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Engineering Prokaryotic Transcriptional Activator XylR as a Xylose-Inducible Biosensor for Transcription Activation in Yeast

Wenping Wei, Yanzhe Shang, Ping Zhang, Yong Liu, Di You, Bincheng Yin, and Bangce Ye*

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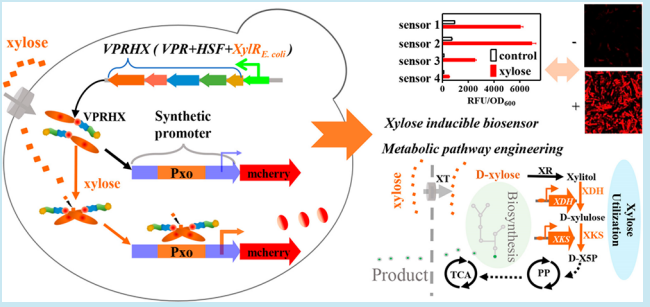
ABSTRACT: Biosensors regulated by specific substrates are needed to develop genetic tools to meet the needs of engineering microbial cell factories. Here, a xylose-inducible biosensor (xylbiosensor), comprising the *Escherichia coli* activation factor XylR, fusion activation domain (AD) VPRH, and a hybrid promoter with operator *xylO*, was established in *Yarrowia lipolytica*. The addition of xylose to an engineered *Y. lipolytica* strain harboring the xylbiosensor could trigger significant transcriptional activation of target genes, such as *mcherry* and the xylose utilization gene. Furthermore, a novel promoter Pleu-Pxo-Ptef was developed to construct a bidirectional expression system. The xylbiosensor showed good portability in *Saccharomyces cerevisiae*, suggesting its potential value in other eukaryotic cells. This study is the first to construct a "turn-on" xylbiosensor induced by xylose addition based on a prokaryotic activator XylR and eukaryotic universal AD. The xylbiosensor exhibits potential in pathway engineering for xylose utilization and xylose-derived product biosynthesis in yeast.



KEYWORDS: *Escherichia coli XylR, hybrid promoter, transcription activator VPRHX, xylose utilization, xylose-inducible biosensor, Yarrowia liapolytica*

Synthetic biology strives to design and build genetic tools that are easier and faster and that enable a predictable and precise regulation of biological processes.[1] Genetic tools, specifically biosensors, have shown good potential for pathway design and screening of specific enzymes.[2] In recent years, biosensors involving carbohydrates have received continued attention in biosynthesis of valuable products. These carbohydrate-inducible sensors can respond to xylose,[3−6] fatty acid,[2] and cellobiose.[7] Among these carbohydrates, xylose is one of the main components of lignocellulosic biomass. It is energetically economical for microorganisms to use xylose to produce useful chemicals.[8] Xylose-responsive transcription factors (TFs) are present in *Escherichia coli*,[6] *Bacillus subtilis*,[5] and *Streptomyces rubiginosus*,[9] and can be used as natural xylose sensor module. However, to the best of our knowledge, yeast does not have this native xylose-inducible biosensor. Construction of a xylose-inducible biosensor (xylbiosensor) in yeast to permit xylose utilization and regulation of biosynthetic gene expression is desirable.

In yeast, the "turn-on" xylbiosensor has been designed by repressor XylR ($XylR^R$) of *Bacillus subtilis*.[3,4] Most xylose-responsive TFs XylR from prokaryotic microbes belong to a repressor,[5,9] which contain a DNA-binding domain and a small-molecule allosteric modulation domain. The natural character of these repressors is to target and bind to their specific promoter in the absence of a corresponding inducer, while the addition of an inducer will hinder the specific binding between the TF and the promoter through allosteric regulation. In yeast, the "turn-on" xylbiosensor from $XylR^R$ functions when the inducer xylose exerts the repressive effect,[3,4] and then the synthetic promoter initiates transcription. The upper limit of its expression depends on the strength of the promoter and assistance of the upstream activation sequence (UAS).[3,10,11] However, in general, the function of "turn-on" biosensors in yeast cells depends on activation domains (ADs) such as GAL4, the universal activation domains VP64 and VPR (the fusion protein of activation domains VP64, P65, and Rta).[12] The activation effect of these biosensors depends on the type and strength of the ADs, not just the strength of the promoter. When we construct a xylbiosensor based on ADs in yeast cells, the feasible solution so far is to construct the complex repressor $XylR^R$-ADs. However, when xylose is present, such $XylR^R$-ADs can only perform a "turn-off" function instead of the desired "turn-on" mode. This is undesirable, especially when building highly efficient activation biosensors that respond to xylose.

There is a "turn-on" biosensor system based on transcriptional activator XylR in response to xylose in *E. coli*.[13] To our knowledge, the native xylose sensing system from the activator

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only exists in *E. coli*. The genes encoding enzymes involved in xylose utilization are controlled and activated by XylR, which can bind to the xylose responsive promoter of those genes only in the presence of xylose.[13,14] We hypothesized that *E. coli* XylR can be used to design xylose-inducible "turn-on" biosensors in yeast, as long as a complex with a universal ADs fused to the N-terminal of XylR is constructed. ADs that have been generally designed include VP64,[15] VPR,[16] and HSF1.[17] Once the ADs are fused to *E. coli* XylR to produce complex ADs-XylR, it is theoretically feasible that the ADs are recruited by XylR to generate reverse parallel dimers induced by xylose;[13] they will then bind to target hybrid promoter with *xylO* site and function as the transcriptional activator.

