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The pentose phosphate pathway and organization of metabolic networks enabling growth programs

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Abstract

'The whole is greater than the sum of its parts', a quote misattributed to Aristotle, is especially true for metabolic networks and the key metabolic reactions within. Here, exemplifying the oxidative and non-oxidative arms of the pentose phosphate pathway (PPP), we separate the roles of key metabolites or precursors generated within, and their roles in the organizations of distinct metabolic networks to drive cell growth. Using recent examples from microbes, plants, and metazoans, we summarize the logic of how the distinct arms of the PPP regulate systems-level metabolism, as well as the reciprocal regulation exerted by diverse metabolic networks on the respective PPP arms. We highlight systems-level consequences of activating/inactivating either arm of the pathway, with a particular emphasis on the inherent coupling of amino acid metabolism with the PPP. We further contextualize how these arms function differently to regulate global metabolic states and the biosynthetic capacity of a cell.

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Introduction

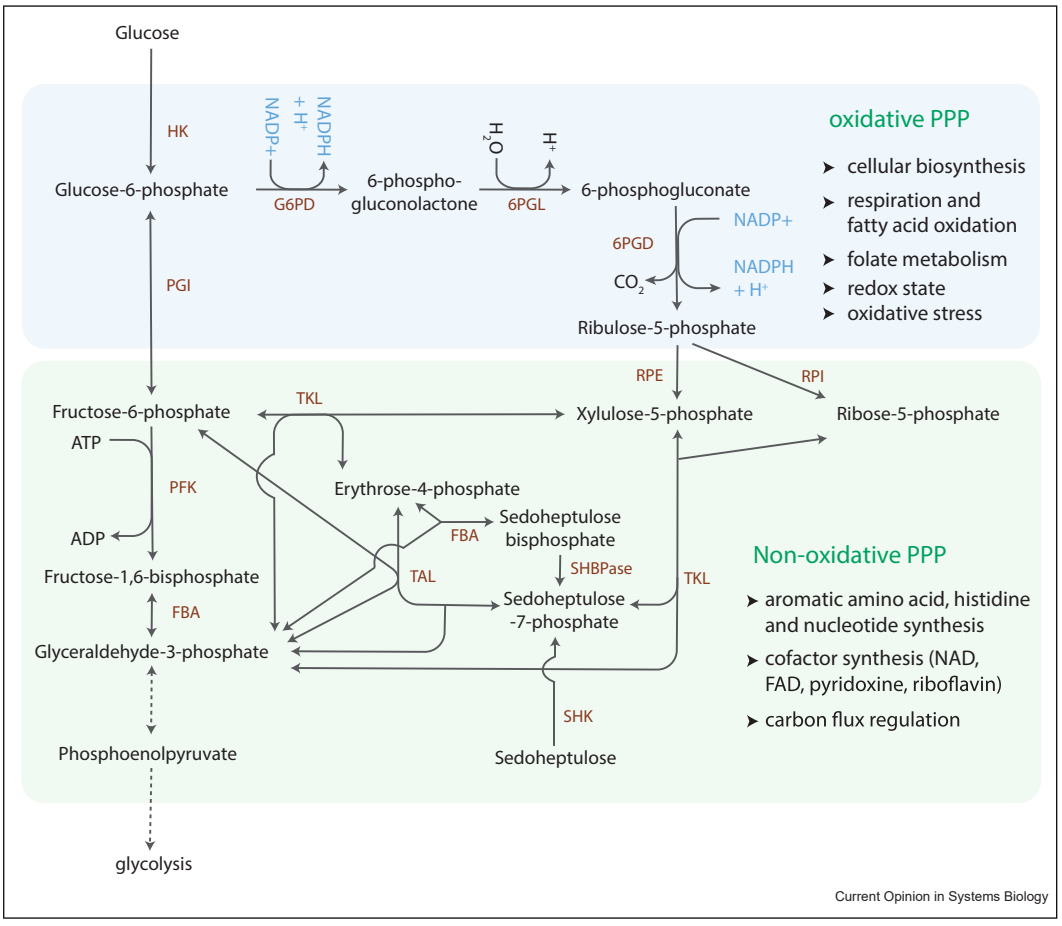
Cell growth inherently depends on balanced carbon flux through interconnected metabolic nodes. This carbon budgeting allows cells to produce sufficient ATP/energy, accumulate biomass, and sustain growth. Biomass accumulation is a consequence of the cellular biosynthetic network, where cells make building blocks like amino acids for proteins and nucleotides, sugar-phosphates for storage carbohydrates and nucleotides, and acetyl-coA for fatty acids synthesis. This 'reductive' biosynthetic capacity is defined by the amounts of NADPH produced by the cells, primarily by the pentose phosphate pathway (PPP) [1]. Concurrently, the PPP produces critical precursors to synthesize various biomolecules. The optimal allocations of carbon flux toward arms of the PPP hence determine the biosynthetic capacity of the cell by balancing flux through multiple metabolic networks to synthesize the required building blocks and co-factors.

Here, we summarize the organization of metabolic networks impacted by allocations of carbon flux toward the PPP and vice-versa in a harmonious cellular economy. We highlight conserved but underappreciated aspects of the importance of the PPP across metabolic networks that are present across kingdoms of life. In particular, we emphasize the reciprocal coupling of the PPP with amino acid networks to collectively impact cell growth programs.

The oxidative and non-oxidative PPP arms and mass-action balance

While the PPP was discovered over 80 years ago, studying it remains as relevant today as it was in the previous millennium. Indeed, its significance to the systems-level organization of metabolic networks is only now crystallizing. Reactions of the PPP are classically divided into two arms — oxidative and non-oxidative [reviewed in 1]. The essentially irreversible oxidative PPP consists of three steps to synthesize ribulose-5-phosphate (Ru5P) from glucose-6-phosphate (G6P). Two of these steps produce NADPH, the reductive currency of cells (Figure 1). The R5P isomerase (RPI) enables Ru5P to isomerize to ribose-5-phosphate (R5P), the primary product of PPP. Contrastingly, the non-oxidative PPP has easily reversible transaldolase and transketolase reactions which, depending on the needs of the cell, either divert flux toward R5P synthesis or help in maintaining glycolytic flux (Figure 1). The non-oxidative PPP also includes sedoheptulose kinase (SHK) in mammals, and sedoheptulose-7-phosphatase (SHBPase) in fungi, algae, and plants, which regulate S7P levels (Figure 1). Thus, the two arms of the PPP serve distinct functions.

