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Proline dehydrogenase: a key enzyme in controlling cellular homeostasis

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1. ABSTRACT

Proline dehydrogenase (ProDH), also called proline oxidase (POX), is a universal enzyme in living organisms. It catalyzes the oxidation of L-proline to delta1-pyrroline-5-carboxylate leading to the release of electrons, which can be transferred to either electron transfer systems or to molecular oxygen. ProDH is not only essential for proline catabolism but also plays key roles in providing energy, shuttling redox potential between cellular compartments and reactive oxygen species production. Structural analysis of prokaryotic ProDHs already gives some insights into the biochemical activity and biological functions of this enzyme, which can be extended to eukaryotic ProDHs based on sequence similarities. Here we report the most recent investigations on the biochemical and regulation of ProDH at transcriptional, post-transcriptional and translational levels. The biological roles of ProDH in cell homeostasis and adaptation through energetic, developmental, adaptive, physiological and pathological processes in eukaryotes are presented and discussed to create a framework for future research direction.

2. INTRODUCTION

Proline is a proteinogenic secondary amino acid, which plays essential roles not only in primary metabolism but also in redox homeostasis, osmotic adjustment, protection against stress and signaling in all organisms (for review see 1). Proline dehydrogenase (ProDH), also known as proline oxidase (POX), catalyzes the first step in the two-step oxidation of proline in bacteria and eukaryotes (Figure 1). ProDH activity is FAD-dependent and produces delta1-pyrroline-5-carboxylate (P5C), which forms a non-enzymatic equilibrium with glutamate semialdehyde (GSA). P5C dehydrogenase (P5CDH) then converts GSA to glutamate (Figure 1). In most bacteria, ProDH and P5CDH activities are combined in a single bifunctional enzyme known as Proline utilization A (PutA) (2). ProDH is a flavoenzyme in prokaryotes and yeast while P5CDH is an NAD^+-dependent reductase (2, 3). Oxidation of proline to glutamate *via* the intermediate GSA/P5C transfers electrons to mitochondrial electron transfer system by ProDH-FAD complex and forms P5C, which is further oxidized to glutamate by P5CDH, that concomitantly reduces NAD^+ or $NADP^+$. In contrast to proline oxidation,

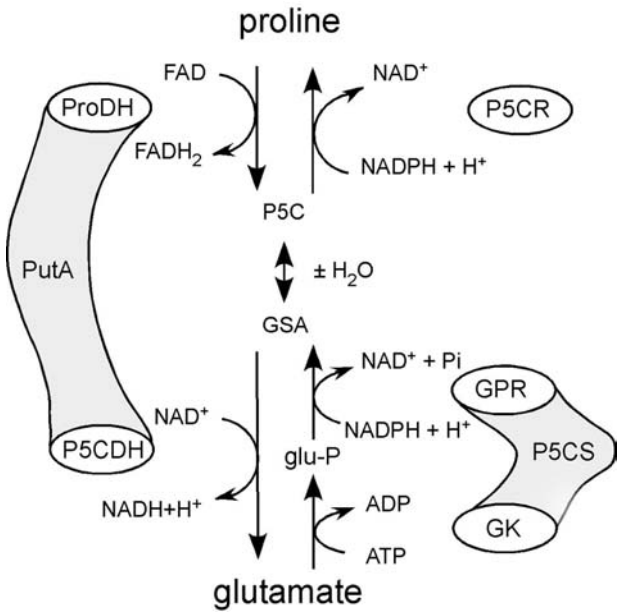

Figure 1. Proline metabolism schematic pathways. Gray area indicate fused enzymes that are present in only certain species. Fused P5CSs are found in both animals and plants although fused PutA enzymes are only present in certain prokaryotes. GK: gamma-glutamyl kinase, glu-P: gamma-glutamyl phsophate , GPR: gamma-glutamyl phosphate reductase, GSA: glutamate semialdehyde, P5C: pyrroline-5-carboxylate, P5CDH: P5C dehydrogenase, P5CR: P5C reductase, P5CS: P5C synthase, ProDH: proline dehydrogenase, PutA: proline utilization A.

which takes place in mitochondria in eukaryotes, proline biosynthesis occurs in different compartments according to species. For example, proline is synthesized in mitochondria in animal cells but in cytosol of fungal and plants cells (1, 4, 5). Recently P5C synthase 1 (P5CS1) accumulation has been reported in chloroplast in stressed plants (6).

So far, most studies have been focused on the biosynthetic pathway and biological roles of proline itself, but the influence of proline oxidation has been underestimated until recently and remains largely unknown. Oxidation of proline not only contributes to lower the amount of proline in the cell, hence abolishing its protective function, but also generates various molecules such as ATP, reactive oxygen species (ROS), $NADH/NADPH+H^+$ and alpha-ketoglutarate (Figure 2). Despite their ubiquity, ProDHs are structurally different with distinct cellular and physiological roles in different species. The aim of this review is to give an overview of what is known about the differences in ProDH structure across kingdoms and to discuss its roles in cell homeostasis adaptation. We therefore collate and compare information on the phylogeny, biochemistry and localization of ProDH in order to understand the physiological consequences of proline oxidation in both prokaryotes and eukaryotes.

3. PRODH, A UNIVERSAL ENZYME IN LIFE KINGDOM

The organization of ProDH enzymes in organisms from all kingdoms has been clarified with the recent availability of several bacterial genomes and comparative structural analysis of the predicted proteins encoded. With the exception of ProDH from Archaea, which is a divergent enzyme (7), ProDH activity is found in mono-, bi- and tri-functional enzymes. Among prokaryotes, Eubacteria possess all three types of enzymes (Figure 3). Bi- and tri-functional enzymes, called PutA, are membrane-associated proteins with 1000-1300 amino acid residues. PutA from *Escherichia coli* was the first protein to be crystallized and is believed to function as a homodimer (8). *E. coli* PutA possesses three functions as revealed by amino acid sequence alignment and crystal structure analysis. A function of regulating transcription of the *Put* regulon is present in the N-terminal part of the protein, followed by the ProDH and the P5CDH enzymatic domains (Figure 3).

Interestingly, eukaryotes possess only monofunctional ProDH enzymes (Figure 3). Structural and spectroscopic analysis of bacterial and yeast ProDHs confirmed the presence of a flavin cofactor bound to the polypeptide (2, 6, 9). This FAD cofactor is also likely to be present in ProDH from animals and plants but to our knowledge this has still to be structurally demonstrated. Protein sequence encoded by *ProDH* genes from 63 eukaryotic species were aligned to build an unrooted phylogenic tree. The analysis reveals distinct clusters of sequence similarities for ProDH originating from algae, fungi, protozoa, invertebrates, vertebrates and plants. Two ProDH isoforms are found in plants and vertebrates whereas, from the available data, algae, fungi and invertebrates only have a single *ProDH* copy per genome (Figure 4). In dicotyledonous plants, the two ProDH isoforms, when known, are closely related (75% identities for *Arabidopsis thaliana* ProDH), in contrast to the

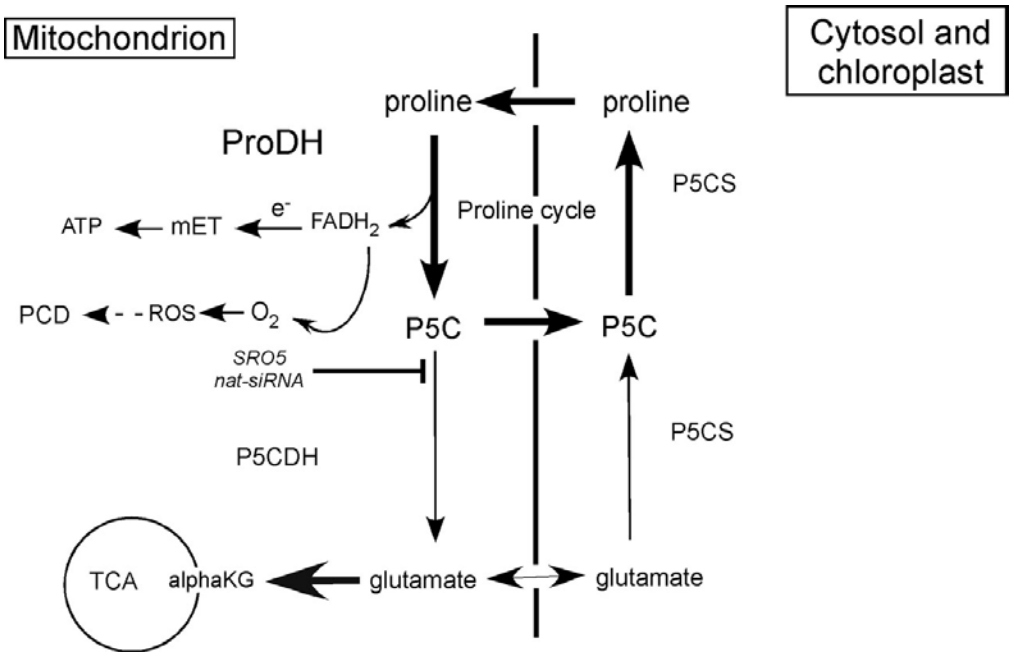

Figure 2. Proline cycle in plants keeps P5C and proline homeostasis. Schematic presentation of proline synthesis and degradation, emphasizing the role of the proline cycle in maintaining proline to P5C constant ratio and the production of reactive oxygen species (ROS) upon hypercycling of Proline-P5C cycle when P5CDH is mutagenized or its transcript level is reduced by the salt-induced production of SRO5-P5CDH nat-siRNA (85). Glutamate serves as the main precursor for proline in the cytosol and likely also in chloroplasts. Thick arrows indicate proline cycle. AlphaKG: alpha-keto-glutarate, mET: mitochondrial electron transfer chain, PCD: programmed cell death, TCA: tricarboxylic acid.

