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Multiplexed CRISPR Activation of Cryptic Sugar Metabolism Enables *Yarrowia Lipolytica* Growth on Cellobiose

*Cory Schwartz, Nicholas Curtis, Ann-Kathrin Löbs, and Ian Wheeldon**

The yeast *Yarrowia lipolytica* has been widely studied for its ability to synthesize and accumulate intracellular lipids to high levels. Recent studies have identified native genes that enable growth on biomass-derived sugars, but these genes are not sufficiently expressed to facilitate robust metabolism. In this work, a CRISPR-dCas9 activation (CRISPRa) system in *Y. lipolytica* is developed and is used it to activate native β-glucosidase expression to support growth on cellobiose. A series of different transcriptional activators are compared for their effectiveness in *Y. lipolytica*, with the synthetic tripartite activator VPR yielding the highest activation. A VPR-dCas9 fusion is then targeted to various locations in a synthetic promoter driving hrGFP expression, and activation is achieved. Subsequently, the CRISPRa system is used to activate transcription of two different native β-glucosidase genes, facilitating enhanced growth on cellobiose as the sole carbon source. This work expands the synthetic biology toolbox for metabolic engineering in *Y. lipolytica* and demonstrates how the programmability of the CRISPR-Cas9 system can enable facile investigation of transcriptionally silent regions of the genome.

1. Introduction

The dimorphic yeast *Yarrowia lipolytica* has been widely studied due to its native ability to synthesize and accumulate lipids, lipid-derived products, and organic acids.[1,2] Over the past several years, a number of studies have achieved success in engineering enhanced lipid production and accumulation.[3–5] Metabolic engineering efforts have also been successful in producing higher value oleochemicals, including fatty alcohols, omega-3 fatty acids, and alkanes.[6,7] Recent studies have expanded the product range to include terpenes, such as limonene, lycopene, and other carotenoids.[8–10] *Y. lipolytica* is an attractive host for the biosynthesis of these products because of its lipogenic metabolism, which is well-suited for synthesizing molecules derived from acetyl-CoA.[11,12]

As a bioprocessing host, *Y. lipolytica* also benefits from its capacity to metabolize a range of different substrates. Native strains have been shown to use glucose, glycerol, alkanes, and a range of different lipid molecules as a carbon source.[13] Despite this, commonly used laboratory strains tend to grow poorly, or not at all, on biomass-derived sugars such as xylose, cellobiose, and galactose.[14–19] To increase the utility of *Y. lipolytica* as an industrial host, several studies have attempted to expand the substrate range to include biomass-derived sugars. Collectively, these studies have found that many of the sugar metabolizing genes are present in the genome, but that their expression is too low to support growth. Several of these works have also shown that overexpressing the cryptic genes confers growth on the target substrate.[14,16,17,19]

The advent and adaptation of the CRISPR-Cas9 system from *Streptococcus pyogenes* for genome editing in eukaryotes has revolutionized strain engineering and enabled the programmable control of gene expression.[20–22] Our group and others have adapted this system for use in *Y. lipolytica*.[23,24] Targeted genetic disruptions and markerless gene integrations are possible with an endonuclease active CRISPR-Cas9 system,[25] while transcriptional knockdowns have been demonstrated with CRISPR interference (CRISPRi).[26] The synthetic biology tools available for engineering *Y. lipolytica* also include well-characterized hybrid and native promoters with constitutive and inducible expression.[27–31]

The primary goal of this work was to design and validate a CRISPR activation (CRISPRa) system for *Y. lipolytica*. We demonstrate and explore its practical utility by upregulating cryptic cellobiose degrading genes that enable growth on the biomass-derived sugar without the need for cloning over-expression cassettes or the expression of heterologous enzymes.[16] We first screened a series of heterologous transcriptional activation domains for use in *Y. lipolytica*, the most successful of which was fused to dCas9 to create a functional CRISPRa system. Validation of the system using a synthetic *S. cerevisiae* GAL1 expression cassette driving hrGFP expression demonstrated positional targeting effects upstream

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of the coding region. Finally, the CRISPRa system was used to activate two extracellular β-glucosidases (singularly and multiplexed) to enable growth with cellobiose as the sole carbon source.

