synthesis of a key pathway component (reviewed in Dubrulle and Pourquié (2004), Goldbeter and Pourquié (2008)).

Not all biochemical oscillations depend on the Notch, Wnt and FGF pathways; many intracellular circuits, including glycolysis, can sustain periodic behavior (Goldbeter 1996). However, because these pathways involve direct cell–cell communication along with intracellular feedback control, they are ideally suited for generation of oscillations on a multicellular scale (Lewis 2003, Monk 2003).

The temporally periodic alteration of a tissue property, such as adhesion, in a growing system, is sufficient to generate segmented or partly segmented forms (Newman 1993). In conjunction with a morphogen gradient (see below), it provides the basis for the well-regulated generation of somites in vertebrate embryos (Pourquié 2003). The Notch signaling pathway is also involved in the segmentation of certain insects and spiders in which segments are generated sequentially over time, but not in the fruit fly *Drosophila*, where they are generated simultaneously (reviewed in Damen (2007)). Significantly, the *hairy* gene, which specifies a Hes-class transcriptional mediator otherwise associated with Notch signaling, is nonetheless expressed in a spatially periodic fashion in the *Drosophila* embryo under the control of a hierarchy of gene products unrelated to the Notch pathway (Howard and Struhl 1990). It has been proposed that the Drosophila 'long germ band' mode of segmentation could have been readily derived from the sequential 'short germ band' mode because of the close dynamical relationship between oscillatory and reaction-diffusion instabilities (Newman 1993, Salazar-Ciudad *et al* 2001). The common use of a Hes modulator in these two segmentation modes may be a remnant of this ancient dynamical connection.

The process of somitogenesis affords an example of how the same DPM components can mediate more than one physical process: the juxtacrine aspect of Notch signaling not only brings about lateral inhibition; the temporal oscillations in its components and thus the cell fates can be synchronized using the same pathway (Lewis 2003, Giudicelli *et al* 2007). Embryonic pattern formation brought about by synchronization of oscillating cell states and molecular concentrations suggests that this synchronized activity may act as a pattern module of its own by creating spot-like or stripe-like patterns in which signaling centers may be surrounded by peripheral fields of cells with synchronized signal-prohibited states (Newman and Bhat 2007).

In the composite DPM-designated OSC+LAT+DAD (table 1), then, a physical process—biochemical oscillation—is mobilized at the aggregate level so as to regulate another DPM-mediated physical process—differential adhesion—in a periodic fashion. While there are limits to the extent that the regulation of differential adhesion alone can mediate very rapid segmentation (like that seen in zebrafish) in typically viscous tissues (Grima and Schnell 2007), the simplicity and versatility of this mechanism for separating domains of tissue from one another suggest that its regulation by oscillatory processes could have been a mechanism of morphological periodicity in primordially segmented metazoa.

3.5. Morphogen gradients

It has long been known that unicellular eukaryotes can communicate with each other by secreting diffusing molecules or by responding to other diffusing signals (Luporini *et al* 2006, Bonner *et al* 1970). It would thus be reasonable to assume that such signaling mechanisms were present even in the earliest unicellular animals. In a multicellular context, a secreted molecule can serve as a patterning signal if it distributes nonuniformly and the cells respond to it in a concentration—or duration-dependent fashion (Lander 2007). Molecules that fit this description are referred to as 'morphogens' (MOR: table 1). In a multicellular environment these molecules diffuse through the extracellular matrix or between cells through interactions with their lipid membranes.

Morphogens can lead to the generation of heterogeneous patterns on a spatial scale of 100 μm–1 mm over tens of hours, based on the time–distance–concentration relationships inherent in macromolecular diffusion (Crick 1970). Bi- or multistable dynamics based on positive feedback of morphogens upon the cells which produce them (Ingolia 2004) or on their mutually antagonistic interaction (Goldbeter *et al* 2007) can govern the switching of cellular states. Some classes of morphogens (e.g., FGFs) typically act on cells that are already different from the producers (perhaps residing in a separate layer), causing them to become different from their unexposed neighbors.

