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Review

Engineering of *Saccharomyces cerevisiae* for co-fermentation of glucose and xylose: Current state and perspectives

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A B S T R A C T

The use of non-food lignocellulosic biomass to produce ethanol fits into the strategy of a global circular economy with low dependence on fossil energy resources. Xylose is the second most abundant sugar in lignocellulosic hydrolysate, and its utilization in fermentation is a key issue in making the full use of raw plant materials for ethanol production and reduce production costs. *Saccharomyces cerevisiae* is the best ethanol producer but the organism is not a native xylose user. In recent years, great efforts have been made in the construction of xylose utilizing *S. cerevisiae* strains by metabolic and evolutionary engineering approaches. In addition, managing global transcriptional regulation works provides an effective means to increase the xylose utilization capacity of recombinant strains. Here we review the common strategies and research advances in the research field in order to facilitate the researches in xylose metabolism and xylose-based fermentation.

1. Introduction

Using renewable lignocellulosic-based fuels and chemicals in the place of non-renewable fossil energy resources is a promising strategy to achieve CO_2 emission reductions and thereby attenuate the related processes responsible for global warming. Bioethanol is considered to be one of the most promising plant-based products. At present, although knowledge of the genetic engineering of metabolic pathways and basic technologies associated with the production and use of bioethanol are available, implementation strategies need to be developed to achieve cost reductions. In general, bioethanol is mixed into gasoline at a volume percentage of ~10%–25% to create a blended fuel [1]. Use of this blended fuel instead of the gasoline fuel helps alleviate energy shortages, reduces CO_2 emissions, and greatly improves the octane rating of fuels. Moreover, the use of bioethanol also helps to reduce environmental pollution caused by use of anti-explosion agents. The first generation of bioethanol was produced from materials rich in starch or sucrose, but doing so caused competition with the consuming by people over food and agricultural land use [2]. However, agricultural waste and other lignocellulosic materials are generated in huge quantities every year, and improper means of disposal, such as incinerating, can cause serious environmental pollution [3]. Producing ethanol from these lignocellulosic materials is therefore a promising way to solve both energy and pollution issues [4].

Saccharomyces cerevisiae is a “generally recognized as safe” microorganism. It is considered to be the best “cell factory” for ethanol production due to its fast fermentation rate, high ethanol yield from glucose, high tolerance to toxic substances in hydrolysates, and high tolerance to ethanol [5]. It is generally recognized that fermentation strains should be modified in two ways to decrease the cost of ethanol production from lignocellulosic materials. First, *S. cerevisiae* strains should be able to ferment xylose, which is the second most abundant sugar present in lignocellulosic hydrolysate, and the fermentation efficiency of xylose would have to be at a level close to glucose [6]. This would enhance the conversion rate of lignocellulose and consequently decrease the cost of raw materials. Second, *S. cerevisiae* strains would have to be more tolerant to the stresses associated with fermentation. These include, among others, acetic acid, furfural, hydroxymethyl furfural, and phenolic compound released from lignocellulose hydrolysis as well as the presence of high concentrations of sugars, various ions, and ethanol in fermentors, all of which are inhibitors of yeast growth and metabolisms [7]. In recent decades, considerable effort has been expended on these two key strategic improvements of *S. cerevisiae* strains. Here we briefly review the work that has been done using metabolic and evolutionary engineering to optimize the characteristics of *S. cerevisiae* to facilitate xylose fermentation and ethanol production. Summaries are also made on the identification of transcription factors and regulatory networks that strongly affect xylose fermentation.

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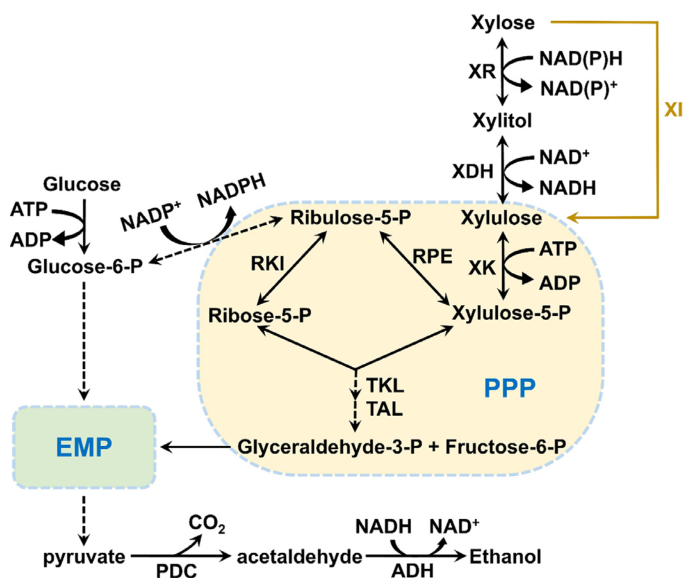

