ACS Synthetic Biology
Cite This: ACS Synth. Biol. 2019, 8, 1968–1975
Letter
pubs.acs.org/synthbio

Expanding the Dynamic Range of a Transcription Factor-Based Biosensor in *Saccharomyces cerevisiae*

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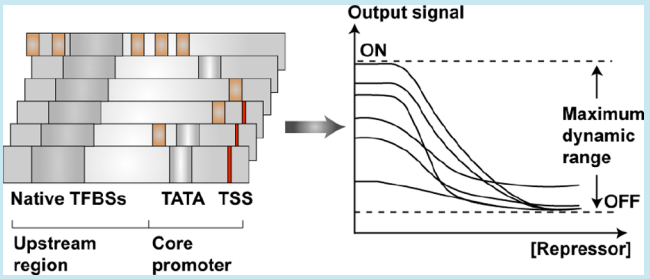
Supporting Information

ABSTRACT: Metabolite biosensors are useful tools for high-throughput screening approaches and pathway regulation approaches. An important feature of biosensors is the dynamic range. To expand the maximum dynamic range of a transcription factor-based biosensor in *Saccharomyces cerevisiae*, using the *fapO*/FapR system from *Bacillus subtilis* as an example case, five native promoters, including constitutive and glucose-regulated ones, were modified. By evaluating different binding site (BS) positions in the core promoters, we identified locations that resulted in a high maximum dynamic range with low expression under repressed conditions. We further identified BS positions in the upstream element region of the *TEF1* promoter that did not influence the native promoter strength but resulted in repression in the presence of a chimeric repressor consisting of FapR and the yeast repressor Mig1. These modified promoters with broad dynamic ranges will provide useful information for the engineering of future biosensors and their use in complex genetic circuits.



KEYWORDS: *Saccharomyces cerevisiae, promoter engineering, biosensor, maximum dynamic range, malonyl-CoA, fapO/FapR*

In the fermentation industry it is important to optimize production titer, rate, and yield of microbial cell factories.[1,2] Such optimization can be achieved by having a well-balanced metabolic system through fine-tuned gene expression levels. A hallmark of such a system is the reduction of metabolic burden, flux imbalance, and toxic intermediate accumulation.[3] One way to achieve this is through dynamic pathway regulation using metabolite biosensors,[4] as exemplified by improving production of fatty acid ethyl esters[5] and 3-hydroxypropionic acid.[6] A common strategy for such purpose centers around promoter engineering. By inserting binding sites (BSs) of a specific metabolite-responsive transcription factor (TF) into a promoter, the TF and the corresponding promoter system can be engineered into biosensors.[7]

To date, the majority of the work on biosensors has been dedicated to develop and engineer new biosensors,[8] whereas less effort has been dedicated to finding generic and transferable ways to enhance the dynamic range of current systems. A high dynamic range is desirable in applications for which biosensors are used in high-throughput screens or in circuits for dynamic pathway regulation. In high-throughput screening of cell libraries, a high dynamic range allows distinguishing the output signal from any potential background noise, thereby reducing the risk of selecting false-positive cells. In sensor circuit designs, biosensors with high dynamic range are suitable when pathways, for example, need to be strongly activated (strong ON state) or when protein expression needs to be tightly regulated (strong OFF state with minor to no leaky expression).[9] In addition, designing complex metabolic circuits may require that each subcircuit exhibits a different output level so that the overall response is balanced.[9] An improved dynamic range can be achieved through various ways, such as modifying the expression level of the TF and changing number and/or position of BSs.[10] Evaluating the number and position of the BSs is often the starting point when constructing TF-based biosensors and is therefore also a good point at which the dynamic range can be improved.[7]

In this study, we developed a set of synthetic promoters, including constitutive and glucose-regulated promoters, with varied maximum dynamic ranges using the well-characterized *fapO*/FapR system as an example in *Saccharomyces cerevisiae*. We demonstrate the importance of BS locations within the promoters and suggest different ways of altering the dynamic range. We believe that the identified BS locations are applicable also for other TF-based biosensors in *S. cerevisiae*.