Figure 1



The reactions of the pentose phosphate pathway. The pentose phosphate pathway is classically divided into the oxidative and non-oxidative arms. The oxidative arm functions to provide NADPH required for cellular biosynthesis and redox balance. The non-oxidative arm provides precursors for amino acids, nucleotide and co-factor synthesis. Together, they support various arms of the greater metabolic network.

The oxidative PPP is a major provider of NADPH and therefore drives reductive biosynthesis. Depending on the environment and cell-type, the oxidative PPP produces ~25–60% of all NADPH, enabling biosynthetic reactions [2–5]. The non-oxidative arm does not directly contribute to NADPH production but still helps maintain NADPH levels. This is because the non-oxidative arm can route excess R5P (produced via the oxidative arm), into glycolysis and G6P recycling, to sustain oxidative PPP. Contrastingly, when ribose demands increase, fructose-bisphosphate aldolase (FBA) diverts glycolytic flux toward SBP and then S7P production, thereby fueling the non-oxidative arm for R5P synthesis, *without* NADPH production [6–8] (Figure 1). This illustrates the intimate connection between glycolysis and PPP in balancing carbon flux. Interestingly, in many microbes present in gut microbiota, as well as yeast, the glycolytic enzyme PFK modulates S7P levels, by phosphorylating S7P to form SBP [8,9]. Mammalian liver fractions enriched in PFK activity also have enhanced S7P kinase activity [10]. S7P acts as a store of carbon, increasing during carbon/nitrogen starvation and oxidative stress [9–12]. S7P, therefore, can provide R5P, glycolytic intermediates, and NADPH (by glucose recycling) depending on the needs of the cell. S7P supply, therefore, becomes an important node that determines flux to and from the non-oxidative PPP [6,10]. The non-oxidative PPP functions to provide precursors like erythrose-4-phosphate (E4P) and R5P for amino acid, nucleotide and co-factor (pyridoxine, riboflavin, NAD, FAD) synthesis (Figure 1). The easy reversibility of the non-oxidative arm enables a dynamic equilibrium between the intermediates, where metabolic needs can be met largely by mass-action balance into various interconnected metabolic pathways based on substrate amounts.

Butterfly effect: PPP and distant nodes in the biosynthetic network

Small changes in different nodes of the PPP result in large, often non-intuitive differences in apparently unrelated metabolic pathways. Classic studies in *Saccharomyces cerevisiae* show that the loss of *G6PD (ZWF1), 6PGL (SOL3/SOL4), 6PGD (GND1/GND2)* reduce cell growth in media without free amino acids, with Gnd1 and Zwf1, the NADPH producing steps, showing the most significant defects [13]. These demonstrated a role for the oxidative-PPP in determining overall biosynthetic capacity. Indeed, *Δzwf1* cells are methionine auxotrophs (discussed later), with altered GSH/GSSG levels and reduced lipid accumulation due to decreased NADPH [2,14]. In contrast, the loss of *GND1(6PGD)* does not change NADPH levels [2,15], likely because of 6PG accumulation, which inhibits glycolysis and promotes flux through the oxidative PPP to produce NADPH. *ZWF1* loss also increases NAD+/NADH ratios, but cells still maintain glycolytic flux [2,13]. Instead, the flux increases through the non-oxidative PPP and tricarboxylic acid (TCA) cycle [13]. Interpreting this requires looking into the 'greater PPP network'. The increased NAD+/NADH ratios most likely come from a combination of altered mitochondrial activity and increased *de novo* NAD synthesis from tryptophan. Consistently, *Δzwf1* cells accumulate tryptophan because of increased non-oxidative PPP flux [2]. Interestingly, while the TCA cycle flux *increases* in *Δzwf1* cells, mitochondrial activity *decreases* [13,16]. This is because respiration itself depends on the PPP for NADPH, which combats increasing ROS levels in respiring cells [16]. The increased flux through the TCA cycle in *Δzwf1* cells can, therefore, be imagined as an adaptive response driving NADPH production through TCA cycle-dependent pathways [13]. Similarly, NADPH from the PPP combats ROS produced during fatty acid oxidation [17]. Finally, the non-oxidative PPP arm can also regulate carbon flux through the TCA cycle. Patients with defects in either transaldolase or transketolase activity accumulate most TCA cycle intermediates [18]. Expectedly, decreased PPP activity also decreases *de novo* nucleotide synthesis, and alters pyridoxine and riboflavin levels, all of which requires R5P/Ru5P as precursors [2,19,20]. These observations emphasize the impact of PPP on multiple nodes of the biosynthetic network.

Feedback, recycling, and allosteric control

Because of its importance to overall carbon balance via regulating multiple networks, the PPP flux is incredibly dynamic and controlled by substrate feedback, recycling, and allosteric control. For example, in yeast growing in low nitrogen conditions, there is a ~2-fold increase in flux toward the PPP pathway [12,21]. Large increases in PPP flux occur when cells shift from fermentative to respiratory metabolism or when quiescent cells adapt to growth [22,23]. In mammalian tissues such as the brain, liver and heart, the PPP consumes up to 16% of glucose taken up [24]. This PPP flux contributes substantially to glycolysis and lactate production. The best-studied divergence of glucose consumption toward the PPP is during oxidative stress, to produce NADPH [11]. Oxidative stress can inhibit glycolysis. G6PD is inhibited by increased NADPH, and in conditions of oxidative stress, sustained PPP activity requires NADP + recycling by ROS dependent oxidation [11]. Generally, however, ROS levels rarely change excessively [16]. So how does the PPP activity remain high given that this will require continuous NADP + recycling? This recycling appears to occur via the greater biosynthetic network coming predominantly from biosynthetic processes [4]. Around 12–20% of NADPH produced by the PPP in mammalian cells is consumed for fatty acid synthesis [5]. The remaining NADPH turnover comes from *other* biosynthetic reactions, especially amino acid biosynthesis [4]. Furthermore, specific nodes of PPP are feedback regulated by various metabolites (Table 1). This extensive feedback underlies the carbon-flux partitioning between PPP and glycolysis, depending on the cellular needs. While the importance of these coupled nodes is still underappreciated, this regulation is likely to become increasingly apparent in many systems, such as in rapidly growing cancer cells.

