Phosphoribosyl Pyrophosphate Synthetase

Subjects: Biochemistry & Molecular Biology

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Phosphoribosyl pyrophosphate synthetase (PRS EC 2.7.6.1) is a rate-limiting enzyme that irreversibly catalyzes the formation of phosphoribosyl pyrophosphate (PRPP) from ribose-5-phosphate and adenosine triphosphate (ATP). This key metabolite is required for the synthesis of purine and pyrimidine nucleotides, the two aromatic amino acids histidine and tryptophan, the cofactors nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$), all of which are essential for various life processes. Despite its ubiquity and essential nature across the plant and animal kingdoms, PRPP synthetase displays species-specific characteristics regarding the number of gene copies and architecture permitting interaction with other areas of cellular metabolism. The impact of mutated *PRS* genes in the model eukaryote *Saccharomyces cerevisiae* on cell signalling and metabolism may be relevant to the human neuropathies associated with *PRPS* mutations. Human *PRPS1* and *PRPS2* gene products are implicated in drug resistance associated with recurrent acute lymphoblastic leukaemia and progression of colorectal cancer and hepatocellular carcinoma.

Keywords: phosphoribosyl pyrophosphate synthetase (PRS/PRPS) ; eukaryotic model organisms ; Saccharomyces cerevisiae ; Danio rerio ; human neuropathies: Arts syndrome ; CMTX5 ; DFN2/DFNX1 ; ageing ; cancer ; cell signalling ; CWI pathway

1. Introduction

Phosphoribosyl pyrophosphate synthetase (PRS; ATP:D-ribose-5-phosphate pyrophosphotransferase, EC 2.7.6.1) is an essential rate-limiting enzyme that catalyzes the irreversible transformation of ribose-5-phosphate in the presence of ATP to produce 5-phospho-D-ribosyl-α-1-pyrophosphate (PRPP) and AMP and is subjected to feedback inhibition by AMP, ADP and GDP [1][2][3][4][5]. PRS links carbon and nitrogen in cellular metabolism producing the high-energy compound PRPP, a key metabolite necessary for the biosynthesis of purine and pyrimidine nucleotides, the cofactors nicotinamide adenine dinucleotide (NAD^+), nicotinamide adenine dinucleotide phosphate ($NADP^+$) and, in specific organisms, e.g., *Escherichia coli*, the amino acids histidine and tryptophan [6][7]. PRS is responsible not only for the *de novo* synthesis of purine nucleotides but also plays a role in the salvage pathways *via* dedicated phosphoribosyl transferases for adenine, guanine and hypoxanthine. However, pyrimidine nucleotides, i.e., thymine and uridine, are salvaged by recycling from degradation products of DNA and RNA [8][9][10][11].

Synthesis of PRPP is essential for all free-living organisms but is lacking in parasites, e.g., *Trypanosoma* and *Leishmania*, which are dependent on the host metabolism for the synthesis of PRPP [12]. PRS enzymes have been identified in numerous organisms including mammals, human and rats [3][13][14][15][16][17]; plants, including *Spinacia oleracea* [18] and *Arabidopsis thaliana* [19][20]; bacteria, including *Bacillus subtilis* [21], and *Bacillus pseudomallei* [22]; yeasts, including *Saccharomyces cerevisiae* [23][24][25][26] and *Schizosaccharomyces pombe* [27]; and more recently, in zebrafish *Danio rerio* [28][29]; confirming the ubiquity of *PRS* genes across the five plant and animal kingdoms. There is now a plethora of *PRS* genes available for investigating the ramifications of altered PRPP synthetase on the physiology of various organisms (**Figure 1**).

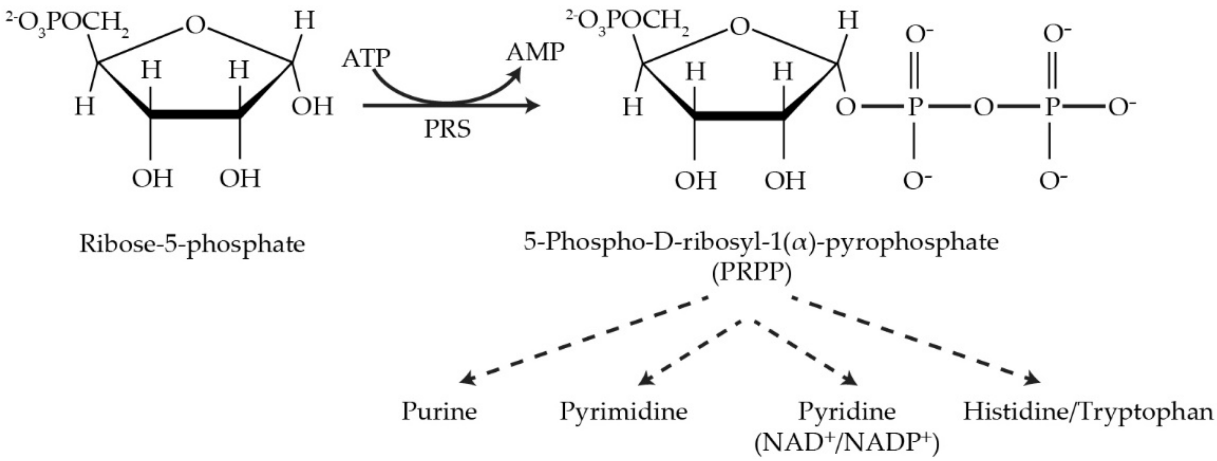

Figure 1. The reaction catalyzed by PRPP synthetase and metabolic pathways utilizing its product, PRPP.

Interestingly, the number of *PRS* genes in the genome varies from organism to organism. For instance, humans (HGNC PRPS), *S. pombe* (PomBase) and *Aspergillus nidulans* (AspGD) each possess three *PRS* genes, whereas *S. oleracea* (Spinach Base) and *Eremothecium* (*Ashbya gossypii* (Ashbya Genome Database)) genomes each contain four *PRS* genes. However, *S. cerevisiae* (Sacharomyces Genome Database (SGD)) and *A. thaliana* (AtGDB) harbour five *PRS* genes in their genomes. Irrespective of the number of *PRS* genes identified in different organisms, each organism must have at least a minimum of one gene coding for *PRS* to ensure survival, as is the case for bacteria [7][21][30]. An interesting discovery is that a gene, Hb*PRS4*, identified in the genome of the rubber tree plant *Hevea brasiliensis* Műll, a member of the family *Euphorbiacaea* [31][32], encodes a protein with 80% identity to Prs4 of *A. thaliana.* A BlastN study revealed a second sequence in the rubber tree genome with 84% similarity to the Hb*PRS4* gene. The expression of both genes responded positively to ethylene treatment, as did the ATP/ADP content of latex cells, thus linking PRPP synthesis to natural rubber production [33][34]. In a similar context, it has been reported that in an industrial yeast strain, the increased expression of the genes required for the synthesis of PRPP correlated with enhanced xylose utilization, emphasizing the significant role of PRPP for biofuel production from lignocellulose [35][36]. More specifically, it has been shown that overexpression of Sc*PRS3* increases the resistance of the yeast cell wall to acetic acid stress, thus illustrating the importance of Prs3 in the enhancement of commercially important pathways, e.g., biofuel production for lignocellulose, possibly as a result of improving cell wall strength [37][38]. *Mycobacterium tuberculosis,* the pathogen responsible for tuberculosis, contains a single *PRS* gene whose product is the only source of PRPP for a precursor of the bacterial cell wall and therefore is essential for the maintenance of cell integrity, thus providing a potential drug target in the treatment of tuberculosis [39][40][41][42].

Numerous scientific reports have shown that mutations in the *PRS* genes in different organisms cause various forms of cellular disruption, including neurological diseases and metabolic disorders. The nomenclature for *PRS* genes is species-specific: the *PRS* genes in humans are designated as h*PRPS*, of which there are three isoforms, h*PRPS1*, h*PRPS2* and h*PRPS3* (h*PRPS1L1*). The genes, h*PRPS1* and h*PRSP2*, are located on the long and short arms of the X-chromosome at positions Xq22.3 (OMIM 311850) and Xp22.2 (OMIM 311860) [43][44], respectively, whereas h*PRPS3* maps to chromosome 7 at position p21.1 (OMIM 611566) and is expressed specifically in the testis [15][17][45]. Missense mutations in the h*PRPS1* gene may lead to a gain-of-function associated with PRPS1 superactivity [46]. Other neurological disorders, such as Arts syndrome [47][48], syndromic and nonsyndromic sensorineural deafness (DFN2/DFNX-2) [49][50] and Charcot-Marie-Tooth disease type 5 (CMTX5) [51] are due to the loss-of-function in the h*PRPS1* gene.

