Chapter 15

Mathematical Models of Pluripotent Stem Cells: At the Dawn of Predictive Regenerative Medicine

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Abstract

Regenerative medicine, ranging from stem cell therapy to organ regeneration, is promising to revolutionize treatments of diseases and aging. These approaches require a perfect understanding of cell reprogramming and differentiation. Predictive modeling of cellular systems has the potential to provide insights about the dynamics of cellular processes, and guide their control. Moreover in many cases, it provides alternative to experimental tests, difficult to perform for practical or ethical reasons. The variety and accuracy of biological processes represented in mathematical models grew in-line with the discovery of underlying molecular mechanisms. High-throughput data generation led to the development of models based on data analysis, as an alternative to more established modeling based on prior mechanistic knowledge. In this chapter, we give an overview of existing mathematical models of pluripotency and cell fate, to illustrate the variety of methods and questions. We conclude that current approaches are yet to overcome a number of limitations: Most of the computational models have so far focused solely on understanding the regulation of pluripotency, and the differentiation of selected cell lineages. In addition, models generally interrogate only a few biological processes. However, a better understanding of the reprogramming process leading to ESCs and iPSCs is required to improve stem-cell therapies. One also needs to understand the links between signaling, metabolism, regulation of gene expression, and the epigenetics machinery.

Key words Regenerative medicine, Systems biology, Mathematical modeling, Predictive models, Stem cells, Pluripotency

1 Introduction

Regenerative medicine aims to repair or regenerate tissues or organs with impaired functions, as an alternative to organ transplantation from donors. Approaches based on the use of the patient's own cells have the potential to overcome obstacles such as ethical concerns, limited donor availability, or transplant rejection. Such approaches often require derivation, generation, or manipulation of stem cells. Establishment of techniques for generation of stem cells to be used in regenerative medicine is a very active field of research [1].

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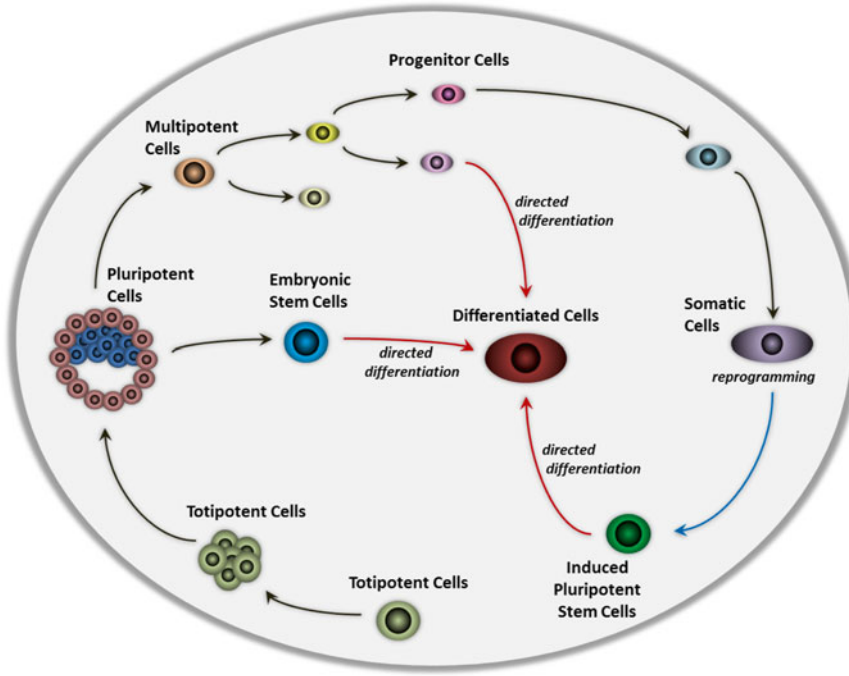

Fig. 1 Differentiation and reprogramming of cells. Embryonic stem cells can be derived from the inner cell mass (illustrated as *blue* cells) at the blastula stage of embryos, and can be differentiated into various cell types under laboratory conditions. Somatic cells can also be re-programmed into induced pluripotent stem cells, which can then be differentiated into desired cell types. See the main text for definition of potency of illustrated cell types

Stem cells have the potential to differentiate into more specialized cells in the multi-cellular organisms (Fig. 1). They also have the ability of self-renewal or proliferation, i.e., to generate cells identical to themselves via mitosis. The differentiation of stem cells is a progressive process, specialization increasing at each step. The zygote and the daughter cells generated via the first couple of cell divisions which can give rise to all cell types in the embryo and the placenta are called *totipotent*. More specialized embryonic stem cells which can give rise to all cell types in the embryo, but cannot contribute to the placenta are called *pluripotent* [2]. The pluripotent cells in pre-implantation embryo and post-implantation have distinct gene expression profiles, developmental and functional characteristics, and are called *naive* and *primed*, respectively [3]. As development of the embryo progresses, more specialized cells are generated, such as *multipotent* stem cells that can give rise to several tissues, *oligopotent* (or *progenitor*) cells that can only generate a set of closely related tissues, and terminally differentiated somatic cells. With the exception of the germinal lineage, mammals are mostly constituted of somatic cells. However, a limited number of adult stem cells keep regenerating tissues throughout the lifetime

of the organism, such as hematopoietic stem cells giving rise to blood cells, muscle satellite cells, or neural stem cells [4].

