Voices

How can Waddington-like landscapes facilitate insights beyond developmental biology?

Nika Shakiba
University of British Columbia

Engineering beyond landscapes

The Waddington landscape has offered an intuitive framework to conceptualize cell fate, grounded in our innate understanding of the physical rules that guide balls rolling down a hill. The hills and valleys of the landscape are molded by the cellular processor—the inner regulatory network of DNA, RNA, and proteins that govern cell "state" —and microenvironmental inputs are forces that guide cells in their probabilistic journey down the hill. While a powerful visual, it's time we break out from the confines of this imaginary landscape.

The reality is that the cellular processor is dynamic. It dances with stochastic fluctuations in the molecular players that define it, as well as regulatory interactions that change the topology of the processor itself. The one-size-fits-all view of the landscape sidesteps the nuance of cellular context: each cell is experiencing a *unique* landscape that is defined by its spatial context. Even cell-cell interactions, akin to bumps against neighbors, can drive appreciable changes to a cell's trajectory—sudden holes in the landscape floor can drive cells to their death. Thus, the metaphor of a rigid landscape is misleading.

Catalyzed by cutting-edge advances in single-cell technologies, it's time to reimagine the landscape. By embracing the power of predictive computational models, we can capture its dynamic dance in space and time. As mammalian synthetic biology comes of age, we can even engineer designer landscapes that guide cell fate along prescribed paths with probabilistic precision. The age of cellular engineering is at our doorstep—it's time we break cells out of this rigid world.

Chunhe Li
Fudan University

Quantifying energy landscapes

The Waddington epigenetic landscape, as a metaphor, has been proposed to explain cellular development. Recently, energy landscape theory has been developed to study stochastic dynamics of cell fate decisions from underlying gene regulatory networks. In the landscape view, cell types are characterized by basins or attractors, reflecting their occurrence probability. A cell fate decision process can be viewed as the transition between attractors in gene expression space. Importantly, Waddington-like landscapes can be quantified from mathematical models combined with experimental data, which provide powerful tools to understand biological systems beyond developmental biology, e.g., for cancer and neuroscience.

Cancer has been viewed as an attractor in gene network space. In principle, cancer cells can be destroyed by altering the stability of the cancer attractor on the landscape, which can be achieved by intervening in genes or regulation of cancer networks. In fact, recent *in vivo* experiments indicate the possible conversion of metastatic cancer cells to non-invasive cells. This opens up an opportunity for a paradigm shift from traditional strategies of killing cancer cells to inducing cell fate transitions from cancer cells to non-malignant cells.

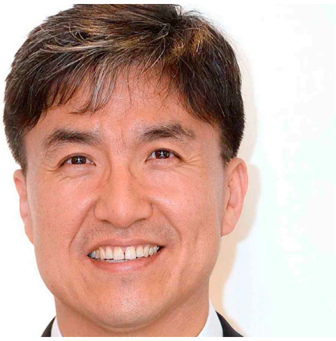
In neuroscience, Hopfield pioneeringly proposed the concept of "energy function" to explore computational properties of neural circuits, such as associative memory. Intriguingly, the landscape for decision making can be quantified based on spiking neural network models. Combined with multiscale brain data, the landscape idea will reveal new insights into cognitive function and related issues such as working memory, decision-making, and brain disease.

Jordi Garcia-Ojalvo
Universitat Pompeu Fabra

Research evolution

Over 20 years ago, the late Sydney Brenner said that ‘‘physics is the art of the optimal,’’ while ‘‘biology is the art of the satisfactory.’’ By contrast, Conrad Waddington has been suggesting for over seven decades, through his metaphorical landscape, that developing organisms are also skilled optimizers, finding surface minima as they self-organize in time and space. We have no reason to assume that such optimization capabilities are restricted to the processes by which living systems mature. Any cellular decision, at any stage in the life cycle of an organism, can be expected to benefit from the proven ability of cells to find an optimal path through their physiological landscape.

Of course, any metaphor has limitations, and the Waddington landscape is no exception. The idea that a single dimension can account for the state of a cell as decisions are made is clearly insufficient, in the light of current high-throughput single-cell surveys. Additionally, we should not ignore the fact that cells actively ‘‘carve out’’ their physiological landscape as they implement their decisions. Optimization in the face of such feedback effects should be examined. Finally, it is well known that mechanics plays a very important role—complementary to that of biochemistry—in normal cell functioning. The landscape is thus not only biochemical, but also mechanical. In any case, finding general optimization principles like those suggested by Waddington might still be our best chance to develop an encompassing theory of living matter.



