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Yeast systems biology in understanding principles of physiology underlying complex human diseases

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Highlights

- Yeast is widely used to study cellular processes underlying complex human diseases
- Yeast systems biology provides holistic insights into disease-associated processes
- Findings in yeast systems biology are readily transferrable to human cell models
- Omics data integration into GEMs can accelerate advances in personalized medicine

Abstract

Complex human diseases commonly arise from deregulation of cell growth, metabolism, and/or gene expression. Yeast is a eukaryal model organism that is widely used to study these processes. Yeast systems biology benefits from the ability to exert fine experimental control over the cell growth rate and nutrient composition, which allows orthogonal experimental design and generation of multi-omics data at high resolution. This has led to several insights on the principles of cellular physiology, including many cellular processes associated with complex human diseases. Here we review these biological insights together with experimental and modeling approaches developed in yeast to study systems biology. The role of yeast systems biology to further advance systems and personalized therapies for complex diseases are discussed.

Introduction

Saccharomyces cerevisiae is a widely used eukaryal model organism that has been instrumental for studying many pathways that are associated with human disease development. For many years yeast has been the go-to organism in functional genomics studies due to the ease by which the yeast genome could be manipulated, and the high degree of conservation in both protein function and regulatory circuitry between yeast and human cells [1]. Knowledge gained by studying yeast in an unbiased approach, eg through a genome-wide screen, can quickly be transferred to humans by confirming the finding(s) via a targeted approach in a human cell model, or by consistent observations in disease symptoms or clinical trials [1]. As such, yeast has been used to model many conditions/processes leading to complex diseases, most notably for several hallmarks of cancer (eg cell cycle control [2●], programmed cell death [3], genome instability [4]); different types of metabolic disorders (eg cholesterol metabolism [5], diabetes [6], mitochondrial dysfunction [7]); factors influencing aging (eg dietary restriction [8]); and etiology of neurodegenerative diseases (amyloid pathologies [9] and lysosomal storage disorders [10]).

At the systems level, many of these multi-factorial diseases arise from a deregulation of one or a combination of three key aspects of cell biology: cell growth, metabolism, and gene expression. While most of the individual components for each of these processes have been identified, currently we lack an understanding of how physiology is shaped by them in a holistic way. To this end, yeast has been used to pioneer the field of systems biology, in the same way that yeast molecular biology paved way for similar studies in human cells [1,11]. Using yeast as a model organism, new concepts and tools have been developed for top-down integration of multi-omics data into mathematical models, which allows cellular functions to be simulated *in silico*. Here, we review the principles of physiology that were discovered using yeast systems biology, which has given insight into the mechanisms of complex diseases in human cells. We also discuss new modeling approaches developed in yeast that will accelerate both human systems biology research and the development of systems and personalized therapeutic interventions.

Cell growth

In a basic model of cell growth, duplication of a cell entails duplication of each of its components [12]. The single largest macromolecular component of the cell biomass is protein, which in yeast represents 40-60% of dry mass depending on the growth condition; while RNA and DNA represent 8-10% and 0.1% of the yeast dry mass, respectively (Fig 1A) [13]. Moreover, protein is the only component that is self-replicating via ribosomes, which places a natural limitation on the cell growth rate. Thus it has long been proposed that cell growth is limited by protein synthesis, and many