Different morphogens also move through different media. Those of the TGF-β/BMP and FGF classes diffuse through the aqueous interstices and extracellular matrices of tissues. The diffusion rates of these molecules vary not only with their size, but also with their capacity to bind specific extracellular molecules, which may themselves be distributed nonuniformly (Ohkawara *et al* 2002). Members of the Hedgehog class of morphogens, in contrast, alternate between being tethered to cell membranes by covalently attached lipid moieties and diffusing through the aqueous interstitial phase beyond the membrane (Goetz *et al* 2006). Because the lipid component of the Hedgehog morphogens limits their spread (Guerrero and Chiang 2007) their diffusion rate is probably intermediate between the TGF-β/BMP class and the Wnts, which diffuse only small distances (Zhu and Scott 2004). The signaling pathways that each of these morphogens activate apparently pre-existed the metazoa (reviewed in Newman and Bhat (2008)).

Cells can respond to morphogen gradients in physical analogy to stationary reactive sites in a solid that change their state in response to local concentrations of a diffusible reactant. They can also respond in physical analogy to particles undergoing Brownian motion (as discussed in relation to differential adhesion in section 3.1, above). In this case, morphogen concentration may act like a temperature, with cells increasing their rate of undirected locomotion at higher gradient values. This is termed 'chemokinesis', a phenomenon that plays a role in a variety of developmental processes, including blood vessel formation (Offermanns *et al* 1997). Finally, cells can respond to morphogens in physical analogy to charged particles in an electric field, moving in a directed fashion toward higher values of the gradient. This phenomenon, termed 'chemotaxis', is employed in numerous developmental processes, and can yield exotic, organized collective behaviors such as paired counter-rotating cell flows in avian gastrulation (Chuai *et al* 2006, Newman 2008).

All morphogens employ the physical principle of diffusion as a common mechanism of transport, although they move through various media with different mechanisms of modulation. In the unicellular context, morphogens would typically act as communicative signals without bringing about a heterogeneity in cellular identity. In the context of the earliest metazoans, however, via the simple effect of setting up molecular gradients across a cluster of initially equivalent but responsive cells, morphogen-based DPMs would have generated organismal forms that ultimately contained nonuniformly distributed cell types. Such forms would have been subject to natural selection, but their structure would have been an expression of diffusion gradients as well as other material properties of such systems.

3.6. Turing- and other LALI-type reactor-diffusion systems

A mathematical demonstration that spatial pattern formation can be brought about by the interaction of two diffusing substances was presented by Turing (1952). This class of systems exhibits reaction-diffusion instability, an experimentally confirmed mechanism for pattern formation in chemical systems (Castets *et al* 1990, Ouyang and Swinney 1991). Such systems are also dynamically related to the temporal oscillatory mechanisms described in section 3.4 (see, for example, Flach *et al* (2007)).

In a generalization of Turing's reaction-diffusion mechanism (Gierer and Meinhardt 1972, Meinhardt and Gierer 1974, 1980, 2000, Meinhardt 2007) known as 'local auto-activation-lateral inhibition' (LALI) systems or networks, a positively autoregulatory morphogen (in a composite DPM) is coupled to a mechanism of lateral inhibition. This will result in peaks of maximal activator morphogen production surrounded by zones in which activator production is suppressed. Newer peaks of activator would only form at loci where effects of inhibition will have attenuated. LALI systems can break compositional symmetry and generate regularly spaced spots or stripes of morphogen concentration (see examples in Nijhout (2003), Forgacs and Newman (2005), Meinhardt (2007) and Newman and Bhat (2007)). The resulting chemical patterns can, in turn, induce primordia of skeletal elements (Newman and Frisch 1979, Hentschel *et al* 2004, Newman 2007) and other serially repeated structures such as teeth (Salazar-Ciudad and Jernvall 2002), feathers (Jiang *et al* 2004) and hair follicles (Sick *et al* 2006). Developmental processes such as the formation of tissue boundaries (Meinhardt and Gierer 1980, von Dassow *et al* 2000) and polarized body axes (Meinhardt 2006, De Robertis 2006) regulated by LALI mechanisms are more robust than those that depend solely on differential adhesion or diffusion gradients (Ingolia 2004).

