Galactose metabolic genes in yeast respond to a ratio of galactose and glucose

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Natural environments are filled with multiple, often competing, signals. In contrast, biological systems are often studied in "well-controlled" environments where only a single input is varied, potentially missing important interactions between signals. Catabolite repression of galactose by glucose is one of the best-studied eukaryotic signal integration systems. In this system, it is believed that galactose metabolic (GAL) genes are induced only when glucose levels drop below a threshold. In contrast, we show that GAL gene induction occurs at a constant external galactose:glucose ratio across a wide range of sugar concentrations. We systematically perturbed the components of the canonical galactose/glucose signaling pathways and found that these components do not account for ratio sensing. Instead we provide evidence that ratio sensing occurs upstream of the canonical signaling pathway and results from the competitive binding of the two sugars to hexose transporters. We show that a mutant that behaves as the classical model expects (i.e., cannot use galactose above a glucose threshold) has a fitness disadvantage compared with wild type. A number of common biological signaling motifs can give rise to ratio sensing, typically through negative interactions between opposing signaling molecules. We therefore suspect that this previously unidentified nutrient sensing paradigm may be common and overlooked in biology.

nutrient signaling | signal integration | gene regulation | ratio sensing | yeast

The ability to integrate multiple cues about nutrient availability from the environment and coordinate uptake, metabolism, and regulatory networks is a major determinant of microbial cell fitness (1–3). The energy and building blocks needed for growth can come from many different sources, leading to a complex combinatorial signal integration problem. In an environment that contains a mixture of sugars, such as glucose and galactose, microbial cells regulate their response according to a carbon hierarchy mediated by catabolite repression. Galactose metabolic genes (GAL genes) are induced to a significant degree only after glucose-based catabolite repression is relieved, resulting in a lag in growth at the point of glucose exhaustion while GAL pathway proteins are produced (1–6). Recent studies of sugar integration in bacteria (7, 8) suggested that in these organisms the combinatorial response results from the multiplication of individual responses to different sugars.

The response of *Saccharomyces cerevisiae* to galactose is one of the best-studied eukaryotic signaling pathways (1, 4–6, 9–12). The GAL response has become a canonical example for combinatorial signal integration based on a genetic switch (10–13). All GAL genes are induced by the activator Gal4p in response to galactose (14) but repressed by Mig1p when glucose is present (15). The inhibition of GAL genes by glucose is thought to occur at a threshold concentration, with signal integration occurring at promoters (11). These conclusions rest on a limited sampling of combinations of concentrations of glucose and galactose (*SI Appendix*, Fig. S6) (16–21). Our goal, therefore, was to use modern high-throughput techniques that allow us to characterize the GAL genes' metabolic response in detail.

Results

GAL Metabolic Genes Respond to the Ratio of Glucose and Galactose. We grew cells in ∼500 combinations of glucose and galactose (Fig. 1 *A* and *B*) spanning a ∼1,000-fold range of glucose and galactose concentrations. We monitored the expression of a *GAL1* promoter yellow fluorescent protein fusion (*GAL1*pr-YFP) in a derivative of the laboratory strain S288C (*SI Appendix*, sections I and II and Table S1). Gal1p, a galactokinase that catalyzes the first step in the Leloir pathway (10) is induced in the presence of galactose. We grew cells at low density so that the extracellular sugar concentrations are nearly constant throughout the course of the experiment, even at low sugar levels (*SI Appendix*, sections III and IV). Previous studies have used the average population expression level from a GAL promoter as a metric for response, which can obscure low but significant expression. To identify the decision to express GAL genes in a manner that is less dependent on absolute expression level, we used flow cytometry to quantify the percentage of cells expressing a *GAL1*pr-YFP reporter above basal levels (Fig. 1*A*). We define basal levels to be the response of cells grown in 2% (wt/vol) glucose in the absence of galactose (Fig. 1*A* and *SI Appendix*, section V).

Significance

Almost all biological systems need to respond to multiple simultaneous inputs. In yeast catabolite repression, a textbook model for signaling integration, the preferred carbohydrate glucose is thought to inhibit the induction of galactose genes when above a threshold concentration. Instead, we show that galactose metabolic genes induction depends on the ratio of galactose and glucose. Surprisingly, a critical portion of the information processing that determines the ratio response occurs upstream of the canonical signaling pathway. The use of modern combinatorial approaches has thus revealed a new signal processing paradigm that may be common throughout biology.

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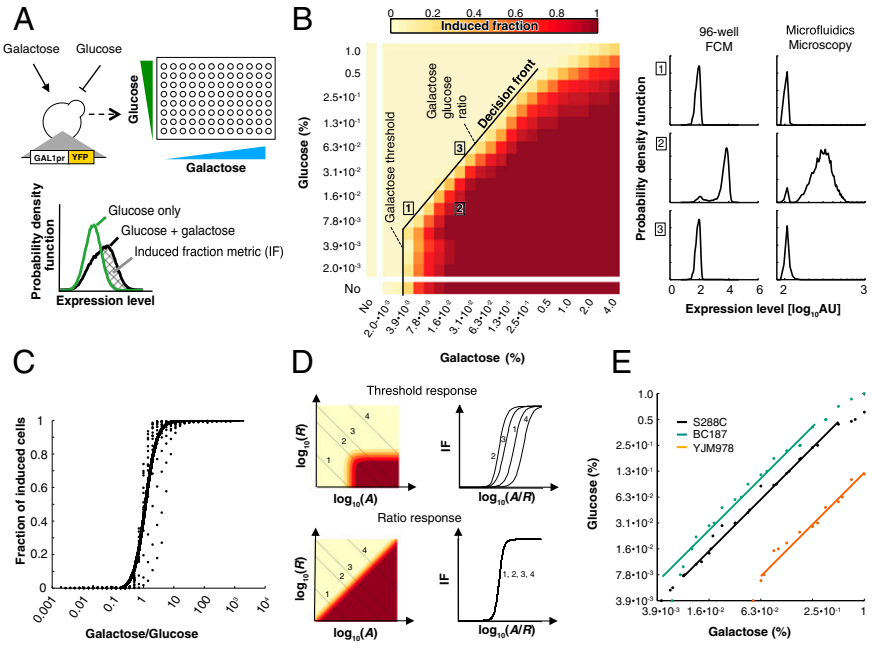

