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The principles of whole-cell modeling

Jonathan R Karr[1], Koichi Takahashi[2,3] and Akira Funahashi[4]

Whole-cell models which comprehensively predict how phenotypes emerge from genotype promise to enable rational bioengineering and precision medicine. Here, we outline the key principles of whole-cell modeling which have emerged from our work developing bacterial whole-cell models: single-cellularity; functional, genetic, molecular, and temporal completeness; biophysical realism including temporal dynamics and stochastic variation; species-specificity; and model integration and reproducibility. We also outline the whole-cell model construction process, highlighting existing resources. Numerous challenges remain to achieving fully complete models including developing new experimental tools to more completely characterize cells and developing a strong theoretical understanding of hybrid mathematics. Solving these challenges requires collaboration among computational and experimental biologists, biophysicists, biochemists, applied mathematicians, computer scientists, and software engineers.

Addresses
[1] Department of Genetics & Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA
[2] RIKEN Quantitative Biology Center, RIKEN, Osaka 565-0874, Japan
[3] Institute for Advanced Biosciences, Keio University, Fujisawa 252-8520, Japan
[4] Department of Biosciences and Informatics, Keio University, Yokohama 223-8522, Japan

Corresponding author: Karr, Jonathan R (karr@mssm.edu)

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Introduction

Whole-cell models are computational models which describe how phenotype arises from genotype [1,2,3•]. The primary goal of whole-cell modeling is to enable rational bioengineering and precision medicine. Combined with genome synthesis [4] and transplantation [5], whole-cell models could enable bioengineers to maximize objectives such as biofuel production by optimizing genomes [6,7]. Such models could also enable clinicians to individualize therapy [8–10]. Furthermore, whole-cell models could be powerful scientific tools.

Shuler *et al.* introduced the first coarse-grained ordinary differential equation whole-cell model in 1979 [11,12•]. Twenty years later, when sequencing provided the first biological parts list, Tomita *et al.* [13•] developed the first large-scale fine-grained dynamical model. Researchers have continued to develop increasingly sophisticated dynamical models [14–16]. In parallel, Varma and Palsson used flux balance analysis (FBA) to create the first static genome-scale metabolic models [17]. The latest FBA models represent over 1000 genes [18]. Researchers have since expanded FBA to represent transcriptional regulation [19], transcription and translation [20•], and signaling [21]. Logical methods have also been used [22]. Recently, we and others used a hybrid methodology to construct the first dynamical model which represents every known molecular species and gene function [23••,24,25]. Simultaneously, Roberts *et al.* developed the first cell-scale structural model [26•].

Here, we describe the core principles of whole-cell modeling. We also outline our model construction process, highlighting existing tools and the challenges to achieving complete models.

The principles of whole-cell modeling

Building on Roberts' discussion [27], we outline 11 fundamental and practical principles of whole-cell modeling to illuminate a path toward complete models (Figure 1).

Single-cellularity

First, whole-cell models should represent individual cells. Single-cell models can account for how temporal dynamics and stochastic variation affect behavior. Single cells are also tractable because they are independent and directly result from molecular biochemistry. Furthermore, single-cell models can take advantage of the growing wealth of single-cell data.

