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Minireview

Modelling of glucose repression signalling in yeast *Saccharomyces cerevisiae*

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One sentence summary: The authors review and discuss modelling approaches that have been used to study the dynamic behaviour of signal transduction pathways with the specific emphasis on the Snf1-regulated glucose signalling and propose potential ways forward that aim at elucidating the dynamics of SNF1 pathway.
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Abstract

Saccharomyces cerevisiae has a sophisticated signalling system that plays a crucial role in cellular adaptation to changing environments. The SNF1 pathway regulates energy homeostasis upon glucose derepression; hence, it plays an important role in various processes, such as metabolism, cell cycle and autophagy. To unravel its behaviour, SNF1 signalling has been extensively studied. However, the pathway components are strongly interconnected and inconstant; therefore, elucidating its dynamic behaviour based on experimental data only is challenging. To tackle this complexity, systems biology approaches have been successfully employed. This review summarizes the progress, advantages and disadvantages of the available mathematical modelling frameworks covering Boolean, dynamic kinetic, single-cell models, which have been used to study processes and phenomena ranging from crosstalks to sources of cell-to-cell variability in the context of SNF1 signalling. Based on the lessons from existing models, we further discuss how to develop a consensus dynamic mechanistic model of the entire SNF1 pathway that can provide novel insights into the dynamics of nutrient signalling.

Keywords: glucose signalling, Boolean, dynamic, single-cell, systems biology

Introduction

To survive in a constantly changing environment, living organisms adapt to new conditions by rapidly responding to the multitude of external stimuli. Once a stimulus is received by the membrane receptors, the signal is further processed through the cascades of biochemical reactions leading to specific alterations that may occur at the level of gene expression, metabolism and growth. In general, cellular response is mediated by spatial and temporal dynamics of signalling networks (Krauss 2006).

Glucose is the preferred energy source that yeast utilize to maintain cellular homeostasis and well-being. In response to altering glucose concentration in the cellular microenvironment, three major glucose signalling pathways—AMPK/SNF1, Rgt2/Snf3 and cAMP/PKA—orchestrate glucose transport, metabolism and overall transcriptional response in order to adapt to new conditions (Kim *et al.* 2013). Glucose sensing and signalling have been primarily studied on the budding yeast *Saccharomyces cerevisiae*, and overall represent a model paradigm of extracellular stimuli transduction resulting in appropriate changes in gene expression (Kim *et al.* 2013).

The AMP-activated protein kinase (AMPK) is conserved throughout all eukaryotes and plays the main role in integrating information about energy source availability and environmental stress factors in order to induce an adaptive response. The yeast AMPK/SNF1 signalling pathway regulates energy homeostasis and is best known for its role in glucose derepression (Celenza and Carlson 1986). One of the main targets of the Snf1 protein kinase is the transcriptional repressor Mig1 that controls the expression of genes essential for the metabolism of carbon sources such as sucrose, maltose and galactose (Nehlin *et al.* 1991, Hu *et al.* 1995, Wu and Trumbly 1998). In brief, upon glucose availability, Mig1 occupies its target promoters resulting in repression of associated genes (Devit *et al.* 1997, Wu and Trumbly 1998). Glucose limitation causes Snf1 phosphorylation, which in turn results in Mig1 phosphorylation and translocation to the cytoplasm, hence gene expression release (Lutfiyya *et al.* 1998, Smith *et al.* 1999, Ahuatzi *et al.* 2007, Shashkova *et al.* 2017). Although the SNF1 signalling has been extensively studied experimentally, and thus well characterized biochemically and genetically, and the crosstalk with other nutrient pathways has been elucidated (Shashkova *et al.* 2015), dynamic aspects of the interplay between the Snf1–Mig1 signalling components remain unclear.

To understand how complex biological systems integrate and coordinate the activity of all their elements, it is not sufficient to study individual components of the system but to take into account molecular interactions and reaction kinetics. To address such challenges, the field of systems biology emerged. It is an interdisciplinary field that merges experimental data collec-

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tion with mathematical and computational methods. Based on a holistic approach, mathematical modelling describes how multiple complex regulatory modules and cellular processes are connected, hence providing an invaluable tool to develop hypotheses and test them by computational simulations as well as targeted experiments (Fischer 2008). The Hohmann group was one of the pioneers in implementing mathematical modelling to describe complex signalling pathways in *S. cerevisiae* and, therefore, to establish the field of yeast systems biology (Krantz *et al.* 2004, Klipp *et al.* 2005, Mustacchi *et al.* 2006, Krantz *et al.* 2009, García-Salcedo *et al.* 2014, Welkenhuysen *et al.* 2017).