Fig. 1. The galactose pathway responds to the ratio of galactose and glucose. All experiments are in at least triplicate; replicates in *SI Appendix*, Fig. S1. (*A*) Schematic of experiment and metric to measure steady-state GAL pathway response in *S. cerevisiae* in hundreds of combinations of glucose and galactose. The induced fraction (IF, hashed area) is computed (*SI Appendix*, section V) by estimating the fluorescence probability distribution for a given well (black curve) and taking the fraction of area outside the probability distribution of cells grown in glucose alone (green curve). (*B*) (*Left*) Flow cytometry (FCM) of response. The decision front is a linear fit to the concentrations at which 20% of all cells in the population show induction (IF >0.2). (*Right*) Comparison of cells monitored by live microscopy to FCM at three sugar mixtures, denoted by numbered squares (*SI Appendix*, Fig. S4). (*C*) Fraction of inducing cells as a function of the ratio of galactose and glucose concentrations. Each well in *B* is represented by a single dot. The line is a 1D sigmoidal curve that depends mainly on the ratio of galactose and glucose. (*D*) Comparison of models of signal integration (*SI Appendix*) by threshold sensing (*Upper*) and ratio sensing (*Lower*), displayed as in *B* and *C*. (*E*) Decision fronts, calculated as in *B*, for three strains of *S. cerevisiae*.

Contrary to the classic view, we found that GAL genes do not respond simply to a threshold concentration of glucose; the decision to induce GAL genes instead depends on the ratio of glucose and galactose (Fig. 1 *B–D*; replicates in *SI Appendix*, Fig. S1). The response was not simply a multiplicative combination of the independent behavior of cells in glucose or galactose (*SI Appendix*, Fig. S2). The value of the ratio was nearly constant over at least a 50-fold range of glucose and galactose concentrations (Fig. 1*B*). Below a glucose concentration of ~0.006%, cells responded solely to a threshold of galactose (Fig. 1*B*). The result was insensitive to decreases in starting inoculum density, confirming that nutrient depletion is not significant in our experiments (*SI Appendix*, section IV and Fig. S3 *A–C*). Furthermore, modeling shows that nutrient depletion would not create the appearance of ratio sensing (*SI Appendix*, section IV and Fig. S3 *D* and *E*). We directly verified that the ratio-sensing behavior was a steady-state, depletion-independent, single-cell phenomenon by monitoring the kinetics of induction for 8 h at several glucose and galactose concentrations in a microfluidic device with constant nutrient replenishment (Fig. 1*B*, *Right* and *SI Appendix*, section VI and Fig. S4). The onset of the decision occurs within 1 h (*SI Appendix*, Fig. S4). Most cells are induced by 4 h, and steady-state is reached by 6 h (*SI Appendix*, Fig. S4). This behavior was also observed in two other strains, BC187 and YJM978, isolated from a vineyard and a clinical sample, respectively (Fig. 1*E*), showing that ratio sensing is not an aberrant behavior in a single laboratory strain. Furthermore, the existence of a ratio is robust to dosage perturbation of GAL genes (*SI Appendix*, Fig. S5).

How can our results be reconciled with previous work that did not report ratio sensing? All previous studies examined a relatively small range of concentrations, such that deviations from the expected threshold behavior were easily interpreted as noise; our study used a concentration range that was approximately 10-fold larger than previous studies (*SI Appendix*, Fig. S6*D*). Many studies also sampled sparsely in the concentration range that they used, obscuring the differences between ratio and threshold sensing (*SI Appendix*, section III and Fig. S6*D*). The metric we use here, which deconvolves expression level from the decision (Fig. 1*A* and *SI Appendix*, section V and Fig. S6), also helps to show the behavior clearly, because it is more responsive at low concentrations where individual cells begin to induce than at high concentrations of sugar where induction is nearly saturated. With a large enough concentration range, however, the ratio-sensing behavior would have been readily observed independent of which metric was used (*SI Appendix*, Fig. S6).

Ratio sensing has not been previously described for carbohydrates but has been phenomenologically described for the sensing of NADH/NAD$^+$, ATP/ADP, and X vs. autosomal chromosome levels (22–24). In the case of ATP/ADP, ratio sensing was proposed to result from mutually exclusive binding to the γ subunit of AMPK (25), but clarity regarding the mechanism is still lacking. In the GAL pathway, an obvious hypothesis would be that ratio sensing might be accomplished at the *GAL1* or other GAL promoters (11). Glucose and galactose signals converge on these promoters through Mig1p and Gal4p, respectively (Fig. 2*A*). Alternatively, ratio sensing could occur upstream of either the canonical glucose or galactose signaling pathways.

