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How plants synthesize coenzyme Q

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ABSTRACT

Coenzyme Q (CoQ) is a conserved redox-active lipid that has a wide distribution across the domains of life. CoQ plays a key role in the oxidative electron transfer chain and serves as a crucial antioxidant in cellular membranes. Our understanding of CoQ biosynthesis in eukaryotes has come mostly from studies of yeast. Recently, significant advances have been made in understanding CoQ biosynthesis in plants. Unique mitochondrial flavin-dependent monooxygenase and benzenoid ring precursor biosynthetic pathways have been discovered, providing new insights into the diversity of CoQ biosynthetic pathways and the evolution of phototrophic eukaryotes. We summarize research progress on CoQ biosynthesis and regulation in plants and recent efforts to increase the CoQ content in plant foods.

Keywords: coenzyme Q, 4-hydroxybenzoic acid, mitochondria, biofortification, plant metabolism

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INTRODUCTION

Coenzyme Q (CoQ), also known as ubiquinone, is an essential electron transporter in the oxidative respiratory chain that generates adenosine triphosphate (ATP). CoQ is synthesized by nearly all eukaryotes and some proteobacteria. Structurally, CoQ is composed of a benzoquinone head group attached to a polyisoprenoid tail whose number of isoprene units varies among species: 10 (CoQ_{10}) in humans and some crops (such as tomato and soybean), CoQ_9 in *Arabidopsis thaliana* and rice, CoQ_8 in *Escherichia coli*, and CoQ_6 in yeast (*Saccharomyces cerevisiae*). The quinone head group of CoQ can exist in three oxidation states: the fully oxidized form (CoQ, ubiquinone), the semi-oxidized form with one electron (CoQH·, ubisemiquinone), and the fully reduced form ($CoQH_2$, ubiquinol).

In eukaryotes, CoQ is a central component in mitochondrial oxidative phosphorylation, mediating the electron transfer from complex I (NADH: ubiquinone oxidoreductase) and II (succinate dehydrogenase) to complex III (cytochrome bc_1 oxidoreductase). CoQ also serves as the electron acceptor for several other mitochondrial inner-membrane dehydrogenases (Banerjee et al., 2021) involved in pyrimidine biosynthesis (Evans and Guy, 2004), sulfide detoxification (Zhang et al., 2008; Ziosi et al., 2017), fatty acid β-oxidation, branched-chain amino acid oxidation (Watmough and Frerman, 2010), and so on. CoQ is also a lipid-soluble antioxidant in all cellular compartments (Baschiera et al., 2021). Recently, CoQ was found to be a cofactor of ferroptosis suppressor protein 1, which reduces CoQ to $CoQH_2$ to suppress ferroptosis (Bersuker et al., 2019; Doll et al., 2019). Because it is essential to human health and important in disease prevention and recovery (Cirilli et al., 2021), CoQ is among the most widely consumed dietary supplements (Arenas-Jal et al., 2020).

Biochemical characterizations of CoQ biosynthetic enzymes in eukaryotes have been focused on the yeast model *S. cerevisiae*. In most cases, human functional orthologs were identified through restoration of the respective yeast mutants. Progress in the characterization of CoQ biosynthesis in yeast, humans, and prokaryotes has been reviewed recently (Stefely and Pagliarini, 2017; Awad et al., 2018; Wang and Hekimi, 2019; Abby et al., 2020). The *S. cerevisiae* and human genes required for CoQ biosynthesis are written in capital letters. The corresponding proteins in humans are in uppercase letters, whereas in *S. cerevisiae*, they are written with only the first letter capitalized. We capitalize only the first letter for both genes and proteins of plants in this review: for example, human gene *COQ3*, human protein COQ3, yeast gene *COQ3*, yeast protein Coq3, *Arabidopsis* gene *Coq3*, and *Arabidopsis* protein Coq3. The prokaryotic proteins of CoQ biosynthesis are typically named with a prefix of "Ubi."

The CoQ biosynthetic pathway can be divided into three parts: formation of the aromatic ring precursor, biosynthesis of the polyisoprenoid tail, and modifications of the aromatic ring (Figure 1).

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Figure 1. The plant CoQ biosynthetic pathway.
The plant-specific enzymes are shown in a green background. The B-ring of kaempferol contributes to the 4-HB pool. For 4-HB biosynthesis, 4-HB, 4-hydroxybenzoic acid; PAL, phenylalanine ammonia-lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumarate CoA ligase; CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone 3-hydroxylase; FLS, flavonol synthase; PXA1, peroxisomal ABC transporter one; and UGTs, uridine diphosphate

(legend continued on next page)

Although the eukaryotic CoQ biosynthetic pathway has not been fully defined to date, some components have been found to be conserved across fungi, metazoans, and plants (Toda et al., 2014). Most plants are photo-autotrophic; although photophosphorylation in the chloroplast is the major source of ATP supply, the mitochondrial oxidative respiratory chain is indispensable for plant survival. In recent years, plants have been reported to have special routes for generating the head group and a unique enzyme in the terminal stage. Here, we outline the CoQ biosynthetic pathway in plants and summarize progress in plant CoQ enhancement.