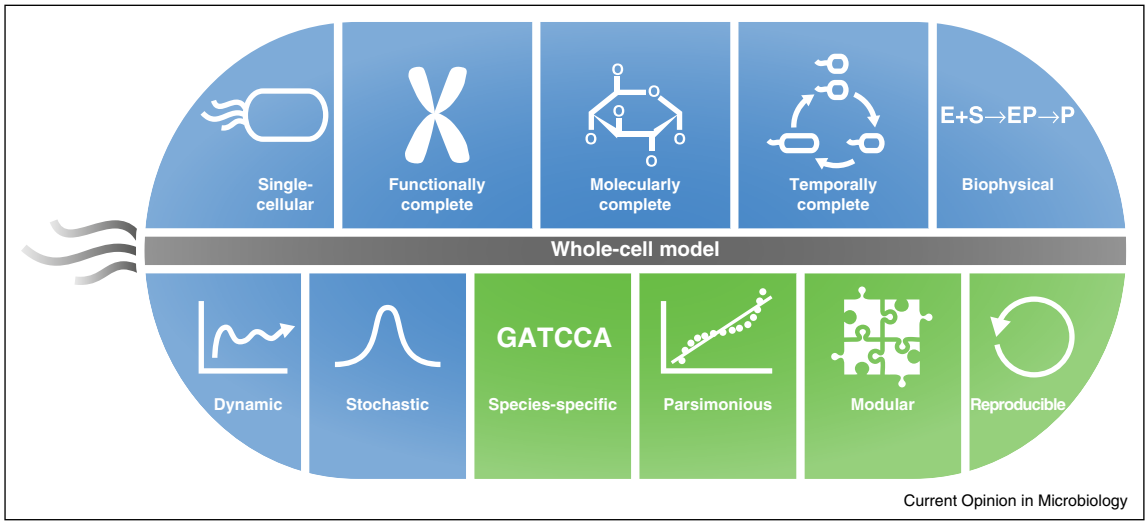
Functional closure

Behavior is determined by interacting pathways and genes. Consequently, whole-cell models should represent every known cellular and gene function. Models which represent every known function are powerful tools. For example, genome-scale metabolic models which represent every known metabolic reaction and enzyme have been used to identify missing reactions and enzymes [28].

Molecular closure

Whole-cell models should represent the cell and its environment as a closed system. Models should explicitly account for exchanges among pathways and the environment and not have arbitrary sources and sinks. This ensures

Figure 1



Fundamental (blue) and practical (green) principles of whole-cell modeling. No existing model satisfies every principle. The most advanced functional models are incomplete and do not fully represent molecular biophysics. The most advanced structural models do not represent cellular-scale behavior. Further work is needed to merge functional and structural modeling and expand their scope.

that models recognize important and often ignored connections such as the common energy carrier ATP. In turn, this enables models to capture pathway interactions that are often missed by studying pathways in isolation, such as how the energy charge affects phosphorylation and signaling.

Temporal closure

Whole-cell models should also represent the entire cell cycle. This ensures that models account for how cells regulate pathways in time to coordinate their life cycle. For example, models should account for how the dynamics of DNA replication affect dNTP concentrations and metabolism. Temporally complete models can also leverage cell theory, the fact that cells come from other cells, to constrain their dynamics. Assuming constant external conditions and absent evolution, cell theory implies that cellular populations are stable across generations. This provides a periodic constraint which enables dynamical modeling with minimal dynamical data.

Biophysics

In addition, whole-cell models should represent cellular biochemistry and biophysics, including mass conservation, thermodynamics, and spatial organization. This provides a recipe for bottom-up model construction, reducing the space of possible models. Takahashi *et al.* have reviewed several mathematical frameworks which are capable of representing cellular biophysics [29].

Dynamics

In particular, whole-cell models should be constructed from differential descriptions of molecular biochemistry and predict the emergence of cellular-scale dynamics. Emergent dynamics are valuable opportunities for experimental validation and discovery.

Stochasticity

Furthermore, whole-cell models should be discrete and stochastic. Stochastic models naturally predict the emergence of cellular variation. For example, stochastic models can account for how stochastic transcription initiation creates variation in gene expression and growth. This variation is another valuable opportunity for experimental validation.

Species-specificity

Whole-cell models must be evaluated by comparison to experimental data. Consequently, whole-cell models should represent specific genomes. This constrains the space of training data.

Parsimony

Despite the explosion in experimental data, limited data is available. For example, there is little data about non-coding RNA. Consequently, models should be parsimonious. This minimizes the need to identify unmeasured parameters.

Modularity

Absent an *ab initio* theory of biochemistry, whole-cell models must be based on many experimental descriptions of molecular biology. Consequently, like other large engineered systems, whole-cell models are best developed by combining multiple pathway submodels. This enables submodels to be developed and tested independently by different investigators using different representations.

Reproducibility

Finally, whole-cell models should be transparent, well-annotated, and reproducible. Researchers should be able to reproduce models from their primary sources, as well as reproduce simulations using multiple simulators. Models should also be described using transparent languages like SBML [30]. This is essential for collaborative modeling.

