Published in final edited form as:
Trends Mol Med. 2011 July ; 17(7): 395–403. doi:10.1016/j.molmed.2011.01.014.

OXIDATIVE STRESS, INFLAMMATION AND CARCINOGENESIS ARE CONTROLLED THROUGH THE PENTOSE PHOSPHATE PATHWAY BY TRANSALDOLASE

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Abstract

Metabolism of glucose through the pentose phosphate pathway (PPP) influences the development of diverse pathologies. Hemolytic anemia due to deficiency of PPP enzyme glucose 6-phosphate dehydrogenase is the most common genetic disease in humans. Recently, inactivation of another PPP enzyme, transaldolase (TAL), has been implicated in male infertility and fatty liver progressing to steatohepatitis and cancer. Hepatocarcinogenesis was associated with activation of aldose reductase and redox-sensitive transcription factors and prevented by N-acetylcysteine. Here, we discuss how alternative formulations of the PPP with and without TAL reflect cell type-specific metabolic control of oxidative stress, a critical source of inflammation and carcinogenesis. Ongoing studies of TAL deficiency will identify new molecular targets for diagnosis and treatment in clinical practice.

Keywords

pentose phosphate pathway; transaldolase; glucose 6-phosphate dehydrogenase; transketolase; aldose reductase; metabolism; mitochondrial transmembrane potential; oxidative stress; apoptosis; necrosis; inflammation; liver; non-alcoholic fatty liver disease; non-alcoholic steatohepatitis; β-catenin; c-jun; alpha fetoprotein; hepatocellular carcinoma; carcinogenesis

Alternative formulations of the PPP and disease pathogenesis

The pentose phosphate pathway (PPP; Glossary) of glucose metabolism was originally conceived in the 1960s based on enzymatic activities and metabolites found in the yeast 1. The PPP fulfills two unique functions: 1) the formation of ribose 5-phosphate (R5P) for the synthesis of nucleotides, RNA, and DNA, supporting cell growth and proliferation; and 2) the formation of NADPH for biosynthetic reactions (Figure 1). NADPH protects against oxidative stress directly by neutralizing reactive oxygen intermediates (ROI) or indirectly via regenerating reduced glutathione (GSH) from its oxidized form GSSG (Figure 1). The PPP is comprised of two separate branches, oxidative and non-oxidative. Reactions in the

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oxidative branch are irreversible, whereas all reactions of the non-oxidative branch are fully reversible. The oxidative branch primarily depends on glucose 6-phosphate dehydrogenase (G6PD) 2, whereas transaldolase (TAL) is the rate-limiting enzyme for the non-oxidative branch 3. While the oxidative branch of the PPP is recognized as the source of NADPH and R5P, the overall contribution of the non-oxidative branch to metabolism and cell survival is poorly understood 3›4. The following sections will review the importance of the PPP for a seemingly diverse variety of oxidative stress-associated human diseases, ranging from hemolytic anemia, to male infertility, acetaminophen-induced liver failure, carcinogenesis, as well as autoimmunune diseases such as lupus and multiple sclerosis.

Mutations in G6PD represent the most common genetic defect in humans 5, affecting 400 million people globally 6. G6PD deficiency causes the depletion of NADPH in red cells 7 and predisposes to oxidative stress-induced hemolytic anemia 8. The high prevalence of G6PD deficiency, which is always partial due to mutations diminishing enzymatic activity, is explained by its protective effects against malaria 9. G6PD deficiency also protects against coronary artery disease (CAD) which has been attributed to the involvement of NADPH in lipid biosynthesis and activity of NADPH oxidase 10. Complete deficiency of G6PD is not compatible with cell survival 11. Diminished activity of transketolase (TK), an enzyme of the non-oxidative branch of the PPP, lead to Wernicke-Korsakoff syndrome due to deficiency of its co-factor, thiamine 12. Similar to complete G6PD deficiency, mice lacking TK are not viable either 13. By contrast, mice lacking the PPP enzyme transaldolase (TAL) normally develop to adulthood, however, males are typically infertile due to structural and functional damage of sperm mitochondria 14. TAL-deficient mice also exhibit oxidative stress and mitochondrial dysfunction in hepatocytes that leads to liver disease progressing from non-alcoholic fatty liver disease (NAFLD) to non-alcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma (HCC) 15.

The phenotype of TAL-deficient mice clearly shows that this PPP enzyme plays a role in oxidative stress of hepatocytes 15 and its deficiency may account for liver disease of variable severity in children carrying inactivating mutations 16. The metabolic basis of liver disease in TAL-deficiency is characterized by the accumulation of sedoheptulose 7-phosphate (S7P) and the failure to recycle ribose 5-phosphate (R5P) through the non-oxidative branch, resulting in NADPH and GSH depletion, increased production of lipid hydroperoxides (LPO), 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA) as well as the loss of the mitochondrial transmembrane potential ($\Delta\psi_m$) and mitochondrial mass 15. The profound influence of TAL on NADPH levels, mitochondrial dysfunction, and oxidative stress is attributed to the blocked recycling of R5P to glucose 6-phosphate (G6P) and to the proposed linkage to aldose reductase (AR) that converts the accumulated C5 sugar phosphates to C5-polyols at the expense of NADPH (Fig. 2).

Human TAL-deficient lymphoblasts also exhibit accumulation of S7P and depletion of G6P, indicating a failure to recycle R5P into G6P through the non-oxidative branch of the PPP, thus reducing NADPH production by the oxidative branch 17. Remarkably, TAL is not expressed in red cells, which lack mitochondria 18, and TAL deficiency does not cause hemolytic anemia in humans or mice. In contrast, overexpression of TAL has been associated with the elevation of $\Delta\psi_m$ or mitochondrial hyperpolarization (MHP) and abnormal activation of T cells in patients with systemic lupus erythematosus 19 as well as with MHP in Jurkat cells following stimulation through the Fas/CD95 death receptor 20. As previously shown, TAL is absent in certain unicellular organisms 21›22 and is expressed in a cell type-specific manner in mammalian tissues 23–25. Therefore, the PPP may operate with (Figure 1) and without TAL which modulates the production of NADPH and thus controls oxidative stress (Figure 2).