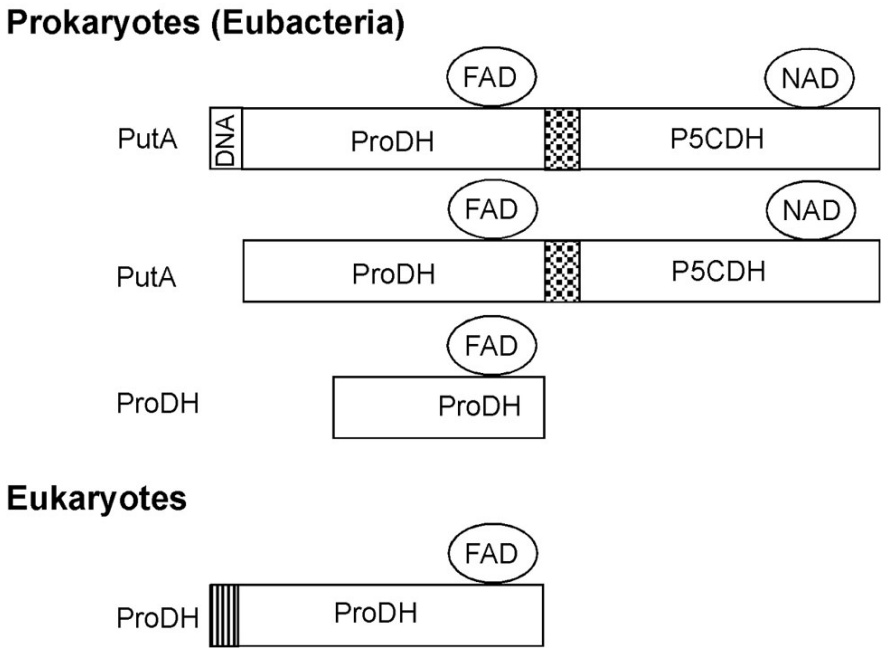

Figure 3. Structural organization of proline dehydrogenase. DNA indicates the DNA-binding domain of PutA, which is involved in the recognition of cis-acting motives in the *Put* regulon. Vertical hatching indicates the mitochondrial transit peptide. Cross-hatching shows the linker that connects the two enzymatic activities. FAD: flavin adenine dinucleotide, NAD: nicotinamide adenine dinucleotide, P5CDH: P5C dehydrogenase, P5CR: P5C reductase, ProDH: proline dehydrogenase, PutA: proline utilization A.

Table 1. Computer prediction and experimental localization of ProDH from selected sequences from plants, fungi and animals.

Protein/Accession	Computer prediction	Computer prediction	Computer prediction	Computer prediction	Experimental localization and methods (References)
	Euk-mPLoc2.0 94	SherLoc 95	TargetP 96	WolF PSORT 97	
Plants					
AtProDH1/ P92983	Mit	Mit	Mit	ER, Cyt	Mit (Imaging (23), Biochemical (21, 22))
AtProDH2/ Q6NKX1	Mit	Mit	Mit	Plas, Mit	Mit, Plas (Imaging (23, 24))
NtProDH1/ AAT57674	Cyt	Cyt, Perox	Mit	Mit, Plas	Nd.
NtProDH2/ AAT57675	Cyt	Cyt	Mit	Mit, Plas	Nd.
OsProDH/ NP_001065321.1	Cyt, Mit	Mit, Plas	Sec, Plas	Plas	Nd.
MsProDH/ AAT45085	Cyt, Nuc	Cyt, Per	Plas , Mit	Mit, Plas	Nd.
GmProDH/ AAR86686	Cyt, Nuc	Cyt, Per	Plas, Mit	Mit, Plas	Nd.
PtProDH/ XP_002329313	Cyt	Cyt, Per	Plas, Mit	Cyt	Nd.
PtProDH/ XP_002305291	Cyt	Cyt, Per	Sec	ER, Cyt	Nd.
ZmProDH/NP_001147577	Cyt, Mit	Cyt, Per	Mit	Mit, Nuc	Mit (Biochemical (19, 20, 98))
Fungi					
AnProDH/ CAK96919	Cyt, Nuc	Cyto, Per	Mit	Mit	Nd.
Pi/ XP_002908376	Mit	Mit, Vac	Mit	Mit	Nd.
ScPut1/ AAB82390	Cyt	Cyt	Mit	Mit	Mit (Biochemical (4))
Animals					
RnProDH1/NP_001129250	Mit	Cyt	Mit	Mit	Mit (Biochemical (17, 18))
RnProDH2/NP_001033677	Cyt	Cyt	Mit	Mit	Nd.
HsProDH1/NP_057419	Mit	Mit	Mit	Mit	Nd.
HsProDH2/NP_067055	Cyt	Cyt	Mit	Mit	Nd.

Abbreviation: Cyt, Cytoplasm; ER, Endoplasmic reticulum; Mit, Mitochondria; Nd., not determined to our knowledge; Nuc, Nucleus; Per, Peroxysome; Plas, Plastid; Sec, Secretory pathway; Vac, Vacuole.

vertebrate ProDH isoforms, which are distantly related (45% identities for human ProDH). Vertebrate ProDH1 from one species is more closely related to ProDH1 from another vertebrate species than the second ProDH within the same species. This is consistent with the distinct activities reported in vertebrates where, for instance, two human ProDH isoforms have evolved to display different substrate specificities (10, 11). Human ProDH1 oxidizes proline whereas the substrate of ProDH2 is hydroxyproline, a non proteinogenic amino acid (12). When the catalytic efficiency of PutA was tested, tyrosine540 residue was found to be an important determinant of substrate specificity in the substrate-binding site. Tyrosine540 may prevent hydroxyproline interacting because of steric clash with the 4-hydroxyl group of hydroxyproline. When this tyrosine540 is substituted with alanine or serine, mutated PutA can bind hydroxyproline (11). Interestingly, all the vertebrate ProDH2 that were analyzed in this study possess a serine residue instead of the tyrosine in this position. It is possible that ProDH2 in vertebrates is important for collagen metabolism, collagen being the most abundant protein in mammals (12). In plants, there is higher homology between ProDH1 and ProDH2 in the same species, with a conserved tyrosine residue in analogous position. No assignment of hydroxyproline specificity is found for plant ProDH2 despite the relative high abundance of hydroxyproline-rich proteins.

4. LOCALIZATION OF PRODH

In prokaryotes, ProDH can have two subcellular localizations. In the absence of proline, PutA is localized in the cytosol where it binds *Put* operators to prevent *Put* gene expression. In presence of proline, PutA conformation is modified allowing its association with the membrane and *Put* gene expression (13, 14). Other studies specify that flavin reduction also influences PutA membrane association (15, 16). In eukaryotes, the oxidation of proline occurs in mitochondria. Mitochondrial transit peptides are predicted in the protein sequence of animal ProDHs (Table 1). Both mitochondrial and plastid transit peptides are predicted in plant ProDH protein sequences (Table 1). Proline oxidase activity has been reported in *Rattus norvegicus* liver mitochondria (17, 18) and in *Saccharomyces cerevisiae* mitochondria (4). Recent studies in *S. cerevisiae* have shown that electron transfer mediated by flavoprotein is not required for yeast to utilize proline as a nitrogen source. This result suggests that after proline oxidation, the enzyme may directly reduce ubiquinones in the mitochondrial membrane (6). In *Zea mays* seedlings, ProDH activity has been detected in the inner mitochondrial membrane (19). The active sites of ProDH have been shown to be exposed to the matrix, as proline-dependent ferricyanide reduction is considerably sensitive to antimycin (20). Using immunodetection, ProDH1 protein has been detected in the mitochondrial fraction of *Arabidopsis thaliana* treated with proline (21, 22). Subcellular localization was also investigated with the help of transgenic plants expressing a ProDH1-GFP fusion protein, which was clearly localized in mitochondria (23). Preliminary studies of ProDH2 fused with GFP also suggest a colocalization with mitochondrial (23, 24) as well as plastid markers (24). Conversely, ProDH1 has not been detected in the chloroplastic fractions of *Arabidopsis* cultured cells (21). Further studies are needed to unravel the possible plastid localization of this enzyme in plants.

