Saccharomyces cerevisiae JEN1 Promoter Activity Is Inversely Related to Concentration of Repressing Sugar

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When carbon sources are changed, Saccharomyces cerevisiae transcriptional patterns drastically change. To identify genes whose transcription can be used to quantitatively measure sugar concentrations, we searched genomic expression databases for a set of genes that are highly induced during the diauxic shift, and we used the promoters from these genes to drive expression of green fluorescent protein (GFP). Certain sugars, including glucose, fructose, and mannose, repress the promoter of JEN1, which encodes a lactate-pyruvate transporter, in a dose-dependent manner. Nonrepressing carbon sources include galactose, raffinose, ethanol, lactate, and glycerol. JEN1 promoter activity is a linear function of glucose concentration when organisms are grown at a steady-state glucose concentration below 1 g/liter. JEN1 promoter repression is specific to carbon source; heat or cold shock, osmotic stress, DNA damage, and nitrogen starvation do not significantly affect promoter activity. Activation of the JEN1 promoter requires the Snf1 protein kinase, but multiple regulatory elements most likely combine to provide the linear relationship between JEN1 promoter activity and sugar concentration. Thus, a JEN1 promoter-reporter system appears to provide a good living cell biosensor for the concentration of certain sugars. The JEN1 promoter also permits quantitative regulation of cellular functions not normally controlled by sugar concentrations. For example, a strain expressing FLO1 under control of the JEN1 promoter flocculates at a low glucose concentration.

Recent advances in measuring genome-wide transcriptional profiles have led to a better understanding of the complex genetic regulatory mechanisms utilized by living cells. When they receive environmental stimuli (e.g., a change in nutrient concentration or cell stress, such as oxidative damage or DNA methylation), cells activate chemical signaling pathways. Often, the signals affect the transcription of certain genes and/or the translation of mRNA to proteins, allowing the cells to adapt to the environmental changes. By understanding how gene expression and protein translation patterns change, we can in theory use cells to assay environmental conditions.

Historically, live microbes have been used as biosensors for cytotoxic environmental conditions based on changes in growth rate. Various classes of toxic compounds inhibit yeast growth in a concentration-dependent manner (8, 17). Similarly, the Ames test is commonly used to assess genotoxicity by measuring Salmonella enterica serovar Typhimurium colony formation on selective medium following exposure to a chemical compound (3, 11). Some compounds also affect cell respiration activity, which can be measured by changes in oxygen concentration or pH. With this method, immobilized Saccharomyces cerevisiae has been used to detect cyanide (26), and bacterial biosensors of carbon- and nitrogen-containing nutrients have been constructed (24, 33).

Molecular genetic manipulation of microorganisms has improved detection thresholds through the use of regulated gene promoters to control reporter genes that possess a fluorescent, chemiluminescent, or enzymatic activity. For example, the Escherichia coli SOS chromotest monitors DNA-damaging agent concentrations through an sfiA::lacZ fusion (32). Similar strategies have been used to monitor chemical concentrations with eukaryotic microbes. Placing a green fluorescent protein (GFP) under control of the DNA damage-sensitive RAD54 or RNR2 promoters permits easy detection of most DNA-damaging agents in S. cerevisiae (1). Likewise, the promoter of the copper-inducible CUP1 gene has been used to regulate expression of the E. coli lacZ gene in S. cerevisiae, and the levels of lacZ activity correspond to copper concentrations over a narrow range (0.5 to 2 mM) (19). An advantage of using a gene promoter as a cellular sensor is that cellular functions, such as growth or metabolic activity, can be engineered based on the stimuli that activate or repress the promoter in addition to driving expression of detection reporters.

In the creation of promoter-reporter or promoter-activator biosensor systems, identification of the proper promoter is perhaps the most crucial decision. In previous work, promoter choice has been based largely upon genes known to be regulated by certain conditions, such as temperature, salts, ions, etc. However, factors other than the desired input also regulate many of the promoters, complicating analysis of reporter activity. For example, low pH, high salt concentrations, DNA damage, and oxidative stresses can induce CUP1 transcription (14, 16, 36). The ideal promoter has the following characteristics: (i) sensitivity to the agent being detected, (ii) a dose-dependent, preferably linear response over a wide range of concentrations, (iii) a high degree of maximal induction or repression, and (iv) specificity for the particular agent.