2. Experimental Section

2.1. Strains and Transformation

The *Escherichia coli* strain DH5α was used for all plasmid construction and propagation, and was cultured in Lysogeny broth with 100 mg L^{-1} ampicillin. Plasmids were isolated from *E. coli* cultures using the Zymo Research Plasmid Miniprep Kit (Irvine, CA). The PO1f strain of *Y. lipolytica* (MatA, leu2-270, ura3-302,xpr2-322, axp-2) was used and grown in YPD medium (2% Bacto peptone, 1% Bacto yeast extract, 2% glucose) or on YPD agar plates (2% agar). Strains transformed with a vector were grown in synthetic defined medium without leucine (SD-leu; 0.069% CSM-leu [Sunrise Science, San Diego, CA], 0.67% Difco yeast nitrogen base without amino acids, and 2% glucose) or on SD-leu agar plates (2% agar). Dual plasmid transformations used synthetic defined media without leucine and uracil (SD-leu-ura; 0.067% CSM-leu-ura [Sunrise Science], 0.67% Difco yeast nitrogen base without amino acids, and 2% glucose). Cellobiose growth experiments used synthetic defined media with cellobiose and without leucine (SC-leu; 0.069% CSM-leu [Sunrise Science], 0.67% Difco yeast nitrogen base without amino acids, and 2% cellobiose).

Transformations were performed using a previously described protocol.[23] Integration of the GAL1-TEF-hrGFP construct into the XPR2 site of the *Y. lipolytica* genome was done using the plasmids pCRISPRyl_XPR2 and pIW709 (Table S1, Supporting Information) as previously described using a 6-day protocol to generate the strain PO1f GAL1-TEF-hrGFP::XPR2.[25] *Y. lipolytica* genomic DNA from the PO1f strain and *S. cerevisiae* genomic DNA from the S288C derived strain BY4742 was isolated using the Zymo Research Yeastar Genomic DNA Kit.

2.2. Culture Conditions

All *Y. lipolytica* cultures were grown at 30 °C, with liquid cultures shaken at 225 RPM. Cultures were grown in 2 mL of liquid media in 14 mL Falcon tubes unless otherwise noted. Cellobiose sgRNA screening growth experiments were done by preculturing transformants in 2 mL of SD-leu for 2 days, and then inoculating 2 mL of SC-leu to an optical density (OD_{600}) of approximately 0.1 and growing for 2 days. Extended cellobiose growth experiments were done in 40 mL of SC-leu in 250 mL baffled flasks, and 1.5 mL samples were taken each day for analysis.

2.3. Cloning

All restriction enzymes, Gibson assembly master mix, and PCR components were purchased from New England Biolabs. The high fidelity DNA Polymerase Q5 was used for all amplifications. DNA purification was done using the Zymo Research Clean and Concentrator-5 and the Zymo Research Zymoclean Gel DNA Recovery kits. All primers (Table S2, Supporting Information) and gBlocks (Table S3, Supporting Information) were ordered from Integrated DNA Technologies (Coralville, IA).

To generate the GAL1-TEF-hrGFP integration plasmid pIW709 (Table S1, Supporting Information), pHR_XPR2_hrGFP was digested with SpeI and BssHII. The GAL1 UAS was amplified from *S. cerevisiae* isolated genomic DNA using primers Cr_863 and Cr_864, and the TEF core promoter amplified from *Y. lipolytica* genomic DNA using Cr860 and Cr862. The resulting fragments were purified and cloned into the digested backbone using Gibson Assembly. Plasmids to express activation domain-GAL4_DBD fusion proteins were generated by first digesting pIW209 with BssHII and NheI.[10] gBlocks consisting of overhangs, the SV40 nuclear localization tag, and the activation domain of interest were combined with a gBlock containing the GAL4_DBD and overhangs were cloned into the digested backbone using Gibson Assembly.