AROMATIC RING PRECURSOR BIOSYNTHESIS

4-hydroxybenzoic acid (4-HB) is the classic benzene quinone ring precursor of CoQ in eukaryotes and prokaryotes. The origins of 4-HB in different species have been reviewed recently (Fernandez-Del-Rio and Clarke, 2021). Here, we discuss 4-HB biosynthetic pathways in plants.

Plants synthesize all proteinogenic amino acids themselves. In plants, 4-HB is derived from either L-phenylalanine or L-tyrosine via independent pathways. Investigations using ^{13}C-labeled compounds demonstrated that phenylalanine is a 2- to 5-fold better precursor than tyrosine in *Arabidopsis* (Block et al., 2014; Soubeyrand et al., 2019). Phenylalanine is deaminated by phenylalanine ammonia-lyase (PAL) and hydroxylated by cinnamate 4-hydroxylase (C4H), producing *p*-coumaric acid in the cytosol (Table 1). The knockout mutant of *C4H* appears to be unable to convert phenylalanine into CoQ (Soubeyrand et al., 2018). Two routes have been shown to convert *p*-coumaric acid into 4-HB. The predominant route is the β-oxidative metabolism of *p*-coumaric acid, unique to plants, in which *p*-coumaric acid is imported into peroxisomes by peroxisomal ATP-binding cassette transporter 1 (PXA1), which also transports other substrates for β-oxidation, including fatty acids and a wide range of bioactive molecules (Bussell et al., 2014; Theodoulou et al., 2005; Zolman et al., 2001). PXA1 has fatty acyl-coenzyme A (CoA) thioesterase activity (De Marcos Lousa et al., 2013), suggesting that the substrates of PXA1 are transported as CoA esters. In peroxisomes, 4-coumarate CoA ligases (AT4G19010 and 4CL8 in *Arabidopsis*) catalyze the formation of *p*-coumaroyl-CoA, initiating β-oxidative side chain shortening. The subsequent steps have been proposed to be similar to the shortening of cinnamoyl-CoA to benzoic acid, involving cinnamoyl-CoA hydratase and dehydrogenase and 3-ketoacyl-CoA thiolase (Widhalm and Dudareva, 2015). However, experimental evidence that these enzymes are responsible for the production of 4-HB is lacking. Loss of function of *PXA1* or *AT4G19010* resulted in a 55%–65% decrease in CoQ content in *Arabidopsis* (Block et al., 2014), suggesting that β-oxidation of *p*-coumaric acid is the dominant route (Figure 1).

In another pathway, *p*-coumaric acid enters flavonoid biosynthesis in the cytosol, as covered and specifically discussed in a recent review (Berger et al., 2022). Genetic analyses and isotopic labeling experiments showed that 4-HB can be derived from kaempferol. First, 4CL3 catalyzes the formation of *p*-coumaroyl-CoA. Chalcone synthase (CHS), the first committed enzyme in flavonoid biosynthesis, carries out the condensation of three malonyl-CoA with *p*-coumaroyl-CoA, leading to the formation of naringenin chalcone, which is converted to kaempferol via the action of chalcone isomerase, flavanone 3-hydroxylase (F3H), and flavonol synthase. Knockout mutants of *4CL3*, *CHS*, and *F3H* displayed a 15%–25% reduction in CoQ content. Then, the B-ring of kaempferol is proposed to be cleaved by heme-dependent peroxidases to generate 4-HB. This reaction requires a free hydroxyl group on *C*3 as well as a *C*2–*C*3 double bond in the C-ring, as naringenin, dihydrokaempferol, and kaempferol 3-β-D-glucopyranoside failed to release 4-HB *in vitro*. The candidate peroxidases have not been identified but are likely to be heme peroxidases because the activity was abolished by azide (Soubeyrand et al., 2018).

In plants, tyrosine also gives rise to 4-HB, but the steps and responsible enzymes have not been identified. Although tyrosine serves as the source of 4-HB in yeast and mammals, the tyrosine-to-4-HB pathway has not been fully defined in any eukaryotes. The first and last reactions in yeast have been characterized (Payet et al., 2016; Stefely et al., 2016). First, the deamination of tyrosine to 4-hydroxyphenylpyruvate (4-HPP) is catalyzed by five redundant aminotransferases (Aro8, Aro9, Bat2, Bna3, and Aat2) (Robinson et al., 2021), and the final step is achieved by aldehyde dehydrogenase Hfd1, which oxidizes 4-hydroxybenzaldehyde to 4-HB. Plants have functional tyrosine aminotransferase (Wang et al., 2016, 2019), but the *in planta* role of tyrosine aminotransferase in 4-HB biosynthesis has not been demonstrated. Hfd1 belongs to the aldehyde dehydrogenase family, and the human enzyme ALDH3A1 restored CoQ biosynthesis of the yeast *Δhfd1* strain (Payet et al., 2016). It is still unknown whether the last step is also conserved in plants. A recent report showed that hydroxyphenylpyruvate dioxygenase-like catalyzes the second reaction in mitochondria of human cells, converting 4-hydroxyphenylpyruvate to 4-hydroxymandelate (Banh et al., 2021). However, *Arabidopsis* has no homolog of hydroxyphenylpyruvate dioxygenase-like. Moreover, it remains unclear whether plant 4-HB production is completed in the mitochondria and how the aromatic ring precursor is transported across the mitochondrial inner membrane.

