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1 Yeast systems biology in understanding 2 principles of physiology underlying 3 complex human diseases

4
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15 16 Highlights

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

21 Abstract

22 Complex human diseases commonly arise from deregulation of cell growth, metabolism, and/or
23 gene expression. Yeast is a eukaryal model organism that is widely used to study these processes.

24 Yeast systems biology benefits from the ability to exert fine experimental control over the cell
25 growth rate and nutrient composition, which allows orthogonal experimental design and generation
26 of multi-omics data at high resolution. This has led to several insights on the principles of cellular
27 physiology, including many cellular processes associated with complex human diseases. Here we
28 review these biological insights together with experimental and modeling approaches developed in
29 yeast to study systems biology. The role of yeast systems biology to further advance systems and
30 personalized therapies for complex diseases are discussed.

31 Introduction

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

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

57 Cell growth

58 In a basic model of cell growth, duplication of a cell entails duplication of each of its components
59 [12]. The single largest macromolecular component of the cell biomass is protein, which in yeast
60 represents 40-60% of dry mass depending on the growth condition; while RNA and DNA represent 8-
61 10% and 0.1% of the yeast dry mass, respectively (Fig 1A) [13]. Moreover, protein is the only
62 component that is self-replicating via ribosomes, which places a natural limitation on the cell growth
63 rate. Thus it has long been proposed that cell growth is limited by protein synthesis, and many

64 “classic” models of cell growth were formulated based on the self-replicating nature of ribosomes
65 [14-16]. Indeed, in bacteria it has been shown that cells finely tune their ribosomal protein content
66 to precisely match the protein synthesis demand at any given growth rate [17]. However, to directly
67 test this hypothesis in fast-growing eukaryal cells such as yeast or human cancer cells, it is necessary
68 to globally profile the expression dynamics of proteins under highly controlled experimental
69 conditions, which has only recently been accomplished in yeast. Yeast is particularly suitable for
70 these types of experiments, because the growth of yeast cells can be controlled very precisely and
71 maintained at steady-state, which is often an important assumption in these models [1]. By growing
72 yeast at steady-state at a controlled growth rate, Lahtvee *et al* [18●●] generated a global matching
73 dataset that includes transcript abundance, protein abundance, protein degradation rates, and
74 calculated protein synthesis rates. Integrative analysis of this data shows that protein synthesis rate
75 matches closely with the rate of protein dilution from cell division, and degradation rate contributes
76 very little to the expression dynamics of most proteins [18●●]. Thus, the physiological task of
77 replicating the proteome places a global constraint on how fast cell replication can occur as a whole.
78 In contrast, multiple transcript expression dynamics datasets [19,20] have shown that globally the
79 mRNA synthesis rate is much faster than the dilution rate, and most mRNAs are synthesized and
80 degraded several times in a cell cycle. However, when growth conditions are experimentally
81 modified to restrict phosphate availability (a critical component of nucleotides), the control of
82 growth rate is shifted towards mRNA synthesis [21●●], presumably because under these conditions
83 it becomes slower to accumulate mRNA to the point of duplication (Fig 1B). From an evolutionary
84 perspective, it has been shown that the abundance of orthologous proteins is highly conserved
85 between *S. cerevisiae* and *S. pombe* (across 400 million years of evolution), while protein
86 degradation rates have significantly diverged [22], indicating that protein abundance is constrained
87 by a strong evolutionary pressure which likely arose from the pressure to maintain a fast growth
88 rate. In contrast, most of the orthologous genes between *S. cerevisiae* and *S. paradoxus* (across 5-10
89 million years of evolution) have diverged in mRNA abundance [23] as well as mRNA synthesis and
90 degradation rates [24], in line with the idea that growth rate is not limited by mRNA abundance and
91 thus there is less selection pressure to conserve transcript expression in evolution.