To investigate whether the *E. coli* XylR can be used to construct a "turn-on" xylbiosensor, we fused the *E. coli* XylR with VPR and HSF1 to form fusion protein VPRH-XylR (VPRHX), and designed a synthetic hybrid promoter with the *xylO* site. Lastly, we combined these components to complete the construction of the xylbiosensor. *Y. lipolytica* is widely used in chemical production processes and is an organism with "Generally Regarded as Safe" (GRAS) status.[18] It lacks a usable xylbiosensor; hence, it was selected as the first host to design the xylbiosensor. The xylbiosensor displayed an obvious response and good specificity to xylose and was then upgraded by designing a xylose-inducible bidirectional expression system (BDES) in *Y. lipolytica*. In addition, the pathway of xylose utilization was engineered through the inducible expression of *XDH* (YALI0E12463) and *XKS* (YALI0F10923) based on the xylbiosensor-mediated transcriptional activation. It is worth mentioning that the xylbiosensor derived from VPRHX also functioned well in *S. cerevisiae*. In yeast, this is the first report of a transcriptional activator biosensor responsive to the inexpensive and abundant biomass-derived carbon source xylose. The xylbiosensor is valuable in pathway engineering for yeast to utilize xylose and to produce xylose-derived value-added natural products.

RESULTS AND DISCUSSION

Design of Hybrid Promoter for Xylbiosensor. In yeast, several biosensors are derived from prokaryotic TFs.[2−4] For biosensors, in addition to the core component of prokaryotic TFs, the promoter is another necessary element. Owing to the differences between prokaryotic and eukaryotic promoters with respect to the RBS, transcription start sites, and regulatory binding sites among others, exogenous prokaryotic promoters paired with prokaryotic TF may not play an effective role in eukaryotic cells for biosensor construction. Therefore, the common approach is to tailor suitable synthetic promoters for TFs based on the native yeast promoter.[2−4,19] Implement the specific TF binding site into a selected yeast core promoter could lead to generation of a new hybrid promoter containing the site for TF regulation and satisfactory functionality in yeast.[1] Four promoters, the TEF promoter, TEF core promoter, Leu core promoter, and ACC1 promoter (Figure 1a-b, Table S3), were screened to construct the specific synthetic hybrid promoter carrying the XylR binding site XylO (shortened to Pxo). The TEF promoter, as the strong promoter that can be widely used for gene expression or as a source to build hybrid synthetic promoters,[20] was used to build the hybrid synthetic promoter Ptefup-Pxo-Ptef through the insertion of the Pxo near the TATA box of the TEF promoter (Figure 1b, Table S3). Pxo-Ptef, the second hybrid synthetic promoter, contains only the Pxo and TEF core promoter. Similarly, the Pxo was inserted near

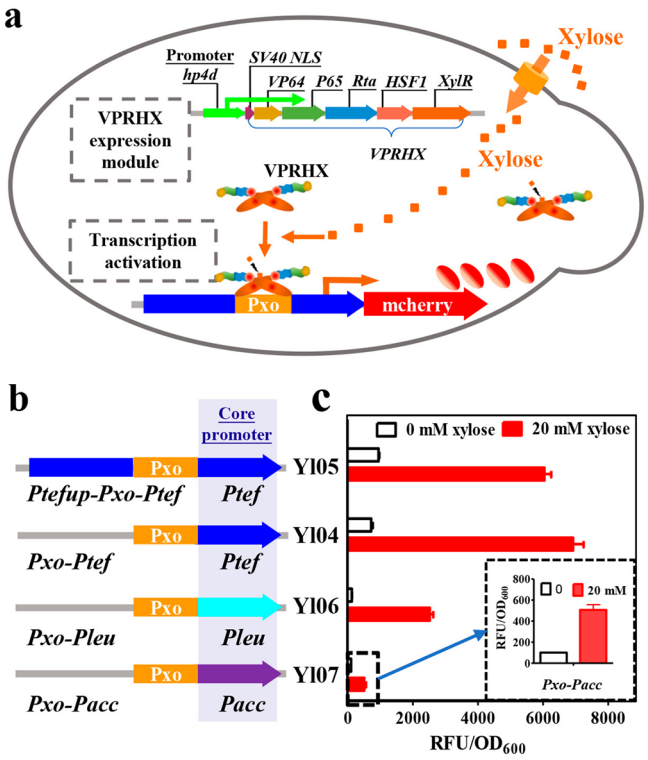

Figure 1. Refactoring the xylbiosensor based on *E. coli* XylR and VPRH in *Y. lipolytica*. (a) Designing the xylbiosensor in *Y. lipolytica*. The xylbiosensor was designed based on four core promoters. *mcherry* was cloned downstream of those promoters as a reporter gene. The transcriptional factor complex VPRHX includes the activation domain (i.e., VP64, P65, Rta, and HSF1 were selected and fused to form the new activation complex VPRH) and xylose-response transcriptional factor XylR from *E. coli*. It was constitutively expressed under the control of the *hp4d* promoter. (b) Design of the hybrid synthetic promoter. The individual *Pxo* sequence (XylR binding site *XylO* from *E. coli*) was fused upstream of core promoter of *Y. lipolytica*. The core promoter selected in this design from TEF, Leu, and ACC1 promoter. (c) mcherry expression response to xylose at 20.0 mM. The strains Yl04−07 were cultivated in YPD for 72 h. The excitation wavelength was 580 nm and the emission wavelength was 608 nm.

the TATA box of the Pleu, the core promoter element of *hp4d*,[18] to generate a third hybrid promoter, Pxo-Pleu. The fourth hybrid promoter Pxo-Pacc1 was designed from Pacc1 (Figure 1b, Table S3), the core promoter of *ACC1*, which controls the expression of enzyme ACC1 to supply malonyl-CoA for lipid biosynthesis.[21] On the basis of these designs, four hybrid promoters were constructed (Table S3).