In the original LALI scheme, lateral inhibition was mediated by a chemical induced by the activator with a faster rate of diffusion than the latter, and a negative effect on its rate of production (Gierer and Meinhardt 1972). Biological systems, however, have additional ways of implementing this inhibitory function. As we have seen above, Notch juxtacrine signaling can mediate lateral inhibition, but since it just acts over short distances, only fine-grained salt-and-pepper type patterns can arise from this alone. The spatial scale of these patterns can be increased by a global coordination of Notch's downstream effects (Newman and Bhat 2007) by synchronization of oscillations in the expression of the Hes transcription factors that mediate those effects (Giudicelli *et al* 2007, Riedel-Kruse *et al* 2007). As noted in section 3.4, above, such oscillations can arise from negative feedback upon, and time-lags in, the production of Hes (Lewis 2003, Monk 2003).

More generally, the notion of a reaction-diffusion system is greatly extended by the capability of cells to function as 'reactors' (Hentschel *et al* 2004) and for the resulting cell clusters and tissues to act as 'excitable media' (Mikhailov 1990). This enables, for example, patterns to form in systems where (in contrast to the classical scheme) the diffusion coefficients of the activator and inhibitor are similar (Rauch and Millonas 2004), or even, as in the above example, where the inhibitor is not literally diffusible.

It should also be noted that whereas the original formulation of this framework presented conditions for the formation of spatial patterns that are stationary in time (Turing 1952, Gierer and Meinhardt 1972), the nonuniformities generated by this class of systems (sometimes referred to as 'dissipative structures'; Nicolis and Prigogine 1977) may be transient, but still impart reliable spatial information to fields of receptive cells, which often integrate both amplitude and duration of exposure to morphogens in deciding what fate to follow (Meinhardt 1978, Jullien and Gurdon 2005, Dessaud *et al* 2007, Lander 2007).

With respect to the question of origination of metazoan forms, we note that the molecular components involved in hypothesized and demonstrated modern-day LALI-pattern-forming systems are virtually all products of the DPM-related toolkit genes. In keeping with our system of three-letter codes for the various DPMs, we use the abbreviation TUR for the one that embodies generalized Turing-type, or LALI, systems (see table 1).

3.7. Epithelial elasticity, epithelial-mesenchymal transformation and global organization of cell polarity

The living tissues we have described up to now, in which cells are directly attached to each other, are termed 'epithelioid'. Such aggregates are analogous to viscoelastic liquids. Viscosity in epithelioid tissues is a function of the ease with which the subunits, cells, slip past one another while maintaining their attachments. Elasticity in a cohesive tissue will primarily be a function of the properties of the cytoskeleton, with cohesivity being determined by the force required to separate the cells. In multicellular metazoan organisms another type of tissue, known as 'mesenchyme', consists of cells that are embedded in a complex macromolecular environment called the *extracellular matrix* (ECM) (Comper 1996). It is the properties of this material that are the main determinants of a mesenchymal tissue's rheological properties, although the cytoskeleton can also play a role. A set of DPMs employing extracellular matrices provides mesenchymal tissues with morphogenetic capabilities additional to those that mold and pattern epithelioid cell aggregates (ECM; table 1).

Morphogenesis of the Porifera, or marine sponges, which are among the most ancient metazoans, depends heavily on the ECM. Sponge cells embedded in a stiff but dynamic collagenous matrix called 'mesohyl' are in a continuous state of locomotion (Bond 1992). Sponges also have epithelial cells (Schröder *et al* 2004) and homologs of type IV-like collagen (Boute *et al* 1996), which in the more elaborate eumetazoa (see below) constitutes a major portion of the *basement membrane*, the sheet-like supporting structure of planar epithelial tissues.

Porifera branched off from the metazoan lineage early on, as a morphological dead-end (Nichols *et al* 2006). It is possible that the low degree of evolvability of the poriferan body plan can be attributed, via its physically compliant ECM, to the property of continual morphogenesis (Bond 1992), which contrasts with the directional, programmed morphogenesis of eumetazoa. In this sense sponge development would be a morphogenetic analog to a dynamical system's randomly exploring state space while trapped in a strange attractor (Strogatz 1994).

The eumetazoan ECM is chemically more complex than that of sponges and a more responsive medium for the actions of additional DPMs. Whereas a true interstitial ECM is found only in triploblasts (metazoans with three fundamental tissue layers) (Huxley-Jones *et al* 2007), cnidarians have a thin sheet-like 'mesoglea' between their two epithelial layers consisting of separate regions with basement membrane-like and interstitial ECM-like properties (Zhang *et al* 2007). A basement membrane reinforces the planar nature of an epithelium and permits it to behave as an elastic sheet in the direction perpendicular to the plane. Because of the potential for cell rearrangement, however, it can retain in-plane liquid-like properties (Mittenthal and Mazo 1983, Newman 1998). Elastic sheet epithelia hence exhibit a range of folding, buckling and wrinkling effects not seen in liquid-like epithelioid tissues. When this arose evolutionarily it set the stage for the emergence of novel mechanisms of gastrulation and for formation of epithelial appendages (Gierer 1977, Newman 1998, Forgacs and Newman 2005).