To study SNF1 signalling, both top-down (Usaite *et al.* 2009, Zhang *et al.* 2010, 2011) and bottom-up systems biology methods have been developed and implemented, enabling our understanding of the functioning of this pathway in more general and fundamental terms. Here, we review and discuss bottom-up modelling approaches (Table 1) that have been used to study the dynamic behaviour of signal transduction pathways with the specific emphasis on the Snf1-regulated glucose signalling. We also propose potential ways forward to elucidate the dynamics of the SNF1 pathway.

Boolean modelling—a tale of two values

To date, a large amount of qualitative data on the SNF1 pathway are available; however, aggregating these data to predict the pathway's response to different stimuli is still challenging. Boolean models (Fig. 1B) represent a simple yet powerful way to create a pathway spanning model based on available qualitative statements, such as 'the Reg1 phosphatase dephosphorylates SNF1' (Saadatpour *et al.* 2010, Wang *et al.* 2012, Saadatpour and Albert 2013). In the past years, three Boolean models have been built on SNF1 signalling (Christensen *et al.* 2009, Lubitz *et al.* 2015, Welkenhuysen *et al.* 2019).

To predict changes in gene expression, Christensen *et al.* (2009) developed the first Boolean model that includes, besides the SNF1, the Snf3/Rgt2 pathway and the MAL and GAL regulatory systems. The model was built upon an extensive literature review, and its relatively weak predictive power (accuracy score of 60% compared with transcriptomic data) strongly suggested gaps in our understanding of the glucose regulatory network. Furthermore, the authors highlighted that a Boolean representation could be inadequate when modelling complex signalling interactions, such as multiple transcription factors acting on the same gene.

Building further and exploiting experimental data available in the literature, Lubitz *et al.* (2015) performed a comprehensive reconstruction of the entire SNF1 pathway based on 440 papers. Apart from representing an extensive summary, the authors proposed a workflow for signalling network reconstruction building on an rxncon language structure (Tiger *et al.* 2012). This approach showed that without filling the knowledge gaps with hypothetical components (so-called gap filling), the proposed model could not predict the observed behaviour of the SNF1 pathway. This suggests the presence of other processes and reactions, besides the ones included in Lubitz's and Christensen's models, that play an important role in the pathway regulation.

Welkenhuysen *et al.* (2019) built a Boolean model to investigate the impact of crosstalk between the SNF1, Snf3/Rgt2 and PKA pathways. By adding two documented crosstalks, the model could predict gene expression patterns more accurately compared with a model without implemented crosstalk. However, the model was still not fully consistent with the expected behaviour of the SNF1 pathway. Following Lubitz's approach, the authors used gap filling to create a testable hypothesis of the missing interactions. In the end, the addition of four hypothetical phosphatases made the model consistent with observed data.

Overall, the Boolean approach shows that when we collect all known interactions within the pathway, the behaviour of the SNF1 signalling cannot be fully explained. Besides knowledge gaps, the predictive power of these models is limited by the Boolean formalism (Christensen *et al.* 2009), which can be inadequate when modelling complicated reactions or non-binary scenarios, such as switching between media with different glucose concentrations. Furthermore, Boolean models have a crude time description, which may cause the discrepancy in the temporal behaviour of the system components (Welkenhuysen *et al.* 2019), demonstrating why such models are not suitable for predicting the dynamics of signalling. Taken together, the Boolean approach imposes the assumption that biological systems are discrete in nature, which limits the predictive power of such models for a complex network like the SNF1 pathway.

How to then move beyond Boolean modelling? One way is qualitative modelling, where system flexibility can be achieved by allowing the components to have various values, such as different activity levels (Schaub *et al.* 2007). This can further be coupled with an asynchronous rule (Garg *et al.* 2008), where reactions within the network are allowed to occur on different time scales. The alternative approach overcoming the shortcomings of Boolean models is kinetic dynamic modelling (Klipp and Liebermeister 2006).