Coupling of the amino acid network with the PPP

a) *PPP* and nitrogen assimilation

It is increasingly clear that carbon and nitrogen metabolism are irrevocably linked, and the PPP is central to this linkage. In microbes and plants, the PPP regulates nitrogen assimilation to control the overall amino acid network. Many microbes and plants must assimilate inorganic nitrogen either as nitrate or ammonia to synthesize amino acids and nitrogenous compounds [25–27]. As a first step, converting nitrate to nitrite and further to ammonia requires NADPH produced via the PPP [26] (Figure 2a). Here, G6PD activity is stimulated by nitrate addition [26,28]. Further ammonia assimilation into glutamate also requires PPP activity [26]. This assimilation is via the function of either a glutamate synthetase (GS) or a glutamate dehydrogenase (GDH) enzyme [25–27] (Figure 2a). In the well-studied model eukaryote *S. cerevisiae*, supplementing NH_4^+ induces a transcriptional response where PPP genes, Gdh1 and other amino acid biosynthesis genes are coincidentally activated, and PPP flux increases [29]. Here, an incredible ~50–60% of the NADPH used for biomass production is through Gdh1 [30]. Furthermore, deleting *GDH1* decreases G6PD (Zwf1) and 6PGD (Gnd1) expression, together with amino acid and nucleotide biosynthetic genes, resulting in a redox imbalance [30]. These observations reveal the tight

Table 1

Specific metabolites that allosterically regulate pentose phosphate pathway activity.

Activity	Molecule	Activating/inhibitory	Organism	References
G6PD	NADPH	Inhibitory	Bacteria, yeasts, plants, mammalian cells	[11,55]
G6PD	ATP	Inhibitory	Bacteria, plants, mammalian cells (RBC, liver enzymes)	[55]
G6PD	Palmitoyl coA	Inhibitory	Bacteria, yeasts, mammalian cells (Bovine mammary G6PD)	[56,57]
G6PD	Sodium octanoate	Inhibitory	Mammalian cells (liver enzymes)	[58]
G6PD	PEP	Inhibitory	Bacteria	[59]
G6PD	6-phosphogluconic acid	Inhibitory	Mammalian cells (liver enzymes)	[60]
G6PD	ADP	Inhibitory	Mammalian cells (liver enzymes)	[60]
G6PD	NADH	Inhibitory	Mammalian cells (liver enzymes)	[60]
6PGD	NADPH	Inhibitory	Mammalian cells (RBC)	[61]
6PGD	ATP	Inhibitory	Mammalian cells (RBC)	[61]
6PGD	3 PG	Inhibitory	Mammalian cells (cancer cells)	[62]
6PGD	4-phosphoerythronate	Inhibitory	Yeast (*S. cerevisiae*), *Trypanosoma brucei*, Mammalian cells (cancer cells)	[63]
6PGD	2,3-BPG	Inhibitory	Mammalian cells (RBC)	[61]
Transaldolase	F16BP	Inhibitory	Bacteria (*E. coli*)	[64]
Overall PPP activity	PEP	Activates PPP by inhibiting glycolysis	Yeast (*S. cerevisiae*)	[16]