In this study we identified the S. cerevisiae JEN1 promoter as a sensitive, quantitative, and specific yeast-based biosensor for measurement of carbon source concentrations. Previously published genomic data on the regulation of transcription during
the diauxic shift were used to identify candidate promoters from the thousands of genes regulated by glucose concentration (9, 13). These candidate promoters were used to drive GFP expression at a variety of fermentable and nonfermentable carbon source concentrations. The JEN1 promoter provided the highest signal level and the broadest dynamic range of all the promoters. Also, JEN1 is specific to repressing carbon sources based on tests of other stresses, such as DNA damage, osmotic stress, and temperature shock. The JEN1 promoter offers a paradigm for quantitatively linking extracellular signals, such as carbon source type and concentration, to intracellular pathways not normally regulated by these signals for cellular engineering applications.

MATERIALS AND METHODS

Yeast strains, plasmids, media, and genetic methods. The yeast strains used in this study are listed in Table 1. All strains were derived from the W303 genetic background by using standard genetic methods. Standard yeast culture media were prepared as previously described (35). Yeast media were obtained from United States Biological (Swampscott, Mass.), and other reagents were obtained from Fisher Scientific (Pittsburgh, Pa.) and Sigma (St. Louis, Mo.). G418 (in-vitrogen) was added to YPD agar at a concentration of 0.2 mg/ml. Media containing different carbon sources were prepared by using rich YEP medium without glucose and adding 40% (wt/vol) stock solutions of glucose, fructose, galactose, sucrose, raffinose, glycerol, ethanol, and lactate to obtain the desired concentrations. Unless indicated otherwise, the carbon source concentration was 2% (wt/vol). Heat shock experiments were performed at 37°C for 8 h, and cold shock experiments were performed at 10°C for 24 h. To test pH sensitivity, the medium pH was adjusted to 4.0 by using 10 N HCl, and cells were incubated in this medium for 8 h. Cells were grown for 8 h in medium containing 1 M NaCl to test the effects of salinity and were grown in YPD medium containing 0.05% ammonia dextrose medium (30). Haploid mating, diploid sporulation, and tetrad dissection were performed as described previously (35). Yeast cells were transduced by using a primer approximately 500 bp upstream of the gene start site and a primer in the kanMX cassette.

To generate a plasmid cassette for replacement of the FLO1 open reading frame with FLO1 (pSP30), the FLO1 open reading frame was amplified by using primers PcfLOI1 and Asc1FLO1t (Table 2). The PCR products were digested with Pae1 and Ascl and ligated into pFA6a-GFP(S65T)-kanMX6, generously provided by John Pringle, was used to prepare the PCR product for transformation, and G418 selection was used to identify transformants. Primers used for the replacement reaction are listed in Table 2. GFP replacements were verified by isolating genomic DNA of transformants and checking for a PCR product by using a primer approximately 500 bp upstream of the gene start site and a primer in the kanMX cassette.

To generate a plasmid cassette for replacement of the JEN1 open reading frame with FLO1 (pSP30), the FLO1 open reading frame was amplified by using primers Pac1FLO1t and Asc1FLO1t (Table 2). The PCR products were digested with Pae1 and Ascl and ligated into pFA6-GFP(S65T)-kanMX6 (22) digested with Pae1 and Ascl to excise GFP. Plasmid pSP30 was used as a template for amplification of a cassette to replace the JEN1 open reading frame from base 1 through base 1851 with the FLO1 open reading frame by using primers JEN1FLO1t and JEN1FLO1t (Table 2).

| Strain | Genotype |
|--------|----------|
| SPY 1001 | MATα ura3-52 his3::GFP::his3 hisG leu2::hisG::kanMX6 |
| SPY 1002 | MATα ura3-52 his3::GFP::his3 hisG leu2::hisG::kanMX6 |
| SPY 1003 | MATα ura3-52 his3::GFP::his3 hisG leu2::hisG::kanMX6 |
| SPY 1004 | MATα ura3-52 his3::GFP::his3 hisG leu2::hisG::kanMX6 |
| SPY 1005 | MATα ura3-52 his3::GFP::his3 hisG leu2::hisG::kanMX6 |
| SPY 1006 | MATα ura3-52 his3::GFP::his3 hisG leu2::hisG::kanMX6 |

* All strains have a W303 background.

