a niche (*11*). Theoretical models that include both cell-cell communication and intracellular expression dynamics have been investigated (*12*, *13*). Extensive simulations of such models over a huge variety of gene expression networks have found that cells that can both proliferate and also differentiate to cell types of different composition generally show temporal oscillations in their gene expressions at the single-cell level (Fig. 2B). In such cases, with the increase in cell number, state differences between cells are amplified by cell-cell communication such that the sensitivity to a signal increases. Some cells at a certain phase of oscillations (i.e., at a certain location within the orange trajectory in Fig. 2B) escape their original attractor in response to a signal and fall into the trough of a different attractor, whereas other cells of different phases remain with the original attractor. Thus, gene expression oscillations are necessary for stemness, potentiality both to proliferate and differentiate, whereas the loss of stemness is characterized by a loss of oscillatory dynamics. Notably, in this mechanism, the timing and pathway of differentiation are robust to noise, a property Waddington termed homeorhesis (*1*). With cell-cell communication, the differentiation frequency of a stem cell is autonomously regulated by the population of each cell type, resulting in a robust population ratio.

Recently, Huang used time-series transcriptome data to experimentally verify the existence of attractors in the dynamics of hematopoietic progenitor cells by demonstrating the robustness of the cellular state (*14*). Additionally, from the fluctuating expression level of stem cell marker Sca1, they found slow-scale changes in cellular states, which was suggested to be regulated by cell-cell communication (*15*).

Single-cell measurements of gene expression dynamics have shown heterologous gene expressions of Rex1, Nanog, and Stella in embryonic stem cell populations (*16*) and Sca1 in hematopoietic stem cells (*15*, *17*), a heterogeneity closely linked to the fate of the stem cell. One possible mechanism for such heterogeneity could be noise in the expression dynamics. Another is oscillatory expression dynamics. Indeed, Kageyama and colleagues found temporal oscillations in the Hes1 expression level of neural precursors and embryonic stem cells, where the phase of the oscillation was potentially seen to control the fate decision (*18*, *19*), whereas existence of a complex dynamic attractor is also suggested (*20*). Furthermore, cell-cell communication via Notch-Delta signaling was suggested to regulate the fate decision of neural progenitors under the control of the oscillatory expression dynamics of Hes1 and other genes (*18*).

Using a dynamical-systems approach to explain the differentiation of stem cells, we have described here how fluctuating and oscillatory gene expressions underlie the essence of stemness. If so, reactivating specific genes may recover these oscillations in differentiated cells to potentially restore potency (*21*). To characterize the attractors of stem and differentiated cells quantitatively, however, further experiments, including systematic sensitivity analysis of gene expressions (*22*), as well as theoretical formulations that go beyond Waddington's epigenetic landscape, are needed.

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PERSPECTIVE

Physico-Genetic Determinants in the Evolution of Development

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Animal bodies and the embryos that generate them exhibit an assortment of stereotypic morphological motifs that first appeared more than half a billion years ago. During development, cells arrange themselves into tissues with interior cavities and multiple layers with immiscible boundaries, containing patterned arrangements of cell types. These tissues go on to elongate, fold, segment, and form appendages. Their motifs are similar to the outcomes of physical processes generic to condensed, chemically excitable, viscoelastic materials, although the embryonic mechanisms that generate them are typically much more complex. I propose that the origins of animal development lay in the mobilization of physical organizational effects that resulted when certain gene products of single-celled ancestors came to operate on the spatial scale of multicellular aggregates.

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 that treat morphological motifs as expected outcomes of physical process that are generic; i.e., pertaining as well to certain nonliving, chemically active, viscoelastic materials (*1*–*4*). 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 (*5*), it is counterintuitive but revealing that the morphological motifs animals began with were carried over to the present, with few additions.

Many developmental events that might be characterized by their simple generic physical properties are, in fact, much more complex. For example, many cells of embryonic tissues are individually mobile while, at the same time, collectively cohesive, as in the formation of distinct layers during gastrulation and of boundaries during later development: behaviors that had been attributed to cell adhesive differentials, with analogy to the phase separation of liquids such as oil and water (*1*). Although differential adhesion is indeed capable of sorting cells into separate

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layers, the embryo is more complicated; for example, with tension being exerted on the cell surface by the cytoskeleton and active cell-cell repulsion (phenomena with no known counterparts in liquids), often contributing more to the configuration of the separated tissues than relative affinities (*6*)

More generally, cells in embryos have the ability, via contractile and protrusive activities, to exert forces on one another and on the extracellular matrices they produce (*7*). Although these mechanical properties can lead to, and in some cases account for, the buckling of epithelial tissues into ridges, as in neurulation, this developmental process actually occurs by several different mechanisms across chordates, only some of which depend on mechanically mediated buckling (*8*).