"classic" models of cell growth were formulated based on the self-replicating nature of ribosomes [14-16]. Indeed, in bacteria it has been shown that cells finely tune their ribosomal protein content to precisely match the protein synthesis demand at any given growth rate [17]. However, to directly test this hypothesis in fast-growing eukaryal cells such as yeast or human cancer cells, it is necessary to globally profile the expression dynamics of proteins under highly controlled experimental conditions, which has only recently been accomplished in yeast. Yeast is particularly suitable for these types of experiments, because the growth of yeast cells can be controlled very precisely and maintained at steady-state, which is often an important assumption in these models [1]. By growing yeast at steady-state at a controlled growth rate, Lahtvee *et al* [18●●] generated a global matching dataset that includes transcript abundance, protein abundance, protein degradation rates, and calculated protein synthesis rates. Integrative analysis of this data shows that protein synthesis rate matches closely with the rate of protein dilution from cell division, and degradation rate contributes very little to the expression dynamics of most proteins [18●●]. Thus, the physiological task of replicating the proteome places a global constraint on how fast cell replication can occur as a whole. In contrast, multiple transcript expression dynamics datasets [19,20] have shown that globally the mRNA synthesis rate is much faster than the dilution rate, and most mRNAs are synthesized and degraded several times in a cell cycle. However, when growth conditions are experimentally modified to restrict phosphate availability (a critical component of nucleotides), the control of growth rate is shifted towards mRNA synthesis [21●●], presumably because under these conditions it becomes slower to accumulate mRNA to the point of duplication (Fig 1B). From an evolutionary perspective, it has been shown that the abundance of orthologous proteins is highly conserved between *S. cerevisiae* and *S. pombe* (across 400 million years of evolution), while protein degradation rates have significantly diverged [22], indicating that protein abundance is constrained by a strong evolutionary pressure which likely arose from the pressure to maintain a fast growth rate. In contrast, most of the orthologous genes between *S. cerevisiae* and *S. paradoxus* (across 5-10 million years of evolution) have diverged in mRNA abundance [23] as well as mRNA synthesis and degradation rates [24], in line with the idea that growth rate is not limited by mRNA abundance and thus there is less selection pressure to conserve transcript expression in evolution.

As yeast systems biology affirms that growth rate is limited by protein synthesis, intuitively growth rate would be maximized when cells only express the minimum amount of proteins necessary to carry out all cell functions. However, in most instances cells employ a trade-off and maintain excess proteins as reserves, while growing at a rate that is lower than the theoretical maximum [25●●,26●]. The size of the protein reserves underlies how quickly cells can respond to changing environments [26●]. For pharmaceuticals that target endogenous enzymes or processes, the sizes of

specific protein reserves may also define the therapeutic window of the drug, whereby the same amount of a drug (eg an enzyme inhibitor) affects the growth of disease cells, but healthy cells remain robust due to the presence of excess enzyme capacity [27]. Thus, quantifying the amount of protein reserves in normal and diseased cells will have significant impact on the design and testing of pharmaceuticals. Recently several approaches have been taken to estimate the protein reserves of various processes in yeast. By generating high-resolution proteomic datasets of yeast growing at different growth rates, Metzl-Raz *et al* [25●●] showed that as growth rate increases, higher proportions of the proteome are allocated to ribosomal proteins and enzymes in amino acid biosynthesis, while lower proportions are allocated to enzymes in central carbon metabolism (CCM). This indicates that at lower growth rates, cells maintain high amounts of CCM enzymes in excess. At faster growth rates, cells forsake these reserves in CCM in order to express more ribosomal proteins and enzymes in amino acid metabolism [25●●], in line with the idea that protein synthesis regulates the cell growth rate. These results also highlight that the size of metabolic reserves can change depending on the growth rate, which means that proteome reserves must be quantified in the context of specific growth rates. Indeed, using an *in silico* approach to compare the amount of enzymes needed in computational simulations of cell growth and the experimentally measured enzyme abundance, Sanchez *et al* [28●●] have estimated that overall, 51% of all enzymes that are expressed in yeast are in active usage during typical laboratory growth (Fig 1C). Moreover, this approach allows enzyme usage to be estimated for each enzyme, which clearly shows that while most enzymes are synthesized in excess to maintain reserve capacities, a small amount of enzymes operate at or close to 100% capacity [28●●], indicating that these may be important rate-limiting control points that regulate the overall flux through the corresponding pathways. It can be anticipated that employing similar computational/experimental approaches in human cell models will allow the identification of similar points of control, which could represent potential drug targets in human diseases. Furthermore, this approach allows patient-specific enzyme abundance to be incorporated, which will enable the design and testing of personalized therapeutics against targeted enzymes.