Kwang-Hyun Cho
Korea Advanced Institute of Science and Technology (KAIST)

Cell fate reprogramming

Owing to the development of systems biology and advancement of measurement technologies, we can now reconstruct molecular regulatory network models that represent crucial mechanisms underlying various biological processes such as cell fate determination. In particular, integrating single-cell multi-omics data helps to infer such molecular network models by following specific pseudo-time trajectories during cell fate determination to dissect molecular dynamics within a cell.

A state of the network comprises a collection of molecular expression levels at different time points, and transitions between states follow the regulatory logic of the network. States that are more stable given certain regulations are visited more frequently, and constitute only a small fraction of possible states. These states are called attractor states and represent particular cellular phenotypes. Moreover, analyzing the relative stability of such attractor states and their resulting epigenetic landscape can further aid the investigation of canalizing paths along which each cell reaches its final destination.

Recent progress in the application of complex network control strategy enables us to identify master molecular switches that determine cell fates. By controlling such master switches embedded within a molecular network model, we can also reprogram lineage-determined cells to another cell type. This can be applied to predicting drug responses of subtype-specific cells or inducing trans-differentiation to a desired cell fate. Waddington’s epigenetic landscape was introduced as a metaphor describing cellular dynamics during development and now brings to life attractor landscapes of actual biological network models for cell fate reprogramming.

Kiran Patil
University of Cambridge

Geometry of intuition

A myriad of molecular players and networks underlying the function of cells and organisms are now known. Yet, the quest to distil the diversity and dynamics of biological systems into basic organizational and operating principles is far from complete. This is where geometric perspectives (and metaphors) can play a fundamental role. A geometric view has been key in numerous fields: optimization (convex spaces), stability theory (saddle points), and enzymology (energy barrier), to name a few. Waddington's landscape metaphor for a developmental process is an excellent case-in-point in biology. It illustrates the power of geometry in providing an intuitive abstraction to a complex process and spurs creativity in data interpretation. The concept of attractors elegantly connects this abstract perspective with complex systems theory.

Geometric perspectives would be equally useful in tackling other biological research questions. An example close to my own interests is how complex microbial communities navigate the hilly landscape of cooperation and competition. While these abstract metaphors often invite criticism due to their apparent disconnect from molecular mechanisms, this is but part of the scientific process. Geometric metaphors will only help in structuring the debate toward uncovering the basic principles. Geometry is intuitively insightful: may it aid our journey through the complex, ever-evolving, landscape of biology.

Aleksandra Walczak
Ecole Normale Supérieure

From attractors to data

Cellular differentiation pathways have diverse molecular, genetic and regulatory implementations that lead to a finite set of (at least quasi) stable states. Mapping this onto the abstract idea of attractors in a landscape, separated by valleys, is a useful metaphor that helps us visualize cell types. But as Corson and Siggia have shown a few years back, it can also simplify molecular description and lead to quantifiable experimental predictions.

More generally, it builds on the intuition we see in many living systems that phenotypic states are stable and reproducible, but the molecular or genetic implementation can vary. Evolution finds many different mutations that lead to similar phenotypes. Ecological communities organize around phenotypic tasks and can be composed of different species, as long as they fulfill similar functions. Similarly, in developmental landscapes, the reproducibility of the final phenotype is important—there is not just one molecular encoding of the trajectory down the developmental pathway.

Landscapes have already contributed enormously to our thinking about living systems, with probably the most known examples, outside of development, coming from neuroscience and protein folding. In neuroscience discrete attractors have been associated to memory encoding, and more recently continuous attractors have been shown to encode orientation in space. In protein folding, the idea that proteins differ from other polymers by having a funnelled folding landscape, which helps them avoid misfolded structures, explains how they can fold in a finite time. Similarly to development, this example conveys the interplay of evolution and function.

In all of these examples, it's finding the right abstraction that allows us to make conceptual progress. For a long time the analogy remained theoretical, but now quantitative experiments make it possible to use results at this phenotypic scale and map them to molecular, genetic or regulatory implementations.

Yang-Yu Liu
Harvard Medical School

Human microbiome landscapes

The notion of Waddington's landscape has been implicitly adopted in human microbiome research. For example, "enterotypes" were originally defined as distinct clusters in the compositional landscape of human gut microbiome that might respond differently to diet and drug intake. Nowadays we usually do not consider enterotypes as distinct clusters ("islands"), but as densely populated areas ("peaks") in the compositional landscape. Mathematically characterizing enterotypes remains challenging.

