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Landscape, bifurcations, geometry for development

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Abstract

We review recent advances in the mathematical modelling of development, with a special focus on the concepts from non linear physics applied to cellular differentiation and metazoan segmentation. Those models suggest that geometric descriptions with few parameters are sufficient to capture many of the non trivial aspects of development. We also describe open questions such as the connections to machine learning, from network enumeration/evolution to landscape reconstruction.

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Introduction

Explosion in data acquisition and progresses from imaging to genetic engineering have triggered a revolution in biology, allowing for the full exploration of biological complexity at multiple scales. But as our knowledge of complexity increases, a more synthetic understanding of many processes has yet to emerge. Can we dream of guiding "theoretical principles" in biology similar to what we have in physics? This idea has been particularly successful in neuroscience, where phenomenological models (such as the Hodgkin-Huxley model [1]) have been routinely used for years [2].

A relevant example is embryonic development. As pointed out by Siggia [3], many phenomena in development can be well described with the help of Morse-Smale systems, defined loosely as systems converging towards a finite number of fixed points or periodic orbits, both forward and backward in time. This also fits very well with Waddington's physical metaphor of the "epigenetic landscape" to describe development [4]. Since the various characteristics of bifurcations are well understood for mathematical systems, observation and quantification of developmental phenotypes might allow us to recognize generic features and, as a consequence, to build predictive models. Pioneers of such approaches include Turing (who coined the word "morphogen" [5]) and Meinhardt who elaborated on Turing's work to propose reaction diffusion models describing specific steps of development [6]. Another famous example is the "clock and wavefront model" proposed by Cooke and Zeeman and inspired by catastrophe theory [7]. With increasing technical progress, those general ideas can be tested, improved, and new approaches integrating theory and experiments are built. Here, we review several recent Examples in developmental biology.

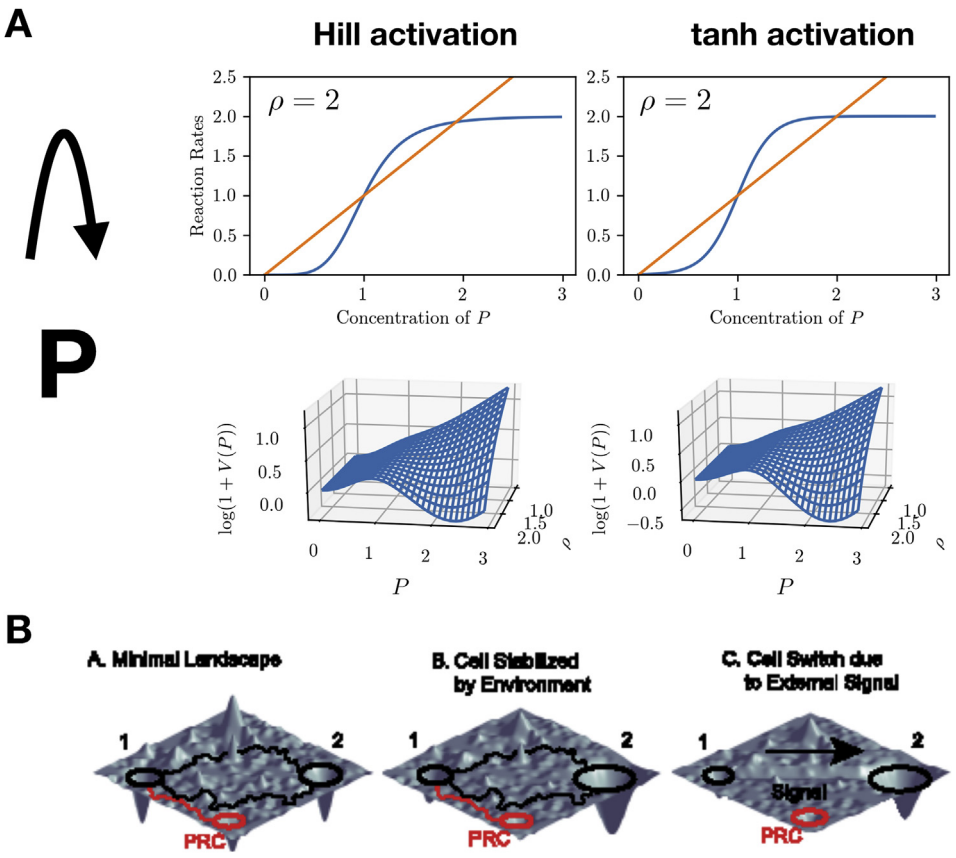
Multistability as the building block

It has been known for some time that multistability (in a dynamical systems sense) is a good model to describe multiple cellular fates. The canonical and simplest example of multistable genetic systems is the self activating gene (Figure 1). This small module ensures that a specific master selector gene (P) sustains its own expression as well as associated targets, locking a cell into a given fate. A simple example will set the picture: assume the dynamics of protein P can be described by a simple equation of the form