The pCRISPRa_VPR_yl plasmid (Addgene, Cambridge, MA, #107677) was generated by first digesting the pCRISPRi_Mxi1_yl plasmid with BssHII and NheI. A dCas9 fragment was then generated by subjecting pCRISPRi_Mxi1_yl to PCR with Cr_1250 and Cr_1251, and a VPR fragment was made by subjecting SV40-VPR to amplification with Cr_1252 and Cr_1253. The resulting fragments were cloned into the digested backbone with Gibson Assembly. All subsequent CRISPRa plasmids were generated by digesting pCRISPRa_VPR_yl with AvrII and using Gibson Assembly to insert two annealed oligonucleotides (such as Cr_1625 and Cr_1626). The multiplexed plasmid activating both BGL1 and BGL2 (pCRISPRa_VPR_yl_BGL1&2) was generated by digesting pCRISPRa_VPR_yl_BGL1-1 with XmaI and inserting the BGL2-1 sgRNA expression cassette after amplification using Cr_272 and Cr_273. All sgRNAs were designed based on location in the promoter of interest and were not based on any sgRNA selection software or algorithm.

2.4. RT-qPCR

Cultures were grown to late exponential phase, and RNA was isolated using the Zymo Research Yeastar RNA kit. The RNA was treated with DNaseI, and cDNA was produced using iScript Reverse Transcription Supermix from Biorad (Hercules, CA). Produced cDNA was diluted tenfold, and qPCR was performed using SsoAdvanced Universal SYBR Green Supermix from Biorad in a Biorad CFX Connect Thermocycler. The relative amount of cDNA was determined as described previously using the primers shown in Table S2, Supporting Information.[26]

2.5. Flow Cytometry

Cultures for analysis of fluorescence were grown to late exponential phase and subjected to flow cytometry. A Biorad S3e was used for all measurements and analysis. For each sample, 15 000 single cell events were collected, and identical

gates were used on all samples based on forward and side scatter. Fluorescence measurements were taken using the GFP channel.

2.6. HPLC Sugar Analysis

For cellobiose analysis, samples were spun down and filtered using a 0.2 µm syringe filter. 10 µL of the filtrate was analyzed using a Shimadzu High Pressure Liquid Chromatography (HPLC) 2040 system equipped with a Phenomenex Rezex ROA-Organic Acid column (300 × 7.8 mm) and a Shimadzu refractive index (RI) detector. The column was heated to 70 °C and analysis was performed using an eluent of 2.5 mM sulfuric acid at a flow rate of 0.8 mL min^{-1}. Peaks were analyzed using the LabSolutions Postrun software (Shimadzu).

3. Results

3.1. Design of a GAL1-TEF Synthetic Hybrid Promoter

A core component of CRISPRa systems is the fusion of a transcriptional activation domain to dCas9. To identify such a domain for use in *Y. lipolytica*, we constructed a synthetic hybrid promoter using the GAL1 UAS from *S. cerevisiae*, its cognate binding domain from Gal4p (Gal4_BD), and the TEF core promoter native to *Y. lipolytica* (see Ref. [31]). The GAL4 system was selected because it has no close homolog in the *Y. lipolytica* genome, while the TEF core promoter was selected because it is well-characterized and has minimal expression in the absence of a UAS.[29,32] The core promoter is a necessary but not sufficient element for RNA polymerase binding and transcription. The GAL UAS provides a heterologous sequence upstream of the core promoter to which synthetic transcription factors can be targeted. A strain containing the GAL1-TEF synthetic promoter driving hrGFP expression was then created via CRISPR-mediated gene integration (**Figure 1**A; *Y. lipolytica* PO1f GAL1-TEF-hrGFP::XPR2). The orthogonality of the GAL1 UAS system to the native *Y. lipolytica* transcriptional machinery was confirmed by flow cytometry, which revealed no significant difference between the mean fluorescence of PO1f GAL1-TEF-hrGFP::XPR2 and un-engineered PO1f (Figure S1, Supporting Information).