Stem cells can be derived from a developing embryo or an adult, and the derived cell lines can be kept proliferating indefinitely or be differentiated into somatic cells in controlled niches. It is also possible to reprogram differentiated somatic cells into pluripotent cells via forced expression of proteins or continued exposure to external cues. Such reprogrammed cells are called *induced pluripotent stem cells* (iPSC) and can subsequently be re-differentiated into tissues [5]. Therefore, stem cells have the potential to be an infinite source of "spare-parts" for regenerative medicine as well as providing a model for drug and disease pathway discovery [1, 6, 7]. Stem cell science recently achieved two milestones, both of which came as a result of decade-long efforts: (1) The generation of human naive iPS cells from somatic cells [8, 9] and (2) the first clinical trial of a stem cell therapy via directed differentiation of human iPS cells into retinal pigment epithelium cells [10]. These milestones increased the confidence in stem cell science and raised the expectations from regenerative medicine.

Computational biology is central to large-scale studies on stem cells, aiding extraction of meaningful information from large data sets, abstraction of biological systems for systematic analysis of their properties and ultimately predicting previously unknown relationships to guide future studies. Mathematical models and simulations have been used for a long time to understand the regulation of stem cell pluripotency and their differentiation and recent developments saw the rise of complex experimentally based models [11, 12]. It is likely that as for any other areas of bioengineering, models will provide major contributions to regenerative medicine research in the near future.

Reprogramming and differentiation of stem cells are regulated by the activity of gene regulatory networks controlled by several signaling pathways and the epigenetics machinery. Signaling pathways are triggered by the signaling molecules that bind their ligands on the cell surface or by the physical forces experienced by the cells; leading to a cascade of intracellular protein–protein interactions which in turn modulate the activity of the transcription factors in the downstream gene regulatory networks. Resulting gene expression profiles establish the composition of the cellular protein pool and ultimately, the phenotype.

The signaling pathways and gene regulatory networks that are actively modulated in pluripotent cell lines and pre-implantation embryos are shown in Fig. 2a. In addition, epigenetic factors may have a role in regulating the gene expression. Factors such as RNA interference, DNA methylation, nucleosome occupancy, and modifications on histone tails correlate with gene expression levels; these epigenetic factors are widely accepted as the barrier between transformation of cells into other cell types [13].