The sequence similarity of hPRPS proteins to those of the established eukaryotic model organisms *S. cerevisiae* (baker's yeast) and *D. rerio* (zebrafish) has the potential to be instrumental in elucidating the ramifications of PRPP metabolism in human neuropathies. Biochemical and genetic studies have also identified the specific roles of h*PRPS1* and h*PRPS2* genes in one of the deadliest diseases, cancer. For instance, an h*PRPS2* knockout in experimental models has resulted in c-Myc-driven tumorigenesis [52][53], suggesting that h*PRPS2* may be a target protein for manipulating cancer cells, whereas it has been reported that a decrease in h*PRPS1* expression impairs the proliferation of tumour cells [54]. Both h*PRPS1* and h*PRPS2* genes have been identified in pluripotent stem cells, where they may contribute to increased stem-cell-associated biosynthetic capacity [55].

2. PRS-Encoding Genes in Saccharomyces cerevisiae

S. cerevisiae, a workhorse eukaryotic model organism which, in addition to its contribution to bread, wine, beer and Marmite® production, has been exploited by biotechnologists for genetic and metabolic engineering by revamping and minimizing its genome through the 'design-build-test-learn' cycle of synthetic biology [56][57][58].

The whole yeast genome duplication that took place approximately 100 million years ago created a tetraploid yeast [59][60]. Over time, this unstable tetraploid yeast lost more than 80% of the duplicated gene copies. Nevertheless, of the remaining 6000 genes that make up the genome of the current species of *S. cerevisiae,* at least 10% are duplicated [61]. This implies that the remaining gene duplications must have made a positive contribution to the ancestral yeast's relative fitness. Gene duplications provide a potential for increasing diversity, thereby permitting adaptation to new environments. If the products of the two gene copies are identical and autonomous, then one of the copies is available for alteration or can be lost. The alteration may provide a new link to the function of the other copy [59][62][63]. When the duplicated genes have interdependent functions, the chance of diversification is limited, but this can be overcome by retaining both the original and the altered copy since they are essential for the survival of the organism. Of the myriad gene products common to both humans and yeast, PRS in yeast is encoded by five unlinked paralogous genes as the result of genome duplication and subsequent evolution. The central role played by *PRS* gene products in cellular metabolism can be exploited to uncover potential therapeutic targets for PRPS-associated human diseases.

2.1. The Role of NHR1-1 in the Provision of PRPP and Maintenance of CWI

The *S. cerevisiae* genome contains five Sc*PRS* genes, namely Sc*PRS1*, Sc*PRS2*, Sc*PRS3*, Sc*PRS4* and Sc*PRS5,* which are located on chromosomes XI, V, VIII, II, and XV, respectively [24][25][26]. Remarkably, Sc*PRS1* and Sc*PRS5* have acquired in-frame non-homologous regions (NHRs), which are not introns since they are still present in the mature polypeptides, as demonstrated by Western blotting with tailor-made antibodies [24][25][64]. The gene products Prs1 and Prs5 differ in length from Prs2 and Prs4 since they contain non-homologous regions NHR1-1, NHR5-1 and NHR5-2. In each instance, one of the component monomers of the essential minimal functional units, Prs1/Prs3, Prs2/Prs5 and Prs4/Prs5, contains an NHR. Both NHR1-1 with a length of 105 aa and NHR5-2 with 65 aa disrupt the catalytic flexible loops of Prs1 and Prs5 (**Figure 2**), respectively, rendering these polypeptides incapable of PRPP synthesis. NHR5-1 has a length of 113 aa and is located within the regulatory flexible loop and contains no less than nine predicted serine, threonine or tyrosine phosphorylation sites (PhosphoGrid and Phosphopep) [65][66]. A distinguishing feature of NHR1-1 and NHR5-1 is that they contain aa (amino acids) motifs associated with phosphorylation or ubiquitination [67]. S_{199} is located just prior to the beginning of the NHR1-1 sequence and has been postulated to play a role in mTORC1 signalling [68]. In the case of NHR5-1 S_{183}, located centrally, it has been identified as being phosphorylated after rapamycin treatment [68], although there are other possible candidate aa residues in the near vicinity [69]. NHR5-2 is a stretch of 65 aa, including five serine residues, of which three, at the neighbouring positions S_{364}, S_{367} and S_{369}, are phosphorylatable [70]. Interestingly, characterization of a rapamycin-sensitive phosphoproteome revealed that Prs5 is hyperphosphorylated following rapamycin treatment [71][72], but the locations of the phosphosites were not specified. A high sequence similarity of 77% has been found for Prs1 between *S. cerevisiae* and *A. gossypii* (Ashbya Genome Database, AER083c) [73]. This similarity is maintained across the N-terminal sections of NHR1-1 in both species up to the midpoint of NHR1-1 of *S. cerevisiae* (unpublished data). Loss of the N-terminal portion of NHR1-1 in *S. cerevisiae* coincides with the inability to respond to heat shock as measured by Rlm1 expression, a transcriptional readout of the CWI (cell wall integrity) pathway [74]. NHR3-1 (**Figure 2**), close to the C-terminus of Prs3, is the pentameric sequence $_{284}$KKCPK$_{288}$, corresponding to an NLS (nuclear localization signal) consensus sequence of two positively charged aa (K (lysine) or R (arginine)) flanking three residues, one of which is P (proline), as described in [75].

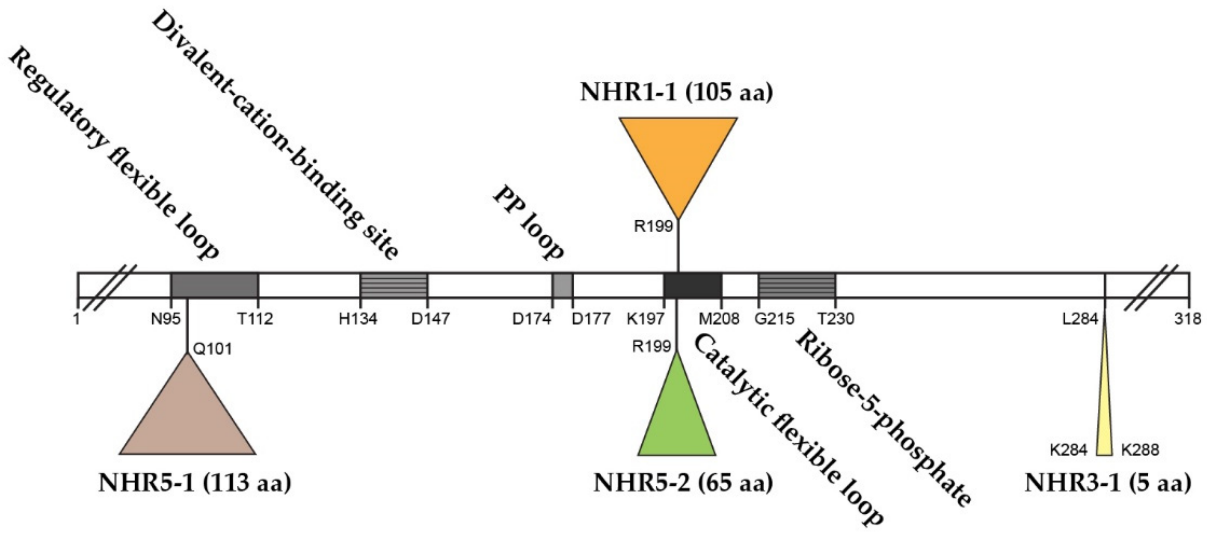

Figure 2. Relative positions of the non-homologous regions—NHR1-1, NHR3-1, NHR5-1 and NHR5-2—in the Prs2 polypeptide as the prototype. The Prs2 polypeptide consists of 318 aa and is indicated as an open bar with characteristic motifs therein. PP = pyrophosphate. The NHRs are positioned in Prs2 as triangles above or below the open bar. The coordinates of the four insertions reflect their lengths in Prs1, Prs3 and Prs5, respectively. The insertion points are defined where the similarity between Prs2 and Prs1 or Prs5 falls off. Sequence comparison was performed using the pairwise sequence alignment programme EMBOSS Needle.

