



The pentose phosphate pathway in industrially relevant fungi: crucial insights for bioprocessing

Audrey Masi^{1,2} · Robert L. Mach² · Astrid R. Mach-Aigner^{1,2}

Received: 4 March 2021 / Revised: 4 March 2021 / Accepted: 20 April 2021 / Published online: 5 May 2021
© The Author(s) 2021

Abstract

The pentose phosphate pathway (PPP) is one of the most targeted pathways in metabolic engineering. This pathway is the primary source of NADPH, and it contributes in fungi to the production of many compounds of interest such as polyols, biofuels, carotenoids, or antibiotics. However, the regulatory mechanisms of the PPP are still not fully known. This review provides an insight into the current comprehension of the PPP in fungi and the limitations of this current understanding. It highlights how this knowledge contributes to targeted engineering of the PPP and thus to better performance of industrially used fungal strains.

Key points

- *Type of carbon and nitrogen source as well as oxidative stress influence the PPP.*
- *A complex network of transcription factors regulates the PPP.*
- *Improved understanding of the PPP will allow to increase yields of bioprocesses.*

Keywords Pentose phosphate pathway · Industrially relevant fungi · Yeast · Filamentous fungi

Introduction

In the early 1920s, Otto Warburg, a German Nobel laureate, investigated the mechanism of oxygen consumption in animal cells. His work was the beginning of the discovery of the pentose phosphate pathway (PPP). In the 1930s, he discovered the first flavoprotein and a non-protein component that is essential for the enzyme activity and acts as an electron carrier: the diphosphopyridine nucleotide DPN (now called NAD⁺ (nicotinamide adenine dinucleotide)) (Warburg 1932; Warburg and Christian 1936). He studied the oxidation of glucose-6-phosphate (G6P) to 6-phosphogluconate (6PG) by an enzyme, which he called *Zwischenferment* (ZWF1) or

intermediate enzyme and is now called glucose-6-phosphate dehydrogenase (G6PDH). He found a second co-enzyme that he named TPN (triphosphopyridine nucleotide, now known as NADP⁺ (nicotinamide adenine dinucleotide phosphate)). The discovery of the requirement of TPN for the oxidation of G6P and also for the oxidation of 6PG prompted Warburg and his team to propose the existence of a “direct oxidative pathway” (Horecker 2002). They formulated the hypothesis that this pathway would be an alternative to glycolysis and is involved in cellular respiration. Franck Dickens and Fritz Lipmann, among other biochemists, who investigated cell respiration, shared this view (Horecker 2002).

This work was pursued in the USA by a member of Warburg’s laboratory, Erwin Haas, joined by Bernard Horecker. During the 1950s, the work of Horecker’s group allowed to elucidate the PPP and to propose a first description of it in animals (Gunsalus et al. 1955). This significant progress was made possible by the development of analytical methods, such as ion chromatography, as well as by discoveries made by other research teams including the teams of Dickens, Racker, Cohen, Lampen, Dickens, and Ashwell (Dickens 1938; Dickens and Glock 1951; Dickens and Williamson 1956; Horecker 2002).

✉ Astrid R. Mach-Aigner
astrid.mach-aigner@tuwien.ac.at

¹ Christian Doppler Laboratory for Optimized Expression of Carbohydrate-Active Enzymes, Institute of Chemical, Environmental and Bioscience Engineering, TU Wien, Gumpendorfer Str. 1a, A-1060 Vienna, Austria

² Institute of Chemical, Environmental and Bioscience Engineering, TU Wien, Gumpendorfer Str. 1a, A-1060 Vienna, Austria

In the 1960s and 1970s, the first research groups started investigating the regulation of the PPP in yeasts and filamentous fungi. The interest in the PPP in fungi increased at the end of the 1990s with the development of metabolic engineering for industrial biotechnology. Due to its central role in carbon metabolism, the PPP became a critical target to increase the production of molecules of interest in industrially used fungal strains (Stincone et al. 2015). Since then, the PPP was investigated in many organisms and was shown to be an ubiquitous pathway present in every eukaryote and most prokaryotes. Figure 1 presents the current understanding of the pathway.

With the exception of archaea, the currently known structure of the PPP is conserved among most prokaryotes and eukaryotes. However, even if archaea have a different pathway organization, it includes similar reactions suggesting a very ancient origin of the PPP in evolution (Bräsen et al. 2014). Table 1 summarizes the enzymes associated with the PPP in different organisms.

Most archaea do not have enzymes for the oxidative pentose phosphate pathway (OPPP) except orthologs of the 6-phosphogluconate dehydrogenase (6PGDH), which were identified mainly in halophilic archaea. And just a few archaea have a complete non-oxidative pentose phosphate pathway (NOPPP) (Soderberg 2005; Oost and van der Siebers 2007). *Methanococci* pose an exception here: their non-oxidative phase is similar to the standard PPP; however, they do not have any of the enzymes of the oxidative phase. The hypothesis is that they do not require the oxidative phase for the synthesis of ribose-5-phosphate (R5P) because it is formed through the reversible reactions of the non-oxidative phase (Soderberg 2005; Oost and van der Siebers 2007). The conservation of the NOPPP among archaea suggests an ancient evolutionary origin of the NOPPP, while the OPPP seems to arise more recent in evolution.

The oxidative phase comprises three irreversible reactions, while the non-oxidative phase is composed of reversible reactions only. The oxidative phase starts with the oxidation of the G6P, a substrate generated during glycolysis. Thus, this first reaction is a metabolic crossroad between the glycolysis and the PPP. The results of the oxidative phase are two molecules of NADPH and ribulose-5-phosphate (Ru5P). Ru5P then enters the non-oxidative phase of the PPP. In the non-oxidative phase, the cells need to control the number of reactions and their orientation. The metabolites involved in this phase, either as substrates or products, are also involved in other pathways. This connection to other pathways assigns a central role in metabolism to the NOPPP. For example, glyceraldehyde-3-phosphate (G3P) and fructose-6-phosphate (F6P) are intermediate products of the glycolysis and linked to the NOPPP, and erythrose-4-phosphate (E4P) is a product of the NOPPP and at the same time a precursor for the shikimate pathway. The latter links carbon metabolism with the biosynthesis of aromatic compounds such as phenylalanine, tryptophan, and

Fig. 1 Schematic drawing of the pentose phosphate pathway, its enzymes, and its connection to glycolysis. Single arrows indicate a non-reversible reaction, and double arrows indicate a reversible reaction. The oxidative pentose phosphate pathway and the non-oxidative pentose phosphate pathway are identified by purple or yellow background, respectively. Red arrows indicate links to other biochemical pathways. Abbreviations of glycolysis enzymes: HK, hexokinase; GPI, glucose-6-phosphate isomerase; PFK, phosphofructokinase 1; FBA, fructose-bisphosphate aldolase; TPI, triosephosphate isomerase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PK, pyruvate kinase (Stincone et al. 2015)

tyrosine. The PPP also leads to the production of R5P, which serves as a precursor for the synthesis of nucleotides. These strong connections between the NOPPP and other biochemical pathways might require a dynamic regulation of the involved enzymes (Stincone et al. 2015).

Summarizing, the PPP has three main functions: (i) increasing the reducing power of the cell through NADPH production; (ii) the production of R5P, which is a precursor for nucleotide biosynthesis; and (iii) synthesis of E4P, a precursor for aromatic amino acid biosynthesis. The PPP also allows the synthesis and degradation of four-, five-, and seven-carbon sugars. Considering such a central role in carbon metabolism, the PPP unsurprisingly is one of the most targeted pathways in metabolic engineering. Obviously, the understanding of the mentioned regulation of the PPP is highly beneficial for knowledge-based pathway engineering.

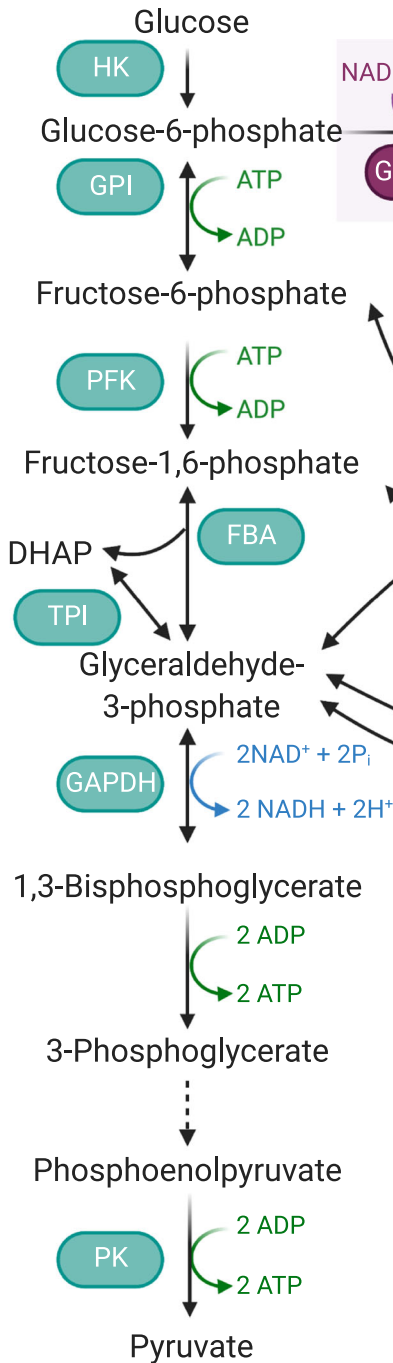
Regulation of the PPP

Earlier studies reported a regulatory influence of carbon sources and nitrogen sources on the PPP and linked this to the NADPH production by the PPP. The role of the PPP as NADPH producer was also the primary explanation for its participation in the redox response. Nevertheless, recent studies have shown that the regulatory mechanisms are more complex than previously believed. Only the capacity of the PPP to produce NADPH is insufficient to explain its involvement in the redox response. Additionally, the regulatory mechanisms seem to operate at multiple levels, i.e., transcription, translation, enzyme activity, and metabolism.