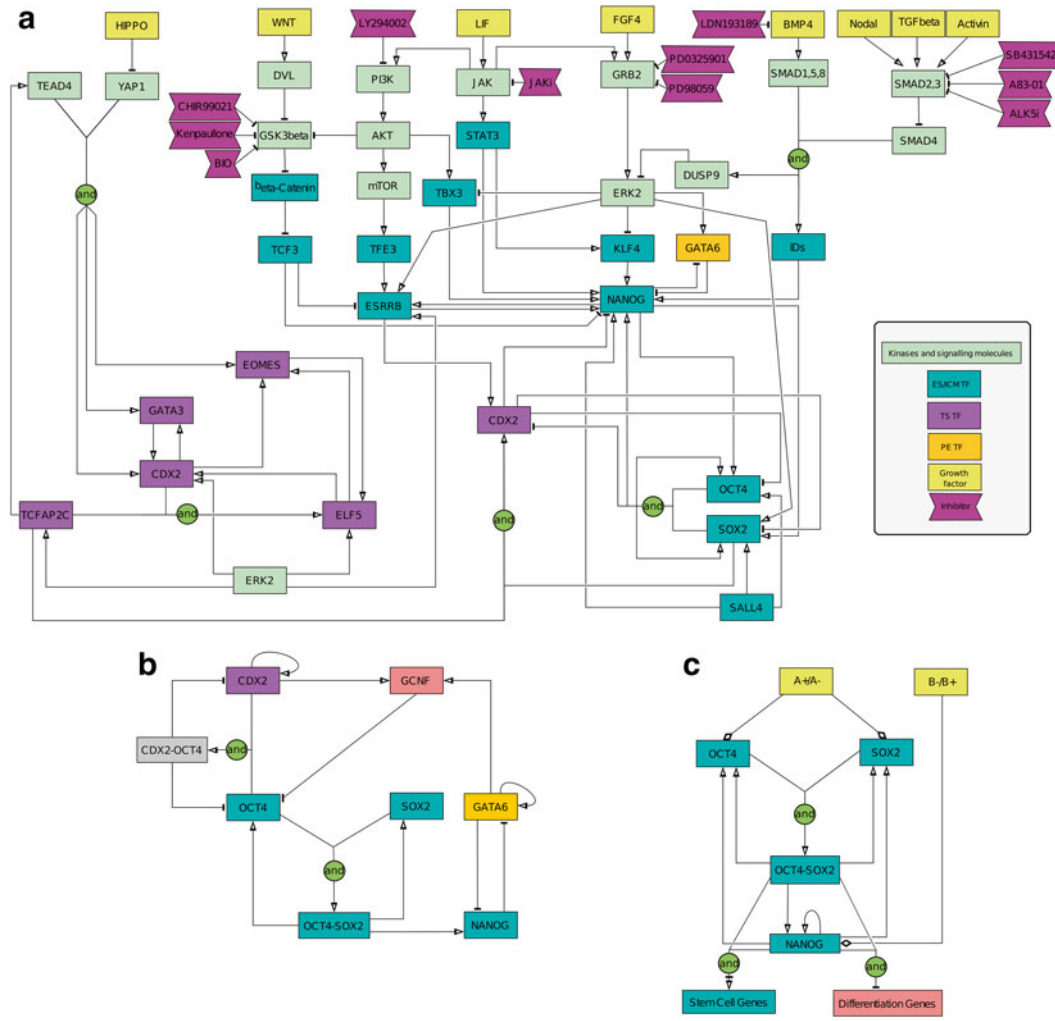

Fig. 2 Pluripotency gene regulatory networks: activity flow map of the pathways regulating pluripotency (using the SBGN Activity Flow notation [110]). The nodes represent activities (of proteins or small chemicals) whereas the edges represent the effect of activities on each other. Proteins typically expressed in embryonic stem cells are shown in *turquoise*, proteins typically expressed in trophoblast stem cells are shown in *violet*, proteins typically expressed in primitive endoderm are shown in *orange* in all three panels. (**a**) Signaling networks and GRN in mouse pluripotent stem cells (modified from [90]) *Yellow boxes* indicate growth factors (or cytokines), whereas *light green boxes* indicate components of signaling pathways downstream to growth factors leading to up- or downregulation of transcription factors. Inhibitors are shown in *fuschia*. (**b**) Core pluripotency network in pre-implantation mouse embryo (modified from [44]) OCT4 forms protein complexes either with CDX2 or SOX2. OCT4–SOX2 protein complex upregulates NANOG expression, whereas CDX2–OCT4 complex leads to reduction of CDX2 and OCT4 pools. CDX2 and GATA6 upregulate the nuclear receptor GCNF, which downregulates OCT4 expression. (**c**) Stem cell box GRN (modified from [46]) OCT4 and SOX2 expression can be regulated by NANOG and external signals such as growth factors. OCT4–SOX2 protein complex takes part in autoregulation of its components and also regulation of NANOG. NANOG also regulates its own expression. The three proteins activate expression of stem cell-related proteins, whereas their absence leads to expression of differentiation-related proteins