Characterization of the Xylbiosensor System in *Y. lipolytica*. Except for hybrid promoters containing the desired TF binding site, a biosensor requires a TF that is responsive to the specific chemical molecule. As previously stated, *E. coli* XylR was chosen as a xylose-sensitive TF. Many prokaryote-derived TFs (i.e., FdeR and BenM) are capable of responding to corresponding small molecules and functioning as a transcriptional activator when present alone in yeast.[19] For this reason, individual XylR and the hybrid promoter Pxo-Ptef were used to generate a biosensor and determine the transcriptional activation of XylR in *Y. lipolytica* Yl01. *mcherry* expression of the xylose response module consisting of *XylR* and Pxo-Ptef indicated that XylR alone cannot function as a transcriptional activation factor in *Y. lipolytica* (Figure S1). Therefore, the transcriptional activation of XylR requires additional ADs. In

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general, transcriptional activation requires ADs such as VPR[16] and HSF1[17] in traditional activation systems, as well as in CRISPRa. In *Y. lipolytica*, VPR-driven gene expression was slightly higher than gene expression driven by the strong promoter UAS1B8-TEF.[12] Here, we compared the xylose-responsive activation effect of the complex transcription factor formed by the fusion of different ADs (i.e., HSF, VPR, and VPRH) with XylR (Figure S2, Table S2). The activation effect of those ADs is characterized by the expression of the reporter gene *mcherry* (Figure S2). The results show that the complex VPRHX formed by VPR, HSF, and XylR has a better xylose-responsive activation effect than HSFX and VPRX. The VPRHX, comprising VPR, HSF, and XylR (Table S2), simultaneously plays a xylose-sensitive role in targeted promoter recognition and transcriptional activation (Figure 1a). The VPRHX contains the SV40-NLS sequence at the N-terminus, which ensures nuclear localization (Table S2). To evaluate the expression *in vivo*, VPRHX carrying the protein tag "Flag" was identified using Western blotting electrophoresis with a Flag-antibody (Figure S3). Results indicated the successful expression of VPRHX in *Y. lipolytica* Yl04.

Figure 1c shows that the RFU (relative fluorescent unit) signal increased by 6.4, 9.6, 20.9, and 5.1-fold when 20.0 mM xylose was added to the strains Yl05, Yl04, Yl06, and Yl07, carrying the hybrid promoter Ptefup-Pxo-Ptef, Pxo-Ptef, Pxo-Pleu, and Pxo-Pacc1, respectively. Generally, the short TEF promoter, only containing its core promoter sequence, has minimal expression in the absence of UAS.[22] Accordingly, the expression of mcherry could be enhanced by the hybrid synthetic promoter, specifically the Pxo-related activation effect. The addition of xylose results in the formation of antiparallel dimers of XylR, binding to operator sites of Pxo, and transcriptional activation in *E. coli*.[13] Here, the VPRHX presented the same xylose-inducible transcriptional activation in *Y. lipolytica*.

Comparison of the four hybrid synthetic promoters, each with different core promoter regions, revealed that the fluorescence intensity of mcherry derived by Pxo-Ptef was higher than that of the Ptefup-Pxo-Ptef and Pxo-Pleu in the presence of xylose (Figure 1c). Pxo-Ptef had a higher signal-to-noise ratio than Ptefup-Pxo-Ptef. In addition, Pxo-Pacc1 had a significant response after xylose was added ($p < 0.05$), although its fluorescence intensity was lower than that of Pxo-Ptef and Pxo-Pleu. On the basis of these data, Pxo-Ptef was selected as the xylose-inducible promoter in subsequent experiments.

High selectivity of biosensors is necessary when they are widely used for detection and response of specific molecules.[23,24] The response to a set of xylose isomer monosaccharides and intermediate metabolites such as xylitol was investigated at the same concentration to determine the specificity of the xylbiosensor (Figure 2a). Xylose significantly induced mcherry expression (Figure 2a). Dynamic time-dependent response curves of mcherry fluorescence intensity from the strain Yl04 in the presence of 20.0 mM xylose further revealed a higher inducible effect throughout the cultivation (Figure S4). It is worth noting that xylose has a 14.8-fold induction for the xylbiosensor in the minimal medium YNB (Figure S5), which was higher than the fold induction in YPD (9.6-fold in Figure 1c). As mentioned above, the xylbiosensor showed a better response to xylose than other moleculars such as xylitol, ribose, arabinose, threose, and galactose. The development of a xylbiosensor in *Y. lipolytica* exhibits good prospects for utilization in industries. In an actual fermentation environment, the strain always grows using a mixed carbon source. Mixed