Fig. 1. The xylose metabolic pathways in recombinant *S. cerevisiae*, including the redox pathway and xylose isomerase pathway [30]. Abbreviations: XI, xylose isomerase; XR, xylose reductase; XDH, xylitol dehydrogenase; XK, xylulokinase; RKI, ribose-5-phosphate ketol-isomerase; RPE, ribulose-5-phosphate 3-epimerase; TKL, transketolase; TAL, transaldolase; PDC, pyruvate decarboxylase; ADH, alcohol dehydrogenase.

2. Building xylose metabolic pathways in recombinant *S. cerevisiae* strains

Most natural *S. cerevisiae* strains cannot use xylose because they lack a functional xylose metabolic pathway [8]. Three different pathways have been tried in metabolic engineering attempts to endow *S. cerevisiae* with the capability to ferment xylose. First, in some yeasts and fungi, xylose is reduced to xylitol by xylose reductase (XR), xylitol is then converted to xylulose by xylitol dehydrogenase (XDH), and xylulose is subsequently phosphorylated by xylulokinase (XK). The resulting xylulose-5-phosphate then follows the pentose phosphate pathway (PPP), ultimately entering glycolysis as the intermediates fructose-6-phosphate and glyceraldehyde-3-phosphate where it is further metabolized (Fig. 1) [9]. Several sources of XR and XDH have been introduced into *S. cerevisiae*. Of these, the most common XR and XDH were cloned from *Schiffersomyces (Pichia) stipites* [10]. However, XR preferentially uses NADPH rather than NADH as a cofactor, while XDH uses only NAD^+. This results in a cofactor imbalance and the accumulation of xylitol during fermentation [11]. At least five strategies have been used to resolve the cofactor imbalance to improve ethanol production from xylose. These are as follows: (i) expression of an NADH-dependent XR mutant [12] or an $NADP^+$-dependent XDH mutant [13,14] instead of the wild -type XR or XDH, respectively; (ii) expression of NADH kinases, which convert NADH/NAD^+ to NADPH/$NADP^+$ in the yeast cytoplasm [15]; (iii) regulating the redox state by overexpression of other endogenous coenzyme-dependent reactions. For example, a transhydrogenase-like shunt can be built by overexpressing malic enzyme, malate dehydrogenase, and pyruvate carboxylase [16]; (iv) coupling the xylose metabolism to the consumption of acetic acid, which is a common inhibitor of lignocellulosic hydrolysate; (v) coupling the xylose metabolism to the reduction of furan compounds (i.e., furfural, hydroxymethyl furfural, etc.), which are also common inhibitors of lignocellulosic hydrolysate [17]. These strategies were found to generally reduce xylitol accumulation and increase ethanol production.

Xylose isomerase (XI), which exists in some bacteria, archaea, and a small minority of fungi, directly converts xylose into xylulose (Fig. 1). The XI pathway is considered to be better than the XR-XDH pathway, which requires coenzymes, both with respect to the complexity of the enzymatic reaction and in terms of the theoretical ethanol yield [18]. This pathway became the preferred choice to construct xylose-utilizing *S. cerevisiae* ever since it was revealed that the XI enzyme found in *Piramyces sp.* [19] could be actively expressed in *S. cerevisiae*. Later, more sources of XI genes that can be actively expressed in *S. cerevisiae* were discovered, such as those cloned from *Clostridium phytofermentans* [20], *Orpinomyces sp.* [21], *Ruminococcus flavefaciens* [22], *Prevotella ruminicola* [23], the bovine rumen metagenome [24], *Bacteroides thetaiotaomicron* [25], *and Reticulitermes speratus* [26]. Furthermore, the XI activity in recombinant strains can be increased by increasing the XI gene copy number [27,28] or by facilitating protein folding by co-expression of molecular chaperones [29]. Thus, making use of the XI pathway is a practical strategy for enhancing the xylose fermentation capacity of recombinant strains.