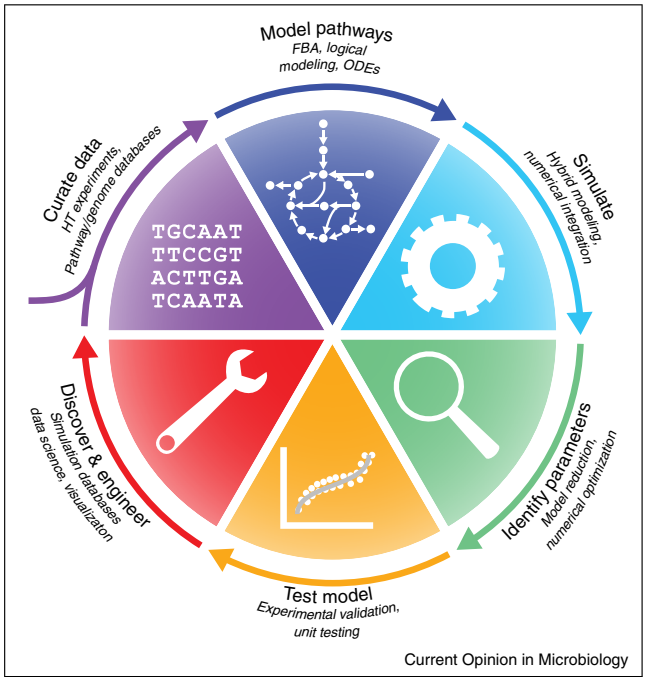
Model construction

Achieving these principles requires new approaches and tools. We briefly outline our approach to constructing whole-cell models (Figure 2), highlighting important areas for further research.

Experimental curation

The first step to constructing a model is to choose an organism and assess the feasibility of modeling it by assembling the available experimental knowledge. We have manually assembled training data from public databases and journal articles. Tables S1 and S2 list the most informative technologies and databases. Higher annotation standards are needed to enable modelers to take more advantage of published data [31]. We have organized our training data using model organism database tools such as Pathway Tools [32], WholeCellKB [33], BioMart [34], and Intermine [35].

Figure 2



Whole-cell modeling process. Experimental data is organized into a database, pathway submodels are constructed, submodels are combined, parameters are identified, the model is simulated and tested, and the model is used to guide discovery and bioengineering. The process is iterated using additional data to refine the model until an accurate model is achieved.

New experimental methods which fully characterize cells are needed to enable more comprehensive and accurate models. Improved metabolomic methods which are capable of quantitating the concentration of every metabolite are needed to train metabolic models. New proteomic methods are needed to characterize macromolecular complexes including their rates of formation and subunit composition dynamics. Improved interaction screens are needed to resolve the function of each individual interaction including every individual chaperone-substrate, protease-substrate, and miRNA–mRNA interaction. New high-throughput methods are needed to comprehensively quantitate reaction kinetics. Additional tools are also needed to comprehensively characterize single-cell temporal and population variance including of metabolite and protein concentrations, as well as of systems properties such as the growth rate, cell cycle phase durations, cell size, and reaction fluxes.

This manual reconstruction approach has been feasible for small bacteria. More scalable approaches will be needed for more complex bacteria and eukaryotes. One potential solution is to automatically reconstruct knowledge using cognitive computing [36] or other machine learning techniques [37–39]. A second potential solution is to engage a large community of scientists. Both of these will require additional molecular databases such as BRENDA [40] and UniProt [41] which are either manually assembled by single researchers, community assembled by self-curation during publication [42], or automatically assembled using natural language processing [43].

Mathematical formulation

Second, a mathematical description of how cells evolve over time must be constructed. We have described cells as thoroughly as possible given our current knowledge, desire to predict cellular behavior, and limited time and resources. This strategy takes full advantage of our existing knowledge, avoids unknown parameters and expensive computations of processes such as diffusion which minimally affect behavior, and enables one model to be used for many scientific questions. In practice, until whole-cell models are complete, modelers will need to focus on the pathways most relevant to their research.

In our experience, the easiest way to construct a whole-cell model, like any other large engineered system, is to assemble multiple pathway submodels. This approach is scalable because it enables pathways to be modeled and tested independently by different investigators using different mathematical formalisms.

Individual submodels must be implemented and/or constructed from experimental data. BioModels [44] and the CellML model repository [45] contain many existing pathway models. However, most pathways have not been

modeled, and most existing models must be modified for integration with other models. The primary obstacle to modeling pathways is the lack of quantitative data. New experimental technologies are needed to characterize more pathways.

Rule-based modeling is a powerful and scalable approach for assembling genome-scale models [46,47]. Several rule-based and conventional tools can be used to construct and modify pathway models including BioNetGen [46], BioUML [48], CellDesigner [49], CobraPy [50], COPASI [51], E-Cell [2], iBioSim [52], and JDesigner [53]. Table S3 lists several additional tools. Further work is needed to scale up these tools for larger models.

