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Physical constraints in cell fate specification. A case in point: Microgravity and phenotypes differentiation

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ABSTRACT

Data obtained by studying mammalian cells in absence of gravity strongly support the notion that cell fate specification cannot be understood according to the current molecular model. A paradigmatic case in point is provided by studying cell populations growing in absence of gravity. When the physical constraint (gravity) is 'experimentally removed', cells spontaneously allocate into two morphologically different phenotypes. Such phenomenon is likely enacted by the intrinsic stochasticity, which, in turn, is successively 'canalized' by a specific gene regulatory network. Both phenotypes are thermodynamically and functionally 'compatibles' with the new, modified environment. However, when the two cell subsets are reseeded into the 1*g* gravity field the two phenotypes collapse into one. Gravity *constraints* the system in adopting only one phenotype, not by selecting a pre-existing configuration, but more precisely shaping it *de-novo* through the modification of the cytoskeleton three-dimensional structure. Overall, those findings highlight how macro-scale features are irreducible to lower-scale explanations. The identification of macroscale control parameters – as those depending on the field (gravity, electromagnetic fields) or emerging from the cooperativity among the field's components (tissue stiffness, cell-to-cell connectivity) – are mandatory for assessing boundary conditions for models at lower scales, thus providing a concrete instantiation of top-down effects.

Contents

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1. Differentiation: a complex issue

A central tenet of modern biology is to understand how a mammalian cell – embryonal or somatic - undergoes differentiation, and how this process will ultimately end up in the development of a tissue, an organ, and an organism (Paldi, 2012).

The current prevailing paradigm in biology posits that biological process can be exhaustively explained according to an 'instructive' molecular model, where, by analogy with the information theory, molecules act as 'signals' and drive the systems in a quasi-deterministic, linear fashion towards specific, irreversible, commitments, ultimately leading the unfolding of a 'program' *already* 'embedded' into genes. However, experimental evidence contradicts strictly deterministic models of cell differentiation (Kupiec, 2009). Furthermore, the theoretical framework behind this approach has been recognized unable in grasping the complexity of living objects (Noble, 2012).

2. Lineage specification

The generation of mature differentiated cells follow the scheme of a tree with branching points. At the top of this hierarchical diagram are totipotent and pluripotent stem cells that can potentially differentiate in any cell types, while endlessly regenerate themselves. Below stem cells, the progenitor cells have a slightly restricted 'differentiating potential', not yet fully committed. They are hence known as multipotent cells, such as the myeloid progenitor within the hematopoietic tree. The lastly remaining branches of that architecture are represented by fully committed (differentiated) cells.

That a single genotype can give rise to more than 200 terminally differentiated cell phenotypes and several times that number of intermediate phenotypes in the human, is usually understood to be due to the 'selective' use of gene products. Yet, how that selection is achieved is far from clear.

According to the classical molecular paradigm, differentiation is viewed as the accomplishment of a 'genetic program' – remnant of the preformationist theory of morphogenesis - where single steps are controlled by linear cascades of regulatory 'signals', supposed to carry 'biological information'. These signals act along a 'chain of command' in which each protein is linearly 'driven' by its upstream controller, and then interacts with its downstream target. The discovery of genetic regulatory elements deemed to be 'tissue-specific promoters (Maniatis et al., 1987), provide a mechanistic rationale for such 'program', which precisely 'instructs' cell how to differentiate by means of the selective activation of a specific pattern of gene expression. This so-called 'instructive' model has fostered the search for 'master regulators', supposed to regulate diachronically the program of phenotypic differentiation through the sequential activation of a small set of integrated transcription factors.

In some cases, ectopic-expression experiments have supported the in*structive* capacity of specific cytokines even though a variety of artifacts biased these models (Rieger et al., 2009). Thereby, the biological meaningfulness of these findings resulted questionable (Orkin and Zon, 2008), while several lines of evidence suggest that a purely 'instructive' program of cell fate determination is unable in capturing the whole picture.

In fact, attempts to genetically manipulate differentiating-related genes to steer cellular differentiation had been ineffective, whereas experiments focused to directly differentiate stem cells in a predetermined direction resulted almost invariably in less than 50% efficiency (Robb, 2007). Moreover, transgenic animals with pivotal genes 'knocked-out' often yield no phenotypes or unpredicted phenotypes (Enver et al., 1998; McArthur et al., 1995). For instance, insertion of the erythropoietin receptor into macrophage precursors allows erythropoietin to stimulate macrophage colony formation, without promoting the *de novo* growth of red blood cells. Conversely, insertion of the macrophage colony-stimulating factor receptor into erythroid precursors allows M-CSF to stimulate the development of erythroid clusters (McArthur et al., 1994). In addition, single cell experiments showed that, in contrast with current beliefs, differentiation could occur even in the absence of growth factors (McArthur et al., 1994, 1995).

Likewise, this approach could hardly accommodate with models provided by classical molecular biology, assuming that phenotypes are 'determined' by the activity of specific signaling pathways. Indeed, 'signaling pathways' are insufficient in 'determining' (only on their own) the high number of differentiated phenotypes we witness in living organisms (Enver et al., 2009). Therefore, it should hypothesized that the same signaling protein/factor can participate in different signaling pathways, where the context – including microenvironmental constraints - ultimately shape the differentiating outcome.

Eventually, the discovery of rare cells (Adolfsson et al., 2005) (at least during hematopoiesis differentiation) that do not fit into the recognized hierarchy of cell types, seem that even more lineage-specific signaling pathways should be hypothesized, thus largely exceeding those till now identified. Pathological phenotypes (including cancer), for which no 'specific' pathways have been discovered to date, should be included among such 'unexpected' phenotypes too (Soto et al., 2008).

As a result, the classical view of a *linear differentiation process* driven by the sequential activation of master regulators has been increasingly challenged in the last few years by both experimental findings and theoretical considerations. Therefore, cell fate specification cannot be longer considered to be encompassed by a strictly deterministic instructive program.

3. Stochasticity and gene regulatory networks

To deal with this complexity, several bottom-up modelling approaches, which focus on the behavior of individual molecular components and their local interactions, have been proposed since the seventies. These models adopted the well-known Waddington's diagram, featured by hills and valleys linked each other through branched pathways to portray the differentiation tree (Waddington, 1957). Both valleys and hills are determined by calculating values of state variables recognized by Gene Regulatory Networks (GRNs) models, where the mean trajectory of the observational data are obtained by numerically solving ordinary differentiated equations (ODE). This procedure usually leads in identifying several activation states, as featured by specific sub-set of gene expression patterns' (Wu et al., 2014). In the seventies, Kaufmann formalized the nebulous notion of landscape by identifying the valleys with attractors in Boolean networks (Kauffman, 1969), while, later, Huang et al. (2005) deepened this concept further into a multidimensional dynamical systems framework.

The GRN allows identifying an 'attractor' – able in "orchestrating" the process of transcription to produce a profile of active gene products - which support the macroscopic emergence of a cell phenotype. The attractors are contingent on the structure of proteins and the target DNA sequences, and are therefore "hard-wired" in the genome.

According to these attempts, Waddington's intuition of genetic control of the landscape is conceptualized by using gene expression profiles projected onto an *n*-dimensional phase space, with vector fields, where stable states are considered as 'attractors'. Activity of

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