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MINI-REVIEW

Metabolic mechanism of lignin-derived aromatics in white-rot fungi

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Abstract

White-rot fungi, such as *Phanerochaete chrysosporium*, play a crucial role in biodegrading lignocellulosic biomass including cellulose, hemicellulose, and lignin. These fungi utilise various extracellular and intracellular enzymes, such as lignin peroxidases, manganese peroxidases, versatile peroxidases, monooxygenases, and dioxygenases, to degrade lignin and lignin-derived aromatics, thereby significantly contributing to the global carbon cycle with potential applications in industrial bioprocessing and bioremediation. Although the metabolism of lignin fragments in *P. chrysosporium* has been studied extensively, the enzymes involved in fragment conversion remain largely unknown. This review provides an overview of the current knowledge regarding the metabolic pathways of lignin and its fragments by white-rot fungi. Recent studies have elucidated the intricate metabolic pathways and regulatory mechanisms of lignin-derived aromatic degradation by focusing on flavoprotein monooxygenases, intradiol dioxygenases, homogentisate dioxygenase-like proteins, and cytochrome P450 monooxygenases. Metabolic regulation of these enzymes demonstrates the adaptability of white-rot fungi in degrading lignin and lignin-derived aromatics. The interplay between the central metabolic pathways, haem biosynthesis, and haem-dependent NAD(P)H regeneration highlights the complexity of lignin degradation in white-rot fungi. These insights improve our understanding of fungal metabolism and pave the way for future studies aimed at leveraging these fungi for sustainable biotechnological applications.

Key points

- *White-rot fungi use enzymes to degrade lignin, and play a role in the carbon cycle.*
- *Oxygenases are key enzymes for converting lignin-derived aromatics.*
- *White-rot fungi adapt to metabolic changes by controlling the TCA/glyoxylate bicycle.*

Keywords Lignin · Monooxygenases · Dioxygenases · TCA cycle · Haem · NAD(P)H regeneration

Introduction

White-rot fungi produce several enzymes to completely degrade lignocellulose including cellulose, hemicellulose, and lignin (Suryadi et al. 2022). Cellulose is the most abundant natural polysaccharide, which comprises a β-1,4-linked linear chain of glucose units and is degraded by several cellulolytic enzymes, including *endo*-glucanases (EC 3.2.1.4), cellobiohydrolases (CBHs; EC 3.2.1.91 and EC 3.2.1.176), and β-glucosidases (EC 3.2.1.74), produced by fungi (Bentil et al. 2018; Okal et al. 2020). Hemicellulose is the second most abundant polysaccharide and is typically associated with cellulose and lignin in plant cell walls (Khodayari et al. 2021). β-Mannans (glucomannan, galactomannan, and galactoglucomannan) along with xylans constitute the major components of hemicellulose (Khodayari et al. 2021). White-rot fungi produce various hemicellulases such as mannanases, xylanases, acetylxylan esterases, pectinases, arabinofuranosidases, and galactosidases (Xiao et al. 2019). Several fungi, including white-rot fungi, can degrade cellulose and hemicellulose (Xiao et al. 2019; Kijpornyongpan et al. 2022).

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Lignin is an abundant aromatic polymer that accounts for 15–35% of the total lignocellulosic biomass found in nature (Chen et al. 2023). It is a complex and heterogeneous polymer composed of phenylpropanoid units linked by various types of chemical bonds including aryl ether (β-O-4), phenylcoumaran (β-5), resinol (β-β), biphenyl ether (5-O-4), and dibenzodioxocin (5-5) (Weng et al. 2021). The complexity and diversity of these linkages make lignin highly recalcitrant to degradation (Weng et al. 2021). As a crucial component of the global carbon cycle, lignin degradation by environmental microorganisms is vital (Weng et al. 2021). White-rot basidiomycetes are renowned for their ability to fully decompose lignin (Suryadi et al. 2022). These fungi secrete extracellular enzymes including lignin peroxidases (LiP), manganese peroxidases (MnP), and versatile peroxidases (Tien and Kirk 1983; Glenn and Gold 1985; Singh and Chen 2008). These enzymes, which are nonspecific and capable of one-electron oxidation, target and break down carbon–carbon and ether linkages in lignin, thereby promoting the production of various lignin-derived aromatics such as *p*-hydroxybenzaldehyde (HBN), vanillin (VN), and syringaldehyde (SN) (Fig. 1) (Kirk et al. 1978; Gold et al. 1989; Hammel and Moen 1991; Wariishi et al. 1991). The resulting lignin fragments can be classified into three categories: guaiacyl units (G-unit) such as VN and vanillic acid (VA); syringyl units (S-unit) including SN and syringic acid (SA); and hydroxyphenyl units (H-unit) such as HBN and *p*-hydroxybenzoic acid (HBA) (Fig. 1) (Weng