Submodel integration

Next, individual submodels must be combined. Mathematically homogeneous submodels can be merged analytically. Heterogeneous submodels must be combined by dividing the state variables into independent subvariables dedicated to each submodel; integrating the individual submodels based on these subvariables; and merging the subvariables to update the global variables. The integration time step should be set faster than the fastest inter-pathway dynamics. Slower time steps will introduce communication delays. We and others have developed hybrid simulators which are capable of integrating heterogeneous submodels [2,3,23••,54–56]. Further work is needed to develop a deeper theoretical understanding of multi-algorithm modeling.

Parameter estimation

Once the model's structure has been implemented, the model's parameters must be identified by matching the model's predictions to experimental data. Identifying whole-cell models is challenging because they are high-dimensional, stochastic, and computationally expensive. We have followed a three-step approach to parameter identification. First, we have only created parameters whose values can be estimated from one or a few experimental observations. Second, we have used public data to estimate each parameter. Third, we have refined parameter values by numerically minimizing the prediction error of a manually constructed reduced model which approximates the full model.

Unfortunately, this approach is not scalable. Building increasingly comprehensive models requires increasingly comprehensive experimental data. Manually constructing reduced models is also not scalable.

There are many other promising local and global parameter identification strategies. Several researchers have reviewed these approaches and their application to smaller models [57–59]. Several innovations are needed to apply these methods to larger, hybrid models. Automated model reduction [60–62] is needed to construct models which are tractable to numerical optimization. Researchers should pursue both statistical and physics-based approaches. Automatic differentiation should be applied to improve the efficiency of gradient-based optimization [63]. Faster, parallelized simulation engines and distributed optimization procedures should be applied to explore parameters more quickly [64,65].

Model refinement and validation

The last step to constructing a whole-cell model is to iteratively evaluate the model's predictions and refine the model. We have focused on evaluating the predicted phenotypes of genetic perturbations. Additional data representing single-cell variation and cell cycle dynamics are needed for more rigorous validation. Experimental design based on predicting the most likely informative experiments [58], robotic and microfluidic experimentation [66], and computational gap filling [67] should be applied to automate model refinement.

Visualization and analysis

The final steps in whole-cell modeling are to simulate the model, analyze simulation results to construct new hypotheses, and conduct experiments to test those hypotheses. We have developed WholeCellSimDB to organize simulation results and facilitate large-scale analyses [68]. We have also developed WholeCellViz [69] and the E-Cell session monitor [70] to visualize simulation results. We have used these tools to gain new insights into cellular energy usage [23••], learn kinetic parameters [71], and analyze the metabolic demands of synthetic gene networks [72]. Numerous other visualization software are available including Cytoscape, Gephi, VANTED, and VisANT [73].

Conclusions

Whole-cell models promise to predict how genotype determines phenotype. Combined with genome synthesis and transplantation, whole-cell models could enable bioengineers to construct cellular factories. Whole-cell models could also enable clinicians to individualize therapy. Furthermore, whole-cell models would be unprecedented scientific tools.

Whole-cell models have several advantages over focused models. They are constructed once, but can drive many scientific, engineering, and medical questions. They can also predict non-intuitive effects by chaining together many individually intuitive interactions. Furthermore, they can systematize biological discovery and unify disparate research.

Whole-cell modeling is a new and exciting field with numerous challenges that require collaboration among computational and experimental biologists, bioinformaticists, and computer scientists. Table S4 lists several efforts to build a whole-cell modeling community.

We have proposed 11 principles to guide what whole-cell models represent and how they presently must be constructed. Our own work has followed most of these principles. However, as detailed by Macklin *et al.*, further work is required to achieve all of these principles even for the simplest bacteria [74]. A high-level declarative language is needed to describe models more transparently, theoretical studies are needed to better understand multi-algorithm models, and more efficient simulators are needed to simulate models more quickly.

To date, we have manually reconstructed and identified whole-cell models which represent hundreds of genes. Achieving models of more complex bacteria and eukaryotes, which represent tens of thousands of genes, demands new automated pathway reconstruction methods based on artificial intelligence techniques such as machine learning, natural language processing, and ontology engineering. These models will contain hundreds of thousands of quantitative parameters such as binding affinities and rate constants. Identifying their values will demand new high-throughput experiments which can quantitate individual molecular interactions, as well as new biophysical models which can accurately predict quantitative parameters from sequences. Ultimately, whole-organism models will require hierarchical modeling approaches which use agent-based modeling to combine multiple whole-cell models of multiple cell types. In addition, alternative incentives are needed to reward collaborative modeling. Solving these challenges will allow whole-cell modeling to fulfill its promise of enabling bioengineering and precision medicine.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.mib.2015.06.004.

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