POLYISOPRENOID TAIL BIOSYNTHESIS

In plants, the isoprene subunits for the CoQ side chain are generated through the mevalonate (MVA) pathway, which also produces precursors for sesquiterpene, triterpene, sterol, and brassinosteroid biosynthesis (Zhou and Pichersky, 2020; Pu et al.,

glycosyltransferases. For the MVA pathway, HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; MVA, mevalonate; MVAP, mevalonate 5-phosphate; MVAPP, mevalonate diphosphate; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; AACT, acetoacetyl-CoA thiolase; HMGS, 3-hydroxy-3-methylglutaryl-CoA synthase; HMGR, 3-hydroxy-3-methylglutaryl-CoA reductase; MK, mevalonate kinase; PMK, phosphomevalonate kinase; MDD, mevalonate diphosphate decarboxylase; and IDI, isopentenyl diphosphate isomerase. For the CoQ pathway in mitochondria, GPP, geranyl diphosphate; FPP, farnesyl diphosphate; GGPP, geranylgeranyl diphosphate; PPPP, polyprenyl-pyrophosphate; PPHB, polyprenyl-hydroxybenzoate; PPDHB, polyprenyl-dihydroxybenzoate; PPVA, polyprenyl-vanillic acid; DDMQ, demethoxy-demethyl-coenzyme Q; DMQ, demethoxy-coenzyme Q; and DMeQ, demethyl-coenzyme Q.

Gene	AGI	Protein	Function	References
C4H	AT2G30490	cinnamate 4-hydroxylase	4-HB biosynthesis from phenylalanine	(Block et al., 2014)
PXA1	AT4G39850	peroxisomal ABC transporter 1		(Block et al., 2014)
AT4G19010	AT4G19010	peroxisomal 4-coumarate CoA ligase	4-HB biosynthesis from phenylalanine	(Block et al., 2014)
4CL8	AT5G38120	peroxisomal 4-coumarate CoA ligase	4-HB biosynthesis from phenylalanine	(Soubeyrand et al., 2019)
4CL3	AT1G65060	cytosolic 4-coumarate CoA ligase	4-HB biosynthesis from phenylalanine	(Soubeyrand et al., 2018)
CHS	AT5G13930	chalcone synthase	4-HB biosynthesis from phenylalanine	(Soubeyrand et al., 2018)
F3H	AT3G51240	flavanone 3-hydroxylase	4-HB biosynthesis from phenylalanine	(Soubeyrand et al., 2018)
IDI1	AT5G16440	isopentenyl diphosphate isomerase	isoprenoid biosynthesis	(Okada et al., 2008; Phillips et al., 2008)
IDI2	AT3G02780	isopentenyl diphosphate isomerase	isoprenoid biosynthesis	(Okada et al., 2008; Phillips et al., 2008)
FPS1	AT5G47770	farnesyl diphosphate synthase	isoprenoid biosynthesis	(Closa et al., 2010; Manzano et al., 2016)
FPS2	AT4G17190	farnesyl diphosphate synthase	isoprenoid biosynthesis	(Closa et al., 2010; Manzano et al., 2016)
Coq1	AT2G34630	polyprenyl diphosphate synthase	isoprene polymerization	(Ducluzeau et al., 2012)
Coq2	AT4G23660	4-hydroxybenzoate polyprenyl diphosphate transferase	*C*3 prenylation	(Okada et al., 2004)
Coq3	AT2G30920	SAM-dependent methyltransferase	*O* methylations	(Avelange-Macherel and Joyard, 1998)
Coq5	AT5G57300	SAM-dependent methyltransferase	*C*2 methylation	(Toda et al., 2014)
Coq6	AT3G24200	flavin-dependent monooxygenase	*C*5 hydroxylation	(Latimer et al., 2021)
CoqF	AT1G24340	flavin-dependent monooxygenase	*C*6 hydroxylation	(Latimer et al., 2021)
Coq4	AT2G03690		scaffold protein?	(Toda et al., 2014)
Coq8	AT4G01660	ATPase		(Toda et al., 2014)
Coq9[a]	AT1G19140	isoprene lipid-binding protein		(Toda et al., 2014)
Coq11A[a]	AT5G10730	atypical short chain dehydrogenase and reductase		(Xu et al., 2021)
Coq11B[a]	AT5G15910	atypical short chain dehydrogenase and reductase		(Xu et al., 2021)

Table 1. Genes involved in CoQ biosynthesis in *Arabidopsis*.
[a]Putative, lacking experimental supporting data.

2021). The mechanism underlying the import of isoprene units into the mitochondria remains uncharacterized.

The polyisoprenoid tail is synthesized in mitochondria by Coq1, a *trans*-polyprenyl diphosphate synthase. The *Arabidopsis* homozygous transfer DNA knockout mutants of *AtCoq1* (also known as *AtSPS3*) are embryo lethal (Ducluzeau et al., 2012). Polyprenyl diphosphate synthases catalyze the condensations of isopentenyl diphosphate with allylic substrates. *In vitro* enzyme assays showed that Coq1 can use dimethylallyl diphosphate, geranyl diphosphate, farnesyl diphosphate (FPP), or geranylgeranyl diphosphate as its allylic diphosphate primers (Ohara et al., 2010; Hsieh et al., 2011). Although the substrates used *in planta* remain uncertain, *Arabidopsis* loss-of-function mutants of *FPS1* or *FPS2* (FPP synthase) had a moderate reduction in CoQ_9 contents (Closa et al., 2010), suggesting that FPP is a potential substrate. *Arabidopsis* contains five genes encoding eranylgeranyl diphosphate synthase (GGPPS), and GGPPS1 (AT1G49530) was shown to be targeted to the mitochondria (Zhu et al., 1997; Okada et al., 2000). It is still unclear whether these GGPPSs are responsible for the biosynthesis of CoQ (Ruiz-Sola et al., 2016), and the means by which the isoprenyl diphosphate substrates are transported into the mitochondria awaits elucidation.