Received: April 2, 2019
Published: August 2, 2019

DOI: 10.1021/acssynbio.9b00144
ACS Synth. Biol. 2019, 8, 1968–1975

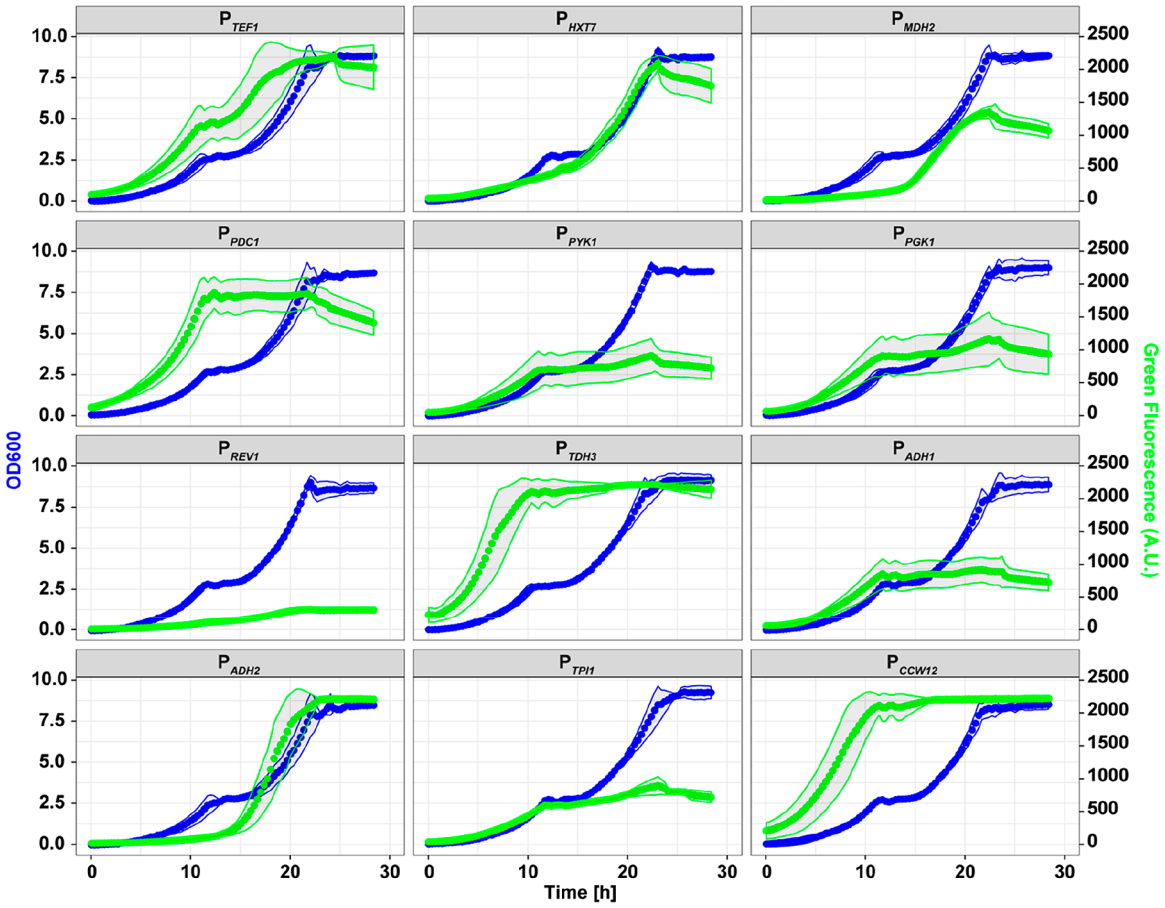


Figure 1. Promoter evaluation through real-time monitoring of growth and fluorescence signal. Constitutive and glucose-regulated *S. cerevisiae* promoters were evaluated by measuring the growth (blue graphs) and the fluorescence intensity (green graphs) employing a Biolector. GFP coupled to the respective promoter was expressed from centromeric plasmid, and the strains were cultured in minimal medium. $n = 3$ and error area = ±SD.

RESULTS AND DISCUSSION

Characterization of Native *S. cerevisiae* Promoters. In *S. cerevisiae*, promoters as the basic transcriptional regulatory elements, have been extensively characterized[11,12] but only a few of these have been used for biosensor construction. For such application, constitutive promoters are commonly used whereas glucose-regulated promoters have received less attention. Glucose-regulated promoters are useful assets in metabolic engineering applications as these can be used to, for example, separate growth and the production phase.[12] We believe this trait will also be valuable for an integration with TF-based biosensors in pathway regulation.

We sought to expand the repertoire of promoters for biosensor development and evaluated nine constitutive (P_{TEF1}, P_{TDH3}, P_{CCW12}, P_{TPI1}, P_{PGK1}, P_{REV1}, P_{PDC1}, P_{PYK1}, P_{ADH1}) and three glucose-regulated (P_{ADH2}, P_{HXT7}, P_{MDH2}) promoters for their transcription efficiency using a GFP-based reporter system expressed from a centromeric plasmid. Each promoter was coupled to a gene encoding GFP, and growth and fluorescence were monitored in real-time (Figure 1). All constitutive promoters showed varied expression strength during growth on glucose. Among these, P_{TDH3}, P_{CCW12}, and P_{PDC1} showed the highest expression levels, with P_{TDH3} and P_{CCW12} reaching the threshold level of the detector after glucose depletion (Figure 1). While P_{TEF1} fluorescence followed the growth curve, the increase of fluorescence on ethanol for P_{PYK1}, P_{PGK1}, P_{ADH1}, and P_{TPI1} was less than the OD increase. This is consistent with previous studies showing decreased promoter activity for glycolytic genes during growth on ethanol.[13] The promoter P_{REV1} showed the weakest expression both on glucose and the ethanol phase. The glucose-regulated promoters led to a negligible GFP signal during growth on glucose and a medium to strong signal after glucose consumption (Figure 1). The overall results of the promoters and their activities are consistent with previous studies.[14,15] From these, three constitutive (P_{TDH3}, P_{CCW12}, P_{TPI1}) and two glucose-regulated (P_{ADH2}, P_{MDH2}) promoters were chosen for the following engineering purposes due to their varied strengths and induction capacity.

