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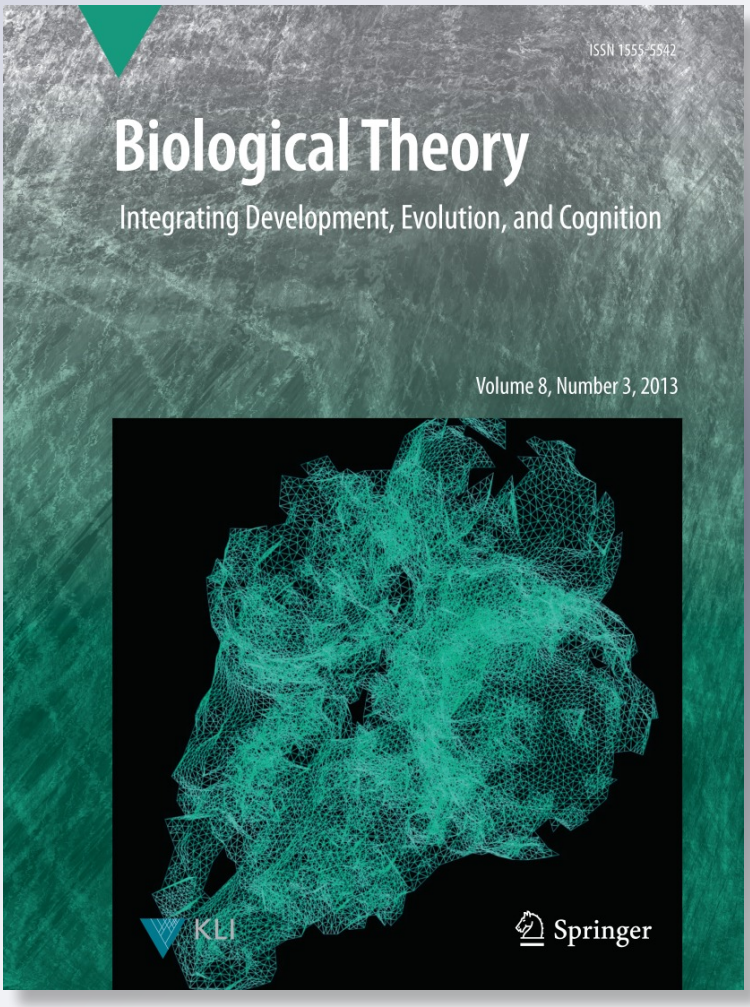

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THEMATIC ISSUE ARTICLE: EMERGENCE OF SHAPE

Physical Determinants in the Emergence and Inheritance of Multicellular Form

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Abstract We argue that the physics of complex materials and self-organizing processes should be made central to the biology of form. Rather than being encoded in genes, form emerges when cells and certain of their molecules mobilize physical forces, effects, and processes in a multicellular context. What is inherited from one generation to the next are not genetic programs for constructing organisms, but generative mechanisms of morphogenesis and pattern formation and the initial and boundary conditions for reproducing the specific traits of a taxon. There is no inherent antagonism between this "physicalist" perspective and genetics, since physics acts on matter, and gene products are essential material components of living systems whose variability affects the systems' parameters. We make this notion concrete by summarizing the concept of "dynamical patterning modules" (DPMs; Newman and Bhat, Phys Biol 5:1–14, 2008; Int J Dev Biol 53:693–705, 2009), an explicit physico-genetic framework for the origin and evolution of multicellular form in animals, as well as (when differences in interaction toolkit genes and applicable physical processes are taken into account) in multicellular plants (Hernández-Hernández et al., Int J Dev Biol 56:661–674, 2012). DPMs provide the missing link between development and evolution by revealing how genes acting in concert with physics can generate and transform morphology in a heritable fashion.

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Evolution and development both depend on processes by which morphological phenotypes—forms—are produced and inherited. Gene-based explanations for these phenomena have predominated over the past century as the respective fields of study were advanced by the recognition that variation in gene sequence and expression are often associated with variations in form. But the privileged role of genetic accounts of multicellular evolution and development is challenged by the finding that the main genes that specify molecules regulating multicellular development in animals were largely present in unicellular ancestors (King et al. 2008; Shalchian-Tabrizi et al. 2008). Indeed, there is no body of experiments or theory that demonstrate how changes in genes, their products, or the interaction of their products can, by themselves, explain developmental morphogenesis and pattern formation, much less the emergence and diversification of animal form during the metazoan radiation.

The development of multicellular organisms involves the reshaping of cell masses and the establishment within them of precise arrangements, over time, of cells of various types. Tissues of animal embryos are soft and pliable (in contrast, for instance, to those of plants, which are solid). Animal (as well as plant) tissues are also "excitable media," in that their mechanical configurations and chemical states (including states of gene expression) of their constituent cells are not merely passive, but draw on energy fluxes and stored energy to self-organize. It therefore seems reasonable to look to the physics of excitable viscoelastic matter, which pre-existed multicellularity, and

indeed all living systems, as a determinant additional to genes of the origin of animal developmental systems more than 600 million years ago.

