How to address cellular heterogeneity by distribution biology

Niko Komin\textsuperscript{a}, Alexander Skupin\textsuperscript{a,b}

\textsuperscript{a}Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Belval, Luxembourg
\textsuperscript{b}University California San Diego, La Jolla, USA

Abstract

Cellular heterogeneity is an immanent property of biological systems that covers very different aspects of life ranging from genetic diversity to cell-to-cell variability driven by stochastic molecular interactions, and noise induced cell differentiation. Here, we review recent developments in characterizing cellular heterogeneity by distributions and argue that understanding multicellular life requires the analysis of heterogeneity dynamics at single cell resolution by integrative approaches that combine methods from non-equilibrium statistical physics, information theory and omics biology.

Introduction

Life is heterogeneous – at nearly all biological scales and levels \cite{1}. Maybe the most obvious heterogeneity can be observed when looking at the different species that evolution has created. But heterogeneity is much further spread in living systems where individuals within one species exhibit unique properties and even cells of the same cell type within the same (multicellular) organism can possess a wide range of divergent characteristics.

An essential function of heterogeneity is to ensure robustness of a biological system in fluctuating environments \cite{2}. Thus, the spread of phenotypic traits within a population of a species allows for a broader niche in which the population as a whole can survive and adapt to different conditions including competition for resources. This central mechanism of life has led to the general perception that "Nothing in Biology Makes Sense Except in the Light of Evolution" as stated by Theodosius Dobzhansky in his landmark essay in 1973 \cite{3}. While Dobzhansky was mainly focusing on the diversity of different organisms and criticizing creationism, his conclusion may be also instructive to address the currently urgent open question on multicellularity as the foundation of multicellular organisms and microorganism colonies.

A central problem in multicellularity arises as to how an organism can give rise to all desired different cell types originating from the same genome in an coordinated manner \cite{4, 5}. Considering the concept of evolution as a general underlying mechanism of life with its two major components, mutation and the interplay of induction and selection, we may scale Dobzhansky’s concept down to the level of cell populations. Thereby, mutations can be generalized to immanent heterogeneity and the interplay between induction and selection represents intra- and intercellular signaling. Hence, understanding (multicellular) life across its different scales relies on investigating cellular nanoevolution - that is the dynamics of cellular heterogeneity.

The origin of cellular heterogeneity (even within clonal populations) is the multiscale organization of life as depicted in Fig. \ref{fig:1}. On the smallest relevant scales, the stochastic nature of molecular interactions induces individual transcription profiles that are subsequently translated into heterogeneous cellular phenotypes. These rather randomly induced phenotypes are subsequently instructed and selected on the level of the cell population by intercellular signaling or cell-cell interactions leading to the coordinated generation of tissues, organs and organisms. This underlying noise-driven cellular heterogeneity is the mechanism to balance cellular robustness and adaptability \cite{6}.

---

Email addresses: niko.komin@uni.lu (Niko Komin), alexander.skupin@uni.lu (Alexander Skupin)

Preprint submitted to Elsevier 15th December 2016
Until recently, cellular heterogeneity was only accessible on rather low dimensional readouts such as specific protein abundance by antibody staining or since more recently by flux cytometry analysis. These limited investigations hindered a more systematic approach to dissect underlying mechanisms. But with the recent developments of several single cell analysis methods [7, 8, 9, 10, 11, 12], we are now able to characterize cellular heterogeneity in great detail. Despite these advancements, a systematic approach how to interpret and use the resulting high-dimensional data for identifying biological principles of multicellular life is still lacking. Currently, methods from statistical physics are intensively discussed to be exploited but the underlying non-equilibrium dynamics and biological complexity of intra- and intercellular interactions make a direct application difficult [6, 13, 14].

While previous reviews summarize potential functions of cellular heterogeneity from an experimental perspective [2, 15], we will review here more recent developments and how mathematical modeling and analysis can be used to reveal general functions and guiding principles from the central descriptor of heterogeneity – the (phenotype) trait distribution.

**Characterization of cellular heterogeneity**

Heterogeneity of cells can have different properties (Fig. 2) and lead to multiple benefits for the population [2, 16, 15]: it ensures robustness of the biological system to fluctuating environments and allows the population to adapt to a wider variety of environments (bet hedging); binary decisions on a cellular scale can yield a fractional or dose-dependent response; rare individuals can coordinate population behavior by emitting local signals; subpopulations can be primed for multiple cell fates; transmitted and encoded information can be more complex.