Existing studies have demonstrated that the gut microbial compositions of healthy adults are highly personalized and stable. But it remains unclear if the gut microbial composition of a healthy adult is just marginally stable or if, in addition, it is asymptotically stable, i.e., displays the feature of "attractivity" so that stable compositions behave as "attractors." Similarly, we do not know whether "true multi-stability" exists for the human gut microbiome (i.e., if there are multiple attractors for any given species collection).

Preliminary results based on functional redundancy analysis suggest that, unlike that of *C. difficile infection* patients, the gut microbiome of patients with chronic diseases (e.g., inflammatory bowel disease, asthma, obesity, autism, and Parkinson's disease) might have been driven to relatively stable disease states. These disease states are resilient to compositional transitions and hence less likely to be susceptible to microbiota-based therapeutics (e.g., fecal microbiota transplantation). For those patients, how to design better microbiota-based therapeutics to restore a healthy microbiota is still a big challenge.

I believe the notion of Waddington-like landscapes will certainly shed light on those challenges in human microbiome research.

Seppe Kuehn
University of Chicago

Learning from natural variation

Waddington proposed that developmental trajectories are canalized to produce consistent outcomes despite genetic and environmental perturbations. In this picture, development is constrained to a landscape so deeply grooved as to permit only those dynamics favored by natural selection. Underlying this landscape are degenerate mechanisms that guide the system into those grooves. Canalization is particular to the wild-type here, but it is lost in mutants created by a geneticist rather than by evolution. One striking observation is that natural selection creates systems that permit a limited set of robust dynamical processes. We observe similar constraints on the dynamics of biological systems in other contexts including ecosystems exhibiting reproducible successions. I take two lessons from Waddington's proposal.

First, Waddington's insight suggests that we must understand the degenerate mechanisms that give rise to constrained dynamics if we hope to successfully design robust biological systems. A dominant approach to biological control is to enumerate, quantify, and perturb system components, without regard for evolution. However, when we attempt to control biological systems without considering natural variation and the canalized dynamics it produces, the resulting synthetic system is often fragile or non-functional. Etching new grooves in the dynamical landscapes of biological systems requires focusing our lens not on mutants, but on the structure and dynamics of natural variation.

Second, while Waddington's paper is grounded in the facts and observations of the day, his emphasis is on presenting an idea rather than a result. Waddington's work reminds us that ideas are key to the scientific endeavor and can have an outsized impact on biology. I hope that we can make space for bold ideas in our scientific discourse.

Qing Nie
University of California, Irvine

Single-cell dynamical landscapes

Advances in single-cell RNA-seq have provided unprecedented resolution in analyzing gene expression heterogeneity of cells. However, such datasets are only static *snapshots* of the cells, which exhibit complex dynamics. Uncovering intrinsic temporal information remains a major challenge for single-cell genomics.

Multiscale reduction of stochastic dynamics enables the upscaling of individual cells to group of cells with similar dynamic properties. By using data-driven dynamical systems methods to construct "stable cell attractors" from high-dimensional single-cell RNA-seq measurements, one obtains saddle points and other upscaled quantities. As a result, a single-cell dynamical landscape emerges, allowing visualization of the measured cells and providing rich dynamics for each cell and the attractors. Transition states, cell lineages, transition trajectories, and marker genes that characterize each transition naturally arise from the landscape, a.k.a. *Dynamical Manifold* in the context of MuTrans, a published method shown to be consistent with the well-established Langevin equation. Such single-cell dynamical landscapes offer unparalleled promise in connecting the static genomics datasets and dynamical systems view of cells.

Splicing dynamics, chromatin accessibility, and other measurements at the single-cell level can be incorporated using similar multiscale reduction methods to generate multiple complementary landscapes. The converging of data-driven and model-based approaches revives the "old" dynamic systems tools in the new era of single-cell biology.

Allon Klein
Harvard Medical School

Single-cell dynamical landscapes

Energy landscapes emerged historically as mathematical representations of physical systems. They occurred in cases where a single local quantity—taken as the height of a "landscape"—determined how a system would behave. For example, the force acting on a ball follows the gradient of a two-dimensional landscape on which it rolls. But when a ball bounces, the landscape no longer simply predicts its motion. Similarly, a molecule with N atoms has a $3N$-dimensional landscape of the energy required to deform it. In equilibrium, thermal fluctuations access each conformation with a frequency that decreases exponentially with the landscape height. Both of these examples use landscapes to encode physical laws, which apply sometimes, or even rarely. When they do apply, they empower us with predictions.