A systematic phenotypic analysis revealed that individual deletion of any of the five *S. cerevisiae PRS* genes (Sc*PRS1*-Sc*PRS5*) did not seriously affect the cell's viability [23][24][25][76]. However, *S. cerevisiae* cannot survive with a single *PRS* gene. Genetic evidence and Y2H (yeast-two-hybrid analysis) have shown that PRS exists as two interacting complexes: one as a heterodimer (Prs1/Prs3) and the other as a heterotrimer (Prs2/Prs4/Prs5). Furthermore, at least one of the three different combinatorial functional units, Prs1/Prs3, Prs2/Prs5 or Prs4/Prs5, must be present to ensure viability [26][76]. Expression of the five possible pairwise combinations of the Sc*PRS* gene products in *E. coli* achieved only 25% of the level of the *E. coli* PRPP synthetase with only the combination, Prs1/Prs3 [77] which correlates with Prs1/Prs3 being the most important heterodimer for yeast survival [25]. Mutation of Sc*PRS1*, Sc*PRS3* and Sc*PRS5* have proven useful for investigating the link between Prs and its specific functions—nucleotide biosynthesis, cell signalling and metabolism [26][76][78][79][80]. Overexpression of Pkc1 had a positive effect on the transcriptional activation of Rlm1 in either *prs1Δ* and *prs3Δ* strains at ambient and elevated temperatures and following α-factor stimulation [78].

A reduction in the capacity to synthesize PRPP, severe loss of enzymatic activity, compromised CWI, increased chitin content and severe growth retardation in *S. cerevisiae* were observed when Sc*PRS1*/Sc*PRS3* were simultaneously deleted in the absence of Sc*PRS2* or Sc*PRS4* (**Figure 3**a). Therefore, the Prs1/Prs3 heterodimer is the most important [25][74] since the mutant strains relying on either Prs2/Prs5 or Prs4/Prs5 are severely impaired in their growth, their PRPP-synthesizing capacities and CWI, thus providing genetic evidence to support the corresponding enzyme activities measured in yeast. Furthermore, yeast PRS enzyme activity was reduced to 80% when Sc*PRS2*, Sc*PRS4* and Sc*PRS5* were deleted simultaneously, but there was no influence on nucleotide content or growth rate (**Figure 3**b) [26][76][80]. Simultaneous deletion of Sc*PRS1* and Sc*PRS5* or Sc*PRS3* and Sc*PRS5* causes synthetic lethality, as does deletion of Sc*PRS2* and Sc*PRS4* in addition to the loss of Sc*PRS1* or Sc*PRS3* [26][76] (**Figure 3**c).

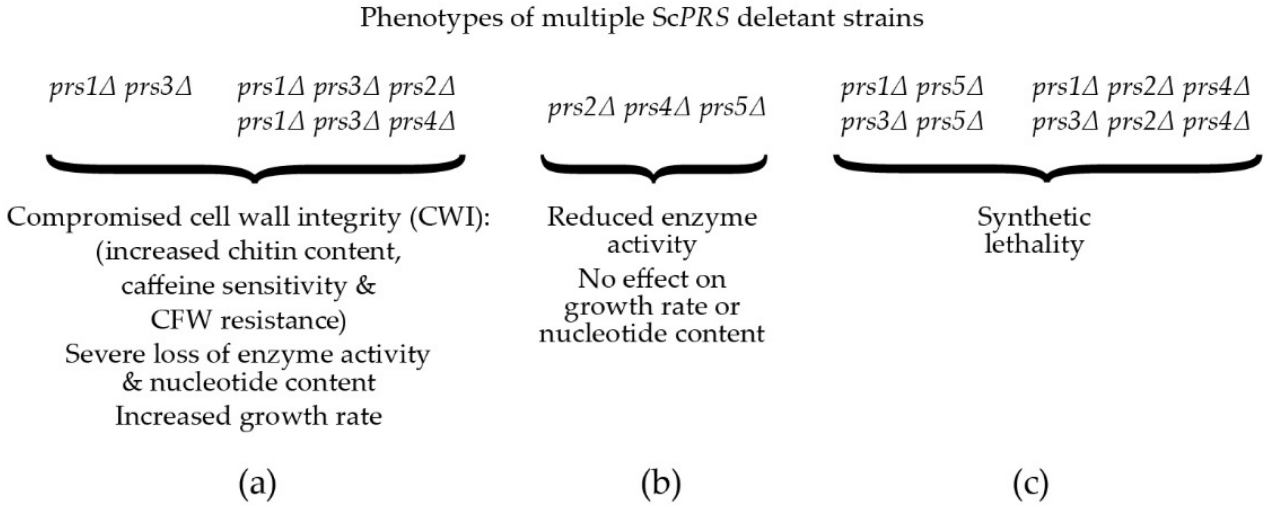

Figure 3. Compromised phenotypes, PRPP synthetase activity and nucleotide content encountered in Sc*PRS* multiple deletant strains: (**a**) phenotypes of defined Sc*PRS* deletant strains. CFW = calcofluor white; (**b**) phenotypes of the triple deletant *prs2Δ prs4Δ prs5Δ*; (**c**) synthetic lethality encountered when either Sc*PRS1* or Sc*PRS3* is deleted from a *prs5Δ* strain or simultaneous deletion of Sc*PRS2* and Sc*PRS4* in combination with loss of either Sc*PRS1* or Sc*PRS3*.

By performing a variety of diagnostic tests, including environmental exposure, e.g., heat shock, caffeine sensitivity, CFW (calcofluor white), Congo red and exposure to mating pheromone, researchers discovered that strains deleted for Sc*PRS1* or Sc*PRS3* are compromised in the maintenance of CWI [64][78][81][82]. The general architecture of the MAPK (mitogen-activating protein kinase) pathway in eukaryotes is illustrated in **Figure 4**a. Excellent reviews of the yeast CWI pathway and the extent of its influence on yeast physiology have been published [83][84][85][86][87][88][89][90][91]. Sc*PRS* mutant strains have consistently shown varying degrees of sensitivity to the purine analogue, caffeine (1,3,7-tri-methylxanthine), thus compromising CWI signalling *via* the MAPK (mitogen-activated protein kinase) [64][74][79][82][92]. It should be noted that caffeine not only interferes with the integrity of the cell wall, but also possibly by the inhibition of mTOR (mechanistic Target Of Rapamycin) mTORC1 and mTORC2, well-known regulators of eukaryotic cellular metabolism [93][94][95].