Attempts to incorporate physics into an understanding of biological form are part of the history of biology, but such efforts—Jean-Baptiste Lamarck's notion of the effects on cell masses of the fluids (both aqueous and "subtle," e.g., heat and electricity) that course through or permeate them (Lamarck [1809] 1984; Newman and Bhat 2011), William Bateson's "vibratory theory" of segmentation and metamerism (Bateson and Bateson 1928), and D'Arcy Thompson's (1942) mechanical, rheological, and allometric determinants of form—have only existed at the scientific margins throughout the 20th century.

During most of that period the Modern Evolutionary Synthesis (of Darwinism and Mendelism), which took changes in gene frequency in populations as the fundamental basis of organismal change, was the dominant evolutionary paradigm, and much of developmental biology was influenced by the notion of the "genetic program." Neither of these conceptual frameworks had any room for explanations that did not start with the gene.

In this article we will argue that the physics of complex materials and of self-organizing processes should be made central to the biology of form. There is no inherent antagonism between this "physicalist" perspective and genetics, since physics acts on matter, and gene products are essential material components of living systems whose variability affects the systems' parameters. Indeed, as we will show, this view provides a way of understanding the specific roles of genetic variation and gene expression in morphological evolution and development. Because of the lack of specificity of gene-centered accounts of form generation and its modification, we devote a substantial portion of our presentation to a description of "dynamical patterning modules" (DPMs; Newman and Bhat 2008, 2009), an explicit physico-genetic framework for the origin and evolution of multicellular form (see also Newman 2012).

Gene-centric models remain entrenched, however, and are unlikely to readily be abandoned. To appreciate the reasons for this it is useful to preface our sketch of a physicalist evolutionary-developmental biology with a brief history of how and why earlier versions of this perspective were thwarted and derailed.

Biology in a Mechanistic World

Physics had been central to Gregor Mendel's early education, and Charles Darwin's thinking was greatly influenced by the new geology of the early 19th century in which the constant and uniform action of known physical processes could bring about large-scale transformations. Both thinkers were therefore steeped in the mechanistic worldview that had taken hold with the predictive successes of Galileo Galilei and Isaac Newton beginning two centuries before. Mendel's materialism was evidenced in his focus on law-like behavior and quantitative prediction, Darwin's by a notion of cause and effect that admitted no surprises or discontinuities, the supposed hallmarks of miracles.

The late 18th and early 19th centuries had seen progress in mechanics, optics, and chemistry. In an industrializing Europe, advances in engineering precepts and in the construction of clocks, pumps, steam-driven ships and rail carriages, provided increasing evidence of the efficacy of mechanical principles in the generation of functional forms and devices. Scientists, philosophers, and many lay people were now ready to accept the proposition that living things were material objects, a notion popularized in such works as Julian Offray de La Mettrie's *L'Homme Machine* (1748).

The problem for 19th-century biologists with materialist aspirations was finding a naturalistic explanation for the *origination* of complex forms, and once they existed, for their *transformation* during development and evolution (often conflated under the latter term; Richards 1992). As noted by Immanuel Kant in his *Critique of Judgment* ([1790] 1966), Newtonian mechanics did not supply an apt model. For Newton, matter was inert and inertial, changing its form and position in a continuous fashion, and only when acted on by *external* forces. Living matter, in contrast, conforms to and reproduces the conditions for the realization of its own complex forms and functions.

Kant's insight that the analysis of life forms requires a "regulative" notion of teleology was not meant to be an argument for the existence of a supernatural creator. But in the hands of religious believers the insufficiency of mechanism to account for organization had additional implications. As William Paley famously pointed out in his *Natural Theology* (1802), anyone encountering a complicated piece of metal-gear machinery like a pocket watch would immediately surmise that it had a designer. Why, he asked, should it be any different for a plant or animal?

Given the state of physics in the 19th century, there were only a limited set of options open for bringing materialist ideas into biology. One strategy was undertaken by a school of scientists termed "teleomechanists" by Timothy Lenoir (1982)—e.g., J. F. Blumenbach (1752–1840), C. F. Kielmeyer (1755–1844), and J. P. Müller (1801–1858), who, while adopting a naturalistic approach to anatomy and physiology, followed Kant's lead by adopting a teleological heuristic with respect to the ultimate organizing principles of living systems. In contrast, the "rational" (or "idealistic") morphologists—e.g., Etienne Geoffroy Saint-Hilaire (1772–1844), and Lorenz Oken

(1779–1851)—took the position that there were laws for the generation of biological form analogous to the laws of gasses and planetary motion (Webster and Goodwin 1996; Amundson 2007). While they thus differed from the teleomechanists in the speculative nature of their hypotheses, in their efforts to discern law-like principles for the organization (and not just the behavior) of matter they were actually working more in the mode of physicists.

A third materialist approach, and the one that prevailed throughout most of the following century, was that of Darwin and his co-originator of the theory of natural selection, Alfred Russel Wallace. Like the teleomechanists (and unlike the rational morphologists), these authors made no conjectures about inherent laws of form. Indeed if such laws existed, much that natural selection purported to explain would be seen to have other causes. Although the origin of biological characters (and not simply their variation) was, in principle, included in the problem-agenda of the Darwin-Wallace theory, it was one that the theory ignored in practice. By adhering to a Newtonian picture of the properties of matter its advocates could ignore the possibilities of inherent ("orthogenetic") and abrupt ("saltational") modes of evolutionary change that might be implied by other, more dynamical, concepts of matter. This lacuna was propagated into the 20th century's Modern Synthesis, which contained the additional claim that genes are the exclusive medium by which distinctive aspects of the phenotype are inherited.