Dynamic modelling—a tale of time-dependent changes

The dynamic of the SNF1 signalling is usually studied with quantitative time-lapse experiments, such as immunoblotting and qPCR. A powerful tool to interpret experimental results in the context of the pathway dynamics is dynamic kinetic modelling (Fig. 1C) based on the ODEs (Chen *et al.* 2004, Klipp *et al.* 2005, Bachmann *et al.* 2011). For SNF1 signalling, two such models have provided insights into the pathway's dynamics (Kuttykrishnan *et al.* 2010) and structure (García-Salcedo *et al.* 2014).

To unravel the structure of the SNF1 pathway, García-Salcedo *et al.* (2014) proposed several hypothetical pathway structures, which they translated into 24 small dynamic models. Each model was then fitted to the available data of, for example, Mig1 localisztion and Snf1 phosphorylation. Based on the fit, the models were then ranked via the Akaike and χ^2 model selection criteria. This systematic approach allowed for the identification of likely pathway structures, and the best-ranked models predicted that phosphatases play a crucial role in regulating SNF1. In addition, the best model, with a small margin in selection criteria, suggested that glucose regulates Mig1 solely via Snf1. Since the models were fitted and subsequently compared based on a limited amount of data, the authors pointed out that the standard selection criteria, like Akaike and χ^2, do not always select the best from similarly scoring models (Vrieze 2012).

While García-Salcedo *et al.* (2014) fitted proposed dynamic models to data to estimate any unknown kinetic parameters, Kuttykrishnan *et al.* (2010) mined parameters from the literature to build a model on the hexose transporters (HXT) gene regulatory layer. The obtained dynamic model of the HXT gene regulation was then

Table 1. Existing mechanistic models of the SNF1 pathway, in the order they appear within this review. The columns are the author(s), model type, cellular pathways included in the model, model size measured by the number of model states/components such as metabolites, proteins, etc. (not counting reactions) and short description of the main aim of the modelling.

Author(s)	Model type	Included pathways	Size	Aim
Christensen *et al.* (2009)	Boolean	GAL–MAL regulatory system and SNF1–Snf3/Rgt2 pathways	Large 72 components	Predict transcriptional responses for various nutrient conditions and/or deletion strains
Lubitz *et al.* (2015)	Boolean	SNF1 components	Large[a] 52 components	Create a comprehensive reconstruction of the SNF1 pathway
Welkenhuysen *et al.* (2019)	Boolean	SNF1, PKA and Snf3/Rgt2 pathways	Large[a] 80 components	Investigate the role of crosstalks between nutrient-sensing pathways
García-Salcedo *et al.* (2014)	Mechanistic ordinary differential equation (ODE) model	SNF1 pathway	Small[b] 8 components	Elucidate how Mig1 and SNF1 are regulated
Kuttykrishnan *et al.* (2010)	Mechanistic ODE model	SNF1–Snf3/Rgt2 pathways, HXT regulatory layer	Medium 24 components	Elucidate the dynamics of how glucose sensing regulates *HXT* genes
Welkenhuysen *et al.* (2017)	Mechanistic single-cell model	SNF1 pathway	Small 8 components	Elucidate sources of cell-to-cell variability in Mig1 localization
Almquist *et al.* (2015)	Mechanistic single-cell model	SNF1 pathway	Small 2 components	Elucidate how Mig1 localization is regulated upon glucose addition to starved cells
Persson *et al.* (2020)	Mechanistic single-cell model	SNF1 pathway	Small[b] 5 components	Elucidate how the *SUC2* gene is regulated upon long-term glucose starvation
Persson *et al.* (2021)	Mechanistic single-cell model	SNF1 pathway	Small[b] 4 components	Elucidate reactions and sources of cell-to-cell variability behind Mig1 localization upon fructose addition to starved cells
Jalihal *et al.* (2021)	Mechanistic ODE model	SNF1, PKA and TOR pathways	Medium 30 components	Create a consensus dynamic model of nutrient sensing in yeast
Österberg *et al.* (2021)	Hybrid model, Boolean (signalling), FBA (metabolism)	Carbon and nitrogen metabolism; the SNF1, PKA and TOR pathways	Large[c] 337 components	Elucidate the impact of nutrient signalling on the metabolism

