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Physical Determinants in the Evolution of Development

Stuart A. Newman

Department of Cell Biology and Anatomy
New York Medical College, Valhalla, NY 10595

(914) 594-4048
newman@jnymc.edu

One sentence summary: Physical processes mobilized by ancient genes in novel multicellular contexts established morphological templates for subsequent animal evolution.

This Week in *Science* summary:

Most animal body plans and morphological motifs arose between 500 and 700 million years ago, during several relatively brief periods of innovation. The genes of the conserved "interaction toolkit," whose products mediate embryonic morphogenesis and pattern formation, were largely present in the unicellular ancestors of the animals; the next half billion years of evolution failed to generate substantial additional morphological novelty. Stuart Newman reconciles these observations by proposing that physical processes characteristic of chemically and mechanically active soft matter, newly mobilized by the interaction toolkit molecules when they came to function in a multicellular context, originated the motifs of animal form and (with the associated genes), have been the underlying basis of their propagation over the course of evolution.

Many of the classic phenomena of early animal development – the formation and folding of distinct germ layers during gastrulation, the convergence and extension movements leading to embryo elongation, the formation of somites (paired blocks of tissue) along the main axis of vertebrate embryos, the generation of the vertebrate limb skeleton, the arrangement of feathers and hairs – have been productively analyzed by mathematical and computational models which treat morphological motifs as expected outcomes of physical process that are *generic*, i.e., pertaining as well to certain nonliving chemically and mechanically active soft materials (*1-6*). Given that the thousands of genes of extant animals have been subject to mutation and (at the organismal level), natural selection, over the more than 600 million years since the Metazoa first emerged (*7*), it is counterintuitive but revealing that the generic morphological motifs animals began with were carried over to the present, with few additions.

In fact, many developmental outcomes that resemble generic physical products turn out not to be, or at least not simply so. Because the cells of embryonic tissues are independently mobile while remaining collectively cohesive, the formation of distinct layers during gastrulation and of boundaries during later development had been attributed to cell adhesive differentials, in analogy to the phase separation of liquids like oil and water (*8*). But while differential adhesion is indeed capable of sorting cells into separate layers, what happens in the embryo is more complicated, with tension exerted on the cell surface by the cytoskeleton and active cell-cell repulsion (phenomena with no analogues in liquids), often contributing more to the configuration of the separated tissues than relative affinities (*9-11*).

More generally, cells in embryos have the ability, via contractile and protrusive activities, to exert forces on one another and upon the extracellular matrices they produce (*12*). While these mechanical properties can lead to, and in some cases account for, the buckling of epithelial tissues into ridges, as in neurulation, the latter actually occurs by several different mechanisms across the chordates, only some of which depend on mechanically mediated buckling (*13*).

An embryo's cells are tiny chemical reactors with stored and exchangeable sources of energy. This is evidenced in their ability to switch among multiple stable compositional states (the basis for cell differentiation) (*2, 14*), and to exhibit biochemical oscillations (the basis of the cell cycle and other cell-physiological periodicities) (*15, 16*). By virtue of this dynamicity, embryonic tissues are chemically "excitable media," the physical properties of which can explain some

enigmatic developmental phenomena. Nonliving chemical oscillators that are weakly coupled readily come into synchrony (*17*). Correspondingly, interactions between adjoining cells an embryonic tissue will synchronize intracellular oscillations, e.g., periodic expression of the transcriptional modulator Hes1, transforming a clump of individual cells into a globally coordinated "embryonic field" (*18*).

While spatial uniformity of biochemical state can thus emerge in embryonic tissues, patterns can also form based on the self-organizing capabilities of interacting diffusible activators and inhibitors of cell differentiation ("morphogens") (*19, 20*). Some periodic and quasi-periodic developmental patterns (distribution of hairs, pigment patches, skeletal structures) clearly depend on such effects (*21*), but others, like the seven stripes of pair-rule proteins in the syncytial *Drosophila* embryo, while exhibiting some self-organizing aspects (*22*), are generated "inelegantly" (*23*), employing stripe-dedicated duplicated promoters.

The operation of generic physical effects in animal embryogenesis along with developmental mechanisms that are complex and non-generic, but nonetheless produce similar stereotypical morphological motifs (multiple layers, interior cavities, segments, folds, etc.), suggests a scenario in which the non-generic mechanisms are evolved embellishments of the generic ones, with selection stabilizing and reinforcing inherent forms rather than inventing new ones (*24*). Hierarchical programs of gene expression during development of modern animals (*25*) regulate shape and form by coordinating, fine-tuning, and constraining the activities of the subset of the conserved developmental "toolkit," products of genes that directly mediate cell-cell interactions (*26*). These molecules (e.g., cadherins, Notch, Wnt, Hedgehog, BMP, collagens) typically served single-cell functions in one or more unicellular ancestors of the multicellular animals before being recruited into developmental roles with the emergence of multicellularity (*27, 28*).

