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Minireview

Multilevel regulation of growth rate in yeast revealed using systems biology

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Abstract

The effect of changing growth rates on the transcriptome, proteome and metabolome has been systematically studied. Measurements made under varying nutrient conditions, corresponding to biochemical pathways that correlate primarily with growth rate, reveal a central role for mitochondrial metabolism and the TOR (target of rapamycin) signaling pathway.

Growth is a fundamental property of living things, and understanding what regulates cell growth has important clinical implications in conditions such as developmental disorders and cancer. Cell growth is, in general, regulated by a linkage between growth rate, cell size and cell division. In some types of growth, however, such as the increase in size of fully differentiated muscle fibers or the outgrowth of neurites from a developing neuron, regulated growth occurs in the absence of cell division. The overall coordination of a complex phenomenon like cell growth, in the context of cell cycle, cell size, nutrients and energy metabolism, must involve an interrelated set of molecular mechanisms. The following questions remain unanswered - at steady state, during every cell cycle, how do cells regulate their energy metabolism and biosynthetic pathways in order to double mass while maintaining the same cell size distribution after cell division? In proliferating cells, with increasing growth rates, in addition to coordinating the length of the cell cycle, what biochemical and signaling pathways need to be modulated by cells to meet increasing energy demands? Flux through biochemical pathways may be altered by transcriptional, post-translational mechanisms, transport and availability of metabolites, or by allosteric regulation by metabolites themselves. Clearly, viewing this problem through a narrow window of a few biological pathways or making limited analytical measurements will be inadequate to see the bigger picture that is necessary for a more complete understanding. A big step towards a better understanding of how cell growth is regulated would be to determine the changes in the transcriptome, the proteome and in cellular physiology as growth rate increases and decreases. In this issue of *Journal of Biology*, Stephen Oliver and colleagues [1] describe the first such study of cellular change at all levels - gene expression, proteins synthesized, and levels of certain metabolites - that accompany alterations in growth rate in the budding yeast *Sacharomyces cerevisiae*.

This is one of the first wave of systematic studies of cell growth in yeast, as opposed to the much-studied relationship between cell size and cell division [2]. Growing cells must reach a critical size before they can enter the cell cycle and undergo cell division, and cell size seems to be under homeostatic control [3-6], as there is a quite limited distribution of cell size in yeast. To analyze the complex network of genes that influence cell-size homeostasis, for example, Jorgensen and colleagues determined the size

distributions of a complete set of yeast deletion strains [2]. They uncovered a dynamic relationship, dependent on the transcription factor Sfp1, between signals that stimulate ribosomal biogenesis and the critical size threshold before cell division.

Growth rate has seldom been studied so systematically. Most transcriptional profiling and systems biology studies in yeast have been performed in batch culture where the nutrient conditions and growth rates are continuously changing. It is therefore difficult to separate the primary effect of changing growth rates on cellular physiology from secondary nutritional and environmental effects. This problem can be avoided by carrying out experiments in continuous culture using a chemostat and limiting nutrients [7-9]. The doubling time (T_d) of a culture is inversely related to the growth rate (μ) by the expression ($T_d = \ln 2/\mu$). In a continuous culture at steady state, the dilution rate (D, defined by the ratio of the rate of addition of the nutrient medium and the volume of the culture) is equal to the growth rate. Therefore, changing the dilution rate can control the growth rate in a continuous culture.

Using this experimental approach, Regenberg *et al*. [10] recently carried out whole-genome transcriptional profiling on continuous cultures of S. *cerevisiae* to uncover the impact of changing growth rates on the transcriptional program in a model eukaryotic cell. With glucose as the limiting nutrient, continuous cultures were grown at six specific growth rates with doubling times ranging from 2 to 35 hours. Using consensus clustering methods [11], the authors analyzed 5,930 yeast transcripts and observed that the levels of half the transcripts changed as growth rate increased. Transcripts of proteins involved in biogenesis, such as amino-acid and lipid biosynthesis, ribosomal protein biosynthesis, nucleotide metabolism, and respiration, were upregulated as a function of growth rate. Transcripts present at higher levels at slower growth rates correlated strongly with those known to be present in cells under stress [12], indicating that common signaling pathways may regulate both cell stress and slow growth rates.