RESULTS

**JEN1 and ACH1 promoter activities correspond to glucose concentrations.** To identify gene promoters sensitive to certain sugars, we analyzed microarray experiment data for diauxic shifts in *S. cerevisiae* (9). One of the hallmarks of the shift in metabolism from fermentation to respiration is the lack of availability of fermentable carbon sources, so the likelihood of identifying a gene regulated by carbon source concentration in these data seemed to be high. First, we searched the microarray data of DeRisi et al. (9) for genes with the highest levels of

**Fluorescence microscopy and flow cytometry.** Cells were grown overnight in YP liquid medium at 30°C and shifted to fresh media at a concentration of 107 cells/ml. The glucose concentration in the media was measured with a glucose analyzer (YSI Life Sciences model 23A).

Fluorescence images were acquired with an Olympus IX70 inverted epifluorescence microscope by using a >100X oil immersion objective with a GFP filter cube. Bright-field images were used to focus on the cells, and a Nikon Spot camera captured 1-s exposures to the 100-W mercury lamp. The MetaVue software was used to control the camera and image acquisition and to analyze images.

For flow cytometry, samples were rinsed five times with sterile phosphate-buffered saline, sonicated, and kept on ice in the dark until analysis. A total of 10^7 cells was analyzed for fluorescence intensity by using a FACSCalibur flow cytometer (Becton Dickinson) and the CellQuest software.

**Northern blot analysis.** Ten-milliliter aliquots of cells from an overnight liquid culture were diluted 50-fold and grown to an optical density at 600 nm (OD600) of 0.8; fresh media were provided hourly. RNA was harvested by phenol-chloroform extraction, followed by ethanol precipitation. For each sample, 10 μg of total RNA was separated by electrophoresis on a formaldehyde gel and transferred by capillary action to a 0.2-μm-pore-size nylon membrane. DNA probes (1,000-bp regions at the 5' ends of the GFP and ACT1 open reading frames) were amplified and radiolabeled with [32P]dATP by PCR, and free [32P]dATP was removed with Sephadex G-50 size exclusion spin columns. Hybridization and washing were performed by the methods described by Sambrook et al. (34), and the expression levels were measured by densitometry following exposure to Biomax MR film (Kodak). Multiple exposure times were used to ensure that the densitometry analysis was not performed with signal-saturated images. GFP expression was normalized to ACT1 expression. At least three independent measurements of gene expression levels were obtained for each strain.

**Cell aggregation assay.** To measure the rate of cell aggregation, yeast strains were grown to saturation in 10 ml of liquid YPD medium overnight at 30°C. Cells were harvested by two washes in 50 mM sodium citrate-5 mM EDTA (pH 3.0) buffer, followed by sonication for 10 min (5). Cells were resuspended in 5 ml of sodium citrate buffer containing 20 mM calcium chloride at a concentration of 10^6 cells/ml to induce flocculation. Culture tubes were inverted 50 times per minute for 10 min and then left standing vertically. After 10 min, 0.2 ml of the cell suspension was removed from just below the meniscus in each tube and added to 1 ml of 250 mM EDTA (pH 8.0) to stop flocculation. The level of flocculation was expressed as the difference between the OD600 of the deflocculated cell sample and the OD600 of the sample after 10 min of settling. The flocculation for each strain was normalized to the flocculation of strain SPY 1001 (MATα ura3-52 his3::GFP::his3 hisG leu2::hisG::kanMX6).

**TABLE 1. Yeast strains**

| Strain | Genotype |
|--------|----------|
| SPY 1001 | MATα ura3-52 his3::GFP::his3 hisG leu2::hisG::kanMX6 |
| SPY 1002 | MATα ura3-52 his3::GFP::his3 hisG leu2::hisG::kanMX6 |
| SPY 1003 | MATα ura3-52 his3::GFP::his3 hisG leu2::hisG::kanMX6 |
| SPY 1004 | MATα ura3-52 his3::GFP::his3 hisG leu2::hisG::kanMX6 |
| SPY 1005 | MATα ura3-52 his3::GFP::his3 hisG leu2::hisG::kanMX6 |
| SPY 1006 | MATα ura3-52 his3::GFP::his3 hisG leu2::hisG::kanMX6 |

* All strains have a W303 background.
induction and repression in wild-type cells at OD_{600} values of 6.90 and 7.30, corresponding to the lowest glucose concentrations. From this set of genes we chose six open reading frames that were strongly induced during the diauxic shift: JEN1, MSC1, SPI1, ACH1, YMR250W, and HAP4.