The third xylose metabolic pathway starts with an oxidation reaction, and exists only in some archaea and bacteria [31]. In this pathway, xylose is first converted to xylonolactone by xylose dehydrogenase, and xylonolactone can then be hydrolyzed to xylonate, either spontaneously or with the help of xylonolactonase. Xylonate then loses a water molecule via the catalyzation of xylonate dehydratase to form 2-keto-3-deoxy-pentonate (KDP, also called 2-keto-3-deoxy-xylonate). KDP has at least three different metabolic fates, producing three distinct sets of products in the presence of specific enzymes, including (a) pyruvate and glycol-aldehyde by 2-keto-3-deoxy-pentonate aldolase [32–34], (b) pyruvate and glycolate by various dehydrogenases and hydrolases [35], and (c) α-ketoglutarate by 2-keto-3-deoxy-xylonate dehydratase and α-ketoglutarate semialdehyde dehydrogenase, a pathway is named the Weimberg pathway [36]. These pathways require fewer reaction steps and operate without any loss of carbon or ATP relative to conventional xylose assimilation pathways [37]. The intact Weimberg pathway has been established in yeast [38] by expressing the *Caulobacter crescentus* genes *xylB* (which encodes xylose dehydrogenase), *xylD* (encoding xylonate dehydratase), *xylX* (encoding 2-keto-3-deoxy-xylonate dehydratase), the *Corynebacterium glutamicum* gene *ksaD* (which encodes α-ketoglutarate semialdehyde dehydrogenase), and the deletion of the endogenous *FRA2* gene (which encodes transcriptional inhibitors). The resulting *S. cerevisiae* strain can grow with xylose as the sole carbon source. Recently, the xylose oxidative pathway has been used by *S. cerevisiae* to produce 3,4-dihydroxybutyrate and 3-hydroxybutyrolactone from xylose, which indicates its potential application [37].

3. Optimizing metabolic pathways to increase ethanol yield

3.1. Increasing the downstream metabolic flux for xylose metabolism

Replenishing its defective metabolic pathway is the most important barrier to xylose use by *S. cerevisiae*, since xylose is not a natural carbon source. However, introducing the initial steps of xylose metabolic pathways into *S. cerevisiae* is not sufficient for efficient xylose fermentation. Downstream pathway flux is also a speed-limiting step for xylose fermentation. For recombinant *S. cerevisiae* strains that express the XR-XDH or XI pathways, it is a routine practice to also increase the yeast xylose utilization capacity by increasing the level of xylulokinase and the flux of the non-oxidative phase of the PPP. The genes *XKS1* (or the *XYL3* gene from *S. stipitis*), *RPE1, RKI1, TAL1*, and *TKL1* have been overexpressed in some strains, where they significantly improved xylose utilization [39,40].

3.2. Reducing the production of xylitol

Reducing by-products is a common strategy for increasing the output of target products. For example, for xylose-based ethanol production by *S. cerevisiae*, xylitol is the main by-product. Xylitol itself is an inhibitor of xylose isomerase [41]. Several non-specific aldose reductases are known, including proteins encoded by *GRE3, GCY1, YPR1,*

YDL124W, and *YJR096W*, show XR activity [42], and they could function in the accumulation of xylitol and thereby decreasing ethanol yield during xylose fermentation. Among these aldose reductases, proteins encoded by *GRE3* are found to be the most important sources of XR in *S. cerevisiae* since knocking out of *GRE3* significantly reduces xylitol production, while knocking out of both *GRE3* and *YPR1* genes has been found to reduce xylitol accumulation only to extremely low levels [27]. For this reason, knocking out *GRE3* represents a common strategy for producing xylose-utilizing *S. cerevisiae* strains [43–45].

3.3. Enhancing the xylose transport capacity of S. cerevisiae cells

The efficiency of transporting xylose into *S. cerevisiae* cells is another limiting step in xylose utilization since *S. cerevisiae* has no dedicated xylose transporter. The cross-membrane transport of xylose therefore mainly depends on hexose transporters. A hxt-null *S. cerevisiae* strain, which lacks all endogenous hexose transporters [46], has been widely used to study xylose transport. The endogenous hexose transporters Hxt1, Hxt2, Hxt3, Hxt4, Hxt5, Hxt7, and Gal2 have all been confirmed as capable of transporting xylose [47–50]. Furthermore, it has also been demonstrated that Hxt4, Hxt5, Hxt7, and Gal2 are the most important xylose transporters at moderate xylose concentrations [50]. Moreover, their relative transport capacities follow the ranking Hxt7 > Hxt5 > Gal2 > Hxt1 > Hxt4 [49]. However, hexose transporters are strongly affected by glucose in two ways. First, glucose regulates the transcription of hexose transporter genes. Glucose activates the expression of *HXT1* and *HXT2*, but inhibits the expression of *HXT5, HXT7*, and *GAL2* [49]. Second, the affinity of these transporters to xylose is much lower than their affinity to glucose. As a result, xylose transport is strongly competitively inhibited by the presence of glucose [51].

