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Single-cell omics analysis with genome-scale metabolic modeling

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Single-cell technologies have been widely used in biological studies and generated a plethora of single-cell data to be interpreted. Due to the inclusion of the priori metabolic network knowledge as well as gene–protein–reaction associations, genome-scale metabolic models (GEMs) have been a powerful tool to integrate and thereby interpret various omics data mostly from bulk samples. Here, we first review two common ways to leverage bulk omics data with GEMs and then discuss advances on integrative analysis of single-cell omics data with GEMs. We end by presenting our views on current challenges and perspectives in this field.

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Introduction

Single-cell technologies, which enable quantifying heterogeneity of cells, are increasingly used in diverse biological studies [1,2]. The wide applications of these technologies have generated a plethora of single-cell data, and thus, the demand for methods of single-cell data analysis and interpretation is growing [3–5]. While single-cell data can be appropriately handled by machine and deep learning methods [6,7], the lack of interpretation remains to be addressed [8]. Meanwhile, mechanistic models built from prior knowledge have been extensively used for the interpretation of omics data from bulk samples [9,10]. If cellular metabolism is of particular interest to investigate, these mechanistic models can be genome-scale metabolic models (GEMs), which have indeed been widely used for integrative analysis [11–13]. Therefore, we conjecture that GEMs are also a valuable platform to handle single-cell data, which is now indeed emerging [8]. Due to the prior metabolic network knowledge in GEMs, the integration of single-cell data within GEMs may bring new insights in wide-ranging fields, including biotechnology and medicine fundamental research.

Here, we first retrospect the methods developed for integrating bulk omics data with GEMs, then review advances in the integration of single-cell data with GEMs, and end with challenges and perspectives in the field.

Methods of integration of bulk omics data with genome-scale metabolic models

At first sight, the integration of single-cell data into GEMs should be relatively straightforward, as it merely requires the adaptation of methods that have been developed for bulk cases to single-cell cases. We will therefore first reflect on the general methods of integrating omics with GEMs before we review in the next section how this has advanced to single-cell data.

A GEM contains all metabolic reactions of an organism with their corresponding gene–protein–reaction associations. It as such links key biological molecules, including genes, transcripts, proteins, and metabolites, within a mathematical framework. Therefore, the GEM should be an ideal platform for integrating genomics, transcriptomics, proteomics, and metabolomics data. Typically, a GEM is reconstructed based on the whole-genome information of the organism of interest and thus is context (e.g. condition, cell type, and organelle) independent. The integration of omics data into GEMs enables generation of context-specific GEMs and

prediction of context-specific metabolic fluxes using methods that can roughly be divided into two categories, although some methods fall into both.

The first category are model extraction methods (MEMs) [14]. While the reference GEM contains the entire set of metabolic reactions that are annotated on the genome, not all reactions are active in various conditions, cell lines, cell types, or organelles. MEMs enable the conversion of the reference GEM into context-specific GEMs, in which a set of reactions are inactive or removed according to the omics data collected from the given contexts. Considering data availability and quality, MEMs have focused on using transcriptomics data for identifying reaction presence [14,15], and the core strategy is to remove from the reference GEM those reactions whose associated genes are at low expression levels according to predefined thresholds. Proteomics data can in principle be handled in the same manner, while metabolomics data might be regarded as supplementary evidence for the presence of certain reactions [16].

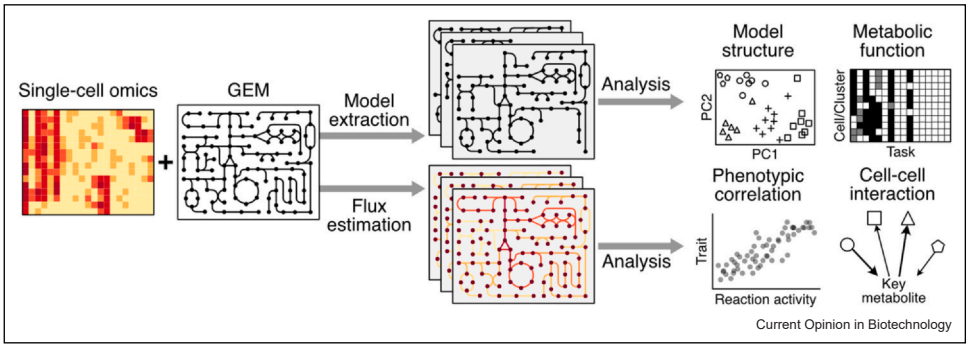
The second category of methods integrating omics data is to improve GEM predictions of metabolic fluxes. GEMs can be utilized to predict metabolic fluxes through constraint-based methods [17], such as flux balance analysis (FBA) [18], which search for flux distributions in the feasible region defined by constraints given by flux balancing around each metabolite. The integration of omics data as constraints can reduce the feasible region and thereby lead to more accurate predictions only if the region is reduced correctly. This reduction of the *feasible reaction fluxes* contrasts with MEMs, where the size of the *network* is reduced by removing inactive reactions. Likewise, methods have mostly been developed for integrating transcriptomics data, and the common strategy is to constrain metabolic fluxes approximately based on gene expression levels [15]. While such methods can also be adapted to impose proteomics constraints on GEMs, emerging frameworks such as enzyme-constrained models enable direct integration and fine-tuning of proteomics data [19]. Metabolomics data can be utilized to define the thermodynamically feasible region [20], and time-series metabolomics data, when available, can be utilized to challenge the steady-state assumption that are normally followed in constraint-based methods [21,22].