**Direct phenotypic heterogeneity**

Cell heterogeneity is observed relatively easily when it directly affects phenotypic traits such as size distributions (e.g. axon diameter variability [17]) or cell survival times or cell division times [18, 19]. The main driver of heterogeneity can be an underlying genetic variability as exemplified in Fig. 2A by the biomass production of yeast strains. The observed trait variability within the two strains YO490 and YO512 is much smaller compared to the distribution of the progenies originating from a cross of these two parental strains. The non-trivial effects of the genetic recombination and the genome-environment interaction lead to a wide range of biomass production for the progenies that is not simply between the parental strains but exhibits more extreme traits.

But cellular heterogeneity does also occur independent of genetic diversity such as in clonal populations due to the multiscale organization depicted in Fig. 1. Here, the stochastic nature of molecular interaction induces random transcription profiles that are subsequently modified in an environment dependent manner including signaling between cells. An related and medically important example of such a coordinated heterogeneity is the epithelial-to-mesenchymal transition (EMT) and its counterpart MET as a mechanism for metastasis [20]. The underlying mechanism is that a cancerous epithelial cell can transdifferentiate into a mobile mesenchymal cells that is subsequently leaving the tumor, can travel with the blood stream to distinct tissue sites where it transforms back into an epithelial cell and initiating a secondary tumor.

The underlying mechanism can also be studied in model cell lines like clonal HMLER cells where e.g. mesenchymal cell identity can be determined by flux cytometry using cell surface markers such as CD44 [21]. The HMLER population exhibits a heterogeneous steady-state distribution as shown in Fig. 2B where a unimodal phenotype distribution (blue) generated by cell sorting is relaxing towards a stable bimodal distribution (gray) that represents a mixture of epithelial (low CD44) and mesenchymal (high CD44) cells. Computational modeling has predicted that partial EMT instead of complete EMT is associated with tumor progression [22] and is currently under experimental investigation. Furthermore, EMT has been recently used as a show case that the dynamics of gene circuits is mainly determined by their topology and not by specific parameters that may explain the stable bimodal trait distribution [23].

2
Dynamical heterogeneity

As illustrated by the example of EMT (Fig. 2B), dynamics is essential for cellular heterogeneity development. But also intracellular dynamics itself can exhibit large variability. The maybe best studied stochastic dynamics in biology is the firing pattern of neurons [17]. The two main drivers of the random dynamics are (i) the channel noise that originates from the stochastic molecular interactions and (ii) the plethora of synaptic connections each neuron has to neighboring cells. Theoretical and computational approaches during the last decades have revealed the rich dynamical spectrum of noise-driven neuronal dynamics where non-linearities lead to non-trivial effects like stochastic and coherent resonance in excitable media [24].

More recently, we have described similar characteristics in Ca$^{2+}$ oscillations [25] of non-electrically excitable cells. Ca$^{2+}$ as a central messenger in eukaryotic cells transmits external signals into the cell by transient increases of the cytosolic Ca$^{2+}$ concentration as those shown in Fig. 2C. While these transients have been referred to as oscillations, we have demonstrated that they occur randomly with a well defined probability density function $P(T)$ shown in Fig. 2C for astrocytes (blue) and HEK cells (red). We combined statistical analysis [26] with multiscale modeling [27] to demonstrate that experimental observations are consistent with an hierarchical signaling system where molecular noise of individual channels are carried onto the level of the cell by diffusion mediated coupling of release channels. Interestingly, the cellular heterogeneity exhibits cell-type and pathway specific signatures (probability distributions $P$) that are also encoded in the signal variability [28].

On a slower temporal scale, the circadian rhythm between sleep and activity of mammals is generated by a number of neurons in the suprachiasmatic nucleus which show phases of spontaneous firing alternating with quiet phases. These sleep-wake cycles show periods distributed over a wide range of durations when dispersed in a culture. Coupled in the tissue however, they generate a rather precise rhythm in synchrony with an external light stimulus [29]. For a model of coupled biochemical clocks, subjected to periodic forcing we showed that an intermediate value of period dispersion actually augments the entrainability to the stimulus [30]. In a similar context an intermediate dispersion in the glutamate/orexin threshold brings the sleep-wake cycle close to the optimal value [31].