5. PRODH HAS MULTIPHYSIOLOGICAL ROLES

5.1. Energy production

Proline oxidation is an excellent source of energy. Oxidation of one molecule of proline can yield 30 ATP equivalents (25). Among prokaryotes for example, *Helicobacter pylori* is a bacterium that occurs in the human gastrointestinal tract (26). This bacterium uses proline as a source of energy giving it greater motility to colonize the gastric mucus layer (27). Studies of *H. pylori* have led to the identification of another ProDH called D-amino acid dehydrogenase, which differs from PutA by its ability to oxidize D-proline (28).

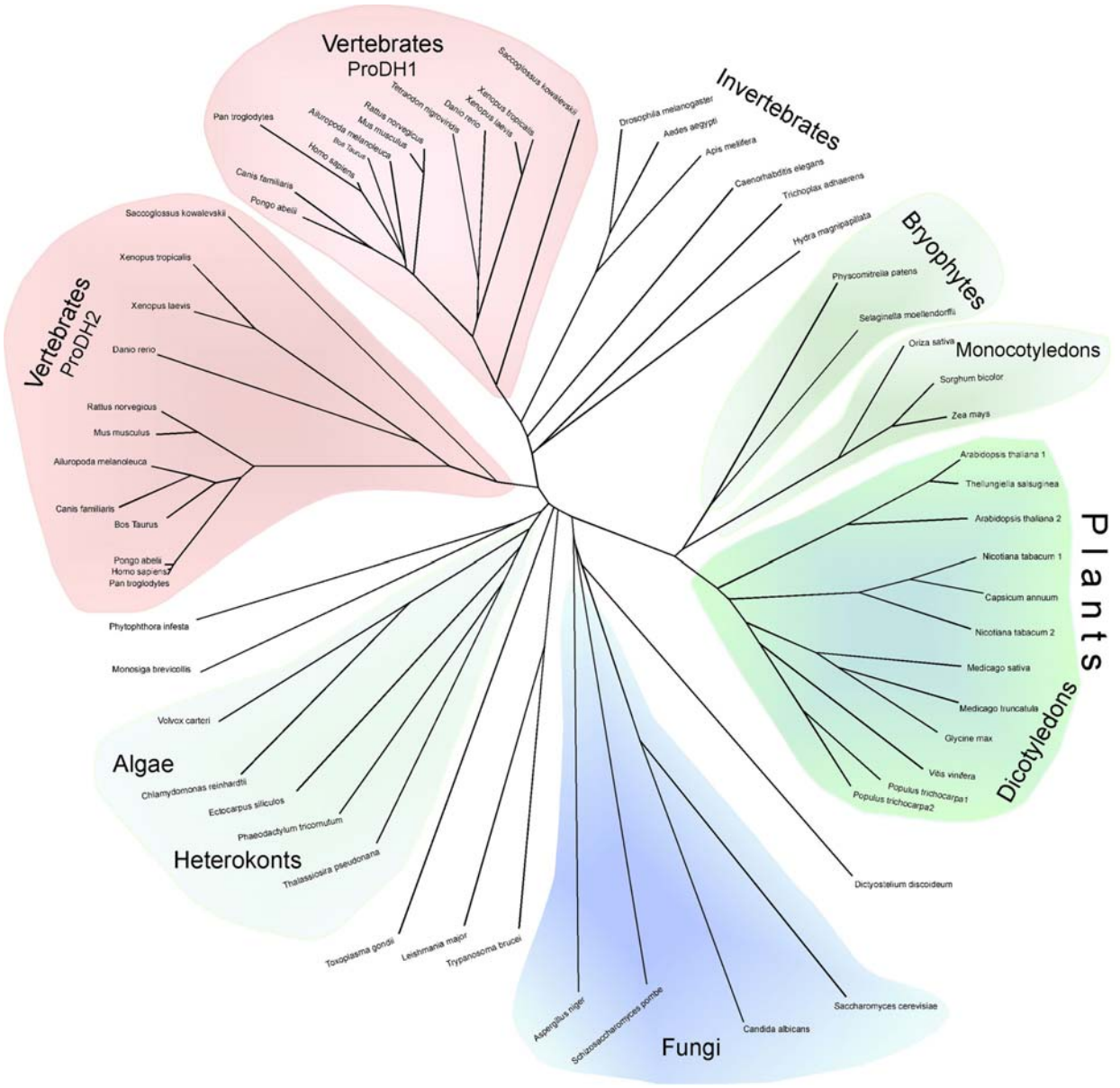

Figure 4. Unrooted phylogenic tree of eukaryotic proline dehydrogenase. Available full length ProDH sequences from eukaryotes were aligned using CLUSTAL W (91) in order to represent unrooted tree using PHYLIP package (92).

In animals, several examples have illustrated the utilization of proline as an energy source. Both *Apis mellifera* (honeybee) and *Glossina morsitans* (tsetse fly) have a high proline content in their hemolymph compared to the other amino acids (29, 30), which is rapidly consumed during flight. After flight, the proline level is low (31) correlating with an increase in ProDH activity in flight muscles (32). This suggests that proline oxidation is necessary during flight. However, other experiments have shown that flight performance by itself is not enhanced by injecting honeybees with proline (33). Possibly proline oxidation generates a rapid energy source just at the beginning of a flight. For long flights, other energy sources produced by the oxidation of carbohydrates may be required. Interestingly, some plants seem to offer proline-rich nectars in order to attract visiting pollinators (34). The role of proline accumulation in pollen is not clear but may be a source of the amino acid for the visiting insects (35). The African trypanosomes (*Trypanosoma brucei*) are of interest as they use different energy sources at different stages of their life cycle (36). Trypanosomes develop into a procyclic form in the Tsetse fly midgut where glucose is scarce. Their procyclics make efficient use of proline as a main energy source. When glucose becomes abundant, trypanosome metabolism switches to preferentially utilize the carbohydrate and this is mediated by the negative control that glucose exerts on ProDH activity (37). In plants, the role of proline as source of energy has not been demonstrated. However in *Arabidopsis*, *ProDH* expression has been detected in lateral and main roots, especially in

central stele and meristem, suggesting that proline catabolism may be necessary to provide energy when roots of rehydrated plants elongate (38).

5.2. ROS production for cell cycle regulation

In animals, many reports show that ProDH1 plays an important role in tumors by acting in apoptotic pathways mediated by the tumor suppressor p53 (for review see 12, 39). Indeed, ProDH1 is one of the 14 genes most strongly induced by p53 (40). ProDH1 has been shown to induce apoptosis through generation of ROS in a variety of cell cancer types (41-43). As mentioned previously, the two human ProDHs display different substrate specificities (44). However in terms of inducing apoptosis, ProDH2 acts in a similar way to ProDH1. An increase in ProDH2 activity has been observed in a wild-type p53 cell line exposed to cytotoxic stress. Conversely, a mutant p53 cell line failed to induce ProDH2 in the same conditions, suggesting that, like human *ProDH1*, human *ProDH2* transcription may require p53 (44). The increase in ProDH2 activity was correlated with an increase in ROS production and with caspase-9 activation. This indicates ProDH2 increases ROS production, which activates caspase leading to apoptosis (44). Thus ProDH1 and ProDH2 are both involved in the p53-induced apoptotic pathway.

Apoptosis or programmed cell death (PCD) is an important component of developmental processes in animals and plants, but no reports link ProDH with this process in plants. However, P5CDH in *Arabidopsis* is known to be involved in PCD by ROS production, suggesting proline degradation may be important in PCD (45). In plants similarly to animal tissues (12), proline cycle controls proline homeostasis, keeping a constant proline to P5C ratio in the cells. This balance is achieved by the uptake of proline, produced in cytosol and perhaps in chloroplast, to the mitochondria where it is oxidized to P5C by ProDH (Figure 2). The latter catalysis provides electrons to the mitochondrial electron transfer chain (mET) (19, 20, 46, 47) and produces P5C, which is either further oxidized to glutamate by P5CDH or exported to the cytosol and reduced back to proline by P5C reductase. When an excess of proline is provided, either during natural recovery from environmental stress or by artificial supply, this cycle is more active and more electrons are delivered to the mET for ATP generation. P5CDH activity compensates the proline-P5C cycling by oxidazing P5C in the mitochondria to glutamate, providing a source of alpha-ketoglutarate to the tricarboxylic acid (TCA) cycle. When P5CDH is mutagenized and its activity is eliminated (45, 48), higher P5C levels promote the operation of the proline cycle and under high cellular proline levels, ProDH delivers an excess of electrons, and also uses oxygen as an electron acceptor leading to ROS formation (49) (Figure 2).