Ratio Sensing Is Generated Upstream of the Canonical Gal Pathway. To identify the mechanism for ratio sensing, we first tested whether glucose signaling is independent of galactose levels, by measuring the fraction of cells with Mig1p-GFP in the nucleus or cytoplasm in different galactose/glucose combinations (Fig. 2*B* and *SI Appendix*, Fig. S7). Mig1p localizes to the nucleus in the presence of glucose and to the cytoplasm in the absence of glucose (26). As expected, Mig1p-GFP localization is independent of galactose concentration (Fig. 2*B*). To further confirm the independence of the glucose branch from galactose we measured the response of a *gal80*Δ strain. Gal80p is a repressor of Gal4p, which in turn induces *GAL1*; in a *gal80*Δ background *GAL1*pr is constitutive (i.e., galactose independent) (27). Indeed, in this background the ratio sensor is broken; the response is converted into a threshold sensor that depends mainly on glucose (Fig. 2*C*; a quantitative comparison of glucose thresholds is shown in *SI Appendix*, Fig. S7). With respect to glucose inhibition, a *gal80*Δ strain therefore mimics the classic threshold expectation.

To test whether ratio sensing occurs in the canonical GAL pathway (i.e., downstream or at Gal3p) (Fig. 2*A*), we monitored the activity of *GAL1*pr-YFP in a *mig1*Δ mutant. Because Mig1p mediates the repression of the GAL pathway by glucose (4, 9),

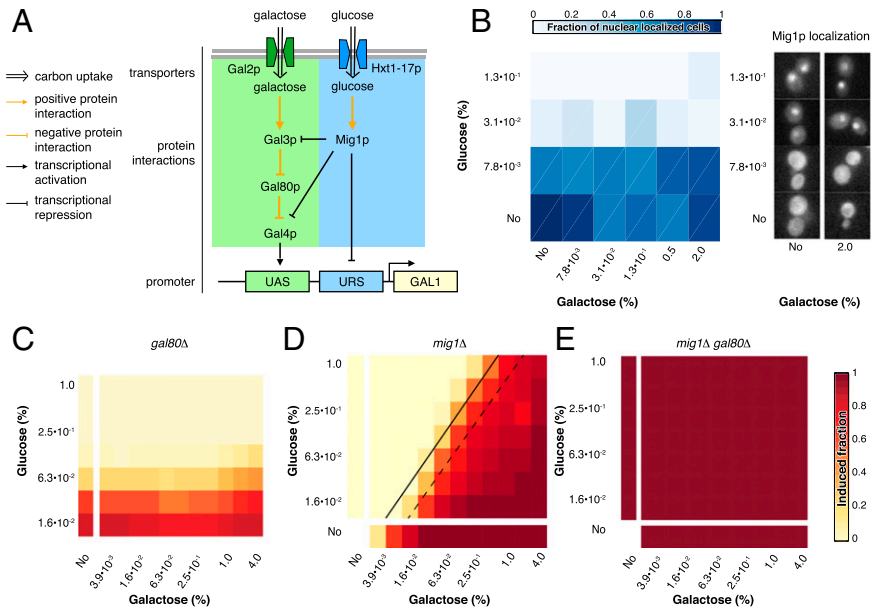

Fig. 2. The GAL pathway senses the ratio of glucose and galactose upstream of known glucose regulation. (*A*) The GAL regulatory network. (*B*) Mig1p localization as a function of glucose and galactose concentrations. Cells expressing Mig1p-GFP were grown under the same conditions as in Fig. 1*B* and imaged after 8 h (*SI Appendix*, section VII); steady-state localization was typically achieved in minutes. Images show representative cells at the indicated sugar concentration. Each concentration is the result of at least 20 cells. The number of cells with nuclear Mig1p-GFP decreases with glucose levels in a galactose-independent manner. (*C*) In a *gal80*Δ background, the ratio response is converted to a threshold response (i.e., in the absence of Gal80p the response is galactose independent). Experiment performed in duplicate. Data for no glucose conditions is not shown for clarity (*Methods*). (*D*) In a *mig1*Δ background, cells continue to respond to the galactose:glucose ratio. Experiment performed in duplicate. Solid line represents the decision front of the *mig1*Δ; dashed line represents the decision front of the wild-type strain (from Fig. 1*B*). (*E*) In a *mig1*Δ *gal80*Δ strain, the response is constitutive and does not dependent on either glucose or galactose.

a *mig1*Δ mutant should be sensitive only to galactose levels, responding as a galactose threshold sensor regardless of whether ratio sensing through Mig1p is achieved directly or indirectly. Surprisingly, we found that even in a *mig1*Δ strain *GAL1*pr-YFP expression is still sensitive to the ratio of galactose and glucose (Fig. 2*D*). The ratio sensing ability of the *mig1*Δ strain is not due to the action of other transcription factors, because a *gal80*Δ *mig1*Δ strain is constitutively active for *GAL1*pr-YFP expression; that is, the activation of the *GAL1* promoter is not dependent on either glucose or galactose in this strain (Fig. 2*E* and *SI Appendix*, Fig. S5*C*). These results are consistent with previous observations showing that glucose represses *GAL1*pr expression even in the absence of Mig1p (20). These results imply that either an intracellular mode by which glucose regulates the galactose pathway has been missed, or that ratio sensing is achieved neither at the *GAL1* promoter nor in the canonical GAL pathway, but upstream of Gal3p.