$$\tau \frac{dP}{dt} = \rho A(P) - P = -\frac{\partial V}{\partial P} \quad (1)$$

where $A(P)$ is a sigmoidal function, taking values between 0 and 1, and τ the typical time-scale of the system. Choices include Hill function $A(P) = \frac{P^m}{P^m + P_0^m}$, effectively modelling binding of P molecules to its own promoter. Generic hyperbolic functions have also been used e.g. $A(P) = \frac{1+\tanh(\lambda(P-P_0))}{2}$ [8]. In both cases, P_0 represents the intermediate P concentration for which $A(P) = 1/2$ and where the sigmoidal function varies more rapidly with P. It is then not difficult to find parameters for which this system is bistable, as illustrated on Figure 1. Intersection of the "nullclines" (where $\rho A(P) = P$) can define up to three fixed points, two of them being stable. We can also define an effective potential $V(P) = -\rho \int_0^P A(u)du + P^2/2$ to explicitly relate this system to physics and Waddington's landscape. The dynamics of P is described by a damped motion in this potential (see Figure 1). Stable fixed points

Figure 1



Two extreme views of multistability as a building block (A) The simplest gene network showing multistability is the self-activating gene. Top panels show non-linear production rates of P as a function of its own concentration, for two different activation functions $A(P)$ (blue curve, see main text for details), as well as degradation rates (orange curves). Intersection of both curves define possible fixed points. We took $P_0 = 1$, for Hill activation we took $m = 5$, for tanh activation $\lambda = 3$. Bottom panels show associated Waddington-like potentials for different values of ρ, for a fixed value of ρ minima of the potential correspond to stable fixed points. (B) Schematic of potential landscape using Hopfield models, reproduced from Ref. [16]. Many random trajectories can exist for those complex problems (black lines), with partially reprogrammed fates (PRC). Landscape can be changed by external signals.

correspond to minima of the potential, and are interpreted as possible cellular fates.

Examples of this motif include MyoD for muscle fate selection [9], or the combinations of terminal selector genes with terminal selector motifs in *Caenorhabditis elegans* neural specification [10]. Generalization of this motif include the famous "toggle switch", where mutually repressing genes encode a global positive feedback loop via repression of a repressor [11,12]. When dealing with quantitative data, a noise component needs to be included to fully account for the observed dynamics, e.g. using the τ leaping algorithm [13], see e.g. Ref. [14] for an explicit example using a mutually repressing system with diffusion.

When many genes define multiple attractors, one issue is that one can not clearly define a potential in the physics sense, so that it is not so easy to relate more complicated gene networks to Waddington's landscapes. For instance, in the 2D case, if we write $\dot{X} = f(X)$ where $X = (x, y)$ and $f = (f_x, f_y)$ for a system of two mutually repressing genes x, y, there is no reason a priori that $\partial_y f_x = \partial_x f_y$ which would be necessary to define $f = -\nabla V$. Much effort has been done to solve this issue, by rather constructing an effective potential, as high throughput data became more available. For instance, Li and Wang attempted to map reprogramming data on 52 genes on simple two-well potentials [15]. Another approach has been proposed by Mehta and coworkers using Hopfield models, traditionally used to model associative memories. Lang et al. [16] used their method to predict the existence of mixed attracting states reminiscent of partially programmed fates (Figure 1B). One can also use such methods to study the dynamics of cell reprogramming and differentiation itself [17]. Strikingly, data from different reprogramming experiments all collapse on a single 1D-manifold,

