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Article Addendum

# Involvement of Arabidopsis Prolyl 4 Hydroxylases in Hypoxia, Anoxia and Mechanical Wounding

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**KEY WORDS**

Arabidopsis, anoxia, gene expression, hypoxia, prolyl 4 hydroxylase, transcription factors

**ABBREVIATIONS**

| | |
|---|---|
| AGPs | arabinogalactan proteins |
| HRGPs | hydroxyproline-rich glycoproteins |
| P4H | prolyl 4 hydroxylase |

Addendum to:

*Arabidopsis Prolyl 4-Hydroxylases are Differentially Expressed in Response to Hypoxia, Anoxia and Mechanical Wounding*

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## ABSTRACT

Arabidopsis prolyl 4 hydroxylases (P4Hs) catalyze an important post-translational modification in plants, though the only information on their patterns of expression is solely based on Arabidopsis microarray analysis data. In addition, the expression patterns of plants P4Hs in response to hypoxia, anoxia and other abiotic stresses such as mechanical wounding have never been studied extensively, despite their central role in hypoxic response of several other organisms through the regulation of stability of the HIF-1$\alpha$ transcription factor, the global regulator of hypoxic response. The 13 putative Arabidopsis P4Hs are low abundance transcripts with differential patterns of expression in response to two hypoxic, 1.5% and 5% $O_2$, anoxic conditions and mechanical wounding. Hypoxia of 1.5% $O_2$ induced the expression of six At-P4Hs while hypoxia of 5% $O_2$ and anoxia induced the expression of three and two At-P4Hs, respectively. Moreover, 308 Arabidopsis genes including 25 transcription factors were identified in silico among the differentially expressed genes under hypoxia that contain proline hydroxylation motifs. These results suggest involvement of this post-translational modification in the processing of hypoxia induced proteins providing an alternative level of regulation for responses to oxygen deficiency conditions.

## P4Hs ARE UPREGULATED IN RESPONSE TO HYPOXIA, ANOXIA AND WOUNDING

Prolyl 4 hydroxylases (P4Hs) belong to the family of 2-oxoglutarate-dependent dioxygenases and catalyze the formation of 4-hydroxyproline requiring 2-oxoglutarate and $O_2$ as cosubstrates, $Fe^{2+}$ as cofactor and ascorbate for optimal activity.[1] Three plant $\alpha$-subunit-like P4Hs, two Arabidopsis and one tobacco, have been cloned and characterized in detail up to now[2-4] while additional P4Hs have been partially characterized from other plant species.[5]

Sequence homology searches of the *Arabidopsis thaliana* genome indicated the presence of thirteen putative P4H genes with conserved residues that bind the $Fe^{2+}$ atom and the C-5 carboxyl group of 2-oxoglutarate.[3] Preliminary analysis of their expression patterns in response to hypoxia, anoxia and wounding indicated low abundance transcripts with higher levels of expression in roots compared to shoots under hypoxia and anoxia.[6] Hypoxic treatment of 1.5% $O_2$ induced the expression of six P4Hs, while hypoxia of 5% $O_2$ and anoxia induced the expression of three and two P4Hs, respectively. Induction of expression under both hypoxic and anoxic conditions in roots was observed only for P4H3, while induction of expression in response to 1.5% and 5% $O_2$ in roots was observed only for P4H4. In addition, approximately half (six) of the P4Hs were upregulated within six hours of leaf blade mechanical wounding since several cell wall proteins such as extensins and extensin-like receptor kinases are reportedly expressed in tissues experiencing mechanical stress.[7,8] This study indicates involvement of proline hydroxylation in hypoxic and anoxic adaptation and mechanical wounding providing an alternative approach to regulate plant responses to abiotic stress.

## FUNCTIONAL SIGNIFICANCE OF P4HS ON HYPOXIC AND ANOXIC RESPONSE

The very low P4H transcript abundance in Arabidopsis indicates tight transcriptional regulation considering they catalyze the formation of 4-hydroxyproline, the site of protein *O*-glycosylation in the plant secretory pathway. This post-translational modification is essential for the function and structure of extracellular matrix proteins that are involved in many aspects of plant growth and development and therefore participation in the hypoxic adaptation mechanism should not be considered unlikely.