An example of suppression of alternatives to the inert materialism of the theory of natural selection is seen in the fate of Darwin's contemporary Richard Owen (1804–1892). The foremost paleontologist and comparative anatomist of the 19th century and the first to conceptually distinguish between analogy and homology, Owen was an unambiguous evolutionist, though this was actively denied by Darwin's advocate, T. H. Huxley, because of Owen's doubts that Darwin's mechanism could effect "transmutation," i.e., macroevolution.

Like the teleomechanists, Owen (1848) supported the notion that living matter was subject to "organizing forces"; like the rational morphologists he believed that organisms conformed to "archetypes," stereotypical morphological formats presumed to arise from natural causes operating during development. Owen's view of the vertebrate limbs, and of the skeleton in general, was that they consisted of series of fundamentally identical segments, each modified according to its position and functions ("serial homologues"; Owen 1848, 1849). He was not mechanistically or mathematically inclined, but by the end of his life another British naturalist, William Bateson, was hypothesizing that such metameric structures were generated during embryonic development by an underlying oscillatory physical process (Bateson 1891 in Bateson and Bateson 1928; see also Newman 2007). Insofar as these ideas were valid (which could not be decided by the experimental embryology or physics of the time), they threatened the privileging of natural selection as an explanation of the emergence of forms, and genes as the exclusive medium for passing characters from one generation to the next.

A New Physics of Condensed, Excitable Materials and its Contributions to Developmental Biology

The physics of the 20th century is best known for theories and experiments relating to phenomena outside normal experience–the very fast (special relativity), very massive (general relativity), and very small (quantum mechanics). Albert Einstein was a prime initiator of all of these areas, but what is less appreciated is that another area to which he made a pioneering contribution (in his 1905 theory of Brownian motion; Einstein 1905), the physics of mesoscopic or "middle scale" materials, which includes (in addition to nonliving examples), cells, embryos, and the organisms they develop into, was as much a departure from the science of the 19th century as was the more familiar "modern physics."

By the third decade of the 20th century the public was made aware by the successes of quantum mechanics that the old adage that "nature does not make jumps" was untrue. But work on differential equations (the best descriptive language for common mesoscale phenomena such as systems of chemical reactions) by Henri Poincaré and Ivar Bendixson beginning in the 1890s had already shown that certain systems can be induced to jump from one stable oscillatory mode to another through unstable intermediate states (Strogatz 1994).

The phenomenon of phase transitions, the familiar abrupt transformations in the physical state of matter that occur, for example, when it is heated or cooled, was analyzed and partly explained early in the 20th century by Paul Ehrenfest (1880–1933) and others in terms of the non-Newtonian sciences of thermodynamics and statistical mechanics developed in the 1800s, though it took the sophisticated mathematics of renormalization group and scaling theories of the late 20th century to formulate more rigorous accounts.

Contemporary mesoscale physics utilizes concepts such as nonlinear oscillations, multistable dynamical systems, excitable media, reaction–diffusion, viscoelastic and other symmetry-breaking instabilities, chaos, and fractals, all of which were unknown to either Newton or Darwin. All of these processes or effects are relevant to forms and patterns potentially assumed by developing tissues. It is in fact physically impossible for the morphology of living systems not to bear the imprint of these effects.

By the mid-20th century scientists began addressing developmental biological questions with this new set of conceptual tools. These efforts quickly found empirical confirmation. To provide just a few examples:

(1) Beginning in the late 1950s, Malcolm Steinberg and his coworkers conducted experiments in which cells derived from different embryonic tissues were mixed with each other and allowed to incubate as co-aggregates for several days (Steinberg 1962a, b). The cells sorted out, first into homotypic islands and lakes and then into two distinct phases. The propensity to sort out appeared to be predictable from the thermodynamics of differential adhesion (DAD): that is, the cells were required to be sufficiently different in the strengths of self-adhesion in order for a distinct interface to form and for the tissues to thus "phase separate." Moreover, the arc of the interface, convex or concave relative to one of the tissues, and the hierarchy of sorting behaviors (e.g., if tissue A engulfs tissue B and tissue B engulfs tissue C, then A will also engulf C), were dependent on the relative cohesivities of the tissues, also apparently predictable a priori from the homotypic adhesive strengths (Steinberg 2007).

These effects are now understood to be not simply the outcome of relative cell affinities, but to depend as well on other tissue boundary-generating physical properties of tissues that derive from complexities of their cellular subunits that are not found in the molecular subunits of non-living liquids. These include tension exerted on the surfaces of cells from within (Brodland 2002; Krieg et al. 2008) and production by certain cells of molecular signals that repel other cells (Winklbauer 2012).