Advances in the integration of single-cell data with genome-scale metabolic models

One of the main issues with adapting the methods developed for bulk to single-cell cases is the sparsity of single-cell omics data. For example, high-throughput single-cell RNA-Seq assays, such as 10X Chromium, typically yield on average about 1500–30 000 molecule counts per cell [23] depending on properties such as cell type, cell size, capture efficiency, and sequencing depth. This can be compared with bulk RNA-Seq data, which typically ranges between 10 and 100 M reads per library [24]. To be able to confidently detect gene expression levels down to a few transcripts/counts per million, which is of the same magnitude as threshold values commonly used for detection of enzyme presence [14], the counts from one cell are simply not enough. Directly applying this approach results in nonfunctional sparse metabolic networks, with many reactions absent due to lack of empirical evidence of their transcript being present. A common solution to the data sparsity is pooling of data across similar single cells, which we review below.

In parallel with bulk data, the integration of single-cell data with GEMs can also fall into the two categories, that is, generation of single-cell GEMs and prediction of single-cell metabolic fluxes (Figure 1).

Figure 1



Single-cell omics analysis with GEMs. There are two categories to integrate single-cell omics (mostly single-cell transcriptomics) data into GEMs. On the one hand, single-cell models can be extracted by mapping single-cell omics data onto a reference GEM, and the resulting single-cell models can be compared to identify differences in network structure and prediction of metabolic tasks [25]. On the other hand, single-cell omics data can be used as constraints to estimate single-cell metabolic fluxes or potential activities, which can subsequently be used to correlate with key phenotypes [26] and infer cell–cell interactions [27].

To generate single-cell GEMs, efforts have focused on directly utilizing the existing methods that have been developed for bulk data. A few studies employed various existing methods to integrate single-cell transcriptomics data with reference GEMs, but the single-cell data were pooled into subtype-specific pseudo-bulk data before the use in extracting submodels, which aimed to handle the low signal-to-noise ratio in single-cell transcriptomics data [28–30]. To further address the data sparsity, a recent study combined the pooling of single-cell transcriptomics data with a bootstrapping strategy and optimized the tINIT method [31] to a faster version, called ftINIT, in order to reduce the compute resource when generating bootstrap-specific models [25]. While these studies claimed that single-cell data were adopted to extract submodels from the reference GEMs, the resulting models did not really represent single-cell resolution. However, this concern is not uncommon for other (nonmetabolic modeling) single-cell data analysis methodologies, in which single cells are clustered to avoid data sparsity [32].

For the prediction of single-cell metabolic fluxes, most of the methods for bulk data cannot be directly transferred to single-cell cases as they require exchange fluxes of extracellular metabolites (e.g. nutrient consumption rate) as baseline constraints, which however are mostly unknown at the single-cell level. As it is easier to obtain the extracellular fluxes from bulk samples, the method scFBA [33] predicts single-cell fluxes constrained by both extracellular bulk fluxes and single-cell transcriptomics. To achieve this, a community model composed of single-cell models is built, in which extracellular bulk fluxes constrain the community model and single-cell transcriptomics constrain intracellular fluxes of each single-cell model. For community model simulations, a biological objective function, for example, maximization of the community growth, is required, and the simulations can point out distinct growth rates of single cells and cell–cell metabolite exchanges. In addition, scFBA can employ bulk transcriptomics to handle data quality issues in single-cell transcriptomics.

Without a priori knowledge of the extracellular fluxes, the method single-cell flux estimation analysis [34] can predict metabolic fluxes at single-cell resolution from single-cell transcriptomics data. This attributes to a graph neural network architecture, which minimizes the total flux imbalance of all intermediates throughout all single cells. It concurrently considers flux non-negativity, coherence between estimated fluxes and gene expression, and flux scale. Rather than individual reactions, single-cell flux estimation analysis focuses on modules by merging multiple reactions in the metabolic network. The advantages include increase the robustness of flux prediction and the computational efficiency; handle the nonlinear dependency between metabolic flux and gene expression — a challenge not only in single-cell but also bulk transcriptomics-based flux prediction; and partially address the dropout events in single-cell transcriptomics data by examining the expression level of a group of genes within the module rather than a single gene.