Expanding the Maximum Dynamic Range of a TF-Based Biosensor by Evaluating the Effect of BS Positioning. Yeast promoters can be divided into two parts, the upstream region and the core promoter, which include the TFBSs and the TATA box and the transcription start site (TSS), respectively.[16] The majority of engineered TF-based biosensors in yeast are based on prokaryotic transcriptional repressors, which are commonly constructed by inserting the BS of a metabolite-responsive TF into the core promoter of a native yeast promoter.[6,17] The aim is to achieve steric hindrance of RNA polymerase, and thereby temporarily disturb transcription initiation or elongation.[7] Although structural information is available for certain promoters, important DNA elements of the core promoters are still

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ACS Synth. Biol. 2019, 8, 1968−1975

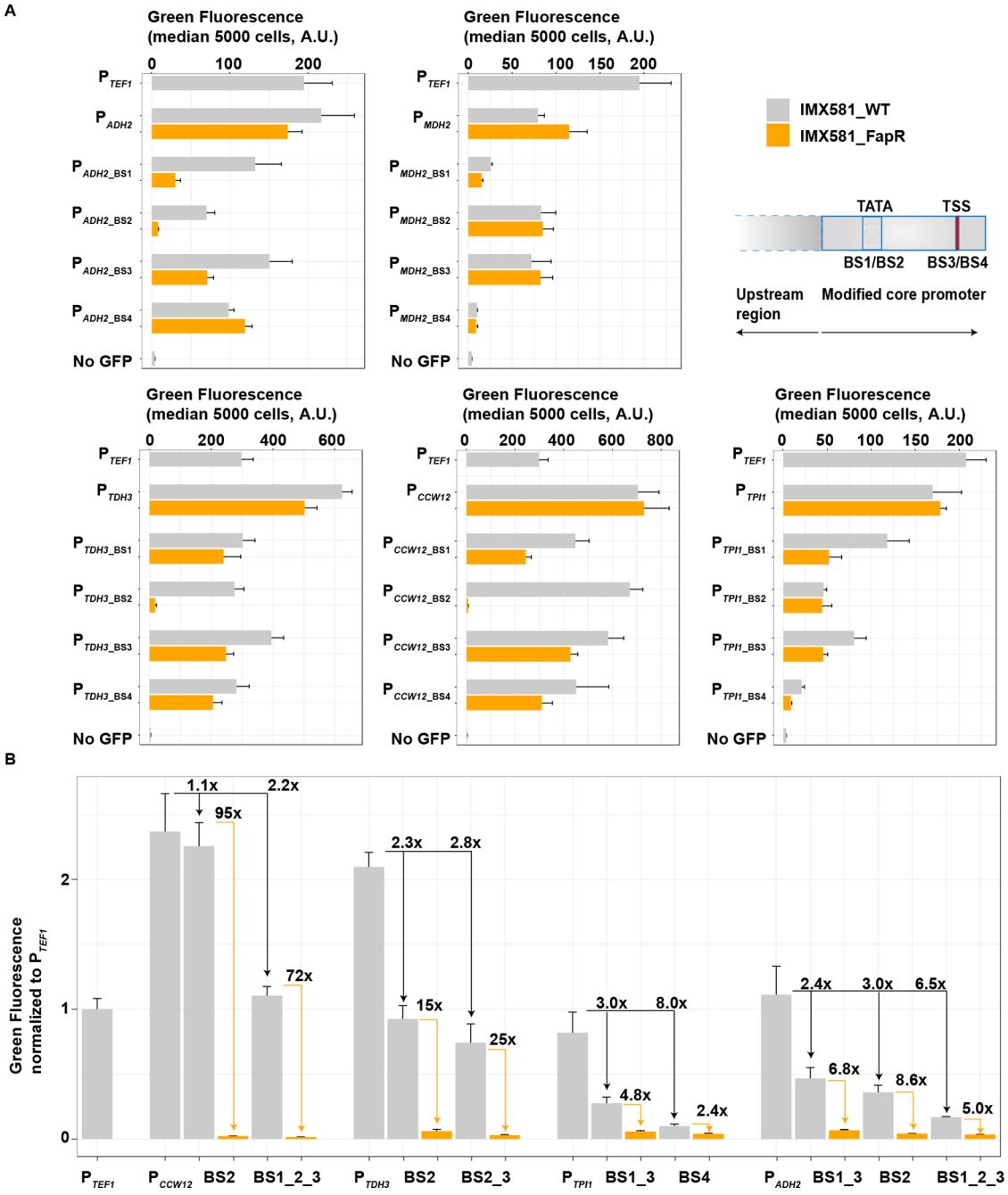

Figure 2. Evaluating the effect of positions and numbers of fapO sites in different promoters. (A) BSs were located individually within the core promoters of P_{ADH2}, P_{MDH2}, P_{TDH3}, P_{CCW12}, and P_{TPI1}, in proximity to the TATA box and the TSS. Their effect was evaluated by placing these modified promoters upstream of GFP and measuring fluorescence employing flow cytometry. (B) The combination of different BS positions was evaluated. Strains were cultured in minimal medium in shake flasks, and fluorescence was measured 8 h after inoculation for P_{TDH3}, P_{CCW12}, and P_{TPI1}, and 24 h after inoculation for P_{ADH2} and P_{MDH2}. n = 3, error bar = ±SD.

uncharacterized. This limited knowledge makes it necessary to systematically evaluate promoters and different BS locations when constructing TF-based biosensors. This is also important for BSs located downstream of the TSS as these can change the RNA secondary structure and therefore influence translation levels.