Cell type-specific regulation of oxidative stress through the PPP by TAL

Oxidative stress depends on the generation of reactive oxygen intermediates during electron transport in mitochondria, peroxisomes, and other organelles 26 as well as on the production of antioxidant pyridine nucleotide NADPH and GSH levels 4 (Fig. 1). Of note, the regeneration of GSH from its oxidized form, GSSG, depends on NADPH produced by the PPP (Fig. 1A). Moreover, the regeneration of the dithiol form of another antioxidant, thioredoxin (TRX) is also dependent on the availability of NADPH 27. The activity of catalase that converts H_2O_2 into water also requires NADPH 28.

TAL activity profoundly impacts the balance between the two branches of PPP and the ultimate output of NADPH and GSH 29. These findings are consistent with earlier predictions that the TAL-catalyzed dihydroxyacetone transfer reactions have an dominant influence on the balance of metabolite flux between the two branches of PPP affecting the production of NADPH and the neutralization of ROI 29 as well as the overall propagation of biochemical signals in the context of a metabolic network (Box 1) 30,31. Enzymatic activity of TAL is regulated in tissue, cell type 25 and developmentally-specific manners 32,33. In Jurkat and H9 human T cell lines, overexpression of TAL diminishes G6PD and 6-phosphogluconate dehydrogenase (6PGD) activities, reduces NADPH and GSH production, and enhances oxidative stress 20,29,34. Suppression of TAL results in opposite effects.

Homozygous deletion of nucleotides 561–563 coding for serine 171 of TAL-H (TALΔS171) has been identified in patients with liver cirrhosis 16,35. TAL mRNA was present in these patients, however, the deletion of S171 caused inactivation and proteasome-mediated degradation of TAL-H 36. S7P, a unique substrate of TAL, was markedly accumulated in TAL-deficient human fibroblasts. By contrast, G6P levels were reduced in these cells, suggesting that TAL deficiency blocked the recycling of G6P through the non-oxidative branch of the PPP. In accordance with these findings, NADPH, which is produced by G6PD in the oxidative branch of the PPP, was diminished in TAL-deficient human lymphoblasts 17. GSH, which is regenerated from its oxidized form at the expense of NADPH, was also diminished in TAL-deficient cells. Spontaneous apoptosis rate of TAL-deficient human lymphoblasts was increased with respect to control donors. H_2O_2-induced apoptosis was also enhanced while Fas apoptosis was decreased in TAL-deficient human cells 17. Increased H_2O_2-induced apoptosis and diminished Fas/CD95-dependent cell death were reversed with normalization of TAL expression, indicating that apoptosis susceptibility of human B cells from a TAL-deficient liver disease patient is regulated by TAL in a pathway-dependent manner 17. Quite similarly, spontaneous or APAP-initiated oxidative stress-dependent death was enhanced, while Fas-induced GSH-dependent apoptosis was diminished in TAL-deficient mouse hepatocytes 15. The effect of TAL on Fas apoptosis was consistent across different cell types, i.e. absent or diminished TAL expression conferred resistance to this apoptosis trigger in human Jurkat and H9 T cells 20,29, human B cells 17 or mouse hepatocytes alike 15. Since the effect of TAL depletion on GSH was discordant among these cells, Fas resistance may not only depend on the availability of GSH. Along this line, the reduction of liver GSH by as much as 75% of baseline has not been associated with spontaneous liver disease, cirrhosis, or hepatomas 37,38. Thus, the metabolic consequences of TAL deficiency go beyond GSH depletion, i.e. accumulation of C5 sugars and polyols (Figure 2 and Box 2). The opposing cell type-specific impact of TAL on activity of enzymes in the oxidative branch of the PPP and GSH output may be related the relative dominance of the forward or reverse reaction catalyzed by TAL (Figures 1 and 2). The changes may be mediated through post-translational modification, such as phosphorylation of TAL, implicated in xeroderma pigmentosum 39. In addition to carcinogenesis, Fas resistance in TAL deficiency may be relevant for the pathogenesis of systemic lupus erythematosus since Fas-deficient mice 40 and humans are predisposed to lupus-like

autoimmunity 41. The relationship TAL overexpression to apoptosis resistance of lupus T cells requires further investigation (Box 3).

The phenotype of mice with a targeted disruption of the TAL-coding genomic locus TALDO1 provides evidence for a controlling role of this enzyme in the PPP and oxidative stress of sperm 14 and hepatocytes 15. Previously, auto-antigenicity of TAL has been implicated in cell and antibody-mediated autoimmunity against oligodendrocytes in patients with multiple sclerosis (MS) 42⁻45. Furthermore, the cleavage and inactivation of TAL by granzyme B may increase the susceptibility of oligodendrocytes to killing by CD8+ cytotoxic T cells 46. A similar mechanism may also confer sensititivity to oxidative stress in other cell types and thus contribute to degenerative diseases.

TAL and autoimmunity

In the brain, TAL is expressed selectively in oligodendrocytes at high levels 42.This is particularly interesting because myelin sheaths are formed by oligodendrocytes and lesions in the most common demyelinating disease of the central nervous system, multiple sclerosis (MS), are characterized by a progressive loss of oligodendrocytes and demyelination 47. Immunohistochemical studies of postmortem brain sections revealed the loss of myelin basic protein (MBP) and TAL in MS plaques, suggesting a concurrent release of these antigens from sites of demyelination 43. A subset of patients with MS has antibodies to TAL 44 and T-cell hyperreactivity, indicating that autoimmunity to TAL could be related to the selective destruction of oligodendrocytes in MS 43. Thus, autoantigenicity to TAL may be secondary to its release from oligodendrocytes in MS. Autoreactivity to TAL was also observed in patients with Crohn's disease 48.