Efforts have also been made to mine new sources of transporters and to construct their mutants with elevated efficacy of xylose transport. Various heterologous transporters, such as *Ci*Gxf1 and *Ci*Gxs1 from *Candida intermedia*, At5g59250/At5g17010 from *Arabidopsis thaliana, Ps*Sut1, *Ps*Sut2, *Ps*Sut3, *Ss*Xut1, *Ss*Xut3, *Ss*Hxt2.6 and *Ss*Qup2 from *S. stipitis, Mgt*05196 from *Meyerozyma (Pichia) guilliermondii, An*HxtB from *Aspergillus nidulans*, and *Bs*AraE from *Bacillus subtilis*, have all been introduced into *S. cerevisiae.* Their relative efficiencies and affinity for xylose have both been recently reviewed [47]. Moreover, it has been revealed that many of these mutants, including *Ss*Xut3^{E538K} [52], Mgt05196^{F432A} and Mgt05196^{N360S} [53], Hxt11^{N366T} [54], and Hxt7^{F79S} [55], among others, all show higher xylose transport capacity than their respective wild-type proteins. Moreover, the mutants Gal2^{N376F} [56], Gal2$^{N376Y/M435I}$ [57], and Mgt05196^{N360F} [53] have shown interesting and promising characteristics. For instance, each of these mutants no longer transport glucose, and their xylose transport activity is not inhibited by glucose. This trait is highly advantageous for glucose-xylose co-fermentation, especially relative to wild-type proteins.

4. Global transcriptional regulation for enhancing xylose utilization

4.1. Evolutionary engineering and inverse metabolic engineering studies of xylose-utilizing S. cerevisiae

In general, recombinant *S. cerevisiae* strains obtained via metabolic engineering can utilize xylose, but not efficiently. Adaptive evolution is a common and effective strategy to subsequently improve xylose utilization [58,59]. This procedure involves continuously transferring recombinant *S. cerevisiae* strains between media containing xylose as the sole carbon source. Cells with increased growth rates are enriched during this process. Furthermore, anaerobic conditions can be included for conducting evolutionary engineering to obtain the strains that can anaerobically ferment xylose and accumulates ethanol. It is known that glucose acts as a fermentative carbon source for *S. cerevisiae*. That is, glucose inhibits respiration, the process on which ethanol metabolism depends. As a result, ethanol accumulates during glucose fermentation, both under aerobic and anaerobic conditions. However, xylose is not a fermentative carbon source for *S. cerevisiae* [30], and it does not inhibit respiration as strongly as glucose does. This means that ethanol can be co-utilized by the respiratory metabolism of *S. cerevisiae* under aerobic conditions during xylose fermentation. Therefore, maintaining anaerobic conditions is necessary for ethanol accumulation. However, when respiration is inhibited by anaerobic conditions, the ATP produced solely by glycolysis may not be sufficient to support cell growth during xylose fermentation if the xylose absorption rate is not fast enough [60]. Sonderegger & Sauer (2003) evolved recombinant *S. cerevisiae*, which expressed the XR and XDH genes from *P. stipitis* and overexpressed the endogenous XK gene, in a medium where xylose was the sole carbon source. During the evolutionary process, they gradually reduced the oxygen supply until fully anaerobic conditions were reached. Using this procedure, they obtained two classes of evolved strains. Class I strains could not anaerobically ferment xylose but produced more ethanol by glucose-xylose co-fermentation. In contrast, class II strains were capable of strictly anaerobic growth when xylose was the sole carbon source but produced less ethanol during glucose-xylose co-fermentation. Destroying the respiratory chain of recombinant *S. cerevisiae* strains before evolving them on a xylose-only medium also a strategy to obtain a strain that can anaerobically ferment xylose. Strains containing a deletion of the *COX4* gene (which encodes subunit IV of cytochrome c oxidase), using either the XR-XDH [58] or XI pathways [61], significantly increased their xylose fermentation and ethanol accumulation capacity after adaptive evolution.

