

# Cell signaling can direct either binary or graded transcriptional responses

Stephen R. Biggar and Gerald R. Crabtree<sup>1</sup>

Department of Developmental Biology, Howard Hughes Medical Institute, Room B211, Beckman Center, 279 Campus Drive, Stanford University School of Medicine, Stanford, CA 94305-5323, USA

<sup>1</sup>Corresponding author  
e-mail: crabtree@cmgm.stanford.edu

**Transcriptional control is generally thought to operate as a binary switch, a behavior that might explain observations such as monoallelic gene expression, stochastic phenotypic changes and bimodal gene activation kinetics. By measuring the activity of the single-copy *GALI* promoter in single cells, we found that changes in the activities of either the transcriptional activator, Gal4 (by simple recruitment with synthetic ligands), or the transcriptional repressor, Mig1, generated graded (non-binary) changes in gene expression that were proportional to signal intensity. However, in the context of the endogenous glucose-responsive signaling pathway, these transcription factors formed part of a binary transcriptional response. Genetic studies demonstrated that this binary response resulted from regulation of a second repressor, Gal80, whereas regulation of Mig1 by a distinct signaling pathway generated graded changes in *GALI* promoter activity. Surprisingly, isogenetic cells can respond to glucose with either binary or graded changes in gene expression, depending on growth conditions. Our studies demonstrate that a given promoter can adapt either binary or graded behavior, and identify the Mig1 and Gal80 genes as necessary for binary versus graded behavior of the Gal1 promoter.**

**Keywords:** CID/FK506/signaling/synthetic ligands/transcription

## Introduction

An unresolved question at the interface of signaling and transcriptional regulation is whether regulators of transcription serve as binary switches between active and inactive promoter states or as graded regulators of transcription. We use the term 'graded' to describe a promoter response that achieves a continuous range of activity from fully on to fully off. In contrast we use 'binary' to refer to the situation when a promoter produces only two levels of activity: on and off.

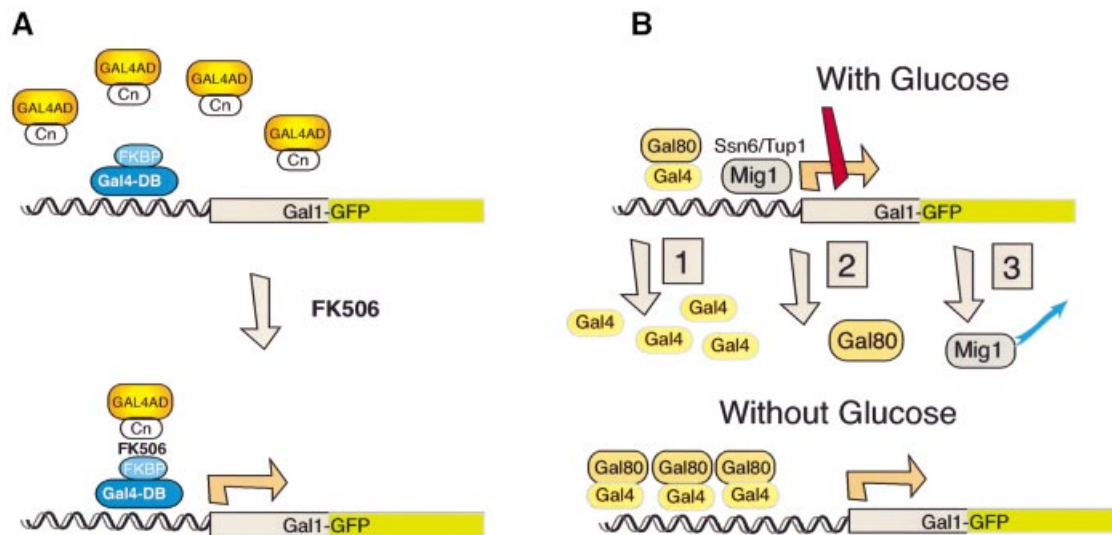
When collections of cells are analyzed experimentally, either graded or binary patterns of transcriptional response would demonstrate similar increases in mRNA levels, but within single cells this distinction underlies fundamental aspects of gene regulation and cellular responses. Binary, all-or-none reactions by individual cells may ensure

efficient cellular responses to fleeting signals, but may also limit the diversity of these responses. The binary pattern of response could also generate distinct populations within a group of cells: one consisting of responsive cells in which expression is turned on, and another comprised of unresponsive cells. Such responses could be critical to cell fate decisions and other all-or-none phenotypic responses to signals. Alternatively, graded alterations in gene expression may allow fine-tuned responses that permit proportionate responses to a stimulus. In the latter case, all cells in a population could respond with similar changes in expression and thereby maintain homogeneity.

The majority of existing evidence suggests that, within individual cells, a gene is either maximally expressed or is not expressed at all (Moreau *et al.*, 1981; Weintraub, 1988; Fiering *et al.*, 1990; Moon and Ley, 1990; Walters *et al.*, 1995, 1996; Magis *et al.*, 1996; Hollander *et al.*, 1998). Under this binary model, transcriptional activators and their corresponding enhancers stimulate expression simply by increasing the likelihood that a promoter will be active, such that promoters containing enhancers express in a larger fraction of cells than promoters lacking enhancers. An important confirmation of this model is the observation that while the proportion of expression-positive cells differs in the presence and absence of the enhancer, the level of expression in individual expression-positive cells is similarly independent of the presence of the enhancer.

Transcriptional activators may also reduce 'silencing' of gene expression in a binary fashion (Magis *et al.*, 1996; Walters *et al.*, 1996). In cells that have been selected for active expression of an integrated reporter gene, removing the selective pressure for expression leads to the accumulation of cells that no longer express the reporter. Single-cell analysis of this process reveals two separate populations of cells: one expressing the reporter at maximal levels and one in which expression is silenced. The presence of enhancers or activators slows the silencing of reporter expression by maintaining the number of expression-positive cells without influencing the level of expression within these cells. These results have been interpreted as support for the idea that transcriptional activators produce binary changes in promoter activity.