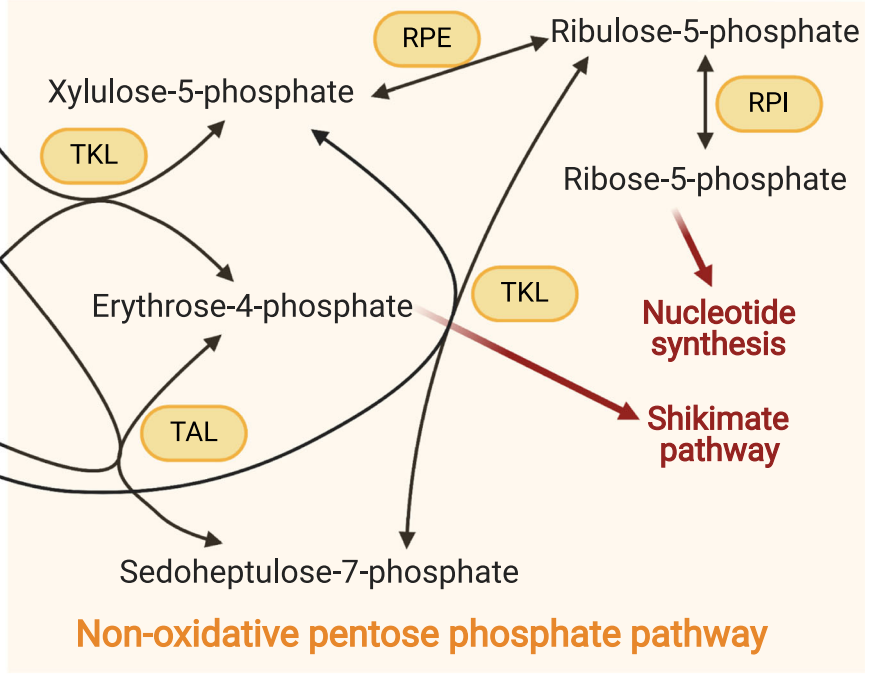
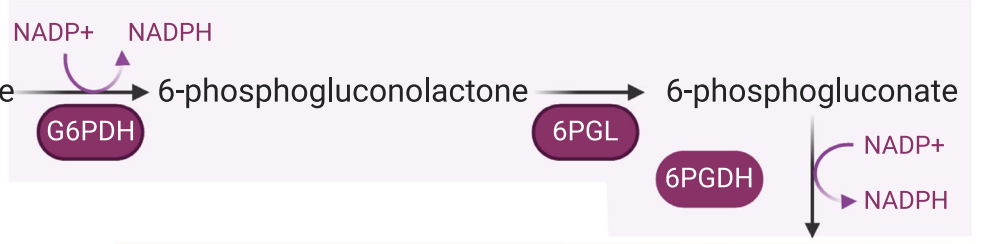
Regulation of the PPP in response to oxidative stress

The PPP produces NADPH during the OPPP and, therefore, is a significant source of NADPH for the cells. NADPH is a primary source of electrons in anabolic reactions, such as fatty acid biosynthesis, nucleotide biosynthesis, and glutathione reduction. Due to its involvement in reducing glutathione, NADPH is essential for resistance of cells to oxidative stress. Thus, the capacity of the PPP to generate NADPH was linked to a role in oxidative stress response (Bruinenberg et al. 1983).

Glycolysis



Oxidative pentose phosphate pathway



Non-oxidative pentose phosphate pathway

Enzymes of the PPP	
G6PDH	Glucose-6-phosphate dehydrogenase
6PGL	6-phosphogluconolactonase
6PGDH	6-phosphogluconate dehydrogenase
RPI	Ribose-5-phosphate isomerase
RPE	Ribulose-5-phosphate 3-epimerase
TKL	Transketolase
TAL	Transaldolase

Roles of the PPP

- Synthesis of precursor of nucleotide synthesis
- Synthesis of precursor of aromatic amino acids synthesis (Shikimate pathway)
- Formation of NADPH

Table 1 Presence of the PPP enzymes among the three domains (Soderberg 2005; Oost and van der Siebers 2007)

Enzyme	Abbr.	EC no.	Present in archaea	Present in bacteria	Present in eukarya
Phosphohexulose isomerase	PHI	5.3.1.27	Except for some halophiles and Thermoplasmatales	Methylotrophs in particular	/
Hexulose phosphate synthase	HPS	4.1.2.43	Except for some halophiles and Thermoplasmatales	Methylotrophs in particular	/
Glucose-6-phosphate dehydrogenase	G6PDH	1.1.1.49	Haloarchaea	✓	✓
6-phosphogluconolactonase	6PGL	3.1.1.31	×	×	✓
6-phosphogluconate dehydrogenase	6PGDH	1.1.1.44	×	✓	✓
Ribose-5-phosphate isomerase	RPI	5.3.1.6	✓	✓	✓
Ribulose-5-phosphate 3-epimerase	RPE	5.1.3.1	<i>Methanococcus</i> spp., Thermoplasmatales, not in most other archaea	✓	✓
Transketolase	TKL	2.2.1.1	<i>Mca. jannaschii</i> , Thermoplasmatales, Thermococcales, Sulfolobales, Thermoproteales, not in most haloarchaea, other methanogens and Archaeoglobales		✓
Transaldolase	TAL	2.2.1.2	<i>Methanococcus</i> spp., Thermoplasmatales	✓	✓

However, several findings from studies performed in *Saccharomyces cerevisiae* suggest that the role of PPP in oxidative stress response goes beyond NADPH generation only.

For example, strains deficient for enzymes of the NOPPP were sensitive to oxidants, although NADPH is only produced in the OPPP (Juhnke et al. 1996; Ralser et al. 2009). On the other hand, an accumulation of intermediates of the NOPPP was observed upon cell exposition to oxidants (Ralser et al. 2007; Ralser et al. 2009).

In another study, deletion strains were constructed in *S. cerevisiae* lacking the G6PDH (which is ZWF1 in *S. cerevisiae*) and/or one of the NOPPP enzymes, i.e., either the transketolase 1 (TKL1) or the ribulose-5-phosphate 3-epimerase 1 (RPE1) or the transaldolase 1 (TAL1) (Krüger et al. 2011). Strains lacking ZWF1 cannot use the PPP to reduce NADP⁺ because this is the first reaction of the PPP and non-reversible. Interestingly, among the double deletion strains, the one lacking TAL1 and ZWF1 was the only viable strain. This strain was exposed to oxidative stress generated via growth on agar plates containing 1 mM H₂O₂ and compared to single deletion strains of ZWF1 and TAL1. The single deletion strain of TAL1 was not sensitive to oxidative stress, the ZWF1 deletion strain was sensitive to oxidative stress, and the double deletion strain was more sensitive to oxidative stress than the ZWF1 single deletion strain. Further, the authors expressed a sedoheptulokinase (SHPK) to increase the flow through the PPP in the cell. SHPK is an enzyme, absent in yeasts, which converts sedoheptulose in sedoheptulose-7-phosphate (S7P), an intermediate of the NOPPP. Strains expressing the SHPK were more resistant to H₂O₂ exposition than the wild type, suggesting that the increase of the flux through the NOPPP increases resistance to oxidative stress. The investigation was continued by generating more strains bearing deletions of enzymes of the PPP and exposing the generated strains to three different

oxidants: H₂O₂, diamide, and cumene-hydroperoxide (CHP). Strains with deletion of ZWF1 or RPE1 or TKL1 were more sensitive to the three tested oxidants than the wild type. A SOL4 deletion strain (deficient in 6-phosphogluconolactonase (6PGL)) was more sensitive to diamide but more resistant to CHP and H₂O₂, while a transketolase 2 (TKL2) deletion strain was more sensitive to H₂O₂ solely. TKL2 is a paralogue of TKL1 and arose from whole genome duplication. A TAL1 deletion strain was more sensitive to H₂O₂ but more resistant to diamide. A SOL3 deletion strain (deficient in 6PGL, *sol3* and *sol4* are two paralogous genes) had an increased resistance to each of the tested oxidants. The deletion strains were also exposed to other environmental stress conditions (NaCl for salt stress, sorbitol for osmotic stress, and MnCl₂ for heavy metal stress) to see whether the response observed before was specific to oxidative stress. Interestingly, none of the mutants exhibited a stress phenotype. Finally, the authors measured the ratio of NADPH/NADP⁺ in cell-free extracts of the strains bearing deletion(s) of NOPPP enzyme(s) and compared it to the ratio in the wild-type exposed to oxidant stress. They found a similar ratio of NADPH/NADP⁺; consequently, this does not explain the changed response to the oxidants by the mutant strains (Krüger et al. 2011).

In the same study, LC-MS/MS (liquid chromatography coupled with tandem mass spectrometry) was used to quantify the PPP intermediates (i.e., 6PGL, R5P, and S7P) produced by the wild-type and the deletion strains exposed to H₂O₂. In the wild-type strain and in the SOL3 deletion strain, H₂O₂ treatment triggered no or only a small increase of the intermediates (below 10-fold; maximum concentration below 10 nmol·ml⁻¹·OD600⁻¹). In contrary, in the TAL1, RPE1, and TKL1 deletion strains, the concentrations of 6PG, R5P, and S7P increased strongly (up to 100-fold; concentrations up to 350 nmol·ml⁻¹·OD600⁻¹) (Krüger et al. 2011).

To understand how these changes are regulated, Krüger et al. investigated the mutant strain MR105. The MR105 strain carries a deletion of the endogenous triosephosphate isomerase (TPI) and expresses a mutant allele. In this strain, the TPI activity is just 30% compared to that of the wild type. It was shown that intermediates of the PPP are increased in MR105. This strain has also an increased resistance to oxidants and an increased NADPH/NADP⁺ ratio (Ralsler et al. 2006; Ralsler et al. 2007).

A transcriptome analysis was performed to compare changes in gene expression caused by the deficiencies of enzymes of the PPP or by H₂O₂ exposition (Krüger et al. 2011). The strain MR105 was used as a control as it has increased concentrations of intermediates of the PPP independent from oxidative stress. The transcriptomes of six conditions were analyzed, i.e., the MR105 strain, the wild-type strain, the wild-type strain treated with H₂O₂, and strains bearing deletion of TAL1, TKL1, or ZWF1, respectively. Transcriptomes of the strain MR105 and the wild-type treated with H₂O₂ shared 40% of the targeted genes, which amount for 140 transcripts. This result is surprising because H₂O₂ treatment creates an oxidative environment, whereas a decrease in TPI activity shifts the NADPH/NADP⁺ ratio towards a reductive environment (Ralsler et al. 2007). Moreover, only 60 of the 140 transcripts were found to correlate with the redox state, i.e., had a different level in the MR105 strain compared to the H₂O₂-treated wild type. Thus, the authors proposed a regulation of the PPP due to the change in primary carbon metabolism rather than to the redox state (Krüger et al. 2011). Finally, the authors performed further investigations on transcript levels employing RT-qPCR and proteomics on targets belonging to the gene ontology categories of cellular respiration and chromatin assembly and disassembly. These measurements were performed with the wild-type and single deletion strains of TKL1, TAL1, or ZWF1. They observed a defect in timing and induction of gene expression during the stress response in the deletion strains. Thus, the regulation of gene expression during the oxidative stress response requires the activation of these enzymes (Krüger et al. 2011).

Regulation of the PPP in response to nitrogen

Osmond and Rees first reported an influence of the nitrogen source on the activity of the PPP enzymes in *Candida utilis* (Osmond and Ap Rees 1969). Nevertheless, their work did not provide any insight into the possible molecular regulatory mechanism. Some years later, Hankinson and Cove observed in *Aspergillus nidulans* an increased activity of the PPP enzymes when the fungus was grown in the presence of nitrate. Their work led to the first description of the PPP regulation by nitrate in filamentous fungi (Hankinson and Cove 1972; Hankinson 1974; Hankinson and Cove 1974).