We replaced each of these open reading frames with the gene encoding GFP, so the endogenous promoter drove GFP expression. One chromosomal copy was replaced in a diploid strain, so the endogenous gene was still expressed from the other chromosome, and the promoter-GFP copy number was strictly regulated (one copy per cell). Overnight cultures of strain, so the endogenous gene was still expressed from the

**TABLE 2. Oligonucleotides**

| Primer | Sequence (5'→3') |
|--------|------------------|
| JEN1-GFP' | ACAGTTTCAAAAGTTTTCTCTCAAAAGAGATATCTGCTACTGAAAAAT |
| JEN1-GFP3 | ATTCGAATTCCATAGAGAAAGCGAAGCGCCCTTCTAGAGAAAATAG |
| GFP R | CAGCGGACCTCTTGTTGCTAC |
| JEN1 check | GTCTAGTCCTTCTCTCCCTAG |
| MSC1-GFP' | GCAATAGAGAGAGGAGACACTAACAAGAAGAGATTTAATTAAGAAAGAAG |
| MSC1-GFP3 | ATGAGGAAAGATTGAGAGAAATGATCTACGGTGCAACCCCAACATCA |
| MSC1 | GACGTTCCGTCGAAGACT |
| SPI1-GFP' | ACCTCAAAAGAGAATCTCAAAGAGGAGCTTCAACATAGAAAAGAAC |
| SPI1-GFP3 | AAAAACACATGTCCTTATATTTAAATAGCACTTAAAGATGACTTATGG |
| SPI1 check | GGAGACCGCTTTGAGGAC |
| ACH1-GFP' | GCAAAACCAAAACCAATTTTCTTTTCTTTATTACACATGGGACTCAAA |
| ACH1-GFP3 | TTTCTCTTCTGTTATATTTTAGTTGTTGTTGTATGCAACATGGCATT |
| ACH1 | ATTAGGGGGCTTAGAGGACAGG |
| HAP4-GFP' | ATTTGTTTTCATCATTATTTTAGTATTTGTTTGGTTTATTGCAACATTTGGCATT |
| HAP4-GFP3 | TTTCTTCTGGAGTTGTCCC |
| HAP4 | CGGTCCCAATTGCTCGAGAG |
| HAP4 check | GGAGAACGTCTTTGGACCAC |
| snf1 | ATTTGCGTGACTCAGATGAGAATGCTTCATAGAAAGAGAACAGTTG |
| snf1 check | ATTGCGTGGCGGCGTGGATG |
| klt | TTTAGTTTACTGACG |
| PacI | GTGTCGACCTCAGATTGCGGAC |
| AscI | GCTAGTCGCTTATGAGAAGAGGAGCTTCAACATAGAAAAGAAC |
| ACH1-P | ACAGTTTCAAAAGTTTTCTCTCAAAAGAGATATCTGCTACTGAAAAAT |
| JEN1-FLO1 | ATTCGAATTCCATAGAGAAAGCGAAGCGCCCTTCTAGAGAAAATAG |
| JEN1-FLO1 check | GTCTAGTCCTTCTCTCCCTAG |
| GFP probe 5' | CTTCAGTCTGAGGATGTTGCC |
| GFP probe 3' | TAGTTCATCCATGAGGAGTG |
| ACT1 probe 5' | CGTCCCAAATGGCTGAGAG |
| ACT1 probe 3' | TGGAAACAAAGCTTGGCGG |