5.3. Adaptive responses to environmental stresses

In animals, the role of proline has been more implicated in response to microenvironmental nutrient stresses (12). In this case, collagen, which is composed of 20-25% proline and hydroxyproline residues, serves as a mobilizable reservoir of amino acids and energy (12). During evolution, plants have developed various adaptive responses to face environmental stresses. Among them, the accumulation of proline, which may participate in the osmotic adjustment of the cell, is a widespread response in many plant species. The accumulation of proline was first reported in wilting *Lolium perenne* (50). Since that first strand of evidence, many studies have demonstrated that proline accumulates in plants in response to abiotic stresses (for review see 51). In *Arabidopsis* for example, free proline level increases more than 10-fold in response to drought or salt stress (52, 53), suggesting that this amino acid may have a physiologically protective role against environmental duress. Conversely, proline content decreases when plants are transferred to recovery conditions (52). Proline content is regulated by the activity of two key enzymes: P5CS implicated in biosynthesis and ProDH involved in its degradation. *P5CS* and *ProDH* genes are inversely regulated during and after osmotic stress in plants. In *Arabidopsis*, transcript levels of *ProDH1,* previously called *Early Responsive to Dehydration 5*, were low during dehydration stress. In contrast, a strong increase in *ProDH1* transcript levels was observed after rehydration (21, 54). *ProDH1* transcript levels correlated with an increase and a decrease in the proline content (21, 52). This shows that ProDH1 is involved in regulating the amount of proline in response to stress. The observation that *ProDH1* antisensing improves tolerance to freezing and salinity in *Arabidopsis* (55) strengthens the hypothesis that ProDH activity interferes with proline accumulation and stress tolerance. However, in transgenic *Nicotiana tabacum* and *Arabidopsis* overexpressing *ProDH*, no change in proline accumulation and stress tolerance was observed (49). Interestingly, when the *ProDH* promoter is fused with the β-Glucuronidase reporter gene (GUS) in *Arabidopsis*, expression is detected in rosette leaves, especially in vascular tissues and hydathodes (38). Correlatively, proline accumulation in the phloem tissue during stress was observed (56), suggesting a role of ProDH in controlling the flux of energy, glutamate and carboxylic acids, all products of proline degradation (Figure 2) in the vascular tissues during rehydration. In *Medicago sativa*, the amount of proline is essentially regulated by ProDH activity during salt stress and recovery. No correlation has been demonstrated between free proline content and *P5CS* transcript levels (57). Furthermore, salt stress did not affect transcript levels of *P5CDH*, the second enzyme of proline catabolism (57), showing that ProDH is particularly important in controlling proline levels. Similar results have been observed in *N. tabacum* in dehydration conditions (58) and in *Brassica napus* during recovery from osmotic stress imposed by PEG treatment (59). In plants, accumulation of proline might act to protect cells against environmental stresses. A high proline content is a physiological trait of some halophyte species that strive in extreme environments. *Thellungiella salsuginea*, previously known as *Thellungiella halophila*, an extremophile plant model, contains proportionally more proline than does *Arabidopsis* when exposed to high salinity and this correlates to improved growth in these conditions (60, 61). The greater accumulation of proline results not only from enhanced biosynthesis but also from a reduction in *ProDH* expression (62). Proline might also be a stress signal

inducing adaptive responses. In recovery conditions, a different signaling mode might then be required for initiating proline pool degradation to produce energy, carbon, nitrogen and redox potential that allows plant growth to restart under favorable conditions (1). Thus, the regulation of *ProDH* expression may be a key in the adaptive response to long-standing environmental variations.

5.4. Development and metabolic disorders

In humans and plants, precise regulation of proline metabolism plays a key role in development. Numerous studies have clearly implicated proline biosynthesis in some developmental processes. For example, mutations in human *P5CS* and *P5CR1* (also known as *PYCR1*) lead to rare diseases with various symptoms of cutis laxa like joint laxity and skin hyperelasticity, progressive neurodegeneration and peripheral neuropathy (63, for review see 64). Some of these symptoms can be explained by the lack of proline, essential for synthesis of brain polypeptides with neuroprotective or neuromodulatory roles (for review see 64). In plants, *p5cs* mutants have developmental defects such as mis-shaped cotyledons and leaves, delayed development of vascular systems and morphologically abnormal epidermal and parenchyma cells and high production of ROS (5, 65).

Equally, modifications in proline catabolism lead to developmental abnormalities in human. ProDH or P5CDH deficiencies may cause hyperprolinemia pathologies leading to various phenotypes like neurological, renal and auditory defects (66). Defects in ProDH may also increase the risk of early schizophrenia (for review see 67). In *Drosophila melanogaster*, the ProDH enzyme is encoded by the *Sluggish-A* gene, which when mutated induces sluggishness in flies (68). *In situ* hybridization revealed that *Sluggish-A* transcripts form a metameric pattern in the developing central nervous system of embryos. This result suggests that ProDH is involved in the formation of neural glutamate pools (68). ProDH has a central function in fetal and placental development in mammals as shown by the synthesis of large amounts of polyamine produced by ProDH in the small intestine and placenta as the piglet fetus grows (69). In plants, the direct role of ProDH in development is not yet well established. Mutants in *ProDH1* gene showed no aberrant phenotype under normal growth conditions (70). However, studies have shown strong expression of *ProDH* transcripts in pollen, stigma, ovules, abscission zones of petals and sepals, and mature seeds with a cut seedcoat (23, 38, 59). In addition, one study has shown a positive correlation between proline content and pollen viability (71). Transgenic RNAi-*ProDH N. tabacum* lines display delayed and asynchronous germination, indicating a key role of ProDH in seed physiology (58). These observations suggest that proline catabolism might be indirectly implicated in development by protecting plant organs during natural dehydration/rehydration cycles and in metabolism by producing energy in developing organs.

6. REGULATION OF PRODH

Because of its involvement in various essential processes, ProDH needs to be precisely regulated probably *via* interactions with other factors. To date, some knowledge has accrued about ProDH protein conformation, function and transcriptional regulation.

6.1. Transcriptional regulation of *ProDH*

In the human *ProDH* promoter, a p53-response element has been identified. This sequence may confer a direct transcriptional regulation of *ProDH* by p53 (72). Recently, *ProDH1* expression was also shown to be enhanced by the peroxisome proliferator activated receptor gamma (PPARgamma) (73). This receptor interacts with the PPAR/retinoid X receptor binding site (PPRE) present in the promoter of the *ProDH* gene allowing activation of its expression. Important regulatory elements in the promoter of the *Arabidopsis ProDH1* gene have been identified. By generating a promoter-GUS reporter fusion, cis-acting elements have been found involved *ProDH1* expression in response to L-Proline and hypoosmotic stress (38). In this promoter, the sequence ACTCAT was found to be necessary and sufficient to induce proline and hypoosmolarity-induced expression of *ProDH1* (74). The same sequence also occurs in the *ProDH* promoter of *M. sativa* (57). Some bZIP transcriptional activators are able to recognize this type of PRE (Proline or hypoosmolarity-Responsive Element) motif and regulate *ProDH* expression. Among them, the bZIP transcription factors AtbZIP2/GBF5 and AtbZIP53 are transiently overexpressed in hypoosmotic conditions, indicative of a role in the regulation of *ProDH1* in the stress response (75). In addition, the partial loss-of-function *atbzip53* mutant shows a significant reduction and delay in *ProDH* expression after hypoosmotic stress (76, 77). Chromatin immunoprecipitation experiments confirmed the physical binding of the bZIP53 transcription factor to the *ProDH* promoter. Another bZIP, bZIP10, can also target the *ProDH* promoter and drive ACTCAT-mediated gene activation. Furthermore a specific synergistic effect is observed when both bZIP factors are together, suggesting bZIP53 and bZIP10 cooperate to regulate hypoosmolarity-induced *ProDH* gene transcription (76).

ProDH2 transcription is also regulated by a bZIP11 transcription factor, which is translationally repressed by sucrose signals in *Arabidopsis* (78). bZIP11 seems to be a direct regulatory link between sucrose-mediated signaling and proline metabolism. However, it is clear whether *ProDH1* is a physiological target of the bZIP11 transcription factor as the two proteins are not co-expressed and bZIP11 does not alter *ProDH1* transcript levels *in planta* (75). Since bZIP transcription factors are involved in regulating *ProDH1* and *ProDH2* expression, we used the Genevestigator server (https://www.genevestigator.com/gv/index.jsp (79)) to predict correlation in expression of bZIP transcription factors and genes involved in proline synthesis and catabolism (Figure 5). Figure 5 summarizes all expression array data sets where *ProDH1* expression is maximal (Figure 5 upper panel) or minimal (Figure 5 lower panel). bZIP1 is the most prominent bZIP transcription factor whose expression correlates with high *ProDH1* transcript levels during exposure to different stresses. Reduction in *ProDH1* expression linearly correlates with increased ABF1 expression during various stresses. Both bZIP1 (80)