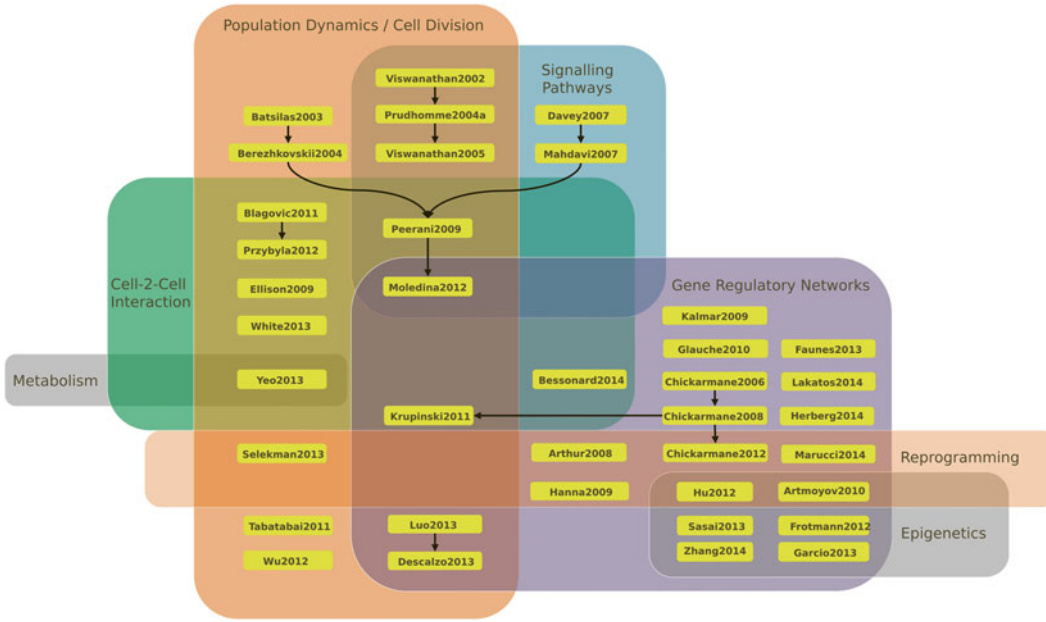

Fig. 3 Classification of the knowledge-based mathematical models of pluripotency. The classification is based on the relevant biological processes represented by transparent *boxes* with different background colors. The models are labeled with the first author's surname and the year of the publication. Models located on overlapping regions represent multiple cellular processes. The *arrows* show models derived from each other

Models of stem cells have been developed for a long time, mostly based on prior knowledge on the signaling pathways, gene regulatory networks, and epigenetic factors (Fig. 3). Recent improvements in omics data, in particular at a single cell level, coupled to ever more accurate computational inference methods, now permit to build and parameterize models directly from experimental results. In this chapter, we provide a critical overview of two general types of mathematical models, namely knowledge-based and data-based models. We focused on models describing the cellular mechanisms that maintain stem cell pluripotency in mammals and the priority was given to models describing well documented in vivo and in vitro systems. Theoretical analysis of cell fate (see for example [14]) can provide interesting insights in system behavior, however models of hypothetical systems were excluded in this chapter due to space limitations. The sections are organized to follow a coarse chronological order, in accordance with the experimental discoveries in the field.

2 Knowledge-Based Models

Most of the mechanistic models of stem cells are built following a bottom-up or knowledge-based approach. Information on the components to include and their relationships is obtained from

scientific literature or public databases, that contain previously generated models or information to incorporate in building blocks. For a general presentation of methods to build models of genes and molecular networks, see Le Novère [15].

2.1 Predicting the Behavior of Stem Cell Populations

The bone marrow contains hematopoietic stem cells (HSC) which are multipotent cells and has self-renewal ability as well as differentiating into blood cells [16–18]. Transplantation of bone marrow has been in clinical use since 1950s as the first successfully established stem cell therapy [19]. In line with clinical applications, early mathematical models of stem cell proliferation and differentiation focused on HSC. These models investigated the cell population dynamics in response to external stimuli such as stress [20] and hypoxia [21]. They aimed at relating the stimuli to the propagation of HSC, and to improve efficiency of the clinical applications via optimization of the parameters. An overview of early predictive mathematical models of hematopoietic cell populations can be found in [22–24].

More recently, population behavior has often been simulated in combination with detailed models of molecular interactions in the stem cells (see sections below); however, minimal models of embryonic stem cell populations omitting the underlying molecular mechanisms were also shown to reproduce the population dynamics [25–28].

2.2 Predicting Cell Fate Control by Signaling Pathways

Pluripotency is defined as the ability of the cells to generate all cell types in an embryo via multiple differentiation steps. Following the derivation of pluripotent stem cells from mouse embryos in 1981 [29], embryonic cells culture has been a fundamental tool in stem cell research and more largely in molecular biology. However, maintenance of the pluripotency in defined growth media has been a challenge until the design of the LIF + 2i medium [30]. This medium contains the cytokine leukemia inhibitory factor (LIF) and inhibitors of ERK and GSK3 pathways (Fig. 2a), underlining how important is the prediction of niche-dependent factors' effect on self-renewal of stem cells, a major aim of modeling efforts since.

The first mathematical model describing the embryonic stem cell renewal as a function of cytokine concentration appeared in the literature in 2002 [31], soon after the discovery of the role of the LIF/JAK/STAT3 pathway in the maintenance of pluripotency [32]. A deterministic model describing the ligand–receptor dynamics on the cell surface was used to determine the cytokine thresholds in cell fate decisions. The simulation results demonstrated that LIF has stronger influence on the maintenance of pluripotency in comparison to a fusion protein derivative of IL-6 (HIL-6) and the differences between the potency of the two cytokines could be explained by receptor binding properties and the stoichiometry of binding.