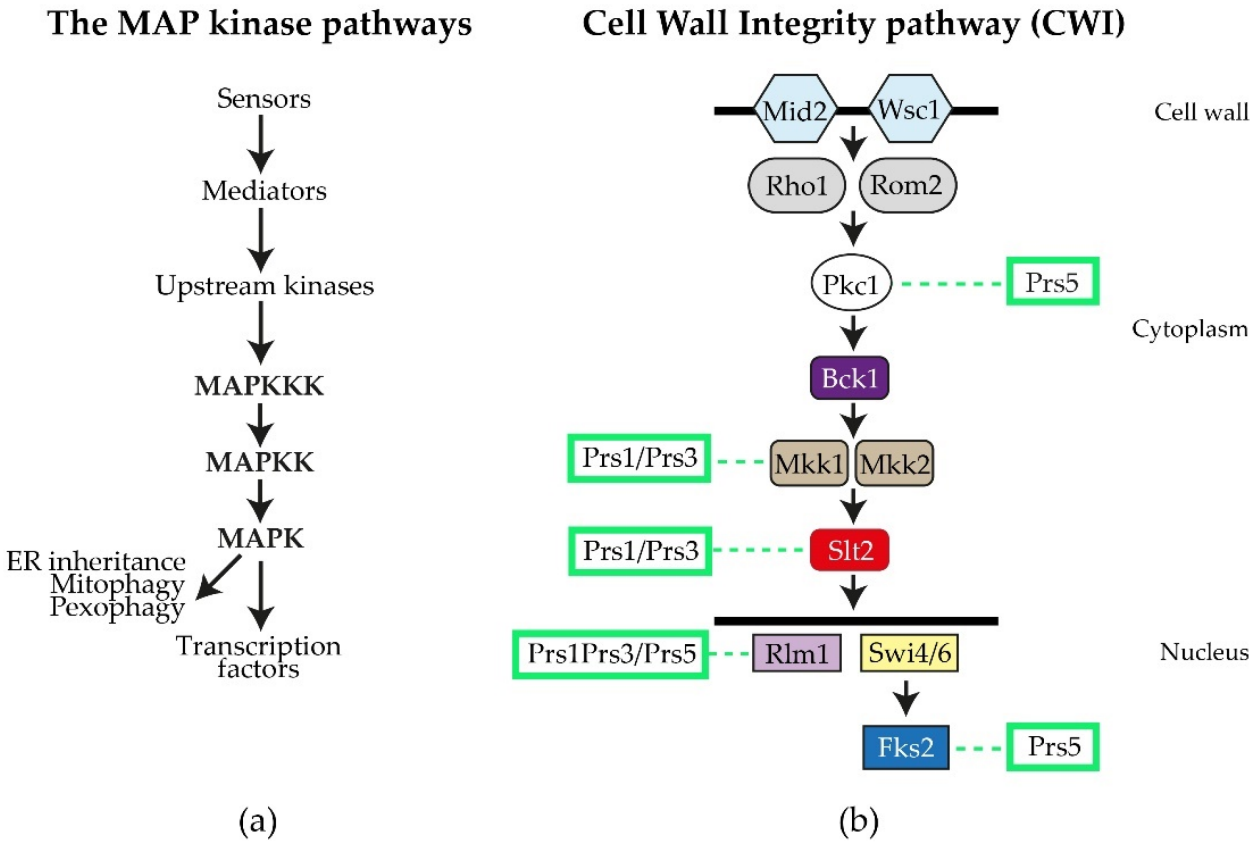

Figure 4. The *S. cerevisiae* CWI (cell wall integrity) pathway: (**a**) the general flow of information for MAP kinase pathways; (**b**) schematic diagram of the yeast CWI pathway. The two sensory proteins Mid2 and Wisc1 span the cell wall and are responsible for receiving signals, e.g., heat shock, that are transmitted to the upstream kinase Pkc1 by the mediators Rho1 and Rom2. Pkc1 then activates Bck1 (MAPKKK), which in turn activates Mkk1/Mkk2 (MAPKK), permitting the activation of Slt2 (MAPK). Slt2, in its phosphorylated state, can enter the nucleus, where it influences gene expression by targeting transcription factors, such as the MADS-box transcription factor Rlm1 and the SBF (Swi4/Swi6) transcriptional complex, as well as impacting on polarized growth and processes associated with mitophagy, pexophagy and ER inheritance. Fks2 is an alternative catalytic subunit of the β-1,3-D-glucan synthase whose expression is regulated by cell wall stress and Rho1 [83][84]. The interactions of Prs polypeptides with components of the CWI pathway or their impact thereon, are collated from the following references [64][74][78][79][80][84][92][95][96][97][98][99] and indicated by dashed lines.

Following gene duplication, yeast can sacrifice sources of PRS enzyme activity caused by the insertions, e.g., NHR1-1 and NHR5-2, since survival depends on the association of two Prs polypeptides, one containing at least one NHR and the other without an NHR. A concrete example of this is the Prs1/Prs3 heterodimer, which is the most important of the three minimal functional units required for yeast viability [27][75]. The insertion NHR1-1 in Prs1 disturbs the catalytic flexible loop, thus increasing its distance from the ribose-5-phosphate loop (**Figure 2**) and impacting its enzyme properties negatively but still permitting an interaction of Prs1 with the components of the CWI pathway, *viz.*, the MAP kinase, Slt2/Mpk1 [92], a functional homologue of human ERK5 (extracellular signal-regulated kinase) [100][101], and Mkk1 (mitogen-activated protein protein-kinase) [78][81][102]. A comparable situation exists for Prs5, which, in combination with either Prs2 or Prs4,

provides the two remaining minimal functional units essential for cell viability. The three insertions referred to above, explain the necessity of retaining at least one of the three minimal functional units, Prs1/Prs3, Prs2/Prs5 or Prs4/Prs5, since only then can the production of PRPP and the maintenance of CWI in the yeast cell be guaranteed. Furthermore, it also explains why simultaneous deletion of Sc*PRS1* and Sc*PRS5* or Sc*PRS3* and Sc*PRS5* is synthetically lethal since neither PRPP production nor maintenance of CWI is sustained (**Figure 3**c).

By Western blotting with human anti-phospho MAPK antibodies, which recognize *S. cerevisiae* phosphorylated Slt2, researchers demonstrated that strains lacking Sc*PRS2*, Sc*PRS4* or Sc*PRS5* can activate Slt2 in response to heat stress in a pattern similar to that of the WT. However, the deletion of *ScPRS1* or *ScPRS3* causes constitutively phosphorylated Slt2 in the absence of heat stress. In a *prs1Δ* strain, there was a strong signal at time zero, which, after heat shock, was maintained over a period of four hours before falling to zero. A *prs3Δ* strain can maintain and increase the level of phosphorylated Slt2 upon exposure to heat stress [78][80][81]. The difference in the pattern of heat-induced Slt2 phosphorylation response in a *prs3Δ* strain is, in fact, the response of a *prs1Δ prs3Δ*, since it has been demonstrated that deletion of Sc*PRS3* or only its $_{284}$KKCPK$_{288}$ motif, which researchers designated NHR3-1 (**Figure 2**), results in simultaneous loss of Prs1 [92]. The sudden disappearance of the phosphorylated form of Slt2 seen in the Sc*PRS1* at five hours of heat exposure suggested an inability of this strain to endure stress over a longer period, possibly due to the breakdown of the Prs1/Prs3 heterodimer [80][81].

Researchers have shown by co-immunoprecipitation that NHR1-1 interacts with the MAP kinase, Slt2, of the CWI pathway only when Slt2 is phosphorylated, thus providing more evidence for linking PRPP synthesis to the maintenance of CWI in yeast. A strain expressing non-phosphorylatable Slt2 (slt2(T_{190}A, Y_{192}F)) does not interact with Prs1; however, the kinase-dead Slt2 mutant (slt2(K_{54}R)) still interacts with Prs1 [79][103]. It has also been possible to demonstrate that Prs1 is bifunctional since a measurable increase in the strength of the Y2H interaction between prs1(ΔNHR1-1) and Prs3 results in a 50% increase in PRS activity in comparison to the WT. A lower, albeit possibly more accurate factor, 15%, is measured when comparing PRS activity with strains carrying plasmid-borne versions of WT Prs1 and prs1(ΔNHR1-1). The inability of a prs1(ΔNHR1-1) strain to increase Rlm1 expression in response to heat stress while maintaining enzyme activity indicates, for the first time, that the two metabolic functions—provision of PRPP and maintenance of CWI—are separable and the presence of NHR1-1 is not essential for the formation of the Prs1/Prs3 heterodimer, as demonstrated by a stronger Y2H interaction with Prs3 [92].

2.5. Yeast Genocopies of PRPS-Associated Human Neuropathies Interfere with CWI

Prs2, Prs3 and Prs4 of yeast and hPRPS1 proteins share a sequence similarity of approximately 70%, which drops to 53% in Prs1 on account of the presence of NHR1-1 (Clustal Omega). Genocopies of mutations in Sc*PRS1* associated with human neuropathies were created to examine their impact on yeast physiology. Missense mutations in the h*PRPS1* gene can lead to a gain-of-function associated with PRPS1 superactivity [104][105][106][107] or a loss-of-function [51][104]. Arts syndrome [47][48] and Charcot-Marie-Tooth disease-X5 (CMTX5) belong to the latter category [51]. Genocopies in Sc*PRS1* of CMTX5 (c.343C > A + c.344T > C (p.L115T) (cf. p.M115T (c.344T > C) in h*PRPS1*) and Arts syndrome (c.398A > C (p.Q133P) (cf. p.Q133P (c.398A > C) in h*PRPS1*) were created. These genocopies are located at 86 and 68 aa, respectively upstream of NHR1-1 in Sc*PRS1*. Furthermore, mutations of the divalent-cation binding site alone or in combination with the ribose-5-phosphate binding site were created [74]. All constructs tested retain NHR1-1 and can increase Rlm1 expression at 37 °C, albeit to varying degrees (**Figure 5**a). Rlm1 expression in the presence of the genocopies p.L115T and p.Q133P was increased with respect to the WT at ambient temperature, indicating that these mutations may influence the folding of Prs1, which does not interfere with NHR1-1/Slt2 interaction [74]. A similar response of Rlm1 expression was observed when researchers tested the effect of mutating the divalent cation-binding site at position H130A (c.388C > G + c.389A > C) and the ribose-5-phosphate binding site, D326A (c.977G > C), singly and in combination, namely, an increased Rlm1 response at 30 °C, which increased further following incubation at 37 °C, suggesting that the folding of the mutated Prs1 polypeptides is altered in such a way that NHR1-1 can interact more readily with Slt2, possibly on account of their locations external to each monomer of the hexameric complex of *B. subtilis* PRPP synthetase complex [21]. Both NHR1-1 and p.H130A are essential for overcoming the synthetic lethality of a *prs1Δ prs5Δ* strain, whereas Prs1 with an altered ribose-5-phosphate binding site, p.D326A, can rescue the synthetic lethality [74][103]. The mutations p.H130A and p.Q133P are separated by only two aa but have significantly different levels of Rlm1 expression at 30 °C, suggesting that the helix breaker mutation, p.Q133P, has a more profound effect on protein folding. The Rlm1 expression data follow the same pattern as PRS activity (**Figure 5**b) [74]. Strains carrying these genocopies could suppress the synthetic lethality of the double mutant *prs1Δ prs5Δ* at 30 °C but did not restore growth at 37 °C. However, the inclusion of 1 M sorbitol as an osmotic stabilizer permitted growth at 37 °C, except for the strain carrying the genocopy of the mutation p.Q133P associated with Arts syndrome. Examining these rescued strains for their caffeine