Coq1 is believed to determine the side-chain length of CoQ, which varies among species. Based on its final product, Coq1 can be named hexaprenyl diphosphate synthase, octaprenyl diphosphate synthase, solanesyl diphosphate synthase, and decaprenyl

Family	Species	Common name	Predominant form of CoQ	References
Poaceae	*Oryza sativa*	Rice	CoQ_9	(Ikeda and Kagei, 1979)
Poaceae	*Triticum aestivum*	Wheat	CoQ_9	(Ikeda and Kagei, 1979)
Poaceae	*Zea mays*	Maize	CoQ_9	(Threlfall and Whistance, 1970)
Cucurbitaceae	*Cucumis melo*	Muskmelon	CoQ_9	(Threlfall and Whistance, 1970)
Cucurbitaceae	*Cucumis sativus*	Cucumber	CoQ_9	(Threlfall and Whistance, 1970)
Asteraceae	*Lactuca sativa*	Cultivated lettuce	CoQ_9	(Threlfall and Whistance, 1970)
Asteraceae	*Cichorium intybus*	Chicory	CoQ_9	(Threlfall and Whistance, 1970)
Ericaceae	*Vaccinium vitis-idaea*	Cowberry	CoQ_9	(Mattila and Kumpulainen, 2001)
Brassicaceae	*Arabidopsis thaliana*	Thale cress	CoQ_9	(Xu et al., 2021)
Brassicaceae	*Brassica oleracea* var. *botrytis*	Cauliflower	CoQ_{10}	(Mattila and Kumpulainen, 2001)
Brassicaceae	*Brassica rapa* subsp. *pekinensis*	Chinese cabbage	CoQ_{10}	(Kettawan et al., 2007)
Fabaceae	*Glycine max*	Soybean	CoQ_{10}	(Ikeda and Kagei, 1979)
Fabaceae	*Pisum sativum*	Pea	CoQ_{10}	(Mattila and Kumpulainen, 2001)
Fabaceae	*Arachis hypogaea*	Peanut	CoQ_{10}	(Ikeda and Kagei, 1979)
Solanaceae	*Solanum lycopersicum*	Tomato	CoQ_{10}	(Mattila and Kumpulainen, 2001)
Solanaceae	*Solanum tuberosum*	Potato	CoQ_{10}	(Mattila and Kumpulainen, 2001)
Solanaceae	*Solanum melongena*	Eggplant	CoQ_{10}	(Kettawan et al., 2007)
Apiaceae	*Daucus carota*	Carrot	CoQ_{10}	(Mattila and Kumpulainen, 2001)
Apiaceae	*Petroselinum crispum*	Parsley	CoQ_{10}	(Kettawan et al., 2007)
Rosaceae	*Malus domestica*	Apple	CoQ_{10}	(Mattila and Kumpulainen, 2001)
Rutaceae	*Citrus clementina*	Clementine	CoQ_{10}	(Mattila and Kumpulainen, 2001)

Table 2. Predominant forms of CoQ in different plant species.

diphosphate synthase, which generate chains of 6 (C30), 8 (C40), 9 (C45), and 10 (C50) isoprene units, respectively. In plants (Table 2), several species of Poaceae, Cucurbitaceae, and Asteraceae have been shown to synthesize mainly CoQ_9, whereas CoQ_{10} is predominant in Fabaceae and Solanaceae (Threlfall and Whistance, 1970; Ikeda and Kagei, 1979). Species in the same family may produce different CoQs. For example, in Brassicaceae, *A. thaliana* contains primarily CoQ_9, but cauliflower (*Brassica oleracea* var. *botrytis*) accumulates CoQ_{10} (Mattila and Kumpulainen, 2001). The mechanism by which Coq1 determines the product length remains enigmatic, although studies in bacteria have proposed a "single-floor" or "double-floor" model for octaprenyl diphosphate synthases (Guo et al., 2004; Han et al., 2015).

AROMATIC RING MODIFICATIONS

The aromatic head is decorated into a fully substituted benzoquinone ring in mitochondria by one prenylation, one decarboxylation, three hydroxylations, and three methylations. First, the isoprene tail is attached to 4-HB, and then the ring is hydroxylated at *C*5, followed by *O*-methylation. After sequential decarboxylation and hydroxylation at *C*1, the ring is further modified via *C*2 methylation, *C*6 hydroxylation, and *O* methylation (Figure 1). The order of these reactions in eukaryotes is still debatable (Acosta Lopez et al., 2019; Fernandez-Del-Rio and Clarke, 2021). Among the enzymes characterized, four (Coq2, Coq3, Coq5, and Coq6) are conserved across plants, fungi, and mammals. The recently identified CoqF is a unique flavin-dependent monooxygenase prevalent in plants and green algae but distinct from its counterpart in fungi and Metazoa. Besides these enzymes, a number of proteins without a clear catalytic role are also involved in CoQ biosynthesis.