3.2. The VPR Transcriptional Activation Domain Drives High Expression in *Y. Lipolytica*

To characterize various activation domains, we designed synthetic transcription factors for the hybrid GAL1-TEF promoter. Each transcription factor followed the general design of "SV40-activation domain-GAL4_BD," with SV40 used to ensure nuclear localization and GAL4_BD for UAS binding. In each case, the designed fusion protein was expressed from an episomal plasmid using the UAS1B8-TEF(136) promoter described by Blazeck et al.[31]

A total of four different activation domains were characterized: the GAL4 activation domain from *S. cerevisiae*; the viral activators VP16 and VP64; and, the synthetic activator VPR. VP16 is a protein

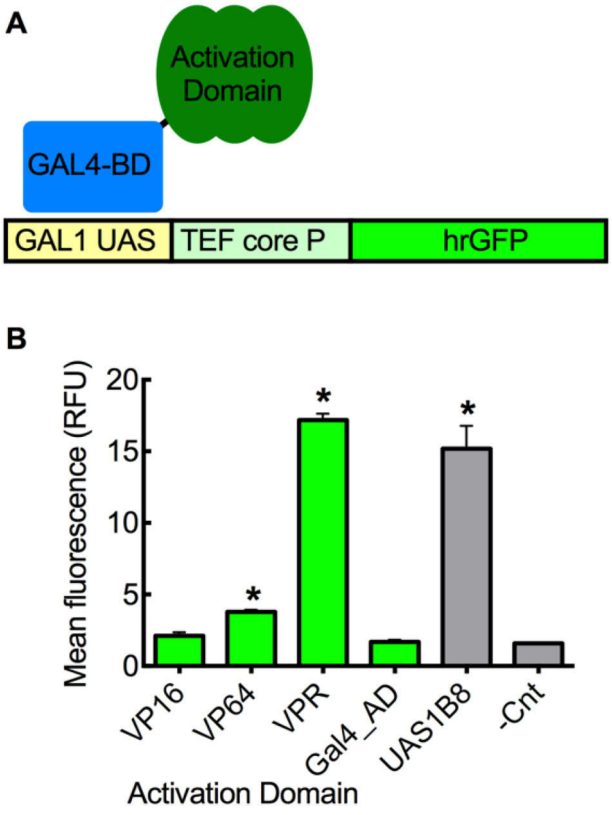

Figure 1. Comparison of activation domains for gene expression in *Y. lipolytica*. A) Schematic diagram depicting the synthetic GAL1-TEF promoter and artificial transcription factor with variable activation domain. B) Flow cytometry measurements of hrGFP fluorescence from a series of transcriptional activators driving expression from the synthetic GAL1-TEF promoter. An empty vector negative control and hrGFP positive control with expression from UAS1B8-TEF(136) are also shown. Data is the mean and standard deviation of biological triplicates, and "*" indicates statistical significance ($p < 0.001$) as determined by 1-way ANOVA and Tukey post-hoc analysis.

domain from the herpes simplex virus that can drive gene expression in a range of eukaryotes.[33] VP64 is the concatenation of four copies of VP16, which has been shown to achieve higher activation levels than a single copy. Finally, the tripartite activation domain VPR, which is a fusion of VP64 and the transcriptional activators p65 and Rta, was also tested.[34] All activation domains were codon optimized for expression in *Y. lipolytica*.

Plasmids expressing each of the designed transcription factors were transformed into PO1f GAL1-TEF-hrGFP::XPR2. Negative and positive control samples were generated by transforming an empty vector and a hrGFP expression vector, respectively. Fusion of VPR to Gal4_BD resulted in the highest expression of hrGFP, while VP64 increased expression above background levels (Figure 1B). Interestingly, VPR driven expression from GAL1-TEF achieved similar or slightly higher fluorescence than the well-characterized UAS1B8-TEF promoter. The GAL4 activation domain from *S. cerevisiae* and VP16 did not show significant increases in fluorescence above the empty vector control. Based on these results, we selected VPR as the activation domain to use in our CRISPRa system.

3.3. CRISPRa Control of Gene Expression From the GAL1-TEF Synthetic Promoter

With a functional activation domain in hand, we converted our previously demonstrated interference system into a CRISPRa system.[26] Specifically, the C-terminal Mxi1 repressor domain used for CRISPRi function was replaced with the VPR activation domain. The other necessary components of the system are: i) sgRNA expression, which was accomplished using a hybrid RNA Polymerase III promoter (SCR1′-tRNA^{gly}) and ii), dCas9-VPR expression from an episomal plasmid. The complete system was expressed from the plasmid pCRISPRa_VPR_yl.