Not all alterations in transcription factor activity generate binary changes in gene expression. In one important exception, mutations in components of the chromatin-remodeling transcription factor, Swi-Snf, reduce expression throughout the entire population of cells to a level that is intermediate to the fully activated and repressed states (Sudarsanam *et al.*, 1999). Also, inducing the function of the MTF transcriptional activator increases expression of an MTF-responsive gene in a population of cells presumed to be universally expressing the reporter (Magis *et al.*, 1996), suggesting that the activator produces



**Fig. 1.** Synthetic and natural modes of transcriptional activation of the integrated Gal1-GFP promoter. Regulation of a single-copy, integrated *GALI-GFP* gene by FK506 and by glucose. (A) Activation with the synthetic ligand FK506. In cells expressing the Gal4BD-FKBP and Gal4AD-CN fusion proteins, FK506 generates an interaction between FKBP and calcineurin at the promoter, and thereby recruits the activation domain and stimulates transcription. The *GALI-GFP* reporter, which consists of the full-length *GALI* promoter fused to the coding sequence of GFP, is shown schematically, with the binding sites for Gal4 and Mig1 indicated. (B) Activation by relief of glucose-induced repression. Glucose regulates *GALI* promoter activity by three mechanisms in wild-type cells (see text). The *GALI-GFP* reporter, which consists of the full-length *GALI* promoter fused to the coding sequence of GFP, is shown schematically, with the binding sites for Gal4 and Mig1 indicated.

graded changes in transcription under these conditions. Recently, the Tet repressor-VP16 fusion was shown to produce a graded response with increasing tetracycline concentrations (Rossi *et al.*, 2000).

Presently, the mechanisms and molecular events underlying either graded or binary modes of transcription are unknown. We set out to study these mechanisms for the well studied Gal1 promoter by exploring the elements comprising the regulatory controls of this promoter, including the changing activities of transcriptional activators and repressors, and the influences of cell signaling. We show that graded and binary modes of transcriptional response are not intrinsic to the promoter, but are determined by specific regulatory mechanisms. Furthermore, we identify for the first time the genes essential to produce these two modes of promoter activity.

## Results

Transcriptional regulation at the single-cell level was studied by dissecting the effects of transcriptional activators and repressors, either individually or in the context of intact signaling pathways, on the activity of a promoter on a cell-by-cell basis. To achieve this, we used both physiological and a small-molecule-based means of regulating the constituents that control expression from the *Saccharomyces cerevisiae* *GALI* promoter. In these experiments, expression was measured by fluorescence-activated cell sorting (FACS) analysis of cells carrying a single-copy, integrated reporter from which the full-length *GALI* promoter directed expression of green fluorescent protein (*GALI-GFP*) (see Figure 1). Previous studies have demonstrated that GFP fluorescence closely correlates with expression levels from several yeast promoters

(Figure 1A; Niedenthal *et al.*, 1996; Sudarsanam *et al.*, 1999).

Using this approach, binary transcriptional responses would be expected to generate two peaks of GFP fluorescence: one of high intensity that corresponds to cells expressing *GALI-GFP*, and one representing background fluorescence corresponding to cells in which *GALI-GFP* is not expressed. In this case, increasing levels of expression would raise the height of the high-intensity peak as the number of expressing cells increased, but the positions (i.e. fluorescence intensities) of the two peaks would not change. Alternatively, graded transcriptional responses would generate a single fluorescence peak that increases in intensity and therefore changes position on a FACS plot as expression levels increase. In this case, the single peak would represent a homogeneous transcriptional response across an entire population of cells.

### **Direct control of Gal4 produced graded changes in *GALI-GFP* expression**

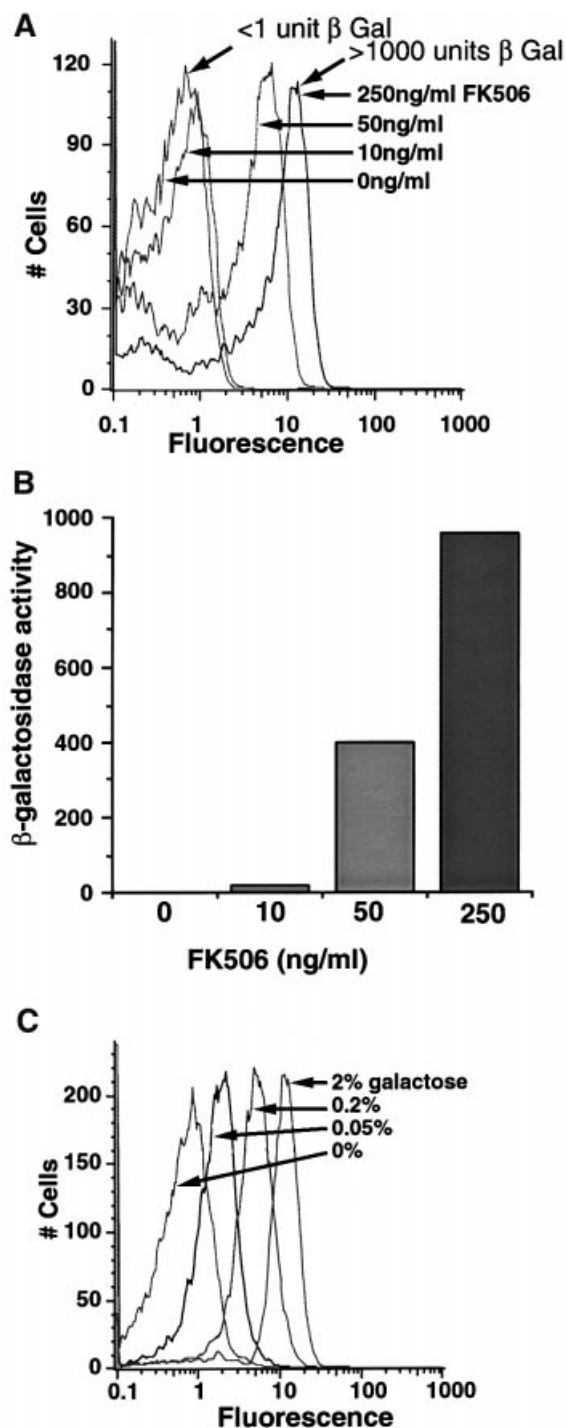
Initially, we addressed the effects of directly recruiting the transcriptional activator, Gal4, on *GALI-GFP* expression. This approach was used to assay the behavior of an activator without the complication of endogenous cellular controls. To assay the behavior of an activator specifically, the Gal4 activation domain was directly recruited to the Gal4 DNA binding domain using FK506 (Figure 1A). Cells expressed the functional domains of Gal4 as two separate fusion proteins: a Gal4 DNA binding domain-FKBP12 (Gal4BD-FKBP) fusion and a Gal4 activation domain-calcineurin A (Gal4AD-CN) fusion. By generating an interaction between calcineurin and FKBP12, FK506 recruited the Gal4 activation domain to Gal4BD-FKBP bound at the *GALI* promoter and thereby activated