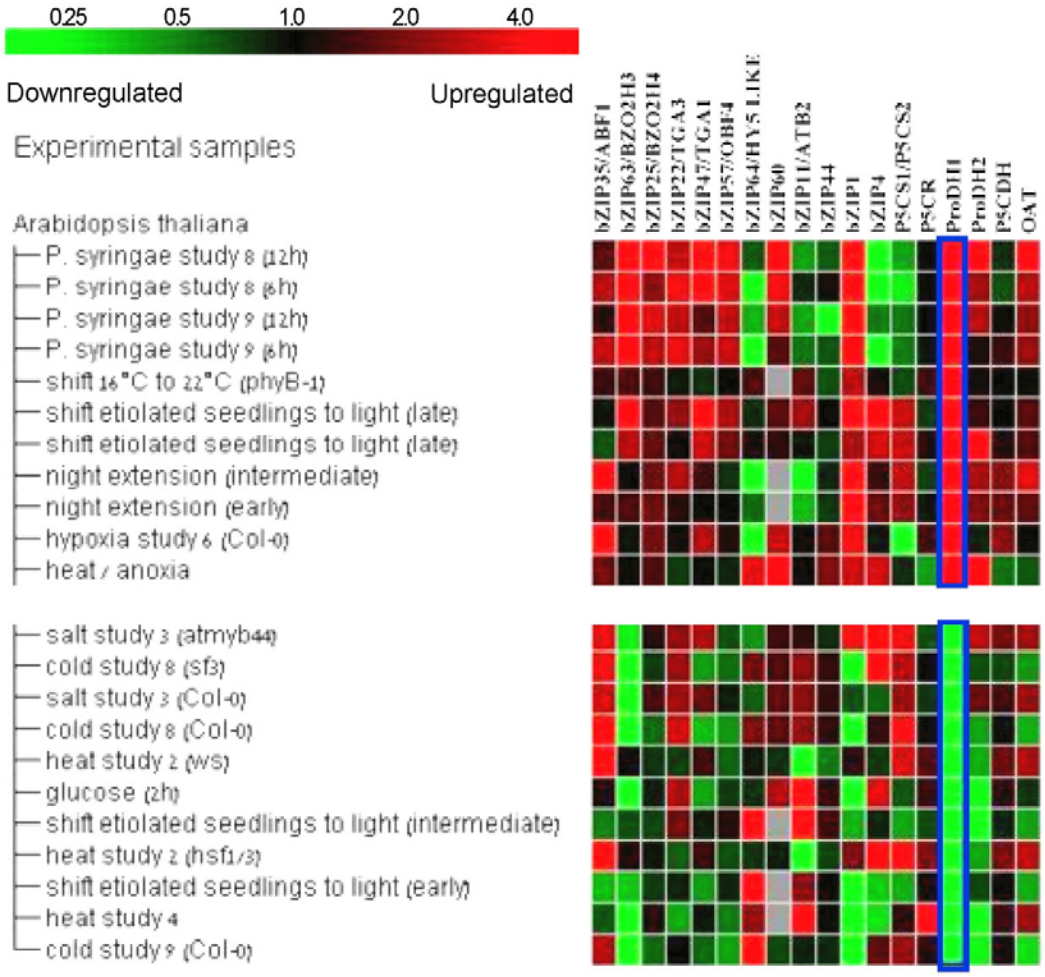

Figure 5. Identification of physiological conditions that support co-expression of *AtProDH1* and *bZIP-TF* transcripts in microarray datasets. Expression patterns of *P5CS1* and *P5CS2*, *P5CR*, *ProDH1*, *ProDH2*, *P5CDH* and *OAT* as well as *bZIP-TF* genes were examined by hierarchical cluster analysis of 4013 microarray datasets deposited in the Genevestigator database (https://www.genevestigator.com 79). The most prominent bZIP transcription factors (93) whose expression correlates with either high or low transcript levels of ProDH1 under different stresses are displayed. The blue frame indicates the expression of ProDH1. Red and green indicate maximal and minimal expression, respectively. All the values are calculated relative to the expression levels obtained in non-treated controls.

and ABF1 (our unpublished results) are regulated by sugar. Hence it seems that several bZIP transcription factors redundantly function in controlling *ProDH* expression in plants. Figure 5 also shows that biotic stress such as inoculation with the bacterial pathogen *Pseudomonas syringae* induces high expression of *ProDH1* and reduces expression of *P5CS* genes, indicating that perhaps bacterial pathogen attack, similarly to lack of sugar (displayed by the extended-night data sets in Figure 5) shifts proline metabolism towards energy providing mode. Since it increases mostly *ProDH* expression and not that of *P5CDH*, it might be assumed that under these circumstances, proline cycle and ROS generation are enhanced in mitochondria.

Some studies have shown that *ProDH* is downregulated. The accumulation of proline during stress conditions such as drought or salinity does not lead to an increase in *ProDH* transcripts. Moreover, *ProDH* transcript levels induced by exogenous application of proline can be lowered by salt treatment (52). This suggests an increase in the turnover of *ProDH* transcripts and/or the activation of a transcriptional repressor in response to stress (52, 54). Transcriptional regulation of *ProDH* appears to be independent of proline in stress conditions (81). Additional studies are required to further unravel the signaling cascades that initiate *ProDH* downregulation in various stresses and its upregulation during the recovery process. In plants, light perception also plays a role in the regulation of *ProDH1* gene expression. In salt stress conditions, proline levels increase in the light and decrease in the dark (82, 83). This effect is correlated respectively with inactivation of *ProDH1* expression in continuous light and activation of transcription in darkness. However the *Arabidopsis ProDH1* promoter does not contain any known motifs for light regulation (83).

6.2. Post-transcriptional regulation

Human *ProDH1* transcript and protein levels are much lower in renal carcinoma cell lines compared to normal cells (84). In these renal cancer cells, expression of several miRNA is higher relative to normal cells. Among them, mir23b is able to significantly reduce ProDH1 protein expression when it is ectopically expressed (84). Experiments confirmed direct targeting of *ProDH1* mRNA by mir23b and a negative correlation between mir23b and ProDH1 protein in tumor compared to normal tissues (84). The percentage of apoptotic cells in renal cancer cell lines increased when they were transfected with a mir23b antagonist associated with an increase in *ProDH* expression. These cells also show an increase in apoptosis and ROS content, consistent with the cancer suppressor role of ProDH mediated by ROS (84). At present, no report describes the post-transcriptional control of *ProDH* transcript levels in plants. However, recently P5CDH was found to be downregulated by a natural cis-antisense transcript called *Similar to RCD One 5*. This regulation is thought to control ROS production and salt stress responses (85).

6.3. Post-translational regulation of ProDH

There are very few studies reporting regulation of ProDH by post-translational modification (PTM). In bacteria, the ProDH PutA was shown to be regulated by phosphorylation (86). This PTM is transient and might modify the activity of the enzyme, its subcellular localization or how it interacts with partners. PutA also autophosphorylates on serine, threonine and tyrosine residues (86), so it might have a dual-specificity kinase activity. The C-terminal part of PutA is similar to some subdomains conserved among protein kinases such as subdomains I and VI (87). Phosphorylation of PutA also occurs *in vivo* as specific antibodies raised against phosphoserine, phosphothreonine or phosphotyrosine are able to detect PutA from crude cell extracts analyzed by western blot (86). Phosphorylation was suggested to regulate the interaction of ProDH with membranes or DNA.

To date, in eukaryotes in general and in plants in particular, no PTM of ProDH has been reported. Nonetheless, it has been postulated that ProDH is regulated by PTM in *A. thaliana*. It has been shown that after 24 h of hyperosmotic stress treatment, proline accumulates and ProDH protein levels are high in *A. thaliana* (88, 89). Thus, *Arabidopsis* ProDH must somehow be negatively regulated by PTM to control proline degradation. This regulation might well be achieved by phosphorylation as comparison of prokaryotic and eukaryotic ProDHs reveals that several serine, threonine and tyrosine residues are conserved across kingdoms (data not shown).

7. CONCLUSIONS AND PROSPECTS

ProDH is found in many organisms from prokaryotes to eukaryotes. Despite its ubiquity, this enzyme displays various structures and isoforms giving rise to diverse cellular and physiological roles. For instance, bacterial ProDH enzymes show different structures containing ProDH enzymatic domain associated with P5CDH activity and transcription factor domain. In contrast, the eukaryotic enzyme displays a single ProDH domain that is very conserved across kingdoms. Vertebrates have evolved two isoforms, ProDH1 and ProDH2, that have distinct functions suggesting an adaptive evolution for hydroxyproline oxidation from collagen (12). Interestingly, only dicotyledonous plants seem to have two ProDH isoforms. Plant ProDHs possesses as vertebrate ProDH1 a conserved tyrosine residue in the catalytic domain, which was proposed to be an important determinant of substrate specificity (11). The presence of this tyrosine residue suggests that ProDH2 cannot hydrolyze this substrate although hydroxyproline-rich glycopeptides are abundant in cell wall (65, 90). Plants may have evolved another enzyme to hydrolyze hydroxyproline.

While the multiple biological roles of proline and its biosynthetic pathway are now known (1), proline catabolism is just starting to be explored. Proline degradation was first considered as a way to restore the amount of proline to basal levels and to cancel the effects of its accumulation. It is only recently that proline degradation has been considered as a way to produce other compounds from the proline pool such as superoxides, reduced cofactors and ATP. In this review, we have reported that proline oxidation is essential for some cellular processes such as apoptosis mediated by ROS in animal cells or plant adaptive response to salt stress. These different roles of ProDHs require a high degree of regulation. Establishing the structure of the eukaryotic ProDHs, similarly to the achieved structure of the prokaryotic enzyme, will help to better understand how these enzymes are regulated. Although ProDH is clearly a key enzyme by dint of its activity and diverse roles, there is much to learn about how ProDH is transcriptionally and post-transcriptionally regulated to allow cell homeostasis.