Prenylation

Coq2, 4-hydroxybenzoate polyprenyl diphosphate transferase, transfers the polyisoprenoid chain to the 4-HB ring (Figure 1), generating the first lipophilic CoQ intermediate. *Arabidopsis Coq2* (also known as *AtPPT1*) was able to complement the yeast *coq2* deletion strain, and the knockout mutant of *Arabidopsis* was embryo lethal at an early stage (Okada et al., 2004).

Hydroxylations

CoQ biosynthesis requires hydroxylation on three positions of the aromatic ring: *C*1, *C*5, and *C*6. In *E. coli*, *C*1 hydroxylation is catalyzed by UbiH, a group A flavin-dependent monooxygenase (Pelosi et al., 2016). The *C*1-hydroxylase is unidentified in all eukaryotes.

The *C*5 hydroxylation is mediated by Coq6, a flavin-dependent monooxygenase that is conserved in eukaryotes, including plants (Toda et al., 2014). In the yeast and *E. coli* mutant strains in which the *C*5-hydroxylase-encoding gene was disrupted, CoQ production was recovered when *Arabidopsis Coq6* was introduced (Latimer et al., 2021); however, the function of AtCoq6 *in planta* has yet to be characterized.

In yeast and humans, the *C*6 hydroxylation is catalyzed by Coq7, a di-iron protein. However, plants do not have a Coq7 homolog.

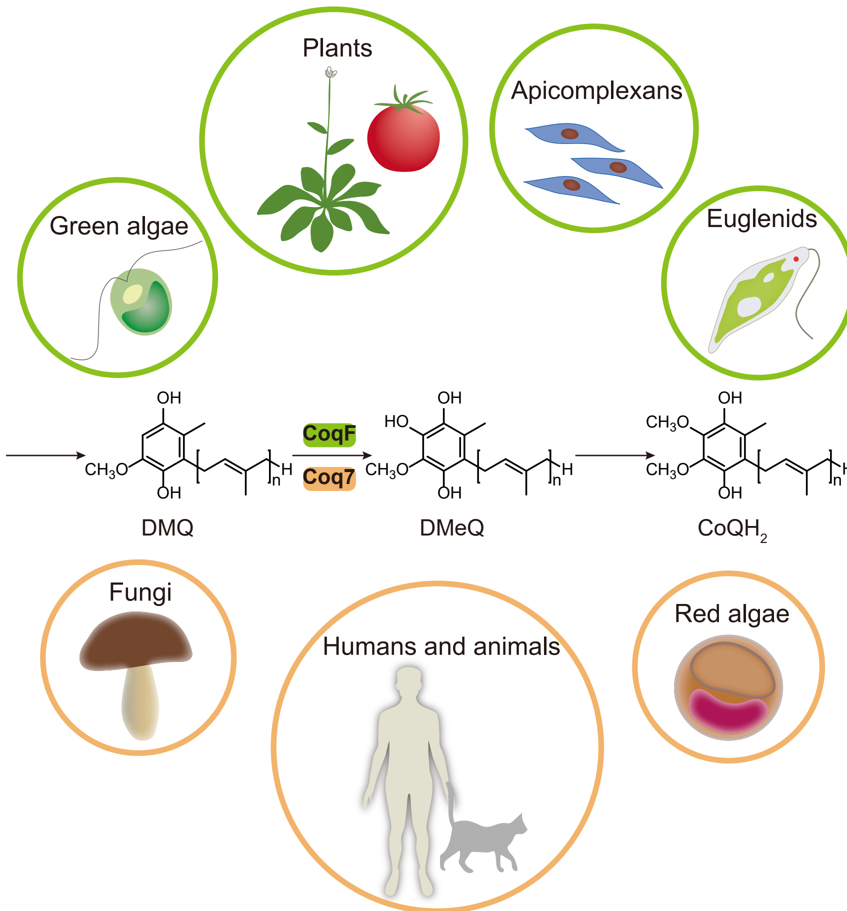

Figure 2. The diversity of CoQ biosynthesis in eukaryotes: Two different enzymes catalyze the penultimate step.
Coq7, a di-iron monooxygenase, occurs in major lineages of eukaryotes, including Metazoa (animals), fungi, red algae, etc. The recently discovered flavin-dependent monooxygenase CoqF catalyzes this reaction in land plants, green algae, apicomplexans, euglenids, and some others. For details, see Xu et al. (2021).