Following an inverse metabolic engineering strategy, the genomic, transcriptomic, and proteomic profiles of evolved strains were compared with parent strains to identify the genetic elements responsible for improvement in xylose fermentation. In the last decade, a number of genes encoding metabolic enzymes or transcription factors have been found to influence the utilization of xylose. For example, several different research groups have independently confirmed that deleting *PHO13*, a gene encoding a phosphatase, can increase xylose utilization in recombinant *S. cerevisiae* strains [61–64]. In addition, the deletion of *BCY1,* which encodes a regulatory subunit of cyclic AMP-dependent protein kinase (PKA) (Fig. 2), decouples growth from metabolism to enable robust fermentation without division [65], and the deletion of the RAS/PKA inhibitor gene *IRA2* was found to promote xylose-based growth and metabolism (Fig. 2) [65]. Moreover, the double deletion of *IRA2* and *HOG1*, which encodes a component of MAP Kinase (MAPK) signaling (Fig. 2), was associated with elevated levels of mitochondrial respiratory proteins and enabled rapid aerobic respiration of xylose. Another double deletion of *IRA2* and *ISU1*, which encodes a scaffolding protein for mitochondrial iron-sulfur (Fe-S) cluster biogenesis (Fig. 3), affects the anaerobic fermentation of xylose [66,67]. Deletion of *THI2,* a gene encoding a transcriptional activator of thiamine biosynthetic genes, was found to promote ribosome synthesis, the growth rate, the specific xylose utilization rate, and the specific ethanol production rate [68,69]. Finally, Li *et al.* confirmed that deletion of *HOG1, KSS1* (encoding an MAPK involved in the signal transduction pathways that control starvation and pheromone response), and *SMK1* (encoding an MAPK involved in signal transduction pathways controlling sporulation), combined with overexpression of *GCN4* (encoding a global transcriptional activator of amino acid biosynthetic genes) shortened the strain lag phase in media containing xylose as the sole carbon source (Fig. 2). They then further investigated the effect of pathways regulated by Hog1 on xylose utilization, and suggested that glycerol-3-P serves as a key regulatory node during xylose metabolism and could be fine-tuned [27]. Last, we note that many additional studies report significant findings related to metabolic engineering of xylose-utilizing recombinant *S. cerevisiae* strains, these research works fall outside the narrative listed above but listed in Table 1.