A malonyl-CoA-responsive sensor based on FapR from *Bacillus subtilis* with its corresponding 34 bp BS (*fapO*) has been widely used in mammalian cells,[18] *S. cerevisiae*,[6,17] and *E. coli*.[19,20] Therefore, the *fapO*/FapR system represents a well-characterized sensor system suitable for demonstration in this study. We first evaluated the effect of four *fapO* sites inserted individually into the core promoters. For promoters P_{TDH3}, P_{CCW12}, P_{ADH2}, and P_{MDH2}, the BSs were placed upstream and downstream of either the TATA box (BS1 and BS2, respectively) or the TSS (BS3 and BS4, respectively) (Figure 2A, and Supporting Information, Sequences). For promoter P_{TPI1}, which has an assigned TSS but no clear TATA box, BSs were placed within the range of 120 bp upstream of the start codon. Since the *TEF1* promoter is one of the most commonly used promoters in metabolic engineering and synthetic biology applications, we used it as a reference promoter. Single BSs

DOI: 10.1021/acssynbio.9b00144
ACS Synth. Biol. 2019, 8, 1968−1975

were not evaluated here as this had been done previously.[6] The promoters were placed upstream of GFP and the fluorescence signal was evaluated in the absence and in the presence of FapR (Figure 2). Since this system senses an endogenous metabolite (malonyl-CoA), we here investigated not the actual dynamic range in response to the input signal, but the maximum dynamic range, which we define here as the ratio of reporter gene expression in the absence and presence of the metabolite-responsive TF.

The evaluated modified promoters can be divided into three groups: promoters with strong repression, promoters with weak repression, and unresponsive promoters. The main biosensor parameters summarizing the quantifiable data across multiple modified promoters are listed in the Supporting Information, Table S1. For instance, BS2 in P_{TDH3}, P_{CCW12}, P_{ADH2}, placed downstream of the TATA box, and BS4 in P_{TPI1} exhibited strong repression in the presence of FapR, less than 3× higher fluorescence intensity compared to the negative control for P_{CCW12}, P_{ADH2}, P_{TPI1}, and less than 6× for P_{TDH3} (Figure 2A and Table S1). In contrast, BS1 upstream of the TATA box did not show as strong repression as BS2, especially BS1 in promoter P_{TDH3}, which led to a low maximum dynamic range (1.3×) (Figure 2A). Weak repression could also be observed in several cases as exemplified by BS3 in P_{TDH3}. In fact, it seems that BS3 and BS4, which are positioned further away from the TATA box and closer to the TSS, all showed less repression compared to BS1 and BS2, except for BS4 in P_{TPI1}. The last group represents promoters with no or minor repression in the presence of FapR, such as BS1 and BS4 in P_{TDH3}. Furthermore, all single BS insertions into promoter P_{MDH2} resulted in negligible repression when FapR was expressed. Insertion of BSs in position 1 and 4 of P_{MDH2} resulted in weak promoter activity, indicating the possibility that these sites are crucial for initiation of transcription or that the architecture of the promoter was severely influenced upon insertion at these positions (Figure 2A). Because of these results, P_{MDH2} was not considered for further engineering purposes. When considering the maximum dynamic range, besides BS2 in promoter P_{ADH2}, P_{CCW12}, and P_{TDH3}, the effect of the other positions did not reach a good consensus when compared to a similar location in other promoters (Figure 2A and Table S1). This indicates that the location effect is promoter-dependent and that similar positions within different promoters might not result in the same output range. On the other hand, using the same BS position for different TFs can lead to similar results. In fact, insertion of a 17 bp FadR BS sequence in the same positions as the 34 bp *fapO* sites in P_{TEF1}, demonstrated in another study,[6] resulted in a similar trend.[21] In addition, it has previously been reported that a highly efficient xylose-sensing/regulation gene circuit was constructed through modifying promoter P_{GPM1}[22] at the same positions used previously for a malonyl-CoA sensor system.[17] In conclusion, the evaluation of BS locations in this work will likely provide useful information for construction of sensors using the same promoters but with different repressor systems.