The mesodermal layer of many triploblasts and some cnidarians (Fritzenwanker *et al* 2004, Seipel and Schmid 2006) is composed of cells that have undergone epithelial–mesenchymal transformation (EMT) (Hay 2005). This is a change in the physical state of cell aggregates that is brought about through their dynamical interaction with the ECM (somewhat analogous to the dispersal of colloids). EMT permits cells that are no longer directly attached to one another to nonetheless remain part of an integral tissue (i.e., by embedment in the matrix). Whereas skeletogenesis and elastic sheet behavior are both based on the stiffness of ECM, its properties of space filling, flexibility (Sarras and Deutzmann 2001) and cell–cell communication are evidenced in EMT.

Because many of the molecules of ECMs are fibrils and fibers which undergo time-dependent changes in aspect ratio, their spontaneous assembly can take the form of a gelation transition. Such phase transformations can be studied *in vitro* by rheometric methods and analyzed using power laws (Forgacs *et al* 2003, Newman *et al* 2004). Unlike epithelioid tissues, whose immiscible behavior is readily explained differential adhesion (see section 3.1, above) immiscibility of mesenchymal tissues, for which there is experimental support (Downie and Newman 1994, Robinson *et al* 2004), is poorly understood (Forgacs and Newman 2005). Phase transformation of the ECM is a physical aspect of the associated DPM that might have been employed in establishing internal tissue boundaries beginning early in metazoan evolution.

Metazoan ECMs can also play additional biological–physical roles. By governing the architecture of an adhesive multicellular microenvironment (Thery *et al* 2006) the ECM influences the polarity of the cells that are embedded in it and therefore interacts closely with the POL_a and POL_p DPMs (see section 3.3). The ECM can act as a mechanical integrator of polarity patterning across a large field of cells and is a means, in addition to morphogen gradients, of promoting directional migration (via *haptotaxis,* Dickinson and Tranquillo (1993)) of cells in multicellular aggregates.

Integrins, the classic metazoan ECM adhesion molecules, have a history that predates the evolution of animals. The free living ameba *Dictyostelium discoideum* encodes an integrin homolog which is involved in adhesion to an external substratum as well as phagocytosis (engulfment of prey) (Cornillon *et al* 2006). Integrin homologs are present in choanoflagellates (King *et al* 2008) and true integrins are found in sponges, where they mediate both adhesion and outside-in signaling (Wimmer *et al* 1999). The emergence of multicellularity and the transformation in organizational architecture by ECM allowed integrins to acquire novel morphogenetic functions, thereby constituting a molecular component of the ECM-related DPMs.

4. Conclusions and outlook

We have described a set of developmental patterning modules, DPMs, which are responsible for generating many of the

morphological features of modern metazoan organisms. The DPMs are closely tied to molecular components that largely pre-existed multicellularity and have changed little in their molecular nature since the rapid burst of origination of animal form during the early Cambrian. It is therefore reasonable to consider the likelihood that factors apart from genetic change alone participated in the evolutionary transformation of simple cell aggregates into complex bodies. As discussed in the previous sections, and in earlier treatments of this question (Newman and Comper 1990, Newman 1994, Newman and Müller 2000, Forgacs and Newman 2005, Newmann *et al* 2006), our proposal for the extra-genetic causative factor for the organization of organismal form is the physics and chemical dynamics of excitable 'soft matter' (Mikhailov 1990, de Gennes 1992).

As a result of the surprising realization over the past decade that the embryogenesis of all metazoans employs a limited set of ancient 'toolkit' gene products, it has now become possible to identify particular molecules of unicellular or proto-metazoan life that became the conduits of novel physical effects when they came to function at the larger, multicellular scale of the first metazoa. The implementation of a physical process by an evolutionarily conserved molecule or molecular network is the defining characteristic of a DPM.