A series of nine sgRNAs spanning the GAL1 UAS and TEF core promoter was designed to test the positional effects of dCas9-VPR mediated activation (**Figure 2**A). Each construct of the series was transformed into PO1f GAL1-TEF-hrGFP::XPR2, transformants were grown to late exponential phase, and hrGFP fluorescence was quantified and compared to a nontargeting sgRNA sample (Figure 2B). Three of the seven sgRNAs targeting the GAL1 UAS resulted in significant activation (sgRNAs-3, -4, and -6), while the other four sgRNAs did not (sgRNAs-1, -2, -5, and -7). Neither of the sgRNAs targeted to the core promoter (sgRNAs-8 and -9) resulted in significant GFP fluorescence.

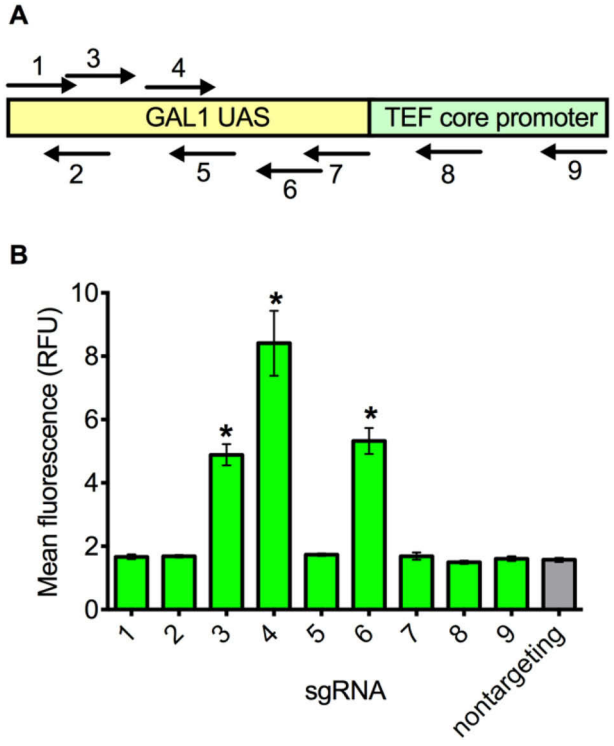

Figure 2. CRISPRa control of the synthetic GAL1-TEF promoter. A) Schematic diagram showing position of tested sgRNAs. B) Comparison of fluorescence by flow cytometry when CRISPRa plasmid containing dCas9-VPR and the indicated sgRNA was transformed into *Y. lipolytica* PO1f GAL1-TEF-hrGFP::XPR2. Data is the mean and standard deviation of biological triplicates, and "*" indicates statistical significance ($p < 0.001$) as determined with 1-way ANOVA and Tukey post-hoc analysis.

3.4. sgRNA Screening for β-Glucosidase Activation

The PO1f strain of *Y. lipolytica* is not capable of growing on cellobiose as a sole carbon source. However, previous work identified two β-glucosidase genes that confer growth on cellobiose when overexpressed.[16,19] BGL1 and BGL2, or YALI1_F21504 and YALI1_B18845, respectively, are putative β-glucosidases. For each gene, we designed five sgRNAs that targeted locations upstream of the native core promoter (we defined the core promoter region as 100 base pairs upstream of the start codon; **Figure 3**A). The effectiveness of each sgRNA was determined by characterizing growth in cellobiose containing media (SC-leu). As shown in Figure 3B, only one sgRNA for BGL1 (sgRNA BGL1-5) resulted in a significant increase in optical density over the nontargeting control. Similarly, only one sgRNA targeting the promoter region of BGL2 (sgRNA BGL2-5) resulted in growth above the background. Of note is that both of the successful sgRNAs were those closest to the core promoter of the gene of interest. These sgRNAs are similar in position to sgRNA-6 show in Figure 2 (CRISPRa activation of hrGFP from the GAL1-TEF synthetic promoter), suggesting that proximity to the core promoter may increase the degree of activation.