transcription. FK506 was chosen because it generates little transcriptional response in yeast at nanomolar concentrations, as determined by studies using yeast whole-genome transcript arrays (Marton *et al.*, 1998; K.Vogel and G.R.Crabtree, unpublished studies). Furthermore, the few genes that are affected are not part of the *GAL1* regulatory network. Titrating FK506 levels produced dose-dependent stimulation of *GAL1* expression (Clipstone *et al.*, 1994; Ho *et al.*, 1996; Biggar and Crabtree, 2000), and FACS analysis showed homogeneous (i.e. a single fluorescence peak) and continuous changes in *GAL1-GFP* expression across the entire population of cells (Figure 2A). Since GFP fluorescence is not quantitative, we determined the magnitude of the difference between the response achieved with no FK506 and that seen with 250 ng/ml (240 nM) FK506 by assaying the relative  $\beta$ -galactosidase levels expressed from a *GAL1-LacZ* reporter also carried by these cells (Figure 2B). Since the two assays could be run on the same population of cells, this allowed us to determine accurately the background level of expression with no FK506. Comparing Figure 2A with B indicates that the background level is undetectable and that the fluorescence seen in Figure 2A in the absence of drug is simply the normal cellular fluorescence. A similar conclusion was reached by FACS analysis of cells not containing a GFP gene (data not shown). Thus, recruitment of the transcriptional activator with FK506 generated graded changes in gene expression over a several thousand-fold range, such that in each cell, *GAL1-GFP* was expressed at levels proportional to drug dose.

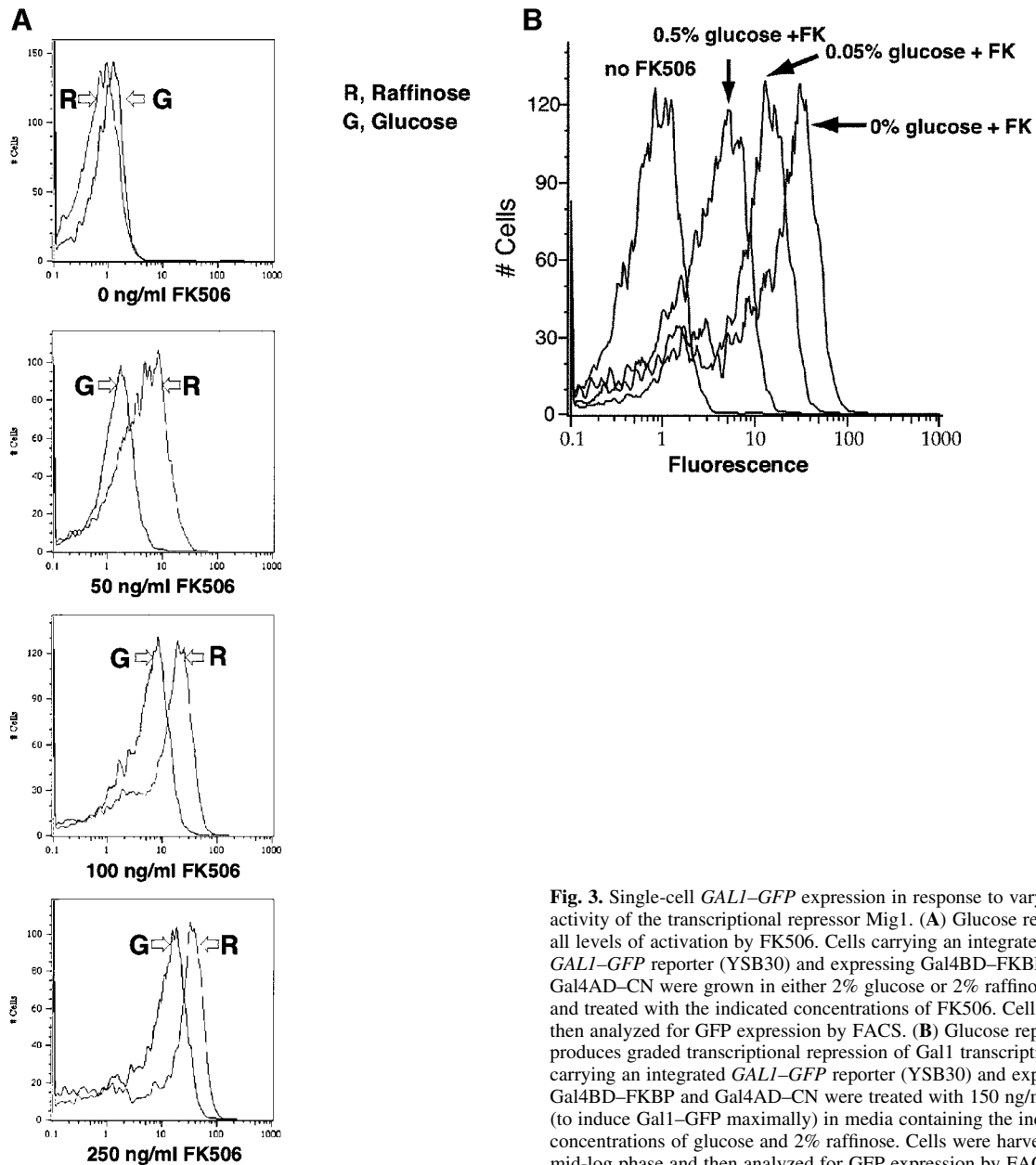
To examine the type of control found under physiological regulation, we examined transcriptional control exerted by the endogenous *GAL1* regulatory mechanisms, including wild-type *GAL4*. Through the action of distinct signals, wild-type yeast repress *GAL1* expression in glucose media and induce *GAL1* expression in galactose (Lohr *et al.*, 1995) (Figure 2C). In the absence of glucose, galactose stimulates *GAL1* expression by increasing the function of Gal4 as a transcriptional activator (Figure 1B). Thus, *GAL1-GFP* expression was measured in cells exposed to varying concentrations of galactose. Similarly to the experiment presented above, in which FK506 directly controlled Gal4 activity (see Figure 2A), physiological regulation of Gal4 activity with galactose produced graded changes in *GAL1* promoter activity over at least a 500-fold range, with the entire populations of cells homogeneously expressing GFP at higher levels in response to increasing galactose concentrations (Figure 2C).