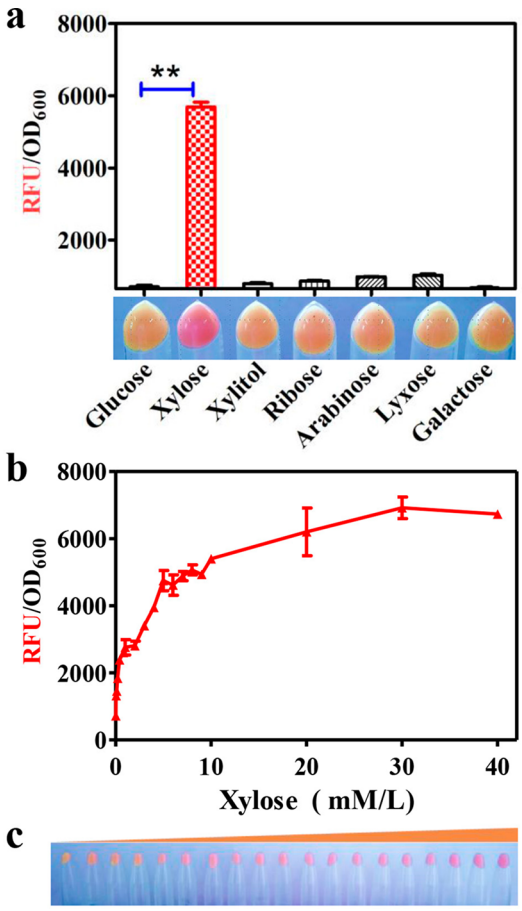

Figure 2. Fluorescence intensity of mcherry at different xylose concentrations from strain Yl04. (a) Ligand specificity of xylbiosensor in strain Yl04 carrying the pVPRHXbios1 plasmid. The ligands used were xylose, xylitol, ribose, arabinose, threose, and galactose, and all were used at 20.0 mM. The ligands were added at 24 h after inoculation, and this is the initial time to determine the fluorescence density. The data collection was performed at 48 h after inoculation. (b) Fluorescence density induced by different concentrations of xylose (0, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 20.0, 30.0, and 40.0 mM/L) at 48 h. The excitation wavelength was 580 nm and the emission wavelength was 608 nm. (c) The image was recorded using a VivoX7 phone at 48 h, the xylose concentration was 0, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 20.0, 30.0, and 40.0 mM/L, respectively. The strain Yl04 was cultured and induced in YPD medium for 72 h.

sugars derived from cereals and hydrolysates are generally used as a carbon source for microorganisms.[25] Specific responses to a single carbon source will contribute to spatiotemporal specific targeting to control transcription and subsequent gene expression.

Moreover, to investigate the response to the signal input relating to xylose concentration, we further studied the sensitivity and operational range of the xylbiosensor by measuring response curves *in vivo*. Strain Yl04 with Pxo-Ptef and VPRHX exhibited a dose-dependent increase in RFU when grown in YPDX with gradually increasing xylose concentrations, which ranged from 0 to 40.0 mM. RFU sensitivity reached saturation between 20.0 and 40.0 mM at 48 h (Figure 2b). A higher output signal was observed and visualized with obvious mcherry expression (Figure 2c, Figure S6). High mcherry expression was induced by xylose. During the culture period, strains cultured in YPDX medium with different concentrations

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of xylose showed no significant difference in growth (Figure S7a). However, its mcherry expression was significantly affected by xylose addition, particularly from 48 to 72 h (Figure S7b). The signal output of xylose response has concentration and time-dependent characteristics that will make the xylbiosensor more suitable for precise regulation of gene expression in *Y. lipolytica*.

Design of a Xylose-Inducible Bidirectional Transcriptional Activation System. In *E. coli*, the xylose-induced specific promoter in the original *xyl* operon is bidirectional[14] (Figure 3a). The phenomenon of bidirectional distribution of such operons is common in prokaryotes and eukaryotes.[26] To verify the possibility of refactoring of the xylose-inducible BDES in *Y. lipolytica*, we designed a transcriptional control model in which the core promoter sequence *Leu* and *TEF* share the same activation site *xylO* (as Pxo in Figure 3b).