Proposed mechanism in *C. utilis* by Osmond and Rees

After growth of the yeast with nitrate or yeast extract as nitrogen source, Osmond and Rees compared the activities of enzymes of the PPP and of glycolysis. The activities of two PPP enzymes, i.e., the G6PDH and the TKL, were 2.5-fold higher in the presence of nitrate. The other enzymes of the PPP showed a smaller increase in activity. Then, the yeast was pre-grown on a complex medium, starved from nitrogen, and separated in two subcultures; one was transferred to a complex medium containing nitrate, the other was transferred to the same medium supplemented with cycloheximide. After this transfer, the activity of the previously mentioned two enzymes increased; however, in the presence of cycloheximide, this was not observed. Further, it was shown that the cycloheximide effect is reversible. Cycloheximide inhibits protein synthesis by inhibiting the translational elongation suggesting that nitrate does not affect the enzyme activities directly but influences their biosynthesis. Unfortunately, Osmond and Rees did not suggest any molecular mechanism. They hypothesized that the need for NADPH regulates the PPP and argued that the formation of amino acids from nitrate requires NADPH, and the primary source of NADPH is the PPP. Thus, when grown on nitrate, yeasts would increase the activity of the G6PDH, this increased activity would lead to a higher flux through the PPP, and consequently, more NADPH would be produced.

In case of the TKL, Osmond and Rees speculated that the increased activity might avoid an accumulation of E4P, which would inhibit the glucose-6-phosphate isomerase (GPI), leading to an inhibition of the glycolysis (Osmond and Ap Rees 1969). However, no experimental proof supported this hypothesis, and the authors again did not provide any molecular regulatory mechanism. Hankinson and Cove, starting with a similar experiment, provided a breakthrough with the first hypothesis of the molecular regulation of the PPP by nitrogen (Hankinson and Cove 1972; Hankinson and Cove 1974).

Proposed mechanism in *A. nidulans* by Hankinson and Cove

Hankinson and Cove grew an *A. nidulans* wild-type strain in the presence of urea or urea and nitrate as nitrogen source. They observed a 2-fold higher activity of four enzymes of the PPP, i.e., the G6PDH, the 6PGDH, the TKL, and the TAL, and a 3-fold higher activity of the GPI compared to growth without nitrate. The ribose-5-phosphate isomerase (RPI) activity was not significantly affected in the presence of nitrate (Hankinson and Cove 1974).

Then, Hankinson and Cove performed the same experiment with mutants for the *niaD* gene (*niaD*-) and the *cnx* gene (*cnx*-). The *niaD* gene encodes a nitrate reductase, and *cnx* encodes a protein required for the synthesis of precursor Z, an intermediate of the molybdopterin cofactor pathway. The molybdopterin cofactor is required for the activity of a nitrate reductase.

Consequently, mutations in the *niaD* locus or in any of the five *cnx* loci cause loss of nitrate reductase activity; hence, these mutants are unable to metabolize nitrate. They also generated a defective mutant for *niiA* (*niiA-4*), which is the nitrite reductase encoding gene, and a mutant for *nirA* (*nirA-1*). NirA positively controls the expression of *niaD* and *niiA* genes.

Surprisingly, in certain *niaD*- and *cnx*- mutants, nitrate increased the activities of PPP enzymes although the mutants cannot metabolize nitrate. Other mutants had high activities of these enzymes even in absence of nitrate. Due to these findings, it was concluded that neither nitrate nor nitrite can directly trigger the increased enzyme activity (Hankinson and Cove 1974). Further, in the *nirA-1* mutants, nitrate did not increase the activity of the PPP enzymes. Other, the *niiA-4* mutants responded to nitrate with increased activity of the PPP enzymes; however, they are only able to reduce nitrate to nitrite. Consequently, nitrate is not a direct inducer of the observed increase of the activity of PPP enzymes but a yet unknown factor that serves as mediator, which was called the *nir* product. These results also suggest that a modification of the concentration of NADP⁺/NADPH is not the cause of the increased activity of the PPP enzymes. Hankinson and Cove proposed the hypothesis that nitrate activates the expression of the *nir* product, which causes the increase in PPP enzyme activity: when the *nir* product is present in an active state, the activities of the PPP enzymes are high; when the *nir* product is present in an inactive state, these activities are low (Hankinson and Cove 1974).

The authors also addressed the point that the obtained results differ from the results reported in *C. utilis*. In *C. utilis* nitrate increased the activity of the G6PDH and the TKL, however, only to a very low extent the 6PGDH activity. Hankinson and Cove hypothesized that this difference can be explained by the reduced contribution of the malic enzyme, the pyridine nucleotide transhydrogenase, and the mannitol dehydrogenase to provide NADPH in *A. nidulans* under the tested growth conditions (Hankinson and Cove 1974). Based on the current knowledge (Horecker 2002), they assumed that the malic enzyme in yeasts might generate more NADPH during growth on glucose than in *A. nidulans*. In *A. nidulans* otherwise, the G6PDH and the 6PGDH might be the main source of NADPH; thus, if an increase of the NADPH concentration is needed, an increased activity of these two enzymes is necessary. A latter work from Hankinson and Cove confirmed that indeed also the mannitol dehydrogenase has a reduced contribution to NADPH generation in *A. nidulans* (Hankinson and Cove 1975).

From the hypothesis of Cove and co-workers to the current model

The current understanding of nitrate regulation of the PPP is summarized and described in Fig. 2 (Hankinson and Cove 1974; Krappmann and Braus 2005; Han et al. 2016).

Regulation of the PPP in response to the carbon source

Several studies reported an effect of the carbon source on the activities of the PPP enzymes; however, most studies propose that this effect is indirect (Bruinenberg et al. 1983; Witteveen et al. 1989; Peleato et al. 1991; David et al. 2005; David et al. 2006; Runquist et al. 2009; Khosravi et al. 2018). As mentioned above, the flux through the PPP might be stimulated by the demand of the cell for NADPH. When D-xylose or L-arabinose are used as carbon source, they are metabolized into xylulose-5-phosphate to enter the glycolysis. Thus, no NADPH is formed; however, the cell still needs NADPH for other biochemical reactions (Witteveen et al. 1989; Peleato et al. 1991; David et al. 2005). Consequently, the cell might activate the PPP to produce more NADPH. The total theoretical need for NADPH of a fungal cell has been modeled for *C. utilis* (Bruinenberg et al. 1983; Bruinenberg 1986). With such a model, it is possible to estimate the demand of NADPH based on the carbon and nitrogen source available and to estimate whether the flow towards the PPP will be increased or decreased under these conditions. Notably, stoichiometric models have been established for *A. niger* and *Penicillium chrysogenum* (Jorgensen et al. 1995; David et al. 2003; Melzer et al. 2007).

In *A. niger*, genes of the PPP are upregulated during growth on D-xylose as a sole carbon source (Battaglia et al. 2014). In this work, the expression of PPP genes was compared on the one hand between an *xlnR* deletion strain and the wild-type grown on D-xylose and on the other hand between an *araR* deletion strain and the wild-type grown on L-arabinose.

XlnR is the transcriptional activator of the xylanolytic and cellulolytic system (van Peij et al. 1998). It notably also controls the pentose catabolic pathway (Hasper et al. 2000). The D-xylose catabolic genes are expressed in response to the presence of D-xylose. However, this induction by D-xylose does not impact the transcript level of *xlnR* (Mach-Aigner et al. 2012). *AraR* encodes the transcriptional activator of the L-arabinose catabolic system (de Groot et al. 2007).

Analysis of gene expression was first performed with RNA microarray. In the *araR* deletion strain grown on L-arabinose, seven genes coding for PPP enzymes showed significantly reduced expression compared to the wild type. In the *xlnR* deletion strain grown on D-xylose, the expression of a transaldolase (*talB*) and a transketolase (*tktB*) decreased significantly and, however, were not affected in the *araR* deletion strain. Only one gene was significantly altered in the *xlnR* and the *araR* deletion strain: the RPI-encoding gene *rpiA*. Based on these initial microarray results, some genes were selected for analysis by Northern blot that confirmed the results. Additionally, a Northern blot was performed with RNA from a double deletion *araR/xlnR* strain cultivated on D-xylose or L-arabinose. In this strain, the expression of five PPP genes