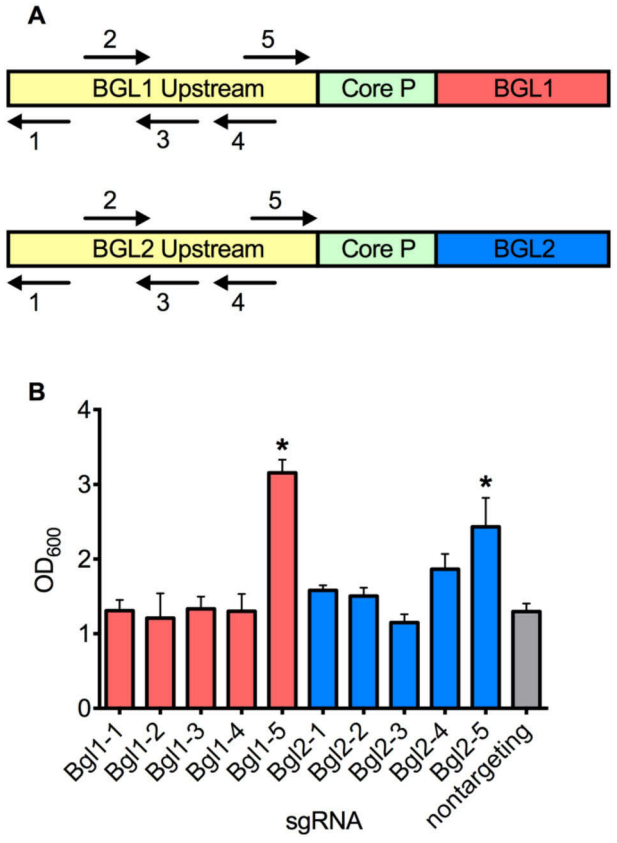

Figure 3. CRISPRa-mediated activation of *Y. lipolytica* β-glucosidase genes BGL1 and BGL2. A) Schematic showing the positions of tested sgRNAs in the native promoters of BGL1 and BGL2. B) Measurement of OD_{600} after 2 days of growth in minimal media with cellobiose as the sole carbon source. Data is the mean and standard deviation of biological triplicates, and "*" indicates statistical significance ($p < 0.001$) as determined with 1-way ANOVA and Tukey post-hoc analysis.

Although the control strain containing a nontargeting sgRNA reached an OD_{600} of ≈1.2 after 48 h at 30 °C, HPLC measurements of cellobiose consumption showed that no cellobiose was metabolized (Figure S2, Supporting Information). This growth was likely due small amounts of glucose present in the cellobiose media (approximately 150 mg L^{-1}; Figure S3, Supporting Information). To confirm that the growth in the nontargeting strain was due to residual glucose, cultures were refreshed in SC-leu media twice and growth of the nontargeting strain was found to be consistent (Figures 3 and S4, Supporting Information). For samples containing sgRNA BGL1-5, the same analysis showed the consumption of ≈1.3 g L^{-1} of cellobiose, while sgRNA BGL2-5 samples only consumed ≈0.4 g L^{-1} (Figure S2, Supporting Information). The activation of BGL1 and BGL2 by these sgRNAs was separately measured and compared to a nontargeting control using RT-qPCR (**Figure 4**B and C). Expression of BGL1 was increased by 112-fold, while BGL2 was increased by 43-fold. This increase in transcript levels confirms that the increased growth and cellobiose consumption was due to CRISPRa-mediated activation of β-glucosidase expression.

3.5. Multiplexed CRISPRa Enhances Growth on Cellobiose

To maximize growth of *Y. lipolytica* on cellobiose, and to expand the application of the system, we attempted simultaneous activation of BGL1 and BGL2 by multiplexing the successful sgRNAs (Figure 4A). Multiplexed sgRNA expression was achieved by cloning two independent expression cassettes in the pCRISPRa_VPR_yl plasmid, each with their own hybrid Pol III promoter and terminator. The success of this strategy was tested via RT-qPCR (Figure 4B and C). Activation of BGL1 from the multiplexed and singularly-targeted systems was equal. BGL2 activation was slightly reduced in the multiplexed format; however, greater than ≈20-fold activation was still achieved.