Tight regulation is often a crucial trait of a sensor, especially when employing it in a synthetic circuit. To obtain a high dynamic range while preventing leaky expression, which we define here as reporter gene expression under full repression compared to a negative control, it is common to combine several BSs.[6,17] Here, BSs at different positions in P_{TDH3}, P_{CCW12}, and P_{TPI1} were combined in order to create tightly regulated promoters. These promoters exhibited different levels of maximum dynamic range, ranging from 95-fold to 2.4-fold for P_{CCW12} and P_{TPI1}, respectively (Figure 2B). Within this interval, the modified promoters exhibited different strengths and output ranges while at the same time displaying minor leaky expression in the presence of FapR. For instance, despite reducing the basal activity of P_{CCW12}_BS2 by almost 50% after additional introduction of BS1 and BS3, the maximum range in the presence of FapR was 72-fold. For P_{TDH3}, the combination of BSs 2 and 3 only reduced the strength of the promoter with 20% compared to the one having only BS2, whereas the maximum dynamic range was improved 1.6-fold. For P_{ADH2}, BS1, BS2, and BS3 were combined, resulting in promoters with three different maximum dynamic range levels in the ethanol phase (Figure 2B). Having a set of modified promoters with different strengths and maximum dynamic ranges at hand is beneficial for several applications, such as in circuit design that might involve several modified promoters that need to exhibit different strengths and yet maintain their regulatory function.

Expanding the Maximum Dynamic Range while Still Retaining the Native Strength of a Promoter. As it is often desirable to retain the native strength of a promoter, we decided to address the, often observed, phenomenon of losing promoter strength upon insertion of BSs in the core promoter by moving the BSs to the upstream region. Prokaryotic transcriptional repressors are believed to mainly function in yeast by sterically hindering RNA polymerase from initiating transcription. Moving the BSs to the upstream region would not allow such steric hindrance to occur. Unlike prokaryotic transcriptional repressors, native yeast transcriptional repressors typically function by recruiting additional repressor complexes to promoters where they directly interact with RNA polymerase or the coactivator complex termed Mediator.[23] This operating mode is different from steric hindrance, which reduces the dependency on positioning the BSs in the core promoter.

To address the limitation of BS positioning, we sought to simulate the yeast native system through fusing FapR with a yeast native repressor. If the chimeric repressor could inhibit transcription while retaining the ability of sensing intracellular malonyl-CoA levels, the localization of the operator would be less restricted. To test this, we used P_{TEF1} as an example case since this is one of the most commonly used promoters in *S. cerevisiae*. Furthermore, it does not contain known BSs for any native repressor. Six FapR BSs were evaluated: BS1 placed outside the defined 405 bp P_{TEF1}, BS2 and BS3 close to the upper activating sequence (UAS), and BS4−BS6, which are closer to the core promoter (Figure 3A and Supporting Information, Sequences).

In yeast, several TFs repress transcription by recruiting the Tup1−Ssn6 complex.[23] Mig1 and Rox1 are two representative TFs involved in repression of glucose-regulated and hypoxic genes, respectively.[24,25] In this study, these repressors were fused at their N-terminus with the C-terminus of FapR to create a chimeric repressor. The modified P_{TEF1} variants were placed upstream of GFP and analyzed in strains without and with expression of FapR-Mig1. From these BSs, insertion of BS2 resulted in complete loss of activity, probably due to interference with the binding of transcriptional activation proteins to the UAS (Figure 3B). BS1 did not negatively influence the activity of the promoter, and repression occurred upon expression of FapR-Mig1. Similar trends could be observed for BS3 and BS4. BS5 and BS6 resulted, however,