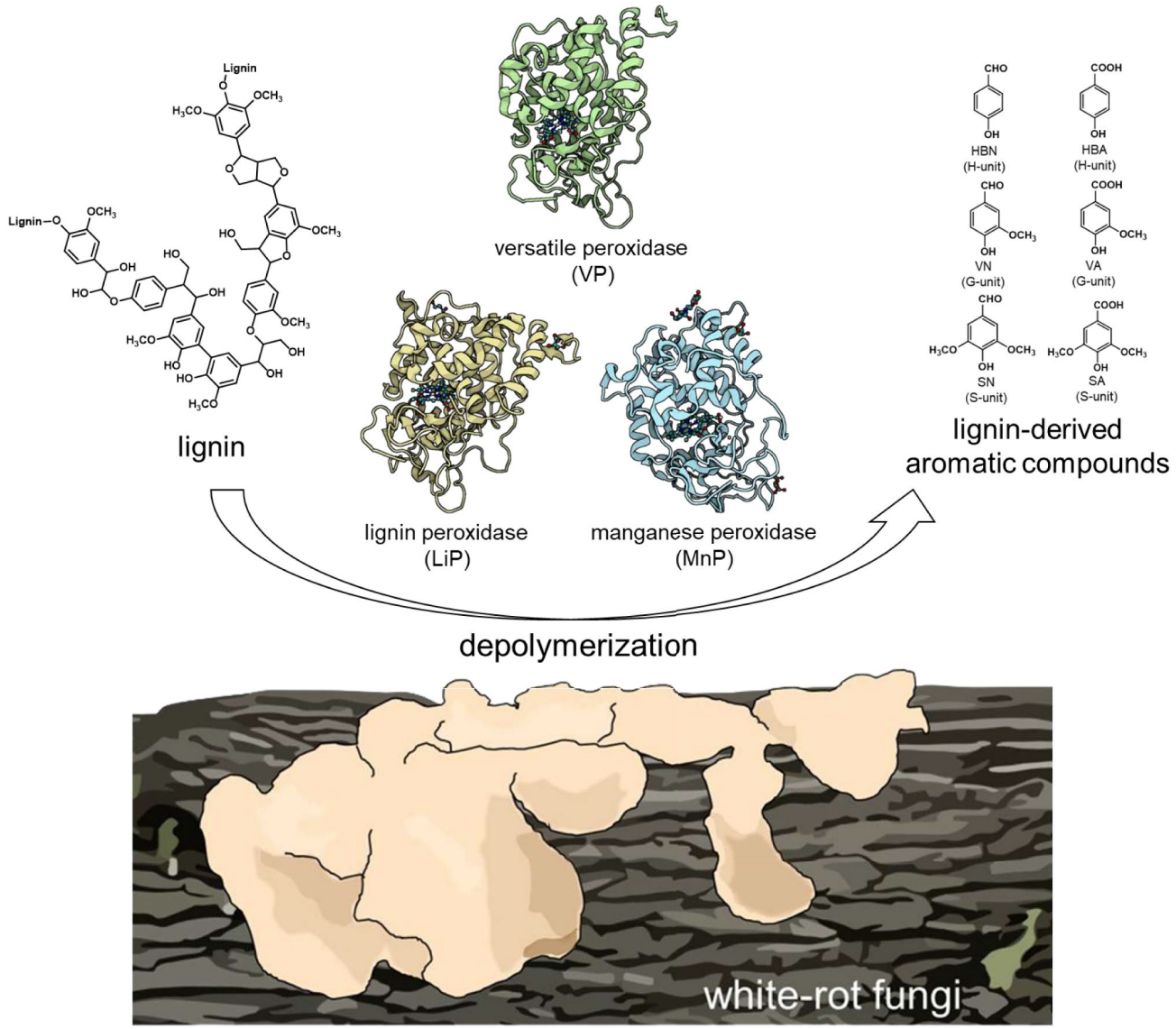

Fig. 1 Depolymerisation of lignin into various lignin-derived aromatics facilitated by MnP, LiP, and VP produced by white-rot fungi. This figure was generated using BioRender (https://biorender.com/)

et al. 2021; Kato et al. 2024). These intermediates undergo intracellular oxidation, decarboxylation, hydroxylation, and/or demethoxylation to produce 1,2,4-trihydroxybenzene (THB) (Yajima et al. 1979; Ander et al. 1983; Kato et al. 2024), which is then subjected to ring cleavage by THB dioxygenases (Rieble et al. 1994; Kato et al. 2022). Despite extensive studies on the metabolism of lignin fragments in *P. chrysosporium* (Yajima et al. 1979; Ander et al. 1983; Rieble et al. 1994; Barnhart-Dailey et al. 2019), the specific enzymes responsible for fragment conversion remain largely unidentified.

This review provides an overview of the current knowledge regarding metabolic regulation of lignin and its fragments by white-rot fungi. Additionally, we discuss the degradation pathways of lignin-derived aromatics generated by ligninolytic enzymes and enzymes, such as monooxygenases and dioxygenases.

Intracellular metabolism of lignin-derived aromatics by white-rot fungi

Flavoprotein monooxygenases

Basidiomycetes can degrade various aromatic compounds, including intermediate fragments generated from lignin decomposition (Matsuzaki and Wariishi 2004; Zhang et al. 2021a, 2021b; Kyrila et al. 2021; Bautista-Zamudio et al. 2023). In *P. chrysosporium*, the G-unit fragment VA undergoes decarboxylation and demethoxylation to form methoxyhydroquinone (MHQ) and THB, respectively, after which degradation occurs via aromatic ring cleavage facilitated by THB dioxygenase (Yajima et al. 1979; Ander et al. 1983; Rieble et al. 1994). The activity of VA-1-decarboxylase has been identified in both brown-rot and white-rot basidiomycetes using cell extracts (Yajima et al. 1979; Tai et al. 1990). Although a VA decarboxylase has not been identified in basidiomycetes, including *P. chrysosporium* (Fig. 2), a 4-hydroxybenzoate 1-hydroxylase (4HB1H: G8B709 from UniProt database) with VA decarboxylase activity, belonging to the flavoprotein monooxygenase (FPMO) group A superfamily, has been noticed (Eppink et al. 1997). This FPMO, initially isolated from *Candida parapsilosis* (Eppink et al. 1997), catalyses an oxidative decarboxylation reaction, producing *p*-hydroquinone (HQ) and MHQ from HBA and VA, respectively. The *P. chrysosporium* genome contains 59 putative FPMO-encoding genes (Suzuki et al. 2023). A BLAST search of the published *P. chrysosporium* RP-78 v4.0 JGI genome database has revealed that 4HB1H shares an amino acid sequence identity of 42.8, 39.2, and 32.7% with protein IDs 6247437, 6220286, and 6212972, respectively, suggesting a potential role of these FPMOs in VA decarboxylation. Recently, omic studies investigating the degradation of lignin and its-derived aromatics in two white-rot fungi, *Trametes versicolor* and *Gelatoporia subvermispora*, have been conducted (del Cerro et al. 2021). Among the FPMOs induced by HBA and lignin, three (TV_32834, GS_90429, and GS_120062 from JGI genome database) have been found to catalyse oxidative decarboxylation of HBA (del Cerro et al. 2021). However, whether these FPMOs also decarboxylate VA and SA remains unclear. Notably, FPMO 6247437 shares 41.9, 34.3, and 69.0% amino acid sequence identity with TV_32834, GS_90429, and GS_120062, respectively. Decarboxylation of HBA, VA, and SA to HQ, MHQ, and dimethoxyhydroquinone (DMHQ), respectively, is crucial for producing aromatic ring-fission compounds, such as THB, during lignin degradation by white-rot fungi (Rieble et al. 1994; Kato et al. 2022).

