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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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 to exhibit biochemical 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 chimeraism (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
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 proto-metazoan 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 (Fig. 1, straight green arrows). The formation of non-intermixed layers, as in placozoans (Fig. 1), depended on differential interfacial tension (mediated by cadherins in conjunction with cytoskeletal mechanics) and apicobasal cell polarity (mediated by the noncanonical Wnt pathway). Lateral inhibition [mediated by the Notch pathway, absent by the canonical Wnt pathway) and apicobasal cell polarity (mediated by cadherins in conjunction with cytoskeletal mechanics) are uncertain because of the possibility of gene loss and lateral transfer.

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 morphotypes are uncertain because of the possibility of gene loss and lateral transfer.

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