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) (*1*, *9*) and to exhibit biochemical oscillations (the basis of the cell cycle and other cell-physiological periodicities) (*10*, *11*). 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 (*12*). Correspondingly, interactions between adjoining cells in an embryonic tissue will synchronize intracellular oscillations; an example is the periodic expression of the transcriptional modulator Hes1 transforming a clump of individual cells into a globally coordinated "embryonic field" (*13*).

Although a 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") (*14*–*16*), Some periodic and quasiperiodic developmental patterns (such as the distribution of hairs, pigment patches, or skeletal structures) clearly depend on such effects (*17*), but others, such as the seven stripes of pair-rule proteins in the syncytial *Drosophila* embryo, although they exhibit some self-organizing aspects (*18*), are generated in a less generic fashion, employing stripe-dedicated duplicated gene promoters (*19*).

The operation of generic physical effects in animal embryogenesis, along with developmental mechanisms that are complex and nongeneric but nonetheless produce similar stereotypical morphological motifs (multiple layers, interior cavities, segments, folds, etc.), suggest a scenario in which the nongeneric mechanisms are evolved embellishments of the generic ones, with selection stabilizing and reinforcing inherent forms rather than inventing new ones (*20*). Hierarchical programs of gene expression during the development of modern animals (*21*) regulate shape and form by coordinating, fine-tuning, and constraining the activities of a subset of the conserved developmental "tool kit," the tools of which are the products of genes that directly mediate cell-cell interactions (*22*). These molecules (such as cadherins, Notch, Wnt, Hedgehog, bone morphogenetic protein, and collagens) typically served single-cell functions in one or more unicellular ancestors of the multicellular animals before being recruited into developmental roles as multicellularity emerged (*23*, *24*).

The morphogenetic and patterning functionalities that arose when "interaction tool kit" molecules, acting in the new multicellular context, mobilized generic physical effects, have been termed dynamical patterning modules (DPMs) (*22*). Although primitive metazoan-type body plans could have quickly arisen in aggregates of genetically variable cells as long as they contained DPM-enabling genes (Fig. 1, aggregation route), without enforced genetic uniformity among the cells of multicellular forms, intraorganismal competition would tend to undermine their persistence (*25*). The emergence of an egg stage of development, with the cell cluster stage of development then generated by cell cleavage, would have obviated such chimerism (Fig. 1, cleavage route), facilitating the generation of evolutionarily stable lineages (*26*) (Fig. 1).

The early products of DPMs would have borne the generic morphological signatures of