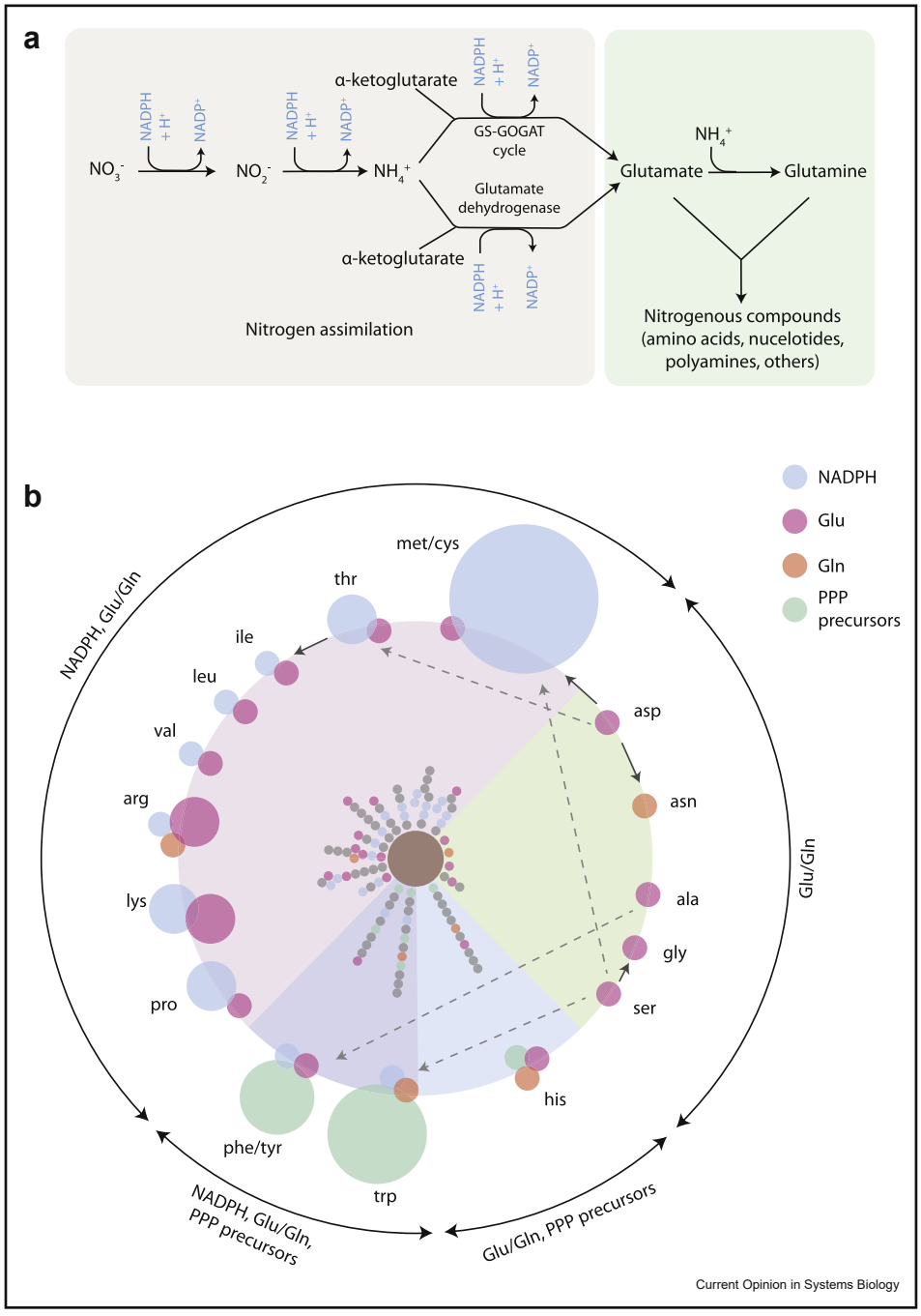
coupling between nitrogen assimilation, amino acid biosynthesis, and the PPP. Glutamate synthesized by GDH further acts as a backbone for ammonia assimilation into glutamine [27], and together glutamate and glutamine are the nitrogen donors required to synthesize all nitrogenous compounds [27] (Figure 2a). This node for glutamate synthesis (with either GDH or GS) and its regulation by PPP, therefore, becomes crucial for regulating biomass increase and growth.

In contrast, in mammals, ammonia assimilation and glutamate synthesis are through an NADH (and not NADPH) consuming GDH. However, this node is critical for biomass accumulation and the *de novo* synthesis of non-essential amino acids and remains linked to the PPP. In neurons, for example, carbon flux toward the TCA cycle and glutamate (neurotransmitter) synthesis is via the PPP [31]. In cancer cells, PPP flux sustains *de novo* amino acid synthesis (by providing NADPH and other precursors) [32]. In some cancer cells, the NADPH turnover by mutated IDH1 (which now depends on NADPH and not NADH) coincident with the conversion of alpha-ketoglutarate to 2-hydroxyglutarate, can support high PPP activity for biomass accumulation [33].

b) *PPP*, and the 'greater' amino acid network

A close inspection brings forth the inseparable coupling of the evolution of amino acid biosynthesis pathways with the reductive capacity of the cell. In organisms that biosynthesize their own amino acids (prototrophs), which include a plurality of plants, bacteria, archaea, algae and fungi, amino acid biosynthesis is irrevocably coupled to PPP. In such organisms, the synthesis of most amino acids requires NADPH, PPP precursors, and glutamate synthesis supported by the PPP (highlighted in the study by Walvekar et al. [34]). The extent of this can be seen in Figure 2b. As these organisms are now complete in their amino acid compositions, evolution has allowed other organisms that feed on these to become auxotrophic for specific amino acids. In mammals, apart from proline, tyrosine, and arginine, all NADPH requiring amino acids are *essential* for survival, meaning that these organisms either cannot synthesize or cannot synthesize enough of these amino acids. Here, *de-novo* synthesis of non-essential amino acids is, therefore, controlled by partitioning carbon flux toward glycolysis and glutamate synthesis by the PPP [32], which, therefore, also directly modulates NADPH production and precursor (R5P and E4P) supply (Figure 2b). Similarly in *Corynebacterium glutamicum*, the split ratio between glycolysis and PPP decides the amino acid preferably synthesized. More PPP flux favors threonine, lysine, and proline synthesis, which require higher NADPH [35–37] (Figure 2b). Contrastingly, increased flux toward glycolysis favors Ala, Val and Leu synthesis, all derived from pyruvate [35]. In yeast, removing 6PGL (*SOL3*) leads to increased Ala and Val, by increasing pyruvate synthesis via glycolysis [38], and the loss of the transaldolase *TAL1* increases Val and Arg levels [38]. Similarly, human patients with transaldolase and transketolase defects show abnormal tryptophan metabolism [18]. Expectedly, to maximize the commercial production of amino acids, the PPP is extensively studied to understand glucose partitioning between glycolysis and PPP [39]. This (as explained

Figure 2



PPP and *de novo* amino acid biosynthesis. (**a**) Nitrogen assimilation and glutamate synthesis in microbes and plants. The reactions of nitrogen assimilation and glutamate synthesis (GS-GOGAT cycle, glutamine synthetase-glutamate synthetase cycle) require NADPH from the PPP. (**b**) Impact of the PPP on general amino acid synthesis in prototrophic organisms. Amino acids can be divided into 4 categories: (i) amino acids which depend on NADPH, Glu/Gln and PPP precursors for their synthesis, (ii) amino acids which depend on NADPH and Glu/Gln for their synthesis, (iii) amino acids which depend on PPP precursors and Glu/Gln for their synthesis (only histidine), and (iv) amino acids which require Glu/Gln for their synthesis. The respective substrates have been color-coded, and scaled in proportion to the number of molecules of the respective substrate utilized. Linear sequence of circles (inside) represent the number of steps in the synthesis of that specific amino acids, and those steps which require either Gln/Glu, NADPH or PPP precursors (E4P, R5P and PEP). The specific reactions and their NADPH requirements are based on the respective pathways in *S. cerevisiae* and *E. coli*, taken from KEGG.