#### **The repressor, Mig1, uniformly reduced *GAL1-GFP* expression in all cells**

As mentioned, glucose represses *GAL1* transcription. To explore the effects of transcriptional repression on gene expression at the single-cell level, yeast carrying the Gal4BD-FKBP and Gal4AD-CN fusions were treated with FK506 to induce *GAL1-GFP* expression and then exposed to glucose. In these cells, glucose directly inhibits *GAL1-GFP* expression by inducing the function of Mig1, a transcriptional repressor that binds to the *GAL1* promoter (Figure 1B; Nehlin *et al.*, 1991). Constitutive expression of the Gal4BD-FKBP and Gal4AD-CN fusion proteins, combined with a deletion of *GAL80*, prevented indirect



**Fig. 2.** Single-cell *GAL1-GFP* expression in response to varying the activity of the transcriptional activator Gal4. (A) Single-cell analysis of the response to directly increasing activity of the Gal4 activator with FK506. Yeast (YSB30) carrying integrated *GAL1-GFP* and *GAL1-LacZ* reporter genes and expressing Gal4BD-FKBP and Gal4AD-CN were treated with 0, 10, 50 and 250 ng/ml FK506. Cells were then analyzed for GFP expression by FACS and for (B)  $\beta$ -galactosidase expression in the entire population of cells. (C) Single-cell expression in response to different galactose concentrations and endogenous regulation. Yeast (YSB31) carrying the integrated *GAL1-GFP* reporter were grown in glucose media, washed, and then exposed to the indicated concentrations of galactose in 2% raffinose media. Cells were harvested at mid-log phase to avoid nutrient depletion and then analyzed for GFP expression by FACS.



**Fig. 3.** Single-cell *GAL1-GFP* expression in response to varying the activity of the transcriptional repressor Mig1. **(A)** Glucose represses at all levels of activation by FK506. Cells carrying an integrated *GAL1-GFP* reporter (YSB30) and expressing Gal4BD-FKBP and Gal4AD-CN were grown in either 2% glucose or 2% raffinose media and treated with the indicated concentrations of FK506. Cells were then analyzed for GFP expression by FACS. **(B)** Glucose repression produces graded transcriptional repression of Gal1 transcription. Cells carrying an integrated *GAL1-GFP* reporter (YSB30) and expressing Gal4BD-FKBP and Gal4AD-CN were treated with 150 ng/ml FK506 (to induce Gal1-GFP maximally) in media containing the indicated concentrations of glucose and 2% raffinose. Cells were harvested at mid-log phase and then analyzed for GFP expression by FACS.

effects of glucose on *GAL1* expression (Lamphier and Ptashne, 1992; Johnston *et al.*, 1994; Biggar and Crabtree, 2000). Comparing GFP levels in cells induced with FK506 and grown in either 2% raffinose (0% glucose) or 2% glucose showed that glucose shifted the peak of expression downward without silencing the promoter (Figure 3A). This result was seen at all FK506 concentrations tested. Furthermore, in cells treated with a given amount of FK506 (150 ng/ml), increasing glucose concentrations shifted the peak of GFP expression downward (Figure 3B). Thus, similar to the transcriptional activator, the actions of the transcriptional repressor, Mig1, produced graded changes in gene expression.

#### **Physiological regulation of *GAL1* generated binary changes in *GAL1-GFP* expression**

The results of these studies of transcription factors differ from the binary transcriptional responses seen in other

systems in response to extracellular signals (Fiering *et al.*, 1990; Walters *et al.*, 1996). We therefore set out to address how, in individual cells, *GAL1-GFP* expression responded to signaling cascades that culminate by altering the activities of both the transcriptional activator, Gal4, and the transcriptional repressor, Mig1. For these experiments, yeast carrying the single-copy, integrated *GAL1-GFP* reporter and the endogenous *GAL1* regulatory network were studied. In wild-type cells, glucose both suppresses the function of the activator, Gal4, and stimulates the function of the repressor, Mig1 (Figure 1; Lamphier and Ptashne, 1992; Johnston *et al.*, 1994). To study the transcriptional response to glucose, cells were grown in 2% galactose media containing varying amounts of glucose. FACS analysis showed that, in contrast to the graded changes seen above, glucose generated a binary response in GFP expression (Figure 4). As the glucose concentration decreased, an accumulating fraction of cells

expressed GFP, and these populations of GFP-positive cells expressed *GAL1-GFP* at levels similar to those in fully activated cells (0% glucose). Conversely, a second subpopulation of cells, which failed to express GFP, declined in number as glucose levels fell. Again, as in previous figures, the quantitative differences in expression from 0.5% glucose to no glucose were at least 500-fold, as measured by  $\beta$ -galactosidase levels. Thus, unlike the graded influences observed when studied in isolation, *GAL4* and *MIG1* combined in the glucose-mediated signaling pathways to form part of a binary control over *GAL1-GFP* expression.