Cellular omics heterogeneity

While the examples on cellular heterogeneity revised above are based on low-dimensional observations, recent developments in single cell analysis methods allow now for characterizing cellular heterogeneity on the genomics and transcriptomics level [12, 32, 33, 34]. Characterizing the resulting high-dimensional information by physiological means is essential for the identification of biological mechanisms. The set of measured genes can be described as a vector in a gene expression space and cluster analysis is commonly used to group genes together by correlation and deducing functional proximity [35]. Other complexity reducing methods based on for example principle component analysis (PCA) [36], stochastic neighboring embedding (tSNE) [37] or diffusion maps including pseudo-time ordering represent useful tools to investigate correlative interactions in the high dimensional data [38].

Despite the detailed characterization of cellular heterogeneity, an integrative approach how to use the resulting high-dimensional data to understand principles of multicellularity is missing. Recently, we applied dynamical system theory to single cell transcription data to investigate blood cell differentiation [39]. By treating hematopoietic stem cells with either EPO or IL-3/GM-CSF we could induce differentiation into erythrocytes (red blood cells) and myeloid (bone marrow) cells, respectively. Using single cell transcription analysis, we obtained distinct gene expression profiles a subset of which is shown in Fig. 2D for stem cells (black), the erythroid (magenta) and myeloid (blue) populations. To investigate the underlying differentiation dynamics we were analyzing correlations between cells and genes in a time and treatment dependent manner. In agreement with insights from dynamical systems theory we could demonstrate that differentiation occurs by the destabilization of an stem cell attractor and the subsequent drug-induced development of the 2 lineage attractors as visualized in Fig. 2E by a data-inferred epigenetic landscape (stem cells shown in gray, erythrocytes in red and myeloid cells in blue). Additional analysis based on properties of imperfect bifurcations validated experimentally that the differentiation dynamics exhibit characteristics of critical transitions [40] including transient increase of heterogeneity and critical slowing down.
In a similar approach to transcription data sets of Drosophila, criticality of a biological system was investigated. The developmental gap gene network in the fruit fly embryo is most likely presented by two mutually repressive genes and this simple genetic network can be tuned to a critical point where one of the eigenvalues vanishes. If spatially coupled, the resulting system exhibits slow dynamics, strong local (anti-)correlations, and long-ranged correlations in space, signs of criticality which can also be validated quantitatively in the experimental data [41].

Use of genetic heterogeneity to identify molecular mechanisms

The scope of the studies mentioned so far was to dissect the origin and function of cellular heterogeneity of different natures by combining quantitative experimental methods with theoretical and computational approaches. But genetic cellular heterogeneity can also be exploited to identify molecular mechanisms of phenotypic traits. For these approaches the awesome power of yeast genetics is often used to introduce genetic heterogeneity in a controlled manner by generating a large number of progenies from two parental strains (Fig. 2A). The phenotypic diversity can then be mapped to specific gene loci by statistical inference or information theory methods [42].

Using such an approach to understand the underlying reason for different morphologies of yeast colonies, we could identify chromosomal copy number variation as a multicellular phenotype switch [43]. Recently, Rabinowitz and colleagues were analyzing metabolic traits across different yeast cultures and inferred the data into Michaelis-Menten relationships between enzyme, substrate and products. This systematic analysis revealed previously unknown cross-pathway regulations and demonstrated that substrate concentrations are the strongest driver of metabolic reactions [44].

While monocausal genetic factors can be identified rather robustly, many phenotypic traits including disease development often result from interactions of several genes. The corresponding combinatorial explosion has to be compensated by large genotype-phenotype data sets that can be analyzed by statistical methods. To address non-linear genetic interactions, we have recently investigated different data sets by information theory based methods [42]. By applying an entropy approach to the phenotype distributions, we were able to identify genetic interactions in the sporulation efficiency of yeast [45] shown in Fig. 2F and introduced the general measure of interaction distance for the identification of genetic synergies.

Conclusion

Cellular heterogeneity is an inmanent property of biological systems originating from the multiscale organization of life shown in Fig. 1. This mechanism of random induction and context dependent adaptation (or selection) can be seen as the establishment of a cellular nanoevolution where cells perform random walks within a general configuration space (the epigenetic landscape) according to a generalized chemotaxis. This analogy emphasizes that evolutionary strategies may represent a generic biological principle and that multicellularity has to consider the dynamics of cellular heterogeneity.

To address this heterogeneity dynamics, we have to integrate the distributions covering the variability at the different biological scales and levels (Fig. 2). While such an integration has already been successfully performed on low dimensional traits like predicting glycogen distributions in vitro based on maximal entropy principles [46] and developing a corresponding theoretical description by open chemical network theory [47], a generalization to heterogeneous and high-dimensional systems is not trivial. A major complication arises therein from the strong non-equilibrium character of life and the different nature of distributions that ranges from genetic heterogeneity [43], noise induced cell-to-cell variability on the transcription and protein level [39] as well as individual dynamic properties that may originate from specific subcellular arrangements [27]. This complexity renders a direct application of distribution based approaches from mathematics and statistical physics complicated.