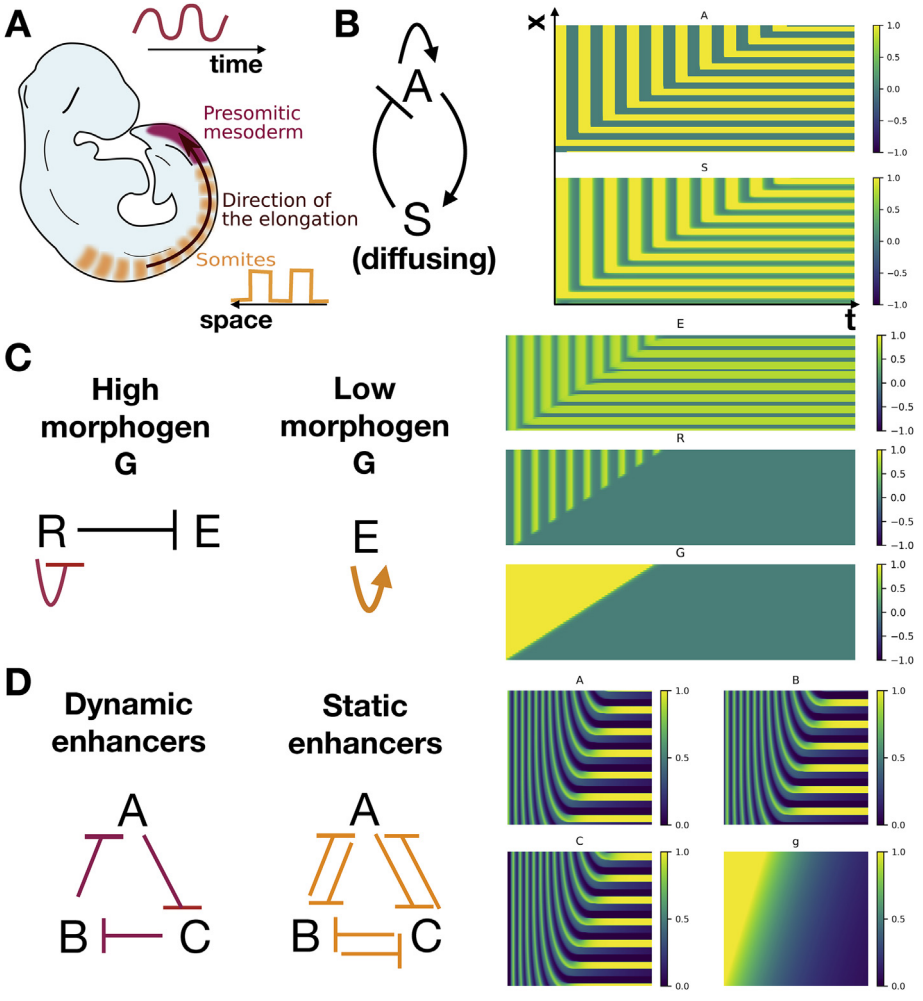
meaning that there is only one coarse-grained pathway to reprogramming, despite the complexity of the system. Stochastic transitions between intermediate fates on this manifold are well described by Poisson rates, in line with what we expect from classical theories of noise-driven dynamics in a landscape with several potential wells.

Changing the landscape

If we zoom out and consider development as a whole, one needs to add a temporal dimension and morphogenetic handles to account for the control of cell differentiation. If cell states are akin to stable states in dynamical system theory, we know that new states should arise through bifurcations. If we further know the controls of the bifurcations (e.g. in the form of morphogens), one might be able to predict the sequence of bifurcations, and to perturb it using external controls.

The original clock and wavefront model for vertebrate segmentation (also called somitogenesis Figure 2A) postulates the existence of an (unknown) moving temporal variable (the wavefront) that interacts with an oscillator, translating the temporal oscillation into a periodic pattern [7]. Meinhardt was the first to propose an explicit biochemical model of the full process where the pattern is encoded through multistability of fates after oscillations in a cellular field [6]. Meinhardt used a

Figure 2



Models of somitogenesis combining oscillations and multistability (A) Schematic of a growing mouse embryo, indicating oscillating (purple) and patterning zones (orange), reproduced from Ref. [28]. (B) Reduced Meinhardt's model (also functionally similar to model proposed in Ref. [30]) with associated kymographs for the two variables *A* and *S*. Segmentation is here initialized by the nucleation of a boundary at low *x*. (C) Somitogenesis model evolved under controlled of a sliding morphogen (re-simulated from Ref. [24]), with kymographs. Colors in the network topology correspond to modules responsible for oscillations (purple) and multistability (orange). (D) Two-enhancer somitogenesis models, inspired by Refs. [31,32]. A graded sliding variable *g* controls the transition from a repressilator oscillator to a multistable system associated to a general slowing down of the system, creating waves of gene expressions.

