Dynamical patterning modules: a "pattern language" for development and evolution of multicellular form

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ABSTRACT **This article considers the role played by a core set of "dynamical patterning modules" (DPMs) in the origination, development and evolution of complex organisms. These consist of the products of a subset of the genes of what has come to be known as the "developmental-genetic toolkit" in association with physical processes they mobilize. The physical processes are those characteristic of chemically and mechanically excitable mesoscopic systems like cell aggregates: cohesion, viscoelasticity, diffusion, spatiotemporal heterogeneity based on activator-inhibitor interaction, and multistable and oscillatory dynamics. We focus on the emergence of the Metazoa, and show how toolkit gene products and pathways that pre-existed the metazoans acquired novel morphogenetic functions simply by virtue of the change in scale and context inherent to multicellularity. We propose that DPMs, acting singly and in combination with each other, constitute a "pattern language" capable of generating all metazoan body plans and organ forms. This concept implies that the multicellular organisms of the late Precambrian-early Cambrian were phenotypically plastic, fluently exploring morphospace in a fashion decoupled from both function-based selection and genotypic change. The relatively stable developmental trajectories and morphological phenotypes of modern organisms, then, are considered to be products of stabilizing selection. This perspective solves the apparent "molecular homology-analogy paradox," whereby widely divergent modern animal types utilize the same molecular toolkit during development, but it does so by inverting the neo-Darwinian principle that phenotypic disparity was generated over long periods of time in concert with, and in proportion to genotypic change.**

KEY WORDS: *body plan, Cambrian explosion, developmental-genetic toolkit, epithelial-mesenchymal transformation, lateral inhibition, macroevolution, morphogenesis, morphospace*

Introduction

Animal body plans of virtually all the modern Metazoan types emerged relatively suddenly ("compressed in time"; Rokas *et al.*, 2005) during the "Cambrian explosion" about 550-530 million years ago (Ma) (Conway Morris, 2006). The metazoans, in turn, may have had roots (Erwin, 2008) in the Ediacaran period, 575-542 Ma (Shen *et al.*, 2008), derived, in part, from simpler sheet-like, or budded, segmented, tube-like multicellular biota found in deposits of the earlier period (Narbonne, 2004; Droser and Gehling, 2008). The early metazoans, variously, had body cavities, multiple tissue layers and appendages, and were elongated and segmented. Significantly, not only was this emergence of disparate forms rapid, it was all but exhaustive. With the possible exception of the bryozoa, which are not seen until the early

Ordovician some 40 million years later (though see Passamaneck and Halanych, 2004), exemplars of all the major metazoan bauplans appeared no later than the early Cambrian.

Rapid morphological evolution is compatible with classic neo-Darwinian evolutionary mechanisms of random genetic change followed by natural selection, whereby existing structures are modified in their size or shape (Weiner, 1994; Losos *et al.*, 2006). Nonetheless, the discovery of the phylum-level diversification

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(with no evident intermediates) that occurred in the early Cambrian is not anticipated by this theory (Müller and Newman, 2005). Even more surprising from the viewpoint of the standard evolutionary model has been the realization that the Metazoa have used a highly conserved set of gene products, the so-called developmental-genetic toolkit, to generate diverse body and organ forms since their inception more than half a billion years ago (Carroll *et al.*, 2005). If morphological change is supposed to be driven by, and to track, genetic change, why were there not dramatic changes in gene content corresponding to innovation of new organismal architectures (e.g., the dorsal location of nerve cord and heart in chordates vs. the ventral location of these structure in annelids and arthropods; Gerhart, 2000), or of new developmental mechanisms for generating morphologically equivalent structures (e.g., segmentation in beetles and fruit flies; Salazar-Ciudad, 2001b)?

The unexpected gives way to the paradoxical in the recognition that disparate organisms use homologous genes when making structurally dissimilar but functionally similar structures traditionally categorized as analogous (e.g., eyes in insects, mollusks and vertebrates; fly and bird wings) (Carroll *et al.*, 2001; Wilkins, 2002). Given that there is nothing eye-like about the twin of eyeless/Pax6 transcription factor (Gehring, 2002), or heart-like about tinman/Nkx-2.5 (Schwartz and Olson, 1999), and considering the immense evolutionary and anatomical divergence between *Drosophila* and the mouse, why are the same gene regulatory proteins used, correspondingly, to initiate the developmental pathways that produce structures that have the same function, but which are evolutionarily unrelated (or have been thought so in the past)? The conflict between the expectations of the neo-Darwinian model and these recent findings from the fields of paleontology, comparative anatomy and genomics, and developmental biology has been termed the "molecular homology-analogy" paradox (Newman, 2006).

In this paper we suggest a way out of these apparent inconsistencies by arguing that pre-metazoans were highly plastic entities and that early morphological diversification was an expression of the primitively loose relationship between phenotype and genotype (Newman, 2005). Our hypothesis entails organizing the toolkit genes of the presumed pre-metazoan genome into two broad categories. The first of these, which we term the "developmental transcription factors" (DTFs), are those gene regulatory molecules which mediate cell type- and region-specific functions: eyeless/Pax6, tinman/Nkx-2.5, Hes, Tbox, Hox, Dlx, Pbx and several others (reviewed in Davidson, 2006 and Newman *et al.*, in press). Our second category of toolkit genes specify products such as cadherins, Notch, Wnt and their ligands, TGF-β/BMP, FGF, Hedgehog, and their receptors, which by mobilizing certain physical processes and effects generic to "soft matter" (de Gennes, 1992) and "excitable media" (Mikhailov, 1990) constitute what we refer to as "dynamical patterning modules" (DPMs) for animal development. The relevant physical processes include adhesion and differential adhesion, induced lateral inhibition of an activated state, dynamical oscillation, cell surface and shape polarization, supracellular gradient formation (reviewed in Forgacs and Newman, 2005; Newman and Bhat, 2008). These physical processes impart a discernable and at least semi-autonomous role to the functions of the gene products; hence the use of the term "module" (see, for example, von Dassow et al., 2000; Wimsatt and

Schank, 2004).

Our rationale for this binary categorization is the following: the several billion years of unicellular evolution that preceded the origin of multicellularity saw the emergence of numerous signaling pathways and transcription factors (King *et al.*, 2003). With great certainty, cells had acquired the capability to modulate and switch between states in various ways, including by means of multistable transcriptional networks. None of these features, however, are distinguishing for complex multicellular forms like the metazoa. The novelty introduced by multicellularity was the potential for more than one cell type and more than one tissue layer or module to co-exist in the same organism, and for the patterning processes of particular arrangements of such cell types and tissues to be propagated from one generation to the next. Transcription factors, though they are important in determining the states that cells assume, do not, in general, act across cell boundaries (though there may be exceptions; Joliot and Prochiantz, 2004).