The Galactose Transporter Gal2p Is Not Required for Ratio Sensing. Because Gal3p directly senses internal galactose levels, ratio sensing upstream of Gal3p suggests a role for sugar transport in ratio sensing. When the GAL pathway is induced, the majority of galactose is imported through the Gal2p transporter, which transports both glucose and galactose with high affinity (K_m ~1 mM) (28, 29). Gal2p is part of the GAL pathway; Gal2p levels are low in glucose media. Nevertheless, it is possible that even the low levels of Gal2p expressed in high glucose are important for ratio sensing. This would not be unprecedented: in the case of Lac induction in *Escherichia coli*, stochastic low-level expression of transporters is critical for the response (30). We therefore measured *GAL1*pr-YFP in a *gal2*Δ strain. Similar to the results with the *mig1*Δ mutant, a *gal2*Δ mutant does not "break" the ratio sensor (Fig. 3*A*); in both cases the mutation affects the ratio sensor, but neither mutant eliminates the ratio-sensing behavior. We interpret these results as strong evidence that the mechanism responsible for ratio sensing involves components outside the canonical galactose sensing pathway (Fig. 2*A*).

In a *gal2*Δ strain, the family of hexose transporters [Hxt1-17p or Mal11p, Mph2p, and Mph3p (31)] are likely to be the main transporters of galactose and a likely source of ratio sensing (Fig. 3*A*). The HXT members transport glucose with various affinities (K_m from ~1 mM to 100 mM) (28, 29), and some also import galactose, albeit with significantly lower affinity (K_m ~250 mM) (28); Hxt14p can even support growth on galactose in a strain where all other hexose transporters have been deleted (31). Thus, ratio sensing might result from competition between the sugars during uptake. In a competitive uptake regime, the intracellular galactose concentration would depend on the ratio of the extracellular galactose and glucose concentrations (*SI Appendix*, section VIII and Figs. S8 and S9).

Galactose Uptake Depends on the Ratio of Extracellular Sugars Concentrations. To directly test whether uptake of galactose through the hexose transporters depends on the extracellular ratio of galactose and glucose concentrations, we measured galactose uptake in mixtures of U-^{13}C-glucose and ^{12}C-galactose. Because intracellular carbohydrates are rapidly metabolized, measuring the incorporation of ^{13}C and ^{12}C into amino acids using gas chromatography mass spectrometry (32) provides information on uptake rates; the ratio of incorporated ^{12}C and ^{13}C is equal to the ratio of galactose and glucose uptake rates (*SI Appendix*, section X). To distinguish the role of hexose transporters from the effects of intracellular regulation, we constructed a *gal2*Δ *gal80*Δ *mig1*Δ strain. This strain is not responsive to glucose or galactose but constitutively expresses GAL genes (Fig. 2*E*). Incorporation of ^{13}C and ^{12}C thus depends solely on the relative sugar uptake rates and not on the induction of the GAL pathway. We pregrew this strain in U-^{13}C-glucose medium and transferred it into media containing mixtures of U-^{13}C-glucose and ^{12}C-galactose for two doublings (*SI Appendix*, Table S3). We found that the ratio between ^{12}C and ^{13}C incorporated into amino acids, and hence galactose uptake, increases as extracellular galactose:glucose ratio is increased (Fig. 3*B*). The ^{12}C:^{13}C ratio increases as extracellular galactose is increased but decreases as extracellular glucose is increased (Fig. 3*B*).

Quantitatively, this result is consistent with a "passive" model of competitive uptake of glucose and galactose by the transporter, which predicts that relative uptake depends on the extracellular sugar ratio multiplied by the relative affinity of the transporter for each sugar (K_m ratio). Our measurements yield a K_m ratio of 170 (Fig. 3*B*), similar to the K_m ratio of 250 calculated from literature reports (28, 29). The concentration of glucose at which the response changes from a ratio sensor to

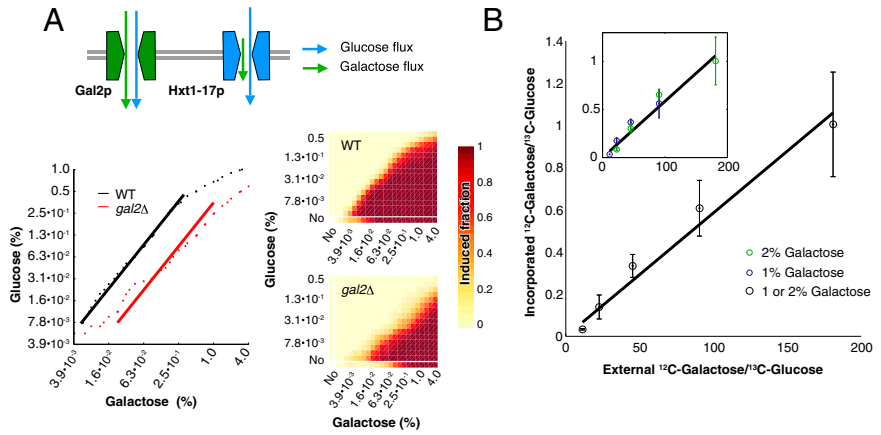

Fig. 3. Galactose uptake depends on the ratio of galactose and glucose even in the absence of Gal2p. (*A*) Gal2p and many of the Hxt1-17p family of hexose transporters import glucose and galactose but have differing relative affinities for the two sugars. Deletion of *GAL2* does not eliminate ratio sensing. Black and red lines are the decision front (Fig. 1*E*). (*B*) Incorporation of ^{12}C-galactose and ^{13}C-glucose into amino acids as measured by GC-MS in a *gal2Δ gal80Δ mig1Δ* strain (*SI Appendix*). This strain constitutively expresses the GAL pathway. Error bars are the SD from three biological replicates (duplicates for the highest glucose to galactose ratio). The slope of the fitted line (black line) is 1/170; the expectation based on literature uptake measurements is 1/250 (28, 29). (*Inset*) Breakdown of the data into two different galactose concentrations.