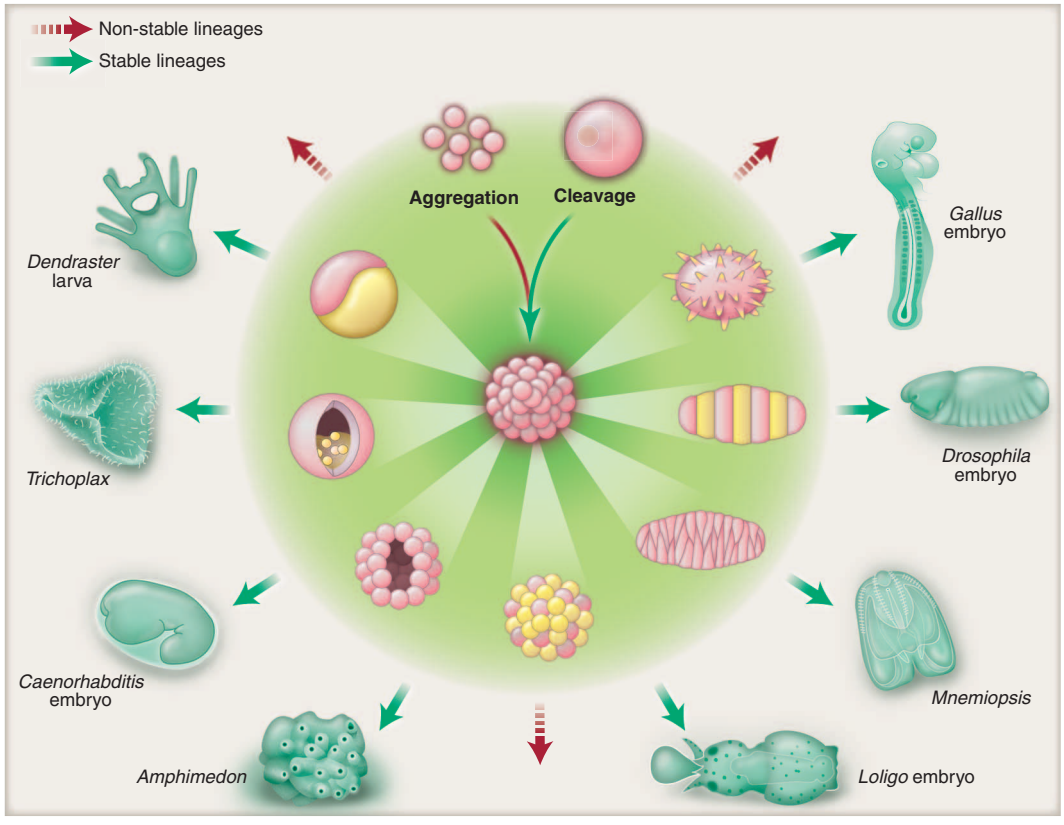

Fig. 1. A core set of physico-genetic modules underlies the morphological evolution of animals. Multicellular entities (center image) were formed by the aggregation of unicellular organisms (red curved arrow) or the cleavage of enlaged cells ["proto-eggs" (*26*) or eventually fertilized eggs] (green curved arrow). The green inner circle shows morphological motifs generated by some of the key DPMs: physical forces and effects relevant to the multicellular scale, mobilized by certain ancient single-cell gene products and pathways. Emergent motifs include (clockwise from top of inner circle) appendages, segments, elongated bodies and primordia, coexisting alternative cell types, interior cavities, dispersed cells, and 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, arthropod (*Drosophila*) embryo, ctenophore (*Mnemiopsis*), cephalopod (*Loligo*) embryo, demosponge (*Amphimedon*), nematode (*Caenorhabditis*) embryo, placozoan (*Trichoplax*), and echinoderm (*Dendraster*) larva.

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, because the relevant genes are not universally present throughout the metazoan phyla. The fundamental DPM is adhesion (mediated mainly by cadherins), which would have generated protometazoan clusters (Fig. 2). Such clusters, with appropriate DPM-enabling genes, could have exhibited more-complex body plans (Fig. 1, curved red arrow), but, as noted above, would have lost out to lineages arising by cleavage (Fig. 1, straight green arrows). The formation of non-intermixed layers, as in placozoans (Fig. 1), depended on differential interfacial tension (meditated by cadherins in conjuction with cytoskeletal mechanics) and apicobasal cell polarity (mediated by the canonical Wnt pathway). Lateral inhibition [mediated by the Notch pathway, absent in *Trichoplax (27)*] and a viscous, generalized (i.e., not epithelial or mesenchymal) extracellular matrix allowed the coexistence and rearrangement of contiguous intertransforming cells, as in sponges (Fig. 1). Planar polarity (mediated by the noncanical Wnt pathway) and a basal lamina-type extracellular matrix [both absent in genetically characterized sponges and placozoans (*27*–*29*)] enabled the formation of elongated bodies and epithelial appendages and ridges, as in ctenophores (Fig. 1) and cndarians. An interstitial extracellular matrix allowed for epithelial-mesenchymal transformation and intereactions, and triploblasty (i.e., three-layered structures). Extracellular matrices with distinctive physical properties (e.g., chitin versus collagen) and heterochrony in the developmental implementation of various shared DPMs led to disparate body plans among the triploblasts (Fig. 2), including vertebrates, arthropods, nematodes, mollusks, and echinoderms (Fig. 1).

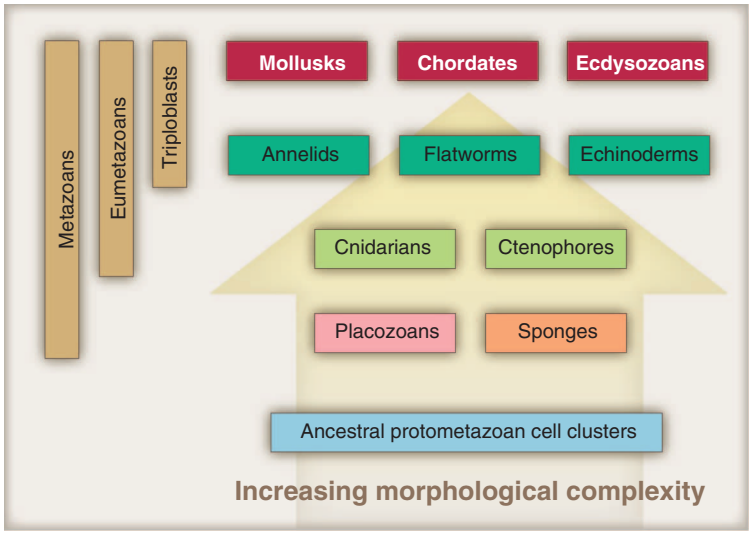

Fig. 2. The increasing complexity of animal body plans during evolution depended on the mobilization of new DPMs. The lines of descent of the various morophotypes are uncertain because of the possibility of gene loss and lateral transfer.

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 (in two episodes of approximately 20 million years each) of nearly all of the metazoan body plans during the late Ediacaran–early Cambrian periods (*5*, *30*); the use of the same genetic tool kit to mediate similar morphogenetic processes in all animal phyla, however disparate (*21*); the recurrent appearance of a limited set of morphological motifs in all animal body plans and organ forms (*20*, *22*); and the relative insensitivity of phylum-associated morphological signatures to variations at stages of development before the multicellular one, when DPMs come into play (*26*).

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