When the effects of cell state dynamics, determined transcriptionally or otherwise, are communicated between cells and manifested in changes of shape or pattern in the multicellular aggregate, processes and forces characteristic of condensed, chemically and mechanically excitable materials are invariably involved (Newman and Comper, 1990; Forgacs and Newman, 2005). We therefore place toolkit gene products that (along with their downstream signaling effectors) mediate cell interaction and thereby mobilize these generic physical processes (see below) as DPMs, in a separate category from the DTFs, which mediate the transcriptional and gene regulatory aspects of the multicellular processes.

The DPMs involve members of the adhesion and receptor-mediated signaling classes of toolkit genes asserted by Nichols *et al.* (2006) to be key elements in the evolution of the metazoans. However, we do not consider signaling and response to the external environment per se, which had many roles in pre-metazoan ancestors, to be decisive in the metazoan-specific burst of morphological diversity. As mentioned above, integral to our definition of DPMs is their role in bringing physical forces and effects, and chemical nonuniformities, to bear on cells within multicellular aggregates and influencing the state of those cells.

Concerning the DTFs, as noted by Gehring, "..there is no functional necessity to use a particular transcription factor like Pax6 for particular function e.g., eye morphogenesis, since a transcription factor can regulate any gene, if this gene is endowed with the appropriate regulatory elements in its enhancer or promoter" (Gehring, 2002). We therefore suggest that the conserved relationships between particular DTFs and particular region- or organ-specific developmental pathways represent "frozen accidents" of the rapid morphological diversification we postulate. The specific roles of the DPMs, in contrast, are tied to the morphogenetic and patterning effects they mediate, making them (as we will show below), more decisive for the origination of the Metazoa than the DTFs and the cis-regulatory networks by which they indirectly regulate one another. Specifically, networks and pathways embodying DPMs, whether or not they incorporate transcriptional regulation, constitute the driving principles of the evolution of development. This collection of molecular-physical modules in effect constitutes a "pattern language"[1] (Alexander *et al.*, 1977) for multicellular form.

In this article we have focused on the molecular-genetic and evolutionary aspects of the major DPMs, and their distinction from the developmental transcription factor-cis-regulatory modules that serve as determinants of cell type. Additional details of the physical aspects of these modules are described in Newman and Bhat (2008).

Dynamical patterning modules, developmental transcription factors and "kernels"

Our model for the origination of animal form departs from purely selectionist accounts, since it is based on the idea that a single genotype can be consistent with a variety of multicellular morphologies. While this phenomenon is rare in modern animals (but see West-Eberhard, 2003 and Borges, 2005), it is well known and extensive in protists (Bonner, 1967), fungi (Magee, 1997), bacteria (Shapiro, 1995) and higher plants (Grime *et al.*, 1986).

Our view is consistent with the interpretation that the Burgess Shale-type fauna of the early Cambrian are "crown group" exemplars of modern phyla (Gould, 1989), but also with the more recent suggestion that they are "stem groups" for the phyla, defined, in Conway Morris's words, as "the series of extinct organisms that possess some, but not (crucial to note) all, of the defining characters that delineate a phylum" (Conway Morris, 2000). If the latter is the case (see also, Budd and Jensen, 2000), then long periods of natural selection of neo-Darwinian gradualist sort could well have followed before the definitive phyla were in place.

Davidson and his coworkers, in contrast, have proposed that the evolution of body plans leading up to the profusion of forms that appeared in the Burgess Shale-type deposits required no novel processes. They postulate that incremental natural selection was at work for tens of millions of years before the Cambrian explosion, but that the pathways and intermediate forms of that evolutionary episode were lost to the fossil record due to their small size and fragility (Davidson, 2006). By the early Cambrian, in this view, a set of cell type-determining transcription factor networks ("kernels") had long been in place, and as a result of selective forces they had also become incorporated into numerous regulatory hierarchies that deployed them regionally during embryogenesis in reliable phylum-specific modes. The seeming burst of morphotypes during the early Cambrian, according to Davidson and his colleagues, arose not from any unique morphogenetic events that happened during that period, but rather from the appearance of additional cell populations in the already morphologically advanced organisms that permitted previously evolved patterning mechanisms to operate on a larger scale.

Davidson and colleagues suggest that morphogenesis and spatial patterning of cells is based on earlier-evolved cell type-specific kernels (Erwin and Davidson, 2002). An example of such a kernel is the gene network that controls heart formation, which involves regulation of cardiac muscle differentiation by the homeobox-containing transcription factor tinman in *Drosophila*, and the homologous protein Nkx-2.5 in mammals (discussed in Davidson, 2006). But while this particular kernel indeed controls cell differentiation, other transcription factors that were similarly recruited early-on into function-specific developmental modules are not associated with specific cell types. The products of the hairy/enhancer of split (Hes) family, which are involved in segmentation in arthropods, annelids and vertebrates (Damen *et al.,* 2005; Song *et al.*, 2004; Giudicelli and Lewis, 2007), and the Pbx1 (Gonzalez-Crespo and Morata, 1996; Selleri *et al.*, 2001) and Dlx families (Panganiban and Rubenstein, 2002; Robledo *et al.*, 2002), which are implicated in marking proximal and distal portions, respectively, of insect and vertebrate appendages, function differently. They participate in establishing spatial relationships among tissue domains, as do the products of the well-known Hox class of transcription factors (Wilkins, 2002).

Although products of the DTF class of toolkit genes became linked to DPMs over the course of evolution to form widely used patterning motifs, we do not consider the cell-type differentiation function of transcription factor networks sufficient to explain morphogenesis and pattern formation. Rather, DPMs, by mediating adhesion and tissue multilayering, the generation of spatially nonuniform and temporally periodic cell states, and cell polarity (with resulting tissue topological and shape transformations), were required in order to turn pre-metazoan multicellular aggregates into metazoan body plans (Newman, 1994; Newman and Müller, 2000; Newman *et al.* 2006). These physical-genetic patterning modules, acting relatively independently and combinatorially during the early stages of metazoan evolution, provide a plausible basis for the rapid generation of morphological diversity evidenced in the fossil record of the early Cambrian.

Components and properties of core dynamical patterning modules

In this section we show how a core group of DPMs serve as elements of a pattern language for modern multicellular organisms. The DPMs, though emerging simultaneously with multicellularity, are nonetheless based on molecules that were present before the split between the architecturally simple poriferans (sponges) (Nielsen, 2008) and placozoans (Miller and Ball, 2005), and the eumetazoans, the evolutionary line that underwent the explosive proliferation of morphologies during the early Cambrian (Nichols *et al.*, 2006). Many of the toolkit genes, therefore, arose first in unicellular organisms, taking on their DPM-associated roles by mobilizing physical processes that only come into play at the "mesoscale"[2] in which multicellular aggregates exist.