To more fully characterize growth of *Y. lipolytica* on cellobiose with CRISPRa-mediated activation of the two β-glucosidases, we performed extended cultures in selective minimal media with cellobiose as the sole carbon source. Control samples with a nontargeting sgRNA were also conducted. As shown in Figure 4D, the OD_{600} of the nontargeting sgRNA increased to slightly over 1 after one day of culture, before beginning a steady decline over time. The strain containing the multiplexed CRISPRa plasmid for BGL1 and -2 activation reached an OD_{600} of ≈10 after 6 days, indicating significant growth over the control sample. A maximum growth rate of 0.042 h^{-1} and biomass yield of 1.4 g L^{-1} was achieved. Cellobiose consumption followed a similar trend (Figure 4E). The nontargeting CRISPRa plasmid resulted in no significant consumption of cellobiose, while cellobiose consumption from samples containing the multiplexed CRISPRa plasmid increased steadily over the 6 days of culture.

4. Discussion

The yeast *Y. lipolytica* is an important and versatile host for metabolic engineering and industrial biotechnology. In this work, we developed a CRISPRa system to add to the collection of synthetic biology tools available for controlling gene transcription in *Y. lipolytica*. CRISPRa has proven valuable in other organisms: for example, it has been used to explore how bacteria can gain antibiotic resistance; tune biosynthetic pathway gene expression in yeast; and, perform genome-wide gain of function studies in mammalian cells.[35–37] This work demonstrates the CRISPRa activation of silent genes to expand the substrate range of an organism, specifically the oleaginous yeast *Y. lipolytica*.

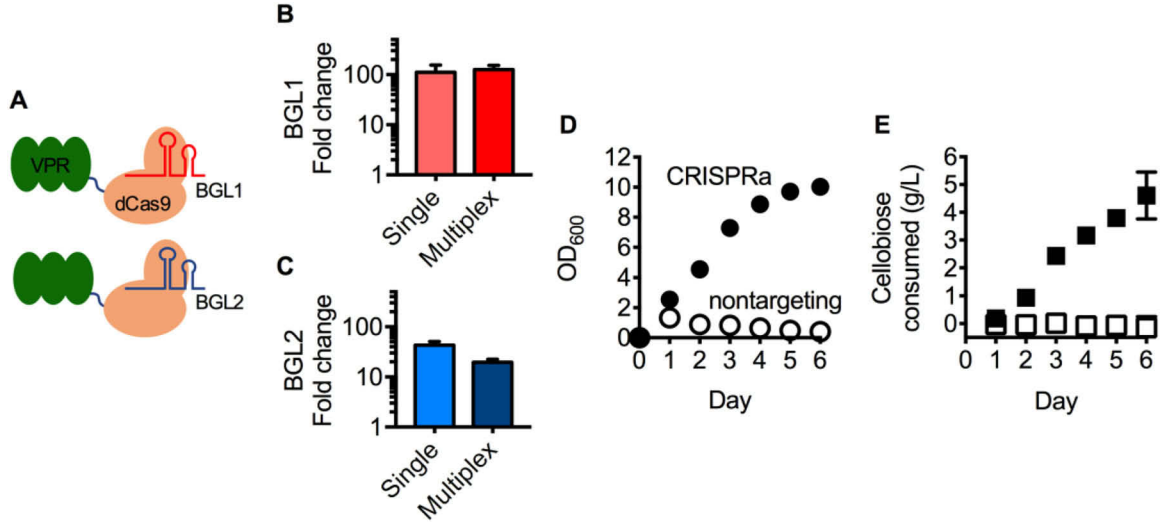

Figure 4. Multiplexed activation of BGL1 and BGL2 for growth on cellobiose. A) Diagram of multiplexed CRISPRa targeting BGL1 and BGL2. B) RT-qPCR data showing transcriptional activation of BGL1 by a CRISPRa plasmid containing a single sgRNA and the multiplexed CRISPRa system. C) RT-qPCR data showing activation of BGL2 by a CRISPRa plasmid containing a single sgRNA and the multiplexed CRISPRa system. D) Growth and E) cellobiose consumption of *Y. lipolytica* PO1f containing the multiplexed CRISPRa plasmid and a nontargeting negative control. In all cases, plotted data is the mean and standard deviation of biological triplicates. Some error bars are not visible because they are within the magnitude of the data point.