Metabolism

The idea that replication of the proteome is a limiting factor in cell growth also manifests in the high energy cost of protein synthesis. In yeast, protein synthesis consumes 50-79% of energy generated in the cell [29-31], and the interaction between energy balance and protein synthesis gives rise to a trade-off between growth rate and biomass yield [31,32]. In human cells, dysregulation of metabolism and energy balance also underlies many diseases, including diabetes, mitochondrial diseases, and the Warburg effect in cancer. Since metabolism is a highly connected network of

thousands of reactions, it is necessary not only to study the molecular mechanisms of individual enzymes and pathways, but also interactions between network components in the whole system. Genome-scale metabolic models (GEMs) represent one such framework of systems biology [33]. GEMs are complete metabolic maps representing the whole metabolic capacity of the cell or organism, where the stoichiometry and enzyme requirement of each metabolic reaction are recorded *in silico*. Today, comprehensive GEMs are available for a large number of organisms, including both yeast [34,35●●] and human cells [36,37]. Many human GEMs that are specific to certain tissue types or cancer cell lines are also available and have been used to study diseases and identify therapeutic targets [38,39].

With the rapid development of omics techniques, recently it has become of interest to integrate omics-level data into the GEM framework, which will allow points of metabolic flux control to be identified. Two general approaches have been used for this endeavour with yeast GEM as a scaffold of metabolic analysis. In the first approach, yeast GEM is used as-is to simulate metabolic flux at a given experimental condition, and how flux through individual enzymes or pathways is controlled can be inferred by comparing the simulated flux with the experimentally measured transcriptomic, proteomic, and/or phospho-proteomic levels (Fig 2A-B). Through this approach it was found that changes in the mRNA abundance of mitochondrial respiration proteins correlates well with changes in flux, while flux through the glycolytic pathway is not correlated with either the mRNA abundance or the protein abundance of glycolytic enzymes [18●●,40]. Instead, glycolysis appears to be engaged in complex regulation circuits involving a combination of substrate concentrations, enzyme allostery, and regulator crosstalk [41●●]. In the second approach, new modeling methodologies are developed to incorporate omics data into GEM simulations themselves. For example, two studies [28●●,42] have directly incorporated experimentally-measured enzyme abundance into GEM simulations, called enzyme-constrained GEM (ecGEM). In ecGEM, the simulated rate of each reaction cannot exceed an upper limit defined by the abundance and the turnover number k_{cat} of the catalyzing enzyme (Fig 2A-B). Through this modeling technique it was found that the Crabtree effect in yeast, analogous to the Warburg effect in human cancer cells [43], is determined by enzyme constraint [28●●,42]: since enzymes in glycolysis and fermentation are smaller and more catalytically efficient compared to enzymes in the TCA cycle and ETC components, at high growth rates cells will trade off the bulky respiratory machinery for fermentation machinery, which has a smaller proteome cost and therefore enables cells to allocate more protein to translation, ie ribosomes, which is necessary for increasing its growth rate [28●●,42]. In a similar approach as direct incorporation of proteomics data in yeast GEM, a number of studies have directly incorporated metabolomics data into models of metabolic subsystems in yeast (Fig 2A-B) [44-46], although to date this approach has not been used

genome-wide. This is in part due to the difficulty in obtaining metabolomics data at high quality and high coverage, but rapidly improving technologies will soon allow these limitations to be lifted [33]. In this modeling concept, the rate of a reaction is estimated based on the Gibbs free energy of the reaction, which is in turn regulated by metabolite concentrations and thermodynamic equilibrium [44-46]. Indeed it has been shown that for most metabolic reactions, calculated flux is better correlated with metabolite concentrations than enzyme abundance [47●●]. Thus, simulations with this modeling concept allows the identification of key flux-controlling metabolites as biomarkers, as exemplified in the identification of cytosolic-mitochondrial NAD^+/NADH ratio as a key regulator of the ethanol-acetaldehyde redox shuttle [46]. In the near future it can be anticipated that multiple layers of omics data will be simultaneously incorporated into the GEM framework to allow for more sophisticated modeling of complex cellular behaviours. Since most yeast proteins and metabolic pathways have homologous counterparts in human cells, these insights obtained in yeast can be readily translated or verified in human cell models of pathophysiology in metabolic diseases.