Fungi and yeasts utilize two metabolic pathways to degrade HBA, both of which ultimately lead to the formation of THB, a critical step in the degradation of H-unit fragments by white-rot fungi (Rieble et al. 1994; Suzuki et al. 2023). One pathway involves hydroxylation of HBA to form PCA that is subsequently decarboxylated to THB (Eppink et al. 1997). The other pathway involves oxidative decarboxylation of HBA to form HQ that is converted to THB by HQ hydroxylase, as observed in yeasts such as *C. parapsilosis* (Berkel et al. 1994; Eppink et al. 2000; Westphal et al. 2021). Fungi and bacteria utilize different types of group A FPMOs for converting HBA and HQ (Lubbers et al. 2019a; Paul et al. 2021). Fungal HBA hydroxylases, such as phhA from *Aspergillus niger* and bacterial pobA from *Pseudomonas fluorescens*, have different geometries of the active sites (Lubbers et al. 2019b; Moriwaki et al. 2019; Katsuki et al. 2024). A BLAST search of the published *P. chrysosporium* RP-78 v4.0 JGI genome database has revealed that phhA (G3Y748 from UniProt database) shares 47.3, 45.7, and 45.2% amino acid sequence identity with protein IDs 6307103, 6203796, and 6441807, respectively. Similarly, a BLAST search using HQ hydroxylase (HQH) from *C. parapsilosis* (G8BGH1 from UniProt database) has revealed identity scores of 58.9, 57.5, and 54.9% with 6307103, 6203796, and 6441807, respectively, in *P. chrysosporium*. BLAST searches using both sequences have yielded the same results, suggesting a potential role of these FPMOs in hydroxylation of HBA and HQ.

Among these FPMOs, PcFPMO1 (6307103 obtained from *P. chrysosporium* RP-78 v.4.0 JGI genome database) and PcFPMO2 (6203796 obtained from *P. chrysosporium* RP-78 v.4.0 JGI genome database), have been identified in *P. chrysosporium* as members of the group A FPMO superfamily. PcFPMO1 shows catalytic activity specifically towards phenol, 4-chrolophenol, and HQ (Nakamura et al. 2012), whereas PcFPMO2 can hydroxylate