In the following subsections we describe several of the most important DPMs, focusing on their major molecular components. We describe each DPM's characteristic initial effects on cells and its distinctiveness in this regard from other DPMs. We also provide examples of how DPMs may combine spatiotemporally to mobilize mesoscale physical phenomena (e.g., biochemical oscillation, reaction-diffusion patterning instabilities) that are novel in a biological context. Each DPM is given a three-letter designa-

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[1] This term was introduced by the architectural theorist Christopher Alexander to describe what he considers to be the elements of successful design in both the natural and artificial realms (Alexander et al., 1977; Alexander, 2002). Except for adopting the term, however, we do not, in this paper, explore the significant relationships between Alexander's "fundamental properties" and the DPMs.

[2] A term referring to the "intermediate scale." Although it has different referents in different scientific fields, here we use it to refer to condensed materials on a scale $\sim 10^{-4} - 10^{-3}$ m; i.e., larger than a typical cell but smaller or equal to a functional unit of a developed organ.
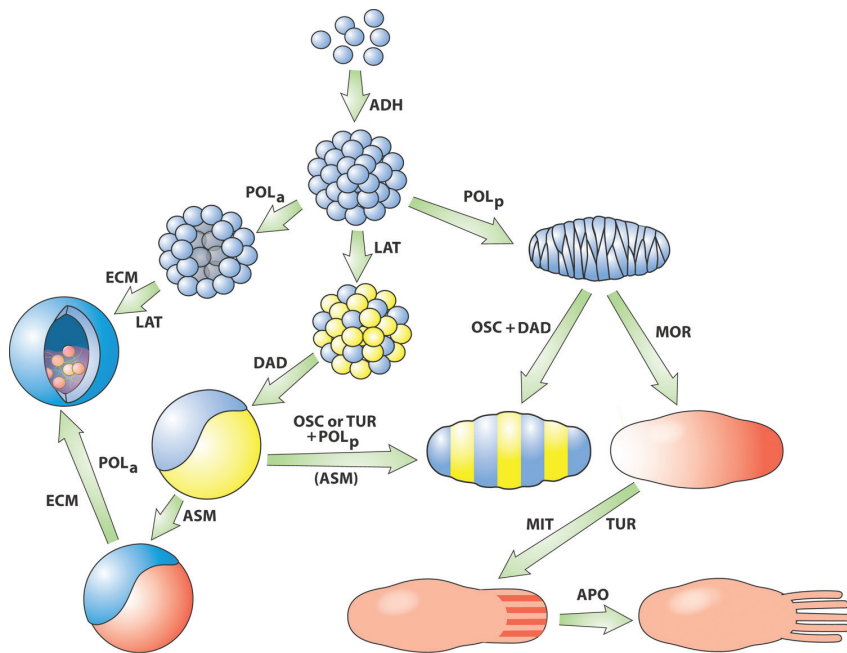
Fig. 1. Schematic representation of metazoan forms potentially generated by single and combinatorial action of dynamical patterning modules (DPMs). *Cells are represented individually in the upper tiers of the diagram, while the middle and lower tiers are shown at the scale of tissues. Beginning at the top, single cells form cell aggregates by the action of the ADH (adhesion, e.g. cadherins, lectins) module. The POL (polarity: Wnt pathway) module has two versions, apical-basal (POL$_a$) and planar (POL$_p$) polarity. POL$_a$ causes cells to have different surface properties at their opposite ends, leading to structurally polarized epithelial sheets and lumens within cell aggregates. POL$_p$, in contrast, causes cells to elongate and intercalate in the plane, which leads to convergent extension and elongation of the cell mass. The LAT (lateral inhibition: Notch pathway) module transforms an aggregate of homotypic cells into one in which two or more cell types coexist in the same aggregate, while the expression of ADH molecules at different quantitative levels lead to sorting out by the action of the differential adhesion (DAD) module. Production of diffusible molecules by cells capable of responding to these same molecules leads to morphogen (MOR, e.g., TGF-β/BMP, hedgehog) gradients, whereas morphogens can also act inductively and asymmetrically (ASM, e.g., FGFs) by being produced by one type of tissue and affecting a different type. Synchronous biochemical oscillation (OSC) of key components of the Notch and Wnt pathways, for example, in conjunction with the DAD module, can generate segments, the best-studied example of this mechanism involving the ASM module as well. Appropriate feedback relationships among activating and inhibitory morphogens can lead to patterns with repetitive elements by Turing-type reaction-diffusion processes (TUR). The action of the MAPK signaling pathway in the context of multicellular aggregates containing morphogen gradients leads to nonuniform growth by the mitogenesis (MIT) module, whereas the apoptosis (APO) module leads to differential cell loss. The secretion of extracellular matrix (ECM, e.g., collagen, fibronectin) between cells or into tissue spaces, creates new microenvironments for cell translocation or novel mechanical properties in cell sheets or masses.*

tion that is also used in Fig. 1 and Table 1. With regard to downstream signaling effects of the DPMs, we will focus on only the most proximal components of such pathways. In particular, in order to highlight the causal differences between DPMs and their associated transcription factors, the DTFs, we will defer discussion of the latter until a subsequent section.

Cell-cell adhesion and differential adhesion (cadherins, lectins)

Cell adhesion is the defining condition of multicellularity. Free-living cells prior to the origin of metazoa contained proteins on their surfaces that had evolved to serve purposes other than adhesion, such as defense against pathogens and recognition and capture of prey (King *et al.*, 2003). Some of these either evolved further or, possibly due to changed external conditions, were recruited to mediate colonial existence (King *et al.*, 2003) and later, true multicellularity (Nichols *et al.*, 2006). 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 the effects of changes in the ionic content of the aqueous medium on previously evolved cell-surface molecules (Kazmierczak and Kempe, 2004).

Homologs of cadherins, a ubiquitous class of metazoan cell adhesion proteins that depend on Ca^{2+} for their homophilic binding function, are present in choanoflagellates (King *et al.*, 2003, 2008; Abedin and King, 2008), 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 relatives (Phillipe *et al.*, 2004). Choanoflagellates are unicellular, or form small colonies, depending on laboratory culture conditions. According to our scenario, then, cadherins, cell surface molecules previously evolved to serve other functions, would have taken on the new role of mediating cell-cell adhesion when single cells bearing them encountered permissive environments, thus becoming what were perhaps the first DPMs (ADH; see Fig. 1, top, downward arrow and Table 1). (This is an example of an "exaptation" in the

TABLE 1

PROPERTIES OF DYNAMICAL PATTERNING MODULES (DPMS) DISCUSSED IN THIS ARTICLE

DPM	Characteristic molecules	Physical principle	Evo-Devo Role
ADH	cadherins	adhesion	multicellularity
DAD	cadherins	differential adhesion	multilayering
LAT	Notch	lateral inhibition	multiple cell types
POL	Wnt	anisotropy	lumen formation; elongation
OSC	Wnt + Notch + Hes	synchronous biochemical oscillation	field formation; segmentation
MOR	TGF-β/BMP; Hh	diffusion	pattern formation
ASM	FGFs	asymmetric interaction	induction; epithelial-mesenchymal interaction
TUR	MOR + Wnt + Notch	chemical waves	periodic patterning
ECM	collagen; chitin; fibronectin	stiffness; dispersal + cohesion	epithelial elasticity; skeletogenesis; epithelial-mesenchymal transformation
MIT	MAPK	mass increase	(differential) growth
APO	Bcl-2	mass decrease	(differential) cell loss

terminology of Gould and Vrba, 1982.) The glycan-binding C-type lectins, which are employed as Ca^{2+}-dependent cell attachment proteins in multicellular organisms (Kaltner and Gabius 2001), are also produced by choanoflagellates (King *et al.,*, 2008) and could thus have provided a basis for additional ADH-class DPMs in metazoan ancestors.