Gene expression

Nearly all diseases are associated with changes in gene expression, either as a driver of disease onset and progression, or as a part of the cellular response to the diseased state. Gene expression changes in multifactorial diseases have received wide attention in clinical research to identify potential targets for clinical intervention. This was pioneered by transcriptomic profiling and is now pushing the bounds of personalized medicine through a rising capability to generate patient-specific datasets and lowering cost [48]. A major underlying assumption of these studies is that changes in transcript-level expression faithfully captures protein-level expression changes, which is the functional product of most genes. However, examinations of matching transcriptomic and proteomic datasets have indicated that this assumption is commonly violated, especially in complex and heterogeneous diseases such as cancer [49,50]. Recently several high-resolution datasets have been generated in yeast with matching transcriptomic and proteomic data across a large number of growth conditions or time series, which allowed the relationship between transcript and protein abundance to be closely examined, revealing several insights in the systems-level regulation of gene expression. In general, most transcripts are expressed at between 1-10 copies per cell, but protein abundance can range from 100 to 1e6 copies per cell [18●●,51]. This indicates that there is extensive control of protein expression at the translation step, and that large dynamic changes in transcript abundance will in most cases be buffered through this translation-level control to produce dampened effects at the protein level (Fig 3A). This appears to be also dependent on the metabolic context of the cell, as some environmental perturbations (eg salinity, temperature, EtOH stress) cause concerted changes in protein and transcript expression, indicative of near-constant and gene-specific translation

efficiencies [18●●]; while others (eg different nutrient limitations) can modulate transcript and protein abundances independently [41●●]. Notably, several multi-omics studies in yeast growing in different contexts have identified two processes that are primarily regulated at the transcription level: enzymes in the respiratory pathway [18●●], and ribosomal proteins [25●●]. Similar systems-level identification of the control points of gene expression will have wide-spread implications in identification of actionable targets and development of therapeutics in human diseases and development of therapeutics.

Gene expression and metabolism also engage in complex reciprocal regulation, as exemplified in a number of subsystems such as the SNF1 (AMPK) and TOR (mTOR) regulation circuits [1,6,52]. Recently it has also become apparent that the metabolic context of the cell has global influences on gene expression beyond the classic feedback control systems. For example, up to 85% of the coding genome in yeast was found to be under epistatic regulation of leucine, histidine, uracil, and/or methionine auxotrophies [53●●], well beyond the genes that would intuitively respond to these metabolic conditions such as enzymes in amino acid synthesis pathways. This represents a major caveat to the use of human cell models in the generation of multi-omics data for systems-level studies, since most cell lines are maintained in conditions that are not representative of the disease microenvironment, and the growth media is often supplemented with components (eg fetal bovine serum) whose exact chemical composition is unknown. Using yeast, it is possible to exert very fine control over both nutrient influx and cell growth, which facilitates modeling and hypothesis generation using high-resolution multi-omic datasets. One such example of this endeavour is the construction of a TRN network in order to delineate the relationship between gene expression and transcription factor (TF) binding in yeast. Preliminary studies mapping the genome-wide binding of 21 TFs across 4 metabolic conditions at a defined growth rate [54●,55●●], have helped uncover both a threshold effect and a saturation effect of TF binding and transcript expression. The threshold effect indicates that a certain minimal level of TF binding must be met before transcription will respond, and the saturation effect indicates that there is a maximum level of transcriptional output beyond which more TF binding is no longer productive [55●●]. Importantly, the use of high-resolution regulomics data in yeast systems biology has allowed these biological phenomena to be quantified in a systematic way, which allows the effect of different TFs on the transcriptional output of a gene or gene set to be directly compared (Fig 3B). These insights can now be validated in human models with very simplistic experimental designs, which is of particular interest since transcription factors are common as both drivers of complex human diseases (eg c-Myc in various cancers) and as drug targets (eg steroid hormone receptors targeted by anti-inflammatory drugs), and to date a full functional accounting of the activities of these TFs remain incomplete. Validation of these insights in

human models will further allow for precise therapeutic interventions to be designed, for example by CRISPR technology, to develop personalized medicine for disease treatment.