[a]Not including the hypothetical components obtained from gap filling.
[b]When the paper includes several models, we refer to the largest.
[c]Counting the number of components in the signalling module, and the number of metabolites and enzymes in the metabolic module.

coupled with a simplified model of the SNF1–Snf3/Rgt2 pathways. Since the kinetic parameters for the regulatory layer were obtained from the literature, only a few parameters for the small SNF1–Snf3/Rgt2 model needed to be estimated. This approach resulted in a model with biologically justifiable kinetic parameters that accurately predicted the expression of the *HXT1–HXT4* genes for varying glucose levels. Furthermore, the model showed that *HXT4* exhibits an expression pulse after the addition of 0.1% glucose to starved cells, which was then confirmed experimentally.

While dynamic modelling goes beyond the Boolean approach, there are also some limitations. Typically, the level of details within the model we can build is defined by the kinetic parameters that either have already been reported or can be estimated based on the published data. However, only a few parameters are available for the SNF1 pathway, and the amount of the quantitative time-lapse or steady state data for estimation is also limited. This restricts the level of details of models with reasonably estimated parameters we can build, impacting how much we can learn about SNF1 signalling by comparing models via selection criteria only. In addition, existing dynamic ODE models of the SNF1 signalling only describe population averages as they are fitted using aggregated data (e.g. western blots). The dynamic behaviour of molecules at the individual level can differ substantially from the ensemble average. To deduce the cell level behaviour of the pathway, several studies have employed single-cell dynamic modelling.

Single-cell dynamic modelling—a tale of individual cells

To reach a complete understanding of the SNF1 pathway, we should study it cell by cell. Experimental techniques like single-cell microscopy can be used to monitor cellular dynamics, for example, in the context of protein variability between cells over time. However, the complexity of the SNF1 pathway makes it challenging to elucidate the dynamics of signalling from experiments. Similarly, determining whether stochastic chemical reactions within the pathway (intrinsic noise) and/or outside signals like cell cycle state (extrinsic noise) are major sources of cell variability is non-trivial via experimental approaches (Elowitz *et al.* 2002, Swain *et al.* 2002). Single-cell models (Fig. 1D) can be applied to rationalize single-cell experiments, and they have been used to study the dynamics (Almquist *et al.* 2015, Persson *et al.* 2020) and sources of cell heterogeneity for the SNF1 pathway (Welkenhuysen *et al.* 2017, Persson *et al.* 2021).

Combining microscopy data of Mig1 localization with mechanistic modelling, Welkenhuysen *et al.* aimed at explaining sources of cell-to-cell variability (Welkenhuysen *et al.* 2017). Cell heterogeneity was assumed to arise from extrinsic noise and was modelled by letting the kinetic parameters vary between cells. This is a common approach, as these parameters often relate to extrinsic noise sources like metabolic activity (Zechner *et al.* 2014). By analysing how changes in rate parameters affect Mig1 local-