If subsets of cells within an aggregate contain sufficiently different amounts of cadherin on their surfaces, the subpopulations will 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 similar to phase separation of two immiscible liquids, like oil and water (Forgacs and Newman, 2005).

Because a more cohesive tissue (one with stronger bonds between its cells) will always be partly or fully engulfed by a less cohesive one, differential adhesion-driven multilayering acts in a goal-directed fashion (Steinberg, 1998). The "goal" is the thermodynamic one of minimization of free energy (Steinberg, 1978; Forgacs and Newman, 2005). Ancient cadherins, 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," in which no interchange or mixing of cells occurs across the common boundary (Crick and Lawrence, 1975; Garcia-Bellido, 1975). This major transition in the history of life can thus have occurred with little genetic change relative to the condition of choanoflagellate-like ancestors.

As components of the DPM designated ADH, cadherins mediate the formation of "solid"[3] cell clusters, with spherical geometry (due to minimization of surface tension in a mass of isotropic, mobile cells; Forgacs and Newman, 2005). By bringing differential adhesion into play (DAD, see Fig. 1, center, leftward arrow and Table 1), the DPM in which subpopulations of cells express different levels of a cadherin or lectin can cause the clusters to become multilayered. But in contrast to modern organisms, in which differential adhesion is under precise spatiotemporal regulation (e.g., Godt and Tepass, 1998; Gonzales-Reyes and St Johnson, 1998; Damon *et al.*, 2008), the ratio of high-expressing to low-expressing cells would most likely have been unregulated in the earliest such forms, so the resulting organisms would have had many different, poorly defined, morphologies. The following subsection describes how molecules carried over from the unicellular ancestors of the metazoa were mobilized in new mechanisms to restrict this morphological profligacy. The most straightforward way this could happen is by a process or pathway that influenced the relative numbers of cells that assume different biosynthetic states.

Cell fate choice and lateral inhibition (the Notch pathway)

The Notch-Delta pathway is an ancient signaling system that depends on the interaction of the single-pass integral membrane protein Notch, which is a receptor for signals that originate outside the cell, and one of a class of other single-pass membrane proteins that act as modulators of Notch activity: Delta, Serrate and Lag2 (the DSL proteins) (Ehebauer *et al.*, 2006). When Notch is activated, an intracellular portion of it is cleaved off and functions in the nucleus to turn a class of transcriptional repressor proteins into transcriptional activators. Notch's effects are therefore entirely dependent on which of the dual-action factors are present in a given cell type. The pathway's role is therefore not to determine the specific fate of a cell, but rather to cause the cell to choose one of two of its potential fates, whatever those may be.

The Notch pathway (present in sponges; Nichols *et al.,* 2006, placozoans, Srivastava *et al.*, 2008 and all eumetazoans, but not in choanoflagellates; King *et al.*, 2008), has been extensively characterized during multicellular development, where it operates in a juxtacrine fashion. That is, the Notch receptor on one cell interacts with a DSL-class protein on an adjacent cell, and the two cells, though initially equivalent, come to assume different fates. Although the original cells in such a population will express both Notch and DSL proteins, the population is initially in a metastable state. The receptor-ligand interaction serves to kick it into a stable state where one or a small contiguous group of cells increase their DSL levels and the cells surrounding them are Notch-activated and are thereby prevented from assuming the same fate as the central group. For this reason, the Notch pathway is usually referred to as mediating lateral inhibition (Simpson, 1997).

Recent work, however, suggests that Notch can interact with DSL proteins in a cell-autonomous fashion; that is, the receptor and "ligand" are on the same cell, rather than on adjacent ones (Sakamoto *et al.*, 2002). This may, indeed be the ancestral state of the Notch pathway, with a cell switching between one state and another (consider, for example, sporulation) due to environmentally induced association between its Notch and DSL proteins. Indeed, the transcriptional mediators of Notch-dependent cell state switching (but not Notch itself) are present in fungi (Prevorovsky *et al.*, 2007). Protein modules found in Notch receptors are present in a choanoflagellate, though they are not all encoded in the same genes (King *et al.,* 2008). With the ADH-dependent transition to multicellularity there would have been selective pressure to take the few steps necessary to turn a cell autonomous mechanism into a juxtacrine one, and thereby convert cell-state switching into lateral inhibition.

The advent of the Notch-related DPM (LAT; see Fig. 1, upper-center, downward arrow and Table 1) (via a change of context and scale, but, in principle, with a minimum of genetic evolution) allowed the relative numbers of cells of different states in an aggregate to be fairly well controlled. Such patterns will be fine-grained, since the spatial scale of juxtacrine signaling is small. If, however, the alternative cell states in question are associated with different levels of cadherin, the subpopulations will sort-out (see previous subsection) and a multilayered aggregate will automatically form.

Apical-basal and planar cell polarity (the Wnt pathway)

As described above, the default morphology of a cell aggre-

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[3] We use the term "solid" here in the topological sense: lacking internal cavities. We also characterize cell aggregates and parcels of tissue as "liquid-like" throughout this paper, but there we refer to the physical state of a material whose subunits (cells in this case) are independently mobile (Forgacs *et al.,* 1998).

gate held together by cadherins (or any other uniformly distributed cell-cell adhesive protein) is solid (i.e., without a lumen) and spherical. Wnt, a family of secreted factors that interact with receptors of the Frizzled family, acting through distinct but related pathways, induces cells to become polarized along their apical-basal (A/B) axis (Karner *et al.*, 2006b), or oriented in a plane perpendicular to this axis (planar cell polarity; Mlodzik, 2002; Karner *et al.*, 2006a). Although these are individual cell behaviors, in a multicellular context they permit cell aggregates to overcome the morphological defaults of solidity and sphericality (see below).