Oligodendrocytes are exquisitely sensitive to oxidative stress generated by the release of ROI, NO, and TNF-α from activated macrophages and astrocytes 49. High expression levels of TAL in oligodendrocytes is possibly linked to production of large amounts of lipids, as a major component of myelin and vulnerability of the vast network of myelin sheaths to oxygen radicals. G6PD activity is also elevated in oligodendrocytes, in accordance with a high requirement for NADPH 50. Interestingly, TAL, but not MBP, is specifically cleaved by human granzyme (GrB) 46. The major C-terminal GrB cleavage product, residues 28–337, had no enzymatic activity but retained the antigenicity of full-length TAL, effectively stimulating the proliferation and cytotoxic T-cell activity in patients with MS. Autoantibodies of MS patients exhibited similar binding affinity to wild-type and GrB-cleaved TAL. Since GrB mediates the killing of target cells and cleavage by GrB is predictive of auto-antigen status of self proteins, GrB-cleaved TAL-specific T cell-mediated cytotoxicity may contribute to the progressive destruction of oligodendrocytes in patients with MS. Inactivation of TAL through cleavage by GrB may facilitate mitochondrial damage, an essential first step during GrB-induced apoptosis 51. In turn, TAL is over-expressed in T cells from patients with the autoimmune disease systemic lupus erythematosus 19, possibly linked to the depletion of GSH20, MHP, and ATP depletion 52. Further downstream, mitochondrial dysfunction leads to the activation of the mammalian target of rapamycin (mTOR), a sensor of $\Delta\psi_m$ and key regulator of inflammation in autoimmune diseases 53 and cancer 54. In contrast, the deficiency of TAL predisposes to a loss of $\Delta\psi_m$ in sperm cells 14.

Mitochondrial damage and sperm dysmotility underlie male infertility in TAL-deficient mice

To investigate the role of TAL in the PPP and mammalian development in general, the TALDO1 genomic locus was inactivated in the mouse. Unlike G6PD 11 and TK deficient

mice which are not viable 13, heterozygous (TAL+/−) or homozygous (TAL−/−) TAL-deficient mice develop normally with the exception of sperm dysmotility and infertility 14. TAL−/− spermatozoa show loss of $\Delta\psi_m$ and mitochondrial membrane integrity due to diminished NADPH, NADH, and GSH. Mitochondria constitute major Ca^{2+} stores, thus, diminished mitochondrial mass accounts for reduced Ca^{2+} fluxing, defective forward motility, and infertility. Reduced forward progression of TAL-deficient spermatozoa is associated with diminished mitochondrial ROI production and Ca^{2+} levels, intracellular acidosis, and compensatory down-regulation of carbonic anhydrase IV and overexpression of NAD hydrolase/CD38 and gamma-glutamyl transferase. Microarray analyses of gene expression in the testis, caput and cauda epididymidis of TAL+/+, TAL+/−, and TAL−/− littermates indicated a dominant impact of TAL deficiency on late stages of sperm cell development, affecting the electron transport chain activity and GSH metabolism. Stimulation of *de novo* GSH synthesis by oral N-acetyl-cysteine (NAC) normalized the low fertility rate of TAL+/− males without affecting the sterility of TAL−/− males. While TAL −/− sperm failed to fertilize TAL+/+ oocytes *in vitro*, sterility of TAL−/− sperm was circumvented by intracytoplasmic sperm injection (ICSI), indicating that TAL deficiency influenced the structure and function of mitochondria without compromising the nucleus and DNA integrity. Collectively, these data reveal an essential role of TAL in sperm cell mitochondrial function. Infertility affects as much as 10% of the adult human male population. Most cases are characterized by reduced or absent movement of spermatozoa due to unknown etiology 55. Since TAL deficiency selectively impacted the structure and function of mitochondria without affecting the nucleus and DNA integrity, it represents a treatable cause of male infertility, by employing ICSI for homozygous deficiency and NAC for heterozygous deficiency. The most robust phenotype of TAL deficiency appears to involve the liver, predisposing to acetaminophen-induced necrosis and hepatocarcinogenesis 15.

Oxidative stress, inflammation, and carcinogenesis in the TAL-deficient liver

Homozygous TAL mutations have now been reported in ten patients with liver disease of varying severity, ranging from steatosis 56 to cirrhosis 16. Since all reported patients were born to consanguineous parents, mutations in genes other than TAL could have influenced the disease manifestations. Most recently, liver failure was also reported in a child with heterozygous TAL mutation 57.

The phenotype of mice with a targeted disruption of the TAL genomic locus TALDO1 provides direct evidence for a protective role of this gene against the development of liver disease 15. Both homozygote (TAL−/−) and heterozygote (TAL+/−) TAL-deficient mice exhibit dramatically increased rates of cirrhosis and HCC 15. Cirrhosis invariably followed the formation of microvesicular and macrovesicular lipid droplets indicating NAFLD and NASH. NAFLD is the most common chronic liver disease, affecting as much as 34% of adults in the US 58. 3–5% of adults develop NASH, a precursor of cirrhosis 58،59; the latter affects ~ 400,000 individuals in the US (http://digestive.niddk.nih.gov/statistics/statistics.htm). HCC is the primary cause of death in both TAL+/− and TAL−/− mice 15 and is also the 3rd leading cause of cancer-related death worldwide 60.