DOI: 10.1021/acssynbio.9b00144
ACS Synth. Biol. 2019, 8, 1968−1975

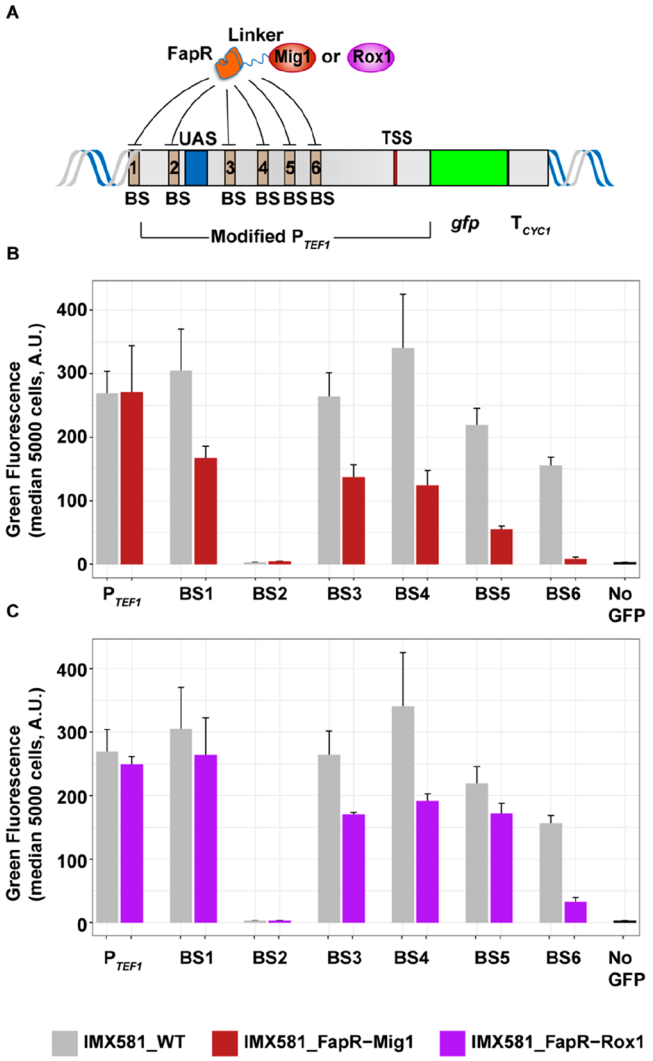

Figure 3. Evaluating the effect of chimeric TFs at different fapO site locations in P_{TEF1}. (A) Schematic design of the BS locations and the chimeric TFs. (B) Evaluation of the effect of the BSs located outside (BS1) of the 405 bp P_{TEF1}, and the BSs within the promoter (BS2−BS6) in a FapR-Mig1 expressing strain and (C) in a FapR-Rox1 expressing strain by measuring the fluorescence intensity. Strains were grown in minimal medium in shake flasks and fluorescence was measured 8 h after inoculation. n = 3, error bar = ± SD.

in decreased promoter activity as well as increased repression, which occurs because these BSs are located closer to the core promoter, thereby creating steric hindrance (Figure 3B). The same BS locations were evaluated in a FapR-Rox1 expressing strain. Fusion with Rox1 showed a lower degree of repression (Figure 3C) compared to the strain carrying FapR-Mig1 (Figure 3B). Therefore, we decided to work further with the Mig1-based TF variant.

As BS1, BS3, and BS4 did not reduce the native strength of P_{TEF1} (Figure 3), we decided to evaluate their combination. Furthermore, we compared these positions with the three BSs located further downstream, as constructed previously[6] (Figure 4A). Combination of the three BSs in the upstream region, which we refer to as BSs_UP, did not reduce the basal strength of P_{TEF1}, whereas insertion of the BSs in the downstream region, which we refer to as BS_DW, decreased the activity of the promoter (Figure 4B,C). Expression of these constructs in both FapR and FapR-Mig1 expressing strains decreased the transcription level, with FapR-Mig1 resulting in the strongest repression in both cases (Figure 4B,C). In conclusion, comparison of the FapR and FapR-Mig1 expressing strains indicates the formation of a functional chimeric repressor of FapR with Mig1 as the repression is more than 5× stronger for the FapR-Mig1 construct than the construct only expressing FapR (Figure 4B).

To validate that the responsiveness of FapR to malonyl-CoA is not damaged upon fusion with Mig1, cerulenin was added to exponentially growing strains 3 h after inoculation to block fatty acid synthesis, thereby increasing intracellular malonyl-CoA levels.[26] Exposure to cerulenin using different concentrations has previously been evaluated at different time-points.[6] Here, fluorescence measurement was performed after an additional 3 h of cultivation. The FapR expressing strain carrying P_{TEF1} with BSs_DW was used for comparison (Figure 5). The addition of cerulenin resulted in increased GFP signal in both FapR and FapR-Mig1 expressing strains, indicating that the sensor system based on FapR-Mig1 retains its responsiveness to the intracellular malonyl-CoA level.

CONCLUSIONS

Engineering of the *fapO*/FapR sensor system in this study provides useful information for designing other TF-based biosensors in *S. cerevisiae* with applications in metabolic engineering and synthetic biology. For example, a low "maximum output" can be used to control proteins that may cause a metabolic burden or toxicity above a certain threshold. The modified promoters with high maximum dynamic range can be advantageous for high-throughput screenings of strain libraries. The promoters with multiple output levels will be useful for the design of complex genetic circuits. In such cases, both constitutive and glucose-regulated promoters are valuable and for each kind we identified suitable BS positions leading to varied output ranges. Besides the conventional construction model, we also demonstrated that the coupling of a heterologous TF with a yeast native repressor can further increase the maximum dynamic range. Although this is based on FapR, we believe that the principles and BS positions are applicable for other prokaryotic transcriptional repressors implemented in *S. cerevisiae*.

MATERIALS AND METHODS

Chemicals and Reagents. All oligonucleotide primers were synthesized at Eurofins. DNA gel extraction- and plasmid purification kits were purchased from Thermo Fischer Scientific. The Gibson Assembly Master mix was purchased from New England Biolabs. Phusion High-Fidelity DNA Polymerase (ThermoFisher Scientific) was routinely used for PCR amplification. All reagents used for media preparation were purchased from Merck Millipore unless otherwise noted. Cerulenin (≥98%) was purchased from Sigma-Aldrich.

Strains, Media, and Culture Conditions. *S. cerevisiae* strain IMX581 (*MATa ura3−52 can1Δ::cas9-natNT2 TRP1 LEU2 HIS3*)[27] was used as the background strain and competent *Escherichia coli* cells, DH5α, were routinely used for standard cloning procedures. *S. cerevisiae* strains and *E. coli* cells were cultured at standard conditions: 30 and 37 °C, respectively, at 200 rpm.