earlier) will require optimal GDH activity. Surprisingly, the physiological roles of the PPP in regulating GDH activity and general amino acid biosynthesis remain poorly explored in most organisms.

c) Interlinked triumvirate: methionine, the PPP, and the folate cycle

Out of all the amino acids, the role of the PPP in regulating *de novo* methionine synthesis is remarkable. As mentioned previously, in yeast, *Δzwf1* cells (deficient in G6PD) are methionine auxotrophs [14], and this auxotrophy is rescued by providing methionine, SAM, cysteine or homocysteine. The auxotrophy appears to arise from the inability to assimilate inorganic sulfur because of decreased NADPH [14]. Synthesizing a single molecule of methionine requires ∼ six molecules of NADPH [34] (Figure 2b). With reduced PPP, the NADPH demands for methionine biosynthesis cannot be easily fulfilled by other NADPH sources. Conversely, *ZWF1* expression decreases when cells are provided with methionine or SAM [14]. Furthermore, through standard feedback regulation, the loss of enzymes in the methionine biosynthesis pathway upregulates the oxidative PPP to produce NADPH [40]. Similarly, Met32, a co-transcriptional regulator of methionine biosynthesis genes, upregulates the expression of PPP enzyme transcripts directly [41]. Methionine biosynthesis, therefore, both depends on and is tightly coupled to NADPH availability via PPP.

Methionine biosynthesis is also linked to folate metabolism, as 5-methyl tetrahydrofolate (THF) is the methyl donor for *de novo* Met biosynthesis. Forming 5-methyl THF from 5,10-methylene THF (a folate cycle intermediate) requires NADPH. During methionine deprivation, this conserved reaction helps synthesize methionine from homocysteine. This requires the PPP, as the loss of G6PD leads to a substantial decrease in 5-methyl THF [3]. Furthermore, NADPH production by the folate cycle and folate-related biomass synthesis remain tightly coupled to the PPP [3].

Why is this important? The folate cycle helps synthesize 5,10-methylene-THF and 10-formyl-THF, needed, respectively, for dTMP and purine biosynthesis. The PPP hence supports *de novo* nucleotide synthesis by providing ribose-5-phosphate, supporting glutamate/glutamine and other amino acid synthesis (which donate nitrogen for nucleotide synthesis), and one-carbon metabolism. In proliferating cells, *de novo* nucleotide biosynthesis via the PPP supports multiple functions beyond DNA and RNA synthesis. ATP synthesis contributes substantially to intracellular SAM pools [42]. Similarly, UTP synthesis supports UDP-GlcNAc formation [43]. This implies that the PPP's role extends beyond *de novo* nucleotide synthesis to include glycosylation (UDP-, GDP-), lipid (CDP-) and storage carbohydrate synthesis (UDP-), methylation potential (via ATP/SAM), and cofactor synthesis (via ATP/NAD/FAD). As a 'behind the scenes' player, the PPP indispensably sustains metabolic networks enabling growth.

d) Amino acid availability/synthesis enhancing PPP flux

Amino acid biosynthesis is perhaps *the* major consumer of NADPH synthesized via the PPP. Probably, for this reason, supplementing amino acids, particularly methionine and lysine in conditions of oxidative stress increases oxidant tolerance in yeast cells [44,45]. The availability of these amino acids enables the channeling of NADPH synthesized by the PPP to counteract oxidative stress rather than amino acid biosynthesis. Notably, these can also drive growth programs. Again, the role of methionine here is striking [34]. Providing excess methionine upregulates 6PGD, RPI and TKL enzymes, increasing glucose recycling into the oxidative PPP to fuel NADPH and R5P production. This subsequently drives increased glutamate synthesis and further amino acid and nucleotide synthesis to enable growth [34]. Cells that have impaired abilities to sense excess methionine rewire carbon flux toward storage carbohydrates, and decrease nucleotide synthesis, with reduced PPP and/or folate activity [46]. Similarly, in cancer cells, glutamate utilization toward fatty acid synthesis is also supported by the PPP [47]. Moreover, inhibiting serine synthesis decreases PPP activity, R5P levels and nucleotide biosynthesis, and the presence of extracellular serine increases PPP activity for *de novo* nucleotide synthesis [42,48]. Separately, cystine transport increases flux toward the PPP to maintain redox balance [49]. These data unravel the intricate, two-way feedback regulations which couple amino acid availability and sustained growth to PPP activity.

Concluding remarks

Proteins constitute ∼50−60% of cellular dry weight, of which ribosomes are the most abundant. Expectedly, biomass accumulation and growth are outputs of ribosomal biogenesis [50]. Ribosomal biogenesis in proliferating cells will depend on the availability of R5P, amino acids and NADPH, for protein and nucleotide synthesis. Probably, for this reason, ribosomal biogenesis is tightly linked to PPP activity during cell-cycle progression [6]. Interestingly, methionine biosynthesis genes and glutamate levels also drive the cell cycle [51,52]. Understanding the direct and indirect roles of the PPP in supporting these pathways during cell-cycle progression will be required to better predict the systems-level organization of growth programs. The most poorly explored, but important context is to understand how cells continue to sustain growth when glucose becomes limiting. In these contexts, the careful carbon flux partitioning toward either amino acid biosynthesis or gluconeogenesis (to support the PPP and biosynthesis) become critical to determine

growth/survival outcomes [53,54]. Understanding the regulation of these pathways in the context of growth outcomes is undoubtedly likely to reveal new surprises about the roles of the PPP within the greater metabolic network, and how it regulates or impacts growth programs.

Conflict of interest statement

Nothing declared.

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References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Stincone A, Prigione A, Cramer T, Wamelink MMC, Campbell K, Cheung E, Olin-Sandoval V, Grüning NM, Krüger A, Tauqeer Alam M, *et al.*: **The return of metabolism: Biochemistry and physiology of the pentose phosphate pathway**. *Biol Rev* 2015, **90**:927–963.