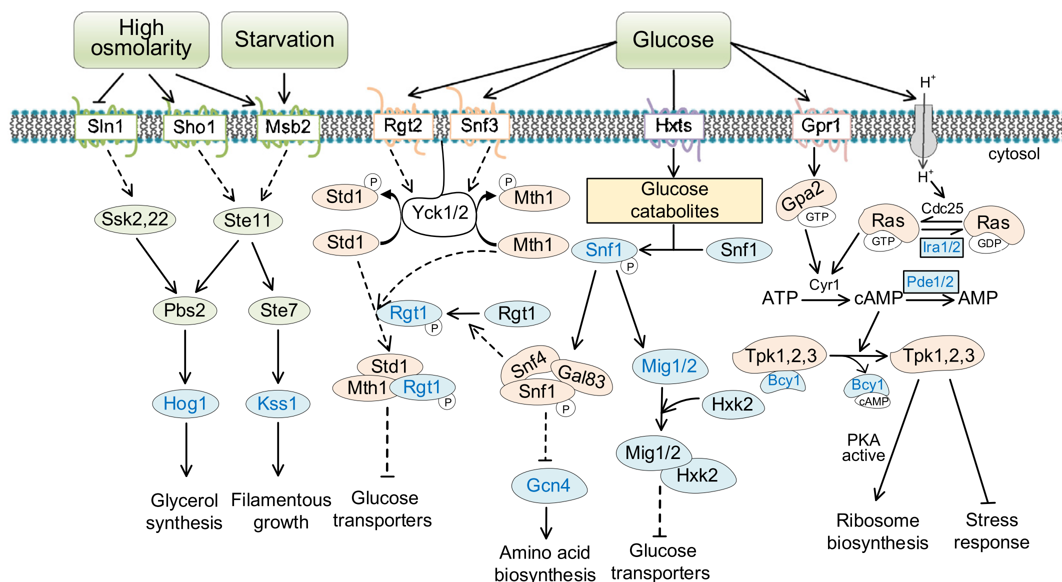

Fig. 2. Signal pathways affecting xylose utilization in *Saccharomyces cerevisiae*. Sln1, Sho1, Msb2, Rgt2, Snf3, Gpr1, and Hxts are cell membrane proteins. Sln1, Sho1, and Msb2 are protein sensors of high osmolarity, and Msb2 also senses starvation. Rgt2, Snf3, and Gpr1 are glucose sensors that signal the Rgt2/Snf3 and cAMP/PKA pathways, respectively. Hxt genes are glucose transporters. Intracellular glucose and glucose metabolites provide regulatory signals for the Snf1/Mig1 pathway. Protein factors with negative effects on xylose utilization are shown in blue font. See the main text for further detail.

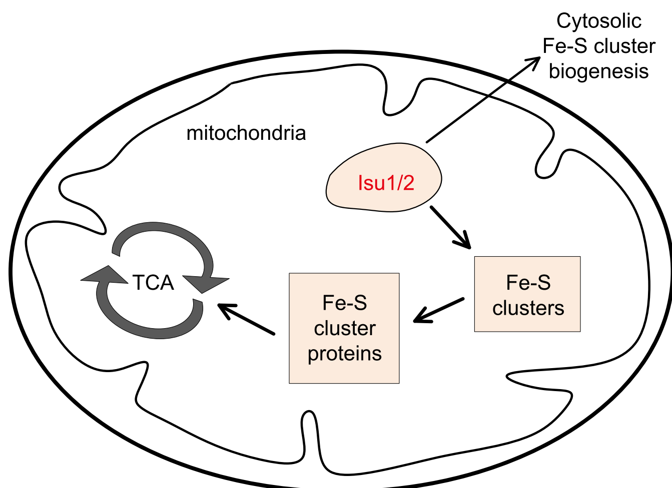

Fig. 3. Mitochondrial genes affect xylose fermentation. For example, Isu1, a scaffolding protein involved in mitochondrial iron-sulfur (Fe-S) cluster biogenesis, was reported to positively affect the anaerobic fermentation of xylose.

4.2. Rational perturbation of transcriptional regulators in central carbon metabolism to achieve efficient xylose utilization

As described in previous sections, efficient xylose utilization requires efficient transporters, the pentose phosphate pathway (PPP), and glycolysis. Furthermore, if the target product is ethanol, the tricarboxylic acid cycle (TCA) and respiration should both be inhibited so that pyruvate, the end product of glycolysis, will be channeled into ethanol instead of completely metabolized to H_2O and CO_2 [67,73]. To coax cells to behave in the desired way, their global gene expression patterns must be modified to the desired state [74]. When glucose is present, *S. cerevisiae* cells maintain a state with highly efficient transporters, PPP, and glycolysis, as well as minimal TCA function. However, after glucose is depleted, cells will shift out this state, which affects xylose utilization. Some glucose response transcriptional regulators have been selected based on their function in central carbon metabolism (Table 1), and their function was then perturbed for efficient xylose utilization.

The transport of xylose into *S. cerevisiae* cells depends on hexose transporters, and the expression of hexose transporter genes is regulated by the Rgt2/Snf3-Rgt1-Mth1/Std1 system (Fig. 2). Rgt2 and Snf3 are cell membrane proteins that sense extracellular glucose and transfer a signal into the cell. Next, co-inhibitors of Rgt1, Mth1, and Std1 are phosphorylated by Yck1 and degraded via the ubiquitin-proteasome system. This relieves the inhibition of Rgt1 on the transcription of hexose transporter genes [80]. Recent work using fluorescent proteins as reporters [85] and real-time fluorescence PCR [80] have independently confirmed that Rgt2 and Snf3 have a very limited capacity to sense xylose and thereby to trigger the expression of hexose transporter genes. Based on these mechanisms, Wu et al. deleted the *RGT1* gene in a xylose-utilizing *S. cerevisiae* strain and confirmed that this deletion positively affects xylose utilization [80].