YPD medium containing 10 g/L yeast extract, 20 g/L casein peptone, and 20 g/L glucose was used when preparing yeast competent cells. All yeast strains were transformed according

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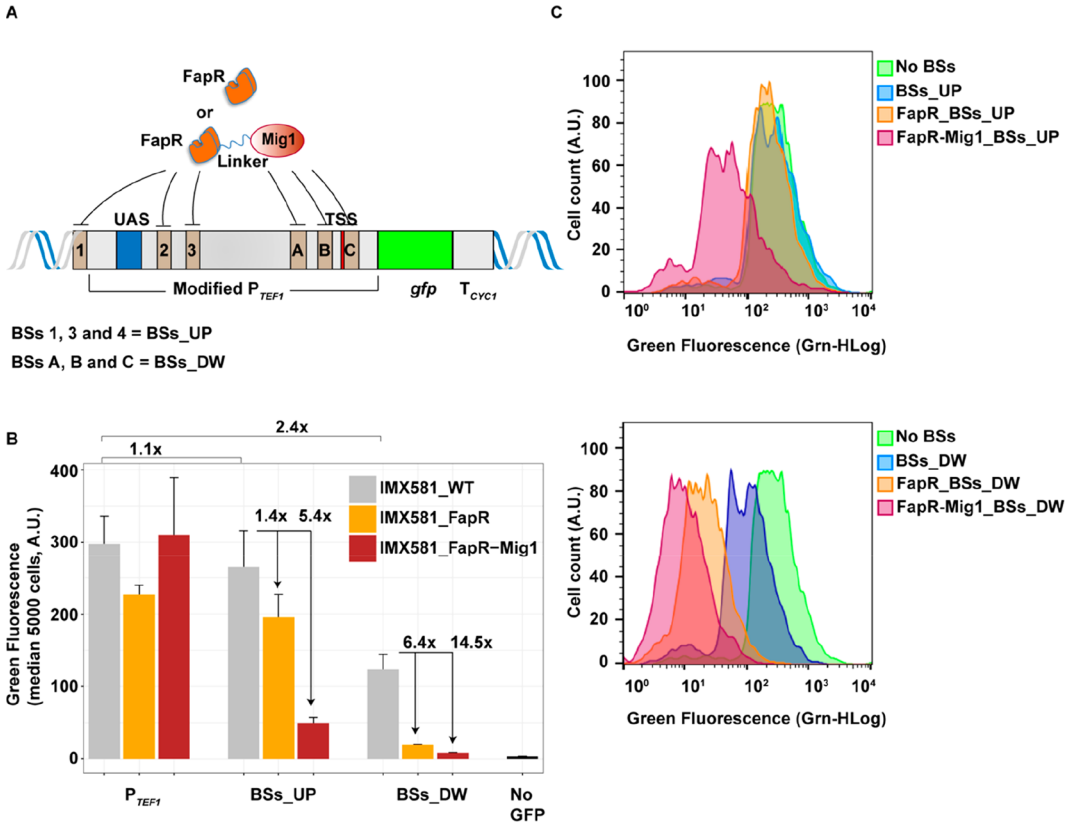

Figure 4. Comparison of two different inhibition modes in P_{TEF1}. (A) Schematic design of the BSs inserted in the upstream and downstream regions. (B) The effect of the BS location was evaluated in FapR and FapR-Mig1 expressing strains as well as in the control strain IMX581 by measuring fluorescence intensity. (C) Histograms of IMX581 carrying plasmid p416TEF-GFP (green), IMX581 (blue), FapR integrated strain (orange), and FapR-Mig1 (purple) carrying a plasmid with GFP under control of the modified *TEF1* promoter. All samples were grown in minimal medium in shake flasks, and the fluorescence was measured 8 h after inoculation. n = 3, error bar = ±SD.

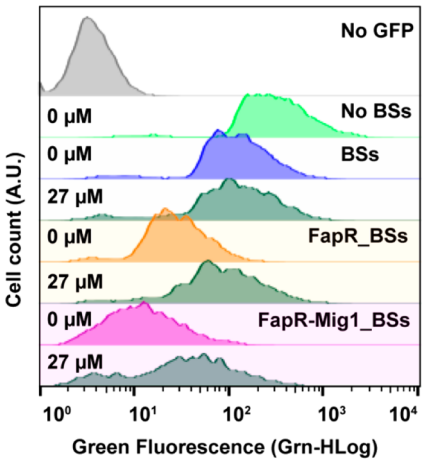

Figure 5. Cerulenin feeding to evaluate FapR-Mig1 responsiveness. To evaluate the responsiveness of FapR-Mig1 to malonyl-CoA, 27 μM cerulenin was added to exponentially growing cells 3 h after inoculation at OD 0.1, and the fluorescence was measured after an additional 3 h of cultivation since exposure. The BSs are the three combined fapO sites in the core promoter, BSs_DW. A strain carrying FapR was used for comparison.

to the lithium acetate method.[28] For selection of yeast transformants, synthetic complete (SC) medium plates containing 6.7 g/L yeast nitrogen base without amino acids, 0.77 g/L complete supplement mixture without uracil (CSM-URA, Formedium), 20 g/L agar, and 20 g/L glucose were used. SC plates supplemented with 0.8 g/L 5-fluoroorotic acid (5-FOA) were routinely used to select against the *URA3*-based gRNA-plasmid. For evaluation of all promoters, yeast strains were cultured in minimal medium[29] containing 7.5 g/L $(NH_4)_2SO_4$, 14.4 g/L KH_2PO_4, 0.5 g/L $MgSO_4{\cdot}7H_2O$, 2% glucose, 2 mL/L trace metals, and 1 mL/L vitamin solutions.[29] The initial pH was adjusted to 6.5 with KOH. For culturing *E. coli* cells, lysogeny broth (LB) supplemented with 100 mg/L ampicillin was used.