The phenomenon of intra-tissue boundary formation thus has some determinants that are straightforwardly "generic" (i.e., in common with non-living materials; Newman and Comper 1990), and others, which drawing on the active properties of living cells, are analogues rather than strict exemplars of generic processes. In combination, these effects produce the abrupt transformations and "goal-directedness" characteristic of many thermodynamically driven systems. They have been demonstrated to play roles in developmental systems as varied as oocyte localization in the insect (Godt and Tepass 1998), generation of pancreatic islets in the mouse (Jia et al. 2007), initiation of limb buds in the chicken (Damon et al. 2008), and gastrulation in the zebrafish (Krieg et al. 2008).

(2) In the 1970s, following the lead of William Bateson's vibratory theory, but using sophisticated concepts of mathematical physics, the developmental biologist Jonathan Cooke and the mathematician Christopher Zeeman proposed an oscillatory mechanism for the generation of somites, paired blocks of tissue that emerge in a sequential cranio-caudal direction during vertebrate embryogenesis (Cooke and Zeeman 1976). In this mechanism, when the periodically changing cell state of the presomitic mesoderm (the "clock") takes on a specific value, it acts as a "gate" for the action of a front of potentially changed cell behavior that sweeps along the embryo's length (the "wavefront"). The interaction of these two factors generates, in the model, a segmental pattern over time.

Then, in the late 1990s, Olivier Pourquié and his colleagues presented compelling experimental evidence for a formally similar mechanism for somitogenesis, involving a demonstrable intracellular biochemical clock, the components of which included the transcriptional switching factor Hes1, and a wavefront consisting of a gradient of the morphogen FGF8, with its source at the embryo's tail tip (Palmeirim et al. 1997). Others provided plausible biochemical dynamics for the underlying oscillation (Lewis 2003; Monk 2003).

Among other things, this physicalist model accounts for the increase in number of somites in snakes by evolutionary alterations in the ratio of parameters characterizing the interaction of the clock and wavefront (Gómez and Pourquié 2009). It is the nature of this mechanism to add or subtract segments in an abrupt fashion, and even, with appropriate tuning of parameters, to turn unsegmented domains of tissue into segmented ones, and vice versa.

(3) Another subfield of mesoscale physics that saw progress in the first half of the 20th century was the interaction of diffusion (first described mathematically by Adolf Fick in 1855) with other processes of material change, such as chemical reactions (Kolmogorov et al. 1937; Rashevsky 1948). By mid-century the mathematician Alan Turing (1952) had published a paper, "The chemical basis of morphogenesis", in which he demonstrated that a balance of positive and negative feedbacks in an open chemical system (essentially identical to networks that generate temporal oscillations like that in the somitogenesis model), coupled with differences in the rates of diffusion of the key reactive molecules, could produce stable nonuniform concentration patterns, often exhibiting spatial periodicities.

Because the vertebrate limb skeleton is arranged in a quasi-periodic fashion (as noted by both Owen and Bateson), it has a natural interpretation in terms of a Turing-type mechanism in which the limb bud mesenchyme behaves as an excitable medium. Insofar as this interpretation is valid (as opposed to alternative models proposing

that the skeleton is mapped out by machine-like genetic programs; Tickle 2003; Zeller et al. 2009), the limb would be expected to exhibit self-organizing capabilities. In fact, isolated and dissociated limb bud tissue can reconstitute limb-like skeletal patterns in vivo (Zwilling 1964; Ros et al. 1994), and patterns of cartilage nodules with similar spacing statistics in vitro (Christley et al. 2007). Moreover, key aspects of the skeletal patterns of mutant and fossil limbs can be accounted for by the assumption that the limb pattern is formed by a reaction–diffusion type mechanism (Miura et al. 2006; Zhu et al. 2010; Sheth et al. 2012), although the biological realization of both "reaction" and "diffusion" are much more complex than the chemical versions discussed by Turing.

Dynamical Patterning Modules and the Physical Origins of Animal Form

While mesoscale physical processes have clear relevance to the generation of form in present-day multicellular organisms, the cell activities and tissue functions mediated by and underlying these processes are often quite elaborate. Is it reasonable to surmise that physics played a role in the very emergence of such forms, at the transition from unicellular life to complex plants and animals?

Regarding the animals (kingdom Metazoa), their appearance in the fossil record suggests a relatively sudden emergence during the late Precambrian and early Cambrian periods (Larroux et al. 2008; Rokas et al. 2005). Sheetlike and hollow spherical forms (Yin et al. 2007), and budding and segmented tubes (Droser and Gehling 2008), possibly representative of the most ancient metazoans, are seen in Precambrian Ediacaran deposits beginning about 630 million years ago. The sponges and diploblastic (body plans consisting of two tissue layers) cnidarians (corals, hydroids) and ctenophores (comb jellies) are thought to have originated in this period, and essentially all the triploblastic (three-layered body plans) metazoans followed within a space of no more than 20 million years, beginning about 542 million years ago (the well-known Cambrian explosion; Conway Morris 2006).

All modern animals are thought to descend from a class of single-celled organisms that also gave rise to the modern choanozoans (Shalchian-Tabrizi et al. 2008). The best-characterized extant choanozoan is the choanoflagellate *Monosiga brevicollis* (King et al. 2008), which is unicellular, although other choanoflagellates are transiently colonial (Lang et al. 2002; Philippe et al. 2004; Wainright et al. 1993).