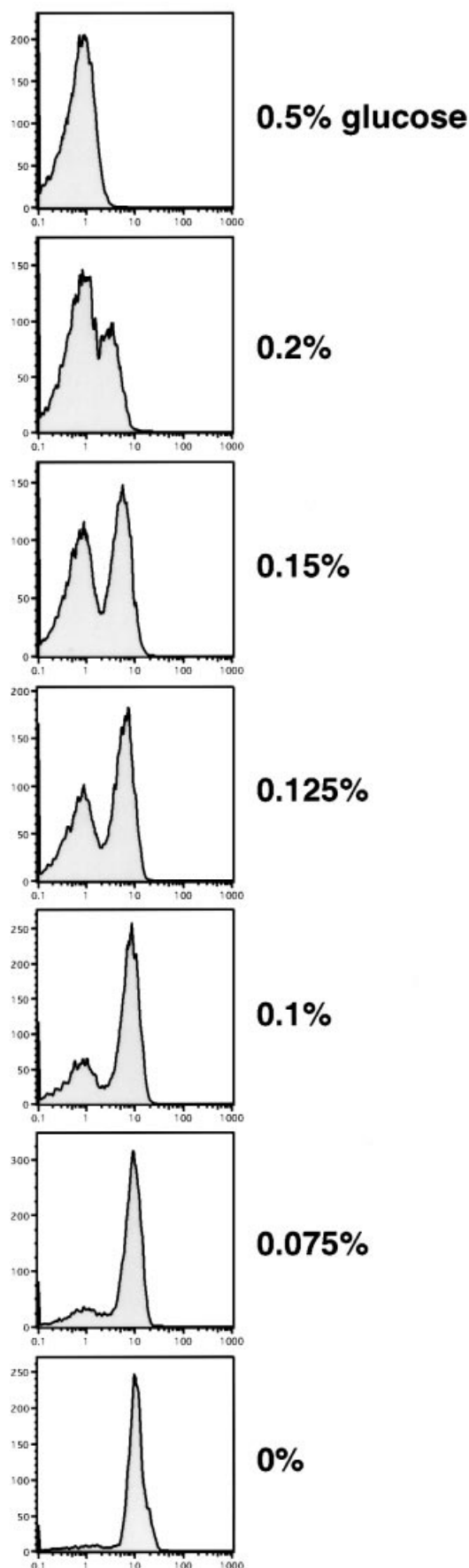
#### ***GAL80* is both necessary and sufficient for a binary transcriptional response**

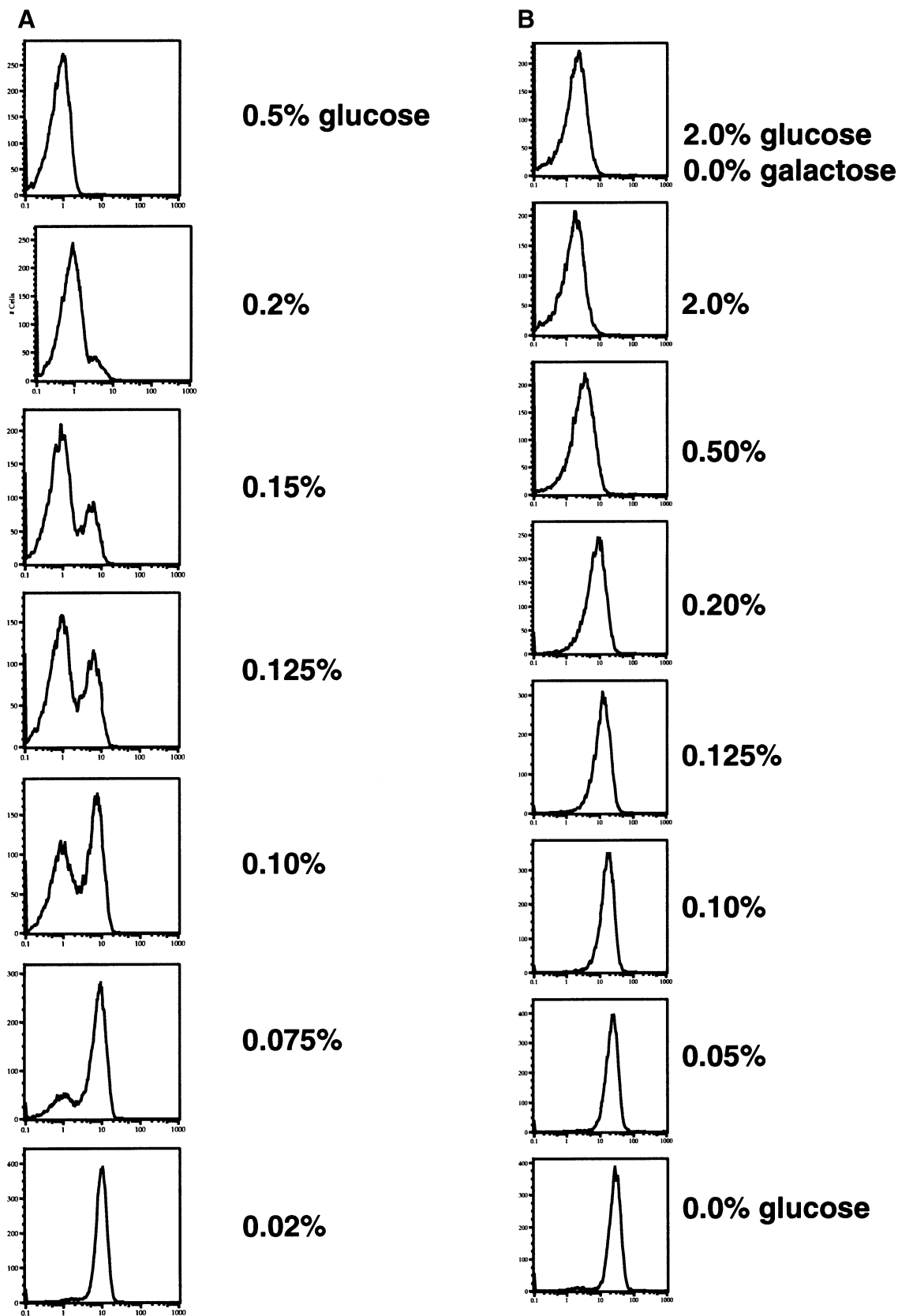
To understand further the mechanism underlying the binary character to the transcriptional response, we tested the effects of glucose under a variety of conditions. Glucose represses *GAL1* expression by three established mechanisms in wild-type cells (Figure 1B; Lamphier and Ptashne, 1992; Johnston *et al.*, 1994). As discussed, glucose activates Mig1 to directly repress transcription from the *GAL1* promoter. Activation of Mig1 by glucose also represses expression of the activator, Gal4. Lastly, glucose activates the expression and function of Gal80, which binds and inactivates Gal4. In cells lacking *MIG1*, *GAL80* assumes the major role in mediating glucose repression of *GAL1* (data not shown; Nehlin *et al.*, 1991). Thus, to test specifically the effects of *GAL80* on expression at the single-cell level, *mig1* $\Delta$  cells were grown in media containing varying concentrations of glucose and analyzed for *GAL1-GFP* expression. Similarly to wild-type cells, *mig1* $\Delta$  mutants showed a binary transcriptional response to glucose (Figure 5A), which when quantitated had a range of several hundred-fold. Thus, *GAL80* sufficed to establish an all-or-none pattern of expression from the *GAL1* promoter. To test whether *GAL80* was also required for this pattern of response, *GAL1-GFP* expression was analyzed in *gal80* $\Delta$  mutants grown in media with varying concentrations of glucose. In this case, FACS analysis showed that glucose induced graded changes in *GAL1-GFP* expression, with a single peak of GFP expression that increased in fluorescence intensity as glucose levels declined (Figure 5B). Thus, *GAL80* was both necessary and sufficient for a binary transcriptional response to glucose.