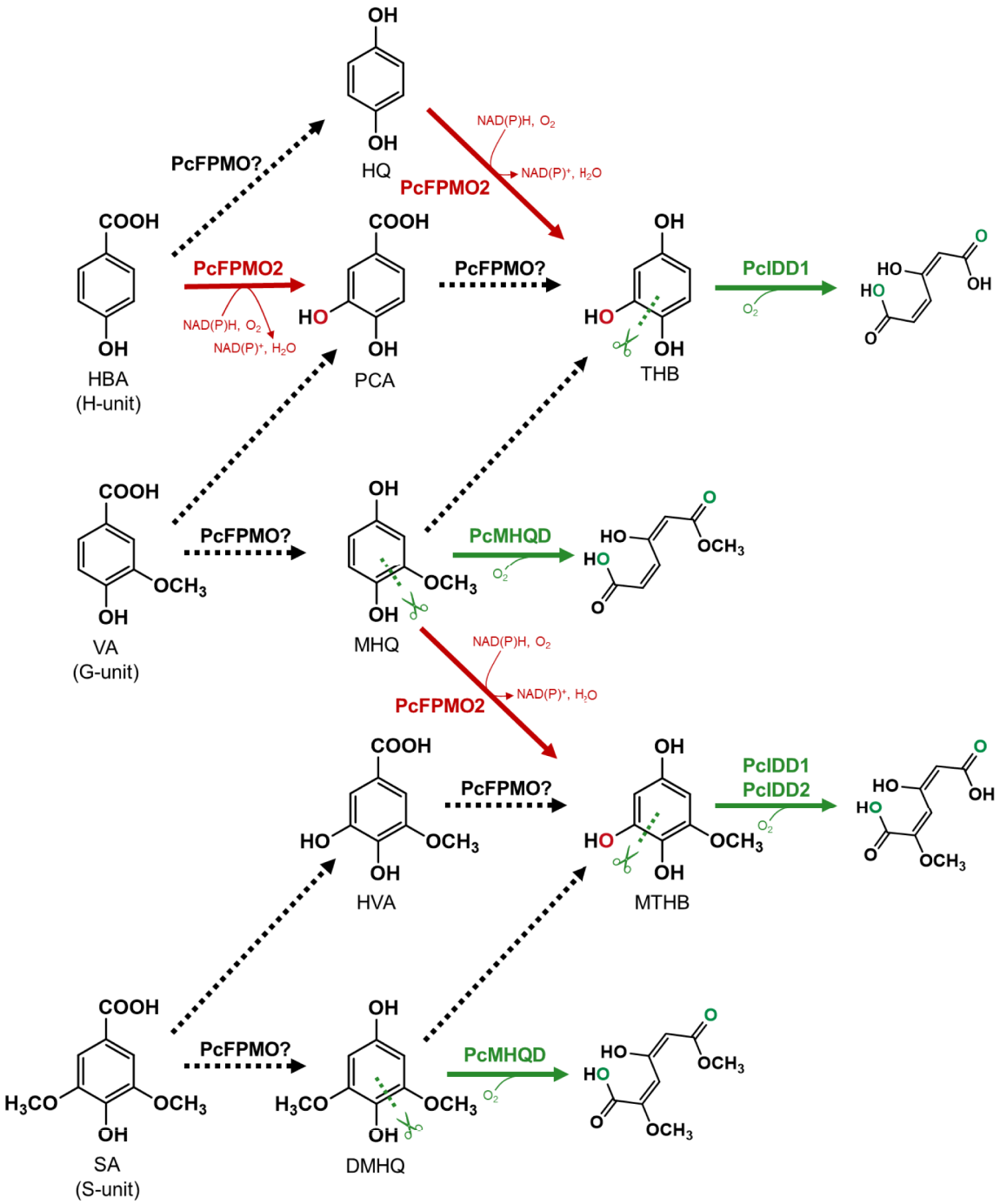

Fig. 2 Metabolic pathways of HBA, VA, and SA in white-rot fungus *P. chrysosporium*. Dotted arrows indicate the estimated reactions, and solid arrows indicate the reactions by identified enzymes including flavoprotein monooxygenase 2 (PcFPMO2), MHQ dioxygenases (PcMHQD), intradiol dioxygenase 1 (PcIDD1), and intradiol dioxygenase 2 (PcIDD2)

several substrates, including H-unit fragments such as HQ and HBA, and the G-unit derivative MHQ (Suzuki et al. 2023). PcFPMO2 is distinct because, unlike ascomycetous FPMOs, such as phhA and HQH, it can catalyse the hydroxylation of both HBA and HQ, suggesting a relatively broad substrate range (Suzuki et al. 2023). The G-unit fragment, VA, is intracellularly decarboxylated to MHQ (Yajima et al. 1979). PcFPMO2, which is induced by VN, hydroxylates MHQ into three methoxytrihydroxybenzene (MTHB) derivatives such as 3-MTHB, 5-MTHB, and 6-MTHB (Fig. 2) (Suzuki et al. 2023). Similar regioselectivity in hydroxylation has been noticed during the

conversion of fluorohydroquinones by HQH (Berkel et al. 1994; Eppink et al. 1997).

Recent multi-omic studies on lignin degradation by two white-rot fungi, *T. versicolor* and *G. subvermispora*, have revealed the involvement of two FPMOs, TV_58730 and GS_82507 (del Cerro et al. 2021). Given the high sequence similarity (PcFPMO2 shares 70.8 and 73.2% amino acid sequence identity with TV_58730 and GS_82507, respectively), these enzymes may have similar hydroxylase activities. They catalyse the hydroxylation of both HBA and HQ, and their substrate specificity is similar to that of FPMO2. Whether these FPMOs can hydroxylate MHQ into the three MTHBs remains unclear. The three MTHBs are known fungal metabolites derived by VN degradation (Suzuki et al. 2023), suggesting that PcFPMO2 may play a role in hydroxylating MHQ during VN degradation. However, the yields of these three MTHBs produced by PcFPMO2 differ from those obtained through fungal metabolism, implying that other FPMOs might be involved in the conversion of MHQ.

Intradiol dioxygenases

The enzymes responsible for the ring-opening step in the β-ketoadipate pathway from catechol (CAT) and THB derivatives have been extensively studied in bacteria and fungi, and their functions are now well understood (Harwood and Parales 1996; Semana and Powlowski 2019; Lubbers et al. 2021; Sgro et al. 2023). Putative intradiol dioxygenases (IDDs) from basidiomycetes have been annotated as ring-cleaving enzymes based on their sequence homologies with distantly related bacterial and fungal counterparts (Latus et al. 1995; Hatta et al. 1999; Semana and Powlowski 2019). Five IDD-like genes (PcIDD1–5) have been found in the white-rot fungus *P. chrysosporium*, and PcIDD1 and PcIDD2 have been characterized (Kato et al. 2022). PcIDD1 catalyses the ring cleavage of eight THB derivatives and acts on a wider range of substrates (Fig. 2) than do bacterial and fungal THB dioxygenases (Latus et al. 1995; Murakami et al. 1999; Hatta et al. 1999; Semana and Powlowski 2019; Kato et al. 2022). PcIDD2 does not act on THB; however, it exhibits high catalytic efficiency for 6-MTHB (Fig. 2) (Kato et al. 2022). The catalytic efficiency of PcIDD1 for 6-MTHB is significantly lower than that for THB (Kato et al. 2022). White-rot basidiomycetes degrade lignin, lignin-derived aromatics, and various aromatic pollutants, which suggests that PcIDD1 plays a role in the lignin degradation pathway via THB formation in *P. chrysosporium* (Rieble et al. 1994). Basidiomycetes can degrade various lignin-derived aromatics, including MHQ and DMHQ (Yajima et al. 1979; Ander et al. 1983; Weng et al. 2021), which are converted to 6-MTHB as a metabolic intermediate of these aromatics (Kato et al. 2022; Suzuki et al. 2023). As mentioned above, MHQ is hydroxylated to 6-MTHB by FPMO2; DMHQ is an intermediate derived from the major lignin-derived aromatic SA that could be demethoxylated to 6-MTHB; however, DMHQ demethylases have not yet been identified in fungi (Kato et al. 2022). The presence of 6-MTHB in the VA and SA degradation pathway suggests that PcIDD1 and/or PcIDD2 may be involved in aromatic ring cleavage via 6-MTHB generated from DMHQ and/or HVA in *P. chrysosporium* (Kato et al. 2022). In lignin-degrading white-rot fungi *T. versicolor* and *G. subvermispora*, some dioxygenases induced by HBA and poplar-derived aromatic compounds catalyse the ring cleavage of THB; however, whether they also cleave 6-MTHB remains unclear (del Cerro et al. 2021). PcIDD1 (6340157 obtained from *P. chrysosporium* RP-78 v.4.0 JGI genome database) shares 71.6 and 73.5% amino acid sequence identity with TV_28066 and GS_116134, respectively. PcIDD2 (6275533 obtained from *P. chrysosporium* RP-78 v4.0 JGI genome database) shares 71.1 and 70.3% amino acid sequence identity with TV_62764 and GS_114110, respectively. Omic analyses of *T. versicolor* and *G. subvermispora* have revealed that TV_28066 and GS_116134 are induced by HBA of the H-unit, while TV_62764 and GS_114110 are induced by SA of the S-unit (del Cerro et al. 2021). These findings indicate that in white-rot fungi, THB and MTHB are employed by PcIDD1 and PcIDD2, respectively, in a selective manner depending on their functional roles.