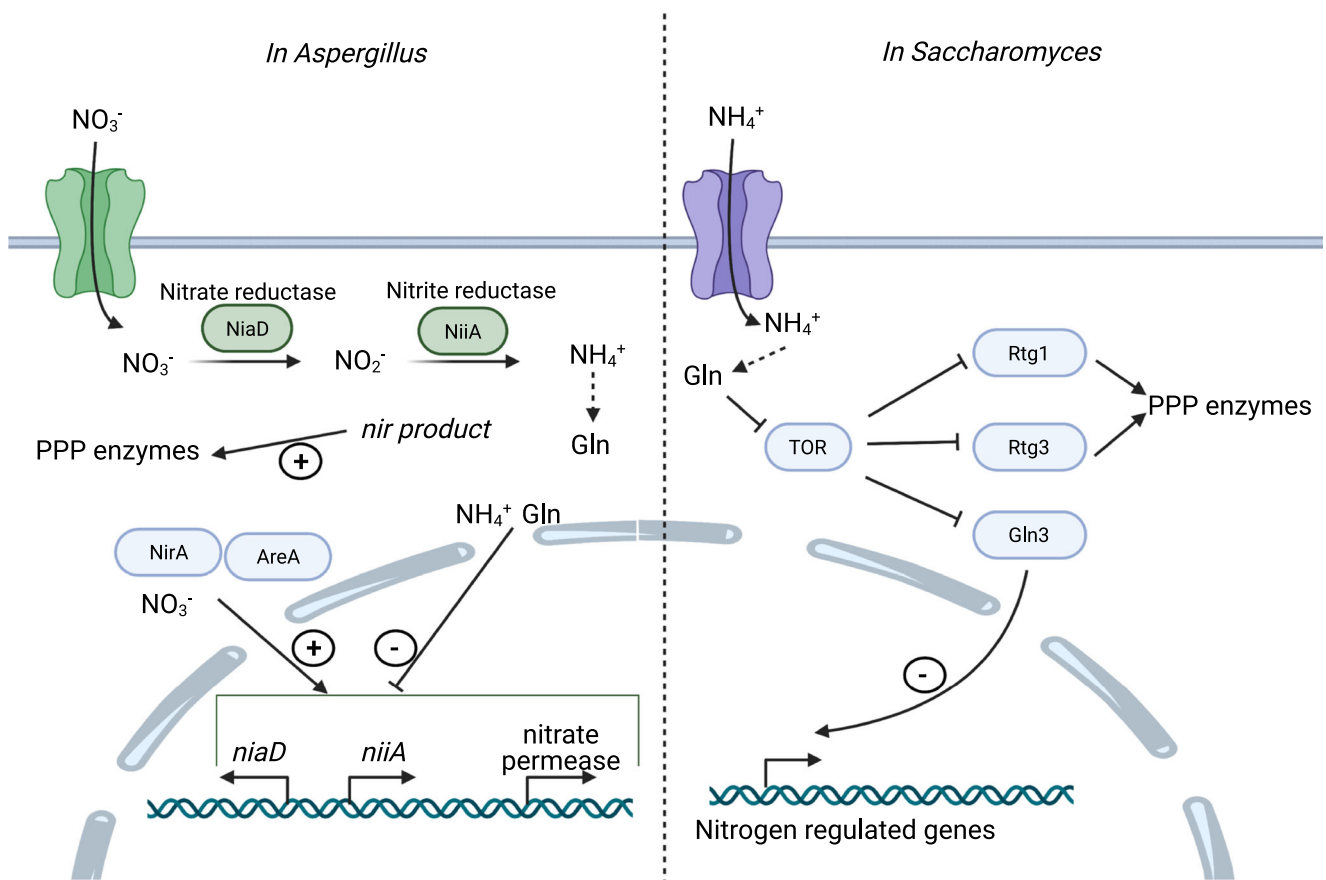


Fig. 2 Schematic drawings of the proposed nitrogen regulation in *Aspergillus* and *Saccharomyces*. In *Aspergillus* nitrate is reduced by consecutive action of two enzymes. Nitrate is reduced to nitrite by the nitrate reductase NiaD, and then nitrite is reduced to ammonium by the nitrite reductase NiiA, also called Nir. Ammonium is further metabolized to glutamine and glutamate. The *nir* product, which is currently not characterized, presumably activates the PPP enzymes through an unknown mechanism. Glutamine and ammonium repress the transcription of *s niaD*, of *niiA*, and of the nitrate permease-encoding gene. The transcription factors NirA and AreA as well as nitrate activate the transcription of the genes involved in nitrate metabolism (Soderberg 2005; Oost and van der Siebers 2007). In *Saccharomyces*, glutamine is produced from different sources, ammonium is one of

them. When present, glutamine inhibits the TOR protein, which itself represses Rtg1, Rtg3, and Gln3 expression. When inhibited by TOR, Gln3 cannot translocate in the nucleus and act as a transcription activator of the nitrogen-regulated genes. Rtg1 and Rtg3 have been shown to have an impact on activity of the PPP enzymes through an unknown mechanism (Soderberg 2005; Oost and van der Siebers 2007). Consequently, in the presence of glutamine, TOR is not expressed resulting in active Rtg1 and Rtg3 as well as Gln3 and the latter to activation of nitrogen metabolism. A hypothesis to explain the effect of nitrate on the PPP enzymes in *Aspergillus* could be a mechanism similar to what has been observed in *Saccharomyces*. It can be hypothesized that the *nir* product is glutamine, and it impacts the PPP through a pathway involving TOR, Rtg1, Rtg3, and Gln3

was reduced on L-arabinose and of four genes on D-xylose. A decrease in the expression of *rpiA* and *talB* was observed in the tested conditions in comparison to the wild type (Battaglia et al. 2014).

The expression of the PPP genes was also analyzed in a strain without D-xylulose kinase activity (*xkiA1* mutant) implicating that D-xylulose cannot be converted in D-xylulose-5-phosphate. Thus, genes, which are induced by D-xylulose-5-phosphate, are expected to show reduced expression, and genes induced by D-xylose or L-arabinose (such as the genes regulated by XlnR and AraR) are expected to show increased expression. Two genes of the PPP had increased levels in the mutant strain, i.e., *talB* in cultures grown on D-xylose and *rpiA* in cultures grown on L-arabinose. The other PPP genes showed reduced or similar expression levels (Battaglia et al. 2014).

Furthermore, Battaglia et al. searched 1kb upstream regions of genes coding for enzymes involved in the pentose catabolic pathway and in the PPP for conserved elements (Battaglia et al. 2014). Six different motifs, of which none was referenced in the JASPAR database, were identified. As these motifs are present in the 1kb upstream region of genes regulated by AraR, they can be considered as putative binding sites for AraR. Further, the consensus sequence of the XlnR DNA-binding site (GGCTA (AG)) was searched in the same set of upstream regions of genes coding for enzymes involved in the pentose catabolic pathway and in the PPP and genes regulated by XlnR. The XlnR DNA-binding site was found in upstream regions of its known target genes and additionally in those of six PPP genes, including *talB* and *rpiA*. This result suggests a regulatory function of XlnR for *talB* and *rpiA*.

Crosslink between carbon utilization and nitrogen source

Trehalose is a sugar metabolized among others by fungi, plants, and bacteria and has been reported as an environmental stress protectant (Argüelles 1994; Thevelein 1996; Argüelles 2000; Tapia et al. 2015; Magalhães et al. 2018). However, several researchers demonstrated that the role of trehalose in the response to environmental stress might not be linked to trehalose *per se* but to the activation of the environmental stress response by trehalose (Winderickx et al. 1996; Gibney et al. 2015). Trehalose is synthesized by two consecutively acting enzymes: the trehalose-6-phosphate synthase TPS1 and the trehalose-6-phosphate phosphatase. In *S. cerevisiae* the deletion of the *tps1* gene leads to a growth inhibition in presence of glucose. This growth inhibition is associated with an increased hexokinase activity leading to an increased flux into glycolysis, depletion of ATP, and accumulation of the sugar phosphate intermediates (van Aelst et al. 1993; Thevelein and Hohmann 1995). It was reported that the deletion of the hexokinase II-encoding gene *hvk2* restores the ability of *tps1* mutants to grow on glucose, suggesting that TPS1 might contribute to the regulation of the hexokinase activity (Hohmann et al. 1993; Gancedo and Flores 2004).

In *Magnaporthe grisea*, it has been demonstrated that the ability of *tps1* mutants to grow on glucose can be restored by the addition of amino acids, suggesting a link between the carbon and the nitrogen metabolism (Foster et al. 2003). This hypothesis was further tested by an investigation of the relationship between TPS1 and the regulation of the glycolysis (Wilson et al. 2007). In this study, a *tps1* deletion strain was compared to a wild-type strain of *M. grisea*. After cultivation, the levels of G6P and F6P were measured in the mycelia. The authors observed significantly higher amounts of sugar phosphates in the *tps1* deletion strain compared to the wild-type when grown on glucose or in a complete medium (5-fold increase of G6P; F6P increased from an undetectable level to 2 mol/g of mycelium) (Wilson et al. 2007). The complete medium contained 1% glucose, 0.2% peptone, 0.1% yeast extract, and 0.1% amino acids, while the glucose medium contained 10% glucose and 0.6% sodium nitrate. No increase in F6P was observed in the *tps1* deletion strain compared to other conditions. Next Wilson and co-workers deleted the *hvk1* gene in *M. grisea* analogous to the experiments of Hohmann and co-workers carried out in *S. cerevisiae* (Hohmann et al. 1993). The wild-type, the *tps1* deletion strain, the *hvk1* deletion strain, and the double deletion *hvk1/tps1* strain were cultivated on the complete medium and on the medium with glucose using nitrate as nitrogen source, and the hexokinase activity was measured. The levels of G6P were reduced by 4-fold in the *hvk1* deletion strain and the double deletion strain, while it was 14-fold increase in the *tps1* deletion strain (Wilson et al. 2007). The ability to grow on glucose

was partially restored in the double deletion strain; however, the growth was still impaired compared to the wild type. This result indicates that in *M. grisea*, the growth phenotype of a *tps1* deletion strain cannot be restored by the deletion of *hvk1*. Consequently, it can be concluded that TPS1 has a different function in *M. grisea* than in yeasts.

Additional growth tests were performed with the *tps1* deletion strain and the wild type using fructose or glucose as carbon source and different nitrogen sources (10 mM each): amino acids, ammonium, nitrite, and nitrate. Interestingly, the deletion strain was able to grow on both carbon sources similar to the wild type in the presence of ammonium, nitrite, and amino acid (except cysteine). In the presence of nitrate, the growth of the deletion strain was impaired with both carbon sources. Thus, the *tps1* mutant can grow on glucose depending on the nitrogen source. Consequently, Wilson and co-workers performed nitrate reductase activity assays of the *tps1* deletion strain and the wild-type cultivated under different nitrogen conditions. In the wild type, the nitrate reductase activity was increased when grown on nitrate compared to growth on ammonium, whereas in the *tps1* mutant, no activity was detectable under both conditions. Thus, the authors concluded that the inability of *M. grisea* mutants to grow on glucose is not linked to glycolytic misregulation but to the inability to use nitrate (Wilson et al. 2007).

The hypothesis suggested by the authors is that the connection between nitrate utilization and sugar metabolism is the availability of NADPH generated in the OPPP by the G6PDH. Growth on nitrate would increase the hexokinase activity and the NADPH levels in a wild type, while in a *tps1* deletion strain, nitrate would reduce G6PDH activity and NADPH production. Additionally, gene expression analysis showed that the *tps1* mutant expresses NMR1 (nitrogen metabolite repressor 1) when cultivated in the presence of nitrate and that the expression of the nitrite and nitrate reductase-encoding genes is reduced (Wilson et al. 2007). The proposed mechanism is that TPS1 derepresses genes involved in nitrate utilization via NMR1. The extracellular nitrate signal might be perceived via a nitrogen sensing mechanism or through the increased levels of G6P resulting from the increased activity of the hexokinase. Wilson and co-workers concluded that TPS1 might integrate carbon and nitrogen metabolism through G6P sensing, increased NADPH production, and induced expression of genes associated with nitrate utilization (Wilson et al. 2007).

A network of transcription factors regulate the PPP

The previous examples show that transcriptional regulation is essential for cell adaptation to environmental conditions. Many transcription factors are involved in the regulation of the PPP, and their activation relies on environmental factors such as the respective carbon and/or nitrogen source.