Nevertheless, distributions provide a promising perspective to tackle cellular heterogeneity and its role in multicellular life because they can bridge between biological complexity and methods from non-equilibrium statistical physics [5] and information theory [49]. Moreover, the recent experimental innovations in single cell analysis approaches enable now the generation of comprehensive data sets needed for the successful
development of a distribution biology framework that goes beyond the established network biology. Thereby the biological complexity may also trigger further theory developments in mathematics and physics indicating that, in accordance with Dobzhansky, "seen in the light of evolution, biology is, perhaps, intellectually the most satisfying and inspiring science" [3].

References

References

[1] Steven J. Altschuler and Lani F. Wu. Cellular heterogeneity: do differences make a difference? Cell, 141(4):559–563, May 2010.
[2] Martin Ackermann. A functional perspective on phenotypic heterogeneity in microorganisms. Nature Reviews Microbiology, 13(8):497–508, Jul 2015.
[3] Theodosius Dobzhansky. Nothing in biology makes sense except in the light of evolution. The American Biology Teacher, 35(3):125–129, 1973.
[4] Sui Huang. Cell lineage determination in state space: A systems view brings flexibility to dogmatic canonical rules. PLOS Biology, 8(5):e1000380+, May 2010.
[5] Sui Huang. Non-genetic heterogeneity of cells in development: more than just noise. Development, 136(23):3853–3862, December 2009.
[6] Jordi Garcia-Ojalvo and Alfonso Martinez Arias. Towards a statistical mechanics of cell fate decisions. Current Opinion in Genetics and Development, 22(6):619–626, Dec 2012.
[7] Akira Sato, Sumito Koshida, and Hiroyuki Takeda. Single-cell analysis of somatotopic map formation in the zebrafish lateral line system. Developmental Dynamics, 239(7):2058–2065, Jul 2010.
[8] Pratik K. Chattopadhyay, Todd M. Gierahn, Mario Roederer, and J Christopher Love. Single-cell technologies for monitoring immune systems. Nature Immunology, 15(2):128–135, Feb 2014.
[9] Anoop P. Patel, Iyai Tirosch, John J. Trombetta, Alex K. Shake, Shawn M. Gillespie, Hiroaki Wakimoto, Daniel P. Cahill, Brian V. Nahed, William T. Curry, Robert L. Martunez, David N. Louis, Orit Rozenblatt-Rosen, Mario L. Suv, Aviv Regev, and Bradley E. Bernstein. Single-cell rna-seq highlights intratumoral heterogeneity in primary glioblastoma. Science, 344(6193):1396–1401, Jun 2014.
[10] Alex K. Shake, Rahul Satija, Joe Shuga, John J. Trombetta, Dave Gennert, Diana Lu, Peilin Chen, Rona S. Gertner, Jellert T. Gaublomme, Nir Yosef, Schraga Schwartz, Brian Fowler, Suzanne Weaver, Jing Wang, Xiaohui Wang, Ruihua Ding, Rakitma Raychowdhury, Nir Friedman, Nir Hacohen, Hongkun Park, Andrew P. May, and Aviv Regev. Single-cell rna-seq reveals dynamic paracrine control of cellular variation. Nature, 510(7505):363–369, Jun 2014.
[11] Jan Philipp Junker and Alexander van Oudenaarden. Every cell is special: genome-wide studies add a new dimension to single-cell biology. Cell, 157(1):8–11, Mar 2014.
[12] Evan Z. Mocasco, Anindita Basu, Rahul Satija, James Nemesh, Karthik Shekhar, Melissa Goldman, Itay Tirosh, Allison R. Bialas, Nolan Kamitaki, Emily M. Martersteck, John J. Trombetta, David A. Weitz, Joshua R. Sanes, Alex K. Shake, Aviv Regev, and Steven A. McCarroll. Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets. Cell, 161(5):1202–1214, May 2015.
[13] Elisabet Pujadas and Andrew P. Feinberg. Regulated noise in the epigenetic landscape of development and disease. Cell, 148(6):1123–1131, Mar 2012.
[14] Ben D. MacArthur and Ihor R. Lemischka. Statistical mechanics of pluripotency. Cell, 154(3):484–489, Aug 2013.
[15] Hannah Dueck, James Elserwine, and Junhyong Kim. Variation is function: Are single cell differences functionally important? BioEssays, 38(2):172–180, February 2016.
[16] Bruno M. C. Martins and James C. W. Locke. Microbial individuality: how single-cell heterogeneity enables population level strategies. Current Opinion in Microbiology, 24:104–112, April 2015.
[17] György Buzsáki and Kenji Mizuseki. The log-dynamic brain: how skewed distributions affect network operations. Nature Reviews Neuroscience, 15(4):264–278, Apr 2014.
[18] Karen E. Gascoigne and Stephen S. Taylor. Cancer Cells Display Profound Intra- and Interline Variation following Prolonged Exposure to Antimitotic Drugs. Cancer Cell, 14(2):111–122, August 2008.
[19] Sabrina L. Spencer, Suzanne Gaudet, John G. Albeck, John M. Burke, and Peter K. Sorger. Non-genetic origins of cell-to-cell variability in TRAIL-induced apoptosis. Nature, 459(7245):428–432, Apr 2009.
[20] Jean Paul Thiery, Hervé Acloque, Ruby Y J. Huang, and M Angela Nieto. Epithelial-mesenchymal transitions in development and disease. Cell, 139(5):871–890, Nov 2009.
[21] Anne Grosse-Wilde, Aymeric Fouquier d’Hérouël, Ellie McIntosh, Gökhan Ertaylan, Alexander Skupin, Rolf E. Kuestner, and Antonio del Sol, Kathie-Anne Walters, and Sui Huang. Stemness of the hybrid epithelial/mesenchymal state in breast cancer and its association with poor survival. PLoS One, 10(5):e0126522, 2015.
[22] Mohit Jolly, Satyendra Tripathi, Dongya Jia, Steven Mooney, Muge Celiktas, Samir Hanash, Sendurai Mani, Kenneth Pienta, Eshel Ben-Jacob, and Herbert Levine. Stability of the hybrid epithelial/mesenchymal phenotype. Oncotarget, 7(19), 2016.
[23] Bin Huang, Mingyang Lu, Dongya Jia, Eshel Ben-Jacob, Herbert Levine, and Jose Onuchic. Interrogating the topological robustness of gene regulatory circuits. bioRxiv, page 084962, 2016.
[50] Sean P. Farris, Robert A. Harris, and Igor Ponomarev. Epigenetic modulation of brain gene networks for cocaine and alcohol abuse. *Frontiers in Neuroscience*, 9:176, 2015.