Conclusion and outlook

Yeast has been widely used as a model eukaryal organism, historically in molecular biology and now also in the rapidly developing field of systems biology. A number of yeast characteristics make it highly suitable for systems biology studies, notably the ability to exert fine control over the nutrient composition, metabolic flux, and growth rate of the cells. This has become especially important in the analysis of multi-omics datasets, not only because it removes these confounding variables thereby improving data quality, but also because orthogonal control of these variables allows new biological insights to be made. An elegant example of this is a recent study of cell cycle progression through the G1 checkpoint Start [56●], using an experimental system which allowed orthogonal control of glycolytic flux in yeast cells in an otherwise unaltered nutrient environment. In this study, Litsios *et al* [56●] revealed that passage through Start is controlled by metabolic oscillations in G1, notably an increase in glycolytic flux, which is coupled to a transient pulse in protein production rate necessary for Start. These insights clearly highlight that while the three key aspects of cell biology – cell growth, metabolism, and gene expression – are highly connected and act in a concerted manner to give rise to biological phenomena, using yeast systems biology it is possible to dissect their dynamics and interactions through careful manipulation of experimental conditions and data analysis (graphical abstract, left). With the high degree of conservation of key cellular processes between yeast and human cells, this further allows human cell models (including primary patient samples) to be used in a more effective manner in simple, well-designed validation experiments. For example, currently cancer cell metabolism is thought to promote oncogenesis via three main axes: bioenergetics, biosynthesis of macromolecules, and redox balance [57]. The results of Litsios *et al* [56●] suggest the possibility of a fourth route of impact of high glycolytic flux on cancer cell growth, by directly driving cell cycle progression through the restriction point (the mammalian equivalent of Start in yeast). This now becomes a relatively simple hypothesis that can be tested in human cancer cell models, and if validated could significantly improve our understanding of cancer as a complex disease (graphical abstract, right). Thus, we anticipate that generation and analysis of high-resolution multi-omics big data in yeast will continue to pioneer systems biology, generating new tools and concepts to accelerate our understanding of the mechanisms of complex human diseases.

Declaration of interest

None.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- ● of special interest
- ●● of outstanding interest

Figure captions

Fig 1. Principles of cell growth. (A) Replication of a cell system is limited by the component that is replicated the slowest, which is typically proteins. P, protein. R, RNA. Asterisk indicates the rate-limiting process. (B) Under conditions where phosphate (a critical component of nucleotides) is limiting, RNA becomes the component that is replicated the slowest and the cell growth rate becomes dependent on the RNA transcription rate. P_i, phosphate. (C) The proteome reserve of a typical yeast cell is approximately 50% but varies depending on the growth condition. Arrowheads represent changes in growth condition.

Fig 2. Use of GEM scaffold for integrative omics data analysis. (A) An example of a reaction in a GEM catalyzed by an enzyme. (B) Mathematical models and omics data analysis approaches for the represented reaction. r, rate of reaction. Asterisk indicates that various measurements of the enzyme can be used: eg mRNA abundance, protein abundance, PTMs, or experimentally measured flux through the enzyme. k_{cat}, enzyme turnover number. σ, enzyme usage. ΔrG^0, standard Gibbs free energy of compound formation. R, universal gas constant. T, temperature.

Fig 3. Gene expression dynamics delineated by yeast systems biology. (A) There is extensive control of gene expression at the translation step which dampens the effect of transcript variability to the protein level. (B) The effect of TF binding on transcriptional output can be directly compared with the construction of TRN using high-resolution omics datasets.