a galactose threshold sensor, 0.006% as measured in Fig. 1*B*, is close to the K_m of the high affinity hexose transporter for glucose ~1 mM, or 0.002% glucose. This is consistent with a competitive uptake model; glucose concentrations below the K_m of the transport have quickly diminishing effects on the uptake of galactose, thereby making galactose uptake glucose independent at low glucose concentration.

Ratio Sensing Can Provide a Fitness Advantage. It is possible that ratio sensing in the sugar metabolism pathways in yeast evolved to compensate for an inevitable lack of perfect discrimination between different sugars in the hexose transporter. Because of substrate competition for the transporter, high galactose will partially inhibit glucose uptake, and cells that do not induce GAL gene expression cannot compensate for the decreased glucose flux by metabolizing galactose. On the other hand, it is also possible that ratio sensing is desirable for other reasons (for example, to allow the cell to sense when using multiple sugars is a better decision than using only a single sugar) and that the lack of discrimination of the hexose transporters is in itself a selected trait. Consistent with the latter possibility, there is a wide variation in the selectivity of HXT family transporters for glucose relative to other sugars, and many do not sustain growth on medium with galactose as the sole carbon source (31). Thus, a cell could evolve to express only highly selective hexose transporters if ratio sensing were undesirable.

The biological advantage of ratio sensing is most likely during a dynamic process such as depletion of glucose in mixed sugar environments. However, no mutant currently exists whose only defect is to convert the ratio response to a threshold response (e.g., a *gal80Δ* has a fitness disadvantage in many media). Therefore, to establish whether ratio sensing can offer a selective advantage relative to a threshold sensing response, we compared the fitness of a *gal4Δ* strain to that of a wild-type strain in two conditions: glucose only, and a glucose/galactose mixture (Fig. 4*A*). A *gal4Δ* strain cannot mount a transcriptional response to galactose and therefore behaves in a glucose/galactose mixture as if it were in glucose alone (33), a behavior that phenocopies a threshold sensing strain in this media regime (Fig. 4*A*). When cocultured in 0.016% glucose the wild-type and *gal4Δ* strains grew comparably (Fig. 4*A*); 0.016% glucose is above the concentration of glucose at which ratio sensing is observed (Fig. 1*B*). When 2% (wt/vol) galactose is added to the 0.016% glucose medium the wild-type strain has a significant fitness advantage of 0.1% per hour ± 0.01% SE of mean, with a *P* value of 0.01 (two-tailed *t* test; Fig. 4*A*). At this concentration all wild-type cells induce the GAL pathway maximally. Given the steady-state advantage to the ratio response we observe here, it is likely that if a true threshold-sensing strain could be constructed we would find that it is at a disadvantage compared with the ratio-sensing strain in dynamic environments as well.

Discussion

We measured the response of yeast to hundreds of mixtures of glucose, the preferred carbon source, and galactose, a less preferred carbon source. Although glucose above a certain concentration threshold is commonly thought to repress other carbohydrate metabolism pathways, our results show that the GAL metabolic genes respond to the ratio of galactose and glucose. We show that ratio sensing is not generated from the interaction of transcription factors on the promoter, as one might expect from the literature, but instead is achieved upstream of the canonical GAL pathway. Moreover, we show that the intracellular galactose concentration depends on the ratio of galactose and glucose, highlighting an information processing step at the level of import.

Our results show that ratio sensing can be achieved even in the absence of the high-affinity galactose transporter Gal2p (Fig. 3*A*). Relative uptake of glucose and galactose suggest that competitive binding of glucose and galactose to hexose transporters is responsible for setting the initial ratio response. GAL2 is induced as part of the GAL response (9, 10, 25). The affinity of Gal2p for both galactose and glucose is approximately 1 mM; Gal2p thus has significantly higher affinity for galactose than the HXT transporters but should still exhibit competitive transport. Hence, even after significant Gal2p expression, cells would be expected to respond to the ratio of glucose and galactose but at a shifted "setpoint." This is consistent with our observation that a *gal2Δ* strain responds to the ratio of glucose and galactose but the setpoint of this ratio is shifted several-fold from a wild-type cell (Fig. 3*A*). These results raise an intriguing hypothesis: because the setpoint of the ratio response can be modulated solely by changing the relative expression of transporters with different affinities, the large number of different hexose transporters may be involved in an intricate and physiologically tunable information processing layer.