Although oxidative stress, mitochondrial dysfunction, and abnormal hepatocyte apoptosis have been implicated in the pathogenesis of APAP-induced acute liver failure 61 and chonic liver diseases (i.e.: NASH, cirrhosis, and HCC) 59, no common underlying genetic and metabolic pathways have been identified. The oxidative stress and mitochondrial dysfunction in TAL-deficient livers were characterized by the accumulation of S7P and the

failure to recycle R5P for the oxidative PPP, leading to NADPH and GSH depletion, increased production of LPO, 4-HNE, and MDA as well as by the loss of $\Delta\psi_m$ and mitochondrial mass 15. Mitochondrial homeostasis is maintained through a balance of *de novo* biogenesis elicited by NADPH-dependent production of NO and mitochondrial autophagy or mitophagy regulated by mTOR 62. Due to diminished NO production and increased expression of autophagy regulator beclin-1, both reduced biogenesis and increased mitophagy may contribute to diminished mitochondrial mass in the TAL-deficient liver 15.

The profound influence of TAL on NADPH levels has been attributed to the blocked recycling of R5P to G6P and to the proposed linkage to AR activity that converts the accumulated C5 sugar phosphates to C5-polyols at the expense of NADPH (Figure 2). AR expression was increased in TAL−/− hepatomas over tumor-free livers from age-matched TAL−/− mice which may not only contribute to NADPH depletion but also neutralize LPO, and thus promote the survival of hepatoma cells 15. While NADPH can be generated by the malic enzyme (ME) using malate metabolized from glutamine through the mitochondrial tricarboxylic acid cycle (TCA) 54, ME is not detectable in mitochondria of the liver 63. Along this line, the genetic deficiency of malic enzyme has no significant influence on hepatic GSH and susceptibility to APAP 64. Thus, the PPP represents a unique source of NADPH in hepatocytes (Figure 3A).

The impact of TAL deficiency on oxidative stress extends beyond the consequences of GSH deficit. Reduction of liver GSH by as much as 75% of baseline has not been associated with spontaneous liver disease, cirrhosis, or hepatomas 37,38. Thus, GSH depletion by itself is not responsible for hepatocarcinogenesis in TAL-deficient mice and emphasizes the potential significance of NADPH depletion as the source of oxidative stress and the primary metabolic switch in carcinogenesis 54. The restoration of GSH in NAC-treated mice was accompanied by the normalization of NAPDH, LPO, MDA, and 4-HNE levels and PCNA and TUNEL-positive nuclei in TAL−/− and TAL+/− livers, suggesting that life-long supplementation of NAC has a sparing effect on the utilization of NADPH and that NAC works as a potent anti-oxidant 15. Of note, GSH-depleted liver of GGT−/− mice was deprived of fat 38. By contrast, fat deposition is increased in the TAL-deficient liver. GSH normalization by NAC treatment failed to prevent steatosis, indicating that lipid deposition was independent of oxidative stress 15. Steatosis is likely to happen through stimulation of lipogenesis by xylulose 5-phosphate (X5P) 65. C5-polyols also accumulated in TAL−/− urine 66 and liver 15. C5-sugars are metabolized to C5-polyols by NADPH-dependent AR 67, which further depletes NADPH (Figure 2). Moreover, JNK activity and c-jun expression are stimulated not only by oxidative stress but also by hyperosmolarity, such as accumulation of sugars and polyols 68. In turn, JNK and c-jun inhibit Fas apoptosis 69. Thus, the accumulation of C5-sugars and C5-polyols may be a unique factor contributing to liver disease in TAL deficiency. Diminished phosphorylation of β-catenin, a known activator of c-jun expression 70, is associated with diffuse accumulation of β-catenin in the cytosol and its translocation into the nucleus of TAL-deficient tumor cells.

β-catenin is the main effector of the canonical Wnt signaling pathway 71. Normally, β-catenin is located in the cytosol and it is associated with E-cadherin near the cell membrane. β-catenin turn-over is dependent on phosphorylation by glycogen synthase kinase 3β (GSK3β) which forms a degradation complex with the scaffolding proteins APC and Axin 1 and Axin 2 72[72]. Increased signaling through Wnt/Frizzled leads to inactivation of GSK3β that limits the phosphorylation of β-catenin. Unphosphorylated β-catenin is protected from ubiquitination and proteosomal degradation and it accumulates in the cytoplasm which is followed by nuclear translocation 71 and activation of T-cell factor and various target genes, such as c-jun 70. Oxidative stress enhances the transcriptional co-activator function of β-catenin 73. Therefore, it is important to determine how NADPH depletion and the resultant

oxidative stress promote the activation of β-catenin, the expression of c-jun and alpha fetoprotein (AFP) and carcinogenesis in TAL−/− livers. Mutations of the β-catenin coding sequence that prevent phosphorylation by GSK3β and proteasomal degradation and expression of Frizzled need to be evaluated 74. C-jun may also be directly activated by oxidative stress. Phosphorylation at Ser 63 and Ser 73 by c-Jun N-terminal kinase (JNK) 75 enhances activity of c-jun which then further stimulates expression of c-jun itself and AP-1 binding activity. JNK activity is stimulated by oxidative stress 75,76 and hyperosmolarity, such as accumulation of sugars and polyols 68. The p46 and p54 isoforms of JNK are 4-HNE targets and are activated by this aldehyde 77. Sugars and aldehydes such as 4-HNE are natural substrates of AR and, interestingly, these metabolites also induce transcription, expression, and activity of AR which in turn further depletes NADPH 78.

The activation of the redox-sensitive transcription factors β-catenin, JNK, and c-jun promote the proliferation of TAL-deficient hepatocytes (Figure 3B). AFP expression, which is dependent on c-jun, is increased in TAL-deficient hepatomas. This is similar to findings of elevated AFP levels in HCC patients 79. β-catenin phosphorylation normalized in the liver of NAC-treated mice which may be an important factor in preventing hepatocarcinogenesis in TAL−/− mice (Fig. 3B). Activation of β-catenin has been demonstrated in up to 90% of human HCC cases 71,80, supporting the notion that TAL-deficiency is a relevant model for human hepatocarcinogenesis. Neoplasms other than HCC, such as B-cell lymphomas appear with increased frequency in TAL-deficient mice 15. Polymorphism of TAL has been recently linked to squamous cell carcinoma of the head and neck 81.