Development in all the metazoan phyla is mediated by a conserved "toolkit" of regulatory molecules (Carroll et al. 2005). This toolkit, comprising gene products or derivatives of their activity (e.g., polysaccharides such as hyaluronan and chitin) can be separated into two general categories: transcription factors, and molecules that mediate cell–cell attachment and other interactions (Newman et al. 2009). Many toolkit genes of each category are present in the genomes of *M. brevicollis* and other choanozoans (King et al. 2008; Shalchian-Tabrizi et al. 2008).

Transcription factors are regulators of gene expression and cell-type switching that long predate multicellularity. The fact that some of them can be traced to the presumed unicellular ancestor of the animals is not surprising. More unexpected is the finding that many molecules of the non-transcription factor "interaction toolkit" are also present in choanozoans. For example, several genes specifying cadherins, whose only known role in metazoans is to mediate cell–cell attachment, are represented in the *M. brevicollis* genome, as are sequences specifying collagens—extracellular matrix proteins—and integrins, which mediate attachment of animal cells to their extracellular matrices (Abedin and King 2008; King et al. 2008; Sebé-Pedrós et al. 2010).

Focusing on the interaction toolkit, there is a rough correspondence between the presence of its members in an organism's genome and the morphological complexity of that organism (Newman 2012). For example, the flat, trilayered placozoan, *Trichoplax adhaerens*, which has no obvious patterned cell arrangement in any of its three epithelioid layers, also lacks the Notch pathway (Srivastava et al. 2008), which mediates "lateral inhibition" of one cell type by another in more elaborate ("eumetazoan") phyla. Sponges, which do not maintain strict boundaries between epithelium-like and mesenchyme-like tissues, lack key components of the basal lamina (Srivastava et al. 2010).

While genes of the interaction toolkit were apparently present in unicellular ancestors of the Metazoa, and others may have entered this group from bacteria, fungi, and algae by horizontal transfer (Tucker 2013), how these "exapted" (Gould and Vrba 1982) molecules were recruited to help produce animal forms via developmental processes that they had not originally evolved to be part of, why the forms fell into a set of recurrent, delimited morphological motifs, and how this happened so rapidly, are not explicable in terms of the gradualist, population-based concepts of the modern synthesis.

Based on insights from the field of mesoscopic physics of condensed, excitable materials discussed in the previous section, we have proposed the following scenario for the origination of animal forms:

(1) Once single-cell ancestors of the metazoans entered into multicellular aggregates, existing non-transcription factor toolkit molecules mobilized physical forces, effects and processes that were largely irrelevant to the

shaping of individual cells. This first got underway when cadherins, or other preexisting surface molecules, acquired (by mutation or a chemical change in the ambient environment) the capacity to mediate cell–cell attachment, a function for which they were unlikely to have evolved to perform.

(2) The clusters produced by this early aggregative event turned cells, which are highly structured internally and have relatively inflexible surfaces, into independently mobile components of larger parcels of matter. These newly constituted materials, analyzed at the appropriate scale, were, in a physical sense, viscoelastic liquids: "soft matter" (de Gennes 1992). Because the cells themselves came to these ancient aggregates as already evolved chemically and mechanically responsive entities that drew on stored energy, the primordial "liquid-tissues" were also excitable media, subject to the physics that pertains to such materials (Levine and Ben-Jacob 2004).

(3) The effectively automatic mobilization of new mesoscale physics by ancient cellular molecules when acting in the multicellular context created entities we have termed "DPMs" (Newman and Bhat 2008, 2009). In particular, the first DPM to come into existence and the precondition for all the others was cell–cell adhesion (abbreviated by the acronym ADH).

(4) A given DPM is defined by the physical process it embodies. Whereas each DPM depends on one or more toolkit molecules, the molecular associations are not unique. For example, whereas cadherins may have been the molecules that first harnessed the force of adhesion for cell–cell attachment, other molecular complexes–a collagen-integrin couple, or one consisting of a lectin and its glycoside ligand–may have served the same function.

(5) The limited set of relevant physical effects that apply to matter on the scale of cell aggregates would have generated a set of stereotypical morphological motifs in the earliest metazoans. These would have included interior spaces, multiple tissue layers, elongated bodies, segments, and appendages.

The key single-cell functions that would inevitably have mobilized novel physical effects when the cells entered into aggregates are depicted in Fig. 1. None of these functions, or the molecules involved in mediating them, originally had any intrinsic connection to multicellularity. Nonetheless, the molecules that evolved in connection with these functions, by virtue of becoming tied to the physical generation of form and pattern at the new multicellular level of organization, thereby became developmental interaction toolkit molecules.