PcIDD3–5 have not been characterized, and their substrate specificity is unknown. The amino acid sequence of the first purified THB dioxygenase from *P. chrysosporium* is unknown; however, PcIDD1 catalyses the ring cleavage of pyrogallol that is not converted by previously reported THB dioxygenase (Rieble et al. 1994). Different activities of PcIDD1 and previously reported THB dioxygenase suggest that one of PcIDD3–5 may correspond to the previously reported enzyme. The roles of IDDs provide a relatively deep insight into the degradation of lignin and lignin-derived aromatics and highlight the functional diversity of IDDs in white-rot fungi.

Homogentisate dioxygenase-like proteins

MHQ and DMHQ are intermediates in the lignin degradation of the major aromatic compounds derived from VA and SA, respectively. As mentioned in the previous section, the fungal demethylases responsible for the degradation of MHQ and DMHQ have not yet been identified (Kato et al. 2022). Alterations in protein expression of *P. chrysosporium* grown with VN have revealed a putative dioxygenase, annotated as homogentisate (HGA) 1,2-dioxygenase (HGD; EC 1.13.11.5), which is strongly induced (Shimizu et al. 2005). Furthermore, among the dioxygenases induced by SA and aromatics derived from lignin and poplar, two homologous enzymes (TV_20432 and TV_124955) known as HGDs are

also upregulated at the mRNA and protein levels (del Cerro et al. 2021).

Recently, two HGD-like enzymes have been identified from *P. chrysosporium*, which catalyse the ring cleavage of MHQ and DMHQ (Kato et al. 2024). These enzymes, now designated PcMHQD1 (6344326 obtained from *P. chrysosporium* RP-78 v.4.0 JGI genome database) and PcMHQD2 (6344291 obtained from *P. chrysosporium* RP-78 v.4.0 JGI genome database) (MHQ dioxygenases 1 and 2), exhibit the highest catalytic efficiency for MHQ (Kato et al. 2024). The presence of ring cleavage products of MHQ and DMHQ using cell extract reactions suggests that these intermediates undergo direct aromatic ring cleavage in fungal cells. While PcMHQD1 and PcMHQD2 do not act on THB, their catalytic efficiencies for MHQ are significantly higher than those for HGA and DMHQ, suggesting that MHQ is the preferred physiological substrate for these HGD homologs (Kato et al. 2024). Whether human and bacterial HGDs ($HGDO_{Hs}$ and $HGDO_{Pp}$, respectively) can also catalyse the ring cleavage of MHQ and DMHQ remains unknown. PcMHQD1 shares 43.4 and 41.1% amino acid sequence identity with HGDs from other organisms ($HGDO_{Hs}$ and $HGDO_{Pp}$, respectively), and PcMHQD2 shares 35.1 and 38.1% sequence identity with these enzymes (Kato et al. 2024). Among these HGDs, two histidine residues and a glutamate residue, which are essential for the coordination of non-haem ferric iron in the active site, are highly conserved (Jeoung et al. 2013). However, a tyrosine residue that interacts with the carboxyl group of HGA in the active site lid of HGD is conserved in these HGDs but is replaced by phenylalanine in PcMHQD2. PcMHQD2 does not convert HGA probably owing to this substitution, highlighting the importance of the tyrosine residue for interaction with HGA. Differences in amino acid compositions of the active site lid between mammalian, plant, fish, insect, and bacterial HGDs and fungal HGDs could account for variations in enzyme activity towards HGA, MHQ, and DMHQ. The interaction between the active site lid and HGA likely influences lid closure upon HGA binding, suggesting that the exact structure of the lid is determined by substrate binding.

PcMHQD1 and PcMHQD2 constitute the first two candidates identified, which exhibit ring cleavage activity for MHQ and DMHQ within the HGD superfamily. PcMHQD homologs have been identified in some white-rot fungi, suggesting their potential role in degrading lignin and lignin-derived aromatics. In contrast, bacteria use an iron (II)-dependent dioxygenase to cleave the aromatic ring of MHQ; however, these bacterial enzymes share low amino acid sequence identity with PcMHQD1 and PcMHQD2 (Kolvenbach et al. 2011). Bacterial and human HGDs involved in the ring-opening step of tyrosine and phenylalanine degradation pathways via HGA are well characterized and understood (Amaya et al. 2004; Veldhuizen et al. 2005). The catalytic efficiency of PcHGDs towards HGA is significantly lower than those of $HGDO_{Pp}$ and $HGDO_{Hs}$, suggesting that *P. chrysosporium* may not degrade aromatic amino acids, such as tyrosine and phenylalanine, as efficiently as do other organisms. This reduced activity may benefit *P. chrysosporium* by allowing relatively efficient production of veratryl alcohol, a metabolite derived from phenylalanine, which serves as a diffusible oxidant and stabilizer for LiP during lignin degradation (Hammel and Cullen 2008).

P. chrysosporium possesses multiple metabolic pathways for MHQ degradation; however, the primary pathway has not yet been fully elucidated. As mentioned above, VN-induced MHQ hydroxylase (PcFPMO2) and MTHB dioxygenases, such as PcIDD1 and PcIDD2, have been identified in *P. chrysosporium* (Kato et al. 2022; Suzuki et al. 2023), further illustrating the complex network of pathways involved in the conversion of lignin fragments by this white-rot fungus.