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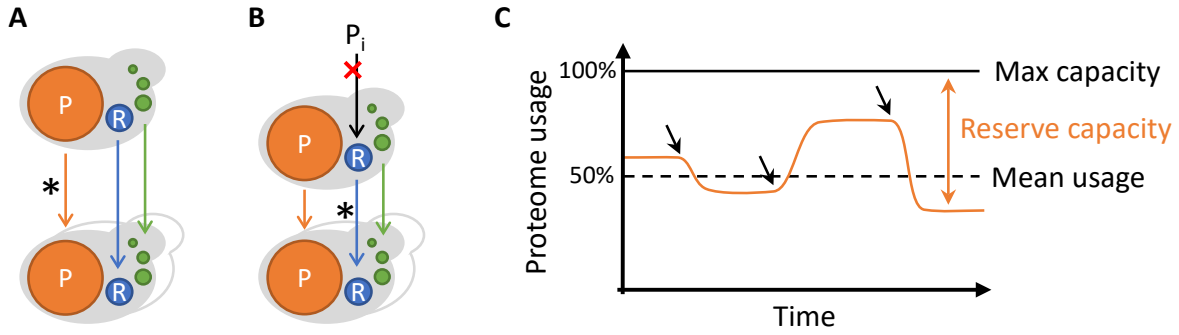
524 Figure 1

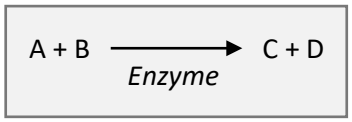


526

527

528 Figure 2

A

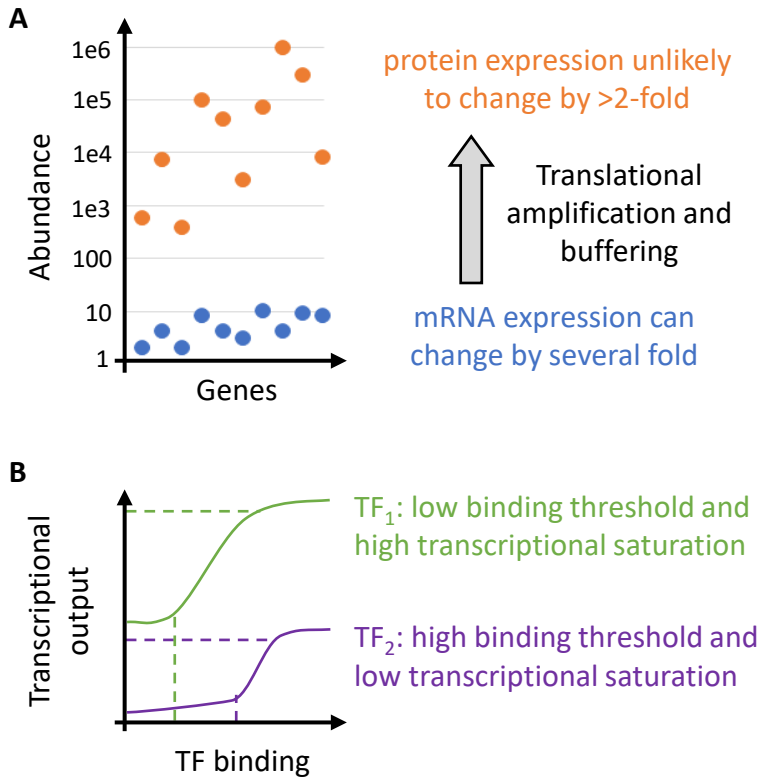


B

Mathematical model	Omics level	Analysis approach	Reference
$\Delta r \propto \Delta E^*$	Various	Comparative	[34, 35●●, 36-40]
$r = k_{cat} \cdot [E] \cdot \sigma$	Proteomics	Integration	[28●●, 42-43]
$r = \Delta_r G^0 + R \cdot T \cdot \ln\left(\frac{[C] \cdot [D]}{[A] \cdot [B]}\right)$	Metabolomics	Integration	[44-46]

530

531 Figure 3


A
1e6
1e5
1e4
1e3
100
10
1
Abundance
Genes
protein expression unlikely to change by >2-fold
Translational amplification and buffering
mRNA expression can change by several fold
B
Transcriptional output
TF binding
TF1: low binding threshold and high transcriptional saturation
TF2: high binding threshold and low transcriptional saturation