#### ***Growth conditions can dictate graded versus binary behavior of the Gal1 promoter***

In the experiments described thus far, cell numbers were grown under repressing conditions in standard media containing 2% glucose prior to exposure to experimental conditions. In a parallel study, cells were grown in non-glucose (2% raffinose) media. In this setting, all three arms of glucose repression were inactive (see Figure 1), but the

**Fig. 4.** Single-cell *GAL1-GFP* expression in response to varying glucose levels in cells carrying the entire *GAL1* regulatory network. Cells carrying the integrated *GAL1-GFP* reporter (YSB31) were grown in 2% glucose media, washed, and transferred to media containing the indicated concentrations of glucose and 2% galactose for 14 h. Cells were harvested at mid-log phase and then analyzed for GFP expression by FACS.





**Fig. 5.** Single-cell *GALI-GFP* expression in response to glucose in cells lacking *MIG1* or *GAL80*. **(A)** Binary responses to glucose in *mig1Δ* mutants. *mig1Δ* cells carrying the integrated *GALI-GFP* reporter (YSB32) were grown in 2% glucose media, washed, and transferred to media containing the indicated concentrations of glucose and 2% galactose for 14 h. Cells were harvested at mid-log phase and then analyzed for GFP expression by FACS. **(B)** Graded responses to glucose in *gal80Δ* mutants. *gal80Δ* cells carrying the integrated *GALI-GFP* reporter (YSB35) were grown in 2% glucose media, washed, and transferred to media containing the indicated concentrations of glucose and 2% galactose for 14 h. Cells were then analyzed for GFP expression by FACS.

absence of galactose prevented *GAL1-GFP* expression because Gal80 binds and inactivates Gal4 in the absence of galactose. These cells were then exposed to galactose and varying concentrations of glucose under conditions similar to those in Figure 3, and GFP expression was measured by FACS. Unlike the binary response seen when cells were grown in glucose media (Figures 3 and 6, left panels), cells grown in raffinose media responded to glucose with graded changes in gene expression (Figure 6). Thus, when pre-grown under different conditions, isogenic cells displayed distinct patterns of *GAL-GFP* expression in response to the same glucose signal.

#### **Low-density cultures were used to measure transcriptional responses**

We considered the possibility that our results would be affected by varying cell densities and degrees of glucose depletion from the media. For this reason, cells were grown logarithmically to low densities (<0.075) at the time of harvest. As a result, continued growth in culture beyond the time points presented here (14 h) produced only minor changes in GFP expression profiles (data not shown). In addition, the observation that *mig1* mutants displayed binary behavior, while *gal80* mutants displayed graded behavior under similar conditions, indicated that binary and graded behavior were not due to culture conditions.

### **Discussion**

Our data demonstrate that cells are equipped with the flexibility to generate either cell-by-cell binary changes in gene expression or population-wide graded changes. We find that specific regulation of a transcriptional activator or a repressor produced graded effects on gene expression. On the other hand, depending on growth conditions, these same transcription factors formed part of either binary or graded transcriptional responses when they functioned as parts of endogenous signaling pathways. Although the integrated glucose-mediated controls over *GAL1* promoter activity are significantly more complicated than specific regulation of individual transcription factors, our studies suggest that the regulation of *GAL80* within the glucose-response pathway imparts binary character to transcriptional responses.