In focusing on the physical aspects of the evolution of development, we have de-emphasized the specific roles of the set of transcription factor-specifying toolkit genes: the Hox, Pax, myogenic and Nkx proteins, and so forth. Although these molecules often control one another's production and stand at the top of hierarchies of the regulation of cell type-specific genes in modern-day developmental systems (Davidson 2006), they all perform the same function, regulation of transcription, albeit at different promoter sites. We have therefore characterized the connection of certain toolkit transcription factors to the generation of particular cell types and states as 'frozen accidents' (Newman and Bhat 2008). This is a different situation from DPM-associated molecules such as cadherins, Notch and its ligands, Wnts and the various morphogens and ECM molecules; in contrast to these cell interaction-mediating molecules, transcription factors do not bring specific physical process directly to bear on the tissues of the developing embryo.

We have suggested that the developmental mechanisms of biologically modern animals can be understood in terms of a 'pattern language' of metazoan form (Newman and Bhat 2008). The elements of this language are used during development and, we speculate, early in evolution, to transform clusters of cells away from their topologically solid, geometrically spherical and spatially uniform default physical condition into morphologically complex body plans. The major transformations include (i) establishment of stable mixtures of cells occupying more than one biochemical state; (ii) formation of distinct non-intermixing cell layers, (iii) formation of internal cavities, (iv) elongation of the multicellular mass, (v) generation of nonuniform patterns of cells occupying different biochemical states and (vi) dispersal of subpopulations of cells without disintegration of the organism.

We have by no means presented an exhaustive list of possible DPMs. For example, a fundamental physical property of any material entity is its mass. In cell aggregates this is controlled by increase and decrease in cell number. Cell division and death are, of course, also properties of free-living unicellular organisms, but these processes have only a quantitative impact on such populations, particularly when all cells have the same or similar biochemical state. In a multicellular context, however, these processes not only change mass, but, when linked to other DPMs, can do so in a nonuniform fashion (Salazar-Ciudad *et al* 2003, Salazar-Ciudad 2006). Thus, the mitogen-activated protein kinase (MAPK) pathway, one of whose functions is to mediate proliferation in response to external signals (Krens *et al* 2006), and the apoptotic pathway, both of which have roots in the premetazoan unicellular world (Widmann *et al* 1999, Blackstone and Green 1999), can be considered to constitute a DPM that modulates the physical mass of a multicellular aggregate in whole or in part. (Note that the graphic representation of the TUR DPM in table 1 also incorporates the 'growth and loss' DPM.) On a more complex level, a quantitative change in an entity's parts can lead to a qualitative change in its overall behavior (Casati and Varzi 1999).

Other morphological motifs not listed in table 1 that are found in some metazoan body plans are 'midlines', a precondition for the evolution of mediolateral organization and a central nervous system (Meinhardt 2004), and branches, seen in some cnidarians and bryozoa. Branching is also well represented in later developmental process, as in the formation of insect and vertebrate respiratory systems (Miura 2007), as well as the vertebrate vascular (Merks *et al* 2006, Czirok *et al* 2007) and urinary systems, and exocrine glands (Nelson *et al* 2006, Lubkin 2007). The physical mechanisms by which branching occurs in cellular systems often lead to structures with fractal geometry (Masters 2004, Miura 2007). The tetrapod appendicular skeleton is based on still another pattern motif, 'spots and stripes' (Alber *et al* 2005, Newman and Bhat 2007). As with segmentation, the DPMs responsible for these formations appear to be composite ones, employing the basic DPMs such as MOR, LAT, POL, ADH, DAD, TUR and ECM in a combinatorial fashion.

In each of the DPMs we have described there is an analogy, at the level of cells and tissues, to physical behaviors that are more familiarly embodied in nonliving condensed materials. These analogies (along with the respective DPMs) include forces of cohesion (ADH) and phase separation (DAD) in liquids, chemical reaction (LAT), self-assembly of anisotropic polymers (POL), chemical oscillation (OSC), molecular diffusion (MOR), reaction-diffusion instability (TUR) and solidification and dispersal of colloids and phase transitions (ECM). We have also referred to active responses of cells in response to gradients in analogy to the behavior of charges particles in electric fields. Our contention is that the physical principles involved often are sufficiently generic that they can be used to adequately describe the biological situation. This has proven correct in many instances (see Forgacs and Newman 2005). Where it fails, there may be interesting physics in explaining the deviations from ideality, as has been

the case (to take an example from within the physical sciences) in understanding the behavior of fluids (Kalikmanov 2001).