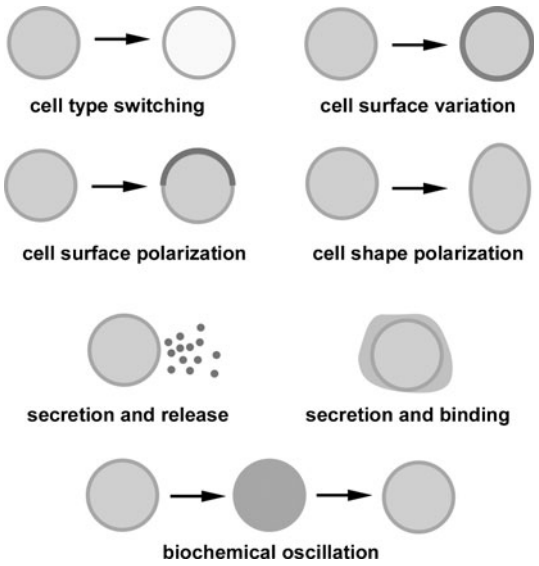

Fig. 1 Single-cell functionalities pre-existing the origin of the metazoans. All of the examples shown have unanticipated morphological consequences in the multicellular context due to the mobilization of mesoscale physical processes and effects. See discussion in the main text and in Newman and Bhat (2008, 2009)

We can consider several examples. As noted above, cell–cell adhesion is the necessary condition for multicellularity. This derives from the capacity of individual cells to exhibit variation in their surface properties (Fig. 1). Surface moieties (e.g., cadherins, lectins) that may originally have served to attach cells to surfaces or to enable them to capture prey could, as a result of mutational, metabolic, or environmental change turn into mediators of cell–cell attachment, bringing into existence the DPM designated by ADH.

If the variable production of adhesion molecules became tied to a cell-type switching mechanism (ancient and inescapable manifestations of the multistable dynamics of intracellular gene regulatory networks: GRNs; Fig. 1), then the multicellular aggregate would contain cells with two or more different surface adhesive properties. A new DPM will then come into play: DAD. By virtue of the phase-separation effect discussed previously, the cell aggregate will become multilayered, much like present-day diploblasts and triploblasts at the beginning stages of their development.

The scenario just described would not work if all the cells in a cluster assumed only one or the other of the possible alternative cell states. There needs to be a way of enforcing a balance between the states of expression (though it does not have to lead to equal numbers of expressers). Present-day metazoans employ "lateral inhibition" during embryogenesis, whereby early differentiating cells signal adjacent cells to take on a different fate

(Rose 1958; Meinhardt and Gierer 2000). Lateral inhibition in metazoans is mediated by the Notch signal transduction pathway, specifically, interaction of the cell surface receptor Notch with members of a class of other integral membrane proteins (Delta, Serrate/Jagged, and Lag2: the DSL proteins) that act as ligands for the receptor and nuclear mediators of Notch activity (Ehebauer et al. 2006). This mechanism, with components tracing back to deeply diverging choanozoans (Shalchian-Tabrizi et al. 2008), does not determine the particular fate of any cell, but instead enforces the coexistence of alternative fates in adjacent cells in the same cluster or aggregate. In the multicellular context it constitutes the DPM which we have designated LAT.

In examples in which the cell states in question reflect surface adhesive differentials, sorting out via DAD will lead to large-scale rearrangements and tissue multilayering, so that the later pattern will not directly reflect the local choices enforced by LAT. However, LAT (and the Notch pathway that mediates it) can also operate with respect to cell type differences that are unrelated to adhesion. In this case it can lead to finer-grained patterns (e.g., neuron-glia associations in present-day organisms).

As indicated above, cell aggregates behave as viscoelastic liquid droplets (reviewed in Forgacs and Newman 2005). Like nonliving liquid droplets, their default morphology is topologically solid (i.e., having no interior spaces), and spherical (the energetically most favorable state of such materials). By employing *cell polarization* (another ancient feature; Fig. 1) in conjunction with adhesive interactions, developing animal embryos can overcome these default morphologies. Cells can be polarized in one of two ways. With surface, or apical-basal (A/B), polarization (Karner et al. 2006b), interior spaces or lumens can arise within aggregates. If such polarization affects surface adhesiveness, cells will preferentially attach to their neighbors on their more adhesive (lateral) portions, leaving the less adhesive (basal) portions adjoining an interior space (Newman 1998). Similarly, A/B polarity is essential in fostering reliably layered tissue arrangements, as in the placozoan *T. adhaerens*, mentioned above.

Tissue elongation may occur when cells individually polarize in shape (rather than surface properties), a phenomenon called planar cell polarity (PCP; Karner et al. 2006a). Planar-polarized cells can intercalate along their long axes, causing the tissue mass to narrow in the direction parallel to the cell's long axis, and consequently elongate in the perpendicular direction. This tissue reshaping is known as "convergent extension" (Keller et al. 2000; Keller 2002).

Both A/B polarity and PCP are mediated by secreted factors of the Wnt family (Karner et al. 2006a, b). The type of polarization that occurs depends on the presence of different accessory proteins. The A/B- and PCP-inducing Wnt pathways are referred to, respectively, as the canonical and noncanonical Wnt pathways. Elements of these pathways are ancient, preceding the origin of the Metazoa. Some components of the Wnt pathway have counterparts in fungi, where they also mediate cell polarity (Mendoza et al. 2005). Sponges, which are characterized by many interior spaces, have genes for Wnt proteins and their ligands (Nichols et al. 2006). Such genes are also present in the placozoan *Trichoplax adhaerens* (Srivastava et al. 2008), which despite containing only four cell types, has them arranged in three distinct layers, which is possible only if the cells are polarized. Sponges and placozoa, however, lack components of the noncanonical Wnt pathway, which accounts for the fact that neither of them exhibit body elongation. The DPMs involving the Wnt pathway are designated POL_a and POL_p.