The strains could be an adequate sensor for glucose depletion. However, several parameters in addition to glucose concentration changed during batch fermentation. Oxygen, amino acid, and other nutrient concentrations decreased. The ethanol concentration increased, and the pH decreased. Any of these factors may have contributed to expression of genes during the diauxic shift. To determine if the GFP expression shown in Fig. 1B was due to changes in the sugar concentration or to some of these other factors, we provided a relatively constant glucose concentration to a growing culture by replacing the medium with fresh 30°C medium every hour. The glucose concentration decreased less than 20% between medium changes (data not shown). After 4 h, cells were harvested and analyzed by fluorescence microscopy and flow cytometry. Representative cells and flow cytometry profiles for JEN1-GFP cells grown at different glucose concentrations are shown in Fig. 2. The mean fluorescence per cell (as measured by flow cytometry) as a function of glucose concentration is shown in Fig. 3. Flow cytometry histograms indicated that an increase in the average fluorescence of a unimodal population, whose mean expression increased in response to decreases in the glucose concentration, was responsible for the increase in fluorescence. At high glucose concentrations a small population of cells did fluoresce, however.

Of the six promoter-GFP systems tested, decreasing the glucose concentration activated the JEN1 and ACH1 promoters to the greatest extent, while the other four promoters were only partially responsive to the glucose concentration (Fig. 3A). Both the JEN1-GFP and ACH1-GFP strains could be used to measure glucose concentrations over the range from...
containing one of the following carbon sources at a concentration of 20 g/liter: glucose, fructose, mannose, galactose, sucrose, raffinose, ethanol, glycerol, or lactate. After 4 h, cells were harvested, and fluorescence was analyzed by flow cytometry and epifluorescence microscopy (Fig. 5). GFP expression on media containing glucose, fructose, and mannose was low. Sucrose, a disaccharide that cells hydrolyze to utilize the glucose and fructose monosaccharides, also inhibited JEN1 promoter activity. On galactose, raffinose, ethanol, glycerol, and lactate the JEN1 promoter activated GFP expression. Lactate appeared to activate the JEN1 promoter to a greater extent than other nonrepressing carbon sources, but this activation was weak. Combining repressing and nonrepressing carbon sources led to very low GFP expression, similar to the expression on glucose. Thus, JEN1 promoter activity is repressed by certain sugars and may be enhanced by the presence of lactate.

_Snf1 is required for JEN1 promoter activity._ To test if the JEN1 promoter operates downstream of the Snf1 protein kinase, which is required for release of many genes from glucose repression (6, 10, 27), we deleted both copies of SNF1 in a diploid strain expressing GFP from the JEN1 promoter. Figure 6 shows that GFP expression from the JEN1 promoter required Snf1. Likewise, growth on nonrepressing carbon sources in the absence of SNF1 did not permit GFP expression from the JEN1 promoter, and the JEN1 promoter activity was very low in an snf1Δ::snf1Δ strain upon glucose depletion during batch fermentation (data not shown).

**JEN1 promoter is not sensitive to most cell stresses.** An important aspect of a promoter-reporter-based biosensor is the specificity for a particular input. To test whether the JEN1 promoter is specific for repressing sugar concentrations, we exposed cells growing with 20 g of glucose per liter (repressing conditions) or 20 g of glycerol per liter (nonrepressing conditions) to a variety of environmental stresses, including heat shock, cold shock, 1 M NaCl, pH 4, DNA damage by MMS, and nitrogen starvation. None of the cell stresses tested significantly induced or repressed GFP expression from the JEN1 promoter (Fig. 7). Based on these experiments, the JEN1-GFP construct appears to be extremely specific for repressing sugar concentrations; however, the strain must be tested further for stresses important in any particular application.

**Kinetics of induction and decay of GFP expression under control of the JEN1 promoter.** An ideal sensor provides an immediate reading of the environmental conditions. However, time delays in signal transduction, transcription, translation, and degradation affect the GFP concentration and the fluorescence in the JEN1-GFP strain as repressing sugar concentrations change. To characterize these time delays, we compared the results of two experiments; in one experiment we examined the slow decrease in the glucose concentration due to cell metabolism, and in the other experiment we examined step changes in the glucose concentration both from a high concentration to a low concentration and from a low concentration to a high concentration. Figure 1 shows that during batch fermentation the glucose concentration decreased exponentially and fluorescence was induced when glucose concentrations below 1 g/liter were reached. As the initial cell inoculation size increased, the lag between glucose depletion and GFP induction remained unchanged (data not shown).