sensitivity indicated that in the absence of an osmotic stabilizer, growth was possible at 30 °C in 2 mM caffeine, although again, the genocopy p.Q133P (Arts syndrome) grew less well than the others. When 1 M sorbitol was included in the media, growth was restored at 30 °C at a concentration of 3 mM caffeine [103].

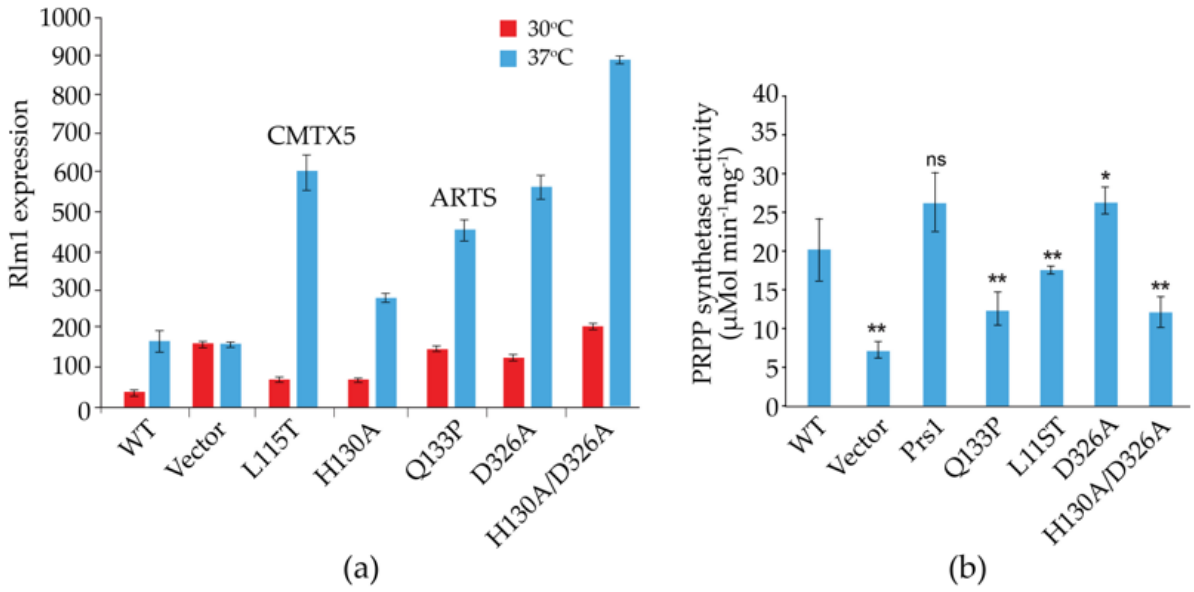

Figure 5. Influence of genocopies p.L115T and p.Q133P in ScPrs1, which correspond to the human neuropathies CMTX5 (p.M115T) and Arts syndrome (p.Q133P), respectively, and characteristic motifs of Prs1 either singly or in combination: H130A, divalent cation-binding site; D326A, PRPP-binding site/Ribose-5-phosphate binding site. It should be noted that the locations of the mutations H130A and D326A differ from those in **Figure 2**, since the length of Prs1 (427 aa) is increased with respect to Prs2 (318 aa) on account of the insertion of NHR1-1; (**a**) on Rlm1 expression following incubation at 30 °C and 37 °C, all values are significantly different from the WT at $p < 0.001$ (n = 12-20); (**b**) on PRS activity. *p*-values: * = $p \leq 0.05$; ** = $p \leq 0.05$, ns = not significant.

3. Human PRPS and Associated Disorders

In humans, the three isoforms of PRS, hPRPS1 (HGNC 9462), hPRPS2 (HGNC 9465) and hPRPS3 (HGNC 9463) [15][44][108][109][110][111][112], share high aa sequence similarity, namely 98.7% between hPRPS1 and hPRPS2, 97.2% between hPRPS1 and hPRPS3 and 96.5% between hPRPS2 and hPRPS3 (EMBOSS Needle). Human *PRPS1* exists in two isoforms. The PRPS1 transcript variant 1 mRNA, RefSeq NM_002764 contains seven exons and gives rise to a protein RefSeq NP_002755 with a MW of 34.8 kDa. The h*PRPS1* transcript variant 2 mRNA, RefSeq NM_001204402 gives rise to a shorter protein variant identical to the last 114 aa of PRPS1 isoform 1 (RefSeq NP_001191331) [112]. For h*PRPS2,* there are also two transcript variants 1 and 2 mRNAs (NM_001039091 and NM_002765), which differ only in length by nine nucleotides due to alternative splicing giving rise to two proteins, one of 321 aa and the other of 318 aa (RefSeq NP_001034180 and NP_002756) [43][45].

There are three classes of PRS enzymes, *viz*. I, II and III, with the hPRPS1 belonging to class I, which require inorganic phosphate (P_i), SO_4^{2-} and Mg^{2+} for enzymatic activity, are dependent on ATP or dATP as the diphosphoryl donor and are allosterically regulated by ADP, AMP and GDP [3][21][113]. Class II enzymes are specific to plants, and they accept a wide range of diphosphoryl donors (ATP, dATP, CTP, GTP, and UTP) and are not allosterically inhibited by ADP or dependent on P_i for their activity [114][115]. Class III enzymes are a novel class of PRPP synthetase found in the archaeon *Methanocaldococcus jannaschii,* which utilize ATP and dATP as a diphosphoryl donor and whose activation is solely dependent on P_i. They are inhibited by ADP in a non-allosteric manner but appear to be competitively inhibited by ATP [116]. The PRPP synthetase from the thermophilic archaeon *Thermoplasma volcanicum* is an interesting case since on the basis of structure, it has a close similarity to *M. jannaschii* and therefore, has been designated as a class III PRPP synthetase, in spite of lacking an allosteric site and existing as a dimer [117]. In addition to the biochemical characterization of PRPP synthetases, they can also be classified according to their three-dimensional structures: class I enzymes have a hexameric quaternary structure found in mammals and bacteria; class II enzymes, including PRPP synthetase isozyme 4 from spinach, have a homo-trimer quaternary structure; and the class III enzyme from *M. jannaschii* is tetrameric [116]. The crystal structure of *B. subtilis* PRS (PDB: 1DKR) [21] has been known for twenty years and has provided the basis for structural analysis of hPRPS1 (PDB: 2H06) and is a homo-hexamer consisting of three tightly interacting homodimers [113]. Each of the homodimers contains a catalytic site and two allosteric sites, I and II, whereas, in the *B. subtilis* PRS, there is only one allosteric site [21][113]. The reactive site binds both ATP and ribose-5-phosphate and is located at the

interface of two domains within one homodimer. The allosteric site I for ADP and P_i is positioned at the interface of the three homodimers of the hexameric structure, whereas the second, novel allosteric site II occupies a position at the interface of two monomers within one homodimer. The crystal structure of hPRPS1 reveals a complex with SO_4^{2-} at the allosteric site II, thus positively impacting on the stability of the catalytic flexible loop and preventing the entry of the inhibitor ADP while maintaining an open conformation for the entry of ATP and activation of the enzyme [113][118].