Several other DPMs are based variously on the ancient unicellular features of "secretion and release" and "secretion and binding" of molecules, and on oscillation in cell state (Fig. 1). These functionalities, associated with relevant mesoscale physical effects in the multicellular context, took the form of morphogens (DPM: MOR), extracellular matrices (DPM: ECM) and synchrony of cell state across a tissue domain (DPM: OSC) (Newman and Bhat 2008, 2009; Newman 2010). OSC and MOR are the main components of the somite-generating mechanism described above (Dequéant et al. 2006). Composite DPMs such as the Turing-type reaction–diffusion network comprised of MOR and LAT (DPM: TUR) can also generate periodic tissue patterns, and in conjunction with a separate instance of MOR and ECM, is the basis for the mechanism of limb development discussed earlier (Newman and Bhat 2007; Zhu et al. 2010). The DPMs discussed here and their associated toolkit molecules and morphogenetic roles are listed in Table 1.

A longstanding puzzle in comparative developmental biology (the "embryonic hourglass") derives from the observation that gene expression patterns at the egg stage of development, as well as the sizes and shapes of eggs themselves, can vary enormously among species within a phylum without affecting the phylum-characteristic body plan or "phylotype" (Richardson 1999). As is clear from the definition and description of the DPMs, these body plan-defining determinants of form only become operative at the stage of development at which the embryo consists of cell clusters (the "morphogenetic stage"), since that is when the relevant mesoscale physical effects first become mobilized. The DPM framework thus predicts that for organismal types characterized by a given set of interaction toolkit genes (and thus DPMs), properties of the egg stage of development, including size, shape, and intracellular "egg-patterning processes," could effect fine-tuning of, but

Table 1 Some major dynamical patterning modules (DPMs)

DPM	Toolkit molecules	Physics	Morphogenetic role
ADH	Cadherins; lectins	Adhesion	Multicellularity; tissue formation
DAD	Cadherins; lectins	Differential adhesion; phase separation	Tissue multilayering
LAT	Notch pathway	Lateral inhibition	Coexistence of alternative cell types
POL_a	Canonical Wnt pathway	Cell surface anisotropy	Topological change; internal cavities
POL_p	Noncanonical Wnt pathway	Cell shape anisotropy	Tissue elongation
ECM	Chitin; collagen; fibronectin	Stiffness, dispersal	Skeleton formation; elasticity; EMT
OSC	Hes + Notch, Wnt	Synchronization of cell state	Developmental fields; periodic spatiotemporal patterning
MOR	Hh; TGF-β/BMP	Diffusive transport	Spatial patterning
TUR	Hh; TGF-β/BMP + Notch	Dissipative structure	Periodic spatial patterning

Based on Newman and Bhat (2008, 2009)

ADH cell–cell adhesion, *DAD* differential adhesion, *LAT* lateral inhibition, POL_a (multicellular) apicobasal polarity, POL_p (multicellular) planar cell polarity, *ECM* (multicellular) extracellular matrix, *OSC* (multicellular) oscillation, *MOR* morphogen, *TUR* turing-type reaction–diffusion process, *Hh* Hedgehog, *TGF-β* transforming growth factor-beta, *BMP* bone morphogenetic protein

not deviations from, the phylotype. This provides a resolution to the embryonic hourglass puzzle (Newman 2011).

Conclusions

The problem of form has been central to the development of biology. In fact the understanding of form in the living realm was the motivation for the birth of biology as a new scientific discipline at the beginning of the 19th century (Lenoir 1982, 1987; Richards 1992). The theory of natural selection was developed by Darwin and Wallace to explain this central problem in the natural sciences.

Any proposed theory of organismal form should address all of its fundamental aspects: its origination, generation (development), and transformation over time (evolution). Although there is no explicit model of morphological development in Darwin's theory, there is an implicit one. Rooted in the Newtonian framework, natural selection was the external "force" proposed by Darwin as an organizing principle for evolutionary transformation (Depew and Weber 1996). But natural selection is the differential sorting of phenotypic variants over time. Since it is development that produces new morphological variants, the purported creative capacity of natural selection relies upon crucial assumptions about how form is generated. The production of morphological variants should be arbitrary (undirected), gradual, and continuous, so that natural selection can impart direction to the process of evolutionary change (Gould 1982, 2002). The implied model of development is thus related to the idea of matter as inert and non-intrinsically organized (Linde-Medina 2010).

Darwin (1868), in his theory of pangenesis, postulated the existence of "gemmules" as the hereditary material transmitted across generations. These were conceived as particles with the capacity to produce the different cell or tissue types of the body's organs. But how gemmules were organized to form the organism during development was left unexplained (Amundson 2007).

With the incorporation of Mendel's ideas into the mainstream of biology at the start of the 20th century the notion took hold that hereditary particles, which according to Darwin only specified cell types, were also responsible for organizing cells into organisms (Allen 1986; Amundson 2007; Robert 2004). This eventually led to the idea of a genetic program for development (Schrödinger 1945). This concept was reinforced when the discovery of the chemical nature of the gene and the genetic code during the 1950s and 1960s endowed the previously hypothetical particles of inheritance with a material identity (Judson 1996; Cobb 2013).