Snf1 is a protein kinase that responds to glucose starvation and regulates growth and metabolism via controlling the expression and/or phosphorylation of transcription factors, such as Mig1, Rgt1, Atg1, etc. (Fig. 2). The effect of the Snf1 regulatory pathway on xylose utilization has been studied intensely. It was first confirmed that this pathway does not response to xylose [85] and that deletion of *SNF1* accelerated xylose utilization when strains were grown in mixed sugar environments [79]. Second, the disruption of Mig1, which is phosphorylated by Snf1 and inhibits the transcription of hexose transporter genes [86], was shown to have little effect on xylose fermentation [87] or on glucose-xylose co-fermentation [79]. However, deleting both of *MIG1* and *MIG2* increased xylose consumption rates by 12% under constant fermentation [87]. Moreover, the overexpression of a mutant of Mig1 was found to promote xylose utilization of a recombinant strain, although the mechanism responsible remains unclear [88]. Finally, deleting both *MIG1* and *SNF1* has been found to accelerate glucose utilization but slow xylose utilization [79]. These inconsistent results suggest that the effect of the Snf1 regulatory pathway on xylose utilization is complex, and additional research is required to reveal the involved regulatory mechanisms.

The cAMP/PKA pathway is another important signaling pathway that responds to glucose (Fig. 2). Extracellular glucose is captured by the cell membrane protein Gpr1 and this signal is then transferred to a G protein Gpa2. Intracellular glucose signaling is also sensed by Ras1/2.

Table 1
Regulatory proteins thought to affect xylose metabolism in recombinant *S. cerevisiae* strains.

Genes	Effect	Description	References
ADR1	Negative	Carbon source-responsive zinc-finger transcription factor	[70,74]
AFT2	Negative	Iron-regulated transcriptional activator	[74]
ASC1	Negative	G-protein beta subunit and guanine dissociation inhibitor for Gpa2p	[75]
BCY1	Negative	Regulatory subunit of the cyclic AMP-dependent protein kinase (PKA)	[65]
CAT8	Negative	Zinc cluster transcriptional activator; binds carbon source responsive elements	[74]
CDC42	Positive	Small rho-like GTPase; essential for establishment and maintenance of cell polarity	[68]
CYC8	Negative	General transcriptional co-repressor; acts together with Tup1p; also acts as part of a transcriptional co-activator complex that recruits the SWI/SNF and SAGA complexes to promoters	[76]
FPS1	Positive	Aquaglyceroporin, plasma membrane channel	[77]
FRA2	Negative	Cytosolic protein involved in repression of iron regulon transcription	[38]
GCN4	Positive	bZIP transcriptional activator of amino acid biosynthetic genes	[27]
GCR2	Negative	Transcriptional activator of genes involved in glycolysis	[72]
GIS1	Negative	Histone demethylase and transcription factor; regulates genes during nutrient limitation	[74]
GPN3	Positive	Putative GTPase with a role in biogenesis of RNA pol II and polIII	[68]
HAA1	Positive	Transcriptional activator involved in adaptation to weak acid stress	[71]
HAP4	Negative	Transcription factor; subunit of the heme-activated, glucose-repressed Hap2p/3p/4p/5p CCAAT-binding complex	[70,74]
HOG1	Negative	Mitogen-activated protein kinase involved in osmoregulation	[65]
HXK2	Negative	Hexokinase isoenzyme 2 activity in the cytosol, a regulator of several genes in the nucleus	[59,78]
IRA2	Negative	GTPase-activating protein; negatively regulates RAS by converting it from the GTP- to the GDP-bound inactive form	[65]
ISU1	Positive	Conserved protein of the mitochondrial matrix; performs a scaffolding function during assembly of iron-sulfur clusters	[66]
KSS1	Negative	Mitogen-activated protein kinase (MAPK); involved in signal transduction pathways that control filamentous growth and pheromone response	[27]
MID2	Positive	O-glycosylated plasma membrane protein; acts as a sensor for cell wall integrity signaling and activates the pathway	[68]
MIG1	No significant effect	Transcription factor involved in glucose repression; sequence specific DNA binding protein containing two Cys2His2 zinc finger motifs	[79]
MSN2/ MSN4	Negative	Stress-responsive transcriptional activator; activated in stochastic pulses of nuclear localization in response to various stress conditions	[74]
NRM1	Positive	Transcriptional co-repressor of MBF-regulated gene expression	[69]
PDE1/ PDE2	Negative	Cyclic AMP phosphodiesterase; controls glucose and intracellular acidification-induced cAMP signaling, target of the cAMP-protein kinase A (PKA) pathway	[80]
PEX34	Positive	Protein that regulates peroxisome populations	[81]
RCK1	Positive	Protein kinase involved in oxidative stress response	[82]
RGI2	Positive	Protein of unknown function; involved in energy metabolism under respiratory conditions	[68]
RGT1	Negative	Glucose-responsive transcription factor; regulates expression of several glucose transporter (HXT) genes in response to glucose	[80]
SIP4	Positive	C6 zinc cluster transcriptional activator; binds to the carbon source-responsive element (CSRE) of gluconeogenic genes; involved in the positive regulation of gluconeogenesis	[70]
SMK1	Negative	Middle sporulation-specific mitogen-activated protein kinase (MAPK); required for prospore membrane development and the production of outer spore wall layers	[27]
SNF1	Negative	AMP-activated S/T protein kinase; complexes with Snf4p and a Sip1p/Sip2p/Gal83p family member; required for glucose-repressed gene transcription, heat shock, sporulation, and peroxisome biogenesis	[79]
TFC3	Positive	Subunit of RNA polymerase III transcription initiation factor complex	[68]
THI2	Negative	Transcriptional activator of thiamine biosynthetic genes; interacts with regulatory factor Thi3p to control expression of thiamine biosynthetic genes with respect to thiamine availability	[68,69]
TUP1	Positive	General repressor of transcription, forms complex with Cyc8p	[70]
TYE7	Positive	Serine-rich protein that contains a bHLH DNA binding motif	[83]
USV1	Negative	Putative transcription factor containing a C2H2 zinc finger	[74]
ZNF1	Positive	Zinc cluster transcription factor that regulates respiratory growth	[84]