A change in carbon source from glucose to glycerol (Fig. 8A)
for JEN1P-GFP cells grown overnight in the presence of a high glucose concentration demonstrated that there was a 40-min lag prior to GFP induction. A medium change from YEP containing glycerol to YPD resulted in a decrease in fluorescence on the time scale of hours (Fig. 8B). The GFP fluorescence half-life was 3.3 h in the JEN1P-GFP strain, assuming that the promoter was completely inactivated when the culture was shifted to medium containing glucose. During growth in the presence of glucose, two populations appeared, one with high GFP expression and the other with low GFP expression (data not shown). As the time of incubation increased, both the fraction of cells in the population with high GFP expression and the fluorescence in this population decreased. The existence of these two populations may be attributable to differences in lag times for release from starvation-induced cell cycle arrest and/or differences in GFP partitioning during cytokinesis. These kinetic experiments indicated that GFP degradation is much slower than transcription and translation. Thus, use of the JEN1P-GFP sensor is best suited for applications where glucose concentrations do not increase.

Also, since previous signals degrade slowly, any readout is a function of the past behavior of the system. Figure 9 demonstrates this concept. A sample grown in the presence of 20 g of glucose liter for 12 h and then switched to a medium contain-
ing 0.05 g of glucose per liter for 60 min had slightly lower fluorescence, at least initially, than a sample grown in the presence of 1 g of glucose per liter for 12 h and then switched to a medium containing 0.05 g of glucose per liter for 60 min. Thus, the calibration curve for amount of fluorescence per cell as a function of the glucose concentration depends on the rate of change of the glucose concentration. For batch fermentations that rapidly progress through low glucose concentrations this effect would be minimal, however.

**Kinetics of cell aggregation upon carbon source shift in cells expressing FLO1 under control of the JEN1 promoter.** A quantitative promoter for carbon source concentration offers the potential for regulating cellular functions in a carbon source dose-dependent manner. To illustrate this concept, we created a strain expressing the cell surface flocculin gene FLO1 under control of the JEN1 promoter and measured flocculation kinetics as a function of pseudosteady glucose concentration (Fig. 10). We found that the flocculation rate increased as the glucose concentration decreased below 0.3 g/liter. When cells were grown in the presence of high glucose concentrations, at which the JEN1 promoter was virtually inactive, we detected no flocculation in the JEN1P-FLO1 strain compared to the flocculation of a wild-type strain. The wild-type W303 strain showed almost no settling at any glucose concentration (data not shown). The increase in flocculation was not directly linearly proportional to glucose concentration or JEN1 promoter strength, probably because cell aggregation is a nonlinear phenomenon and our assay to measure aggregation was not linear with respect to aggregate size. However, the JEN1P-FLO1 construct did demonstrate that we can quantitatively control cellular behavior as a function of carbon source concentration.

**DISCUSSION**

The *S. cerevisiae* JEN1 promoter is able to quantitatively respond to changes in repressing sugar concentrations. By using the JEN1 promoter to drive GFP expression, we can construct a cellular biosensor for sugars. In addition, we can use the JEN1 promoter to quantitatively modify gene transcription patterns in response to changes in sugar concentrations. JEN1 promoter activity is induced at glucose concentrations below 3 g/liter and is maximal at a concentration of approximately 0.1 g/liter. Repressing carbon sources inhibit JEN1 promoter activity via the Snf1 protein kinase. In the presence of non-repressing carbon sources that permit cell growth, the JEN1...
The promoter is active. The \textit{JEN1} promoter is insensitive to many cell stresses, making it a specific sensor for repressing sugars. The kinetics of \textit{JEN1} promoter activity and GFP translation are favorable for use of this system as a sensor for a decreasing glucose concentration, but the degradation kinetics are slow and the half-life is 3.3 h.