Recently, two teams independently reported that plants use a unique enzyme, which we named CoqF, to complete this last hydroxylation step (Latimer et al., 2021; Xu et al., 2021). CoqF restored CoQ biosynthesis when expressed in the yeast *coq7* mutant and the *E. coli ubiF* (encoding *C6* hydroxylase in some Gammaproteobacteria; Pelosi et al., 2016) mutant. Insertional mutations of *AtCoqF* resulted in embryo lethality that could be rescued by either human COQ7 or *E. coli* UbiF. In addition, suppression of *CoqF* expression in *Nicotiana benthamiana* via virus-induced gene silencing (VIGS) resulted in CoQ deficiency. CoqF is a unique flavin-dependent monooxygenase, in that it has a C-terminal extension (~200 amino acids), distinct from other flavin-dependent enzymes previously identified to participate in CoQ biosynthesis. Besides being ubiquitous in land plants, CoqF is also widely distributed in several other major groups of eukaryotes, such as green algae, Cryptista, Haptista, Stramenopiles, Alveolata, and Rhizaria, and it occurs sporadically in other eukaryotic domains owing to lateral gene transfers, providing an excellent marker for distinguishing eukaryotes (Xu et al., 2021). Notably, apicomplexan parasites such as *Plasmodium falciparum* and *Toxoplasma gondii* all have CoqF instead of Coq7 (Figure 2). Thus, CoqF represents an interesting antiparasitic drug target for further exploration.

Methylations

There are two methoxyl groups at *C5* and *C6* of the benzoquinone ring, both of which are methylated by Coq3 in eukaryotes (Poon et al., 1999). It is likely that these reactions require the substrate in reduced form (quinol). *Arabidopsis Coq3* restored CoQ production when expressed in a yeast *coq3* deletion mutant (Avelange-Macherel and Joyard, 1998). A transfer DNA insertion line of *AtCoq3* was characterized as an embryo-defective mutant with an early stage of arrest by the SeedGenes project (Meinke, 2020).

Coq5 catalyzes the *C2* methylation (*C* methylation) in eukaryotes. *Arabidopsis Coq5* was shown to be functional in the fission yeast *Schizosaccharomyces pombe* (Toda et al., 2014), but its function *in planta* has not been confirmed.

Decarboxylation

In *E. coli*, the decarboxylation is catalyzed by the decarboxylase UbiD (Cox et al., 1969), supported by flavin prenyltransferase UbiX that produces a highly modified flavin cofactor for UbiD activity (White et al., 2015). The *C1* decarboxylation in eukaryotes is still a mystery.

Other proteins

Besides the enzymes discussed above, several yeast proteins have been found to be required for efficient biosynthesis of CoQ, including Coq4, Coq8, Coq9, and Coq11; their homologs all exist in *Arabidopsis*.

Coq4 is thought to act as a scaffold protein that organizes the CoQ biosynthetic complex (Marbois et al., 2009), but the precise mechanisms are unclear. Coq8 is a member of an atypical kinase family, and it exhibits a conserved ATPase activity that is activated by CoQ-like phenolic compounds and cardiolipin-containing liposomes (Reidenbach et al., 2018). Overexpression of Coq8 in yeast *coq*-null mutants stabilized the remaining CoQ biosynthesis polypeptides (Xie et al., 2011, 2012). Expression of *Arabidopsis* Coq4 and Coq8 recovered CoQ biosynthesis in the *S. pombe coq4*- and *coq8*-null mutants, respectively (Toda et al., 2014).

Coq9 is a lipid-binding protein. In human models, it has been proposed that COQ9 may act as a "lipid presenter" to deliver intermediates directly to COQ7 (Lohman et al., 2019). For instance, COQ9 physically interacts with COQ7 and is required for *C6*

hydroxylation catalyzed by COQ7 (Lohman et al., 2014, 2019). However, plants use the flavin-dependent CoqF instead of the di-iron Coq7 as the *C*6 hydroxylase, and the *Arabidopsis* homolog of Coq9 failed to complement the *S. pombe coq9* deletion (Toda et al., 2014). Whether and how Coq9 participates in plant CoQ biosynthesis is an open question.

Coq11, a member of the atypical short-chain dehydrogenase and reductase superfamily, was identified as a constituent of the CoQ biosynthetic complex via affinity purification of tagged Coq proteins in yeast (Allan et al., 2015). The genome of *Arabidopsis* encodes several homologs of *Coq11*, two of which (*Coq11A* and *Coq11B*) show co-expression with CoQ biosynthesis (Xu et al., 2021). Again, their function has not been demonstrated.

Biosynthetic complex

Enzymes that act downstream of polyprenyl-hydroxybenzoate and additional associated proteins form a biosynthetic complex known as the CoQ-synthome or Complex Q. In yeast, Coq3–Coq9 and Coq11 are members of the complex. The CoQ-synthome also exists in humans (Floyd et al., 2016). In *E. coli*, seven Ubi proteins form a stable metabolon that synthesizes ubiquinone in the cytosol (Hajj Chehade et al., 2019). In plants, such a complex awaits exploration.