Human PRPS1 and PRPS2 are class 1 PRPS enzymes that, despite their high degree of sequence similarity, differ in function and regulation. It has been observed that *PRPS1* is expressed constitutively, whereas *PRPS2* is expressed inter alia in colorectal cancer (CRC) metastasis and hepatocellular carcinoma (HCC) [119][120]. In addition to hPRPS1 and hPRPS2, human PRPS-associated proteins (PAPs) (PAP39 (hPRPSAP1, HGNC 9466) at position 17.q25.1 and PAP41 (hPRPSAP2, HGNC 9467) at position 17p11.2 have been discovered [13][17]. Human PAPs share high amino acid sequence similarity and interact physically with hPRPS1 [121][122][123][124][125]. However, PAPs, PRSPSAP1 and PRPSAP2 do not contain an ATP-catalytic binding site and, therefore, cannot be directly involved in the phosphoribosyl transferase reaction but may act as negative regulators of PRPP synthetase [13][17]. It should be noted that the insertions in rat *PRSPSAP1* and rat *PRPSAP2* are at similar positions to NHR1-1 of Sc*PRS1* and NHR5-2 of Sc*PRS5* (cf. **Figure 2**) [125]. Two processed pseudogenes for h*PRPS1* (HGNC 39427 and HGNC 9464) are located on chromosomes 2q24.3 and 9q33.3, respectively, and both are transcribed but not translated. Human PRPS1-related diseases can be characterized by a continuous spectrum of features spanning neuropathies, hearing loss, optic atrophy, ataxia, cognitive impairment, and recurrent upper-respiratory infections [104]. It is possible to correlate mutations in h*PRPS1* with the severity of the disease, which ranges across varying degrees of loss of PRPS activity attributable to minor changes in the architecture of the hexameric structure. For instance, PRPS activity in the neuropathy CMTX5 patients is reduced in comparison to patients presenting with DFN2-associated hearing loss because of the nature of the local structural changes of the enzyme. There is also the possibility that epigenetic modification affects h*PRPS1* expression [126][127]. The neurological symptoms of h*PRPS*-related diseases may be associated with the degree of myelination of neuronal structures since the synthesis of myelin is dependent on lipid esters of pyrimidine nucleotides and CDP-choline and requires SAM (*S*-adenosylmethionine) [104]. Researchers have observed that in the *prs1Δ* strain, the combined CDP and CTP levels were extremely low [26][76][80], only 12% of the level in the WT, thus connecting PRPP synthesis to both *de novo* and salvage synthesis of phospholipids which require CTP. The osmotic sensitive phenotype of a *prs1Δ* strain is rescued by overexpression of CTP synthetase [80][128]. Neurons have a high energy demand and are therefore susceptible to any reduction in GTP and ATP supply. There is a parallel scenario in yeast that CTP pools are important for maintaining the supply of CDP-choline and CDP-ethanolamine for phospholipid biosynthesis, essential for membrane integrity [104][129]—a further argument for the use of yeast as a eukaryotic model organism. The role of pyridine nucleotides in energy production cannot be ignored since they are involved in energy storage, cell signalling, nucleic acid structure and enzyme cofactors, e.g., NAD^+ [104]. The conflicting evidence that PRPS1 superactivity can lead to purine depletion and overproduction of uric acid reflects the spectrum of mutations encountered in the product of the h*PRPS1* gene, which can affect local enzyme structure or impinge on the integrity of the quaternary structure and the stability and overall activity of the enzyme.

3.1. PRPP Synthetase Superactivity: hPRPS1 (*OMIM 300661*)

Missense mutations of h*PRPS1*, the more highly expressed isoform of the two h*PRPS* genes, lead to gain-of-function mutants [46][104][130]. These changes cause critical alterations in PRPS levels that may have a significant influence on many life processes, such as nucleotide biosynthesis and cell signalling. The gain-of-function of h*PRPS1* results in superactivity of the enzyme associated with hyperuricaemia and hyperurocosuria—overproduction and accumulation of uric acid in the blood and urine, respectively. Overproduction of uric acid can lead to gouty arthritis in joints and impairment of kidney function because of excessive production of purine nucleotides and their subsequent conversion to uric acid [50] and references therein [105][107][131][132]. Specifically, PRPS1 superactivity is a rare X-linked disorder of purine metabolism that results in an increase in enzyme activity due to regulatory defects or alteration in catalytic properties [14][130][133][134][135][136][137]. Overexpression of h*PRPS1* with altered kinetic enzyme characteristics has been observed in white cells, erythrocytes and fibroblasts of patients [131][136][137] and is characterized by several physiological disorders, including, in addition to gouty arthritis, hearing impairment or deafness, hyperuricaemia, hyperuricosuria, urolithiasis in men, ataxia, hypotonia, developmental delay, intellectual impairment and recurrence of infectious diseases in the upper respiratory tract [104][105][131][138][139][140][141]. Since the h*PRPS1* gene is located on the X-chromosome, if one of the mother's two X-chromosomes carries the mutation for PRPS superactivity and this chromosome is inherited by the son, he will suffer this disease, and he can pass the affected chromosome to a daughter. The daughter will not necessarily display symptoms since she may have inherited an unaffected chromosome from her mother. Women are less likely to suffer from PRPS1 superactivity since random X-chromosome inactivation [142][143] may reduce the dosage or eliminate the mutated version of the h*PRPS1* gene.

Patients with severe or extremely high PRPS1 superactivity may exhibit one or more of the following nine missense *PRPS1* mutations, p.D52H (c.154G > C), p.L129I (c.385C > A), p.A190V (c.569C > T), p.H193Q (c.579C > G) [133], p.N114S (c.341A > G) and p.D183H (c.547G > C) [104][133], p.H193L (c.578A > T) [132][133], p.G174V (c.521G > T) [144] and p. G174R (c.520G > A) [145]. Specifically, the missense mutations of p.D52H and p.L129I result in the disruption of local structure close to the allosteric sites and therefore inhibit feedback regulation [104][133][146]. The p.D52H mutation causes destabilization of the enzyme structure around Asp52 and directly disturbs allosteric site I [104][106][127], thus reducing the sensitivity to the inhibitor ATP in vivo [146], whereas the p.L129I mutation leads to steric hindrance with both the protein backbone at Ala131 and Ile134 disturbing allosteric site II [104], leading to a neuropathy [127][133].

The other missense mutations are located in the homodimer interface, thereby disrupting the homodimer and affecting the allosteric sites [105][132]. The p.N114S mutation also destabilizes the hydrogen bonding of the homodimer. The p.A190V mutation leads to disruption of the hydrophobic environment in the homodimer interface, whereas p.D183H and p.H193L/Q mutations break the hydrogen bond interactions between Asp183 of one homodimer partner and His193 of the other polypeptide chain of the homodimer, thereby destabilizing the homodimer interface [104][127]. Interestingly, the h*PRPS1* mutations p.D183H and p.N114S in both hemizygous males and affected females caused neurological deficiency, e.g., sensorineural deafness [50][106], whereas p.D52H leads to increased production of PRPP levels and induces hyperuricaemia and gout [146]. In males, PRPS1 superactivity resulted in an alteration in the allosteric control of PRPP synthesis with high purine nucleotide and uric acid production [17]. However, the study of Zikánová et al. (2018) [145] showed that female carriers of PRPS1 superactivity had elevated levels of uric acid and gout since random X-chromosome inactivation may result in them retaining only the mutated allele p.G174R (c.520G > C).

3.2. Reduced PRPP Synthetase in Humans

Reduced activity in *PRPS1* is associated with neurological disorders, such as Arts syndrome, Charcot-Marie-Tooth disease type 5 (CMTX5), X-linked syndromic/nonsyndromic sensorineural deafness (DFN2/DFNX2) and retinal dystrophy.

3.2.1. Arts Syndrome

This disorder (OMIM 301835) is associated with X-linked missense mutations in h*PRPS1*, resulting in loss-of-function of PRPS1. It affects mainly males with a prevalence of less than 1 in 10^6 and is lethal, with symptoms appearing before two years of age. Unfortunately, 80% of patients die in childhood. It is a severe phenotype caused by the missense mutation in c.455T > C (p.L152P), which was first identified in a Dutch family. A further missense mutation c.398A > C (p.Q133P) was identified in an Australian family [47][48]. MD revealed that the p.Q113P variant destabilizes the allosteric site II and the ATP binding pocket of hPRPS1 and that p.L152P alters only the ATP binding site [48]. An autopsy of the Dutch Arts syndrome patient revealed a total lack of myelin in the posterior columns of the spinal cord, and a sural nerve biopsy of the Australian patient showed mild paranodal demyelination. Arts syndrome is characterized by profound delayed motor development, early-onset hypotonia, hearing impairment, mental retardation, optic atrophy, intellectual disability, a compromised immune system, recurrent infection, and early childhood mortality [47][48][104].