The morphogenetic and patterning functionalities that arose when "interaction toolkit" molecules, acting in the new multicellular context, mobilized generic physical effects, have been termed "dynamical patterning modules" (DPMs) (*26*). Although primitive metazoan-type body plans could have quickly arisen in aggregates of cells containing DPM-enabling genes, genetic heterogeneity would have compromised the evolutionary stability of such forms (*29*). The emergence of an egg stage of development, with cell clusters generated by cleavage, would have led to genetically uniform embryos and populationally stable lineages (*30*) (Fig. 1).

The early products of DPMs would have borne the generic morphological signatures of chemically and mechanically active soft materials. However, just as nonliving materials do not equally engage every physical effect, not every DPM appears in each animal lineage, since the relevant genes are not universally present throughout the metazoan phyla. Thus, the morphological simplicity of the placozoan *Trichoplax adhaerens*, which consists of three cell layers but no patterns of differentiation within the layers, and the sponges, exemplified by *Amphimedon queenslandica*, which lacks an elongated body axis and a true epithelium, are likely connected to the absence of planar polarity pathway and basement membrane constituents, and in *Trichoplax*, the Notch-Delta pathway, all of which are present in eumetazoans (*31-33*) (Fig. 2).

The idea that physics acted on early multicellular forms to define in broad strokes the patterns of development resolves several seemingly paradoxical aspects of the evolution of the animal phyla. These include the rapid emergence (i.e., in two episodes of approximately 20 million years each) of nearly all of the metazoan body plans during the late Ediacaran-early Cambrian periods (*7, 34*); the use of the same genetic toolkit to mediate similar morphogenetic processes in all animal phyla, however disparate (*25, 35*); the recurrent appearance of a limited set of morphological motifs in all animal body plans and organ forms (*26, 36*); and the relative insensitivity of phylum-associated morphological signatures to variations at stages of development prior to the multicellular one when DPMs come into play (*30*).

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Figure Legends

Fig. 1. A core set of physico-genetic modules underlies morphological evolution of animals. The inner circle shows morphological motifs generated by some of the key dynamical patterning modules (DPMs): physical forces and effects relevant to the multicellular scale mobilized by certain ancient single-cell gene products and pathways. Multicellular entities (center image) were formed by the aggregation of unicellular organism (red curved arrow) or the cleavage of eggs (green curved arrow). Emergent motifs include (clockwise from top of inner circle) appendages, segments, elongated bodies and primordia, coexisting alternative cell types, interior cavities, dispersed cells, multiple layers. Genetically uniform clusters produced stable lineages (straight green arrows), whereas chimeric clusters did not (broken red arrows). Contemporary organisms containing some or all of these motifs are shown in the outer circle. Clockwise from top right: vertebrate (*Gallus*) embryo; insect (*Drosophila*) embryo; brachiopod (*Capitella*) embryo; cephalopod (*Loligo*) embryo; demosponge (*Amphimedon*); nematode (*Caenorhabditis*) embryo; placozoan (*Trichoplax*); echinoderm (*Dendraster*) larva.

Fig. 2. Increasing complexity of animal body plans over evolution depended on mobilization of new dynamical patterning modules. Ancestral cell clusters would have contained subsets of interaction toolkit genes. The fundamental DPM is cell adhesion (ADH). Formation of non-intermixed layers, as in placozoans, depended on differential interfacial tension (DIT) and apicobasal cell polarity (POL_a). Addition of lateral inhibition (LAT) and a generalized extracellular matrix (ECM_g) allowed coexistence and rearrangement of contiguous inter-transforming cells, as in sponges. Planar polarity (POL_p) and basal lamina-type extracellular matrix (ECM_b), enabled formation of elongated bodies and epithelial appendages and ridges. Interstitial extracellular matrix (ECM_i) allowed for epithelial-mesenchymal transformation and intereaction, and triploblasty. Physically different ECM_is and heterochrony in the developmental implementation of various shared DPMs led to disparate body plan. Lines of descent of the various morophotypes are uncertain owing to the possibility of gene loss and lateral transfer. (See refs. 26 and 30 for additional details.)