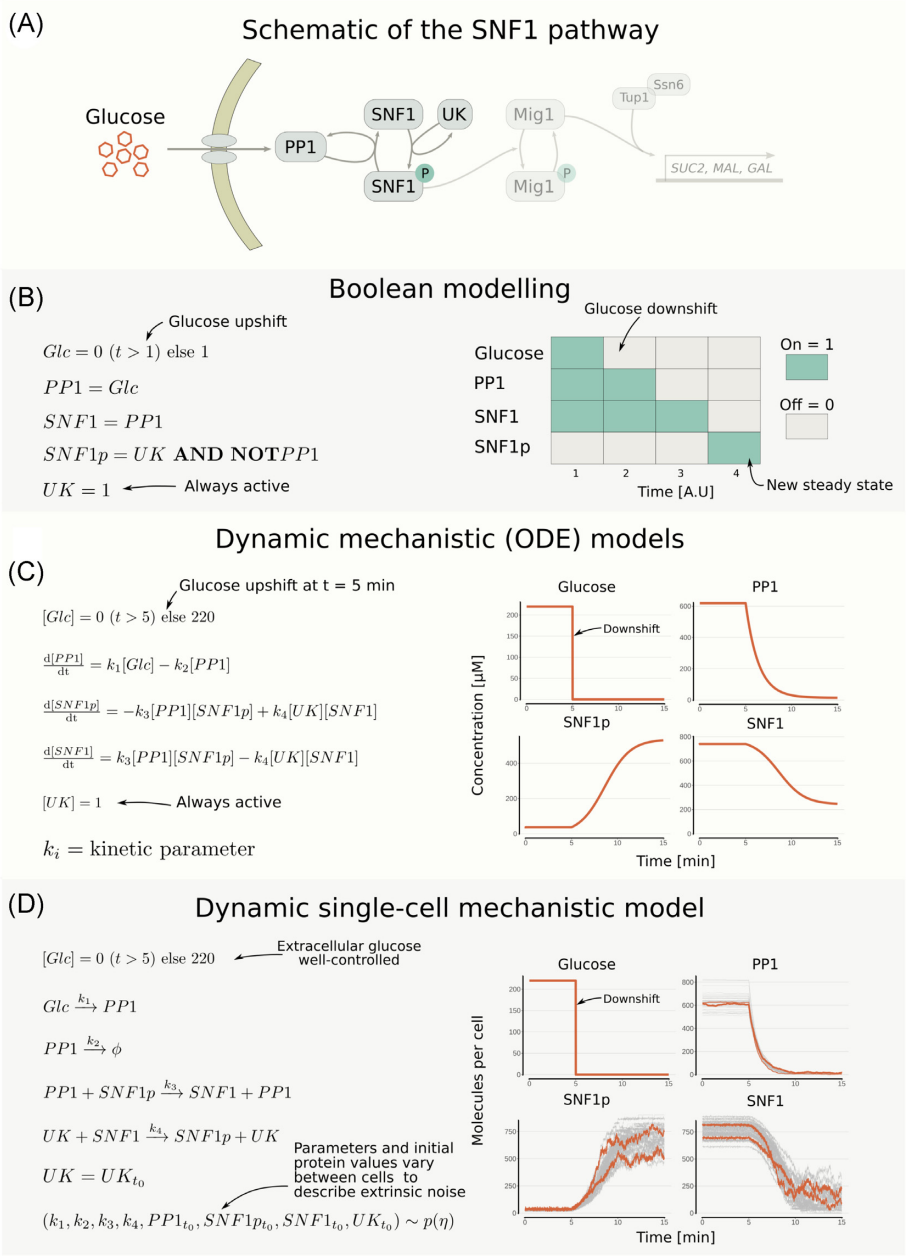

Figure 1. The three types of mathematical models used to model SNF1 signalling. Demonstration of how the **(A)** none-faded part in the simplified description of the SNF1 pathway can be modelled upon a glucose downshift using **(B)** Boolean, **(C)** dynamic kinetic and **(D)** single-cell mechanistic modelling. Note that the models here are purely demonstrative. (B) A Boolean model is composed of Boolean statements, which can be simulated to understand how a network moves from one steady state to another. Here, we simulated the model using a synchronous update scheme. (C) A dynamic mechanistic (ODE) model consists of ordinary differential equations (ODEs) that are based on reaction rates. By solving these ODEs, for a given set of kinetic parameters k_i, the concentrations of species can be simulated over time (right). (D) A single-cell mechanistic model is composed of chemical reactions. These are simulated using ODEs if intrinsic noise is assumed negligible, else an appropriate stochastic simulator is used (reviewed in Gillespie 2007). Extrinsic noise is often modelled by letting a subset, or all kinetic rates and initial protein values, vary between cells by following a probability distribution $p(\eta)$. By accounting for sources of cell-to-cell variability, a single-cell model simulates the dynamics of an entire cell population over time (right).

ization, it was suggested that the Mig1 shuttling in and out of the nucleus is a source of cell heterogeneity. Due to the lack of experimental data, most kinetic rates were arbitrarily estimated. However, to base conclusions on such parameters, it is important that they are accurately estimated, and that the estimation uncertainty is assessed by likelihood-based statistical methods.