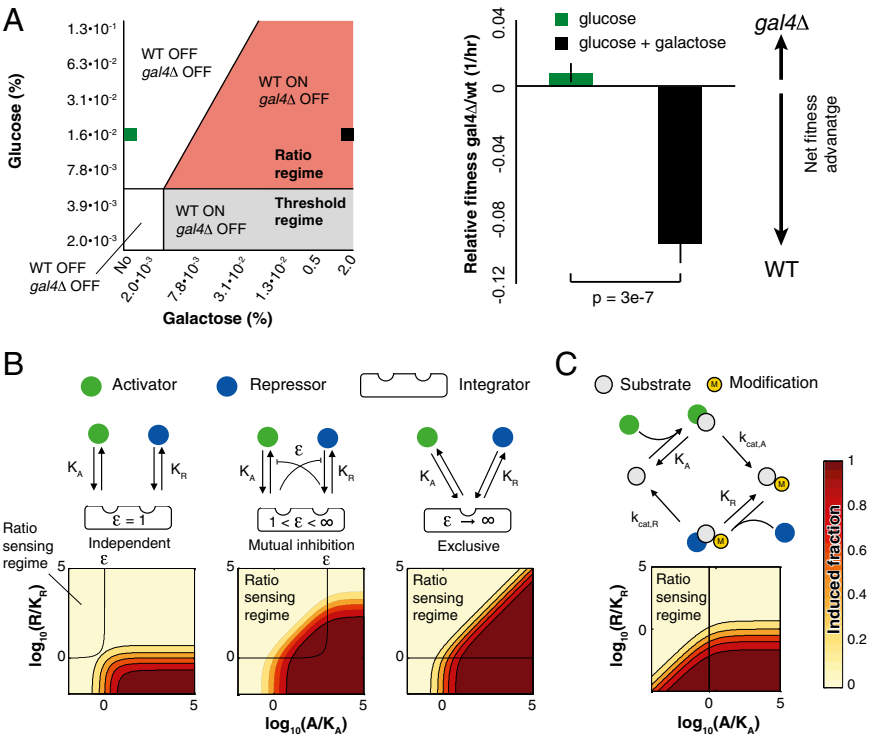

Fig. 4. Biological implications, implementations, and regimes for ratio sensing. (*A*) The ability to use galactose even in the presence of glucose gives cells a fitness advantage. A wild-type strain was competed against a *gal4*Δ in two concentrations of carbon: (*i*) 0.016% glucose or (*ii*) 0.016% glucose and 2% (wt/vol) galactose. The 0.016% glucose concentration places cells in the ratio sensing regime (*Left*, green and black squares). Ten independent replicates were grown until gene expression reached steady state (8 h), then samples were taken every 2 h for 10 h to calculate cellular fitness. Error bars are the SEM. (*B*) Ratio output can be generally achieved by a simple module in which two input molecules, an activator (green) and a repressor (blue), bind to an integrator molecule—a promoter, transporter, scaffold protein, etc. Mutual inhibition, ε, is necessary for a robust ratio response. (*C*) Futile cycles, such as phosphorylation and dephosphorylation or acetylation and deacetylation, can also create ratio sensors. Ratio sensing is achieved when the enzymes are not saturated.

Yeast cells respond both to extracellular cues, using transmembrane sensors such as Snf3p and Rgt2p that bind extracellular glucose, and to intracellular cues, using internal sensors such as Gal3p and Hxk2p (11) that bind to galactose and glucose, respectively. The fact that galactose is sensed intracellularly is crucial for our model; competitive transport will only affect an internal sensor. Our data thus identify a previously unidentified layer of regulation above the regulation represented by previous mechanistic models of glucose and galactose integration. Because intracellular sensing is common in all domains of life from bacteria through mammals (34, 35), information processing through competitive transport could be a common mechanism.

This phenomenon of ratio sensing through competitive binding at a transporter can be generalized to any situation in which an activator and a repressor both bind to a third molecule and affect its function, provided that there is some mutual exclusion (Fig. 4*B* and *SI Appendix*, section VIII and Figs. S8 and S9). Mutual exclusion through competitive binding can occur in almost any biological system and can be mediated by any substrate (e.g., DNA, RNA, or protein), opening up the possibility that ratio sensing could occur in a wide range of situations. Another common biological process that can create a ratio sensor is a futile cycle (Fig. 4*C*), in which two reactions run simultaneously in opposite directions, burning energy. When the enzymes are unsaturated the steady-state amount of modified substrate is a function of the ratio of the activities of the kinase and phosphatase. In both classes of modules the molecule being bound or modified needs to be limiting (*SI Appendix*, section XI and Fig. S10). These are common and simple motifs that can give rise to ratio sensing, although many other methods of creating a ratio sensor may exist (36).

Although we found that ratio sensing gives cells a fitness advantage in a specific steady-state environment, in natural settings the ratio response is more likely to be relevant in the context of dynamic processes, such as the depletion of glucose in a mixed sugar environment. We observed a ratio response in multiple strains (Fig. 1*E*), but the specific ratio of galactose and glucose at which a given strain starts to induce *GAL1* varies between strains. These differences might affect the duration of the diauxic (37); strains that induce at a higher galactose to glucose ratio might be expected to begin to induce galactose genes before glucose is fully depleted. Indeed, strains that respond at a lower galactose to glucose ratio (BC187 and S288C in Fig. 1*E*) have a shorter diauxic lag than a strain that responds at a higher galactose to glucose ratio (YJM978; Fig. 1*E*) (38).