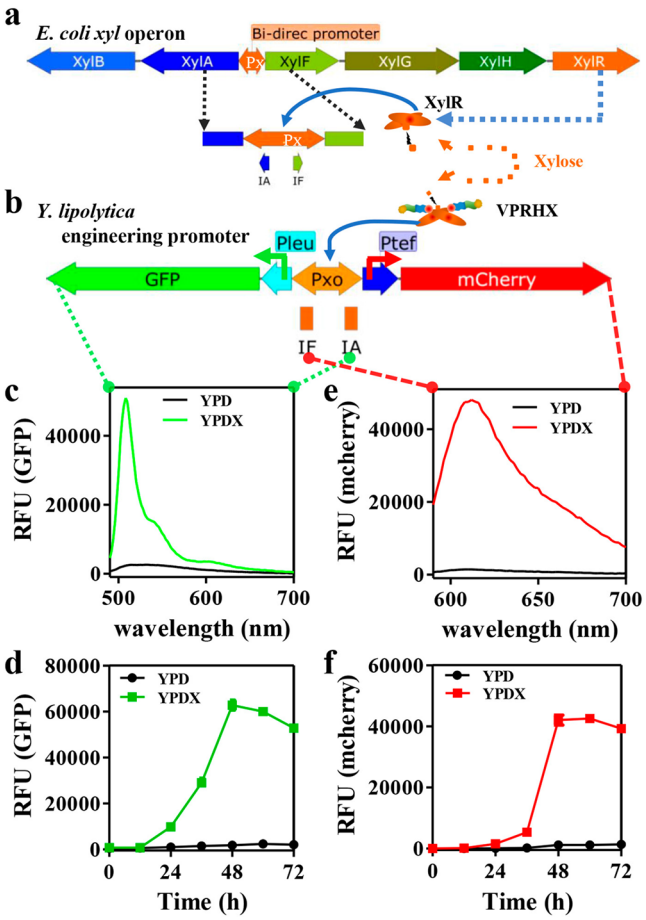

Figure 3. Construction of the xylose-inducible bidirectional expression system (BDES) in *Y. lipolytica*. (a) Native structure of *xyl* operon in *E. coli*. The *xyl* operon is involved in the reverse expression of *xylAB* and *xylFGH*. (b) Refactoring xylose-inducible BDES using Pxo, core promoter Pleu and Ptef in *Y. lipolytica*. Pleu and Ptef are distributed in opposite directions on both sides of Pxo, and the expression of *GFP* and *mcherry* indicated unilateral promoter activity. (c) RFU of GFP for the strain Yl08. (d) Dynamic change in *GFP* expression BDES. (e) RFU of mcherry for the strain Yl08. (f) Dynamic change in *mcherry* expression BDES. The strain Yl08 carrying the pVPRHXBDES plasmid was cultivated in YPD containing 3.0 g/L xylose (YPDX).

It has been demonstrated that both Pxo-Ptef and Pxo-Pleu can successfully achieve xylose-responsive transcriptional activation (Figure 1). In theory, when a transcriptional activator binds to the same TF binding site Pxo, the promoters on both sides share the change in transcriptional activity from the activator VPRHX (Figure 3b). Artificially constructed BDES, GFP, and mcherry were highly expressed in the presence of xylose compared with the expression in the blank control (Figure 3c–f). BDES produced an approximately 22.9-fold RFU increase in GFP expression (Figure 3c) and an approximately 33.4-fold RFU increase in mcherry expression (Figure 3e). The time nodes of the growth trend and fluorescence change were essentially identical for Pxo-Ptef and Pxo-Pleu (Figure 3e,f). The fold induction of gene expression in this system was higher than that of a single promoter (Figure 1). This difference may arise from the composition of the promoter and expressed gene sequences (Table S3), and shows that optimizing the adaptability between different exogenous operators, core promoters and target genes is particularly important for obtaining better fold induction in the later applied research of biosensor. Similar to this artificial BDES, generation of an artificial promoter by the addition of a cis-acting element is universal in eukaryotes.[27,28] As an exogenous cis-acting element, Pxo regulates the activity of BDES, which results in bidirectional transcription activation. As with yeast's native divergent transcription,[29] this artificial transcription control system may have a potential impact on targeted gene regulation. The application of BDES can activate the simultaneous expression of genes on both sides, particularly genes that are equally important for a synthesis process.

Inducible Utilization of Xylose Based on Xylbiosensor. As a novel and effective transcriptional activation biosensor, the xylbiosensor should have the potential to simultaneously activate the expression of multiple genes. Excluding the high expression of mcherry, VPRHX would enable *Y. lipolytica* to utilize xylose by carrying related enzymes derived by the Pxo-Ptef promoter (Figure 4a). On the basis of the above assumptions, we overexpressed two native genes *XDH* and *XKS*, encoding the major enzymes in the xylose utilization pathway, by using the xylbiosensor. Specifically, XDH catalyzes the conversion of xylose to xylitol, and XKS can catalyze xylulose to xylulose-5-phosphate (Figure 4a), which enters the pentose phosphate pathway.[8] Growth of Yl12 in the YNB–xylose medium was satisfactory only when *XDH* and *XKS* were simultaneously overexpressed (Figure 4b). This finding is consistent with prior results from other studies.[8] The OD_{600} (biomass) of Yl12 was approximately 2.0 at 72 h, which is higher than that of the engineered *Y. lipolytica* YlSR02, in which *XDH* was overexpressed under the transcriptional control of the constitutive promoter Ptef (minimum medium, 10 g/L xylose as carbon source, OD_{600} < 1.0 at 72 h).[30] From this point, xylose-inducible expression may offer greater advantages for enhancing the xylose metabolism capacity. However, the biomass in the xylose medium was still considerably less than that obtained in YPD (Figure S7, OD_{600} > 10.0). This difference may be attributed to the available nitrogen sources in the minimum medium YNBX15 that were not abundant enough compared to that in YPD, and compared to glucose, xylose is far from being a carbon source that yeast cells prefer to utilize before systematic engineering. In addition, the intracellularly loaded reporter gene *mcherry* expression module also functioned normally (Figure 4b), indicating that the xylbiosensor has the potential to regulate multiple processes, not just xylose utilization. Only strain Yl12 grew in the YNB–xylose medium, while Yl11, which lacked plasmid pVPRHXbios1, lacked the capability to grow in this medium. These data indicated that the xylose-inducible utilization system of xylose can function well when the