92 As yeast systems biology affirms that growth rate is limited by protein synthesis, intuitively growth
93 rate would be maximized when cells only express the minimum amount of proteins necessary to
94 carry out all cell functions. However, in most instances cells employ a trade-off and maintain excess
95 proteins as reserves, while growing at a rate that is lower than the theoretical maximum
96 [25●●,26●]. The size of the protein reserves underlies how quickly cells can respond to changing
97 environments [26●]. For pharmaceuticals that target endogenous enzymes or processes, the sizes of

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

125 Metabolism

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

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

141 With the rapid development of omics techniques, recently it has become of interest to integrate
142 omics-level data into the GEM framework, which will allow points of metabolic flux control to be
143 identified. Two general approaches have been used for this endeavour with yeast GEM as a scaffold
144 of metabolic analysis. In the first approach, yeast GEM is used as-is to simulate metabolic flux at a
145 given experimental condition, and how flux through individual enzymes or pathways is controlled
146 can be inferred by comparing the simulated flux with the experimentally measured transcriptomic,
147 proteomic, and/or phospho-proteomic levels (Fig 2A-B). Through this approach it was found that
148 changes in the mRNA abundance of mitochondrial respiration proteins correlates well with changes
149 in flux, while flux through the glycolytic pathway is not correlated with either the mRNA abundance
150 or the protein abundance of glycolytic enzymes [18●●,40]. Instead, glycolysis appears to be engaged
151 in complex regulation circuits involving a combination of substrate concentrations, enzyme allostery,
152 and regulator crosstalk [41●●]. In the second approach, new modeling methodologies are developed
153 to incorporate omics data into GEM simulations themselves. For example, two studies [28●●,42]
154 have directly incorporated experimentally-measured enzyme abundance into GEM simulations,
155 called enzyme-constrained GEM (ecGEM). In ecGEM, the simulated rate of each reaction cannot
156 exceed an upper limit defined by the abundance and the turnover number k_{cat} of the catalyzing
157 enzyme (Fig 2A-B). Through this modeling technique it was found that the Crabtree effect in yeast,
158 analogous to the Warburg effect in human cancer cells [43], is determined by enzyme constraint
159 [28●●,42]: since enzymes in glycolysis and fermentation are smaller and more catalytically efficient
160 compared to enzymes in the TCA cycle and ETC components, at high growth rates cells will trade off
161 the bulky respiratory machinery for fermentation machinery, which has a smaller proteome cost and
162 therefore enables cells to allocate more protein to translation, ie ribosomes, which is necessary for
163 increasing its growth rate [28●●,42]. In a similar approach as direct incorporation of proteomics data
164 in yeast GEM, a number of studies have directly incorporated metabolomics data into models of
165 metabolic subsystems in yeast (Fig 2A-B) [44-46], although to date this approach has not been used

166 genome-wide. This is in part due to the difficulty in obtaining metabolomics data at high quality and
167 high coverage, but rapidly improving technologies will soon allow these limitations to be lifted [33].
168 In this modeling concept, the rate of a reaction is estimated based on the Gibbs free energy of the
169 reaction, which is in turn regulated by metabolite concentrations and thermodynamic equilibrium
170 [44-46]. Indeed it has been shown that for most metabolic reactions, calculated flux is better
171 correlated with metabolite concentrations than enzyme abundance [47●●]. Thus, simulations with
172 this modeling concept allows the identification of key flux-controlling metabolites as biomarkers, as
173 exemplified in the identification of cytosolic-mitochondrial NAD^+/NADH ratio as a key regulator of
174 the ethanol-acetaldehyde redox shuttle [46]. In the near future it can be anticipated that multiple
175 layers of omics data will be simultaneously incorporated into the GEM framework to allow for more
176 sophisticated modeling of complex cellular behaviours. Since most yeast proteins and metabolic
177 pathways have homologous counterparts in human cells, these insights obtained in yeast can be
178 readily translated or verified in human cell models of pathophysiology in metabolic diseases.