Our results add a previously unidentified layer to the standard description of glucose repression in yeast and highlight the possibility that ratio sensing is frequent, and potentially useful, in biology. In many situations ratios may be more biologically robust than absolute concentrations. As techniques allowing for multidimensional analyses are becoming more readily available, we anticipate that ratio sensing will be identified in many other settings. Our work suggests that a critical portion of information processing in a major metabolic decision is made upstream of the canonical signaling network and highlights the dual role transporters can play in both nutrient uptake and signal integration.

Methods

Growth Conditions and Media. Cells were grown for ~14–16 h in synthetic minimal media with 2% (wt/vol) raffinose, to an OD of ~0.3, and then diluted 1:100 in mixtures of glucose and galactose. Cells were grown for 8 h at 30 °C in flasks or 96-well plates and then washed twice in TE (10 mM Tris,

1 mM EDTA, pH 7.5) in preparation for flow cytometry. Cells to be imaged by microscopy were transferred to a micro-well plate (Matriplate from Metrical Bioscience) coated with concanavin A grown in synthetic media with different mixtures of glucose and galactose. A gal80Δ strain growing in raffinose constitutively activates GAL genes. Therefore, the gal80Δ strain was pregrown in 2% (wt/vol) glucose. When switched from glucose to no glucose plus very low galactose media, the *gal80*Δ strain does not grow and marginally induces. Hence, we do not show data for no glucose in this strain.

Flow Cytometry. Samples were measured by flow cytometer (LSRII with high-throughput sampler; Beckton Dickinson) as previously described (39).

Fluorescence Microscopy. Images were captured with a Ti Eclipse inverted Nikon microscope using Micromanager. A Hamamatsu Orca-R2 camera was used to capture fluorescent images and a Scion CFW-1612M for bright-field images. Nuclear and cytoplasmic of Mig1p-GFP localization was analyzed using custom MATLAB software.

Microfluidics and Live Cell Imaging. Cells grown in 2% (wt/vol) raffinose to an OD of 0.3 were loaded into a Y04C yeast microfluidic plate (CellASIC ONIX system). Cells were maintained in 0.011% glucose and 0.0039% galactose; 0.011% glucose and 0.0221% galactose; 0.0625% glucose and 0.0039% galactose; or 0.0625% glucose and 0.0221% galactose for ∼12 h with images taken, as described above, every 15 min.

Strain Construction. The reporter *GAL1*pr-YFP, and the constitutively expressed fluorophore *TDH3*pr-mCherry, *TDH3*pr-BFP, or *TDH3*pr-TagBFP2 was transformed into the *HO* locus (Homothallic switching endonuclease) of the prototrophic S288C, BC187, and/or YJM978 strains. Hemizygous deletion strains were made by mating a haploid deletion strain containing the reporter *GAL1*pr-YFP and a haploid S288C containing *TDH3*pr-mCherry or a *TDH3*pr-TagBFP2. Other deletion strains (*SI Appendix*, Table S1) were constructed by integrating a KanMX cassette in each of the loci using standard PCR and yeast transformation protocols.

Data Analysis. Analysis of flow cytometry data were performed using custom-written MATLAB code (available upon request). A 2D Gaussian mixture model fit to the mCherry and BFP or the mCherry and nonfluorescent populations was used to segment the different competing populations.

GC-Mass Spectrometry. Cells were grown for 16 h in 1% U-^{13}C-glucose, then diluted into fresh 1% U-^{13}C-glucose until cells reached exponential phase. After 6 h cells were transferred to different combinations of U-^{13}C-glucose and ^{12}C-galactose and grown until the cells had doubled once. Cells were spun down and pellets collected for processing in the mass spectrometer as described by Zamboni et al. (32).

Fitness Measurements. A diploid *gal4*Δ strain tagged with a fluorescent mCherry (or BFP) was grown with the wild-type strain S288C tagged with BFP (or mCherry) in synthetic 2% (wt/vol) raffinose media for 14 h to an OD of ∼0.3. Cells were washed three times in synthetic media with no carbon and then inoculated in synthetic media with 0.0156% glucose and 2% (wt/vol) galactose or with 0.0156% glucose. Samples were collected every 2 h in TE (pH 7.5) plus 0.1% sodium azide and read by flow cytometry.