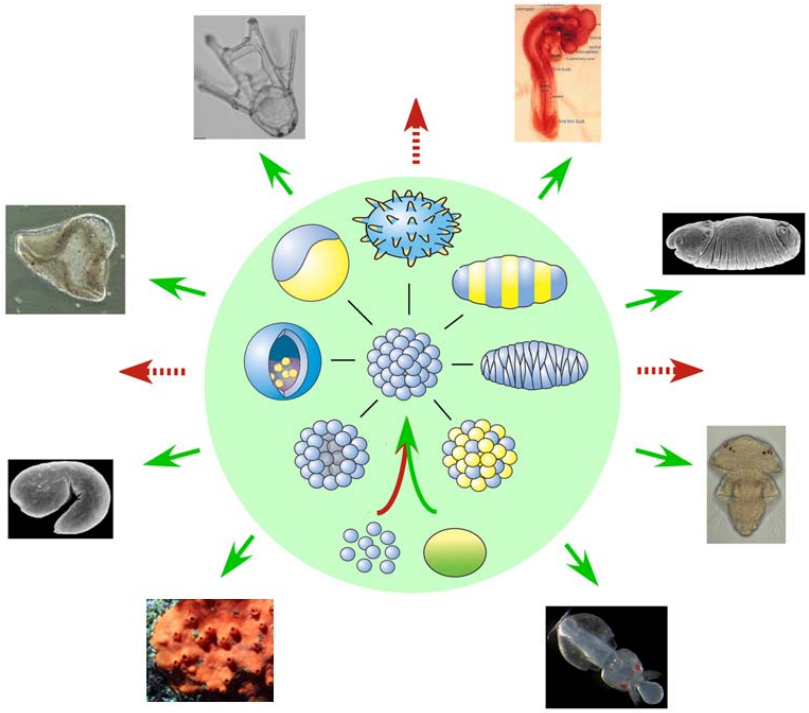

Fig. 1

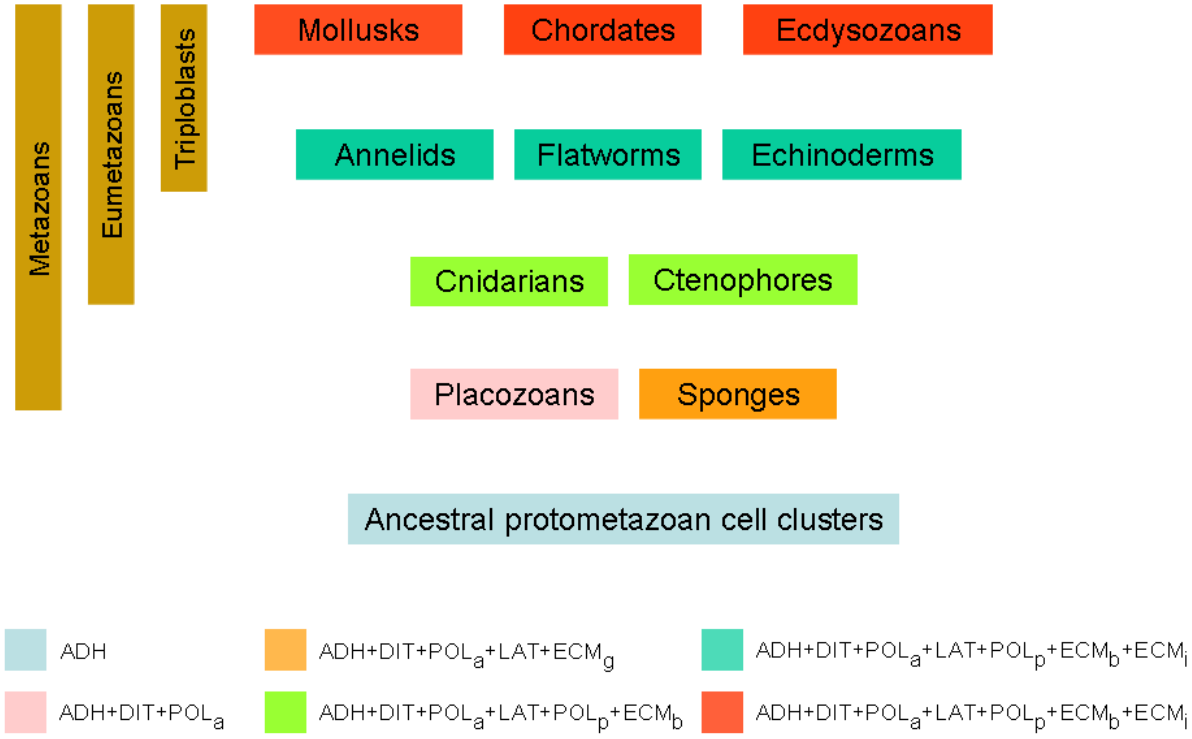
Metazoans
Eumetazoans
Triploblasts
Mollusks
Chordates
Ecdysozoans
Annelids
Flatworms
Echinoderms
Cnidarians
Ctenophores
Placozoans
Sponges
Ancestral protometazoan cell clusters
ADH
ADH+DIT+POLa
ADH+DIT+POLa+LAT+ECMg
ADH+DIT+POLa+LAT+POLp+ECMb
ADH+DIT+POLa+LAT+POLp+ECMb+ECMi
ADH+DIT+POLa+LAT+POLp+ECMb+ECMi

Fig. 2