An important observation in this study was the relationship between carbon metabolism and growth rate. At high D (greater than 0.3/hour), yeast cells switch their metabolism from pure respiration to a mix of fermentation and respiration. This phenomenon of simultaneous respiration and fermentation is known as the Crabtree effect after the physiologist H.G. Crabtree, who showed that tumor cells under aerobic conditions have lower rates of respiration after the addition of glucose [13,14]. In his 1929 paper Crabtree stated, "the glycolytic activity of tumors exerts a checking effect on their respiration" [13]. These results followed the closely related observations by Otto Warburg on increased aerobic glycolysis compared to respiration in tumors [15]. In their study, Regenberg *et al*. [10] found that in conjunction with this metabolic switch at high growth rates, there were abrupt changes in the levels of some yeast transcripts, with increases in expression of key genes involved in glucose transport and vesicle transport and repression of those involved in ethanol metabolism and gluconeogenesis. The metabolic shift from oxidative to fermentative growth has also been investigated using ^{13}C flux analysis by Frick *et al*. [16], who studied three different growth rates in continuous culture. They found that this shift was accompanied by a change in carbon flux from the pentose phosphate pathway towards glycolysis, a decrease in flux through the tricarboxylic acid (TCA) cycle, and an increase through pyruvate carboxylase and ethanol production.

Despite the insights gained from these studies, we lack a systems-level understanding of eukaryotic growth-rate control. The experiments carried out so far have been mostly confined to transcriptional analysis; there is, however, strong evidence for the importance of translational and post-translational control of eukaryotic growth [17,18]. To uncover these effects requires comprehensive analysis of the impact of growth rate at the level of transcripts, proteins, and metabolites. In addition, carrying out separate experiments with ammonium, phosphate or sulfate as limiting nutrients, and not just glucose, would help to further differentiate purely growth-related effects from nutrient-related effects.

In this issue Castrillo *et al*. [1] now describe such a comprehensive study of eukaryotic growth-rate control. They used continuous cultures of *S. cerevisiae* to measure the impact of the change in growth rate on the transcriptome, proteome and metabolome under four different nutrient-limiting conditions - glucose, ammonium, phosphate, and sulfate. It is useful to visualize the details of the experiment in the form of a matrix, as shown in Figure 1. This experimental design is able to separate out the control of cell growth rate in terms of gene expression, protein levels, and metabolic flux. To reveal components related to growth-rate regulation, Castrillo *et al*. [1] looked for changes common to all four nutrient-limiting conditions.

A striking insight from their study is that all three global measurements revealed growth-rate correlated trends, suggesting a multilevel (transcriptome, proteome, and metabolome) control of growth rate. Initially, they examined changes in transcript levels correlating with increasing growth rates using analysis of covariance (ANCOVA) after a multiple testing correction. They found 493 transcripts that were upregulated and 398 that were downregulated with