Although Wnt genes and their cognate secreted proteins are not present in choanoflagellates (King *et al.,* 2008), they and their Frizzled receptors are found in sponges (Nichols *et al.,* 2006) and placozoans (Srivastava *et al.*, 2008). The Wnt pathway has even deeper roots in cellular evolution, however. In the fission yeast *Schizosaccharomyces pombe* the protein Pmo25p is essential for polar growth; in its absence the actin cytoskeleton becomes depolarized and cells adopt a round morphology (Mendoza *et al.*, 2005). Pmo25p is a homolog of the metazoan MO25 family of proteins, which in nematode, insect and vertebrate cells act as cofactors of the serine-threonine kinase Lkb-1. The latter, by regulating the activity of the cytoplasmic enzyme glycogen synthase kinase 3β (GSK3β), acts as a fulcrum of the "canonical" (that is, mediated through the transcriptional co-regulator β-catenin) Wnt pathway (Green, 2004).

The regulation of A/B polarity by Wnt in the metazoa is thus based on an ancient mechanism of polarity control that existed before the fungi and choanoflagellates split off from each other. Furthermore, even in modern metazoa, this pathway, while inducible by the secreted Wnt ligand, remains a property of individual cells. Indeed, the entire A/B polarity program is triggered in isolated animal cells if Lkb-1 is activated (Karner *et al.*, 2006a).

Polarization along the A/B axis has a clear role in the regulation of cell division in unicellular organisms like *S. pombe* (Mendoza *et al.*, 2005), and the same likely held for the single-celled antecedents of the metazoa. In multicellular aggregates, however, it can mediate some entirely unprecedented morphogenetic effects. If cells are polarized in their distribution of an attachment protein, for example, they will not form a solid mass when aggregated. The energetically most favorable configuration would be achieved when the more adhesive portions of the cell membranes bind to each other while the less adhesive regions are freed up to enclose an interior lumen (Newman, 1998; Forgacs and Newman, 2005), as seen in the sponges and all eumetazoans. Alternatively, if cells are polarized in different attachment proteins they can form multiple non-mixing layers, such as seen in the three-layered placozoan, *Trichoplax* (Miller and Ball, 2005).

The characteristic multicellular organisms of the Precambrian had earlier been described as solid-bodied (Seilacher, 1992), but the discovery of small, hollow, cell clusters from the Doushantuo Formation, China (Chen *et al.*, 2004) has qualified this description. Although these lumen-containing forms have been termed "embryos" (Chen *et al.*, 2004; Hagadorn *et al.*, 2006), the evidence is also consistent with their being definitive adult forms of their time. The Wnt pathway is very ancient, likely originating in the Precambrian (Erwin, 2008). Because A/B polarization accompanies and appears to be a necessary condition for the formation of interior cavities in cell aggregates, it is plausible that the advent of the DPM enabling A/B polarization helped drive the transition

between the Ediacaran biota and those of the Cambrian explosion (POL$_a$; Fig. 1, upper-center, leftward arrow and Table 1).

The utilization of β-catenin as a transcriptional co-factor in the POL$_a$ DPM and certain other Wnt-mediated functions (proliferation, alteration of gene expression), and its use in the ADH and DAD DPMs as a submembrane component of cadherin-based adhesion complexes, provides the basis of regulated switching among Wnt-based DPMs (Nelson and Nusse, 2004). Such alternative deployment of DPMs is important in a variety of complex morphogenetic changes (Jamora *et al.*, 2003; de Melker *et al.*, 2004).

Planar cell polarity (PCP) is also initiated at Frizzled receptors and is therefore usually described as employing the Wnt pathway (noncanonical in this case, since β-catenin is not involved). Despite the involvement of Frizzled, however, Wnt ligand is not involved in all cases of PCP induction (Veeman *et al.*, 2003). Thus, like A/B polarization, PCP may be based on a cellular mechanism that predates cell-cell communication.

The consequences of PCP in a multicellular context are unintuitive, but highly significant. Elongated cells with anisotropic adhesive properties are predicted on the basis of physical principles to spontaneously align and intercalate among one another, leading to the tissue mass narrowing in one direction and elongating in the orthogonal direction (Zajac *et al.*, 2003; Keller *et al.,* 2008). These cellular rearrangements and tissue reshaping effects are seen in "convergent extension," which establishes the elongated body axis during gastrulation in the amphibian embryo, and related phenomena throughout the Metazoa (Keller, 2002) (POL$_p$; Fig. 1, upper-center, rightward arrow and Table 1).

The cell polarization effects associated with the Wnt pathways, then, are based on the mobilization of mechanisms of cytoskeletal rearrangement that appear to have evolved to serve single-cell functions. With the arrival of multicellularity, the existence of these mechanisms enabled those tissue masses in which they were present to acquire internal lumens and elongated shapes, i.e., to move beyond the default solid, spherical morphology of adhesive, mobile cells. They could have done this, we again note, mainly as a result of change in scale and context. Substantial genetic change was not required.

Field formation by synchronized biochemical oscillations (Hes, Notch and Wnt)

A balance of positive and negative feedback interactions in a gene regulatory network can lead to temporal oscillations in concentration of gene products (Goldbeter, 1996; Reinke and Gatfield, 2006). In individual cells, such as the common ancestors of metazoans and choanoflagellates, such oscillations would not have a lasting morphological effect. In a multicellular context, where they can be coordinated across cell boundaries by juxtacrine (e.g., Notch pathway) and short-range paracrine (e.g., Wnt pathway) signaling, however, such oscillations have the potential to drive morphogenetic change.

The composite DPM we refer to as OSC (Table 1; Fig 1, upper-right, left-downward arrow) is used, for example, in somitogenesis, the process by which blocks of tissue, the primordia of the vertebrae and associated muscles, form in a progressive spatiotemporal order along the central axis of vertebrate embryos. In the presomitic mesoderm of chicken and other vertebrate embryos, the expression of certain genes (particularly that specifying

the Notch pathway mediator Hes1), undergo temporal oscillation with a period similar to the formation of the somites (Pourquié *et al.*, 2003). These oscillations then become synchronized by Notch-mediated juxtacrine signaling (Giudicelli *et al.*, 2007; Kageyama *et al.*, 2007; Riedel-Kruse *et al.*, 2007). In conjunction with an FGF8 morphogen gradient (see below) with its source at one end of the extended embryo, the Hes1- and associated oscillations provide the basis for the generation of somites in vertebrate embryos (Pourquié, 2003).

The recurrent nature of the oscillatory state is an important feature that may utilized in a developmental system, where, as in somitogenesis, temporal periodicity is converted to spatial periodicity. But another key property of oscillators is their ability to become synchronized (Garcia-Ojalvo *et al.,* 2004; Masamizu *et al.,* 2006). This highlights a general effect of the OSC DPM that can be developmentally important over durations shorter than the oscillator's period. The coordination of cell state (e.g., with respect to the oscillator's components) over a broad tissue domain, a phenomenon described in the older embryological literature as a "morphogenetic field" (reviewed in Haraway, 1976; Gilbert, 2006), has long eluded mechanistic explanation but can now be understood as a manifestation of this DPM.