Using assumed bounds on extracellular fluxes, the method Compass enables prediction of metabolic states of single cells [26] without the consideration of cell–cell metabolic communications. Instead of predicting fluxes, Compass assigns scores to each reaction in every cell, reflecting the consistency between the single-cell transcriptome and the flux distribution while maintaining a relatively high flux through that reaction. In other words, the score indicates if the reaction is likely to be active in the cell and is for each reaction based on the entire transcriptome throughout the whole metabolic network, thereby reducing the effects of data sparsity and cases where gene expression does not reflect flux. To further mitigate the sparsity of single-cell transcriptomics data, Compass adopts information-sharing between cells with similar transcriptional profiles using k-nearest neighbor graph, and statistical analysis of the large number of the predicted single-cell metabolic states can further add robustness.

A recent method METAFlux [27] can integrate both bulk and single-cell transcriptomics data into GEMs to predict metabolic fluxes. In dealing with single-cell data, METAFlux merges all single cells as a community and optimizes the whole community growth under nutritional constraints, similar to scFBA. The major difference is that METAFlux clusters the same cell types before merging to form a community, which aims to handle noise and sparsity of single-cell transcriptomics data, and bootstrapping for single-cell data is required for cluster-wise metabolic statistics.

Challenges and perspectives

Single-cell technologies combined with GEMs hold promise to unravel numerous new findings in the field of cell metabolism, allowing for investigating the differences in metabolism between cell types and even single cells within complex tissues. To what extent these differences can be investigated is largely governed by technical limitations, and parallels can be drawn between the problem addressed in this paper and the more established use of single-cell technologies within other biological fields. Single-cell technologies are today mainly used to investigate clusters of cells with similar behavior and are seldom used to investigate single cells, which is mostly due to technical limitations and that investigations at cluster level are sufficient to address many biological questions. The technical limit of the granularity of the clustering that can be reached, where maximum granularity is at single-cell level, depends mainly on the ability to separate cells confidently into

relevant clusters and the signal-to-noise ratio within each cluster, which both can be improved by increasing the number of cells and molecules per cell. For genome-scale modeling purposes, the challenges are largely the same, and improvements to the single-cell techniques will allow for the investigation of differences between more similar cell types and states.

Another challenge with predicting metabolic fluxes of cell types or single cells is to estimate the availability of metabolites for single cells or groups of cells. The availability of metabolites in, for example, human tissue depends on the distance to blood vessels and is complex to estimate. Although estimates based on diffusion coefficients and metabolite concentrations in blood have been suggested [35], it is difficult to map such estimates to single cells when the spatial context is lost. In addition, the behavior of nearby cells may affect the nutrient availability. Spatial transcriptomics [36], where the transcriptomes of small spots are measured in a spatial context, offers a possibility to partly resolve these challenges, especially in combination with single-cell RNA-Seq in which the position of single cells can be estimated [37], but how to combine this data type with GEMs remains to be explored. While scFBA groups all single cells into a community model and thereby only needs to set constraints on the total metabolite consumption/production, it does not take spatial context into account, both in terms of nutrient availability and spatial cell-to-cell interaction. Methods to measure metabolite uptake rates in single cells are in their infancy; future approaches may include microfluidics-based single-cell metabolomics [38] potentially even combined with stable-isotope labeling experiments [39].

A third challenge is how to estimate the objectives of cells. While not all methods require an objective, many do, and the objective of cells is sometimes difficult to estimate, regardless of if the modeling is based on bulk or single-cell data. While cell lines, cancer cells, and a few other cell types can be expected to be optimized for growth [40], the objective of many other cell types, for example, immune cells, is more difficult to estimate and requires deep knowledge of the behavior of these cells. This problem is still largely unsolved and is even harder in a single-cell context as individual cells might behave differently, that is, with distinct objectives. Methods that fit or score fluxes to omics data (e.g. Compass) have an advantage in that this problem does not need to be addressed but has the disadvantage that they tend to estimate flux capacity rather than actual fluxes.

While the use of single-cell and spatial omics together with GEMs brings many challenges, it also makes possible many types of analyses that are difficult to do with bulk omics, for example, investigations of specific cell types and states, differentiation trajectories, transitions between cell states, and use of spatial context. Currently, single-cell and spatial RNA-Seq are the main techniques considered for modeling purposes — however, this may soon change. Recent advances in single-cell proteomics and metabolomics show promise for new modeling approaches, and in the future, it may be possible to combine several of these data types in a single simulation. While single-cell omics is still more costly than bulk, the costs are decreasing with time. With lowered sequencing costs and improved single-cell techniques, the number of sequenced cells per data set and the number of captured molecules per cell will increase and thereby enable separation of the cells into clusters with more subtle differences. However, despite the rapid advances in the different fields of single-cell omics, the goal to generate context-specific models and predict metabolic states from single cells with reasonably low uncertainty will likely still require a few more years to be reached.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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