reaction-diffusion model that shows relaxation oscillations in the absence of diffusion. If two compartments characterized respectively by two steady states (A/P for anterior/posterior), mutually exclude on short-range but reinforce on longer ranges, a sequential pattern APAPAP… appears, that can be interpreted as a segmented embryo. Meinhardt's original model can be easily reduced into a model with only two variables, corresponding to a relaxation oscillator with one fast activator and one slow diffusing repressor (Figure 2B).[1] When the slow repressor diffuses, boundary effects induce a bifurcation from an oscillatory to a bistable phase. Propagation of this bistable phase into the oscillatory phase takes time so that the oscillator can go on cycling in most of the embryo; when the transition happens, the oscillator stops and the chosen steady state (A or not A in reduced variable) depends on the phase of the oscillation. The wavefront here can then be formally defined as the "front" of diffusion of the bistable system.

Cellular differentiation and positional information have further been shown in various contexts to be regulated by both the level and the duration of morphogens, e.g. in the somitogenesis context, modifications of somite positions can be induced by FGF [18,19]. A recent example in another context is neural tube patterning, where Shh level and timing has been shown to control fates [20]. This is an example where theoretical modelling has been crucial to explain the observed dynamics: depending on the level of Shh, the system can undergo bifurcations towards different steady states. This led the authors to suggest the so-called AC-DC topology [21], that is able to implement two different dynamical behaviours depending on parameters [22]: either a repressilator-based oscillation [23], or a bistable system based on mutual repression (or toggle-switch [11,12]).

The idea that specific gene network topologies could specifically lead to non-trivial dynamical behaviour also suggests an inverse problem approach where, starting from the non-trivial observed phenotype, underlying network structure is inferred [24–27]. As an example, we applied in silico evolution to the problem of metazoan segmentation, under the control of a moving morphogen (simulating embryonic growth) [24]. An example of evolved network topology with kymographs is displayed in Figure 2C (model has been evolved with φ-evo software [28]). Network selection favouring emergence of patterns converges towards simple implementations of clock and wavefront models. Evolved networks typically contain oscillators with delayed negative feedback loops or repressilators [23]. Decay of the morphogen triggers transition to a bistable phase controlled by a self activating gene [24,29]. Importantly, the combination of oscillation with multistable states is not explicitly selected, only patterning is, suggesting that such clock and wavefront models are generic and easy to evolve. Another fruitful approach is to systematically enumerate and study small networks performing segmentation [25]; interestingly a mechanism very close to Meinhardt's original proposal combining a relaxation oscillator, a self activating gene, a diffusing repressor and stabilization of pattern by diffusion has been rediscovered with such approach [30].

Riding the wave

Another layer of complexity is added when studying how the transition from a dynamical phase to multistability occurs. Very often, as development moves from a dynamic phase to a more static one, this happens gradually with a general slowing down of the dynamics. In the context of somitogenesis, it is well known that as cells mature in the presomitic mesoderm, the period of the Notch segmentation oscillator increases [33,34], which creates posterior to anterior kinematic waves of genetic expression coupled to segmentation. Waves of segmentation genes are also observed in arthropod segmentation e.g. in *Tribolium* [35] and various Dipterans [36].

Period/wave control in somitogenesis is still not well understood. The period of the segmentation clock was first suggested to diverge to infinity in embryos [33], but the origin of such extreme behaviour is still mysterious since very strong experimental perturbations of Notch signalling only lead to modest changes of periods [37]. Single layer cultures of mice presomitic mesoderm cells display striking exponential increases of period accompanied with "spatial scaling" [38]. This complex behaviour can be explained by a cell-autonomous non-linear slowing down of the period (appendix of [38]) to be contrasted with the more classical model where a morphogen level explicitly controls the period (see also [39] for single cell oscillations in zebrafish). Consistent with this idea, Hubaud also showed that suppression of FGF leads to a gradual slowing down of the period, which is faster as FGF is more stringently suppressed [40]. A given FGF level is thus not associated to a period value; rather it seems that FGF might rather control the slowing down program itself, or in mathematical terms, some time derivative of the period (rather than the period itself). For insect segmentation, controls of the wave might be more direct. In *Tribolium*, changing the level of Caudal has been shown to influence the speed of waves of expression of both gap genes and eve, with lower levels of Caudal corresponding to slower time-scales [32,35]. In long germ band insects such as *Drosophila*, Caudal has been suggested to control the timing of the gap genes shifts [41].