Cytochrome P450 monooxygenases

White-rot fungi can degrade various recalcitrant aromatic compounds, including lignin and persistent environmental pollutants (Asgher et al. 2008; Swathy et al. 2023). Fungal cytochrome P450 monooxygenases (P450s) play crucial roles in these degradation processes (Ichinose 2012; Khan et al. 2023). *P. chrysosporium* has been a principal focus of research owing to its ligninolytic abilities and its metabolism of xenobiotics (Hatakeyama et al. 2016; Sakai et al. 2018; Wang et al. 2019; Mori et al. 2021). The genome sequence of *P. chrysosporium* highlights extensive genetic diversity of its P450s, with as many as 154 P450 genes identified (Doddapaneni et al. 2005; Syed and Yadav 2012). This diversity suggests that some P450s may play key roles in the metabolism of lignin-derived aromatic compounds; however, specific P450 enzymes involved in these conversions have not yet been identified.

Bacterial systems, such as the GcoAB cytochrome P450 system, including a coupled monooxygenase (GcoA) and reductase (GcoB) catalyse *O*-demethylation of 3-methoxycatechol to produce pyrogallol (Mallinson et al. 2018). Similarly, the VanAB methyltransferase system, comprising VanA and VanB enzymes, catalyses demethylation of VA to PCA, playing a key role in lignin degradation (Priefert et al. 1997; Wolf et al. 2024). In the *P. chrysosporium* genome, the identification of 154 P450-encoding genes underscores the genetic diversity of its P450 enzymes (Syed and Yadav 2012), suggesting that some of these P450s may be responsible for *O*-demethylation of MHQ and DMHQ, leading to the formation of THB and MTHB, respectively (Fig. 2).

Quinone reductases and aryl-aldehyde and aryl-alcohol dehydrogenases

VA significantly enhances the activity of 1,4-benzoquinone reductases (QRs) (Akileswaran et al. 1999; Brock et al. 1995; Brock and Gold 1996; Mori et al. 2016). These enzymes are a broadly distributed family of flavoprotein QRs, which are believed to detoxify intracellular quinones by keeping them in their reduced forms (Akileswaran et al. 1999; Brock and Gold 1996; Mori et al. 2016). QRs from *P. chrysosporium* have been purified and characterized, demonstrating their role in converting quinones derived from VN into their corresponding hydroquinones (Brock and Gold 1996).

Aryl-aldehyde dehydrogenase (AALDH) and aryl-alcohol dehydrogenase (AADH) are induced by lignin and its-derived aromatic compounds (Ferreira et al. 2023). These enzymes facilitate the conversion of aromatic alcohols into aromatic acids, with aromatic aldehydes serving as intermediates (Muheim et al. 1991; Yang et al. 2012). As mentioned earlier, the expression of genes encoding AADH and AALDH that are thought to be involved in lignin degradation are upregulated in response to the presence of various aromatics (Shimizu et al. 2005; Matsuzaki et al. 2008; Ferreira et al. 2023).

Metabolic regulation by lignin and lignin-derived aromatics

Transcriptional regulation of enzymes involved in metabolising lignin and lignin-derived aromatics

White-rot fungi produce enzymes that convert lignin and its-derived aromatics in response to various aromatic compounds (Wang et al. 2024). However, the mechanisms, by which fungi sense these aromatic compounds, transmit the corresponding signals to the nucleus, and regulate the expression of genes encoding ligninolytic enzymes, remain unexplored (Wang et al. 2024). The upstream sequences of *lip* and *mnp* in *P. chrysosporium* reveal the presence of activator protein-2 (AP-2) recognition sequences in several genes (Dhawale 1993). *lip* and *mnp* have AP-2-binding sites, suggesting that AP-2-like factors may control the expression of these gene families (Dhawale 1993). Furthermore, MnP production depends on the presence of Mn^{2+} in the culture (Li et al. 1995; Ma et al. 2004). Several sequences in the promoter regions of *mnp* of *P. chrysosporium* conserves *cis*-acting promoter elements, including inverted CCAAT boxes, Mn^{2+}-responsive *cis* elements, heat shock elements, and a binding site for AP-2 (Ma et al. 2004). The Mn^{2+}-responsive element in the promoter sequence of *mnp1* has been characterised; however, the transcription factors that bind to these promoters and their involvement in regulating *mnp* expression have not been demonstrated (Ma et al. 2004). Transcriptional regulators of laccases and P450s have been studies in white-rot fungi. Zn_2Cys_6-type TH8421 and TH4300 are key regulators in white-rot basidiomycete *Trametes hirsuta* AH28-2 (Wang et al. 2024). They function as heterodimers to simultaneously trigger the expression of laccases and P450s, thereby positively regulating gene expression. Additionally, ThhspA1 regulates laccase A (*lacA*) transcription by directly interacting with its promoter region (Zhang et al. 2022). TH8421, TH4300, and ThhspA1 cooperatively activate *lacA* transcription (Wang et al. 2024; Zhang et al. 2022). In *Trametes trogii*, TtHSF2α, a heat shock transcription factor 2 that is induced by Cu^{2+}, directly binds to the promoter regions of representative laccase genes (Zhang et al. 2021a, 2021b). These findings highlight the intricate regulatory networks involving various transcription factors and promoter elements of genes encoding ligninolytic enzymes in white-rot fungi. However, transcriptional regulation of FPMO, IDD, and MHQD genes remain unclear. Further studies are necessary to fully elucidate the molecular mechanisms underlying gene expression of these enzymes.

Switching between the short-cut tricarboxylic acid (TCA)/glyoxylate bicycle and classical TCA cycle

Basidiomycetes possess a unique metabolic system known as the shortcut TCA/glyoxylate bicycle. In this cycle, basidiomycetes bypass the conversion of isocitrate to α-ketoglutarate and subsequently to succinyl-CoA, instead forming succinate directly from isocitrate via isocitrate lyase (ICL) (Fig. 3). ICL has a much higher activity than that of isocitrate dehydrogenase (IDH). Furthermore, glyoxylate is produced as a counterpart to succinate (Takao 1965). The glyoxylate cycle is typically associated with acetate metabolism; however, it is also present in plants and certain microorganisms (Cozzone 1998). Interestingly, the glyoxylate cycle is generally found in white-rot and brown-rot fungi, even when these fungi are grown in glucose-containing media without acetate (Munir et al. 2001a). In addition, the fungal glyoxylate cycle may be associated with oxalate production (Munir et al. 2001b).