Table 1 **Differentially expressed Arabidopsis transcription factor genes in response to hypoxia that contain proline hydroxylation motif(s)**

| Description of Transcription Factor Genes | Locus |
|---|---|
| bZIP transcription factor family protein | AT2G22850 |
| Basic helix-loop-helix (bHLH) family protein | AT4G28790 AT2G18300 AT3G19860 AT4G29930 |
| WRKY family transcription factor | AT2G30590 AT1G80840 AT5G52830 AT5G15130 |
| Auxin-responsive factor (ARF9) | AT4G23980 |
| Basic helix-loop-helix (bHLH) protein (RAP-1) | AT1G32640 |
| AP2 domain-containing transcription factor, putative | AT4G13620 AT2G20880 |
| zinc finger (CCCH-type) family protein | AT2G25900 AT5G58620 AT1G04990 AT5G12850 |
| G-box binding factor 1 (GBF1) | AT4G36730 |
| myb-related transcription factor (CCA1) | AT2G46830 |
| Dof-type zinc finger domain-containing protein | AT1G69570 AT1G64620 |
| myb family transcription factor | AT5G02840 |
| squamosa promoter-binding protein, putative | AT5G50570 |
| homeobox-leucine zipper family protein / lipid-binding START domain-containing protein | AT1G05230 |
| AP2 domain-containing transcription factor TINY, putative | AT1G44830 |

Putative hydroxyproline-rich glycoprotein (HRGPs) and, arabinogalactan (AGPs) genes are differentially expressed under hypoxic conditions according to transcriptome analysis of hypoxic response in Arabidopsis plants suggesting possible involvement in hypoxic adaptation.[9] In addition, proline hydroxylation and hydroxyproline arabinogalactosylation motifs were experimentally determined recently.[10] To this direction, Arabidopsis proteins were surveyed for the hydroxylation motif[10] using the Patmatch search engine and subsequently were filtered for the genes encoding these proteins for differential expression in response to hypoxia.[9] This search resulted in the identification of 308 genes that were classified into functional categories using the MIPS Arabidopsis Database (MAtDB).[6] Among them, 22 were identified as HRGPs with (10 genes) and without (12 genes) hydroxylation motifs.[6] This finding implies extensive P4H activity during hypoxia to accommodate demands for this post-translational modification by a portion of the 308 encoded proteins.

## IS PROLINE HYDROXYLATION INVOLVED IN THE REGULATION OF TRANSCRIPTION FACTORS?

A large portion of the transcription functional class that contain proline hydroxylation motif(s) comprised of 25 transcription factors with various expression patterns in response to hypoxia (Table 1).

In mammalian systems, cells respond to changes in oxygen availability through a pathway that is regulated by the transcriptional complex hypoxia inducible factor (HIF), which plays a central role in both local and systemic responses to hypoxia.[11] The stability of the alpha subunit of HIF, and thus HIF activity, is regulated through prolyl hydroxylation by a family of P4H proteins that has also been identified as playing a similar role in *C. elegans* and *Drosophila melanogaster*.[12] Identification of the HIF system in nematode worms indicates that the system evolved before the development of complex systemic oxygen delivery systems, presumably to regulate responses to oxygen availability at the cellular level.[12] This is an indication that a similar system might also be present in plants.

There are no reports on similar mechanisms for regulation of transcription factors involved in hypoxia in higher plants or on the identification of plant HIF-1α homologues. However, we cannot exclude the possibility that P4Hs play a similar role as HIF-P4Hs in response to low oxygen tension or other biotic and abiotic stress and that the formation of 4-hydroxyproline is also involved in protein stability of transcription factors. A recombinant Arabidopsis P4H (At-P4H-1) was found to effectively hydroxylate synthetic peptides representing the two hydroxylated sequences in human transcription factor HIF-1α indicating substrate specificity for a transcription factor from a heterologous organisms.[3]

Proline residues are also involved in the stability of Aux/IAA repressors since it has been shown that prolyl isomerization of the Aux/IAA proteins catalyzed by peptidyl prolyl cis/trans isomerase is required for recognition by the SCF ubiquitin protein ligase E3 complex and subsequent proteosomal degradation.[13]

However, the significance of a post translational modification such as proline hydroxylation in the regulation of hypoxic responses in plants remains to be seen through the functional analysis of Arabidopsis P4H knock out mutants.

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