THE EVOLUTION OF PLANT CoQ BIOSYNTHESIS

Plants have evolved the unique ability to synthesize 4-HB from phenylalanine via two parallel routes. The phenylpropanoid pathway also serves as a starting point for the production of a variety of metabolites such as lignin, flavonoids, coumarins, and lignans. A recent analysis of the evolutionary history of key enzymes in the phenylpropanoid pathway showed that the enzymes involved in making *p*-coumaroyl-CoA from phenylalanine (PAL, C4H, and 4CL), are generally present across Embryophyta (de Vries et al., 2021). Homologs of the genes encoding PAL and 4CL can even be found in streptophyte algae, the algal sisters of land plants. The main route that opens up after the synthesis of *p*-coumaroyl-CoA is β-oxidative metabolism in the peroxisomes. In *Arabidopsis*, two peroxisomal 4CLs (AT4G19010 and 4CL8) have been identified to participate in this pathway. In the acyl-activating enzyme superfamily, AT4G19010 and 4CL8 belong to clade V (Shockey and Browse, 2011), which also contains several members that exhibit high activities toward fatty acids (Kienow et al., 2008). A phylogenetic analysis showed that the clade V members of *Arabidopsis* fell into a clade that included sequences from major lineages of land plants. It would be interesting to see whether peroxisomal 4CLs are widely distributed in plants. The second route is the conversion of *p*-coumaric acid into kaempferol. Among flavonoids, kaempferol is a flavonol that is widely present in land plant species ranging from bryophytes and ferns to seed plants, often in the form of glycosides (Iwashina and Murai, 2013). Notably, mammalian cells also have the ability to use exogenous kaempferol as a precursor for CoQ biosynthesis (Fernandez-Del-Rio et al., 2017). The mechanism of release of a CoQ ring precursor from kaempferol is likely to be conserved between plants and mammals (Fernandez-Del-Rio et al., 2020).

Unlike animals and fungi, in which the *C*6 hydroxylation is catalyzed by Coq7, plants use CoqF (Figure 2). Although the two enzymes are functional counterparts, they are evolutionarily unrelated and have different origins: Coq7 is a di-iron hydroxylase, whereas CoqF belongs to an isolated subfamily of flavoenzymes. It has been proposed that CoqF emerged early during eukaryotic diversification and then became dominant among photosynthetic and related organisms. Both CoqF and Coq7 were found in Prasinodermophyta and Chlorophyta, but usually in different sublineages. In streptophyte algae and land plants, CoqF is the sole *C*6 hydroxylase, implying an adaptive advantage of this unique flavoenzyme in aerobic respiration during plant terrestrialization (Xu et al., 2021).

The bioactive compound shikonin and its derivatives are a group of red-pigmented naphthoquinones produced in many members of the family Boraginaceae. The shikonin and CoQ pathways share precursors and contain similar biochemical architectures, and evolutionary links between CoQ and shikonin biosynthesis have been identified in red gromwell, *Lithospermum erythrorhizon* (Auber et al., 2020; Suttiyut et al., 2022). The *p*-hydroxybenzoate:geranyltransferase genes and several gene candidates for shikonin biosynthesis were found to have evolved via duplication of the CoQ pathway genes. Further investigation of CoQ biosynthesis is likely to help gene discovery for the biosynthesis of shikonins and other quinone-bearing specialized metabolites.

REGULATION OF CoQ BIOSYNTHESIS

Regulation of CoQ biosynthesis in yeast and mammals has been reviewed recently, and Coq7 seems to be a key regulatory hub that integrates endogenous and environmental signals (Villalba and Navas, 2021). Far less is known about the regulation of CoQ biosynthesis in plants. Since plants use a distinct flavoenzyme in place of Coq7, it is likely that plants employ a different regulatory mechanism.

According to the *Arabidopsis* Electronic Fluorescent Pictograph Browser (Winter et al., 2007), *Coq* genes are highly expressed in seeds. Congruently, CoQ is distributed throughout the plant and more abundant in seeds (Xu et al., 2021). Analysis of *Arabidopsis* demonstrated the co-expression of genes involved in CoQ biosynthesis in mitochondria (Ducluzeau et al., 2012). In addition, genes involved in 4-HB biosynthesis (*AT4G19010*, *C4H*, *4CL3*, *CHS*, *CHI*, *F3H*, and *FLS1*) and the MVA pathway (*HMGR1*, *HMGR2*, and *HMGS*) in the cytosol were also co-expressed with the *Coq* genes (Ducluzeau et al., 2012; Soubeyrand et al., 2018). It will be interesting to analyze the regulatory mechanisms at the transcriptional level.

It remains elusive how plants synthesize CoQ in response to environmental stimuli. A recent report showed that continuous high-light treatments promoted the *de novo* biosynthesis of CoQ in *Arabidopsis* (Soubeyrand et al., 2019); the underlying signaling pathway is worthy of investigation.

CoQ_{10} BIOFORTIFICATION

CoQ is endogenously synthesized in the human body, and CoQ levels decrease as people age (Ernster and Forsmark-Andree,

1993). In addition, certain cholesterol-lowering drugs, such as statins, inhibit 3-hydroxy-3-methylglutaryl-CoA reductase activity, resulting in a decrease in CoQ (Bhagavan and Chopra, 2006). Dietary intake from food is another source of CoQ; however, this supply is not always sufficient because plant-based foods, particularly vegetables, fruits, and cereals, are generally low in CoQ contents (Pravst et al., 2010; Parmar et al., 2015). In addition, many plants synthesize CoQ_9 instead of CoQ_{10} as the principal CoQ molecule. Thus, there is a great need for engineering CoQ_{10} production in plants. Major strategies used for CoQ_{10} biofortification include increasing precursor availability and overexpressing rate-limiting enzymes in mitochondria.