In *P. chrysosporium*, a significant shift in the central pathway upon exposure to aromatics, such as VN and benzoic acid, has been observed (Shimizu et al. 2005; Matsuzaki et al. 2008). Notably, the classical TCA cycle becomes active in response to exogenous aromatics (Shimizu et al. 2005; Matsuzaki et al. 2008). This pathway switch at the branch point of succinyl-CoA is crucial for (i) generating NAD(P)H for energy production through IDH and α-ketoglutarate dehydrogenases (ODH) and (ii) upregulating enzymes, such as 5'-aminolevulinate synthase, uroporphyrinogen decarboxylase, and coproporphyrinogen oxidase, in the haem

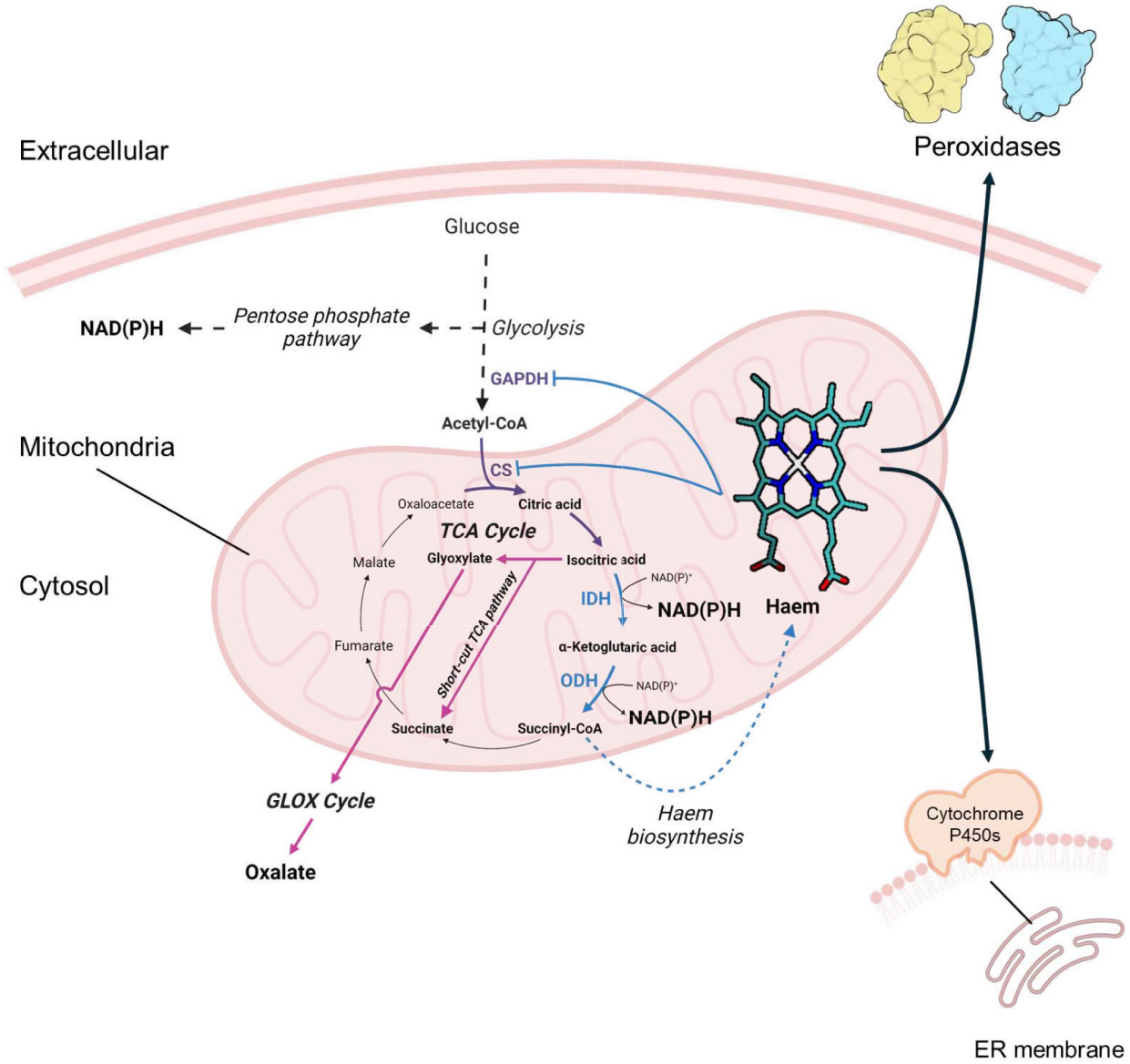

Fig. 3 Central metabolic regulation of white-rot fungi in lignin degradation. During degradation of lignin and lignin-derived aromatics, production of isocitrate dehydrogenase (IDH) and α-ketoglutarate dehydrogenase (ODH) significantly increases, resulting in a shift in metabolic flux from a short-cut tricarboxylic acid (TCA)/glyoxylate bicycle system (magenta) to the classical TCA cycle (cyan), which activates the haem biosynthetic pathway by providing succinyl-CoA for effective production of haem enzymes. In contrast, excessive haem production directly inhibits citrate synthase (PcCS) and glyceraldehyde 3-phosphate dehydrogenase (PcGAPDH), thereby regulating haem synthesis, ATP synthesis, flux of the TCA cycle, and NADPH production. Through this metabolic regulation, effective production of haem enzymes, including lignin and manganese peroxidases and cytochrome P450s, takes place. This figure was generated using BioRender (https://biorender.com/)

biosynthetic pathway (Shimizu et al. 2005). Therefore, the addition of exogenous aromatics to *P. chrysosporium* culture may induce a metabolic shift from the short-cut TCA/glyoxylate bicycle to the classical TCA cycle, potentially activating the haem biosynthetic pathway by providing succinyl-CoA for efficient production of haem-containing enzymes such as LiPs, MnPs, and P450s (Fig. 3). The upregulation of these peroxidases and P450s by exogenous aromatics has also been reported (Korripally et al. 2015; Qin et al. 2018; Kowalczyk et al. 2019; Ribeiro Tomé et al. 2024).