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9. REFERENCES

1. L. Szabados and A. Savouré: Proline: a multifunctional amino acid. *Trends Plant Sci*, 15(2), 89-97 (2010)

2. J. J. Tanner: Structural biology of proline catabolism. *Amino Acids*, 35(4), 719-30 (2008)

3. S. Wanduragala, N. Sanyal, X. Liang and D. F. Becker: Purification and characterization of Put1p from *Saccharomyces cerevisiae*. *Arch Biochem Biophys*, 498(2), 136-42 (2010)

4. S. Lehmann, D. Funck, L. Szabados and D. Rentsch: Proline metabolism and transport in plant development. *Amino Acids*, 39(4), 949-62 (2010)

5. S. S. Wang and M. C. Brandriss: Proline utilization in *Saccharomyces cerevisiae*: sequence, regulation, and mitochondrial localization of the *PUT1* gene product. *Mol Cell Biol*, 7(12), 4431-40 (1987)

6. G. Szekely, E. Abraham, A. Cseplo, G. Rigo, L. Zsigmond, J. Csiszar, F. Ayaydin, N. Strizhov, J. Jasik, E. Schmelzer, C. Koncz and L. Szabados: Duplicated *P5CS* genes of Arabidopsis play distinct roles in stress regulation and developmental control of proline biosynthesis. *Plant J*, 53(1), 11-28 (2008)

7. H. Tsuge, R. Kawakami, H. Sakuraba, H. Ago, M. Miyano, K. Aki, N. Katunuma and T. Ohshima: Crystal structure of a novel FAD-, FMN-, and ATP-containing L-proline dehydrogenase complex from *Pyrococcus horikoshii*. *J Biol Chem*, 280(35), 31045-9 (2005)

8. Y. H. Lee, S. Nadaraia, D. Gu, D. F. Becker and J. J. Tanner: Structure of the proline dehydrogenase domain of the multifunctional PutA flavoprotein. *Nat Struct Biol*, 10(2), 109-14 (2003)

9. T. A. White, N. Krishnan, D. F. Becker and J. J. Tanner: Structure and kinetics of monofunctional proline dehydrogenase from *Thermus thermophilus*. *J Biol Chem*, 282(19), 14316-27 (2007)

10. S. J. Downing, J. M. Phang, E. M. Kowaloff, D. Valle and R. J. Smith: Proline oxidase in cultured mammalian cells. *J Cell Physiol*, 91(3), 369-76 (1977)

11. E. L. Ostrander, J. D. Larson, J. P. Schuermann and J. J. Tanner: A conserved active site tyrosine residue of proline dehydrogenase helps enforce the preference for proline over hydroxyproline as the substrate. *Biochemistry*, 48(5), 951-9 (2009)

12. J. M. Phang, W. Liu and O. Zabirnyk: Proline metabolism and microenvironmental stress. *Annu Rev Nutr*, 30, 441-63 (2010)

13. P. Ostrovsky de Spicer and S. Maloy: PutA protein, a membrane-associated flavin dehydrogenase, acts as a redox-dependent transcriptional regulator. *Proc Natl Acad Sci U S A*, 90(9), 4295-8 (1993)

14. P. Ostrovsky de Spicer, K. O'Brien and S. Maloy: Regulation of proline utilization in *Salmonella typhimurium*: a membrane-associated dehydrogenase binds DNA *in vitro*. *J Bacteriol*, 173(1), 211-9 (1991)

15. E. D. Brown and J. M. Wood: Conformational change and membrane association of the PutA protein are coincident with reduction of its FAD cofactor by proline. *J Biol Chem*, 268(12), 8972-9 (1993)

16. M. W. Surber and S. Maloy: Regulation of flavin dehydrogenase compartmentalization: requirements for PutA-membrane association in *Salmonella typhimurium*. *Biochim Biophys Acta*, 1421(1), 5-18 (1999)

17. A. B. Johnson and H. J. Strecker: The interconversion of glutamic acid and proline. IV. The oxidation of proline by rat liver mitochondria. *J Biol Chem*, 237, 1876-82 (1962)

18. E. M. Kowaloff, J. M. Phang, A. S. Granger and S. J. Downing: Regulation of proline oxidase activity by lactate. *Proc Natl Acad Sci U S A*, 74(12), 5368-71 (1977)

19. T. E. Elthon and C. R. Stewart: Submitochondrial location and electron transport characteristics of enzymes involved in proline oxidation. *Plant Physiol*, 67(4), 780-4 (1981)

20. T. E. Elthon and C. R. Stewart: Proline Oxidation in Corn Mitochondria : Involvement of NAD, Relationship to Ornithine Metabolism, and Sidedness on the Inner Membrane. *Plant Physiol*, 70(2), 567-72 (1982)

21. T. Kiyosue, Y. Yoshiba, K. Yamaguchi-Shinozaki and K. Shinozaki: A nuclear gene encoding mitochondrial proline dehydrogenase, an enzyme involved in proline metabolism, is upregulated by proline but downregulated by dehydration in Arabidopsis. *Plant Cell*, 8(8), 1323-35 (1996)

22. S. Mani, B. Van De Cotte, M. Van Montagu and N. Verbruggen: Altered levels of proline dehydrogenase cause hypersensitivity to proline and its analogs in Arabidopsis. *Plant Physiol*, 128(1), 73-83 (2002)

23. D. Funck, S. Eckard and G. Muller: Non-redundant functions of two proline dehydrogenase isoforms in Arabidopsis. *BMC Plant Biol*, 10, 70 (2010)

24. O. Van Aken, B. Zhang, C. Carrie, V. Uggalla, E. Paynter, E. Giraud and J. Whelan: Defining the Mitochondrial Stress Response in *Arabidopsis thaliana*. *Mol Plant*, 2(6), 1310-24 (2009)

25. D. E. Atkinson: Cellular energy metabolism and its regulation. *NY Acadamic Press* (1977)

26. D. J. Kelly: The physiology and metabolism of the human gastric pathogen *Helicobacter pylori*. *Adv Microb Physiol*, 40, 137-89 (1998)

27. K. Nakajima, S. Inatsu, T. Mizote, Y. Nagata, K. Aoyama, Y. Fukuda and K. Nagata: Possible involvement of *Put A* gene in *Helicobacter pylori* colonization in the stomach and motility. *Biomed Res*, 29(1), 9-18 (2008)

28. M. Tanigawa, T. Shinohara, M. Saito, K. Nishimura, Y. Hasegawa, S. Wakabayashi, M. Ishizuka and Y. Nagata: D-Amino acid dehydrogenase from *Helicobacter pylori* NCTC 11637. *Amino Acids*, 38(1), 247-55 (2010)

29. R. A. Balogun: Studies on the amino acids of the tsetse fly, *Glossina morsitans*, maintained on *in vitro* and *in vivo* feeding systems. *Comp Biochem Physiol A Comp Physiol*, 49(2A), 215-22 (1974)

30. B. Berger, K. Crailsheim and B. Leonhard: Proline, leucine and phenylalanine metabolism in adult honeybee drones (*Apis mellifica carnica Pollm*). *Insect Biochem Mol Biol* 27, 587–93 (1997)

31. R. J. Barker and Y. Lehner: Free amino acids in the thoraces of flown honey bees, *Apis melifera L. Comp Biochem Physiol*, 43B, 163-9 (1972)

32. B. Crabtree and E. A. Newsholme: The activities of proline dehydrogenase, glutamate dehydrogenase, aspartate-oxoglutarate aminotransferase and alanine-oxoglutarate aminotransferase in some insect flight muscles. *Biochem J*, 117(5), 1019-21 (1970)

33. S. Micheu, K. Crailsheim and B. Leonhard: Importance of proline and other amino acids during honeybee flight--*Apis mellifera carnica* POLLMANN). *Amino Acids*, 18(2), 157-75 (2000)

34. C. Carter, S. Shafir, L. Yehonatan, R. G. Palmer and R. Thornburg: A novel role for proline in plant floral nectars. *Naturwissenschaften*, 93(2), 72-9 (2006)

35. D. M. O'Brien, C. L. Boggs and M. L. Fogel: Pollen feeding in the butterfly *Heliconius charitonia*: isotopic evidence for essential amino acid transfer from pollen to eggs. *Proc Biol Sci*, 270(1533), 2631-6 (2003)

36. V. Coustou, M. Biran, M. Breton, F. Guegan, L. Riviere, N. Plazolles, D. Nolan, M. P. Barrett, J. M. Franconi and F. Bringaud: Glucose-induced remodeling of intermediary and energy metabolism in procyclic *Trypanosoma brucei*. *J Biol Chem*, 283(24), 16342-54 (2008)

37. N. Lamour, L. Riviere, V. Coustou, G. H. Coombs, M. P. Barrett and F. Bringaud: Proline metabolism in procyclic *Trypanosoma brucei* is down-regulated in the presence of glucose. *J Biol Chem*, 280(12), 11902-10 (2005)

38. K. Nakashima, R. Satoh, T. Kiyosue, K. Yamaguchi-Shinozaki and K. Shinozaki: A gene encoding proline dehydrogenase is not only induced by proline and hypoosmolarity, but is also developmentally regulated in the reproductive organs of Arabidopsis. *Plant Physiol*, 118(4), 1233-41 (1998)