In an alternative strategy to construct a sugar biosensor-response system, a Jen1-GFP fusion protein could be used to assess \textit{JEN1} promoter activity. A potential advantage of this system is that the Jen1 protein level would more closely approximate the level in a wild-type cell. Our results have shown that this is not important for \textit{JEN1}, but it might be a critical issue if promoters for genes whose concentrations are rate limiting for cell growth are used. However, using a fusion protein as a sensor has disadvantages. GFP signal localization or RNA and protein synthesis and degradation kinetics may be affected. For example, Jen1-GFP localizes to the plasma membrane and is endocytosed and degraded upon exposure of a cell to glucose (28). Upon exposure to glucose, the fluorescence intensity remains intact for hours in the Jen1-GFP strain (28), which is similar to our results obtained with GFP under control of the \textit{JEN1} promoter. This may be due to the relatively high stability of GFP in the vacuole. The change in localization from

FIG. 5. Relative fluorescence of the \textit{JEN1P}-GFP strain as a function of carbon source. A total of 10^8 cells from a YPD medium culture were inoculated into 100 ml of YEP medium containing a carbon source at a concentration of 2% (wt/vol), unless indicated otherwise. Cells were grown for 6 h at 30°C. The error bars indicate the standard errors of the means for three independent measurements.

FIG. 6. Induction of GFP expression by the \textit{JEN1} promoter during glucose depletion requires \textit{SNF1}. The mean fluorescence per cell for \textit{JEN1P}-GFP \textit{SNF1}/\textit{SNF1} and \textit{JEN1P}-GFP \textit{snf1}/\textit{snf1} strains, normalized to expression in the \textit{JEN1P}-GFP \textit{SNF1}/\textit{SNF1} strain grown with 20 g of glucose per liter, is expressed as a function of glucose concentration. A total of 10^8 cells from a YPD medium culture were inoculated into 100 ml of YEP medium containing glucose at one of the concentrations indicated. Cells were grown for 6 h at 30°C, and the medium was replaced every hour to maintain a relatively constant medium concentration. The error bars indicate the standard errors of the means for three independent measurements.

FIG. 7. Mean fluorescence per cell for the \textit{JEN1P}-GFP strain grown at 30°C in YEP medium containing either 20 g of glucose per liter or 20 g of glycerol per liter. Cells were exposed to heat shock at 37°C, cold shock at 10°C, 1 M NaCl, pH 4, 0.1% MMS, or growth on low-nitrogen medium. The error bars indicate the standard errors of the means for three independent trials.

FIG. 8. Kinetics of GFP induction and decay in the \textit{JEN1P}-GFP strain. (A) A total of 10^8 cells from a pseudo-steady-state YPD medium culture were inoculated into 100 ml of YEP medium containing 20 g of glycerol per liter at time zero. At each time point, fluorescence was determined by flow cytometry and was normalized to the fluorescence at zero time. The mean fluorescence was normalized to the fluorescence at 24 h. The error bars indicate the standard errors of the means for three independent trials.
the plasma membrane to vacuoles would be difficult to detect in a biosensor application, so we prefer the JEN1-GFP system to the Jen1-GFP fusion protein.

An ideal sensor system would be tunable. Strain-dependent variations in metabolism or mutations in metabolic pathways offer promise for further regulating JEN1 promoter activity. Mutations that block import, sensing, or utilization of certain repressing sugars may prevent the sugars from repressing JEN1 promoter activity or may alter the relationship between activity and concentration.

The stability of GFP is an advantage for detecting steady-state or decreasing concentrations of repressing sugars by amplifying the signal and increasing the sensitivity of the sensor. However, the stability becomes a disadvantage when we try to detect increasing concentrations of repressing sugars. One potential solution is to use a GFP destabilized with the C-terminal PEST-containing residues of Cln2 (23). This mutation decreases the half-life of GFP 15-fold (23) and should increase the dynamic response of the sensor at the cost of sensitivity. For applications where the sugar concentration might increase over time, this tradeoff would be necessary.

The existence of gene promoters with dose-dependent responses to an environmental input indicates the feasibility of cell-based transcriptional and/or translational sensors. The number of promoters with such characteristics remains unknown, however. Also, the mechanisms responsible for the dose-dependent responses are of interest. We propose that a combination of multiple regulatory elements and stochastic activation of the promoters may contribute to complex promoter sensitivities. Multiple repressive and activating signals operating independently and targeted at different input concentrations may provide a concentration-dependent promoter response. For example, the Cat8 and Hap2/3/4/5 complexes both activate JEN1 transcription (4, 13), and a hap2 cat8 mutant contains less JEN1 mRNA than either single mutant contains (21). Also, mutation of the transcription factors Pip2 and Oaf1, which regulate enzymes involved in peroxisomal metabolism, increases JEN1 expression in cells grown on oleate (15).