At least two molecular mechanisms could explain the combination of binary and graded characteristics of promoter activity. Levels of initiation at a promoter could be pulsatile and set to function at only fully on or fully off, and graded regulation would be achieved by controlling the pulse length. Alternatively, and perhaps more likely, rates of initiation could simply vary continuously over a wide range, and binary responses depend on all-or-none changes in the functions of trans-regulatory factors. Present technical approaches do not permit these differences to be distinguished. We noted that under conditions that produced binary changes in expression, the peak of expression in GFP-positive cells shifted slightly (~3-fold) with varying glucose concentrations (Figures 4, 5A and 6). Thus, a binary response may not exclude underlying graded changes in promoter activity.

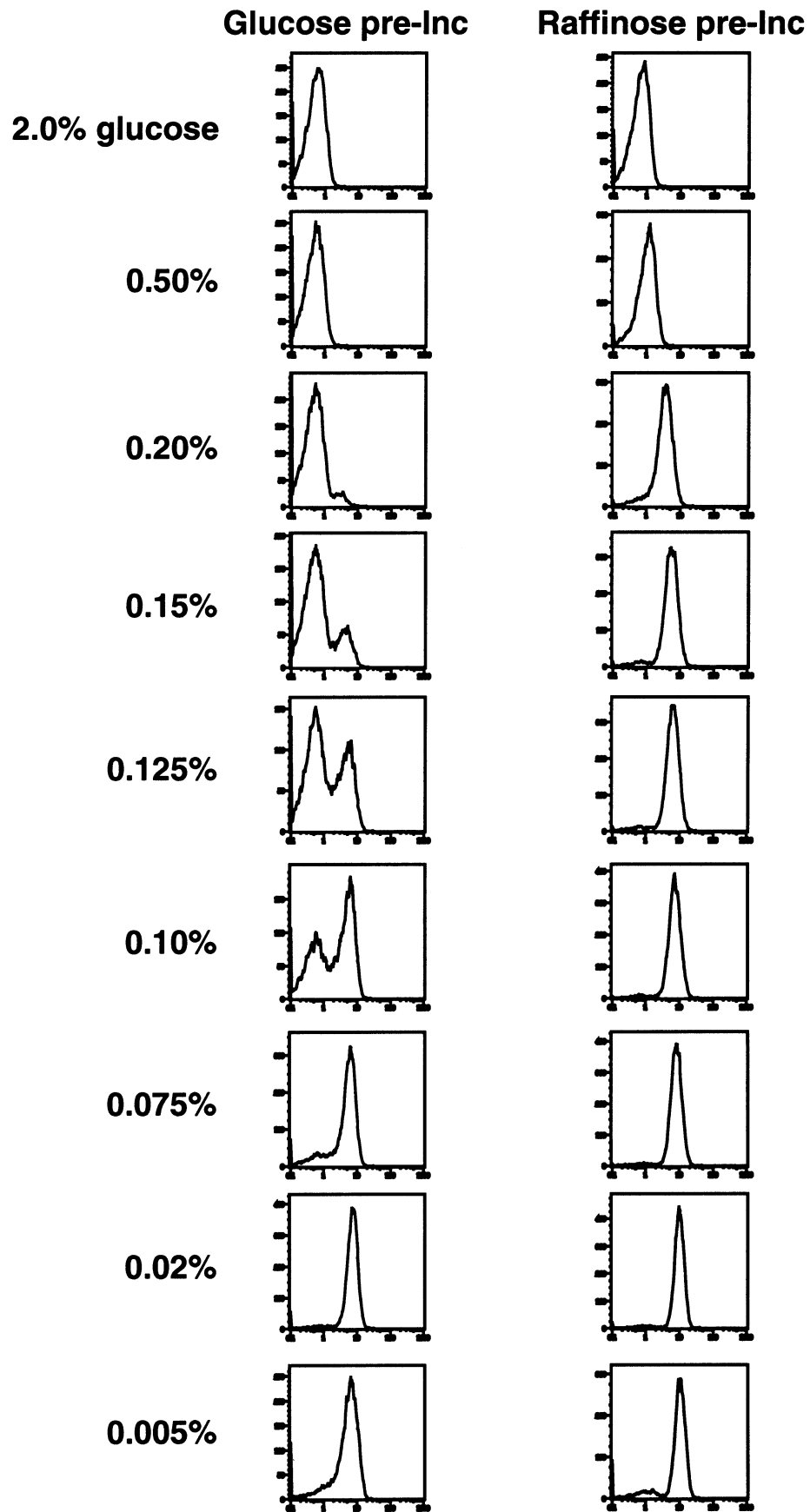
Our data indicate that different signaling mechanisms impart distinct modes of transcriptional response. Glucose-mediated repression of the *GAL1* promoter

depends almost entirely on two transcriptional repressors, *MIG1* and *GAL80* (data not shown; Nehlin *et al.*, 1991), and distinct signaling pathways control the functions of these two repressors in response to glucose (reviewed by Carlson, 1999). A balance between a kinase and a phosphatase controls Mig1 function (Jiang and Carlson, 1996). Regulation of Gal80 by glucose is less well defined, but appears to involve changes in the levels and activities of Gal80, Gal3 and Gal1 (Zenke *et al.*, 1996). Interestingly, cells lacking *GAL80* responded to glucose in a graded fashion, while cells lacking *MIG1* responded in a binary way. In separate experiments, titrating the function of Gal80 with galactose produced graded changes in expression, demonstrating that binary responses were not due to properties inherent to Gal80 itself. Instead, the results suggest that the two glucose-mediated signaling pathways controlling Mig1 and Gal80 ultimately produce either gradual changes in gene expression or all-or-none changes, respectively.