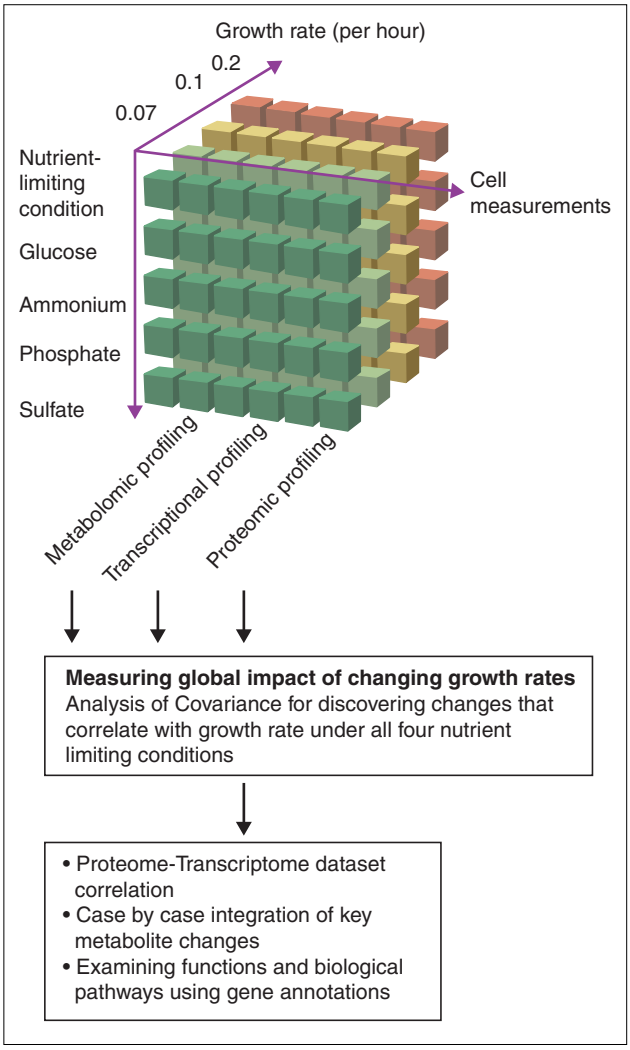

Figure 1
The experimental approach used by Castrillo *et al.* [1]. The regulation of eukaryotic growth is described in the form of a matrix, with growth rates, nutrient-limiting conditions, and cell measurements as the three axes. Yeast continuous cultures were grown at three growth rates under four different nutrient limiting conditions (glucose, ammonium, sulfate and phosphate). Transcriptome, proteome and metabolome measurements were made for each member of this matrix. Changes that correlated with growth rate under all four nutrient-limiting conditions were attributed to intrinsic growth-related processes. Relative changes in transcriptome, proteome, and a few key metabolite measurements were correlated to identify biological pathways that play a critical role in growth-rate regulation.

increasing growth rates in all four nutrient-limiting conditions. Transcripts that were up- and downregulated with growth rate were similar to those found in the study by Regenberg *et al.* [10]. Castrillo *et al.* also observed up-regulation of stress-responsive genes with lower growth rates. In this study, transcription of genes involved in autophagy, a response to starvation, was shown to increase with decreasing growth rates. Genes involved in autophagy are highly conserved from yeast to mammals, and the autophagic response to starvation is mediated by the target of rapamycin (TOR), a protein kinase that is important in the control of eukaryotic cell growth [19]. Further emphasizing the importance of TOR in growth-rate control, the study found that 72.5% of growth-rate-regulated genes were also responsive to rapamycin, which inhibits the TOR complex 1 (TORC1) signaling pathway.

Castrillo *et al.* [1] extended their study to the level of the proteome, using isotope tags for multiplexed relative and absolute quantifications (iTRAQ) to measure levels of particular proteins at two growth rates. The transcriptional and proteomic profiling were performed on samples from the same culture, and after sample normalization and statistical treatment, the datasets could be analyzed for correlation between the levels of proteins and their mRNAs. The paper introduces a useful metric to measure mechanisms of control at the level of RNA or protein, called 'translational control efficiency', which is defined by the ratio of the level of a protein to that of its RNA. For each nutrient condition, the authors found that 35% of transcripts have significant changes in translational control efficiency, indicating post-transcriptional or post-translational control mechanisms. Integrating these datasets with metabolite measurements is more difficult, and for this study Castrillo *et al.* integrated levels of a selected subset of important metabolites with the transcriptome data [20].