The loss of Δψm is a critical checkpoint of death signal processing 15. TAL-deficient mouse hepatocytes show resistance to Fas apoptosis 15. In contrast, TAL-deficient mice show increased liver cell necrosis and increased mortality after exposure to acetaminophen (acetyl-p-aminophenol or APAP/Tylenol), which is the leading cause of acute liver failure in the US 82. Lifelong administration of NAC blocked APAP susceptibility, restored Fas apoptosis, phosphorylation of β-catenin, activation of JNK and c-jun and prevented the development of NASH, cirrhosis, and hepatocarcinogenesis in TAL-deficient mice (Fig. 3B). Thus, the TAL-controlled non-oxidative branch of the PPP plays a protective role against APAP-induced liver failure as well as NASH, cirrhosis, and hepatocarcinogenesis 15.

Inhibiting apoptosis allows the survival of hepatocytes with genetic mutations that provide growth advantage in the context of oxidative stress 8384. Depletion of GSH, which is required for activity of caspases, can abrogate Fas-dependent apoptosis of the liver 85. Alternatively, ATP concentration is key factor in the decision between apoptosis and necrosis and depletion of ATP shifts susceptibility from apoptosis to necrosis 86. Thus, the loss of $\Delta\psi_m$ and reduced ATP/ADP ratio may contribute to inhibited apoptosis. NADPH, GSH and NO production were all corrected in NAC-fed mice, suggesting that these metabolic changes are the consequences of diminished reducing power in TAL deficiency. The success of NAC therapy in normalizing of GSH and preventing APAP susceptibility and hepatocarcinogenesis in TAL-deficient mice suggests that a similar approach may benefit TAL-deficient patients.

Concluding remarks and future directions

This review provides a rationale for alternative formulations of the PPP with and without TAL as well as for connecting the PPP to AR activity and polyol metabolism. In erythrocytes lacking TAL, mitochondria, or nuclei, the PPP is controlled by the activity of G6PD; its partial deficiency predisposes to hemolytic anemia. In nucleated cells, TAL-dependent NADPH production through the PPP controls mitochondrial function, oxidative

stress, inflammation, death pathway selection, as well as proliferation via the activation of the β-catenin/JNK/c-jun pathway (Fig. 3B). The deficiency of TAL has been linked to a widening spectrum of clinical manifestations, including male infertility, acetaminophen-induced acute liver failure, chronic liver diseases progressing from NAFLD to NASH, cirrhosis, and HCC, and possibly to autoimmune inflammatory diseases and extra-hepatic malignancies. Thus, the TAL-regulated production of NADPH is proposed as a key metabolic switch in inflammation and carcinogenesis. Importantly, NAC prevents acetaminophen-induced acute liver failure and hepatocarcinogenesis in TAL deficiency. Ongoing efforts focus on determining the prevalence of TAL deficiency in healthy individuals and in patients with a history of APAP-induced acute liver injury and chronic liver diseases (Box 3). The formulation of the PPP needs to be further examined in mice with cell type-specific inactivation of TALDO1. Comprehensive metabolomic and gene expression analysis of TAL-deficient cells and tissues will be essential for understanding the role of PPP-dependent NADPH production in the metabolic bases of inflammation and carcinogenesis, ranging from mitochondrial dysfunction to oxidative stress, enhanced glycolysis and generation of the Warburg effect, and activation of mTOR 54. These studies will be critical for identifying new metabolic targets for the prevention and treatment of inflammatory diseases and cancer.

Box 1. Cell type-specific regulation of oxidative stress by the PPP

- The pentose phosphate pathway (PPP) controls oxidative stress via the production of NADPH in a cell type-specific manner.
- NADPH production in red cells is dependent on the activity of glucose 6-phosphate dehydrogenase (G6PD), an enzyme in the oxidative branch of the PPP.
- Diminished G6PD activity due the genetic mutations resulting in amino acid substitutions causes hemolytic anemia.
- G6PD deficiency protects against malaria and CAD.
- G6PD is essential for NADPH production and its complete deficiency is incompatible with cell survival.
- Transketolase (TK), an enzyme in the non-oxidative branch of the PPP, is essential for cell survival.
- Diminished TK activity due the thiamine co-factor deficiency causes Wernicke-Korsakoff encephalopathy.
- Deficiency of transaldolase (TAL), an enzyme in the non-oxidative branch of the PPP, is compatible with normal development in humans and mice.
- TAL regulates the PPP and causes NADPH depletion and oxidative in a cell type-specific manner.
- TAL deficiency has been associated with a widening spectrum of diseases in humans and mice.

Box 2. Consequences of TAL deficiency

- Deficiency of transaldolase (TAL), an enzyme in the non-oxidative branch of the PPP, blocks the recycling of ribose 5-phosphate (R5P) into glucose 6-phosphate (G6P) and thus reduces the availability of substrate and the

production of NADPH by G6PD in nucleated cells, such as sperm, B lymphocytes, and hepatocytes.

- TAL does not limit the production of R5P, another unique product of the PPP, which is required for the synthesis of nucleotides.
- In the absence of TAL, R5P accumulates and metabolized to C5-polyols by aldose reductase (AR), thus further depleting NADPH. AR enzymatic activity is functionally linked to the non-oxidative phase of the PPP.
- Through NADPH production by the PPP, TAL controls mitochondrial function, oxidative stress, inflammation, death pathway selection (i.e. resistance to Fas apoptosis and susceptibility to acetaminophen-induced necrosis), as well as proliferation via the activation of the β-catenin/JNK/c-jun pathway in hepatocytes and possibly in other nucleated cells.
- TAL deficiency causes male infertility, acetaminophen-induced acute liver failure, chronic fatty changes and inflammatory disease in the liver progressing from NAFLD to NASH, cirrhosis, and HCC.