Binary changes in gene expression may result from the catalytic nature of many signaling cascades. Hence, the signal amplification that results may lead to activation of an entire cellular pool of transcription factors. Thus, only cells that initiate the signaling cascade will alter expression, and they will do so with a maximal response, thereby generating a binary pattern of transcriptional regulation. An all-or-none response to graded signaling could also arise from the positive and negative feedback mechanisms that exist in many signaling pathways. Similarly, transcriptional activators stimulate promoters in a synergistic fashion, with minimal or no expression seen when one activator binds a promoter, but with an unexpectedly high level of expression seen when several activators bind (Carey *et al.*, 1990). Even with gradual or graded regulation of transcription factor activity, this synergy among activators may lead to apparent binary responses in gene expression, since transcription will reflect the probability of the filling of independent transcription factor binding sites.

Chromatin remodeling may be an additional cause of binary transcriptional responses. Promoters bound into chromatin become unresponsive to the transcriptional machinery (Weintraub, 1985; Lorch *et al.*, 1987; Matsui, 1987; Workman and Roeder, 1987), and the change between the repressed and remodeled configurations of chromatin may serve as a switch between transcriptionally incompetent and competent states. This switch could impart a binary character to transcriptional responses involving alterations in chromatin structure, such as responses at the *GAL1* promoter (Axelrod *et al.*, 1993; Cavalli and Thoma, 1993; Lohr and Lopez, 1995). However, mutations in components of the chromatin remodeling transcription factor Swi-Snf reduced expression in entire populations of cells in a graded fashion (Sudarsanam *et al.*, 1999). Thus, the role of chromatin in determining binary responses in gene expression may be limited.

Recently, Blau and colleagues have shown that competition between an activator and a repressor can produce a binary pattern of transcription (Rossi *et al.*, 2000). They found that viral infection of cells with a TetR-VP16 fusion and a TetR can generate binary patterns of expression, while the activator alone produces graded changes in



**Fig. 6.** Single-cell *GALI-GFP* expression in response to glucose in cells expanded in non-glucose media. Cells carrying the integrated *GALI-GFP* reporter (YSB31) were grown overnight in 2% raffinose media, washed, and transferred to media containing the indicated concentrations of glucose and 2% galactose for 14 h. Cells were harvested at mid-log phase and then analyzed for GFP expression by FACS.



expression. This is thought to reflect the probability of occupancy of the single site for a repressor or activator. In our case, the repressor (Mig1) and the activator (Gal4) bind at separate sites on the Gal1 promoter. Hence, competition between activators and repressors in a population of cells is unlikely to account for the two forms of regulation that we describe and the shift between the graded and binary behavior found with growth conditions.

*GAL80* is necessary and sufficient to impart a binary response to glucose, but the aspect(s) of *GAL80* regulation responsible for binary changes in transcription remains unknown. Possibilities include all-or-none propagation of cellular signals that control Gal80 function, binary changes in expression of *GAL80* itself, and/or binary interactions between Gal80 and Gal4. Interestingly, unlike cells expanded in glucose media, cells grown in raffinose media showed graded responses to glucose signaling. Gal80 was still active in cells grown in raffinose because *GAL1-GFP* expression required galactose (data not shown). Furthermore, the differences between growing cells in glucose or raffinose did not result only from inactivation of Mig1 in raffinose, since *mig1Δ* cells showed binary responses to glucose. Thus, pre-growth in the absence of glucose appeared to alter the context in which glucose-mediated signals control Gal80 function, resulting in a switch between binary and graded modes of regulation. This difference in response patterns should provide a means of defining the component(s) of Gal80 regulation responsible for binary transcriptional responses.

In summary, analysis of the regulation of a single-copy *GAL1* promoter in individual yeast cells has identified some of the molecular components of binary and graded transcriptional control. Most surprisingly, isogenetic cells possess the flexibility to generate either binary or graded changes in gene expression in response to the same glucose signal.

## Materials and methods

### Yeast strains and growth conditions

Cells were grown in standard media at 30°C (Guthrie and Fink, 1991). YSB30 (*Mata. leu2-3,112 ura3-52 trp1-901 his3-Δ200 ade2-101 gal4Δ gal80Δ fpr1Δ::ADE2 TOR1-1 LY2::GAL→HIS3 GAL→LacZ URA3::GAL1→GFP*) was derived by transforming YSB7 (Biggar and Crabtree, 2000) with pGAL-GFP cut with *NcoI* with the *URA3* gene. YSB31 (*Mata. ura3-52 lys2-801 ade2-101 trp1-63 his3-Δ200 leu2-Δ1 URA3::GAL1→GFP*) was derived by transforming YPH499 with pGAL-GFP cut with *NcoI* with the *URA3* gene. YSB32 (*YSB31 mig1Δ::TRP1*) was derived by transforming YSB31 with pMIG1-ΔTRP1 cut with *EcoRI*. YSB35 (*Mata. ura3-52 lys2-801 ade2-101 trp1 his3-200 leu2-1 metΔ can1Δ gal80Δ538 GAL1-GFP::URA3 Gal-LacZ::LEU2*) was derived from a cross between YSB36 [YPH500 (Sikorski and Hieter, 1989) carrying pGAL-GFP] and YM3733 (Lutfiyya and Johnston, 1996).

### Plasmids

To create the *GAL-GFP* reporter, enhanced GFP was cloned as a *XhoI-EcoRI* fragment into pRS306 (Sikorski and Hieter, 1989) along with an *EcoRI-NotI* fragment containing the *ADCI* transcriptional terminator to form pYSB113. The full-length *GAL1/10* promoter was cloned as a *KpnI* fragment into pYSB113 to generate pGAL-GFP. Orientation of the *GAL1/10* promoter fragment placed GFP under the *GAL1* promoter. pMIG1-ΔTRP1 replaced +170 to +1430 of the *MIG1* open reading frame with *TRP1*.

### FACS analysis

All yeast strains were grown for at least 14 h under inducing conditions prior to FACS. Cells were sonicated briefly prior to analysis, and at least 10 000 cells were analyzed per sample. β-galactosidase levels were

measured using methyl-umbelliferol-galactoside (MUG) (Biggar and Crabtree, 1999).

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