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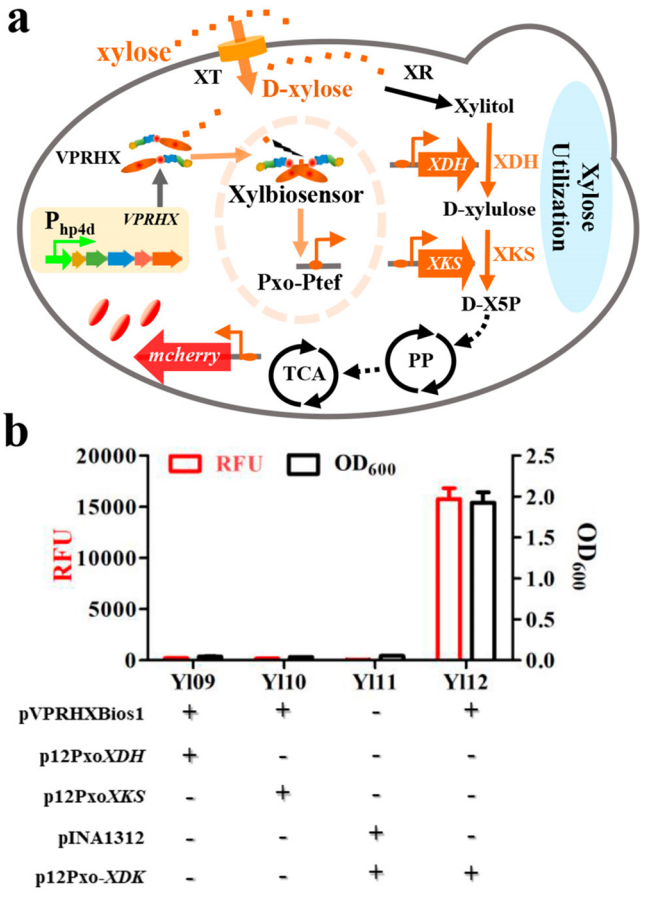

Figure 4. Xylose utilization and mcherry expression mediated by xylbiosensor. (a) The xylose-inducible activator VPRHX mediates the utilization of xylose and the expression of targeted protein. (b) Strain growth and target protein expression by using the xylose as the sole carbon. Utilization of xylose by the xylose-inducible expression of *XDH* and *XKS*. The culture medium was YNB−xylose (6.7 g/L YNB, 15.0 g/L xylose). Growth conditions were 30 °C for 72 h. Abbreviation: XT, xylose transporter (YALI0B00396). XR, xylose reductase (YALI0D07634). X5P, xylulose-5-phosphate. PP, pentose phosphate pathway. TCA, tricarboxylic acid cycle.

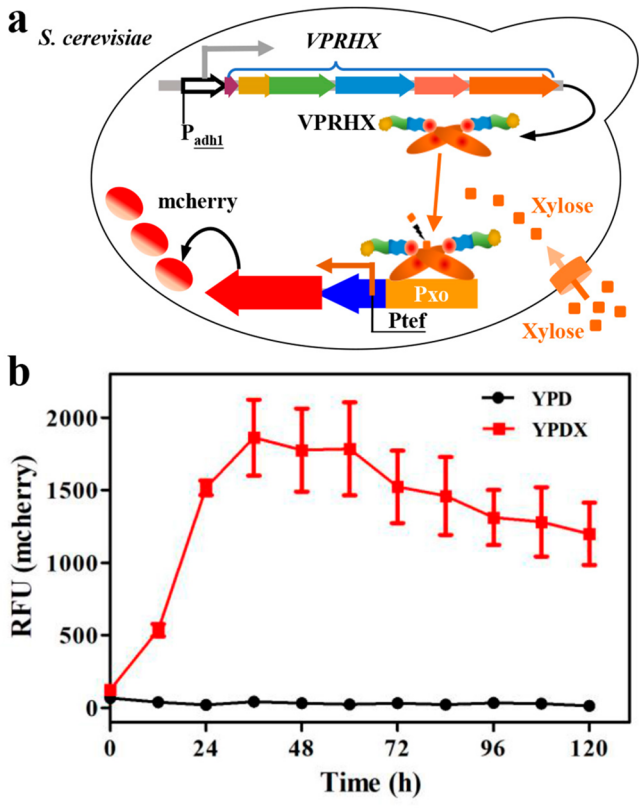

Figure 5. Xylbiosensor responsiveness in *S.cerevisiae*. (a) Schematic representation of xylose-inducible biosensor in *S. cerevisiae* BY-Xbios. The VPRHX expression module was controlled by the constitutive *ADH1* promoter. The synthetic promoter PxoPtef consists of the *E. coli xylO* and the *S. cerevisiae* TEF core promoter, with *mcherry* as the reporter gene. (b) The dynamic responsiveness of the xylbiosensor to xylose in strain BY-Xbios. The medium was YPD-His-Met-Leu (YPD medium with 0.5 g/L each of His, Met, and Leu). Xylose (3.0 g/L) was the inducer (as YPDX medium). For the mcherry fluorescence detection, the excitation and emission wavelengths were 580 and 608 nm, respectively.