A natural question for physics is the origin of the continuously slowing time-scale explaining the

[1] Jupyter Notebooks used for simulations of Figure 2 are available at https://gitlab.com/pfrancois/review_curr_system_bio.

kinematic waves, and more generally, the nature of the slowing down program suggested by some experiments. Again, dynamical system theory might suggest simple answers. We should first point out that it is a priori unexpected that such period modulation and divergence is observed, since in the standard Hopf bifurcation case, oscillations should disappear with finite period [29]. A first possibility is that there is some external timer, e.g. in the form of an accumulating morphogen, directly controlling transitions between states, which would also naturally explain evolutionary transitions between different modes of development (e.g. short to long germ band in insect) [42,43]. Another possibility is that the slowing down of the system might be the signature of a bifurcation towards multistability *before* it occurs. This so-called "critical slowing down" has been observed in many natural contexts from ecology to neuroscience [2,44]. The very same phenomenon has thus been suggested to control timing of differentiation under control of a dynamic morphogen [31]. Recently, two enhancers systems have been suggested to allow for the refined temporal control of gap genes by morphogenetic information [32]. Those models assume the system continuously interpolates between a "dynamic" and a "static" set of enhancers, under the control of a graded morphogen (Caudal in *Tribolium*). As a consequence, the typical time-scale of the system interpolates between the time-scale of the dynamic phase and an infinite one corresponding to the static system. Those models are close to criticality, and include the non trivial prediction of variability in the speed of the waves in different mutants. Figure 2D illustrates what happens for such a two enhancer model where the dynamic enhancers encode a clock, adapted from Refs. [31,32]: wave dynamics visibly appears (compare Figure 2B–D), providing robust patterning (Jutras-Dubé et al., in preparation).

Another open question is the functional role of such waves in terms of positional information. Meinhardt was again the first to point out that a simple alternation of two fates (A/P) at steady state is not enough to define polarity in the tissue [6]. Wave propagations might then be exploited to correctly pattern the embryo, adding an effective extra state (distinguishing between A/P and P/A boundaries). While this problem is still open in vertebrates, recent works in insects have clarified this question. In Ref. [45], based on evolutionary simulations bridging different Dipteran patterning, it was suggested that so-called secondary pair-rule genes might indeed read the phase of *eve* waves in insects to define polarity. Detailed studies combining theory and experiments by Erik Clark and Michael Akam have indeed established the functional role for the dynamic shift of pair-rule stripes controlled by gap inputs in fly, possibly connecting segmentation in short-germ and long-germ arthropods [43,46].

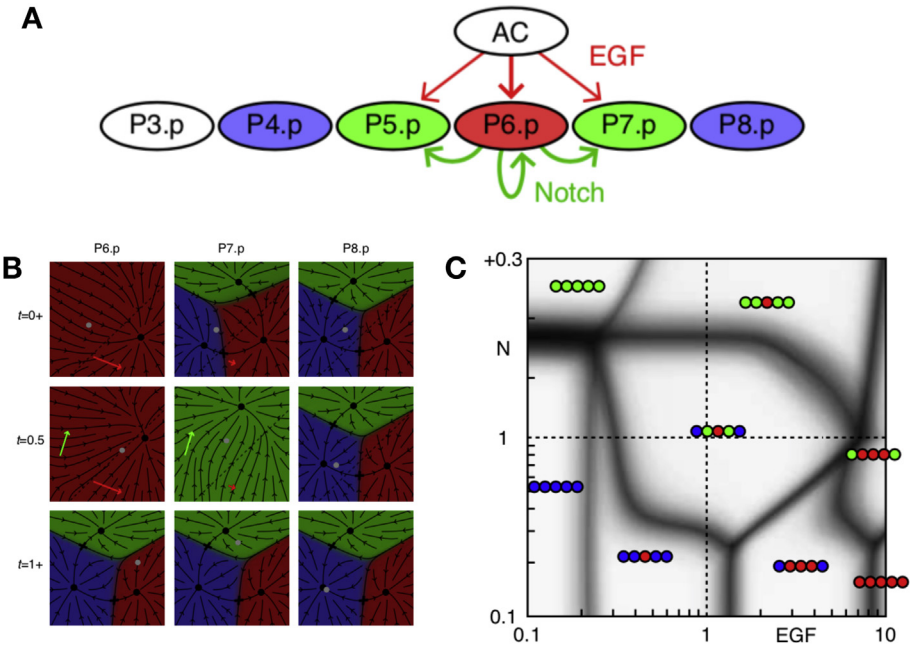
Criticality has also been characterized in stem cell differentiation. Commitment of blood progenitor cells to the erythroid or myeloid lineage present aspects similar to a critical transitions from one steady-state to two new ones [47]. Those observations rely on single-cell measurements of cellular states, and the two criteria to identify a critical transition are an increase of cell-to-cell variability (expected from a "flattening" of the epigenetic landscape) combined to an increase in the gene-to-gene coupling (expected from the appearance of new stable attractors). A last signature of those transitions is the existence of "rogue cells", where cells seem to randomly escape their predefined attractor to stabilize in a well-defined alternative fate, suggesting active negative feedback between mutual fates. Similar signatures have been observed in differentiation of induced pluripotent stem cells toward endodermal or mesodermal lineage [48]. Another signature of criticality might be seen in long range correlations during developmental patterning, as proposed for fly development [49].