2. * Kwolek-Mirek M, Maslanka R, Molon M: **Disorders in NADPH generation via pentose phosphate pathway influence the reproductive potential of the Saccharomyces cerevisiae yeast due to changes in redox status**. *J Cell Biochem* 2019, **120**:8521–8533.
This study illustrates the metabolic phenotypes observed due to loss of oxidative pentose phosphate pathway.

3. ** Chen L, Zhang Z, Hoshino A, Zheng HD, Morley M, Arany Z, Rabinowitz JD: **NADPH production by the oxidative pentose-phosphate pathway supports folate metabolism**. *Nat Metab* 2019, **1**:404–415.
This study identifies a role of PPP in supporting biosynthesis and NADPH production via one-carbon metabolism.

4. Fan J, Ye J, Kamphorst JJ, Shlomi T, Thompson CB, Rabinowitz JD: **Quantitative flux analysis reveals folate-dependent NADPH production**. *Nature* 2014, **510**:298–302.

5. Lewis CA, Parker SJ, Fiske BP, McCloskey D, Gui DY, Green CR, Vokes NI, Feist AM, Vander Heiden MG, Metallo CM: **Tracing compartmentalized NADPH metabolism in the Cytosol and Mitochondria of mammalian cells**. *Mol Cell* 2014, **55**:253–263.

6. Clasquin MF, Melamud E, Singer A, Gooding JR, Xu X, Dong A, Cui H, Campagna SR, Savchenko A, Yakunin AF, *et al.*: **Riboneogenesis in yeast**. *Cell* 2011, **145**:969–980.

7. Li Q, Qin T, Bi Z, Hong H, Ding L, Chen J, Wu W, Lin X, Fu W, Zheng F, *et al.*: **Rac1 activates non-oxidative pentose phosphate pathway to induce chemoresistance of breast cancer**. *Nat Commun* 2020, **11**:1–18.

8. * Garschagen LS, Franke T, Deppenmeier U: **An alternative pentose phosphate pathway in human gut bacteria for the degradation of C5 sugars in dietary fibers**. *FEBS J* 2021, **288**:1839–1858.
These important studies (7,8) illusrates the role of Aldolase (FBA) in regulating PPP activity in mammalian cells and bacteria suggesting that FBA regulates PPP activity in all kingdoms of life.

9. Xu YF, Létisse F, Absalan F, Lu W, Kuznetsova E, Brown G, Caudy AA, Yakunin AF, Broach JR, Rabinowitz JD: **Nucleotide degradation and ribose salvage in yeast**. *Mol Syst Biol* 2013, **9**.

10. Nagy C, Haschemi A: **Sedoheptulose kinase regulates cellular carbohydrate metabolism by sedoheptulose 7-phosphate supply**. *Biochem Soc Trans* 2013, **41**:674–680.

11. Christodoulou D, Kuehne A, Estermann A, Fuhrer T, Lang P, Sauer U: **Reserve flux capacity in the pentose phosphate pathway by NADPH binding is conserved across kingdoms**. *iScience* 2019, **19**:1133–1144.

12. * Røst LM, Thorfinnsdottir LB, Kumar K, Fuchino K, Langørgen IE, Bartosova Z, Kristiansen KA, Bruheim P: **Absolute quantification of the central carbon metabolome in eight commonly applied prokaryotic and eukaryotic model systems**. *Metabolites* 2020, **10**.
This paper shows an increase in PPP flux when cells are cultured in minimal medium, requiring higher amounts of biosynthesis.

13. Blank LM, Kuepfer L, Sauer U: **Large-scale 13C-flux analysis reveals mechanistic principles of metabolic network robustness to null mutations in yeast**. *Genome Biol* 2005, **6**:1–16.

14. Thomas D, Cherest H, Surdin-Kerjan Y: **Identification of the structural gene of glucose-6-phosphate dehydrogenase in yeast. Inactivation leads to a nutritional requirement for organic sulfur**. *EMBO J* 1991, **10**:547–553.

15. Dubreuil MM, Morgens DW, Okumoto K, Honsho M, Contrepois K, Lee-McMullen B, Traber GMA, Sood RS, Dixon SJ, Snyder MP, *et al.*: **Systematic identification of regulators of oxidative stress reveals non-canonical roles for peroxisomal Import and the pentose phosphate pathway**. *Cell Rep* 2020, **30**:1417–1433.e7.

16. Grüning NM, Rinnerthaler M, Bluemlein K, Mülleder M, Wamelink MMC, Lehrach H, Jakobs C, Breitenbach M, Ralser M: **Pyruvate kinase triggers a metabolic feedback loop that controls redox metabolism in respiring cells**. *Cell Metabol* 2011, **14**:415–427.

17. Minard KI, McAlister-Henn L: **Dependence of peroxisomal β-oxidation on cytosolic sources of NADPH**. *J Biol Chem* 1999, **274**:3402–3406.

18. Shayota BJ, Donti TR, Xiao J, Gijavanekar C, Kennedy AD, Hubert L, Rodan L, Vanderpluym C, Nowak C, Bjornsson HT, *et al.*: **Untargeted metabolomics as an unbiased approach to the diagnosis of inborn errors of metabolism of the non-oxidative branch of the pentose phosphate pathway**. *Mol Genet Metabol* 2020, **131**:147–154.

19. Kondo H, Nakamura Y, Dong YX, Nikawa JI, Sueda S: **Pyridoxine biosynthesis in yeast: Participation of ribose 5-phosphate ketol-isomerase**. *Biochem J* 2004, **379**:65–70.

20. Lin R, Elf S, Shan C, Kang HB, Ji Q, Zhou L, Hitosugi T, Zhang L, Zhang S, Seo JH, *et al.*: **6-Phosphogluconate dehydrogenase links oxidative PPP, lipogenesis and tumour growth by inhibiting LKB1-AMPK signalling**. *Nat Cell Biol* 2015, **17**:1484–1496.