Because of the generic nature of DPM-related physical processes, and the capability of biological systems to evolve novel molecules, the standard evolutionary model would not have predicted that metazoan forms as disparate as arthropods, nematodes, echinoderms and chordates would use the same molecular toolkit, building what have classically been considered analogous structures by homologous means. After all, plants employ an entirely different set of 'interaction molecules' for developmental morphogenesis and pattern formation (Meyerowitz 2002).

Our 'physicalist' perspective on the origination and development of biological form, based on the inherent organizational plasticity of material systems, provides a way out of this apparent paradox (Newman 2006). With respect to multicellular organisms, organizational plasticity translates into phenotypic and developmental plasticity (understood as the possibility of generating more than one form from a single genotype; see Newman and Müller 2000, West-Eberhard 2003, Jablonka and Lamb 2005). And although continued utilization of DPMs guarantee a degree of developmental plasticity in modern-day metazoa, ancient metazoa were likely to have been even more plastic (Newman and Müller 2000, Newman *et al* 2006). Consequently, the first metazoans would, with minimal genetic change, have taken on many forms in variable physical environments. Natural selection could then act on this morphological variation to sharpen the means by which some of the variants were generated, a process that has been termed 'stabilizing' (Schmalhausen 1949) or 'canalizing' (Waddington 1942) selection. The apparently precisely engineered developmental 'machines' (Istrail *et al* 2007) studied by Davidson and co-workers would thus have a physically comprehensible origin in this 'phenotype first' scenario.

Although GRNs can potentially evolve to arbitrarily high degrees of complexity, DPMs, despite the plasticity they embody, are limited in the forms they can generate. Although they have the potential to transform different anatomies from one into the other with little or no genetic change, DPMs, by mediating adhesion and tissue multilayering, the generation of spatially nonuniform and temporally periodic cell states, and cell polarity are constrained to mold cell masses into only those morphologies which are characteristic of chemically and mechanically excitable mesoscopic materials, e.g., hollow, multilayered, elongated, segmented and branched forms. But these are, in fact, the common morphological motifs of all metazoan body plans and organ forms, both in the invertebrates and vertebrates, appearing repeatedly over the course of evolution despite there frequently being no common ancestor between organisms with the same feature.

In the interplay between genetics and physics in the production of organismal form, then, genes ensure the perpetuation of variations on biological themes but they do not define the themes themselves (Müller 2007). Concerning generation of living form, it is the physics pertaining to the materials and scales in question that establishes the range of possibilities. The rapid profusion of metazoan forms during the early Cambrian, the striking conservation of the proteins and pathways that mediate these forms, and the nature of the forms themselves, can be accounted for together by the recognition that a set of proteins and pathways, most of them with roots in the unicellular world, upon coming to operate on the multicellular scale took on new roles as the conduits and mobilizers of the physical laws pertaining to soft matter and excitable media.

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Glossary

Body plan. A prototype for the characteristic morphological organization of an organism in relation to a specific (phylum or class) taxonomic level.

Morphospace. The complete set of morphological variations achieved by a protypic body plan or organ form under the influence of developmental plasticity, stocahsticity and external environments.

Developmental-genetic toolkit. A small, highly conserved set of gene products (a few dozen to a hundred or more, depending on the exact criteria) that participate in coordinating basic developmental mechanisms across all the metazoan phyla.

Gene regulatory network (GRN). A relatively isolable set of genes within a cell that interact via their expression products to regulate each other's transcriptional activities.

Juxtacrine. Mode of communication between cells involving direct cell–cell contact.

Paracrine. Mode of communication between cells involving locally released factors.

Convergent extension. The rearrangement of a tissue mass characterized by intercalation of individual cells in one direction resulting in the elongation of the mass in an orthogonal direction.

Segmentation clock. A temporally oscillating molecule/molecular network that mediates the transformation of a uniform tissue mass into sequentially arranged tissue blocks.

Epithelial–mesenchymal transformation (EMT). The dispersal of directly attached cells in a tissue mass into a loosely packed state in which they are separated from one another by an extracellular matrix.

Haptotaxis. The directional movement of cells in response to contact with an insoluble molecular gradient.

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