39. M. Farnebo, V. J. Bykov and K. G. Wiman: The p53 tumor suppressor: a master regulator of diverse cellular processes and therapeutic target in cancer. *Biochem Biophys Res Commun*, 396(1), 85-9 (2010)

40. K. Polyak, Y. Xia, J. L. Zweier, K. W. Kinzler and B. Vogelstein: A model for p53-induced apoptosis. *Nature*, 389(6648), 300-5 (1997)

41. S. P. Donald, X. Y. Sun, C. A. Hu, J. Yu, J. M. Mei, D. Valle and J. M. Phang: Proline oxidase, encoded by p53-induced gene-6, catalyzes the generation of proline-dependent reactive oxygen species. *Cancer Res*, 61(5), 1810-5 (2001)

42. Y. Liu, G. L. Borchert, S. P. Donald, A. Surazynski, C. A. Hu, C. J. Weydert, L. W. Oberley and J. M. Phang: MnSOD inhibits proline oxidase-induced apoptosis in colorectal cancer cells. *Carcinogenesis*, 26(8), 1335-42 (2005)

43. Y. Liu, G. L. Borchert, S. P. Donald, B. A. Diwan, M. Anver and J. M. Phang: Proline oxidase functions as a mitochondrial tumor suppressor in human cancers. *Cancer Res*, 69(16), 6414-22 (2009)

44. S. K. Cooper, J. Pandhare, S. P. Donald and J. M. Phang: A novel function for hydroxyproline oxidase in apoptosis through generation of reactive oxygen species. *J Biol Chem*, 283(16), 10485-92 (2008)

45. K. Deuschle, D. Funck, G. Forlani, H. Stransky, A. Biehl, D. Leister, E. van der Graaff, R. Kunze and W. B. Frommer: The role of [Delta]1-pyrroline-5-carboxylate dehydrogenase in proline degradation. *Plant Cell*, 16(12), 3413-25 (2004)

46. S. F. Boggess and D. E. Koeppe: Oxidation of proline by plant mitochondria. *Plant Physiol*, 62(1), 22-5 (1978)

47. G. D. Sells and D. E. Koeppe: Oxidation of proline by mitochondria isolated from water-stressed maize shoots. *Plant Physiol*, 68(5), 1058-63 (1981)

48. K. Deuschle, D. Funck, H. Hellmann, K. Daschner, S. Binder and W. B. Frommer: A nuclear gene encoding mitochondrial Delta-pyrroline-5-carboxylate dehydrogenase and its potential role in protection from proline toxicity. *Plant J*, 27(4), 345-56 (2001)

49. G. Miller, A. Honig, H. Stein, N. Suzuki, R. Mittler and A. Zilberstein: Unraveling delta1-pyrroline-5-carboxylate-proline cycle in plants by uncoupled expression of proline oxidation enzymes. *J Biol Chem*, 284(39), 26482-92 (2009)

50. A. R. Kemble and H. T. Macpherson: Liberation of amino acids in perennial rye grass during wilting. *Biochem J*, 58(1), 46-9 (1954)

51. A. J. Delauney and D. P. Verma: Proline biosynthesis and osmoregulation in plants. *Plant J*, 4(2), 215-23 (1993)

52. Z. Peng, Q. Lu and D. P. Verma: Reciprocal regulation of delta 1-pyrroline-5-carboxylate synthetase and proline dehydrogenase genes controls proline levels during and after osmotic stress in plants. *Mol Gen Genet*, 253(3), 334-41 (1996)

53. A. Savouré, S. Jaoua, X. J. Hua, W. Ardiles, M. Montagu and N. Verbruggen: Isolation, characterization, and chromosomal location of a gene encoding the Δ1-pyrroline-5-carboxylate synthetase in *Arabidopsis thaliana*. *FEBS Lett*, 372, 13–9 (1995)

54. N. Verbruggen, X. J. Hua, M. May and M. Van Montagu: Environmental and developmental signals modulate proline homeostasis: evidence for a negative

transcriptional regulator. *Proc Natl Acad Sci U S A*, 93(16), 8787-91 (1996)

55. T. Nanjo, M. Kobayashi, Y. Yoshiba, Y. Kakubari, K. Yamaguchi-Shinozaki and K. Shinozaki: Antisense suppression of proline degradation improves tolerance to freezing and salinity in *Arabidopsis thaliana*. *FEBS Lett*, 461(3), 205-10 (1999)

56. C. Girousse, R. Bournoville and J. L. Bonnemain: Water Deficit-Induced Changes in Concentrations in Proline and Some Other Amino Acids in the Phloem Sap of Alfalfa. *Plant Physiol*, 111(1), 109-113 (1996)

57. G. Miller, H. Stein, A. Honig, Y. Kapulnik and A. Zilberstein: Responsive modes of *Medicago sativa* proline dehydrogenase genes during salt stress and recovery dictate free proline accumulation. *Planta*, 222(1), 70-9 (2005)

58. A. Ribarits, A. Abdullaev, A. Tashpulatov, A. Richter, E. Heberle-Bors and A. Touraev: Two tobacco proline dehydrogenases are differentially regulated and play a role in early plant development. *Planta*, 225(5), 1313-24 (2007)

59. X. Xue, A. Liu and X. Hua: Proline accumulation and transcriptional regulation of proline biosynthesis and degradation in *Brassica napus*. *BMB Rep*, 42(1), 28-34 (2009)

60. M. A. Ghars, E. Parre, A. Debez, M. Bordenave, L. Richard, L. Leport, A. Bouchereau, A. Savouré and C. Abdelly: Comparative salt tolerance analysis between Arabidopsis thaliana and Thellungiella halophila, with special emphasis on K(+)/Na(+) selectivity and proline accumulation. *J Plant Physiol*, 165(6), 588-99 (2008)

61. E. P. Murakeozy, Z. Nagy, C. Duhaze, A. Bouchereau and Z. Tuba: Seasonal changes in the levels of compatible osmolytes in three halophytic species of inland saline vegetation in Hungary. *J Plant Physiol*, 160(4), 395-401 (2003)

62. S. Kant, P. Kant, E. Raveh and S. Barak: Evidence that differential gene expression between the halophyte, *Thellungiella halophila*, and *Arabidopsis thaliana* is responsible for higher levels of the compatible osmolyte proline and tight control of Na+ uptake in *T. halophila*. *Plant Cell Environ*, 29(7), 1220-34 (2006)

63. B. Reversade, N. Escande-Beillard, A. Dimopoulou, B. Fischer, S. C. Chng, Y. Li, M. Shboul, P. Y. Tham, H. Kayserili, L. Al-Gazali, M. Shahwan, F. Brancati, H. Lee, B. D. O'Connor, M. Schmidt-von Kegler, B. Merriman, S. F. Nelson, A. Masri, F. Alkazaleh, D. Guerra, P. Ferrari, A. Nanda, A. Rajab, D. Markie, M. Gray, J. Nelson, A. Grix, A. Sommer, R. Savarirayan, A. R. Janecke, E. Steichen, D. Sillence, I. Hausser, B. Budde, G. Nurnberg, P. Nurnberg, P. Seemann, D. Kunkel, G. Zambruno, B. Dallapiccola, M. Schuelke, S. Robertson, H. Hamamy, B. Wollnik, L. Van Maldergem, S. Mundlos and U. Kornak: Mutations in *PYCR1* cause cutis laxa with progeroid features. *Nat Genet*, 41(9), 1016-21 (2009)

64. I. Perez-Arellano, F. Carmona-Alvarez, A. I. Martinez, J. Rodriguez-Diaz and J. Cervera: Pyrroline-5-carboxylate synthase and proline biosynthesis: from osmotolerance to rare metabolic disease. *Protein Sci*, 19(3), 372-82 (2010)

65. T. Nanjo, M. Kobayashi, Y. Yoshiba, Y. Sanada, K. Wada, H. Tsukaya, Y. Kakubari, K. Yamaguchi-Shinozaki and K. Shinozaki: Biological functions of proline in morphogenesis and osmotolerance revealed in antisense transgenic *Arabidopsis thaliana*. *Plant J*, 18(2), 185-93 (1999)

66. H. Mitsubuchi, K. Nakamura, S. Matsumoto and F. Endo: Inborn errors of proline metabolism. *J Nutr*, 138(10), 2016S-2020S (2008)

67. C. M. O'Tuathaigh, D. Babovic, G. O'Meara, J. J. Clifford, D. T. Croke and J. L. Waddington: Susceptibility genes for schizophrenia: characterisation of mutant mouse models at the level of phenotypic behaviour. *Neurosci Biobehav Rev*, 31(1), 60-78 (2007)

68. D. C. Hayward, S. J. Delaney, H. D. Campbell, A. Ghysen, S. Benzer, A. B. Kasprzak, J. N. Cotsell, I. G. Young and G. L. Miklos: The sluggish-A gene of *Drosophila melanogaster* is expressed in the nervous system and encodes proline oxidase, a mitochondrial enzyme involved in glutamate biosynthesis. *Proc Natl Acad Sci U S A*, 90(7), 2979-83 (1993)