In the course of this study, we constructed a synthetic hybrid promoter consisting of the GAL1 UAS from *S. cerevisiae* and the TEF core promoter from *Y. lipolytica* (Figure 1). Interestingly, this promoter achieved slightly higher expression than the widely used hybrid UAS1B8-TEF(136) promoter, despite being significantly smaller in length (235-1036 bp).[31] A caveat is that the GAL1-TEF promoter required separate expression of the synthetic transcription factor GAL4_BD-VPR to drive expression. This result demonstrates that heterologous and orthogonal promoter systems could serve as an alternative to re-engineering native promoters. The VPR activation domain, which is a concatenation of the VP64 domain and two additional activation domains (the p65 subunit from the human NF-kappa-B transcription factor and the activation domain from the Epstein-Barr virus R transactivator protein), showed the highest level of activation among all tested domains. This matches results in other eukaryotes, and is likely due to VPR containing three unique activators working in parallel.

The capacity to grow on a range of different biomass-derived sugars is an important trait for industrial biotechnology hosts.[38] While wild type strains do not typically grow with cellobiose, galactose, or xylose as a carbon source, engineering of *Y. lipolytica* has produced strains with these phenotypes.[14–19] Genome analysis shows that genes encoding enzymes that can degrade and metabolize these sugars are present, but that they are silent and/or not sufficiently expressed to support growth. By using a CRISPRa system to upregulate BGL1 and BGL2 (β-glucosidase genes that are transcriptionally silent), we validated a strategy to enhance cryptic sugar metabolism. Using simple growth experiments, we screened a series of sgRNAs targeting the upstream regions of BGL1 and BGL2 for transcriptional activation. In each case, the sgRNA closest to the core promoter increased transcript abundance (Figure 4B and C) and conferred enhanced growth with cellobiose as the sole carbon source (Figure 3B). Multiplexing of the two functional sgRNAs successfully activated both genes and produced growth comparable to previous reports of BGL1 and BGL2 overexpression using traditional metabolic engineering.[16,19] Our CRISPRa strategy could be expanded to activate additional genes, such as a cellobiose transporter, to further improve growth, and cellobiose assimilation.[16] Multiplexing could also expand experimental scope and facilitate the screening of more complex combinatorial space than traditional metabolic engineering strategies, as has been shown in *S. cerevisiae*.[39,40]

In this work, we used a CRISPRa system to activate native β-glucosidases to facilitate growth of *Y. lipolytica* on cellobiose. The tripartite VPR activator was identified as a potent activation domain in *Y. lipolytica*, and fusion to dCas9 enabled targeted gene activation. A panel of sgRNAs was tested for activation of two separate β-glucosidases, and successful sgRNAs were identified using a growth screen. The functional sgRNAs were then multiplexed, and the cellobiose-assimilation capacity of a strain transformed with the multiplexed plasmid was characterized. This CRISPRa system represents a valuable addition to the set of synthetic biology tools available for metabolic engineering of *Y. lipolytica*, and will help advance *Y. lipolytica* as a host for industrial biotechnology.

Abbreviations

CRISPRa, CRISPR dCas9 activation; CRISPRi, CRISPR-dCas9 interference; dCas9, catalytically inactive Cas9; Gal4_BD, Gal4p DNA binding domain; HPLC, high-performance liquid chromatography; hrGFP, humanized Renilla green fluorescent protein; OD_{600}, optical density at 600 nm; RT-qPCR, quantitative reverse transcription PCR; sgRNA, single guide RNA; UAS, upstream activating sequence.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no commercial or financial conflict of interest.

Keywords

cellobiose, CRISPR activation, metabolic engineering, synthetic biology, transcription, *Yarrowia lipolytica*

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