21. Bertels L-K, Fernández Murillo L, Heinisch JJ: **The pentose phosphate pathway in yeasts-more than a poor Cousin of glycolysis**. *Biomolecules* 2021, **11**.

22. Gombert AK, Dos Santos MM, Christensen B, Nielsen J: **Network identification and flux quantification in the central metabolism of Saccharomyces cerevisiae under different conditions of glucose repression**. *J Bacteriol* 2001, **183**:1441–1451.

23. * Zhang J, Martinez-Gomez K, Heinzle E, Wahl SA: **Metabolic switches from quiescence to growth in synchronized Saccharomyces cerevisiae**. *Metabolomics* 2019, **15**:121.
This study illustartes increase in PPP activity when cells switch from quiscent o growth.

24. Lee MH, Malloy CR, Corbin IR, Li J, Jin ES: **Assessing the pentose phosphate pathway using [2, 3-13C2]glucose**. *NMR Biomed* 2019, **32**:1–10.

25. van Heeswijk WC, Westerhoff HV, Boogerd FC: **Nitrogen assimilation in Escherichia coli: putting molecular data into a systems perspective**. *Microbiol Mol Biol Rev* 2013, **77**:628–695.

26. Esposito S: **Nitrogen assimilation, abiotic stress and glucose 6-phosphate dehydrogenase: the full circle of reductants**. *Plants* 2016, **5**:236–246.

27. Magasanik B: **Ammonia assimilation by Saccharomyces cerevisiae**. *Eukaryot Cell* 2003, **2**:827–829.

28. Wilson RA, Jenkinson JM, Gibson RP, Littlechild JA, Wang ZY, Talbot NJ: **Tps1 regulates the pentose phosphate pathway, nitrogen metabolism and fungal virulence**. *EMBO J* 2007, **26**: 3673–3685.

29. Jiménez-Martí E, del Olmo M l-: **Addition of ammonia or amino acids to a nitrogen-depleted medium affects gene expression patterns in yeast cells during alcoholic fermentation**. *FEMS Yeast Res* 2008, **8**:245–256.

30. Bro C, Regenberg B, Nielsen J: **Genome-wide transcriptional response of a Saccharomyces cerevisiae strain with an altered redox metabolism**. *Biotechnol Bioeng* 2004, **85**: 269–276.

31. Brekke EMF, Walls AB, Schousboe A, Waagepetersen HS, Sonnewald U: **Quantitative importance of the pentose phosphate pathway determined by incorporation of 13 C from 2-13 C-and 3-13 Cglucose into TCA cycle intermediates and neurotransmitter amino acids in functionally intact neurons**. *J Cerebr Blood Flow Metabol* 2012, **32**:1788–1799.

32. Ghergurovich JM, Lang JD, Levin MK, Briones N, Facista SJ, Mueller C, Cowan AJ, McBride MJ, San Roman Rodriguez E, Killian A, *et al.*: **Local production of lactate, ribose phosphate, and amino acids by human triple-negative breast cancer**. *Med* 2021, https://doi.org/10.1016/j.medj.2021.03.009.

33. Gelman SJ, Naser F, Mahieu NG, McKenzie LD, Dunn GP, Chheda MG, Patti GJ: **Consumption of NADPH for 2-HG synthesis increases pentose phosphate pathway flux and Sensitizes cells to oxidative stress**. *Cell Rep* 2018, **22**:512–522.

34. ** Walvekar AS, Srinivasan R, Gupta R, Laxman S: **Methionine coordinates a hierarchically organized anabolic program enabling proliferation**. *Mol Biol Cell* 2018, **29**:3183–3200.
This important study describes the role of methionine in activating PPP for increased biomass production.

35. * Murai K, Sasaki D, Kobayashi S, Yamaguchi A, Uchikura H, Shirai T, Sasaki K, Kondo A, Tsuge Y: **Optimal ratio of carbon flux between glycolysis and the pentose phosphate pathway for amino acid accumulation in Corynebacterium glutamicum**. *ACS Synth Biol* 2020, **9**:1615–1622.
This study shows how different proportioning of carbon flux between PPP and glycolysis leads to synthesis of different amino acids.

36. Kobayashi S, Kawaguchi H, Shirai T, Ninomiya K, Takahashi K, Kondo A, Tsuge Y: **Automatic redirection of carbon flux between glycolysis and pentose phosphate pathway using an oxygen-responsive metabolic switch in Corynebacterium glutamicum**. *ACS Synth Biol* 2020, **9**:814–826.

37. Zhang J, Qian F, Dong F, Wang Q, Yang J, Jiang Y, Yang S: **De novo engineering of Corynebacterium glutamicum for l -proline production**. *ACS Synth Biol* 2020, **9**:1897–1906.

38. Mülleder M, Calvani E, Alam MT, Wang RK, Eckerstorfer F, Zelezniak A, Ralser M: **Functional metabolomics describes the yeast biosynthetic regulome**. *Cell* 2016, **167**:553–565.e12.

39. Becker J, Wittmann C: **Systems and synthetic metabolic engineering for amino acid production - the heartbeat of industrial strain development**. *Curr Opin Biotechnol* 2012, **23**: 718–726.

40. Petti AA, Crutchfield CA, Rabinowitz JD, Botstein D: **Survival of starving yeast is correlated with oxidative stress response and nonrespiratory mitochondrial function**. *Proc Natl Acad Sci U S A* 2011, **108**:E1089.

41. Carrillo E, Ben-Ari G, Wildenhain J, Tyers M, Grammentz D, Lee TA: **Characterizing the roles of Met31 and Met32 in coordinating met4-activated transcription in the absence of Met30**. *Mol Biol Cell* 2012, **23**:1928–1942.

42. ** Yu W, Wang Z, Zhang K, Chi Z, Xu T, Jiang D, Chen S, Li W, Yang X, Zhang X, *et al.*: **One-carbon metabolism supports S-adenosylmethionine and Histone methylation to drive inflammatory macrophages**. *Mol Cell* 2019, **75**:1147–1160.e5.
This study describes how extracellular serine supports increases PPP activity for *de novo* nucleotide synthesis.