While many gene regulatory networks are capable of sustaining oscillatory behavior, Hes-type transcriptional modulators are particularly suitable as elements of the OSC DPM. This is because they are downstream effectors of the Notch pathway, a mediator of juxtacrine cell communication in the multicellular context. Interactions of the Notch pathway with the Wnt pathway (Hofmann *et al.*, 2004; Ishikawa *et al.*, 2004; Hayward *et al.*, 2006) can also mobilize paracrine interactions in the synchronization of Hes oscillations. Hes-type transcription factors are ancient members of the developmental-genetic toolkit, with two representatives in the placozoan genome (Srivastava *et al.* 2008).

Single- and dual-tissue morphogen gradients (the TGF-β/ BMP superfamily, Hedgehog and FGFs)

Unicellular eukaryotes such as protozoan ciliates have the ability to change their physiological state in response to molecules secreted into the microenvironment by other such cells (Luporini *et al.*, 2006). It is a reasonable assumption that this capacity had already evolved in the unicellular progenitors of the metazoa. In the multicellular context, a secreted molecule for which these ancient cells had evolved concentration-dependent responses could serve as a patterning molecule or "morphogen" (MOR; Fig. 1, upper-right, right-downward arrow and Table 1). To perform this function, however, it would need to have the capacity to spread a significant distance across a cluster of cells using their lipid membranes or the extracellular space as a medium for diffusion. The locally acting Wnts have a limited capacity to act as morphogens (Sick *et al.*, 2006); in general they stabilize, rather than specify, cell fate (Martinez-Arias, 2003).

The ability of one or a small group of cells to influence other cells via morphogens enables the generation of heterogeneous patterns on a spatial scale of 100 µm - 1 mm over tens of hours, consistent with the time-distance-concentration relationships inherent in macromolecular diffusion (Crick, 1970). Morphogens fall into two main classes. Some act on cells similar to those that produce them, causing them to assume one of several new states depending on the concentration of morphogen to which they are exposed. Others 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 in the aqueous interstices and extracellular matrix at variable rates, dependent on their capacity to bind to specific extracellular molecules, which may themselves be distributed nonuniformly (Ohkawara *et al.*, 2002). Molecules of the Hedgehog class of morphogens are alternately tethered to cell membranes by covalently attached lipid moieties and diffusible 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 very short-range Wnts.

While each of these morphogens activates signaling pathways that apparently preexisted the metazoa, the morphogen molecules themselves arose at various times during the metazoan radiation. For example, while demosponges, the most primitive metazoan group, have a Hedgehog-type morphogen, they lack TGF-β/BMP and FGFs (though they have a receptor homolog for the latter class; Nichols *et al.*, 2006). Morphogens of all three classes are present in cnidarians (Rentzsch *et al.*, 2006; 2008; Matus *et al.*, 2008) and echinoderms (Lapraz *et al.*, 2006; Walton *et al.*, 2006), while the placozoan *Trichoplax* appears to only contain the TGF-β/BMP pathway (Srivastava *et al.*, 2008).

FGFs in arthropods and chordates have multiplied by gene duplication and their receptors are alternatively spliced in such a fashion that an FGF produced by one tissue layer typically only affect cells in a different layer, and not those cells that produce it (Huang and Stern, 2005). This type of tissue interaction constitutes a novel and fruitful developmental principle not seen in other animal groups, nor, generally, with respect to other morphogen systems. In particular, it enables asymmetric interaction between different tissue layers, exemplified by induction and epithelial-mesenchymal interaction (ASM; Fig. 1, left-center, left-downward arrow and Table 1).

Morphogen function is inextricably tied to the physical principle of diffusion, a mechanism that signifies a different biological phenomenon in multicellular and unicellular contexts. By the simple effect of setting up molecular gradients across a cluster of initially equivalent but responsive cells, morphogen-based DPMs generated organismal forms that ultimately contained heterogeneously distributed cell types. Whether or not any given form was functionally compatible with survival was a matter subject to natural selection. But as with the DPMs described earlier, the potential to generate a panoply of morphologies is tied to material properties of the system rather than to incremental selective advantage.

Spot, stripe and boundary patterns arising from local autoactivation-lateral inhibition

Positively autoregulatory morphogens, that is, those which directly or indirectly stimulate their own synthesis as part of the state change they promote in target cells, will not be maintained as gradients, since in such cases the morphogen will convert the entire cluster into the "induced" type. This effect could have produced variety among organisms during early evolution, but not internal heterogeneity or patterning in any single organism. A

positively autoregulatory morphogen that became linked, however, in a composite DPM, to a mechanism of lateral inhibition (like the LAT DPM associated with Notch signaling), will induce a zone around any peak of its activity within which no local activation can occur (Gierer and Meinhardt, 1972; Meinhardt and Gierer, 2000; Meinhardt, 2008). New peaks of activation would only form at distances sufficiently far from other peaks so that the inhibitor's effects will have attenuated. Such systems, involving short-range local activation and long-range lateral inhibition (similar to the chemical pattern forming systems described by Turing, 1952), or formally equivalent local autoactivation-lateral inhibition (LALI) systems (Nijhout 2003; Newman and Bhat, 2007), can produce regularly spaced spots or stripes of morphogen concentration (TUR; Fig. 1, far-right-center, left downward arrow and Table 1). These, in turn, can induce primordia of skeletal elements (Newman and Frisch, 1979; Hentschel *et al.*, 2004) and other serially repeated structures such as teeth (Salazar-Ciudad and Jernvall, 2002), feather germs (Jiang *et al.*, 2004) and hair follicles (Sick *et al.*, 2006). Tissue boundary formation (von Dassow *et al.*, 2000) and axial polarization (De Robertis, 2006) regulated by such mechanisms are more robust than those that depend solely on differential adhesion or diffusion gradients (Ingolia, 2004).

Epithelial elasticity, epithelial-mesenchymal transformation and global organization of cell polarity (extracellular matrix)

Up to this point we have described "epithelioid" cell clusters in which cells are directly attached to each other. The *viscosity* of such clusters depends on the ease with which cells slip past one another while maintaining their attachments. (Cell-cell bonds, even if strong, can have short lifetimes). Their *elasticity* is primarily a function of the cytoskeleton, and their *cohesivity* is determined by the force required to separate the cells. The other major category of cell aggregate or tissue in multicellular metazoan forms is termed "mesenchyme." In mesenchymal aggregates the constituent cells secrete a complex macromolecular microenvironment, the extracellular matrix (ECM) (Comper, 1996), and it is the properties of this material that determine the aggregate's viscoelasticity and cohesivity, collectively, its *rheological* properties. ECMs are often quite elaborate on the molecular scale and mesenchymes thus have different morphogenetic capabilities from the epithelioid cell aggregates subject to the DPMs discussed above.