A growth-rate-based deterministic model of differentiating and self-renewing stem cell populations was developed to predict the response to the cytokines LIF and FGF4 in addition to the extracellular matrix components laminin and fibronectin [33]. Each factor was hypothesized to have a dose effect on cell growth rate and kinetic parameters were estimated based on measured growth-rates. The model was validated by comparing the simulation results to the fraction of cells expressing high levels of OCT4 in a 4-factor culture. Its applicability to predicting the kinase activity level of the JAK/STAT3 pathway was demonstrated [34]. The experimental data on the kinase activity and the 4-factor growth experiment of embryonic stem cells (ESCs) were further used to construct a Bayesian network model of the same system [35] and the network was used to identify causal interactions between the components of the JAK/STAT3, AKT, and MAPK signaling pathways. This study is unique among the ES pluripotency network inference models in the sense that components of the signaling pathways have been predicted rather than gene regulatory network (GRN, see sections below). Predictions of the model were in accordance with known interactions, however the data collected at steady-state conditions were limiting the models' potential to represent timescales of the responses. For instance, phosphorylation of Stat3 is expected to be much faster than cell fate determination.

The timescale of the responses was addressed by parameterizing a compartmental model of JAK/STAT3 signaling pathway [36] using time course data of protein phosphorylation and gene expression [37]. The model was shown to reproduce the response of the pathway to its inhibitors and to predict the stem cell fate decisions. The sensitivity analysis indicated that ESC self-renewal was controlled by the frequency of the LIF stimulation. Further, this model was merged with a stochastic spatial model [38] to describe the colonial behavior in response to autocrine and paracrine signals, which is LIF in this particular case. An optimal colony size was suggested based on the simulations to maintain ES cells in niche [39].

Reproducible, robust, and efficient expansion of stem cells in well-controlled growth niches is one of the essential stages in stem cell research. Coarse-grained models relating the external cues directly to the cellular phenotype found applicability in the design of such bioprocesses. The variability of the micro-environment in a microfluidics system was modeled dynamically to predict the expansion of ES cells in response to signals [28, 40, 41]. A growth rate kinetics-based model to predict the response of ES cells to toxic material accumulation was shown to represent the population dynamics in batch and continuous ES cultures [42]. The spatial JAK/STAT3 model mentioned above [39] was extended further to analyze cell growth in a microfluidics system, and impact of the

system parameters like flow rate, position in the flow field, and local cell organization was demonstrated [43].

Activation of signaling pathways by external cues is a major determinant of cell fate. However, gene transcription is the stage where fate decision is "executed," by providing the cells with the specific set of proteins required to generate the cellular phenotype.

2.3 Predicting Cell Fate Control by Gene Regulatory Networks

Transcription factors (TFs) are the major actors in gene expression regulation. TFs often bind to each others' promoters, therefore construct a regulatory network with feedback loops. Genes targeted by gene regulatory networks (GRN) can be inferred from differential mRNA expression levels, as well as the detection of DNA–TF complexes. The differentiation of morula cells into trophectoderm (TS), inner cell mass (ICM) and further differentiation of ICM into primitive endoderm (PE) and epiblast (EPI) was used as an experimental model in the discovery of key pluripotency TFs. The identification of GRNs that regulate pluripotency and cell differentiation allowed to represent a new level of complexity in mathematical models of stem cells. The excellent review by Niwa ([44] and the references therein) gives an overview of the early discoveries in pluripotency GRN in the pre-implantation mouse embryo. The core GRN described by Niwa, including NANOG, OCT4, SOX2, CDX2, GATA6 and GCNF, has been the basis of most models since (Fig. 2b).

The first model of the core GRN in embryonic stem cells [45] focused on the "stem cell box" (Fig. 2c), the tight regulation loop between NANOG, OCT4, and SOX2 which maintains the pluripotency. The model was used to analyze the response of the network to external signals as a function of model parameters such as strength of the feedback loops. The model was extended [46] to represent a larger network including the TFs CDX2, GATA6, and GCNF (Fig. 2b), which regulates the differentiation of stem cells into trophectoderm or endoderm in the mouse embryo. The network was analyzed to identify the factors that mediate the reprogramming and concluded that NANOG overexpression is a more robust way of reprogramming as opposed to suppression of its repressor GATA6. This model was further extended [47] to take into account the cell–cell interactions and asymmetric cell division that play important roles in regulating the GRN in the development of mouse embryos [48]. The model was able to reproduce the early development of the embryo in 3D and capture the experimentally observed patterns in cell fate decisions together with the heterogeneity in the embryo as a function of spatial mechanical forces, signaling pathways, and GRN. The effect of the FGF4 signal, via the MAPK pathway, was investigated with a 2D 25-cell model [49] where cells can receive FGF4 from neighboring cells. The simulations indicated the existence of a tri-stable network in

pre-implantation mouse embryos, which corresponds to ICM, EPI, and PE cells.