Increasing the precursor supply

4-HB serves as the skeleton for the CoQ head group. When 4-HB was fed to *Arabidopsis* seedlings, CoQ accumulated to as much as 150% of the control level (Soubeyrand et al., 2021). Overexpression of *AT4G19010* or *4CL8*, which encode 4-coumarate CoA ligases responsible for 4-HB production in peroxisomes, increased CoQ accumulation to ~150% of the wild-type level (Block et al., 2014; Soubeyrand et al., 2019). In knockout mutants of genes encoding kaempferol 3-*O*-glycosyltransferase that restricts the supply of 4-HB, CoQ content was elevated to 160% of the wild-type level (Soubeyrand et al., 2021). These results indicated that 4-HB supply limits plant CoQ biosynthesis.

Precursors of the isoprene tail are produced from the cytosolic MVA pathway. In *Nicotiana tabacum*, expression of a bacterial phosphomevalonate decarboxylase that increases available isopentenyl phosphate resulted in increased production of MVA-derived terpenoids; however, CoQ accumulation was not affected (Henry et al., 2018). One reason might be that the isoprenoid subunits produced in the cytosol were not efficiently transported to mitochondria.

Overcoming downstream rate-limiting steps

In mitochondria, transfer of the polyprenyl chain to 4-HB by Coq2 is considered to be a rate-limiting step in CoQ biosynthesis. Overexpression of *Coq2* in *Salvia miltiorrhiza* resulted in up to a 3-fold increase in CoQ content (Liu et al., 2019). Heterologous expression of a yeast *Coq2* in tobacco led to elevation of CoQ content, which was two times higher than that of the wild-type control (Ohara et al., 2004).

Recently, encouraging progress was achieved in tomato, which showed a 7-fold increase in CoQ_{10} production following overexpression of four genes encoding *Arabidopsis* 3-hydroxy-3-methylglutaryl-CoA reductase, *E. coli* chorismate pyruvate-lyase UbiC (which catalyzes the removal of pyruvate from chorismate to produce 4-HB), and tobacco Coq1 and Coq2, all driven by the tomato-fruit-specific *E8* promoter (Fan et al., 2021). The results provide evidence that Coq1 and Coq2, which catalyze the decaprenyl chain formation and attachment in mitochondria, play a key role in determining the final CoQ_{10} yield.

Modifying the side-chain length

Another problem encountered in engineering CoQ in plants is that most cereal crops, as well as some vegetables and fruits, predominantly produce CoQ_9, whereas human mitochondria synthesize CoQ_{10}. Side-chain length has been shown to be a critical factor for CoQ biological activity, and the efficacies of CoQ analogs for medical purposes have been reviewed recently (Suarez-Rivero et al., 2021). Wang and Hekimi generated conditional *Coq7* (also known as *Mclk1*) knockout mouse embryonic fibroblasts in which CoQ_9 was undetectable and exogenously applied CoQs of varying isoprenoid chain length. CoQ_9, which is the original species of CoQ in mice, appeared to improve respiratory chain activity more effectively than other CoQs tested (Wang and Hekimi, 2013). Another investigation, based on the kinetics of bovine respiratory complex I catalysis with a series of CoQs of different isoprenoid side-chain lengths (from 1 to 10 units), suggested that CoQ_{10} has both the highest binding affinity and the fastest binding rate (Fedor et al., 2017).

The length of the side chain is determined by Coq1. In rice and *Panicum meyerianum*, expression of decaprenyl diphosphate synthase from *Gluconobacter suboxydans* modified the length of the CoQ side chain from 9 to 10 isoprene units (Takahashi et al., 2006, 2009, 2010; Seo et al., 2011). The mechanism of Coq1-mediated chain-length determination is, however, poorly understood. Characterization of the catalytic steps will facilitate the development of CoQ_{10}-enriched crops by selection of appropriate natural variations and genome editing.

FUTURE PERSPECTIVES

CoQ biosynthesis has been studied mainly in yeast and human cells, and thus our understanding of the diversity of the CoQ biosynthetic pathway in eukaryotes is highly limited. Recent investigations in plants have identified several unique enzymes in the plant CoQ biosynthetic pathway. It will be of great interest to search for the enzymes and cofactors that act in the steps that have not been identified in plants, and this should help us to understand the evolution of oxidative respiration from bacteria to eukaryotes.

In plants, CoQ is important for growth and development. *Arabidopsis* mutants lacking Coq1, Coq2, Coq3, or CoqF are embryo lethal (Avelange-Macherel and Joyard, 1998; Okada et al., 2004; Ducluzeau et al., 2012; Xu et al., 2021). Because CoQ is an essential component in mitochondrial oxidative phosphorylation, plant-specific enzymes of CoQ biosynthesis are potential targets for herbicide development.

At the moment, microbial fermentation is the major industrial source of CoQ_{10}. Enhancement in plant foods can be a cost-effective and environmentally friendly strategy for improving CoQ_{10} supply. However, to date, little attention has been paid to CoQ_{10} biofortification, and current engineering strategies have had a relatively modest impact on CoQ_{10} production in plants. Recently, a genome-wide genetic screen of yeast identified 30 previously unknown regulators of CoQ accumulation; phospholipid metabolism was confirmed to be a key player, as deficiency in phosphatidylethanolamine methylation resulted in a 5-fold increase in CoQ (Ayer et al., 2021). It would be particularly interesting to test whether some of these regulators are functionally conserved in plants. A better understanding of the ubiquinone biosynthetic pathway and its regulatory mechanisms in plants will facilitate the breeding of CoQ_{10}-enriched crop cultivars, hopefully in the near future.

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