Both Ras1/2 and Gpa2 can active the cAMP/PKA pathway, which regulates cell growth, metabolism, and stress response in *S. cerevisiae*. However, neither Ras1/2 nor Gpr1-Gpa2 responds to xylose [85]. To perturb this pathway, Wu et al. expressed the constitutively active mutants Gpa2^{G132V} and Ras2^{G19V}, respectively, or deleted both *Pde1* and *Pde2* genes, which increase PKA signaling by catalyzing the conversion of cAMP to AMP. These operations were found to improve xylose utilization and ethanol production [80].

To improve the validity of regulatory targets, Michael et al. (2016) presented a broadly applicable algorithm for transcriptome engineering, which was defined as “designing deletion or overexpression of transcription factors to move cells to a gene expression state that is associated with a desired phenotype.” Based on this algorithm, they proposed that the knockout of eight transcription factors—i.e., Cat8 (catabolite repression), Hap4 (heme activator protein), Adr1 (alcohol dehydrogenase II synthesis regulator), Msn2 and Msn4 (multicopy suppressor of SNF1 mutation 2 and 4), Gis1 (GIg1–2 suppressor), Aft2 (activator of Fe (iron) transcription), and Usv1 (up in starvation)—may force cells to improve xylose utilization. Their experimental results showed that knockout of these transcription factors brought the cells closer to the designed transcriptional state; for example, the knockout of *CAT8* changes metabolic gene transcription levels, reduced the Euclidean distance to the goal state by 60%. Unfortunately, the carbon flow to both biomass and ethanol decreased [74]. Furthermore, a recent study also reported that the deletion of *HAP4* lead to a 1.8-fold increase in ethanol production from xylose compare to its parental strain [70]. Although the current algorithm is not precise, the transcriptome engineering model has great potential to become a powerful research tool in the future, given continuous improvement in knowledge of the metabolic regulatory system and further optimization of the algorithm.

5. Conclusions

Utilization of xylose is an unavoidable issue in the production of biofuels and chemicals from lignocellulosic materials. In recent decades, great progresses have been made both in the study of metabolic mechanisms and in genetic engineering techniques. The construction of recombinant strains of *S. cerevisiae* that co-ferment glucose and xylose to produce the fuel ethanol provides a good example for how to design research strategies. A general scheme for such research includes 4 aspects: (a) identification of specific metabolic enzymes, (b) optimizing connected metabolic pathways, (c) improving xylose utilization through irrational adaptive evolution, and (d) global transcriptional regulation by disturbing specific regulatory proteins. An algorithm has been designed for selecting target regulatory proteins, and more accurate algorithms can be expected as the understanding of regulatory networks deepens. A number of new regulators effecting xylose metabolism have been identified. Intriguingly, some of the regulators of the xylose metabolism also function in stress-responsive regulation, such as Hog1 and Msn2/4. Future studies may put a focus on the coordination between cellular metabolisms and stress resistance pathways to further facilitate the cell biology research of *S. cerevisiae* and to enhance its efficacy and robustness in ethanol production using plant raw materials.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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