Heterogeneity in NANOG expression has been shown to be a property of stem cell populations [50]. Understanding the origin of such heterogeneity is important since homogeneous cell populations are more desirable in cell therapies. LIF + 2i growth medium, designed for maintenance of stem cells in culture [30], activates NANOG expression via activation of the JAK/STAT3 pathway, inhibition of the MAPK pathway, and inhibition of the GSK3β phosphorylation. LIF + 2i was shown to reduce the heterogeneity in NANOG levels [51]. These experimental discoveries were complemented by mathematical models investigating the origin and consequences of NANOG heterogeneity in stem cells [52–54]. Simulation of the "stem cell box" together with the autocrine signaling of FGF4 demonstrated that the autocrine feedback loops are also a likely source of heterogeneity in NANOG levels [55].

Serum has often been used as a growth media component together with LIF before LIF + 2i was designed. Comparison of the two media has been of interest as serum is known to have BMP4, which is a signal that regulates the pluripotency GRN via SMAD signaling pathways (Fig. 2a). A model which represents the regulation of the stem cell box with all four external factors was used to analyze the three steady states of NANOG, effect of inhibitors, and noise in the cells growing in media with combinations of the external factors [56]. In another study, relative levels of the protein complexes formed between the components of the pluripotency network have been shown to regulate the OCT4 levels in LIF + 2i and LIF + serum [57], at the ground state and exit from pluripotency. The minimal model, representing the dynamics of the GRN and post-transcriptional regulation mediated by the protein complexes, was able to recapitulate the effect of gene deletions in the GRN. A recent model [58] has combined the mathematical modeling of the "stem cell box" with experimental validation via a downstream reporter, REX1 in LIF + 2i. The mechanism of stem cell box regulation by beta-catenin in serum + LIF and 2i was shown to be significant in differentiation compared to maintenance of pluripotency by modeling the Wnt/beta-catenin pathway together with the stem cell box [59]. The impact of beta-catenin on NANOG during reprogramming was also investigated [60].

2.4 Predicting Cell Reprogramming Trajectories Controlled by Epigenetics

Epigenetics has been long known to have an important role in the cell fate as conceptually described by Waddington [61–63], also see [64] for an excellent review of Waddington`s work in a philosophical context of systems biology and mathematical modeling. Currently, epigenetics is defined as relatively stable and potentially heritable changes in the transcriptional potential of the cells without any changes in the DNA sequence. Epigenetic factors are being defined as small molecules such as methyl- groups deposited on

DNA, the 3-D chromatin structure dictated by a set of DNA-binding proteins such as histones, together with the small molecules deposited on the DNA-binding proteins, and non-coding RNA with regulatory functions [65]. Identification of molecular details of epigenetic factors in embryonic stem cells followed shortly after the identification of the core pluripotency GRN. It has been shown that the enzymes and structural components of the epigenetic machinery work together with the signaling pathways and GRN on the gene regulation (see for example [66]), and further mediate transmitting the phenotype of the mother to its daughter cells via silencing the transcription of a set of genes.

Following the Yamanaka's and Gurdon's discoveries on reprogramming of somatic cells into stem cells [67], identification of the factors that increase the efficiency of reprogramming has been a new avenue for mathematical modeling of pluripotency. Understanding the underlying epigenetic profiles in stem cells became even more crucial with the recognition that epigenetic factors are the barrier in the reprogramming of somatic cells into pluripotent cells [13].

A GRN-based stochastic model for reprogramming of differentiated cells into pluripotent state was proposed [68]. The noise in gene expression levels was shown to be adequate for reprogramming if the level of the noise is large enough to overcome the silencing in stem cell box genes imposed by differentiation genes. The reprogramming efficiency has been described by a mathematical model as a function of the cell doubling time [69]. The results have suggested that all cells in a given population can be reprogrammed into induced pluripotent stem cells (iPS cells), whereas the number of cell divisions required for reprogramming differs between the cells. These two models explained the reprogramming trajectories without taking the epigenetic factors into account explicitly.

A first model explicitly representing the activity in GRNs in interaction with epigenetic profiles in pluripotent stem cells was constructed as a cell-cycle-based binary model [70] by simplifying the GRN and epigenetic networks into cell-type-specific modules. The simulations assumed that gene expression takes place only in the interphase of a two-phase cell cycle, whereas changes in epigenetic profiles take place only in the telophase. Active, silent, and poised states were represented in epigenetic modules where genes were represented either as being expressed or silenced. The model was able to reproduce the observed trajectories of differentiation and reprogramming. This model was modified as a Markov model with simplified rules in comparison to the original model and was shown to reproduce reprogramming and gene expression profiles [71].

Another Boolean model representing both the pluripotency GRN and epigenetics in finer detail [72] was able to reproduce the

profiles observed in cell differentiation and reprogramming as response to external modifications such as gene silencing or inhibition of epigenetic regulation. Optimization of reprogramming speed and efficiency was proposed as a function of dynamics of DNA methylation and chromatin structure.