VPRHX factor is present *in vivo*. Xylose was able to autoinduce its own utilization, allowing *Y. lipolytica* to initiate efficient expression of the xylose utilization gene in the presence of xylose, rather than the continuously constitutive expression independent of xylose. This feature may allow a more economical coordination of spatiotemporal specific expression of target genes and could promote the rational distribution of intracellular energy flow and metabolic flux according to the xylose concentration.

Xylose-Inducible Transcription Activator Functions Well in *S. cerevisiae*. Drawing on the successful construction of the xylbiosensor in *Y. lipolytica*, we applied it to *S. cerevisiae* (Figure 5a). The Padh1 promoter drives the expression of VPRHX. The ability of VPRHX to target Pxo in synthetic promoters has been verified in *Y. lipolytica* (Figure 1 and Figure 3). The results of the xylose-induced expression of mcherry and the signal output (Figure 5b) indicated that the targeted binding of VPRHX to the newly constructed promoter PxoPtef in *S. cerevisiae* was also successful. Mcherry expression in YPDX was notably higher than that of YPD during culture (Figure 5b). The signal-to-noise ratio of the xylbiosensor in BY-Xbios reached 43.3 at 36 h, indicating the significance of xylose-induced activation ($p < 0.01$). The transplantation of the xylbiosensor from *Y. lipolytica* to *S. cerevisiae* proved to be feasible after designing a synthetic promoter based on an effective combination of the yeast endogenous core promoter and the exogenous binding site Pxo. The realization of this activation effect is attributed to the versatility of activating factors such as VPR in yeast[12,16] and mammalian cells.[15] The re-emergence of transcriptional activation effects in *S. cerevisiae* indicated that the xylbiosensor developed in this study has the potential to be utilized in other eukaryotic cells.

CONCLUSION

In this study, we constructed a xylose-responsive transcriptional activation biosensor termed the xylbiosensor, which shows a dynamic range of expression upon xylose induction and broadens the genetic tools available for engineering of yeast. The expanded development of xylbiosensor involved the creation of a BDES and refactoring the inducible xylose utilization pathway. On the basis of this xylbiosensor, the dual function of xylose as a carbon source and a molecular inducer was exploited to achieve a synchronized process, which combined utilization of substrates and inducible gene expression. The successful transplantation of the xylbiosensor

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Table 1. Plasmids and Strains Used in the Current Study

name	description	original plasmid	source
	plasmid		
pINA1312	integration vector with *hp4d* promoter, XPR2 terminator, selection marker URA3, KmR		18
pINA1269	integration vector with *hp4d* promoter, XPR2 terminator, selection marker LEU2, AmpR		18
PUC57-VPRH	PUC57 with the codon-optimized *VPR* and *HSF1*	PUC57	lab collection
PUC57-mCh	harboring *mcherry* fragment	PUC57	lab collection
pmCh	harboring *mcherry* fragment	pINA1312	this study
pHSFX	harboring *HSF1-XylR* controlled by *hp4d* promoter	pINA1312	this study
pVPRX	harboring *VPR-XylR* controlled by *hp4d* promoter	pINA1312	this study
pVPRHX[a]	harboring *VPRHX* controlled by *hp4d* promoter	pINA1312	this study
pHSFXbios	Pxo-Ptef and *mcherry* were inserted into *Sal*I and *Stu*I site	pHSFX	this study
pVPRXbios	Pxo-Ptef and *mcherry* were inserted into *Sal*I and *Stu*I site	pVPRX	this study
pVPRHXbios1[b]	Pxo-Ptef and *mcherry* inserted into *Sal*I and *Stu*I site	pVPRHX	this study
pVPRHXbios2	Ptefup-Pxo-Ptef and *mcherry* inserted into *Sal*I and *Stu*I site	pVPRHX	this study
pVPRHXbios3	Pxo-Pleu and *mcherry* inserted into *Sal*I and *Stu*I site	pVPRHX	this study
pVPRHXbios4	Pxo-Pacc1 and *mcherry* inserted into *Sal*I and *Stu*I site	pVPRHX	this study
pX	harboring *E. coli XylR* with the *Flag* and *SV40-NLS* sequence located at 5′-End	pINA1312	this study
pXbios	pX with Pxo-Ptef and *mcherry* inserted into *Sal*I and *Stu*I site	pX	this study
pVPRHXBDES[c]	xylose-responsive bidirectional promoter Pleu-Pxo-Ptef. The Pleu-Pxo to control *GFP*, and the Pxo-Ptef to control *mcherry*	pVPRHX	this study
pL1269	linearized plasmid amplified from pINA1269 without native *hp4d* promoter	pINA1269	this study
p12P*xoXDH*[d]	with Pxo-Ptef and *XDH*	pL1269	this study
p12P*xoXKS*[e]	with Pxo-Ptef and *XKS*	pL1269	this study
p12P*xo-XDK*[f]	with Pxo*XKS* inserted into *Kpn*I site	p12P*xoXDH*	
pYES2	with GAL1 promoter and CYC terminator	-	lab collection
pLYES	linearized pYES2 amplified by PCR, *GAL1* promoter was removed	pYES2	this study
pYES2-*mch*	harboring *mcherry* fragment	pYES2	this study
pLYES-P*adh1-VPRHX*-Pxo-Ptef-*mch*	with P*adh1-VPRHX*-Pxo-Ptef-*mcherry* fragment	pLYES	this study
	Strains		
Po1f	auxotrophic strain (*Leu-*, *Ura-*). The derivatives *Y. lipolytica* strain W29 (CLIB89)		18
Yl01[g]	with pXbios	Po1f	this study
Yl02	with pHSFXbios	Po1f	this study
Yl03	with pVPRXbios	Po1f	this study
Yl04	with pVPRHXbios1	Po1f	this study
Yl05	with pVPRHXbios2	Po1f	this study
Yl06	with pVPRHXbios3	Po1f	this study
Yl07	with pVPRHXbios4	Po1f	this study
Yl08	with pVPRHXBDES	Po1f	this study
Yl09	p12P*xoXDH*	Yl04	this study
Yl10	p12P*xoXKS*	Yl04	this study
Yl11	pINA1312 and p12Pxo-*XDK*	Po1f	this study
Yl12	p12P*xo-XDK*	Yl04	this study
BY4741	commonly used auxotrophic *S. cerevisiae* strain (his3-, leu2-, met-, ura3-.)		lab collection
BY-Xbios	with pLYES-P*adh1-VPRHX*-Pxo-Ptef-*mch* plasmid	BY4741	This study