Waddington's landscape emerged not as a tool, but rather as a challenge: might we one day, perhaps, understand biological systems well enough to define them mathematically, perhaps even as a landscape? This challenge has inspired many, but there are few cases where landscapes have captured reductionist laws in biology.

Still, Waddington's landscape also captured a lasting insight: that biological systems are very confined. Of the many configurations that biological systems could occupy, only a few occur reproducibly and are compatible with life. Further, transitions between states appear stereotyped and continuous. A range of tools from graph theory and machine learning, applied to high-dimensional single cell measurements, now help to visualize the few continuous states that cells occupy in development. The resulting representations suggest rushing rivers flowing down mountain valleys: landscapes.

So what lessons generalize? Are biological systems continuous and constrained outside of developmental biology? Most certainly so. Many biological processes—such as disease onset, bacterial ecosystems, inflammatory responses—are likely to show highly constrained dynamics. These lend themselves to visualization and analysis as landscapes. But here, too, the laws that predict which way the ball rolls may not often reducible to a gradient.

Gustavo Deco and Morten Kringelbach
Universitat Pompeu Fabra and University of Oxford

Brain attractor landscapes

Beyond Waddington's epigenetic implementation, attractor landscapes also play a key role in macro systems such as the human brain over a lifetime. The brain consists of many billions of neurons and synapses interacting to bring about the complex dynamics giving rise to many different brain states, whether awake, sleeping or dreaming. Paradoxically, however, this complexity can be reduced to a lower-dimensional manifold which is sufficient to describe the main aspects of the dynamical repertoire.

Precisely given this low-dimensionality we are able to capture the necessary and essential features of the dynamical feature by means of a simple landscape of attractors. Whole-brain models integrate anatomy and local dynamics to successfully describe the main features of brain activity in different brain states in health and disease (https://doi.org/10.1016/j.celrep.2020.108471). Research has shown that the so-called resting state networks measured with functional magnetic resonance imaging result from structured noise fluctuations around the trivial low firing equilibrium state induced at the edge of a bifurcation by the presence of latent "ghost" multi-stable attractors corresponding to distinct foci of high firing activity in particular brain areas (https://doi.org/10.1523/JNEUROSCI.2523-11.2012). The global attractor structure represents the brain's dynamic repertoire and shows the highest signal complexity as measured by entropy.

Srividya Iyer-Biswas
Purdue University

Beyond the evocative picture

The essence of Waddington's visual metaphor: a smoothly varying complex signaling input leads to a choice between a finite number of possible cell fates, such that the choice is robust to perturbations within some tolerance in input variability. Reminiscent of systems in which some "potential" decreases as the system evolves, the specific local minimum the system eventually ends in is determined by the starting point and the topography of the potential. These local minima (attractors) correspond to the finite cell fates.

This geometric framework provides a heuristic route to dimensional reduction. For instance, greatly reducing the vast number of parameters typically involved in genetic models to just the essential set which affects the cell's fate in a given context, say during development.

The mathematical framework encapsulated by the visual metaphor is widely applicable provided the (biological or other) process: (1) involves selection of a distinct output from a finite and discrete set of possibilities; (2) does not have chaotic behavior in the underlying dynamics; and (3) can be represented by models in which some potential decreases as the system evolves.

Waddington believed that the development of phenotypic characteristics was driven by the interaction of genetic and environmental factors. Highly canalized valleys are robust to perturbations (i.e., the same phenotype will result despite changes in determinant factors). This led naturally to a conceptual leap: this picture describes a dynamic system following *homeorhesis*, which represents the return to a stable trajectory (a "set line") after perturbation (unlike *homeostasis*, which returns to a set point, i.e., a particular state).

Waddington's compelling and prescient metaphor has since been applied to diverse phenomena, not only in stem cell, cancer and evolutionary biology, but also in developmental psychology, topology, and cultural anthropology. A promising avenue of future inquiry, which builds on Waddington's writings (rather than the seductive but often misleading imagery) is in the context of individual bacterial cell dynamics: encompassing death, aging, adaptation to changing environments, and related phenomenology. Excitingly, these excursions may unequivocally answer a question first identified as fundamental by Waddington: What *precisely* is a phenotype?