Box 3. Outstanding questions

- To determine the molecular bases of cell type-specific regulation of the PPP in TAL deficiency, i.e. down-regulation of G6PD activity and NADPH production and GSH in sperm cells, hepatocytes, and B cells but not in Jurkat or H9 T cells.
- To clarify the role of Fas resistance and activation of the β-catenin/JNK/c-jun pathway in proliferation and malignant transformation of hepatocytes, lymphocytes, and possibly other nucleated cells.
- To understand the relevance of Fas resistance in TAL deficiency for the pathogenesis of lupus and other autoimmune diseases.
- To determine the relationship of TAL overexpression in lupus T cells to increased nitric oxide production, MHP, oxidative stress, GSH depletion, mTOR activation, increased spontaneous apoptosis, decreased activation-induced apoptosis, and predisposition to necrosis.
- To determine the role of TAL as an autoantigen and metabolic regulator of apoptosis in destruction of oligodendrocytes in multiple sclerosis.
- To determine the role of DNA damage, endoplasmic reticulum stress, mTOR activation, autophagy, and altered behavior of other enzymes due to modification by ROI in TAL deficiency.
- To determine the prevalence of TAL deficiency in healthy subjects and patients with diseases suspected to be associated with TAL deficiency.

Acknowledgments

This work was supported in part by grants DK78922 and AI72678 from the National Institutes of Health, the Hendricks Foundation, the Children's Miracle Network, and the Central New York Community Foundation.

Glossary

4-HNE 4-hydroxynonenal

6PGD	6-phosphogluconate dehydrogenase
AR	aldose reductase
CAD	coronary artery disease
GSH	reduced glutathione
GSSG	oxidized glutathione
G6P	glucose 6-phosphate
G6PD	glucose 6-phosphate dehydrogenase
HCC	hepatocellular carcinoma
LPO	lipid hydroperoxides
MDA	malondialdehyde
MHP	mitochondrial hyperpolarization
NAC	N-acetylcysteine
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
PCD	programmed cell death
PPP	pentose phosphate pathway
R5P	ribose 5-phosphate
ROI	reactive oxygen intermediates
S7P	Sedoheptulose 7-phosphate
TAL	transaldolase
TK	transketolase
TRX	thioredoxin
mTOR	mammalian target of rapamycin
X5P	xylulose 5-phosphate
$\Delta\psi_m$	mitochondrial transmembrane potential

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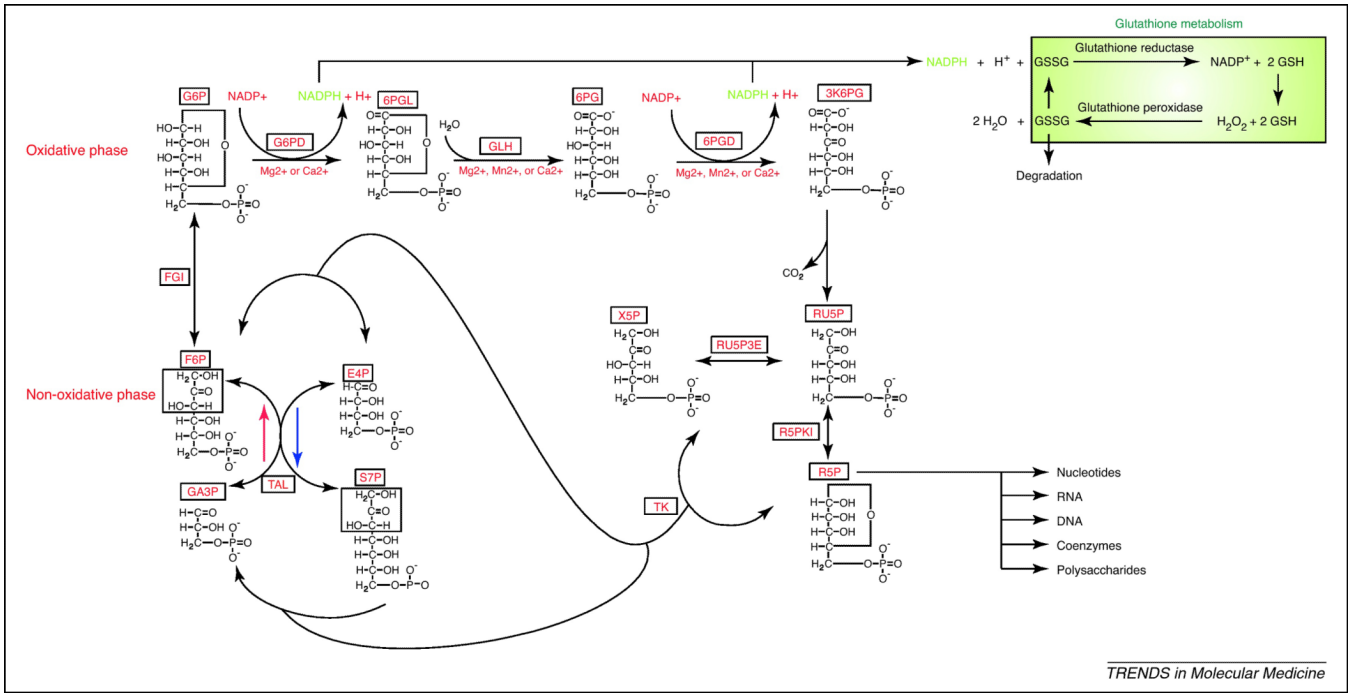

Figure 1.