An ODE-based model of the stem cell box with most studied epigenetic marks H3K4me3 and H3K27me3 (tri-methylation on Lysine4 and Lysine27 residues on Histone3 proteins) was constructed [73] and simulated stochastically. The model was used to analyze the observed bistability, inducibility, stochasticity, and reprogramming profiles of the cells as response to external stimuli.

Recently, *Nanog* expression in pre-implantation mouse embryos and ES cells grown in LIF + 2i was shown to be under allelic regulation via differential epigenetic silencing between the alleles [74]. The allelic regulation of *Nanog* was investigated in a mathematical model based on the core GRN of pre-implantation mouse embryos extended with epigenetic regulation of gene expression [75]. The model did not take into account any external signals, however was able to demonstrate the bistable behavior of *Nanog* expression as a function of slow epigenetic dynamics that leads to differentiation or self-renewal of ES cells. Impact of slow epigenetic kinetics on stochastic cell fate decisions was shown with another GRN model, an extended pluripotency network with KLF4 and PBX1 [76].

Reprogramming and directed differentiation of stem cells usually require step-wise protocols. Recently, few models were proposed to represent the discrete changes taking place in signaling, GRN, and epigenetics profiles of the cells depending on the stage of the process [77, 78].

Representation of epigenetic effects in the mathematical models of stem cells is still in it infancy, however it can be envisioned that the cell commitment and reprogramming models with epigenetics will be superior in terms of their accuracy in making predictions, given the fact that epigenetics is the barrier that has to be overcome by transforming cells.

3 Data-Based Models

The models we have mentioned so far were built on a priori information about the systems to be investigated, and aimed to predict the phenotype as a function of selected factors and parameters. Building knowledge-based models can be tedious as the relevant molecular interactions have to be curated from the literature. In an emerging field like stem cell research, there is also the possibility that the list of interactions derived from the literature will be incomplete. Further, available measurements and biochemical information may not be adequate to parameterize the models.

In the following section we will be giving examples of models built without a priori information, i.e., built using data-based methods to identify network components or network structures in the pluripotent stem cells. Data-based methods are becoming more and more attractive in mathematical model reconstruction as they do not require tedious literature surveys, but indeed rely on analysis of high-throughput data which is accumulating with a tremendous speed in databases.

A partial least squares regression model (PLS, a singular value decomposition-based linear regression method) [79] relates the external factors to signaling pathways and the cellular responses (i.e., growth rate kinetics determined in [33]). The predicted effect of the tested factors on the phospho-proteome and growth patterns agreed well with the literature, demonstrating that data-driven unsupervised models can be used to build plausible models. While this model is a reconstruction of signaling pathways based on phospho-protein measurements, GRNs can be reconstructed based on gene expression data. The reconstructed GRN models are built using the predictive power of gene expression levels on other relevant readouts [80–83]. Gene expression levels can be further combined with data providing evidence on gene regulation, such as DNA–protein binding data [84] or miRNA [85] for reconstruction of GRNs. On the other hand, gene expression levels can be used to validate models built using other types of high-throughput data such as histone modifications [86] or chromatin structure [87].

Data-based models can also improve our understanding of the barriers hindering the reprogramming efficiency, for example, gene expression-based GRNs supplemented by known interactions were analyzed to identify the response pathways that may hinder reprogramming efficiency [88, 89]. Differences between human and mouse ESC have been a matter of debate [90], the active signaling pathways in mouse and human ESC were compared using gene expression-based GRNs [84, 91] to answer the open questions in the field.

Hybrid models of pluripotency were proposed as an alternative to purely qualitative or quantitative models. For example, a meta-model derived from an ODE-based model of the human PI3K/AKT pathway was used for efficient parameter sensitivity analysis of the steady state of the pathway in hESC [92], whereas complementing a gene expression-based unsupervised GRN with literature-curated regulatory interactions was shown to lead to more accurate predictions on the active GRN of mESCs differentiating into the three germ lines, in parallel with the gastrulation process in the embryo [93].

The high-throughput data-driven nature of unsupervised methods has the advantage that their predictions are genome-wide and open to context-dependent interpretation. Further, the

algorithms underlying the models can be readily implemented to new systems or datasets. Therefore, taking the extra mile to provide a web application or software package facilitates the re-use of the models and their predictions by the stem cell community. Few such applications were specifically validated for pluripotent stem cells: CellNet is a collection of tools for construction and analysis of gene expression-based GRNs in stem cells [94]. RE:IN [11] is another web-based application for gene expression-based GRN reconstruction. RE:IN was used to propose a minimal pluripotency network in mES which was shown to correctly predict the phenotype of double knock-out mutants. ExprEssence [95] has been developed as a Cytoscape [96] plugin to build networks of gene expression data; the tool was verified with pluripotency GRN.