[51] James W. Putney Jr. and Gary St. J. Bird. The inositol phosphate-calcium signaling system in nonexcitable cells. *Endocrine Reviews*, 14(5):610–631, Oct 1993.
Figure 1: **Multiscale organization of life.** Considering the different scales of multicellular cell fate from noisy gene expression to intercellular signaling enables adaptation and reliable morphogenesis. (Some figure elements are taken from [50][51] and permissions are currently pending.)
Figure 2: Different aspects of heterogeneity. A: The biomass production of two different yeast strains and progenies from a cross of these exhibit different variability where the heterogeneity of the progenies is increased due to the genetic variability [43]. B: Heterogeneity of a cell surface marker (CD44) in a clonal population of HMLER cells [21] characterized by flux cytometry. After sorting for mesenchymal cells (blue bottom) the cell population relaxes towards a stable mixture of epithelial and mesenchymal cells (gray) by mesenchymal-to-epithelial transition over 6 weeks. C: Dynamic heterogeneity in cell signaling illustrated by calcium imaging within astrocytes exhibits random spiking behavior [25]. Thus, spikes occur randomly with the shown probability density for astrocytes (blue) and HEK cells (red). The stochastic process can be further characterized by the Fano factor (inlet) [20]. D: Recent developments in single cell analysis methods allow for characterization of single cell transcription profiles as shown here for blood cell development from stem cells (black) to erythrocytes (magenta) and myeloid cells (blue) that exhibit distinct gene expression signatures [39]. E: To characterize differentiation dynamics, single cell transcriptomics data can be combined with dynamic systems theory to identify transitions between distinct cell state attractors as shown here by an inferred epigenetic landscape based on a PCA analysis of blood cell development where gray spheres correspond to stem cells, blue to myeloid cells and red to erythrocytes. F: Genetic heterogeneity such as generated by yeast crosses (A) can be used to identify genetic basis of phenotypic traits by statistical and information theory based methods [42].