Maruyama et al. (2016) [147] also reported a transient neurogenic proximal weakness and loss of muscle strength caused by the mutation p.H123D (c.367C > G) in Arts syndrome patients. The premature death of Arts syndrome patients is most commonly due to infections of the upper respiratory tract [47]. In this disorder, low serum uric acid concentrations and undetectable hypoxanthine with normal range xanthine and uric acid concentrations in the urine purine profile have been observed [48]. In affected males, PRPS enzyme activity is significantly decreased, while obligate female carriers show mild symptoms [148]. PRPP synthetase activity, as measured in fibroblasts from the affected members of the Dutch family, was reduced 10-fold in comparison to the activity from controls [47]. In a recent study, Puusepp et al. (2020) [149] reported no hearing loss and normal levels of both serum uric acid, purines and pyrimidines in a hemizygous variant c.130A > G (p.I44V) of the h*PRPS1* gene in a 6-year-old male patient presenting with clinical features of Arts syndrome, as described above. However, a marked reduction in PRPS1 activity in erythrocytes was recorded in this patient, emphasizing the necessity of testing for all parameters associated with Arts syndrome. It is noteworthy that the p.I44 residue is conserved in mammals and amphibia, *Drosophila* spp., *Caenorhabditis elegans* and *S. cerevisiae*.

Another mutation, c.424G > C (p.V142L), was found in a patient presenting with classical symptoms, including hyperuricaemia and hyperuricosuria but with no gouty arthritis, developmental delay, hypotonia, and bilateral hearing loss, indicative of both the severe form of hPRPS1 superactivity and symptoms of Arts syndrome, e.g., recurrent respiratory infections, myopia, glaucoma and motor neuropathy [139]. This valine-to-leucine change arising from a transversion is predicted to alter the allosteric sites I and II and the ATP binding site, thus impairing hPRPS1 inhibition and resulting in hPRPS1 overexpression. Furthermore, this substitution is postulated to influence the interaction of Val142 with Lys100 in a flexible loop, which is part of the ATP-binding site of the other PRPS1 subunit of the homodimer. The two-fold effect of

this mutation on nucleotide concentrations is reflected in the difference in PRPS activity measured in fibroblasts and postmitotic cells, e.g., erythrocytes and brain cells. In the former, there is increased PRPS1 activity due to loss of feedback inhibition, whereas in the latter, there is loss of PRPS1 activity over time. This combination of gain-of-function and loss-of-function of PRPS1 activity in the same patient was revealed by molecular modelling and enzyme activity measurement in different cell types and illustrated the range of features associated with an imbalance of PRPP synthetase underlying the clinical features of various non-syndromic postlingual hearing loss neuropathies.

In summary, there are several manifestations, e.g., PRPS1 superactivity and recurrent infections, which can be traced back to the p.V142L mutation resulting in intermediate phenotypes lacking the severity of those associated with Arts syndrome (p.Q133P or p.L152P). There was a difference in PRPP synthetase activity as measured in erythrocytes and fibroblasts in the proband p.V142L, which could be due to the altered regulatory properties of the enzyme. Supporting evidence for this suggestion is that the GDP/GTP levels in the proband's erythrocytes were reduced in comparison to the controls, but ATP/ADP levels were normal. Unfortunately, there was no data concerning GTP/GDP levels in fibroblasts, although enzyme activity in fibroblasts carrying the mutation did respond to the level of phosphate, whereas the control did not. It could be that intracellular purine levels are sensed by an as-yet-unknown mechanism(s) involving GTP interaction with mTORC1 *via* Rheb [150].

Overlapping phenotypes are also described in a male/female sibling study [127]. A male subject, aged 36, p.Q277P (c.830A > C), displayed overlapping features of CMTX5 and Arts syndrome, prelingual hearing loss, recurrent severe infections and progressive visual loss due to optic atrophy, as well as undetectable PRPS1 activity in erythrocytes. His sister had reduced PRPS1 activity in addition to prelingual nonsyndromic hearing loss and deafness (DFN2/DFNX1), whereas their mother had normal PRPS1 activity and no hearing loss. Brain MRI (magenetic resonance imaging) of the two siblings revealed cerebellar atrophy. X-chromosome inactivation was extremely skewed in the sister but only moderately skewed in the mother. Recently, in 2021 [151], a novel Arts syndrome h*PRPS1* deficiency missense mutant p.R84T (c.250C > T) was reported, which disturbed the interface regions between the subunits, thus negatively impacting the ligand binding of the allosteric site I. An Australian male patient presented in his first year with symptoms congruent with Arts syndrome, including congenital sensorineural deafness, recurrent severe infections, visual impairment inter alia with substantially reduced hPRPS1 activity in erythrocytes. It was decided with parental consent to start feeding studies with SAM (*S*-adenosylmethionine) and NR (nicotinamide riboside) at the age of three. Unlike most purines, SAM can cross both the gut and the blood-brain barrier and has been used successfully in the treatment of depression and dementia [152]. It was also shown that SAM, which can supply ATP and GTP independently of PRPP [104], may alleviate some of the symptoms of patients with Arts syndrome. NR can form NADP(H) independently of PRPP by virtue of NR kinases 1 and 2 to produce NMN (nicotinamide mononucleotide), which is adenylated to NAD^+ [153][154]. Shortly after commencing treatment, the intervals between respiratory infections increased and recovery was improved. The patient's muscular strength, speech, ability to play and overall well-being improved markedly. No adverse treatment effects were recorded, and homocysteine/methionine concentrations were normal. Despite these improvements, audio-visual impairment and ataxia persisted, but nevertheless, the treatment reduced the strain on the patient and his family. ATP and GTP improved sustainably, but NAD^+ measurements could not be repeated with higher levels of NR [104][151].

4. Roles of PRPS in Cancer

A recent development is the discovery of the involvement of PRPP synthetases 1 and 2—hPRPS1 & hPRPS2—in cancer progression, management, and treatment. Human PRPS1 and PRPS2 have been identified in acute lymphoblastic leukaemia (ALL) as the major cause of drug resistance to the prodrugs thioguanine and thiopurine, which are used in cancer therapy [155][156][157][158]. The way in which cancer cells become resistant to the prodrugs is through the acquisition of hyper-activating h*PRPS1* mutations, e.g., c.568G > A (p.A190T), p.A190V, c.340A > G (N114D), p.D183H, c.521G > A (p.G174E), c.528A > T/C (p.K176N), c.571C > T + c.573G > T/C (p.L191F), c.308G > C (p.S103T) ([159] and other mutations cited therein). All these mutations destabilize the dimer interface, causing the loss of ADP and GDP allosteric inhibition. Large-scale sequencing of DNA from patients with therapy-resistant mutations in relapsed ALL showed the presence of h*PRPS2* hyper-activating mutations c.400G > A (p.A134T) and c.523G > A (p.A175T) [156]. h*PRPS1* and h*PRPS2* have been implicated in the metastasis and proliferation of cancer [159][160]. Mammalian PRPS2 is associated with oncogene Myc-driven tumorigenesis activation [52] and promotes metastasis of neuroblastoma cancer [160]. The translationally regulated enzyme PRPS2 may play a role in the proliferation of oncogenic signalling-driven cancers. This is due to an eIF-4E cis-regulatory pyrimidine-rich translational element (PRTE) found in m*PRPS2* and h*PRPS2* [52][161], but not in the corresponding *PRPS1* genes, which renders PRSP2 resistant to feedback inhibition by ADP and GDP and may also explain the therapy-resistant human cell lines associated with ALL [156]. The distinguishing features of the *PRPS2* isoform are found in many translationally regulated transcripts because of mTOR hyperactivation, allowing nucleotide and protein biosynthesis to increase concomitantly with higher protein synthetic capacity protein of cancer cells

[52][162][163]. *PRPS2*-knockout mice are viable and fertile with no discernible developmental defects, which supports the hypothesis that mPRPS2 is not essential for development but contributes to Myc-dependent cancer formation. However, a recent publication [164] has correlated the depletion of murine *PRPS2* by sh RNA lentivirus with hypospermatogenesis, suggesting that PRPS2 may be a potential biomarker for male infertility. Human *PRPS2* may well be an attractive diagnostic target for Myc-driven cancers in osteosarcoma [165]. The c-Myc-PRPS2 interaction promotes metabolic reprogramming of biosynthetic processes leading to the proliferation of cancer cells. There is statistical evidence linking a longer survival time with negative PRPS2/p-mTOR staining in comparison to positive PRPS2/p-mTOR staining, implying that Myc-driven PRPS2 expression *via* the eukaryotic translation initiation factor 4E (eIF-4E) allowing interaction with mTOR results in tumour progression.