The recent developments in the technology allow generation of high-throughput data on a variety of cellular features relevant to maintenance of pluripotency, such as profiles of epigenetic marks and 3-D chromosome structure [97], in addition to proving more efficient and more accurate techniques over the established techniques of collecting transcriptome, proteome, metabolome, protein–protein, and DNA–protein interaction data. The potential of such features in unsupervised modeling of pluripotency has not been fully utilized yet, although few recent applications initiated such efforts: StemSight [98] integrates gene expression and DNA–protein binding data to provide a verified network of mouse pluripotent stem cells. StemSight has been further extended to include the human pluripotency network [99]. The ESCAPE database (former iScMiD, [100]) stores data from sources such as phosphoproteins, miRNA interactions, histone modifications, and gene expression. ESCAPE also provides a collection of data analysis and network construction tools for mouse and human pluripotent stem cells, verified for pluripotency network in mESCs [12]. Repositories such as multi-organism STRING [101] and mouse-specific MouseNET [102] integrate data from diverse sources including gene expression levels, protein–protein interaction, text mining, and functional relatedness to build interaction maps of known and predicted interactions between the genes.

4 Perspectives on Predictive Models in Regenerative Medicine

In this chapter, we tried to give an overview of computational modeling of pluripotent stem cells in line with the experimental discoveries in the field. The pioneering modeling efforts have started from minimal probabilistic population dynamics models, and progressed to represent most levels of regulation in cell fate decisions. The approach and granularity of the models have also progressed, a range of approaches from deterministic ODE-based models to unsupervised logical models were adopted, whereas

building models in finer granularity became feasible with increasing availability of quantitative data, evidence on molecular interactions, and more powerful computational resources.

4.1 All Regulatory Levels Need to Be Represented

It is becoming clear that the pluripotency is regulated at many levels of cellular activities, i.e., signaling, gene regulatory networks, epigenetics, and metabolism [103]. The existing computational models of pluripotency often focus on only one or two of these levels. For instance, metabolism has not yet been taken into consideration in modeling the pluripotency although metabolism dependency of embryos and induced pluripotent stem cells has been reported [104–106]. Dependency of the cell cycle on GRNs, post-transcriptional regulation by non-coding RNAs, and post-translational regulation of protein activities are other phenomena that have been overlooked by the current models. The success of next-generation predictive models of pluripotency will rely on the representation of cell fate regulation at *all* levels.

4.2 Modularity Needed for Integrating Different Modeling Approaches

The choice of the modeling approach depends on the nature of the cellular activities to be modeled. For example, gene expression is a noisy process; the molecular crowd in the nucleus and low copy number of the regulatory proteins introduce an intrinsic stochasticity that leads to stochastic cell fate decisions and heterogeneous cell populations. Cell division and unequal partition of low copy number species between the daughter cells is another source of heterogeneity in cell populations. However, metabolism and signaling are faster processes and take place in a relatively homogeneous environment, where assumption of continuity and steady state may hold in most cases. Therefore, a complete model representing all levels of cellular activities may require the use of different approaches for each level.

4.3 Re-usability of the Models Needs to Be Promoted

Re-usability of the models needs to be taken into consideration by the computational biologists. Distribution of the models in standard formats with adequate documentation is crucial for efficient use of resources. Only three of the models we have mentioned in this chapter are available at the BioModels database [107], and none could be found in the CellML [108] or JWS [109] model repositories, which demonstrates that re-use of the pluripotency models is currently not straightforward and potentially a time-consuming task.

The computational cost of parameterizing and simulating models increases with increasing granularity. Therefore, the molecular interactions represented in the model have to be chosen with care to provide enough details to answer the biological question being asked while omitting the details which are not relevant for the current question or observable with the current experimental

tools. Re-use of existing models to build larger and modular models can keep the computational cost at feasible levels.

Software tools and databases (particularly specialized on stem cells) are invaluable resources for computational biologists. However, often very useful tools and databases are not maintained or updated, therefore become obsolete quickly. Continued maintenance by dedicated teams could prevent the waste of resources and provide the community with reliable and up-to-date tools and databases.

4.4 Validation of the Predictive Power Missing for Most Models

Mathematical models aim to explain the biological phenomena observed and predict the outcome under the conditions not yet experimentally tested. Validation of models can rely on use of existing experimental observations, however accuracy of the predictions often are not tested. Lack of follow-up experiments often hinders the usefulness of the models. An iterative systems biology approach is needed to utilize the potential of predictive models, where model construction, prediction, and validation have to be designed a priory for the biological question being asked. Such iterative approaches require close collaboration between experimental and computational biologists.

We would like to conclude with the observation that predictive modeling in regenerative medicine is at its dawn. Availability of detailed knowledge and genome-wide data on cellular and organismal level will promote the construction of larger and modular models with higher predictive power. The predictions from the models will lead to targeted clinical applications with higher rates of success in regenerative medicine.

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