Moreover, the PPP is interconnected with the glycolysis and other pathways such as the shikimate pathway. Thus, understanding how transcription factors control the distribution of metabolic fluxes under a given condition is critical to understand the overall regulation of metabolism at the cellular level. This question has been mainly investigated by studying the changes in transcripts levels or/and in protein abundances. However, these parameters do not necessarily allow to predict changes in a cell phenotype or corresponding activities. A study on 119 transcription factors (TFs) performed in *S. cerevisiae* under five growth conditions tackled this gap (Fendt et al. 2010). Basically, this study linked growth conditions to changes in the metabolic flux and to modifications in the expression of TFs. Among 275 genes annotated as “transcriptional regulatory active” in the yeast genome database, the authors selected 119 TFs either related to metabolism or stress response. Then, single mutant strains of these 119 TFs were generated, and the strains were cultivated under five conditions: glucose, galactose, glucose combined with low pH, glucose combined with urea as nitrogen source, and glucose combined with high osmolarity. The chosen conditions cover two common stress factors, reduced and maximal carbon catabolite repression, and two nitrogen sources (urea versus ammonium sulfate). Cultivation was performed with 20% ^{13}C -labeled glucose or galactose, and then the transcriptome (by next-generation sequencing), the metabolome (by GC-MS), and the proteome (nano LC-MS/MS) were determined (Fendt et al. 2010). Fendt and co-workers evaluated six flux ratios corresponding to (i) gluconeogenesis, (ii) glycine production through C1 metabolism, (iii) transport of mitochondrial oxaloacetate into the cytosol, (iv) the relative contribution of glycolysis versus the PPP, (v) the relative contribution of the backward flux from glycine to serine versus the forward flux from 3-phosphoglycerate to serine, and (vi) the relative contribution of the respiratory tricarboxylic acid (TCA) cycle flux versus the replenishment of the biosynthetic precursor. Only three mutants of the 119 mutants exhibited an altered pathway usage of glycolysis versus the PPP. This alteration on the fluxes did not correlate with the specific growth rates; thus, the mutated TFs are responsible for the observed changes. Among all the tested transcription factors, 23 were affected by at least one tested growth condition. All these 23 are involved in controlling the TCA cycle, of which only two regulate the TCA cycle and the PPP, i.e., the retrograde regulation transcription factor 1 and 3 (Rtg1 and Rtg3) (Fendt et al. 2010). Rtg1 and Rtg3 are known as regulators of the mitochondrial retrograde response (Liao and Butow 1993; Chelstowska and Butow 1995).

While 55 tested TFs have at least one target in glycolysis or the PPP, only two altered the flux distribution between these two pathways, namely, PHO2 and BAS1. Mutants of these TFs exhibited an increase of several glycolysis enzymes; however, this did not alter the relative usage of glycolysis versus

the PPP (Fendt et al. 2010). PHO2 is a phosphate metabolism homeobox transcription factor regulating genes involved in phosphate metabolism, and BAS1 is a myb-related transcription factor regulating basal and induced expression of genes of the purine and histidine biosynthetic pathways (Arndt et al. 1987; Berben et al. 1988; Lenburg and O’Shea 1996).

These data demonstrate the robustness of the regulation of the PPP and central metabolic fluxes in general. Several hypotheses try to explain this phenomenon. The favored hypothesis is that changes in functional fluxes require several transcription factors acting on a number of target genes. Different signaling pathways might transmit environmental signals to these transcription factors. Interestingly, it has been demonstrated that Rtg1 and Rtg3 are controlled by TOR (target of rapamycin)-mediated control in response to glutamine in *S. cerevisiae*, suggesting an integration of nitrogen and carbon metabolism by the TOR protein (Crespo et al. 2002). Figure 2 provides a schematic drawing of this regulatory cascade. Additionally, it allows a comparison of the nitrogen-related regulation of the PPP between *Aspergillus* and *Saccharomyces* and suggests a hypothesis about the nature of the *nir* product.

Engineering the PPP in industrially relevant fungi

Fungi have a broad range of applications in biotechnologies: production of food and beverages as well as biofuels, chemicals, enzymes, and also polysaccharides, antibiotics, and medicines (Demain 2014; Katz and Baltz 2016; Meyer et al. 2016; Silber et al. 2016). Fungi used in the biotechnological industry mainly comprise yeasts and filamentous fungi. They produce a vast array of secondary metabolites and an extensive catalogue of proteins, specifically enzymes. The mostly used yeast in the industry is *S. cerevisiae*, while the mostly used filamentous fungi are *Aspergillus* spp., *Penicillium* spp., and *T. reesei*. The genomes of industrially used filamentous fungi, such as *A. niger*, *P. chrysogenum*, or *T. reesei*, have been sequenced, which might facilitate the understanding of the PPP and its regulation in these strains (Pel et al. 2007; Martinez et al. 2008; van den Berg et al. 2008).

Another industrially applied fungal family worth to be mentioned is the C1 expression platform. The company Dyadic developed this fungal expression platform from *Myceliophthora thermophila*. The C1 technology is a “robust and versatile fungal expression system for gene discovery, development, expression and production of enzymes and other proteins” according to Dyadic (Visser et al. 2011). Nevertheless, this technology will not be further discussed in this review because very few information has been made public. For example, no insights in the metabolism of this fungus nor in its PPP are available.

Due to its central role in carbon metabolism, the PPP is one of the main targets for metabolic engineering in fungal biotechnologies. The motivation for engineering the PPP can be seen in three major aims: (i) increasing the resistance of the cells to environmental stress, (ii) rerouting the metabolism towards a specific metabolite of interest, and (iii) increasing the NADPH concentration. This review article addresses these three aims by three studies and highlights how these strategies benefit from understanding of the PPP and, in turn, how these reports enlarge the current knowledge.

Modifications to increase resistance of the cells to stress

Industrial production setups and the requirement for cultivation in bioreactors cause diverse stress factors on fungal strains, such as stirring stress, toxic products synthesized during the process, and oxidative conditions. An example of such a situation is the process for biofuel production. Due to current concerns about climate change, significant efforts are put on developing processes to produce these fuels from renewable feedstocks. A diversity of yeasts can produce alcoholic biofuels, but these alcohols are also toxic for them. Several fungi naturally produce branched-chained alcohol; thus, fungi are a good alternative for developing alcoholic fuel production processes. However, fungi are also sensitive to alcohol resulting in low yields of the processes. Nevertheless, reaching high yields is a prerequisite for cost-effective production and the sustainability of such processes. A strategy to increase the yield is to increase the resistance of the cell to the toxic product (Mukhopadhyay 2015). Modifications of the PPP can help to overcome this challenge.

An example is the research work performed in *S. cerevisiae* to increase its tolerance to isobutanol (Kuroda et al. 2019). As a starting point, the research team studied the BY4741 gene deletion library (Winzeler et al. 1999; Giaever et al. 2002) to identify deletions causing changes in cell growth using medium containing isobutanol. Based on these data, cultivations were performed to confirm an increased sensitivity or increased tolerance of the strains to isobutanol and ethanol. Three strains with a unique phenotype were identified: they were more sensitive to isobutanol, but not to ethanol than the wild type. These strains had deletions of 6PGDH (GND1 in *Saccharomyces*), ZWF1, and the Na⁺/H⁺ antiporter NHA1. Further experiments demonstrated that strains with a deletion of GND1 or ZWF1 (NHA1 deletion strains were not tested) have higher sensitivity to C4–C6 alcohols regardless of their branching (Kuroda et al. 2019).

GND1 and ZWF1, respectively, code for the 6PGDH and the 6PDH, two enzymes of the PPP that catalyze reactions generating NADPH. NADPH/NADP⁺ ratios were lower in the ZWF1 deletion strain and unchanged in the GND1 deletion strain compared to the wild type. Kuroda and co-workers also

tested the isobutanol-specific sensitivity of deletion strains lacking an enzyme of the PPP that is not involved in NADPH generating reactions, such as TKL1 or RPE1. The single deletion strains for TKL1 and RPE1 had an isobutanol-specific sensitivity at 1.4% isobutanol similar to the ZWF1 and the GND1 deletion strains (Kuroda et al. 2019). Thus, the reduced tolerance to isobutanol cannot be linked only to a reduced amount of NADPH as often stated in studies on yeast tolerance to environmental stress (Gorsich et al. 2006; Matsufuji et al. 2008). The role of the PPP concerning the sensitivity to isobutanol cannot be limited only to the provision of NADPH. This is consistent with the fact that the role of the PPP in the oxidative stress response is also not limited to NADPH production (Park et al. 2019). The work from Kuroda and co-workers provides a link between sensitivity to environmental stress and the deletion of enzymes of the PPP (Park et al. 2019). However, no mechanism was proposed to explain this phenomenon. It rather demonstrated the complexity of the regulation networks involving the PPP. A lack of an enzyme of the PPP might impact more than just the product of the catalyzed reaction.

Modification to reroute metabolism towards a metabolite of interest

Besides the natural ability of fungi to produce a wide range of useful molecules, they can use a diversity of carbon and nitrogen sources, an asset in the context of focusing on sustainable nutrient resources. Moreover, some fungi and yeasts are also classified as safe, making them ideal industrial production hosts. Many of these microorganisms can be engineered to gain additional functions. In the following example, *S. cerevisiae* has been engineered to produce shinorine (Park et al. 2019).

Shinorine is a mycosporine, or mycosporine-like amino acid, used as sunscreen material in biobased products. The demand for these products increases, but the production yield of the natural producers (cyanobacteria and red algae, e.g., *Porphyra umbilicalis*) is low. The developed strategy used knowledge about the PPP for several purposes. Briefly, the PPP was modified to increase the formation of the product of interest by directing the cell metabolism towards a specific carbon source, namely, D-xylose, and at the same time, to reduce the formation of side products. First, shinorine biosynthetic genes from cyanobacterium *Nostoc punctiforme* were introduced into *S. cerevisiae* leading to a strain able to synthesize shinorine at a concentration of 0.67 mg/l at best. To enhance the production yield, it was aimed to increase the formation of the PPP intermediate S7P; S7P is a precursor in the shinorine synthesis pathway. The usage of D-xylose was supposed to increase the S7P production because D-xylose cannot enter glycolysis; thus, its use as carbon source allows redirecting carbons towards the PPP and avoids competition with the glycolysis. The genomic introduction of a gene from *Scheffersomyces stipitis* enables the reduction and metabolism

of D-xylose. The obtained strain reached concentrations of 17.99 mg/l shinorine in a medium with 18 g/l D-xylose and 2 g/l glucose (Park et al. 2019).

To further increase the shinorine production, the authors deleted TAL1 in order to prevent the conversion from S7P to G3P. The obtained strain showed a severe growth defect in rich D-xylose medium, presumably because TAL1 is necessary for D-xylose assimilation. Nevertheless, by reducing the amount of D-xylose in the medium, a yield of shinorine of 21.9 mg/l was reached. To compensate for the deletion of TAL1 and to enhance the flux through the PPP, the sin three binding protein 5 (STB5) and the TKL1 were overexpressed. STB5 is a transcription factor involved in response to oxidative stress. In oxidative conditions, it activates PPP genes, including ZWF1, GND1, and TKL1, leading to increased NADPH synthesis. TKL1 was chosen due to its involvement in D-xylose metabolism. The effects of overexpression of the single genes were compared to an empty vector strain and a strain with overexpression of both STB5 and TKL1. The double overexpression strain had increased D-xylose consumption and shinorine production. Finally, by medium optimization, a shinorine production of 31.0 mg/l was reached in a medium containing 8 g/l of D-xylose and 12 g/l of glucose with the latter strain. In this study, the understanding of the PPP was successfully employed to increase the production of a compound of interest. The impact of the carbon source and the known function of TFs were also taken into account. The consequence of the deletion of TAL1 emphasizes the limitations of the strategy to modify the expression of a particular enzyme of the PPP without offering any compensation. The involvement of the PPP in multiple regulation networks makes outcomes of such a modification approach hard to predict.