Overexpression of hPRPS1 elevates cancer cell proliferation and inhibits apoptosis in B-cell acute lymphoblastic leukaemia cell lines (B-ALL) as well as increasing the expression of Bcl-2, a gene in the mitochondrial-associated apoptosis pathway, at the RNA and protein levels [166]. The same authors suggested that h*PRPS1* silencing has no effect on the regulators of the cell cycle but elevates apoptosis in B-ALL cell lines. He et al. (2017) [167] found that h*PRPS1* silencing increases apoptosis in breast cancer. The expression of h*PRPS1* and h*PRPS2* is reduced by hypoxia in glioma cells. Furthermore, the expression of *PRPS1*, *PRPS2*, *PRPSAP1* (*PAP39*) and *PRPSAP2* (*PAP41)* genes in U87 glioma cells is not inhibited by ERN1 (EC 2.7.11, endoribonuclease activity of endoplasmic reticulum to nuclei-1). Endoplasmic reticulum-induced stress *via* tunicamycin in glioma cells, together with the suppression of ERN1, downregulates *PRPS1* and *PRPS2* gene expression levels [168]. The combined deletion of *PRPS1* and *PRPS2*, *PPAT* (PRPP-amidotransferase, EC 2.4.2.14) (HGNC 9238) and *CAD* (carbamoylphosphate synthetase II, EC 6.3.5.5) (HGNC 1424) suppresses cell proliferation in Kaposi's sarcoma-associated herpes virus (KSHV), by redirecting glutamine and asparagine, which provide the nitrogen source for purine and pyrimidine biosynthesis from the TCA cycle to the biosynthesis of nucleotides and non-essential amino acids, suggesting potential therapeutic application for this pathway [169].

Interestingly, in a recent study conducted by Miao and Wang (2019) [119], it has been reported that hPRPS2 promotes the movement and invasion of colorectal cancer cells (CRC), suggesting that the upregulation of PRPS2 observed in CRC was induced by the *MYC* proto-oncogene, thus implicating PRPS2 in CRC metastasis. Previously, Qiu et al. (2015) [54] demonstrated that the mRNA and protein levels of h*PRPS1* are upregulated in CRC. Knockdown of h*PRPS1* or upregulation of the micro-RNA, miR-124, a tumour suppressor, leads to reduced DNA synthesis and cell proliferation in CRC cell lines, revealing potential therapeutic targets. There are miRNA integrated signatures for h*PRPS1* mRNA and h*PRPS2* mRNA interactions, which may represent potential biomarkers and therapeutic targets for CRC [170].

MD simulation of four missense mutations p.D52H, p.M115T, p.L152P and c.607G > C (p.D203H) in h*PRPS1* predicted to be disease-causing mutations [171], revealed that the CMTX5 mutation p.M115T affected protein stability and is consistent with previously published experimental results documenting 60% reduction in hPRPP synthetase activity due to the disruption of the ATP binding region and allosteric site I of the PRPS1 active site [51]. Interestingly, a further h*PRPS1* missense mutation c.335T > C (p.V112A) spans the reference SNP (rs11541075) for the BOR (Branchio-Oto-Renal) syndrome. This syndrome, which is associated with hearing loss and other hearing abnormalities, in addition to abnormalities in kidney structure and function, may affect approximately 1 in 40,000 people (MedlinePlus). These data show the far-reaching effects of missense mutations in the h*PRPS1* gene which, on reflection, are not unexpected in a gene whose product is required to produce essential metabolites involved in nucleotide synthesis as well as contributing to cellular signalling and energy metabolism [171][172].

KHK-A (ketohexokinase-A), a protein kinase that is a splicing variant of KHK-C, an enzyme involved in fructose metabolism, phosphorylates and activates hPRPS1 at T_{225} *via* the inhibition of ADP, AMP, and GDP by sterical blocking allosteric site I to ADP, thus leading to an increase in nucleotide synthesis and progression of HCC. The oncogenic transcription factor c-Myc upregulates hnRNPH1/2 expression, thereby favouring splicing from KHK-C to KHK-A which then phosphorylates PRPS1 at T_{225}, increasing PRPP synthesis. By in vitro phosphorylation assays it was shown that KHK-A functions as a protein kinase rather than phosphorylating fructose, thus constitutively activating PRPP production in HCC which has been linked to a considerable risk of poor prognosis in HCC [173]. Clinical relevance has been demonstrated by immuno-histochemical comparative studies of matched tumour and non-tumour liver tissue samples supporting the molecular biological studies [173]. The upregulation of PRPS1 by KHK-A has also been shown to increase the growth of oesophageal squamous cell carcinoma [174]. Furthermore, the suppressed tumour formation of glioma $CD133^+$ cells by reduction in PRPS1 expression is a result of increased cell apoptosis [175]. Screening of a miRNA-targeting database identified *PRPS1* mRNA as being negatively regulated by the expression of miR-154 in glioma $CD133^+$ cells [176]. This result has been echoed in neuroblastoma cells [177], adding weight to the potential of PRPS1 targeted therapy in glioblastoma multiforme and neuroblastoma cell proliferation.

Jing et al. (2019) [178] observed a delay in the cell cycle and a reduction in CRC proliferation due to the lack of PRPS1 phosphorylation site at S_{103}. CDK1 (cyclin-dependent kinase 1)-driven cell proliferation is dependent on the phosphorylation of PRPS1 at S_{103}, possibly by blocking the binding of ADP to the allosteric sites of PRPS1. Upregulation of PRPS1 synthetase activity in the S-phase allows CRC cells to overcome cell cycle progression. Manipulation of this residue may be a promising strategy to combat CRC, especially since the mutations p.S103N (c.308G > A) and p.S103T have been shown to affect PRPS1 synthetase activity in ALL [159]. PRPS1 synthetase is regulated by the ratio of ATP/ADP which inter alia, indicates the metabolic capacity of a cell. ADP is the key allosteric inhibitor for PRPS1 in the presence of the substrate ribose-5-phosphate [113]. However, at a low concentration of ribose-5-phosphate ADP is a competitive inhibitor with respect to ATP, resulting in reduced PRPS1 activity and slowing down the progression of CRC. Nevertheless, further investigation is required prior to exploiting p.S_{103} for therapeutic purposes.

Notably, PRPS2 can be arginylated on Asn3 (asparagine), distinguishing it from PRPS1 by conferring differential degradation for the two proteins. Arginylation affects both the activity and stability of PRPS2 since different molecular mechanisms are responsible for its degradation [179]. The discovery that the p300 KIX-(kinase-inducible) domain interacted with PRPS1 indicates that, in addition to its interplay with cAMP regulation, p300 may play a co-activator role in the regulation of DNA synthesis [180].

Qiao et al. (2020) [181] postulated that the knockdown of h*PRPS2* blocks the progress of prostate cancer *via* the suppression of the cell cycle and induction of apoptosis. They revealed that PRPS2 upregulates prostate adenocarcinoma tissues, whereas knockdown of h*PRPS2* suppresses cell growth in both in vitro and in vivo studies. Li et al. (2019) [177] reported that the down-regulation of h*PRPS1* in vitro and in vivo blocks neuroblastoma cell proliferation and tumour growth. Qian et al. (2018) [182] reported that glucose deprivation causes AMPK-mediated direct phosphorylation of PRPS1 at S_{180} and PRPS2 at $S_{183,}$ which leads to brain tumorigenesis. Interestingly, under these conditions, the collapse of the hexameric structures of PRPS1 and PRPS2 to the constituent monomeric states occurs, along with concomitant loss of PRPS1 and PRPS2 synthetase activities, nucleic acid synthesis and NAD^+ synthesis.

The study conducted by Lei et al. (2015) [183] demonstrated that the expression of PRPS2 blocks the apoptosis of Sertoli cells, which correlates with Sertoli cell-only syndrome (SCOS). PRPS1 was found to be active in brain tumour-initiating cells (BTICs), indicating that PRPS1 is vital for maintaining BTIC linking MYC with *de novo* purine synthesis [184]. Wang et al. (2018) [185] reported that h*PRPS1* mutants elevate intracellular PRPP levels, which potentially enhances the transformation of 5-fluorouracil to fluorodeoxyuridine monophosphate and fluordeoxyuridine triphosphate, which are known metabolites that can induce DNA damage and apoptosis. The study suggests that ALL patients with h*PRPS1* mutations are likely to benefit from 5-fluorouracil-based chemotherapy.

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