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1. Chubukov V, Gerosa L, Kochanowski K, Sauer U (2014) Coordination of microbial metabolism. *Nat Rev Microbiol* 12(5):327–340.
2. Cai L, Tu BP (2012) Driving the cell cycle through metabolism. *Annu Rev Cell Dev Biol* 28:59–87.
3. Broach JR (2012) Nutritional control of growth and development in yeast. *Genetics* 192(1):73–105.
4. Gancedo JM (1998) Yeast carbon catabolite repression. *Microbiol Mol Biol Rev* 62(2): 334–361.
5. Jacob F, Monod J (1961) Genetic regulatory mechanisms in the synthesis of proteins. *J Mol Biol* 3(3):318–356.
6. Dienert F (1900) *Sur la fermentation du galactose et sur l'accoutumance des levures à ce sucre* (Ann Inst Pasteur, Paris), pp 139–189. French.
7. Kaplan S, Bren A, Zaslaver A, Dekel E, Alon U (2008) Diverse two-dimensional input functions control bacterial sugar genes. *Mol Cell* 29(6):786–792.
8. Setty Y, Mayo AE, Surette MG, Alon U (2003) Detailed map of a cis-regulatory input function. *Proc Natl Acad Sci USA* 100(13):7702–7707.
9. Timson D (2007) Galactose metabolism in *Saccharomyces cerevisiae*. *Dyn Biochem Process Biotechnol Mol Biol* 11:63–73.
10. Bhat PJ (2008) *Galactose Regulon of Yeast: From Genetics to Systems Biology* (Springer, Berlin).
11. Ptashne M, Gann A (2002) *Genes and Signals* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY).
12. Douglas HC, Hawthorne DC (1966) Regulation of genes controlling synthesis of the galactose pathway enzymes in yeast. *Genetics* 54(3):911–916.
13. Johnston M (1987) A model fungal gene regulatory mechanism: The GAL genes of *Saccharomyces cerevisiae*. *Microbiol Rev* 51(4):458–476.
14. Douglas HC, Hawthorne DC (1964) Enzymatic expression and genetic linkage of genes controlling galactose utilization in *Saccharomyces*. *Genetics* 49:837–844.
15. Nehlin JO, Carlberg M, Ronne H (1991) Control of yeast GAL genes by MIG1 repressor: A transcriptional cascade in the glucose response. *EMBO J* 10(11):3373–3377.
16. Acar M, Pando BF, Arnold FH, Elowitz MB, van Oudenaarden A (2010) A general mechanism for network-dosage compensation in gene circuits. *Science* 329(5999): 1656–1660.
17. Acar M, Becskei A, van Oudenaarden A (2005) Enhancement of cellular memory by reducing stochastic transitions. *Nature* 435(7039):228–232.
18. Avendaño MS, Leidy C, Pedraza JM (2013) Tuning the range and stability of multiple phenotypic states with coupled positive-negative feedback loops. *Nat Commun* 4:2605.
19. Venturelli OS, El-Samad H, Murray RM (2012) Synergistic dual positive feedback loops established by molecular sequestration generate robust bimodal response. *Proc Natl Acad Sci USA* 109(48):E3324–E3333.
20. Biggar SR, Crabtree GR (2001) Cell signaling can direct either binary or graded transcriptional responses. *EMBO J* 20(12):3167–3176.
21. Bennett MR, et al. (2008) Metabolic gene regulation in a dynamically changing environment. *Nature* 454(7208):1119–1122.
22. Atkinson DE (1968) The energy charge of the adenylate pool as a regulatory parameter. Interaction with feedback modifiers. *Biochemistry* 7(11):4030–4034.
23. Berg J, Hung YP, Yellen G (2009) A genetically encoded fluorescent reporter of ATP: ADP ratio. *Nat Methods* 6(2):161–166.
24. Madl JE, Herman RK (1979) Polyploids and sex determination in *Caenorhabditis elegans*. *Genetics* 93(2):393–402.
25. Scott JW, et al. (2004) CBS domains form energy-sensing modules whose binding of adenosine ligands is disrupted by disease mutations. *J Clin Invest* 113(2):274–284.
26. De Vit MJ, Waddle JA, Johnston M (1997) Regulated nuclear translocation of the Mig1 glucose repressor. *Mol Biol Cell* 8(8):1603–1618.
27. Torchia TE, Hamilton RW, Cano CL, Hopper JE (1984) Disruption of regulatory gene GAL80 in *Saccharomyces cerevisiae*: Effects on carbon-controlled regulation of the galactose/melibiose pathway genes. *Mol Cell Biol* 4(8):1521–1527.
28. Ramos J, Szkutnicka K, Cirillo VP (1989) Characteristics of galactose transport in *Saccharomyces cerevisiae* cells and reconstituted lipid vesicles. *J Bacteriol* 171(6): 3539–3544.
29. Reifenberger E, Boles E, Ciriacy M (1997) Kinetic characterization of individual hexose transporters of *Saccharomyces cerevisiae* and their relation to the triggering mechanisms of glucose repression. *Eur J Biochem* 245(2):324–333.
30. Choi PJ, Cai L, Frieda K, Xie XS (2008) A stochastic single-molecule event triggers phenotype switching of a bacterial cell. *Science* 322(5900):442–446.
31. Wieczorke R, et al. (1999) Concurrent knock-out of at least 20 transporter genes is required to block uptake of hexoses in *Saccharomyces cerevisiae*. *FEBS Lett* 464(3): 123–128.
32. Zamboni N, Fendt SM, Rühl M, Sauer U (2009) (13)C-based metabolic flux analysis. *Nat Protoc* 4(6):878–892.
33. Gill G, Ptashne M (1988) Negative effect of the transcriptional activator GAL4. *Nature* 334(6184):721–724.
34. Schlax PJ, Capp MW, Record MT, Jr (1995) Inhibition of transcription initiation by lac repressor. *J Mol Biol* 245(4):331–350.
35. Yamamoto KR (1997) Intracellular receptors: New instruments for a symphony of signals. *Molecular Biology of Steroid and Nuclear Hormone Receptors*, ed Freedman LP (Birkhauser, Boston, MA), pp vii–x.
36. Daniel R, Rubens JR, Sarpeshkar R, Lu TK (2013) Synthetic analog computation in living cells. *Nature* 497(7451):619–623.
37. Monod J (1949) The growth of bacterial cultures. *Annu Rev Microbiol* 3:371–394.
38. Wang J, et al. A cost-benefit tradeoff underlies natural variation in preparation for nutrient depletion. *PLoS Biol*, 10.1371/journal.pbio.1002041.
39. Springer M, Weissman JS, Kirschner MW (2010) A general lack of compensation for gene dosage in yeast. *Mol Syst Biol* 6:368.