43. Chi F, Sharpley MS, Nagaraj R, Roy S Sen, Banerjee U: **Glycolysis-independent glucose metabolism Distinguishes TE from ICM fate during mammalian embryogenesis**. *Dev Cell* 2020, **53**:9–26.e4.

44. Campbell K, Vowinckel J, Keller MA, Ralser M: **Methionine metabolism alters oxidative stress resistance via the pentose phosphate pathway**. *Antioxidants Redox Signal* 2015, **24**: 543–547.

45. Olin-Sandoval V, Yu JSL, Miller-Fleming L, Alam MT, Kamrad S, Correia-Melo C, Haas R, Segal J, Peña Navarro DA, Herrera-Dominguez L, *et al.*: **Lysine harvesting is an antioxidant strategy and triggers underground polyamine metabolism**. *Nature* 2019, **572**:249–253.

46. Gupta R, Walvekar AS, Liang S, Rashida Z, Shah P, Laxman S: **A tRNA modification balances carbon and nitrogen metabolism by regulating phosphate homeostasis**. *Elife* 2019, **8**: 1–33.

47. Zhong B, Jiang D, Hong Y, Li L, Qiu L, Yang R, Jin X, Song Y, Chen C, Li B: **Glucose-6-phosphate dehydrogenase neutralizes stresses by supporting reductive glutamine metabolism and AMPK activation**. *Signal Transduct Target Ther* 2021, **6**: 1–4.

48. ** Reid MA, Allen AE, Liu S, Liberti MV, Liu P, Liu X, Dai Z, Gao X, Wang Q, Liu Y, *et al.*: **Serine synthesis through PHGDH coordinates nucleotide levels by maintaining central carbon metabolism**. *Nat Commun* 2018, **9**.
This important study illustrates the role of serine synthesis in regulating carbon flux through the PPP pathway for nucleotide synthesis.

49. Liu X, Olszewski K, Zhang Y, Lim EW, Shi J, Zhang X, Zhang J, Lee H, Koppula P, Lei G, *et al.*: **Cystine transporter regulation of pentose phosphate pathway dependency and disulfide stress exposes a targetable metabolic vulnerability in cancer**. *Nat Cell Biol* 2020, **22**:476–486.

50. Jorgensen P, Nishikawa JL, Breitkreutz BJ, Tyers M: **Systematic identification of pathways that couple cell growth and division in yeast**. *Science* 2002, **297**:395–400.

51. Tu BP, Mohler RE, Liu JC, Dombek KM, Young ET, Synovec RE, McKnight SL: **Cyclic changes in metabolic state during the life of a yeast cell**. *Proc Natl Acad Sci U S A* 2007, **104**: 16886–16891.

52. Spellman PT, Sherlock G, Zhang MQ, Iyer VR, Anders K, Eisen MB, Brown PO, Botstein D, Futcher B: **Comprehensive identification of cell cycle-regulated genes of the yeast Saccharomyces cerevisiae by microarray hybridization**. *Mol Biol Cell* 1998, **9**:3273–3297.

53. Varahan S, Walvekar A, Sinha V, Krishna S, Laxman S: **Metabolic constraints drive self-organization of specialized cell groups**. *Elife* 2019, **8**.

54. Rashida Z, Srinivasan R, Cyanam M, Laxman S: **Kog1/Raptor mediates metabolic rewiring during nutrient limitation by controlling SNF1/AMPK activity**. *Sci Adv* 2021, **7**:1–18.

55. Elhefny A, Kuliyev A, Gyulakhmedov S: **Regulation of glucose-6-phosphate dehydrogenase in plants (review Article)**. *J Plant Prod* 2011, **2**:949–957.

56. Kawaguchi A, Bloch K: **Inhibition of glucose 6 phosphate dehydrogenase by palmitoyl coenzyme A**. *J Biol Chem* 1974, **249**:5793–5800.

57. AJ Y, WB S, LV S: **In vitro regulation of mammary glucose-6-phosphate dehydrogenase activity by palmitoyl coenzyme A, acetate, and polyamines**. *Proc Soc Exp Biol Med* 1990, **193**: 274–279.

58. Weber G, Convery HJH, Lea MA, Stamm NB: **Feedback inhibition of key glycolytic enzymes in liver: action of free fatty acids**. *Science* 1966, **154**:1357–1360.

59. Opitz R, Schlegel HG: **Allosteric inhibition by phosphoenolpyruvate of glucose-6-phosphate dehydrogenase from bacteria and its taxonomic importance**. *Biochem Systemat Ecol* 1978, **6**:149–155.

60. Ibrahim MA, Ghazy AHM, Salem AMH, Ghazy MA, Abdel-Monsef MM: **Purification and characterization of glucose-6-phosphate dehydrogenase from camel liver**. *Enzym Res* 2014, **2014**.

61. Yoshida A, Lin M: **Regulation of glucose-6-phosphate dehydrogenase activity in red Blood cells from hemolytic and nonhemolytic variant subjects**. *Blood* 1973, **41**:877–891.

62. Hitosugi T, Zhou L, Elf S, Fan J, Kang HB, Seo JH, Shan C, Dai Q, Zhang L, Xie J, *et al.*: **Phosphoglycerate Mutase 1 coordinates glycolysis and biosynthesis to promote tumor growth**. *Cancer Cell* 2012, **22**:585–600.

63. Collard F, Baldin F, Gerin I, Bolsée J, Noël G, Graff J, Veiga-Da-Cunha M, Stroobant V, Vertommen D, Houddane A, *et al.*: **A conserved phosphatase destroys toxic glycolytic side products in mammals and yeast**. *Nat Chem Biol* 2016, **12**:601–607.

64. Ogawa T, Murakami K, Yoshino M: **Inhibition by fructose 1,6-bisphosphate of transaldolase from Escherichia coli**. *FEMS Microbiol Lett* 2016, **363**:183.