Conventional PPP. Conventional formulation of the pentose phosphate pathway (PPP) that allows the oxidative branch to produce two NADPH molecules per each molecule of glucose 6-phosphate (G6P). The non-oxidative branch recycles ribose 5-phosphate (R5P) back into G6P for the oxidative branch. R5P is required for the synthesis of nucleotides, RNA, and DNA in support of cell growth while NADPH is needed for biosynthetic reactions and protects against oxidative stress through neutralizing reactive oxygen intermediates (ROI) directly or indirectly via regeneration of reduced glutathione (GSH) from its oxidized form GSSG.

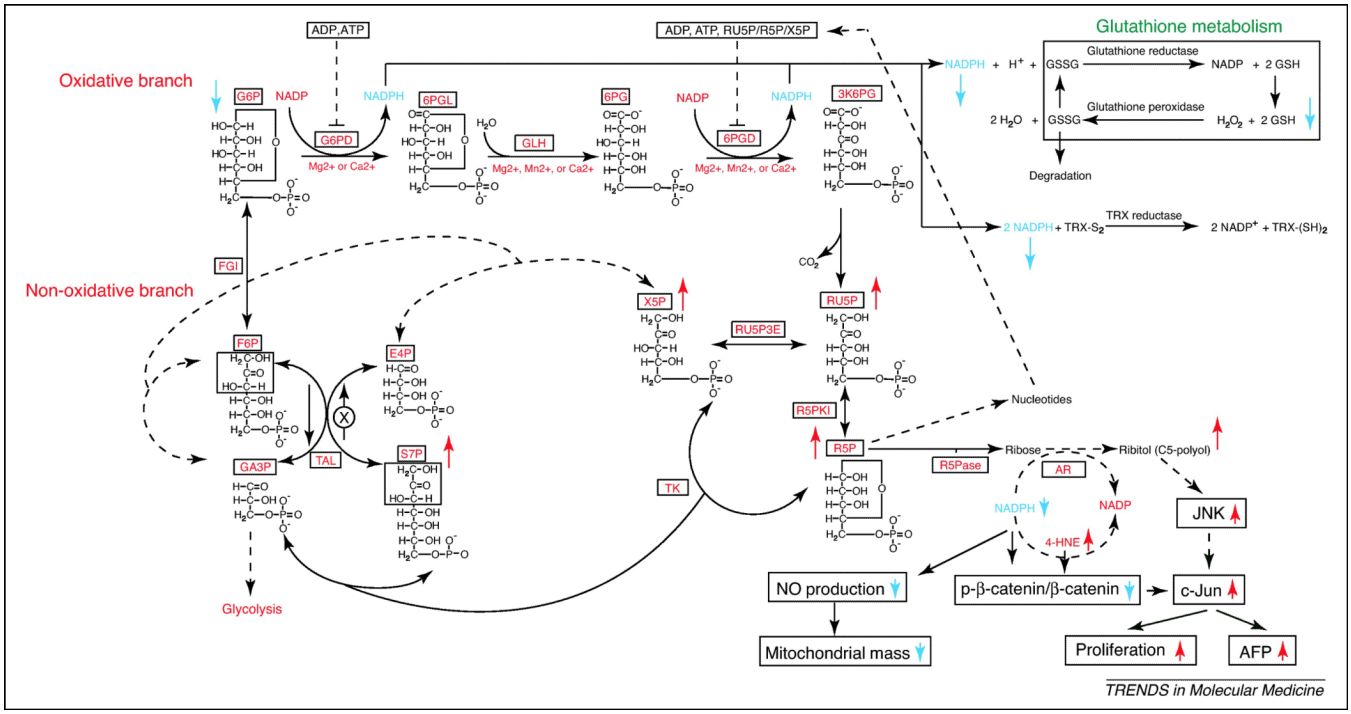

Figure 2.

Alternative PPP. Alternative formulation of the PPP in the absence of transaldolase (TAL) leading to NADPH depletion, oxidative stress, and activation of β-catenin and c-jun. TAL, an enzyme of the non-oxidative branch, catalyzes the transfer of dihydroxyacetone from sedoheptulose 7-phosphate (S7P) and fructose 6-phosphate (F6P) to glyceraldehyde 3-phosphate (GA3P) and erythrose 4-phosphate (E4P), respectively. GA3P generated by TAL and TK connects the PPP to glycolysis. The forward reaction of TAL favors the generation of G6P; the reverse TAL reaction promotes the metabolism of G6P into R5P. Formulation of the PPP without TAL leads to the accumulation of S7P, R5P, X5P, RU5P, C5-polyols D-ribitol/D-arabitol/D-xylitol (red/upward arrows), and depletion of G6P and NADPH (blue/downward arrows).R5P, X5P, and RU5P inhibit 6PGD 87, while elevation of the total adenine nucleotide pool (AMP, ADP and ATP) inhibit both G6PD and 6PGD 88. Excess R5P (X5P and RU5P) can be converted to ribose by ribose-5-phosphatase 89, which is then reduced to ribitol by AR, thus further depleting NADPH 67. GSH reductase uses NADPH to regenerate GSH from GSSG. In the absence of TAL, the accumulation of S7P, C5 sugar phosphates R5P and X5P and the depletion of G6P indicate a failure to recycle R5P into G6P through the non-oxidative branch, thus reducing NADPH production by the oxidative branch 14,15,17. Diminished production of NADPH leads to secondary depletion of GSH and oxidative stress marked by increased levels of lipid hydroperoxides (LPO), 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA). Redox-sensitive genetic changes, reduced β-catenin phosphorylation and increased c-jun N-terminal kinase (JNK) activity and c-jun expression occur in TAL-deficient livers, while expression of alpha-fetoprotein (AFP) and AR are increased in hepatomas 15. In addition to converting C5 sugars to C5 polyols, AR also neutralizes LPO at the expense of NADPH.