69. G. Wu, N. E. Flynn, D. A. Knabe and L. A. Jaeger: A cortisol surge mediates the enhanced polyamine synthesis in porcine enterocytes during weaning. *Am J Physiol Regul Integr Comp Physiol*, 279(2), R554-9 (2000)

70. T. Nanjo, M. Fujita, M. Seki, T. Kato, S. Tabata and K. Shinozaki: Toxicity of free proline revealed in an arabidopsis T-DNA-tagged mutant deficient in proline dehydrogenase. *Plant Cell Physiol*, 44(5), 541-8 (2003)

71. A. R. Lansac, C. Y. Sullivan and B. E. Johnson: Accumulation of free proline in sorghum (*Sorghum bicolor*) pollen. *Can J Bot* 74, 40-5 (1996)

72. S. A. Maxwell and G. J. Kochevar: Identification of a p53-response element in the promoter of the proline oxidase gene. *Biochem Biophys Res Commun*, 369(2), 308-13 (2008)

73. J. Pandhare, S. K. Cooper and J. M. Phang: Proline oxidase, a proapoptotic gene, is induced by troglitazone: evidence for both peroxisome proliferator-activated receptor gamma-dependent and -independent mechanisms. *J Biol Chem*, 281(4), 2044-52 (2006)

74. R. Satoh, K. Nakashima, M. Seki, K. Shinozaki and K. Yamaguchi-Shinozaki: ACTCAT, a novel cis-acting element for proline- and hypoosmolarity-responsive expression of the *ProDH* gene encoding proline dehydrogenase in Arabidopsis. *Plant Physiol*, 130(2), 709-19 (2002)

75. R. Satoh, Y. Fujita, K. Nakashima, K. Shinozaki and K. Yamaguchi-Shinozaki: A novel subgroup of bZIP proteins functions as transcriptional activators in hypoosmolarity-responsive expression of the *ProDH* gene in Arabidopsis. *Plant Cell Physiol*, 45(3), 309-17 (2004)

76. F. Weltmeier, A. Ehlert, C. S. Mayer, K. Dietrich, X. Wang, K. Schutze, R. Alonso, K. Harter, J. Vicente-Carbajosa and W. Droge-Laser: Combinatorial control of Arabidopsis proline dehydrogenase transcription by specific heterodimerisation of bZIP transcription factors. *EMBO J*, 25(13), 3133-43 (2006)

77. K. Dietrich, F. Weltmeier, A. Ehlert, C. Weiste, M. Stahl, K. Harter and W. Droge-Laser: Heterodimers of the Arabidopsis Transcription Factors bZIP1 and bZIP53 Reprogram Amino Acid Metabolism during Low Energy Stress. *Plant Cell* (2011)

78. J. Hanson, M. Hanssen, A. Wiese, M. M. Hendriks and S. Smeekens: The sucrose regulated transcription factor bZIP11 affects amino acid metabolism by regulating the expression of *ASPARAGINE SYNTHETASE1* and *PROLINE DEHYDROGENASE2*. *Plant J*, 53(6), 935-49 (2008)

79. T. Hruz, O. Laule, G. Szabo, F. Wessendorp, S. Bleuler, L. Oertle, P. Widmayer, W. Gruissem and P. Zimmermann: Genevestigator v3: a reference expression database for the meta-analysis of transcriptomes. *Adv Bioinformatics*, 2008, 420747 (2008)

80. S. G. Kang, J. Price, P. C. Lin, J. C. Hong and J. C. Jang: The arabidopsis bZIP1 transcription factor is involved in sugar signaling, protein networking, and DNA binding. *Mol Plant*, 3(2), 361-73 (2010)

81. S. Sharma and P. E. Verslues: Mechanisms independent of abscisic acid (ABA) or proline feedback have a predominant role in transcriptional regulation of proline metabolism during low water potential and stress recovery. *Plant Cell Environ*, 33(11), 1838-51 (2010)

82. E. Abraham, G. Rigo, G. Szekely, R. Nagy, C. Koncz and L. Szabados: Light-dependent induction of proline biosynthesis by abscisic acid and salt stress is inhibited by brassinosteroid in Arabidopsis. *Plant Mol Biol*, 51(3), 363-72 (2003)

83. F. Hayashi, T. Ichino, M. Osanai and K. Wada: Oscillation and regulation of proline content by *P5CS* and *ProDH* gene expressions in the light/dark cycles in *Arabidopsis thaliana* L. *Plant Cell Physiol*, 41(10), 1096-101 (2000)

84. W. Liu, O. Zabirnyk, H. Wang, Y. H. Shiao, M. L. Nickerson, S. Khalil, L. M. Anderson, A. O. Perantoni and J. M. Phang: miR-23b targets proline oxidase, a novel tumor suppressor protein in renal cancer. *Oncogene*, 29(35), 4914-24 (2010)

85. O. Borsani, J. Zhu, P. E. Verslues, R. Sunkar and J. K. Zhu: Endogenous siRNAs derived from a pair of natural cis-antisense transcripts regulate salt tolerance in Arabidopsis. *Cell*, 123(7), 1279-91 (2005)

86. P. C. Ostrovsky and S. Maloy: Protein phosphorylation on serine, threonine, and tyrosine residues modulates membrane-protein interactions and transcriptional regulation in Salmonella typhimurium. *Genes Dev*, 9(16), 2034-41 (1995)

87. S. K. Hanks and T. Hunter: Protein kinases 6. The eukaryotic protein kinase superfamily: kinase (catalytic) domain structure and classification. *FASEB J*, 9(8), 576-96 (1995)

88. L. Thiery, A. S. Leprince, D. Lefebvre, M. A. Ghars, E. Debarbieux and A. Savouré: Phospholipase D is a negative regulator of proline biosynthesis in *Arabidopsis thaliana*. *J Biol Chem*, 279(15), 14812-8 (2004)

89. E. Parre, M. A. Ghars, A. S. Leprince, L. Thiery, D. Lefebvre, M. Bordenave, L. Richard, C. Mazars, C. Abdelly and A. Savouré: Calcium signaling via phospholipase C is essential for proline accumulation upon ionic but not nonionic hyperosmotic stresses in Arabidopsis. *Plant Physiol*, 144(1), 503-12 (2007)

90. J. Narvaez-Vasquez, M. L. Orozco-Cardenas and C. A. Ryan: Systemic wound signaling in tomato leaves is cooperatively regulated by systemin and hydroxyproline-rich glycopeptide signals. *Plant Mol Biol*, 65(6), 711-8 (2007)

91. J. D. Thompson, D. G. Higgins and T. J. Gibson: CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res*, 22(22), 4673-80 (1994)

92. J. Felsenstein: Phylogenies from molecular sequences: inference and reliability. *Annu Rev Genet*, 22, 521-65 (1988)

93. M. Jakoby, B. Weisshaar, W. Droge-Laser, J. Vicente-Carbajosa, J. Tiedemann, T. Kroj and F. Parcy: bZIP transcription factors in *Arabidopsis*. *Trends Plant Sci*, 7(3), 106-11 (2002)

94. K. C. Chou and H. B. Shen: A new method for predicting the subcellular localization of eukaryotic proteins with both single and multiple sites: Euk-mPLoc 2.0. *PLoS One*, 5(4), e9931 (2010)

95. H. Shatkay, A. Hoglund, S. Brady, T. Blum, P. Donnes and O. Kohlbacher: SherLoc: high-accuracy prediction of protein subcellular localization by integrating text and protein sequence data. *Bioinformatics*, 23(11), 1410-7 (2007)

96. O. Emanuelsson, H. Nielsen, S. Brunak and G. von Heijne: Predicting subcellular localization of proteins based on their N-terminal amino acid sequence. *J Mol Biol*, 300(4), 1005-16 (2000)

97. P. Horton, K. J. Park, T. Obayashi, N. Fujita, H. Harada, C. J. Adams-Collier and K. Nakai: WoLF PSORT: protein localization predictor. *Nucleic Acids Res*, 35(Web Server issue), W585-7 (2007)

98. P. J. Rayapati and C. R. Stewart: Solubilization of a Proline Dehydrogenase from Maize (Zea mays L.) Mitochondria. *Plant Physiol*, 95(3), 787-91 (1991)

Abbreviations: ATP: adenosine triphosphate, bZIP: basic leucine zipper, FAD: flavin adenine dinucleotide, GFP: green fluorescent protein, GSA: glutamate semialdehyde, GUS: β-glucuronidase, mET: mitochondrial electron transfer chain, NAD: nicotinamide adenine dinucleotide, OH-POX: hydroxyproline-specific proline oxidase, P5C: delta-pyrroline-5-carboxylate, P5CDH: delta-pyrroline-5-carboxylate dehydrogenase, P5CR: delta-pyrroline-5-carboxylate reductase, P5CS: delta-pyrroline-5-carboxylate dehydrogenase synthetase, POX: proline oxidase, PRODH: proline dehydrogenase, PTM: post-translational modification, PutA: Proline utilization A, ROS: reactive oxygen species, TCA: tricarboxylic acid.

Key Words: Proline dehydrogenase, Proline oxidase, Proline, Hydroxyproline, Metabolism, Mitochondria, Review

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