Modifications towards NADPH production

The availability of NADPH is a determining factor for many biochemical reactions, and the PPP is the primary source of NADPH. Consequently, increasing the flux through the PPP could be a strategy to generate more NADPH and, thus, can increase the yield of biochemical metabolites. Poulsen et al. have used a strategy to engineer the PPP in order to increase NADPH concentration in *A. niger* (Poulsen et al. 2005).

In this study, three enzymes of the PPP were overexpressed, i.e., the G6PDH, the 6PGDH, and the TKL. The wild-type and the engineered strains were cultured in bioreactors with ammonium or nitrate as the nitrogen source. The data were analyzed by multivariate data analysis using the following variables: G6PDH, 6PGDH, TKL, mannitol-1-phosphate dehydrogenase, S7P, dihydroxyacetone phosphate, Xu5P, F6P, pyruvate, R5P, G3P, 6PG, NADP, NADPH, NADH, i-erythritol, i-arabitol, i-mannitol, i-arabitol, e-trehalose, oxalate, NADH, aldolase (EC 4.1.2.13), TAL (EC 2.2.1.2), PGI, glycerol dehydrogenase (EC 1.1.1.156), G6P, ADP, AMP, NAD, catabolic reduction charge, i-glycerol, i-

trehalose, glycerol, e-erythritol, and e-mannitol (i-, intracellular; e-, extracellular). The primary outcome of the study was that the strain overproducing 6PGDH is the only one with an increased NADPH concentration, while no effect of the overproduction of G6PDH and TKL was observed. The increased NADPH concentration did not affect the overall strain physiology: the specific growth rate and the spore formation were not changed compared to the wild type.

These findings suggest that engineering to increase NADPH concentration can be a relevant target to increase the synthesis of a biochemical metabolite. However, the latter was not demonstrated in this work. Indeed, no metabolite of which the biosynthesis requires NADPH was investigated.

Conclusion

The current understanding of the PPP suggests a complex regulatory control of the PPP and a role of this pathway beyond the provision of NADPH. The studies discussed in this review suggest that nutrient sources such as carbon, nitrogen, and glutamine impact the regulation of the PPP and might interact in their regulation of the PPP. The current knowledge suggests a common regulation through the TOR pathway which controls a complex network of TFs. However, experiments are needed to confirm this hypothesis and to find the missing links between the regulatory impacts of carbon sources, nitrogen sources, glutamine, and the TFs involved.

The metabolic engineering approaches described in the last part of this review demonstrate the potential of engineering the PPP for improvement of biotechnological processes. They also revealed how much is still to learn about the PPP and the difficulty to optimize the production of a metabolite without a complete understanding of the metabolic regulations involved. Fungi have already been in the past significant providers of industrial or medical products, even despite the lack of a full comprehension of their metabolism. Improved knowledge and according modification of the PPP could start a new era in industrial processes and lead to further increase in yields of manufacturing processes.

Finally, solving the regulation of the PPP in fungi might improve insights in the PPP of higher eukaryotes as humans. The PPP is studied in humans, especially in relation to cancer and diabetes, where enzymes of the PPP are targets for possible treatments. The study of fungal cells is easier to carry out and poses less ethical and safety concerns than the work on human or animal cells. Obtained insights from fungi could lead to a progress in the understanding of relevant issues related to the PPP in humans.

Abbreviations 6PG, 6-phosphogluconate; 6PGDH, 6-phosphogluconate dehydrogenase; 6PGL, 6-phosphogluconolactonase;

CHP, cumene-hydroperoxide; *cmx*, gene coding for an activator of the enzyme-like protein involved in molybdopterin biosynthesis; *cmx-*, *cmx*-deficient mutant strain; *DHAP*, dihydroxyacetone phosphate; *DPN*, diphosphopyridine nucleotide; *E4P*, erythrose-4-phosphate; *F6P*, fructose-6-phosphate; *G3P*, glyceraldehyde-3-phosphate; *G6P*, glucose-6-phosphate; *G6PDH*, glucose-6-phosphate dehydrogenase; *GND1*, 6-phosphogluconatedehydrogenase in *Saccharomyces* (same as G6PDH); *GPI*, glucose-6-phosphate isomerase; *HXK1*, hexokinase 1; *LC-MS/MS*, liquid chromatography coupled with tandem mass spectrometry; *NAD+*, nicotinamide adenine dinucleotide (same as DPN); *NADH*, nicotinamide adenine dinucleotide + hydrogen; *NADP+*, nicotinamide adenine dinucleotide phosphate (same as TPN); *NADPH*, nicotinamide adenine dinucleotide phosphate + hydrogen; *NHA1*, Na⁺/H⁺ antiporter 1; *niaD*, gene coding for the nitrate reductase; *niaD-*, *niaD*-deficient mutant strain; *niia*, gene coding for the nitrite reductase; *niia-4*, *niia*-deficient mutant strain; *nirA*, gene coding for the transactivator NirA, activates genes associated to nitrate metabolism; *nirA-1*, *nirA*-deficient mutant strain; *NMR1*, nitrogen metabolism repressor 1; *NOPPP*, non-oxidative pentose phosphate pathway; *OPPP*, oxidative pentose phosphate pathway; *PHO2*, phosphate metabolism 2; *PPP*, pentose phosphate pathway; *R5P*, ribose-5-phosphate; *RPE*, ribulose-5-phosphate 3-epimerase; *RPI*, ribose-5-phosphate isomerase; *rpiA*, gene coding for the ribose-5-phosphate isomerase A; *Rtg1*, retrograde regulation transcription factor 1; *Rtg3*, retrograde regulation transcription factor 3; *Ru5P*, ribulose-5-phosphate; *S7P*, sedoheptulose-7-phosphate; *SHPK*, sedoheptulose kinase; *SOL4*, suppressor of *Los1-1 4*; *STB5*, sin three binding protein 5; *TAL*, transaldolase; *TCA*, tricarboxylic acid; *TFs*, transcription factors; *TKL*, transketolase; *TOR*, target of rapamycin; *TPI*, triose phosphate isomerase; *TPS1*, trehalose-6-phosphate synthase 1; *TPN*, triphosphopyridine nucleotide; *XlnR*, transcriptional activator of the xylanolytic system (in *Aspergillus*); *ZWF1*, Zwischenferment (in *Saccharomyces*, same as G6PDH)

Acknowledgments Figure 1 and Fig. 2 have been created using [Biorender.com](https://biorender.com).

Figure 1 has been adapted from the following template: <https://app.biorender.com/biorender-templates/t-5ed8278771111200ab35257a-glycolysis-and-glycolytic-enzymes>.

Author contribution AM proposed the idea of the manuscript, performed the literature search, and drafted the manuscript. ARMA and RLM critically revised and corrected the manuscript. All authors read and approved the manuscript.

Funding Open access funding provided by TU Wien (TUW). The financial support provided by the Christian Doppler Research Association, the Austrian Federal Ministry for Digital and Economic Affairs, the National Foundation for Research, Technology and Development, and Konzil Estate GmbH is gratefully acknowledged.

Declarations

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included

in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Argüelles JC (1994) Heat-shock response in a yeast *tps1* mutant deficient in trehalose synthesis. *FEBS Lett* 350:266–270. [https://doi.org/10.1016/0014-5793\(94\)00786-1](https://doi.org/10.1016/0014-5793(94)00786-1)
- Argüelles JC (2000) Physiological roles of trehalose in bacteria and yeasts: a comparative analysis. *Arch Microbiol* 174:217–224
- Arndt KT, Styles C, Fink GR (1987) Multiple global regulators control *HIS4* transcription in yeast. *Science* 237:874–880. <https://doi.org/10.1126/science.3303332>
- Battaglia E, Zhou M, de Vries RP (2014) The transcriptional activators AraR and XlnR from *Aspergillus niger* regulate expression of pentose catabolic and pentose phosphate pathway genes. *Res Microbiol* 165:531–540. <https://doi.org/10.1016/j.resmic.2014.07.013>
- Berben G, Legrain M, Hilger F (1988) Studies on the structure, expression and function of the yeast regulatory gene *PHO2*. *Gene* 66:307–312. [https://doi.org/10.1016/0378-1119\(88\)90367-8](https://doi.org/10.1016/0378-1119(88)90367-8)
- Bräsen C, Esser D, Rauch B, Siebers B (2014) Carbohydrate metabolism in *Archaea*: current insights into unusual enzymes and pathways and their regulation. *Microbiol Mol Biol Rev* 78:89–175. <https://doi.org/10.1128/MMBR.00041-13>
- Bruinenberg PM (1986) The NADP(H) redox couple in yeast metabolism. *Antonie Van Leeuwenhoek* 52:411–429. <https://doi.org/10.1007/BF00393469>
- Bruinenberg PM, van Dijken JP, Scheffers WA (1983) An enzymic analysis of NADPH production and consumption in *Candida utilis*. *J Gen Microbiol* 129:965–971. <https://doi.org/10.1099/00221287-129-4-965>
- Chelstowska A, Butow RA (1995) RTG genes in yeast that function in communication between mitochondria and the nucleus are also required for expression of genes encoding peroxisomal proteins. *J Biol Chem* 270:18141–18146. <https://doi.org/10.1074/jbc.270.30.18141>
- Crespo JL, Powers T, Fowler B, Hall MN (2002) The TOR-controlled transcription activators GLN3, RTG1, and RTG3 are regulated in response to intracellular levels of glutamine. *Proc Natl Acad Sci USA* 99:6784–6789. <https://doi.org/10.1073/pnas.102687599>
- David H, Åkesson M, Nielsen J (2003) Reconstruction of the central carbon metabolism of *Aspergillus niger*. *Eur J Biochem* 270:4243–4253. <https://doi.org/10.1046/j.1432-1033.2003.03798.x>
- David H, Krogh AM, Roca C, Åkesson M, Nielsen J (2005) CreA influences the metabolic fluxes of *Aspergillus nidulans* during growth on glucose and xylose. *Microbiology* 151:2209–2221. <https://doi.org/10.1099/mic.0.27787-0>
- David H, Hofmann G, Oliveira AP, Jarmer H, Nielsen J (2006) Metabolic network driven analysis of genome-wide transcription data from *Aspergillus nidulans*. *Genome Biol* 7:R108. <https://doi.org/10.1186/gb-2006-7-11-r108>
- de Groot MJL, van den Dool C, Wösten HAB, Levisson M, Vankuyk PA, Ruijter GJG, de Vries RP (2007) Regulation of pentose catabolic pathway genes of *Aspergillus niger*. *Food Technol Biotechnol* 45:134–138
- Demain AL (2014) Importance of microbial natural products and the need to revitalize their discovery. *J Ind Microbiol* 41:185–201. <https://doi.org/10.1007/s10295-013-1325-z>
- Dickens F (1938) Oxidation of phosphohexonate and pentose phosphoric acids by yeast enzymes. *Biochem J* 32:1626–1644. <https://doi.org/10.1042/bj0321626>