179 Gene expression

180 Nearly all diseases are associated with changes in gene expression, either as a driver of disease onset
181 and progression, or as a part of the cellular response to the diseased state. Gene expression changes
182 in multifactorial diseases have received wide attention in clinical research to identify potential
183 targets for clinical intervention. This was pioneered by transcriptomic profiling and is now pushing
184 the bounds of personalized medicine through a rising capability to generate patient-specific datasets
185 and lowering cost [48]. A major underlying assumption of these studies is that changes in transcript-
186 level expression faithfully captures protein-level expression changes, which is the functional product
187 of most genes. However, examinations of matching transcriptomic and proteomic datasets have
188 indicated that this assumption is commonly violated, especially in complex and heterogeneous
189 diseases such as cancer [49,50]. Recently several high-resolution datasets have been generated in
190 yeast with matching transcriptomic and proteomic data across a large number of growth conditions
191 or time series, which allowed the relationship between transcript and protein abundance to be
192 closely examined, revealing several insights in the systems-level regulation of gene expression. In
193 general, most transcripts are expressed at between 1-10 copies per cell, but protein abundance can
194 range from 100 to $1\text{e}6$ copies per cell [18●●,51]. This indicates that there is extensive control of
195 protein expression at the translation step, and that large dynamic changes in transcript abundance
196 will in most cases be buffered through this translation-level control to produce dampened effects at
197 the protein level (Fig 3A). This appears to be also dependent on the metabolic context of the cell, as
198 some environmental perturbations (eg salinity, temperature, EtOH stress) cause concerted changes
199 in protein and transcript expression, indicative of near-constant and gene-specific translation

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

207 Gene expression and metabolism also engage in complex reciprocal regulation, as exemplified in a
208 number of subsystems such as the SNF1 (AMPK) and TOR (mTOR) regulation circuits [1,6,52].
209 Recently it has also become apparent that the metabolic context of the cell has global influences on
210 gene expression beyond the classic feedback control systems. For example, up to 85% of the coding
211 genome in yeast was found to be under epistatic regulation of leucine, histidine, uracil, and/or
212 methionine auxotrophies [53●●], well beyond the genes that would intuitively respond to these
213 metabolic conditions such as enzymes in amino acid synthesis pathways. This represents a major
214 caveat to the use of human cell models in the generation of multi-omics data for systems-level
215 studies, since most cell lines are maintained in conditions that are not representative of the disease
216 microenvironment, and the growth media is often supplemented with components (eg fetal bovine
217 serum) whose exact chemical composition is unknown. Using yeast, it is possible to exert very fine
218 control over both nutrient influx and cell growth, which facilitates modeling and hypothesis
219 generation using high-resolution multi-omic datasets. One such example of this endeavour is the
220 construction of a TRN network in order to delineate the relationship between gene expression and
221 transcription factor (TF) binding in yeast. Preliminary studies mapping the genome-wide binding of
222 21 TFs across 4 metabolic conditions at a defined growth rate [54●,55●●], have helped uncover both
223 a threshold effect and a saturation effect of TF binding and transcript expression. The threshold
224 effect indicates that a certain minimal level of TF binding must be met before transcription will
225 respond, and the saturation effect indicates that there is a maximum level of transcriptional output
226 beyond which more TF binding is no longer productive [55●●]. Importantly, the use of high-
227 resolution regulomics data in yeast systems biology has allowed these biological phenomena to be
228 quantified in a systematic way, which allows the effect of different TFs on the transcriptional output
229 of a gene or gene set to be directly compared (Fig 3B). These insights can now be validated in human
230 models with very simplistic experimental designs, which is of particular interest since transcription
231 factors are common as both drivers of complex human diseases (eg c-Myc in various cancers) and as
232 drug targets (eg steroid hormone receptors targeted by anti-inflammatory drugs), and to date a full
233 functional accounting of the activities of these TFs remain incomplete. Validation of these insights in