To rationalize microscopy time-lapse data on Mig1 localization (Almquist *et al.* 2015) and *SUC2* expression (Persson *et al.* 2020), Almquist *et al.* and Persson *et al.* translated plausible hypotheses of the SNF1 pathway structure into mechanistic models. By fitting models to each individual cell within their respective data sets (50–100 cells were observed per set), it was determined which hypotheses were capable of describing the observed experimental dynamics. By constructing minimalistic models, Almquist *et al.* showed that delayed positive feedback could explain why Mig1 re-enters the nucleus after a glucose upshift and that this feedback is co-regulated with the process regulating Mig1 nuclear exit. Furthermore, by combining modelling with additional ex-

periments, Persson *et al.* showed that the *SUC2* expression likely decreases upon long-term glucose starvation due to a partial recovery in cellular energy levels, which reduces the activity of the SNF1 complex.

These two approaches demonstrated how modelling could help interpret single-cell experiments; however, in both studies, negligible intrinsic noise was assumed. This is often used to facilitate kinetic parameter estimation, which, however, may cause bias in such evaluations (Munsky *et al.* 2018, Wiqvist *et al.* 2021). To circumvent this, a Bayesian framework for estimating kinetic parameters in models that account for both intrinsic and extrinsic noise has been recently introduced (Persson *et al.* 2021).

Using this framework, Persson *et al.* (2021) studied Mig1 localization upon fructose addition to starved cells. To build four small but not minimalistic models with interpretable kinetic parameters, the authors included prior information about potential values into their Bayesian parameter estimation. These models were then ranked based on their ability to describe Mig1 localization data for all observed cells, and the best model suggests that Mig1 starts to leave the nucleus 5 min after a fructose upshift due to metabolism-dependent feedback. Moreover, by analysing the estimated kinetic parameters between cells, the authors suggest, in line with their experiments, that a potential source of cellular heterogeneity in Mig1 localization is the activity of hexokinases.

Available single-cell dynamic models are typically small and sometimes even minimalistic, where most of the biological players are black-boxed. This is not ideal, as such models often cannot predict which pathway components are key regulators behind observed data. Indeed, Almquist's model revealed delayed feedback but not the specific pathway components regulating it. This can be circumvented by adding more details to a model. However, as illustrated by Welkenhuysen *et al.* (2017), this may lead to arbitrary estimated parameters that cannot be fully interpreted and can also negatively impact the model's predictions (Gutenkunst *et al.* 2007, Chis *et al.* 2016). To build more detailed models with non-arbitrarily estimated parameters requires extensive quantitative single-cell time-course data. However, even if such data sets could be gathered, methods for estimating kinetic parameters have limitations (reviewed in Loos and Hasenauer 2019). In essence, the development of methods for both data collection and kinetic parameter estimation is needed to allow for more detailed single-cell models.

Even though the single-cell models zoom in to a specific part of the pathway while black-boxing the surroundings, they can still reveal sources of cell heterogeneity. Modelling these black boxes often involves several assumptions that may affect a model's predictive power. To ease this approach, we propose to initially construct a pathway-spanning kinetic ODE model of the SNF1 pathway. More specifically, a large-scale kinetic model can help us understand the surrounding population average input to the subsystem we are modelling at the cell level. Naturally, the premise of this methodology is the existence of a kinetic model on the entire SNF1 pathway.

Thinking big with dynamic models

Most of the models we discussed earlier have focused on a small part of the SNF pathway, limiting their ability to predict its response to previously undescribed scenarios. To fully account for the dynamic behaviour of the SNF1 pathway, we need a holistic approach that considers crosstalks with other nutrient pathways. Addressing all of these, Jalihal *et al.* (2021) recently developed a kinetic dynamic model of nutrient signalling in yeast integrating the SNF1, TOR and PKA pathways. To estimate the kinetic parameters, the authors scourged the literature for quantitative time-lapse and steady state data. The estimated model fits these data, which noticeably spans across various nutrient conditions. Moreover, it predicts the response of nutrient-sensing regulated transcription factors in deletion strains, such as *gcn4*Δ.

However, while around 18 000 sets of kinetic parameters adequately fit the data, it cannot be distinguished which ones produce the best predictions. Since different parameter sets sometimes produce different model dynamics, there are scenarios where model predictions are highly uncertain. To address this, additional quantitative experimental data are required to constrain the kinetic parameters, and the proposed Jalihal's model can help design these. Relevant experiments are those where parameter sets yield conflicting predictions, such as activation of transcription factors in deletion strains. Moreover, parameter estimation has classically been based on quantitative data, but recently frameworks that consider qualitative data were introduced (Mitra *et al.* 2018).