- Dickens F, Glock GE (1951) Direct oxidation of glucose-6-phosphate, 6-phosphogluconate and pentose-5-phosphates by enzymes of animal origin. *Biochem J* 50:81–95. <https://doi.org/10.1042/bj0500081>
- Dickens F, Williamson DH (1956) Pentose phosphate isomerase and epimerase from animal tissues. *Biochem J* 64:567–578. <https://doi.org/10.1042/bj0640567>
- Fendt SM, Oliveira AP, Christen S, Picotti P, Dechant RC, Sauer U (2010) Unraveling condition-dependent networks of transcription factors that control metabolic pathway activity in yeast. *Mol Syst Biol* 6:1–11. <https://doi.org/10.1038/msb.2010.91>
- Foster AJ, Jenkinson JM, Talbot NJ (2003) Trehalose synthesis and metabolism are required at different stages of plant infection by *Magnaporthe grisea*. *EMBO J* 22:225–235. <https://doi.org/10.1093/emboj/cdg018>
- Gancedo C, Flores CL (2004) The importance of a functional trehalose biosynthetic pathway for the life of yeasts and fungi. *FEMS Yeast Res* 4:351–359
- Giaever G, Chu AM, Ni L, Connelly C, Riles L, Véronneau S, Dow S, Lucanu-Danila A, Anderson K, André B, Arkin AP, Astromoff A, el Bakkoury M, Bangham R, Benito R, Brachat S, Campanaro S, Curtiss M, Davis K, Deutschbauer A, Entian KD, Flaherty P, Foury F, Garfinkel DJ, Gerstein M, Gotte D, Güldener U, Hegemann JH, Hempel S, Herman Z, Jaramillo DF, Kelly DE, Kelly SL, Kötter P, LaBonte D, Lamb DC, Lan N, Liang H, Liao H, Liu L, Luo C, Lussier M, Mao R, Menard P, Ooi SL, Revuelta JL, Roberts CJ, Rose M, Ross-Macdonald P, Scherens B, Schimmack G, Shafer B, Shoemaker DD, Sookhai-Mahadeo S, Storms RK, Strathern JN, Valle G, Voet M, Volckaert G, Wang CY, Ward TR, Wilhelmy J, Winzler EA, Yang Y, Yen G, Youngman E, Yu K, Bussey H, Boeke JD, Snyder M, Philippsen P, Davis RW, Johnston M (2002) Functional profiling of the *Saccharomyces cerevisiae* genome. *Nature* 418:387–391. <https://doi.org/10.1038/nature00935>
- Gibney PA, Schieler A, Chen JC, Rabinowitz JD, Botstein D (2015) Characterizing the in vivo role of trehalose in *Saccharomyces cerevisiae* using the AGT1 transporter. *Proc Natl Acad Sci USA* 112:6116–6121. <https://doi.org/10.1073/pnas.1506289112>
- Gorsich SW, Dien BS, Nichols NN, Slininger PJ, Liu ZL, Skory CD (2006) Tolerance to furfural-induced stress is associated with pentose phosphate pathway genes ZWF1, GND1, RPE1, and TKL1 in *Saccharomyces cerevisiae*. *Appl Microbiol Biotechnol* 71:339–349. <https://doi.org/10.1007/s00253-005-0142-3>
- Gunsalus IC, Horecker BL, Wood WA (1955) Pathways of carbohydrate metabolism in microorganisms. *Bacteriol Rev* 19:79–128
- Han X, Qiu M, Wang B, Yin W-B, Nie X, Qin Q, Ren S, Yang K, Zhang F, Zhuang Z, Wang S (2016) Functional analysis of the nitrogen metabolite repression regulator gene nmrA in *Aspergillus flavus*. *Front Microbiol* 7:1794. <https://doi.org/10.3389/fmicb.2016.01794>
- Hankinson O (1974) Mutants of the pentose phosphate pathway in *Aspergillus nidulans*. *J Bacteriol* 117:1121–1130
- Hankinson O, Cove DJ (1972) Regulation of pentose phosphate pathway in fungus *Aspergillus nidulans*. *Biochem J* 127:18–19. <https://doi.org/10.1042/bj1270018pb>
- Hankinson O, Cove DJ (1974) Regulation of the pentose phosphate pathway in the fungus *Aspergillus nidulans*. The effect of growth with nitrate. *J Biol Chem* 249:2344–2353
- Hankinson O, Cove DJ (1975) Regulation of mannitol 1 phosphate dehydrogenase in *Aspergillus nidulans*. *Can J Microbiol* 21:99–101. <https://doi.org/10.1139/m75-014>
- Hasper AA, Visser J, de Graaff LH (2000) The *Aspergillus niger* transcriptional activator XlnR, which is involved in the degradation of the polysaccharides xylan and cellulose, also regulates D-xylose reductase gene expression. *Mol Microbiol* 36:193–200. <https://doi.org/10.1046/j.1365-2958.2000.01843.x>
- Hohmann S, Neves MJ, de Koning W, Alijo R, Ramos J, Thevelein JM (1993) The growth and signalling defects of the ggs1 (fdp1/byp1) deletion mutant on glucose are suppressed by a deletion of the gene encoding hexokinase PII. *Curr Genet* 23:281–289. <https://doi.org/10.1007/BF00310888>
- Horecker BL (2002) The pentose phosphate pathway. *J Biol Chem* 277:47965–47971. <https://doi.org/10.1074/jbc.X200007200>
- Jorgensen H, Nielsen J, Villadsen J, Mollgaard H (1995) Metabolic flux distributions in *Penicillium chrysogenum* during fed-batch cultivations. *Biotechnol Bioeng* 46:117–131. <https://doi.org/10.1002/bit.260460205>
- Juhnke H, Krems B, Kotter P, Entian KD (1996) Mutants that show increased sensitivity to hydrogen peroxide reveal an important role for the pentose phosphate pathway in protection of yeast against oxidative stress. *Mol Gen Genet* 252:456–464. <https://doi.org/10.1007/Bf02173011>
- Katz L, Baltz RH (2016) Natural product discovery: past, present, and future. *J Ind Microbiol Biotechnol* 43:155–176. <https://doi.org/10.1007/s10295-015-1723-5>
- Khosravi C, Battaglia E, Kun RS, Dalhuijsen S, Visser J, Aguilar-Pontes M V, Zhou M, Heyman HM, Kim YM, Ronald SEB, de Vries RP (2018) Blocking hexose entry into glycolysis activates alternative metabolic conversion of these sugars and upregulates pentose metabolism in *Aspergillus nidulans*. *Bmc Genomics* 19. ARTN 214 10.1186/s12864-018-4609-x
- Krappmann S, Braus GH (2005) Nitrogen metabolism of *Aspergillus* and its role in pathogenicity. *Med Mycol* 43:31–40. <https://doi.org/10.1080/13693780400024271>
- Krüger A, Grüning NM, Wamelink MMC, Kerick M, Kirpy A, Parkhomchuk D, Bluemlein K, Schweiger MR, Soldatov A, Lehrach H, Jakobs C, Ralser M (2011) The pentose phosphate pathway is a metabolic redox sensor and regulates transcription during the antioxidant response. *Antioxid Redox Signal* 15:311–324. <https://doi.org/10.1089/ars.2010.3797>
- Kuroda K, Hammer SK, Watanabe Y, Montaña López J, Fink GR, Stephanopoulos G, Ueda M, Avalos JL (2019) Critical roles of the pentose phosphate pathway and GLN3 in isobutanol-specific tolerance in yeast. *Cell Syst* 9:534–547.e5. <https://doi.org/10.1016/j.cels.2019.10.006>
- Lenburg ME, O’Shea EK (1996) Signaling phosphate starvation. *Trends Biochem Sci* 21:383–387
- Liao X, Butow RA (1993) RTG1 and RTG2: Two yeast genes required for a novel path of communication from mitochondria to the nucleus. *Cell* 72:61–71. [https://doi.org/10.1016/0092-8674\(93\)90050-Z](https://doi.org/10.1016/0092-8674(93)90050-Z)
- Mach-Aigner AR, Omony J, Jovanovic B, van Boxel AJ, de Graaff LH (2012) d-Xylose concentration-dependent hydrolase expression profiles and the function of CreA and XlnR in *Aspergillus niger*. *Appl Environ Microbiol* 78:3145–3155. <https://doi.org/10.1128/AEM.07772-11>
- Magalhães RSS, Popova B, Braus GH, Outeiro TF, Eleutherio ECA (2018) The trehalose protective mechanism during thermal stress in *Saccharomyces cerevisiae*: the roles of Ath1 and Agt1. *FEMS Yeast Res* 18. <https://doi.org/10.1093/femsyr/foy066>
- Martinez D, Berka RM, Henrissat B, Saloheimo M, Arvas M, Baker SE, Chapman J, Chertkov O, Coutinho PM, Cullen D, Danchin EG, Grigoriev IV, Harris P, Jackson M, Kubicek CP, Han CS, Ho I, Larrondo LF, de Leon AL, Magnuson JK, Merino S, Misra M, Nelson B, Putnam N, Robbertse B, Salamov AA, Schmolli M, Terry A, Thayer N, Westerholm-Parvinen A, Schoch CL, Yao J, Barabote R, Nelson MA, Detter C, Bruce D, Kuske CR, Xie G, Richardson P, Rokhsar DS, Lucas SM, Rubin EM, Dunn-Coleman