Plasmid and Strain Construction. All primers, plasmids and strains used in this study are listed in Tables S2–S4. Plasmid p416TEF-GFP[6] was previously constructed and was used as the backbone plasmid for all plasmid constructions used in this study. Primers pSensor-F and pSensor-R were used for amplifying the backbone such that the P_{TEF1} was removed. The backbone was subsequently assembled with the promoter of choice. All promoters were amplified from the genomic DNA of strain IMX581 using their corresponding primers (Table S2), and assembled with the backbone plasmid using the Gibson Assembly Master mix. Similarly, the synthetically modified promoters with the BSs integrated were constructed using their corresponding primers (Table S2). All plasmids were verified through sequencing at Eurofins.

For expression of the transcription factor variants, strains were engineered through insertion of cassettes at integration site X-3[30] in strain IMX581. The overlapping homologous regions were amplified using primers HR_UP-F and HR_UP-R for the upstream region and HR_DW-F and HR_DW-R for the downstream region. The fragment containing P_{TEF1} and the

DOI: 10.1021/acssynbio.9b00144
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gene encoding FapR with an NLS sequence at its N-terminus was amplified from pFDA9,[6] using primers pTEF1-F and pFapR-R1. The terminator T_{ADH1} was amplified from genomic DNA using primers tADH1-F and tADH1-R. The cassette containing HR_UP-P_{TEF1}-NLS-*fapR*-T_{ADH1}-HR_DW was constructed from the amplified fragments using a two-step fusion PCR protocol.[31] Integration of this cassette resulted in strain Y&X01. Strain Y&X02 was similarly constructed by integrating the cassette HR_UP-P_{TEF1}-*fapR*-L-*MIG1*-T_{ADH1}_HR_DW. Here, *fapR* was amplified using primers pTEF1-F and pFapR-R2 and assembled with *MIG1*, which was amplified using primers pMig1-F and pMig1-R such that a linker, L, (GSGSGS)[32] was introduced at the N-terminus of the gene encoding Mig1 in order to create a fusion between FapR and Mig1. Strain Y&X03 was constructed by integrating cassette HR_UP-P_{TEF1}-*fapR*-L-*ROX1*-T_{ADH1}-HR_DW, where *fapR* was amplified using primers pTEF1-F and pFapR-R3, and *ROXI* was amplified using primers pRox1-F and pRox1-R. All cassettes were verified for correct integration through colony PCR using primers pCPCR-F and pCPCR-R, and these were subsequently sequenced at Eurofins. Transformation of plasmids resulted in strains Y&X04−Y&X62.

Fluorescence Measurements. For evaluating promoter activity throughout the growth phase, strains Y&X05−Y&X16 carrying the different promoters coupled to GFP were analyzed in a 48-well FlowerPlate at 800 rpm, employing a Biolector (m2p-laboratories GmbH). Cultures were started, from precultures grown for 24 h, at an OD_{600} of 0.1 in 1 mL minimal of medium. For measuring the biomass, excitation and emission at 600 nm was used as well as a gain of 20. For measuring the GFP signal, excitation at 488 nm and emission at 520 nm was used as well as a gain of 100.

Strains were analyzed employing flow cytometry, a Guava easyCyte 8HT system (Merck Millipore). Cultures were inoculated from precultures at an OD_{600} of 0.1 in 10 mL minimal of medium in 100 mL shake flasks. All samples, except strains with P_{ADH2} and P_{MDH2} modifications, were analyzed after 8 h, during the glucose exponential growth phase, whereas the remaining samples were analyzed after 24 h, during the ethanol respiration phase. All samples were, prior to analysis, diluted to an OD_{600} of 0.02 in 200 μL of water. FlowJoX software was used to calculate the median values of 5000 cells and to create the histogram images.

For evaluating whether Mig1 influences the responsiveness of FapR to malonyl-CoA, exponentially growing cells, 3 h after inoculation, were exposed to 27 μM cerulenin, and the samples were analyzed after another 3 h of cultivation. The cerulenin stock of 13.5 mM was prepared in 96% ethanol.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssynbio.9b00144.

Table summarizing data of some main biosensor parameters, oligonucleotide primers, plasmids, and strains used in this study (Table S1−S4); promoter sequences including BS positions (PDF)

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Author Contributions

Y.D. and X.L. designed the research; Y.D. and X.L. performed the experiments; Y.D. and X.L. analyzed the results. All authors discussed the results; Y.D., X.L., and V.S. prepared the manuscript.

Author Contributions

#These authors contributed equally

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was financially supported by The Novo Nordisk Foundation (Grant No. NNF10CC1016517) and Ångpanneföreningen's Foundation for Research and Development. The authors would also like to acknowledge Kate Campbell, Lucy Fang-I Chao, and Quanli Liu for fruitful discussions. We are also thankful to Quanli Liu for providing the gRNA plasmid pQC005.

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