In addition, a relevant area of model improvement is the time span. The model currently describes the nutrient network up to 30 min after a nutritional shift; however, there are scenarios where the long-term response is relevant to study, for example when investigating the role of nutrient sensing on ageing (Coccetti *et al.* 2018) or when trying to rewire signalling for metabolic engineering purposes (Nielsen and Keasling 2016). However, modelling the long-term response of nutrient signalling is complicated since it is regulated by feedback that acts via the metabolism (Conrad *et al.* 2014); hence, an integrated signalling and metabolic model is required (Jalihal *et al.* 2021).

Modelling nutrient sensing + metabolism = profit?

During long-term signalling, SNF1 regulates the metabolism in several ways, such as by modulating transcription factors or directly through post-translational modifications (Woods *et al.* 1994). In turn, the metabolism regulates SNF1 signalling; for example, ADP binds to and subsequently protects the SNF1 complex from dephosphorylation (Chandrashekarappa *et al.* 2011). To understand this interplay, Österberg *et al.* (2021) recently developed a framework that integrates a Boolean model of the SNF1, TOR and PKA pathways, with an enzyme constraint flux balance model of the central carbon metabolism. Within this hybrid model, the intracellular glucose level is obtained from the metabolism module and then used as an input to the nutrient-sensing module. This module then regulates the transcription factors, which further controls the enzyme levels in the metabolic module. Overall, this integration of signalling and metabolism yields improved predictions of the metabolic enzyme levels compared with previous models and theoretically can be used to explore long-term signalling.

Due to the inconsistencies in available proteomics data (Arike *et al.* 2012, Sánchez *et al.* 2021), validation of hybrid models like that of Österberg remains challenging. Moreover, there are uncertainties in the cellular physiology, growth, metabolism and dynamic data used to calibrate the model parameters. This is limiting, as the constrained model used in the metabolic module can often not be simulated/solved if it tries to match uncertain data on enzyme activities. One way to allow for the incorporation of useful but uncertain data is to develop metabolic modelling frameworks that can, to a certain degree, deviate from measured enzyme activities. Another hardship is modelling the gene reg-

ulatory layer because it is not fully understood. The prospects of gaining enough knowledge to properly model gene regulation are promising since the amount of available data under various conditions is increasing, together with improvements in methods to infer regulatory effects from such data (Wang *et al.* 2017).

Further, the signalling module in Österberg's framework is a Boolean model. To gain more knowledge on how metabolic feedback affects nutrient signalling, a fine-grained representation of the signalling module in the form of a dynamic model is desirable. This is because not all metabolic feedback signals are likely to cause a discrete switch of protein activity.

Outlook

The SNF1 pathway is a central part of the nutrient signalling system in *S. cerevisiae*. Systems biology approaches have been employed to improve our knowledge about the behaviour of SNF1 signalling and reveal the key gaps in understanding its complex interactions. To date, numerous types of models have been developed (Table 1), ranging from a Boolean model to understand the role of crosstalk to a dynamic model to elucidate the time-dependent regulation of genes, to single-cell models to reveal sources of cell heterogeneity. To capture the highly dynamic behaviour of the pathway, dynamic models have proven to be particularly useful, and with recent advances (Jalihal *et al.* 2021), the field is moving in a direction to develop a consensus dynamic model on the entire SNF1 pathway. Ideally, the aim is to have a model with non-arbitrary estimated kinetic parameters, hence non-arbitrary predictive power, which requires high-quality experimental data. Moreover, we must consider crosstalks with other nutrient-sensing pathways as well as the interplay between signalling and metabolism to enable the study of long-term signalling.

A challenge, but if such a modelling framework can be developed for yeast, it can, like the yeast cell cycle model (Chen *et al.* 2004), act as a foundation to reason about the entire pathway's behaviour, providing insights into the dynamics of nutrient signalling and the interplay of signalling with metabolism. Finally, this approach could potentially be scaled to multicellular organisms to shed light and provide direct clues to underlying mechanisms of multiple chronic and age-related diseases.

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