Deletion of SNF1 keeps JEN1 transcription repressed, even in the presence of nonrepressing carbon sources and at low glucose concentrations (Fig. 6) (21, 29). Snf1-mediated derepression acts through multiple mechanisms, which may contribute to the dose-dependent response of the JEN1 promoter. Snf1 inhibits the Mig1 transcriptional repressor, which represses JEN1 transcription in the presence of glucose (4), and the Nrg1 and Nrg2 transcriptional repressors (18). Snf1 may also act indirectly by relieving repression of transcriptional activators and histone phosphorylation (20) or by stimulating the RNA polymerase II holoenzyme.

Specificity for the desired input is one of the most important design parameters for a biosensor. The JEN1 promoter appears to be quite specific for the carbon source since temperature, salinity, DNA damage, and nitrogen availability do not activate or repress its activity. Lodi et al. (21) reported that JEN1 expression in cells utilizing raffinose as a carbon source decreases as the oxygen concentration approaches zero. Therefore, this sensor would be less effective in anaerobic cultures. Also, JEN1 expression appears to decrease in the presence of isoctane in a strain selected for isoctane tolerance (25). Conversely, several other glucose-sensitive promoters are very sensitive to many general cell stresses. Compilations of genomics experiments should facilitate identification of promoters specific for particular environmental changes.

The main drawback of using the JEN1-GFP system as a fermentation monitor is the costly monitoring system required to measure fluorescence. However, a number of other reporter genes may be used to monitor JEN1 promoter activity, including genes encoding enzymes that catalyze a colorimetric reaction (e.g., β-galactosidase) or cell adhesion proteins that induce aggregation (e.g., FLO1). Such reporters may be less accurate or precise than GFP, but they would also be less expensive to monitor.

Glucose and other sugar concentrations are easily measured by chemical and optical methods, and we do not propose that JEN1-GFP strains should supplant these methods in most applications. However, the JEN1-GFP system has sensory advantages over other assays in one key respect: JEN1-GFP strains can distinguish between glucose-repressing and nonrepressing carbon sources in a particular strain. Typically, S. cerevisiae cells metabolize repressing sugars before they utilize nonrepressing carbon sources, so a JEN1-GFP strain also can act as a monitor for fermentation progress, independent of other process variables. Therefore, a JEN1-GFP strain could
be useful for monitoring carbon source metabolism in (i) systems containing complex mixtures of repressing and nonrepressing carbon sources and (ii) systems in which repressing carbon source concentrations decrease over a time scale longer than the GFP expression time scale (e.g., hours).

The true advantage of the JEN1 promoter system is that it can be used for carbon source concentration-dependent control of other cellular processes. By driving FLO1 expression with the JEN1 promoter we were able to construct a strain whose flocculation rate is quantitatively controlled by the repressible sugar concentration. Such a construct could be useful in industrial batch fermentations in which cell dispersion is desired during glucose utilization and flocculation is beneficial upon completion of the reaction to facilitate cell separation (31). We anticipate that metabolic enzymes could be placed under control of the JEN1 promoter to transition from a cell growth phase on glucose to a product formation phase on nonrepressible carbon sources for metabolic engineering applications. Also, cell cycle regulators controlled by the JEN1 promoter may be used to permit growth on repressible carbon sources by not nonrepressible carbon sources or vice versa. In addition, global gene transcription patterns might be regulated by placing one or more transcription factors under control of the JEN1 promoter.

The existence of a dose-dependent glucose-responsive promoter for sugars suggests a paradigm for identification and development of cell-based sensors for other compounds or environmental conditions that may not be as easy to directly measure as sugar concentrations. Genomic studies (i.e., microarray experiments) offer the opportunity to identify specific promoters whose activities are quantitatively related to a particular stimulus. Reporter genes can be used to easily quantify promoter activity. Multiple promoter-reporter systems could be combined to cover a larger dynamic range or measure multiple stimuli. This approach will likely provide effective measurements of nutrients or toxins for which reliable assays do not exist. Such promoter systems should also be valuable in redesigning cells so that they have a desired response to changes in a particular stimulus.

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