The most ancient metazoans, the Porifera or marine sponges, produce skeletal structures whose morphology is, in part, a function of environmental factors (Uriz *et al.*, 2003). Most sponge cells (sclerocytes) reside within an ECM called the "mesohyl," which constitutes the bulk of the organism's body. These cells bind to the ECM via integrin transmembrane proteins (Wimmer *et al.*, 1999), as in eumetazoans. Sponges also contain epithelial cells (Schröder *et al.*, 2004) and homologs of type IV-like collagen (Boute *et al.,* 1996), which in eumetazoans forms a major component of the sheet-like supporting structure for epithelia, the basal lamina. Despite the active remodeling of the internal anatomy of sponges by the continuous movement of their cells (Bond, 1992), and the presence of most of the molecular ingredients of the major DPMs (see above), poriferans branched off from the metazoan lineage early on, as a morphological dead-end (Nichols *et al.*, 2006). One reason for this may have been their great reliance on ECM, a stiff, relatively non-dynamic medium at the mesoscopic

scale, as their major morphogenetic component. While sponges produce both metalloproteinases (Arreguin *et al.*, 1995) and metalloproteinase inhibitors (Fujita *et al.*, 2003), two classes of molecules used by eumetazoans to break down and remodel their ECMs, it is possible that these functions never became integrated in these groups of organisms into a composite DPM that would have facilitated the mobilization of other DPMs.

In the eumetazoa ECM is employed in a more limited fashion, one that permits the other DPMs to have freer play. Cnidarians have a thin sheet-like *mesoglea* consisting of separate regions of basement membrane-like and interstitial matrix-like properties (Zhang *et al.*, 2007), whereas true interstitial ECM is found only in triploblasts (Huxley-Jones *et al.*, 2006). A basement membrane permits an epithelium to behave as an elastic sheet in the direction perpendicular to the plane, though it may retain in-plane liquid-like properties (Mittenthal and Mazo, 1983; Newman, 1998). Elastic sheet epithelia exhibit a range of folding, buckling and wrinkling effects that are not seen in liquid-like epithelioid tissues, and which provide the basis for some modes of gastrulation and for formation of appendages (Gierer, 1977; 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 change in the physical state of tissues is enabled by the ECM, which permits cells that are not directly attached to one another to remain part of an integral tissue. Whereas skeletogenesis and elastic sheet behavior are both based on the stiffness of ECM, EMT employs its space-filling properties.

Cell polarity, which we have seen above is mediated by the Wnt signaling pathway, can be oriented in preferred directions by interaction with previously deposited ECM (Thery *et al.*, 2006). In analogy to the effects of diffusible morphogens in organizing spatial patterns of cell state across a cluster of cells (see above), ECM cues can help determine global patterns of cell polarity in multicellular aggregates. Although ECM molecules and integrins pre-existed the origins of the metazoa – homologs are present in the non-colonial choanoflagellate *M. brevicollis* (King *et al.*, 2008) – the emergence of multicellularity recruited these molecules into "internal substrata," constituting a distinct DPM (ECM; Fig. 1, bottom-left, left upward arrow and Table 1), with new capacities for generating pattern motifs.

Developmental function-dedicated transcription factors as "frozen accidents"

Transcriptional regulation is a key determinant of cell state, and the changes thereof, during development. But cells can change their functional states, and embryos their three-dimensional forms, independently of transcriptional changes. This suggests that the transcription factor and cis-regulatory module-based "kernels" (Davidson, 2006) may not, as claimed, constitute the fundamental constructional elements of metazoan development. The mutual regulation of transcription factors across cell boundaries involves more than the transcription factors themselves. Specifically, it is mediated by juxtacrine or paracrine interactions between the cells which, in turn, are critically influenced by changing geometrical relationships among the signaling

and responding centers (Salazar-Ciudad *et al.*, 2003; Salazar-Ciudad, 2006).

Certain of the transcription factors included among the "toolkit" gene products (the developmental transcription factors, or DTFs, in our terminology), were tied to functions in single-celled ancestors that were later utilized in differentiated cells of metazoans (cell contractility, light sensitivity). But in other cases (participation in segmentation, anteroposterior or proximodistal tissue identity) they were unlikely to have corresponding functional roots in the unicellular world. Given the scenario we have described above, we can speculate that in the first metazoa, fortuitous associations of one or more DPMs with the DTFs existing at the time produced "generalized" eyes, appendages, heart-like contractile tubes and segments. If, as seems likely, they were loosely interacting, subject to external conditions, and not yet organized into the hierarchical regulatory schemes that came with subsequent evolution (Salazar-Ciudad *et al.*, 2001a,b), these DPM-DTF complexes would not have programmed unique, definitive body plans and organs, but rather versions of these morphological motifs that were plastic within populations and even individual proto-organisms. In this view, radically different types of eyes (Gehring, 2002) and appendages, and even wholesale inversions of the body axis (Gerhart, 2000), were possible for organisms of fixed genotype, in different environments and across generations.

With subsequent selection for reliability of developmental outcome (Waddington, 1942; Schmalhausen, 1949) the "proto"-bodies and -organs resulting from particular DPM-DTF associations would have become uniquely associated with specific programs of gene expression having homologous molecular bases and analogous morphological outcomes (Newman *et al.*, 2006). Examples of developmental outcomes based on such "homologous-analogous" DTF-DPM associations across metazoan phyla include skeletogenesis, appendage formation, eye formation, and heart development.

Conclusion: a pattern language for development and morphological evolution

We have proposed that the developmental mechanisms of biologically modern animals can be understood in terms of a pattern language of metazoan form. The elements of this language are transformations away from the physical default of an aggregate of undifferentiated cells: topologically solid, geometrically spherical, and spatially uniform. The major transformations include (i) formation of stable mixtures of cells in more than one biochemical state, (ii) stable formation of distinct cell layers, (iii) formation of internal cavities, (iv) elongation of cell masses, (v) generation of nonuniform patterns of cell type, and (vi) dispersal of subpopulations of cells without disintegration of the organism.

We have further proposed that each pattern-language element is tied to a set of gene activities of the so-called developmental-genetic toolkit many of which pre-evolved the metazoa in single-celled ancestors. The elements of the pattern language, however, are not genes, gene products, or even merely gene networks. They are, rather, what we term dynamical patterning modules or DPMs, in which a complex of the toolkit gene products mobilizes a mesoscale physical process. Thus, cell adhesion is mobilized by cadherins, lateral inhibition enabling maintenance of stable cell mixtures by the Notch pathway, tissue multilayering by differential expression of cadherins, cell polarization, leading to cavity formation and elongation, induced by the Wnt pathway, coordination of cell state by the Notch- and Wnt-dependent synchronization of Hes oscillations, and so forth.