The genetic program filled the gap in the Darwinian–Mendelian synthesis left by rejection of the nebulous theory of pangenesis and provided a conception of development guided by "information" that persuaded many that natural selection could be the primary cause of form (Kay 2000). If form is in the genes (i.e., there exist genetic programs for development), the origin of forms, like the outcomes of computer simulations implementing capricious rules, is essentially arbitrary. Traits could change in any direction and any combination of different traits could be possible, with the only constraints being the past history of the lineage. The sorting process then selects and permits the accumulation of small changes in form, generation after generation, forging the organism.

Because the sorting process selects the fitter variants, resulting organismal characters would mainly be built to serve external demands. The implication is that the organization of living beings does not reflect any intrinsic order

stemming from natural morphogenetic processes, but is rather entirely opportunistic, resulting in machines programmed to extract as much advantage as possible from their surroundings (Linde-Medina 2010).

This prevailing conceptualization of modern biology is, in fact, the opposite of what its pre-Darwinian founders had in mind (Amundson 2007; Lenoir 1982, 1987). As in other sciences, the principal goal was the search of governing principles, in this case, of biological organization. But if, as indicated above, genes dictate form, form is arbitrary and the order we observe today is contingent, rather than inherent. The construction of a science of biological form thus becomes a questionable enterprise. However, despite its wide acceptance, the notion of the genetic program has also been criticized as an inapt metaphor (Goodwin 1985; Nijhout 1990; Newman 2002). This is why the search for the laws of form has continued, despite changes in fashion, from the 19th century to the present.

The modern evolutionary synthesis purports to account for all aspects of organisms' phenotypes, including their forms. By discounting embryonic development in favor of a gene-centric determinism, it incorporated a model, however non-explicit, for how genes produce form. As a theory that was produced in the mid-20th century within a 19th century mechanistic framework, the Synthesis conceived of the organism's matter as inert and separate from its form in an almost Aristotelian sense, needing instructions from the genome to become organized (Delbrück 1976).

The modern sciences of mesoscopic condensed excitable materials, however, show living matter (like other condensed, excitable systems) to be capable of self-organization by virtue of its intrinsic physical and chemical properties. But if inherent physical properties are the primary determinants of form, natural selection cannot play this central role.

While genes are essential components in this dynamical physicalist framework they do not perform the role assigned to them in the mechanistic framework of the modern synthesis. Genes specify some of the components (RNAs, proteins) that participate in the complex physicochemical reaction networks, and binding, viscoelastic, disaggregative, and solidification transitions that constitute embryogenesis. Their mutational change over time is followed by selection of the resulting variants not only, or even primarily, under the Darwinian constraint of marginally superior functioning in the external environment. Much genetic novelty is also selected because it enhances the reliability of generation of functionally adequate forms by more primitive versions of the developmental systems in which the gene products participate. Novelties that are adaptations may sometimes only become so after the fact, when the altered form has settled into a new niche (Odling-Smee et al. 2003).

The Synthesis of course never postulated an equivalence between genes and biological characters. As stated by one of its architects, George Gaylord Simpson:

> Characters, as such, are not inherited, whether they be acquired characters or not. It is a series of determiners for a developmental system that is inherited. What characters result from this depends on the interplay of the inherited determiners, the activities of the organism, and the environment during development. (Simpson 1950, p. 247).

This was just before the notion of a computer program was put in place as the exclusive paradigm for "the interplay of inherited determiners" (i.e., genes). What was missed in this move was the fact that the material properties of embryonic tissues are propagated from parent to offspring as inevitably as genes. In actuality, form is propagated (inherited) from generation to generation first by the generic behaviors of living materials as physical systems, and increasingly by the canalization of developmental outcomes (among the limited possibilities available to these plastic systems) against environmental and developmental noise and subsequent mutation (Newman et al. 2006). In no manner, however, are genes the "cause" of form, and there is no reason to expect that evolution of form or biological kinds will be reflected in the evolution of genes (Schwartz and Maresca 2006).

In summary, form is not arbitrary; for multicellular systems the physical principles pertaining to soft, chemically and mechanically excitable material determine the spectrum of available forms and how they can be transformed (Newman and Comper 1990; Newman and Forgacs 1993; Newman 1994).

Rather than being encoded in genes, form emerges when cells and certain of their molecules mobilize physical forces, effects and processes in a multicellular context. Genes do not encode traits but stabilize and actualize potential morphological motifs. What is inherited from one generation to the next are not genetic programs for constructing organisms, but generative mechanisms of morphogenesis and pattern formation and the initial and boundary conditions for reproducing the specific traits of a taxon.

The DPMs described above are the shared mechanisms of form generation in the animals, as well as (when differences in interaction toolkit genes and applicable physical processes are taken into account) in multicellular plants (Hernández-Hernández et al. 2012). They provide the missing link between development and evolution by revealing how genes acting in concert with physics can generate and transform morphology in a heritable fashion. In addition, they account for the origination and innovation of form, which the earlier synthesis, despite its claims, was unable to do (Müller and Newman 2005). DPMs thus

represent a unifying principle of morphological diversity and facilitate the characterization of organismal types (e.g., phyla, genera; see Newman 2011) and recurrent morphological motifs (homologs) in a materialist, non-essentialist fashion.

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