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

236 Conclusion and outlook

237 Yeast has been widely used as a model eukaryal organism, historically in molecular biology and now
238 also in the rapidly developing field of systems biology. A number of yeast characteristics make it
239 highly suitable for systems biology studies, notably the ability to exert fine control over the nutrient
240 composition, metabolic flux, and growth rate of the cells. This has become especially important in
241 the analysis of multi-omics datasets, not only because it removes these confounding variables
242 thereby improving data quality, but also because orthogonal control of these variables allows new
243 biological insights to be made. An elegant example of this is a recent study of cell cycle progression
244 through the G1 checkpoint Start [56●], using an experimental system which allowed orthogonal
245 control of glycolytic flux in yeast cells in an otherwise unaltered nutrient environment. In this study,
246 Litsios *et al* [56●] revealed that passage through Start is controlled by metabolic oscillations in G1,
247 notably an increase in glycolytic flux, which is coupled to a transient pulse in protein production rate
248 necessary for Start. These insights clearly highlight that while the three key aspects of cell biology –
249 cell growth, metabolism, and gene expression – are highly connected and act in a concerted manner
250 to give rise to biological phenomena, using yeast systems biology it is possible to dissect their
251 dynamics and interactions through careful manipulation of experimental conditions and data
252 analysis (graphical abstract, left). With the high degree of conservation of key cellular processes
253 between yeast and human cells, this further allows human cell models (including primary patient
254 samples) to be used in a more effective manner in simple, well-designed validation experiments. For
255 example, currently cancer cell metabolism is thought to promote oncogenesis via three main axes:
256 bioenergetics, biosynthesis of macromolecules, and redox balance [57]. The results of Litsios *et al*
257 [56●] suggest the possibility of a fourth route of impact of high glycolytic flux on cancer cell growth,
258 by directly driving cell cycle progression through the restriction point (the mammalian equivalent of
259 Start in yeast). This now becomes a relatively simple hypothesis that can be tested in human cancer
260 cell models, and if validated could significantly improve our understanding of cancer as a complex
261 disease (graphical abstract, right). Thus, we anticipate that generation and analysis of high-
262 resolution multi-omics big data in yeast will continue to pioneer systems biology, generating new
263 tools and concepts to accelerate our understanding of the mechanisms of complex human diseases.

264

265 Declaration of interest

266 None.

267 References and recommended reading

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

269 ● of special interest

270 ●● of outstanding interest

271 Figure captions

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

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

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

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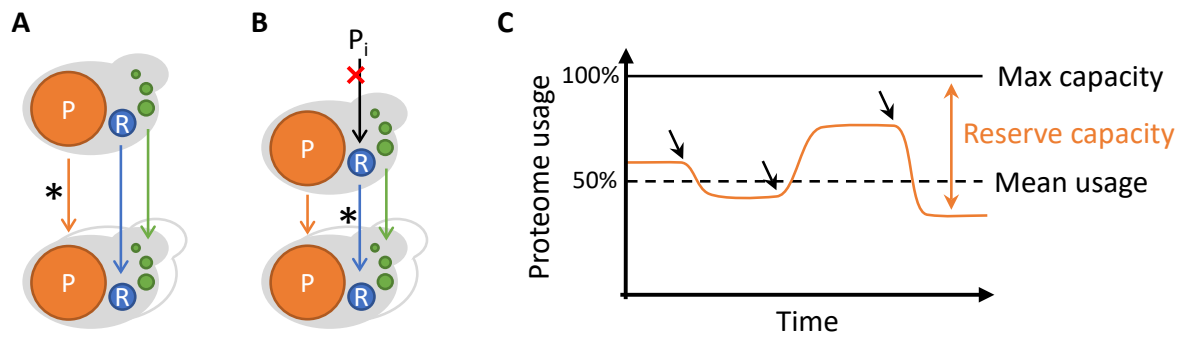
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524 Figure 1

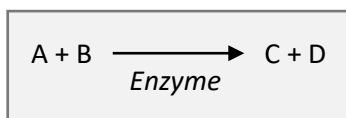


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528 Figure 2

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]
$r = \Delta_r G^{\circ} + R \cdot T \cdot \ln \left(\frac{[C] \cdot [D]}{[A] \cdot [B]} \right)$	Metabolomics	Integration	[44-46]

530

531 Figure 3