The collection of DPMs we have described here is relatively comprehensive, but not exhaustive. Increase and decrease in cell number, for example, have only a quantitative impact on populations of free-living protists. In a multicellular context, however, these processes constitute reciprocal elements of a patterning system in which organismal shape and form are altered, but not cell state (Salazar-Ciudad *et al.*, 2003; Salazar-Ciudad, 2006). For this reason 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 DPMs which modulate the physical mass of a multicellular aggregate (MIT, Fig. 1, center-right, left downward arrow, and APO; Fig. 1, bottom-center, rightward arrow and Table 1).

An additional aspect of our hypothesis is that the earliest metazoan developmental mechanisms did not arise primarily by incremental natural selection. We suggest instead that the change in context and spatial scale inherent to the multicellular state led to the relatively abrupt appearance of such mechanisms. Thus, for example, Notch signaling, which plausibly evolved in unicellular forms to elicit transitions in cell state in response to environmental cues, would come to mediate the coexistence of alternative cell states in a single colony, where cells serve as each other's microenvironmental determinants.

Finally, autoregulatory networks of transcription factors and cis-regulatory modules, a major mechanism in the specification of cell fate in modern-day developmental systems (Davidson, 2006), are nonetheless not central to our concept of a pattern language for multicellular form. To paraphrase the statement by Gehring (2002) quoted in the introduction to this paper, transcription factors are, in principle, interchangeable in their roles and are not intrinsically connected to the functions of the genes they happen to regulate. This contrasts with the gene products that mediate the DPMs, which perform not one common gene expression-associated function (transcription), but qualitatively different, physically-associated functions (adhesion, diffusion, geometric polarization, etc.).

We suggest that the mobilization of physical processes capable of producing patterns and forms (not only of differentiated cells types, but of cells and tissue modules in three-space: segments, tubes, vesicles, placodes; Salazar-Ciudad *et al.,* 2003), was the basis of the Precambrian-Cambrian explosion. The utilization of homologous transcription factors in seemingly analogous organ systems across the range of metazoan phyla (the apparent molecular homology-analogy paradox; Wilkins, 2002; Newman, 2006) is, in our view, presumptive evidence for the facile interconvertibility of disparate metazoan body plans once multicellularity had been achieved, but before canalizing evolution (Waddington, 1942; Schmalhausen, 1949) had locked phylotypic differences into place (Newman, 2006; Newman *et al.*, 2006). This implies that a comparative genomic analysis of the origination of metazoan form that does not take into account the generic self-organizing properties of multicellular aggregates

(e.g., Ruiz-Trillo *et al.*, 2007) cannot be entirely successful.

In general, the recruitment of toolkit gene products and pathways into basic and composite DPMs must lead to very different outcomes depending on the topology (i.e., connectivity) and the relative strengths of interaction among the components (Salazar-Ciudad, 2006). In extreme cases, only certain DPMs might have become active in certain lineages and other DPMs largely foreclosed. We may speculate, for example, that with multicellularity in place, the Wnt-mediated DPM in the ancestors of sponges, via its induction of apical-basal polarity, enabled the generation of multicellular forms with simple and branched interior cavities, but that an overactive integrin-ECM-mediated DPM (or underactive metalloproteinases), may have rendered the tissue microenvironment nonpermissive for employment of other DPMs.

In the ancestors of the eumetazoa, in contrast, the action of TGF-β/BMP and hedgehog-mediated DPMs readily produced tissue masses nonuniform in cell state, and Notch-mediated lateral inhibition must have stabilized these patterns. One result of this would have been cell aggregates compartmentalized into distinct layers. Then, heterotypic tissue interactions, mediated by FGFs and their receptors, would have promoted the emergence of sophisticated diploblastic forms, such as the Cnidaria. Next, the functional linking of simple DPMs, such as the cadherin and Wnt pathways (via their common effector, β-catenin), and the Notch and Wnt pathways (via their common mediator, GSK3β), into composite DPMs, opened the way for unprecedented dynamical processes acting on cell masses: global coordination of cell states via synchronized oscillations and reaction-diffusion patterning, to name two important ones. Finally, secreted ECMs, assembled on the mesoscopic scale of multicellular aggregates, became DPMs that mobilized mechanical effects, introducing elastic properties and solid skeletons to tissue masses and sheets. Once established, the DPMs remained highly conserved across the diploblasts and triploblasts, protostomes and deuterostomes, invertebrates and vertebrates.

Development of modern organisms exhibits extensive plasticity, permitting alternative outcomes depending on different externalities (West-Eberhard, 2003). By our hypothesis, development in metazoan ancestors would have been even more plastic, since prior to the evolution of canalizing mechanisms the physical processes mobilized by the various DPMs would have been highly sensitive to environmental conditions (see also Newman, 1994; Newman and Müller, 2000; Newman *et al.,* in press). This bears on recent attempts to relate mechanisms of axis formation and arrangement of molecular signaling centers in the embryos of metazoan groups to presumed ancestral relationships among those groups (e.g., chordates, tunicates, hemichordates, cephalochordates; Passamaneck and Di Gregorio, 2005; Lowe *et al.*, 2006; Yu *et al.*, 2007). Because the DPMs could have been deployed combinatorially and non-hierarchically in ancient form the taxonomic relationships among these groups may not be direct, nor would they necessarily conform either to anatomical affinities or a straightforward developmental logic.

The deployment of DPMs, in modern metazoa, but even in their primitive ancestors, cannot generate an unlimited array of forms. Despite their potential (presumably greater in early metazoan evolution than at present) to transform different anatomies one into the other with little or no genetic change, DPMs are constrained to mold pre-metazoan cell masses into only those morphologies characteristic of chemically and mechanically excitable mesoscopic materials, among these hollow, multilayered, elongated, segmented forms. If the "tape of life" were to be replayed (Gould, 1989) things would probably turn out not too differently at the level of bauplan.

Comparative anatomists have long recognized that animal bodies share a common morphological phrase book. More recently, molecular evolutionists have discovered that the metazoa share a common developmental-genetic vocabulary. Both of these findings, as we have shown, stem from the existence of a pattern language for animal development. The grammar of this language emerged abruptly more than 500 million years ago when a group of proteins and pathways of the unicellular world, by coming to operate on the mesoscale, mobilized the physical laws pertaining to soft-matter and excitable media in the construction of multicellular organisms.

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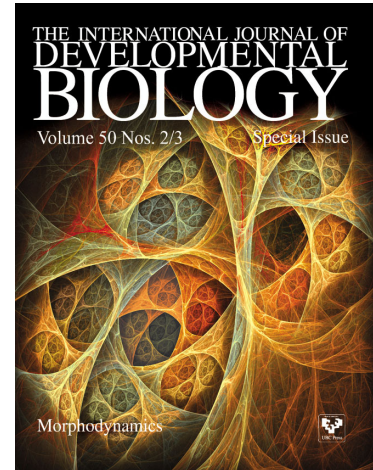**Evo-Devo: the long and winding road.**
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