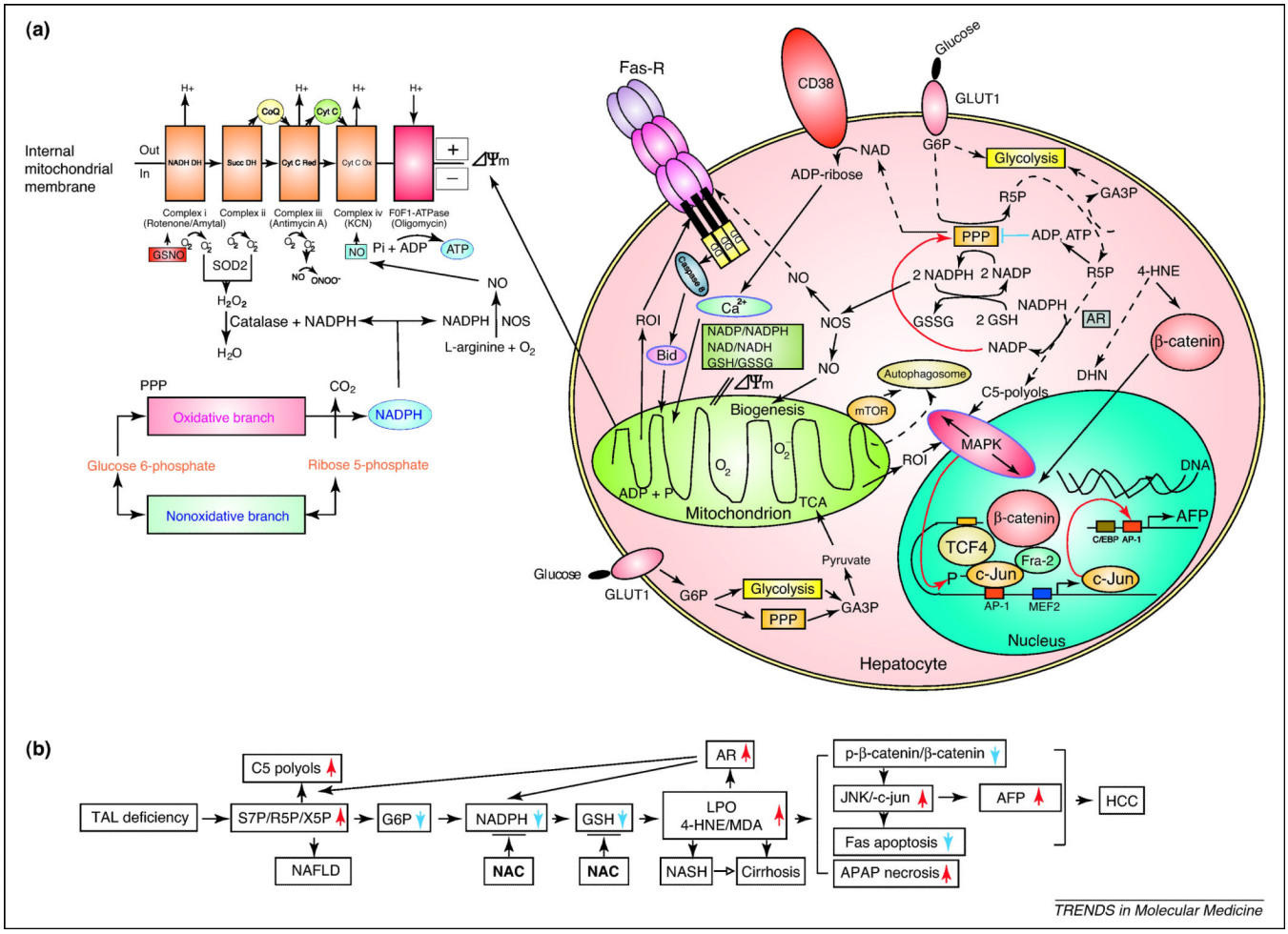

Figure 3.

A) Schematic outline of mitochondrial and metabolic pathways connected to the PPP in hepatocytes. The PPP regulates the $\Delta\psi_m$ by providing i) NADPH that serves as a reducing equivalent for the activity of catalase and nitric oxide synthase (NOS) and for GSH regeneration from its oxidized form GSSG and ii) R5P for biosynthesis of nucleotides. Mitochondrial electron transport chain activity is reversibly inhibited by NO at complex IV/ cytochrome c oxidase 90and irreversibly inhibited via S-nitrosylation of complex I in a state of GSH depletion 91. Blocked electron transport leads to the transfer of electron to molecular oxygen (O_2^-) and the formation of reactive oxygen intermediates (ROI). Mitochondrial ROI production is neutralized through the activities of superoxide dismutase 2 (SOD2) and catalase at the expense of NADPH. Glucose is taken up by hepatocytes through the transporter GLUT1 and enters glycolysis or the PPP as G6P. The PPP and glycolysis are also connected via the common substrate GA3P. The glycolysis product pyruvate is converted to acetyl-CoA and thus enters the tricarboxylic acid cycle (TCA) in mitochondria. The mammalian target of rapamycin (mTOR) senses $\Delta\psi_m$ 92 and ATP depletion and controls cellular metabolism via protein translation and authophagy 62. TAL-deficient hepatocytes have reduced mitochondrial mass. Mitochondrial homeostasis is maintained through a balance between de novo biogenesis elicited by NO and mitochondrial autophagy or mitophagy regulated by mTOR. B) Metabolic and genetic checkpoints of progressive liver disease leading from nonalcoholic fatty liver disease (NAFLD) to non-alcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma (HCC) in TAL

deficiency. Hepatocytes of TAL-deficient mice exhibit increased susceptibility to APAP-induced necrosis and resistance to Fas apoptosis. Life-long supplementation of N-acetylcysteine (NAC) increases GSH levels, normalizes NADPH, blockes APAP susceptibility and restores Fas apoptosis, phosphorylation of β-catenin, and activation of c-jun and prevents the development of NASH, cirrhosis and HCC 15. However, NAC does not prevent NAFLD which may be attributed to stimulation of lipogenesis by X5P 65.