Beyond the gene-centric view

Finally, if we take the geometric view of development seriously, it might be possible to think in terms of geometry without modelling explicitly genes. Corson and Siggia have pioneered this approach, in the context of *C. elegans* vulva formation [50,51], with recent applications to other systems such as sensory organ patterns in *Drosophila* [8]. The general idea is to focus on the geometry of the process in an abstract phase space. Defining cellular states as fixed points related by continuous flows, topological properties constrain changes of the flows, basins, and landscape to account for developmental dynamics. It is then possible to fit data to minimally parametrize the flows between the different states and to relate them to known morphogenetic controls. Such models are validated by prediction of mutants and various developmental perturbations. As an example, Corson and Siggia recently produced a "phase diagram" of cell fate choices in a plane defined by EGF and Notch signalling levels [51]. The most non-trivial predictions happen close to critical points in the diagram, where small changes lead to very strong epistatic interactions between EGF and Notch, that are consistent with experiments (Figure 3).

Other approaches have leveraged dynamical system theory concepts to explore dynamics of patterning, to suggest new mechanisms and predictions. A recent example can be found in Sonnen et al. [52] who have successfully entrained two different somitogenesis oscillators (Notch and Wnt). Importantly, they have uncovered a biological role of the relative phase of those oscillators for clock stopping. This suggests that development itself might take cues at a higher phenotypic levels (here a phase shift), validating in retrospect a

Figure 3



Gene-free models for vulva development, reproduced from Ref. [51]. (A) Schematic of the vulva cells, with WT fates and associated signalling pathways. (B) Geometric model of vulva development: as a function of time (different rows), the attractor basin changes and cells reach their final states. (C) Phase diagram showing the expected fates of the P4.p to P8.p cells displayed on panel A as a function of relative signalling pathways level.

more coarse-grained view of developmental dynamics. This is also in line with the puzzling observations that somitogenesis networks are not well conserved between species [53]. The observed genotypic and phenotypic variability between species [54] might thus be better explained and captured by low dimension, coarse grained modelling, rather than by a gene-centric view.

Conclusions and future directions

There is no doubt that dynamical systems theory will remain a major tool for the theoretical understanding of embryonic development. The Examples discussed in this review have confirmed that beyond "data fitting", biological insights can be gained from geometric discussions, e.g. in terms of bifurcations. The approaches described here are fully consistent with the paradigm that a relatively small number of "dimensions" are enough to describe key aspects of development. An open question is the nature of those: are they "emergent" directions from the biological hairball or are there explicit genetic controls (reminiscent of the "omnigenics vs polygenics" debate in population genetics [55])? In some classical examples such as insect segmentation, much can be understood through the observation of a relatively small number of gap and segmentation genes, consistent with the latter hypothesis. Similarly, in gene-free modelling described above, few controls can be identified corresponding to well-known pathways. Epistatic effect could rather be some manifestation of the underlying phase space geometry, close to critical points. In vertebrates, the situation is less clear e.g. a vast number of genes seem to play a role in segmentation. That said controlled experiments of differentiation again suggest that a small number of handles are sufficient [56,57], which is also in line with the (then surprising) fact that stem cells state could be induced with only 4 transcription factors [58]. Even if we can not have a description with a small number of genes, this does not mean that generic rules from dynamical systems theory do not apply. Effective variables could be built using recent advances in machine learning (see e.g. recent experimental and theoretical works on the construction of landscapes from single cell sequencing data [59,60]). As often with machine learning, interpretability of those effective variables might not be straightforward, and methods for model inference and reduction might further help to disentangle the actual biological handles [61–63].

Conflict of interest

Nothing declared.

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