[a]*VPRHX* is the fusion DNA fragment of *VPRH* and *E. coli XylR*. [b]Derived vector from pVPRHX, with the Pxo-Ptef to control mcherry and to form a biosensor plasmid ("bios"). [c]BDES, bidirectional expression system. [d]*XDH* gene (YALI0E12463). [e]*XKS* gene (YALI0F10923). [f]*XDK*, the abbreviation of *XDH* and *XKS*. [g]Yl, abbreviation of *Y. lipolytica*.

from *Y. lipolytica* to *S. cerevisiae*, based on the synthetic promoter and xylose-inducible transcription activator VPRHX, indicated that the xylbiosensor model developed in this study has the potential to be applied in other eukaryotic cells. This study successfully demonstrates the transformation of prokaryotic transcriptional activators into eukaryotic cells to construct a "turn-on" xylbiosensor induced by xylose addition, based on prokaryotic activator XylR and eukaryotic universal AD. In addition, the xylbiosensor displayed the potential to applied in engineering pathways involved in xylose utilization and xylose-derived chemical biosynthesis in yeast.

MATERIALS AND METHODS

The original *Y. lipolytica* strain was po1f and the *S. cerevisiae* strain was BY4741. The strains and plasmids used in this study are summarized in Table 1. The complete "Materials and Methods" section is provided in the Supporting Information.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssynbio.0c00122.

Materials and methods: cultivation conditions; DNA manipulation; yeast transformation and construction of strains; determination of VPRHX expression; quantifica-

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tion of biomass and fluorescence; cell imaging; primers used in this study; sequence of *HSFX, VPRX* and *VPRHX;* sequence of artificial hybrid promoter; biosensor construction based on *E. coli* xylose-responsive transcription factor XylR in *Y. lipolytica*; activation domains used for transcription activation in *Y. lipolytica*; determination of VPRHX expression *in vivo* by Western Blotting; response kinetics curves of strain Yl04 in the YPD with different ligands; ligand specificity of xylbiosensor in minimal nutrient medium YNB; imaging of a single *Y. lipolytica* cell Yl04 in the presence of xylose using laser scanning confocal microscopy; time-dependent change of mcherry expression response to xylose in YPD (PDF)

AUTHOR INFORMATION

Corresponding Author

Bangce Ye − *Laboratory of Biosystems and Microanalysis, State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, China; Institute of Engineering Biology and Health, Collaborative Innovation Center of Yangtze River Delta Region Green Pharmaceuticals, College of Pharmaceutical Sciences, Zhejiang University of Technology, Hangzhou, Zhejiang 310014, China; School of Chemistry and Chemical Engineering, Shihezi University, Xinjiang 832000, China;* orcid.org/0000-0002-5555-5359; Email: bcye@ecust.edu.cn

Authors

Wenping Wei − *Laboratory of Biosystems and Microanalysis, State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, China*

Yanzhe Shang − *Laboratory of Biosystems and Microanalysis, State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, China*

Ping Zhang − *Laboratory of Biosystems and Microanalysis, State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, China*

Yong Liu − *Laboratory of Biosystems and Microanalysis, State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, China*

Di You − *Laboratory of Biosystems and Microanalysis, State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, China*

Bincheng Yin − *Laboratory of Biosystems and Microanalysis, State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, China;* orcid.org/0000-0002-4011-4307

Complete contact information is available at: https://pubs.acs.org/10.1021/acssynbio.0c00122

Author Contributions

W.W. was responsible for experimental design, data collection, and writing manuscript. Y.S. and P.Z. participated in plasmid construction. Y.L., D.Y., and B.Yin provided advice for experimental data analysis. B. Ye was responsible for experimental design, data analysis, and final approval of the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

xylbiosensor, xylose-inducible biosensor; VPRHX, VP64-P65-Rta-HSF1-XylR; TF, transcription factors; AD, activation domain; UAS, upstream activation sequence; NLS, nuclear localization signal; BDES, bidirectional expression system; YPD, yeast extract peptone dextrose; YPDX, Yeast extract peptone dextrose xylose; YNB, yeast nitrogen base; RFU, relative fluorescent unit.

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