Transcriptomic analysis of *P. chrysosporium* during synthetic lignin degradation suggests that bond cleavage of synthetic lignin is facilitated by extracellular enzymes, such as LiPs and MnPs, while the released aromatics are further processed by intracellular enzymes such as P450s, QRs, AADHs, and dioxygenases (Korripally et al. 2015; Qin et al.

2018; Kowalczyk et al. 2019; Ribeiro Tomé et al. 2024). These ring-cleaved aromatic compounds are metabolized in *P. chrysosporium* via both the shortcut and classical TCA cycles (Hong et al. 2017). This intracellular metabolic regulation, switching from the short-cut TCA/glyoxylate bicycle to the classical TCA cycle, is crucial for activating the haem biosynthetic pathway and production of NAD(P)H during lignin degradation (Fig. 3).

Crosstalk between the central metabolic and haem biosynthetic pathways

Cellular metabolism shifts from basic energy production to a highly productive mode by the introduction of exogenous aromatic compounds. This metabolic change is characterized by increased glucose uptake and consumption and increased production of NAD(P)H and coenzyme A, resulting in the activation of the haem biosynthesis pathway (Fig. 3) (Shimizu et al. 2005; Matsuzaki et al. 2008). Rapid activation of haem synthesis is essential for the production of enzymes that degrade aromatic compounds. In *P. chrysosporium*, haem acts as a critical cofactor for ligninolytic enzymes such as LiPs, MnPs, and P450s (Weng et al. 2021). However, free haem is toxic to cells and can cause oxidative stress, particularly in mitochondria.

A comprehensive analysis of haem-binding proteins in *P. chrysosporium* has identified mitochondrial citrate synthase (PcCS) and cytosolic glyceraldehyde-3-phosphate dehydrogenase (PcGAPDH) as haem-binding proteins (Miura et al. 2024). The enzymatic activities of both PcGAPDH and PcCS are strongly inhibited by haem, with an inhibition constant K_i value of 5.8 μM for PcCS and 1.0 μM for PcGAPDH, highlighting their physiological relevance (Miura et al. 2024). PcCS inhibition by haem probably functions as a buffering mechanism against metabolic overflow in the TCA cycle, which provides succinyl-CoA, a precursor for haem synthesis (Srere 1975; Weitzman and Danson 1976) (Fig. 3). Inhibition of GAPDH by haem promotes a shift to the pentose-phosphate pathway (PPP), increasing NAD(P)H production while suppressing glycolysis. This shift is critical for maintaining cellular redox balance, particularly during the degradation of lignin and its fragments, an oxidative process that requires significant reducing equivalents. In addition, haem bound to PcGAPDH may protect cells from oxidative damage by forming inactive complexes with excess free haem (Miura et al. 2024; Sirover 1999). The suppression of TMB-oxidizing capacity of haem when bound to PcGAPDH further supports this protective role (Miura et al. 2024).

The transfer of haem from the mitochondrial matrix to the cytosol is essential for haemoprotein production, but haem concentrations can remain relatively high within the matrix (Mcalister-Henn and Small 1997; Obi et al. 2022). The binding of haem to PcCS and PcGAPDH is critical for its detoxification and for maintaining metabolic and redox balances (Fig. 3). This novel crosstalk between the central metabolic and haem biosynthetic pathways mediated by haem is essential for optimizing the degradation pathways of aromatic compounds in *P. chrysosporium* (Miura et al. 2024).

NAD(P)H regeneration

The catalytic activities of IDH, ODH, and PPP, which are upregulated by VN and synthetic lignin, lead to the production of NAD(P)H (Fig. 3) (Shimizu et al. 2005; Hong et al. 2017). Since fungal metabolism of aromatics involves multiple oxidative steps, the production of NAD(P)H in response to the metabolism of these compounds is crucial for maintaining the redox balance. The degradation of lignin and lignin-derived aromatics, such as VN, by *P. chrysosporium* follows the β-ketoadipate pathway that depends on NAD(P)H (Shimizu et al. 2005; Hong et al. 2017). Moreover, enzymes like AADH, VA decarboxylase (FPMO), hydroquinone hydroxylase (FPMO2), MHQ demethoxylase (P450), and QR require NAD(P)H as an electron donor (Fig. 2) (Shimizu et al. 2005; Hong et al. 2017). Therefore, the generation of NAD(P)H is essential for supporting the fungal redox systems and sustaining the metabolism of aromatic compounds.

Conclusion

Recent studies have highlighted the intricate metabolic pathways and regulatory mechanisms involved in lignin degradation. Understanding these processes, particularly the roles of FPMOs, IDDs, MHQDs, and P450s, is crucial for optimising the biodegradation of lignin and its-derived aromatics. Additionally, transcriptional regulation of these enzymes and metabolic regulation of the short-cut TCA/glyoxylate bicycle system and classical TCA cycle underline the adaptability of white-rot fungi for degrading lignin and lignin-derived aromatics and regenerating NAD(P)H.

White-rot fungi are essential for biodegradation of lignocellulosic biomass, including lignin. These fungi employ a wide range of extracellular and intracellular enzymes, such as LiPs, MnPs, monooxygenases, and dioxygenases, to break down complex polymer lignin into simple molecules. This process significantly contributes to the global carbon cycle and offers potential applications in industrial bioprocessing and bioremediation.

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Declarations

Competing interest The authors have no competing interests to declare that are relevant to the content of this article.

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