- N, Ward M, Brettin TS (2008) Genome sequencing and analysis of the biomass-degrading fungus *Trichoderma reesei* (syn. *Hypocrea jecorina*). *Nat Biotechnol* 26:553–560. <https://doi.org/10.1038/nbt1403>
- Matsufuji Y, Fujimura S, Ito T, Nishizawa M, Miyaji T, Nakagawa J, Ohyama T, Tomizuka N, Nakagawa T (2008) Acetaldehyde tolerance in *Saccharomyces cerevisiae* involves the pentose phosphate pathway and oleic acid biosynthesis. *Yeast* 25:825–833. <https://doi.org/10.1002/yea.1637>
- Melzer G, Dalpiaz A, Grote A, Kucklick M, Göcke Y, Jonas R, Dersch P, Franco-Lara E, Nörtemann B, Hempel DC (2007) Metabolic flux analysis using stoichiometric models for *Aspergillus niger*: comparison under glucoamylase-producing and non-producing conditions. *J Biotechnol* 132:405–417. <https://doi.org/10.1016/j.jbiotec.2007.08.034>
- Meyer V, Andersen MR, Brakhage AA, Braus GH, Caddick MX, Cairns TC, de Vries RP, Haarmann T, Hansen K, Hertz-Fowler C, Krappmann S, Mortensen UH, Penalva MA, Ram AFJ, Head RM (2016) Current challenges of research on filamentous fungi in relation to human welfare and a sustainable bio-economy: a white paper. *Fungal Biol Biotechnol* 3:6. <https://doi.org/10.1186/s40694-016-0024-8>
- Mukhopadhyay A (2015) Tolerance engineering in bacteria for the production of advanced biofuels and chemicals. *Trends Microbiol* 23: 498–508
- Oost J van der Siebers B (2007) The glycolytic pathways of *Archaea*. In: *Archaea: Evolution, Physiology, and Molecular Biology*. Blackwell Publishing Ltd, pp 247–259
- Osmond CB, Ap Rees T (1969) Control of the pentose-phosphate pathway in yeast. *Biochim Biophys Acta* 184:35–42. [https://doi.org/10.1016/0304-4165\(69\)90095-6](https://doi.org/10.1016/0304-4165(69)90095-6)
- Park SH, Lee K, Jang JW, Hahn JS (2019) Metabolic engineering of *Saccharomyces cerevisiae* for production of shinorine, a sunscreen material, from xylose. *acs synth biol* 8: 346–357. <https://doi.org/10.1021/acssynbio.8b00388>
- Pel HJ, de Winde JH, Archer DB, Dyer PS, Hofmann G, Schaap PJ, Turner G, de Vries RP, Albang R, Albermann K, Andersen MR, Bendtsen JD, JAE B, van den Berg M, Breestraat S, Caddick MX, Contreras R, Cornell M, Coutinho PM, Danchin EGJ, Debets AJM, Dekker P, van Dijk PWM, van Dijk A, Dijkhuizen L, Driessen AJM, D'Enfert C, Geysens S, Goosen C, Groot GSP, de Groot PWJ, Guillemette T, Henrissat B, Herweijer M, van den Hombergh JPTW, van den Hondel CAMJJ, van der Heijden RTJM, van der Kaaij RM, Klis FM, Kools HJ, Kubicek CP, van Kuyk PA, Lauber J, Lu X, van der Maarel MJEC, Meulenber R, Menke H, Mortimer MA, Nielsen J, Oliver SG, Olsthoorn M, Pal K, van Peij NNME, Ram AFJ, Rinas U, Roubos JA, Sagt CMJ, Schmoll M, Sun J, Ussery D, Varga J, Verwecken W, van de Vondervoort PJJ, Wedler H, Wösten HAB, Zeng AP, van Ooyen AJJ, Visser J, Stam H (2007) Genome sequencing and analysis of the versatile cell factory *Aspergillus niger* CBS 513.88. *Nat Biotechnol* 25:221–231. <https://doi.org/10.1038/nbt1282>
- Peleato ML, Muiño-Blanco T, Cebrian Pérez JA, Lopez-Pérez MJ (1991) Significance of the non-oxidative pentose phosphate pathway in *Aspergillus oryzae* grown on different carbon sources. *Z Naturforsch C* 46:223–227. <https://doi.org/10.1515/znc-1991-3-411>
- Poulsen BR, Nohr J, Douthwaite S, Hansen LV, Iversen JJJ, Visser J, Ruijter GJG (2005) Increased NADPH concentration obtained by metabolic engineering of the pentose phosphate pathway in *Aspergillus niger*. *FEBS J* 272:1313–1325. <https://doi.org/10.1111/j.1742-4658.2005.04554.x>
- Ralser M, Heeren G, Breitenbach M, Lehrach H, Krobitsch S (2006) Triose phosphate isomerase deficiency is caused by altered dimerization-not catalytic inactivity-of the mutant enzymes. *PLoS One* 1:e30. <https://doi.org/10.1371/journal.pone.0000030>
- Ralser M, Wamelink MM, Kowald A, Gerisch B, Heeren G, Struys EA, Klipp E, Jakobs C, Breitenbach M, Lehrach H, Krobitsch S (2007) Dynamic rerouting of the carbohydrate flux is key to counteracting oxidative stress. *J Biol* 6:10. <https://doi.org/10.1186/jbiol61>
- Ralser M, Wamelink MMC, Latkolik S, Jansen EEW, Lehrach H, Jakobs C (2009) Metabolic reconfiguration precedes transcriptional regulation in the antioxidant response. *Nat Biotechnol* 27:604–605. <https://doi.org/10.1038/nbt0709-604>
- Runquist D, Hahn-Hagerdal B, Bettiga M (2009) Increased expression of the oxidative pentose phosphate pathway and gluconeogenesis in anaerobically growing xylose-utilizing *Saccharomyces cerevisiae*. *Microb Cell Factories* 8:49. <https://doi.org/10.1186/1475-2859-8-49>
- Silber J, Kramer A, Labes A, Tasdemir D (2016) From discovery to production: biotechnology of marine fungi for the production of new antibiotics. *Mar Drugs* 14:7. <https://doi.org/10.3390/md14070137>
- Soderberg T (2005) Biosynthesis of ribose-5-phosphate and erythrose-4-phosphate in *archaea*: a phylogenetic analysis of archaeal genomes. *Archaea* 1:347–352. <https://doi.org/10.1155/2005/314760>
- Stincione A, Prigione A, Cramer T, Wamelink MM, Campbell K, Cheung E, Olin-Sandoval V, Gruning NM, Kruger A, Tauqeer Alam M, Keller MA, Breitenbach M, Brindle KM, Rabinowitz JD, Ralser M (2015) The return of metabolism: biochemistry and physiology of the pentose phosphate pathway. *Biol Rev Camb Philos Soc* 90: 927–963. <https://doi.org/10.1111/brv.12140>
- Tapia H, Young L, Fox D, Bertozzi CR, Koshland D (2015) Increasing intracellular trehalose is sufficient to confer desiccation tolerance to *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA* 112:6122–6127. <https://doi.org/10.1073/pnas.1506415112>
- Thevelein JM (1996) Regulation of trehalose metabolism and its relevance to cell growth and function. In: *Biochemistry and Molecular Biology*. Springer, Berlin Heidelberg, pp 395–420
- Thevelein JM, Hohmann S (1995) Trehalose synthase: guard to the gate of glycolysis in yeast? *Trends Biochem Sci* 20:3–10. [https://doi.org/10.1016/S0968-0004\(00\)88938-0](https://doi.org/10.1016/S0968-0004(00)88938-0)
- van Aelst L, Hohmann S, Bulaya B, de Koning W, Sierkstra L, Neves MJ, Luyten K, Alijo R, Ramos J, Coccetti P, Martegani E, de Magalhães-Rocha NM, Brandão RL, van Dijk P, Vanhalewyn M, Dumez P, Arnold WHJ, Thevelein JM (1993) Molecular cloning of a gene involved in glucose sensing in the yeast *Saccharomyces cerevisiae*. *Mol Microbiol* 8:927–943. <https://doi.org/10.1111/j.1365-2958.1993.tb01638.x>
- van den Berg MA, Albang R, Albermann K, Badger JH, Daran JM, Driessen AJ M, Garcia-Estrada C, Fedorova ND, Harris DM, Heijne WHM, Joardar VW, Kiel JAK, Kovalchuk A, Martin JF, Niernan WC, Nijland JG, Pronk JT, Roubos JA, van der Klei IJ, van Peij NNME, Veenhuis M, von Döhren H, Wagner C, Wortman J, Bovenberg RAL (2008) Genome sequencing and analysis of the filamentous fungus *Penicillium chrysogenum*. *Nature Biotechnol* 26:1161–1168. <https://doi.org/10.1038/nbt.1498>
- van Peij NNME, Gielkens MMC, de Vries RP, Visser J, de Graaff LH (1998) The transcriptional activator XlnR regulates both xylanolytic and endoglucanase gene expression in *Aspergillus niger*. *Appl Environ Microbiol* 64:3615–3619. <https://doi.org/10.1128/aem.64.10.3615-3619.1998>
- Visser H, Joosten V, Punt PJ, Gusakov AV, Olson PT, Joosten R, Bartels J, Visser J, Sinitzyn AP, Emalfarb MA, Verdoes JC, Wery J (2011) Development of a mature fungal technology and production platform for industrial enzymes based on a *Myceliophthora thermophila*

- isolate, previously known as *Chrysosporium lucknowense* C1. Ind Biotechnol 7:214–223. <https://doi.org/10.1089/ind.2011.7.214>
- Warburg O (1932) Das sauerstoffübertragende Ferment der Atmung. Angew Chem 45:1–6. <https://doi.org/10.1002/ange.19320450102>
- Warburg O, Christian W (1936) Pyridin, der wasserstoffübertragende Bestandteil von Gärungsfermenten. Helv Chim Acta 19:E79–E88. <https://doi.org/10.1002/hlca.193601901199>
- Wilson RA, Jenkinson JM, Gibson RP, Littlechild JA, Wang ZY, Talbot NJ (2007) Tps1 regulates the pentose phosphate pathway, nitrogen metabolism and fungal virulence. EMBO J 26:3673–3685. <https://doi.org/10.1038/sj.emboj.7601795>
- Winderickx J, de Winde JH, Crauwels M, Hino A, Hohmann S, van Dijk P, Thevelein JM (1996) Regulation of genes encoding subunits of the trehalose synthase complex in *Saccharomyces cerevisiae*: novel variations of STRE-mediated transcription control? Mol Gen Genet MGG 252:470–482. <https://doi.org/10.1007/bf02173013>
- Winzeler EA, Shoemaker DD, Astromoff A, Liang H, Anderson K, Andre B, Bangham R, Benito R, Boeke JD, Bussey H, Chu AM, Connelly C, Davis K, Dietrich F, Dow SW, el Bakkoury M, Foury F, Friend SH, Gentalen E, Giaever G, Hegemann JH, Jones T, Laub M, Liao H, Liebundguth N, Lockhart DJ, Lucau-Danila A, Lussier M, M'Rabet N, Menard P, Mittmann M, Pai C, Rebischung C, Revuelta JL, Riles L, Roberts CJ, Ross-MacDonald P, Scherens B, Snyder M, Sookhai-Mahadeo S, Storms RK, Véronneau S, Voet M, Volckaert G, Ward TR, Wysocki R, Yen GS, Yu K, Zimmermann K, Philippsen P, Johnston M, Davis RW (1999) Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. Science 285:901–906. <https://doi.org/10.1126/science.285.5429.901>
- Witteveen CFB, Busink R, van de Vondervoort P, Dijkema C, Swart K, Visser J (1989) L-Arabinose and D-xylose catabolism in *Aspergillus niger*. Microbiology 135:2163–2171. <https://doi.org/10.1099/00221287-135-8-2163>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.