One of their key findings is that within a metabolic pathway, such as the biosynthesis of leucine, both transcriptional and translational control may be operating, with only a subset of enzymes being regulated at the protein level. They also looked at the metabolite *S*-adenosylmethionine (SAM) and inferred a role for SAM metabolism in growth-rate regulation from the fact that the level of the metabolite itself changes with growth rate, along with an increase in the levels of the Sam2 *S*-adenosylmethionine synthase (but not of Sam1) and of other enzymes involved in SAM biosynthesis. An increasing level of SAM with increased growth rate was accompanied by upregulation of rRNA and tRNA methyltransferases. Thus, Castrillo *et al.* [1] were able to specifically implicate Sam2 as mediating the flux of *S*-adenosylmethionine towards RNA methylation.

The efficient regulation of flux between carbon and nitrogen in amino-acid biosynthesis can be analyzed by measuring the levels of key metabolic intermediates and relevant proteins. Castrillo *et al.* [1] found that with increasing growth rates, transcription of the gene encoding the TCA cycle enzyme *cis*-aconitase increased, and levels of its

substrate, citric acid, decreased. Similarly, increased levels of the TCA cycle enzyme succinate dehydrogenase were accompanied by a corresponding decrease in succinate. From these observations, an increased flux though the TCA cycle could be inferred. 2-Oxoglutarate, an intermediate in the TCA cycle, is critical in coupling carbon metabolism to nitrogen metabolism via glutamate dehydrogenase and glutamine synthase. It is interesting to note that in spite of the inferred increased flux through the TCA cycle, steady-state metabolite levels of both 2-oxoglutarate and glutamine did not change, and there was a decrease in levels of glutamate. Instead, there was a corresponding trend in the increase in the transcript and protein levels of the mitochondrial 2-oxoglutarate transporters, and an increase in protein levels of glutamine synthase, further corroborating the coupling between carbon and nitrogen metabolism with increasing growth rates.

This study is one of the first to provide evidence of a comprehensive set of biochemical pathways that are regulated at the transcript, protein and metabolite level. In addition to making important strides in the application of analytical technologies for global measurements, the authors use these measurements to gain insights into the importance of mitochondrial energy and nitrogen metabolism in affecting growth rates. These insights were possible only by integrating information from transcripts, protein levels and relevant mitochondrial metabolites, and could not have been obtained from any one of these aspects alone. Pathways central to mitochondrial and nitrogen metabolism are regulated by TOR-mediated signaling [21], and metabolite levels that change with growth rate, like glutamate, are known to modulate the mTOR pathway [22]. Therefore, an important trend that emerges from this study is to direct future research towards a more detailed study of the effect of TOR signaling in the regulation of growth rate.

Finally, this work also addresses important challenges for the integration of metabolomic data with proteomic and transcriptional datasets. Castrillo *et al.* [1] integrate the metabolome data on an *ad hoc* basis in an empirical fashion; for example, levels of metabolites involved in the TCA cycle were related to levels of transcripts and enzymes that connect to them as substrates or products. The difficulty in a systematic analysis of many metabolites arises from the fact that there is not necessarily a simple one-to-one relationship between metabolites and a given protein or transcript. Some metabolites (such as ATP) can be connected with a very large number of pathways, as they are substrates or products of a number of different biochemical reactions. Furthermore, when measuring steady-state metabolite levels it can be difficult to interpret flux through the pathway without additional measurements, such as glucose or oxygen consumption rates, or by using isotope-labeled substrates. Systematic data integration has been attempted for a small number of metabolites such as glucose or ethanol, where using a partial least square method a matrix of metabolite measurements was modeled as a function of a matrix of transcriptional measurements. Using this mathematical approach, a set of genes that corresponded to changes in metabolite data was discovered [20].

The work of Castrillo *et al.* [1] essentially varied the growth rate and identified its systemic impact. The next stage will be to study the impact on growth rate of altering gene copy numbers or transcript levels, which will provide further enlightenment on the complex multilevel regulation of eukaryotic growth rate.

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