REVIEW

Apocarotenoids: hormones, mycorrhizal metabolites and aroma volatiles

Michael H. Walter · Daniela S. Floss · Dieter Strack

Abstract Apocarotenoids are tailored from carotenoids by oxidative enzymes [carotenoid cleavage oxygenases (CCOs)], cleaving specific double bonds of the polyene chain. The cleavage products can act as hormones, signaling compounds, chromophores and scent/aroma constituents. Recent advances were the identification of strigolactones as apocarotenoids and the description of their novel role as shoot branching inhibitor hormones. Strigolactones are also involved in plant signaling to both harmful (parasitic weeds) and beneficial [arbuscular mycorrhizal (AM) fungi] rhizosphere residents. This review describes the progress in the characterization of CCOs, termed CCDs and NCEDs, in plants. It highlights the importance of sequential cleavage reactions of C_{40} carotenoid precursors, the apocarotenoid cleavage oxygenase (ACO) nature of several CCOs and the topic of compartmentation. Work on the biosynthesis of abundant C_{13} cyclohexenone and C_{14} mycorradicin apocarotenoids in mycorrhizal roots has revealed a new role of CCD1 as an ACO of C_{27} apocarotenoid intermediates, following their predicted export from plastid to cytosol. Manipulation of the AM-induced apocarotenoid pathway further suggests novel roles of C_{13} apocarotenoids in controlling arbuscule turnover in the AM symbiosis. CCD7 has been established as a biosynthetic crosspoint, controlling both strigolactone and AM-induced C_{13} apocarotenoid biosynthesis. Interdependence of the two apocarotenoid pathways may thus play a role in AM-mediated reduction of parasitic weed infestations. Potential scenarios of C_{13} scent/aroma volatile biogenesis are discussed, including the novel mechanism revealed from mycorrhizal roots. The recent progress in apocarotenoid research opens up new perspectives for fundamental work, but has also great application potential for the horticulture, food and fragrance industries.

Abbreviations

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ACO	Apocarotenoid cleavage oxygenase
AM	Arbuscular mycorrhiza(l)
CCD	Carotenoid cleavage dioxygenase
CCO	Carotenoid cleavage oxygenase
MEP	Methylerythritol phosphate
DXS	1-Deoxy-D-xylulose 5-phosphate synthase
NCED	Nine-*cis*-epoxycarotenoid dioxygenase

Introduction

Carotenoids are vital not only in their intact form, but also constitute an important precursor reservoir for the biosynthesis of bioactive compounds in bacteria, fungi, animals and plants. The multifaceted carotenoid cleavage products (apocarotenoids) of plants include pigments, aroma and scent compounds, as well as regulatory molecules and compounds with yet unknown functions (Giuliano et al. 2003; Bouvier et al. 2005; Auldridge et al. 2006b; Walter et al. 2007; Ohmiya 2009). Specific tailoring of carotenoids to

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Fig. 1 Diversity of carotenoid cleavage products including several polyene chromophores. The double bond cleaved for specific products is indicated in the C_{40} carotenoid precursors. Moieties of unknown metabolic fate are indicated in *gray*
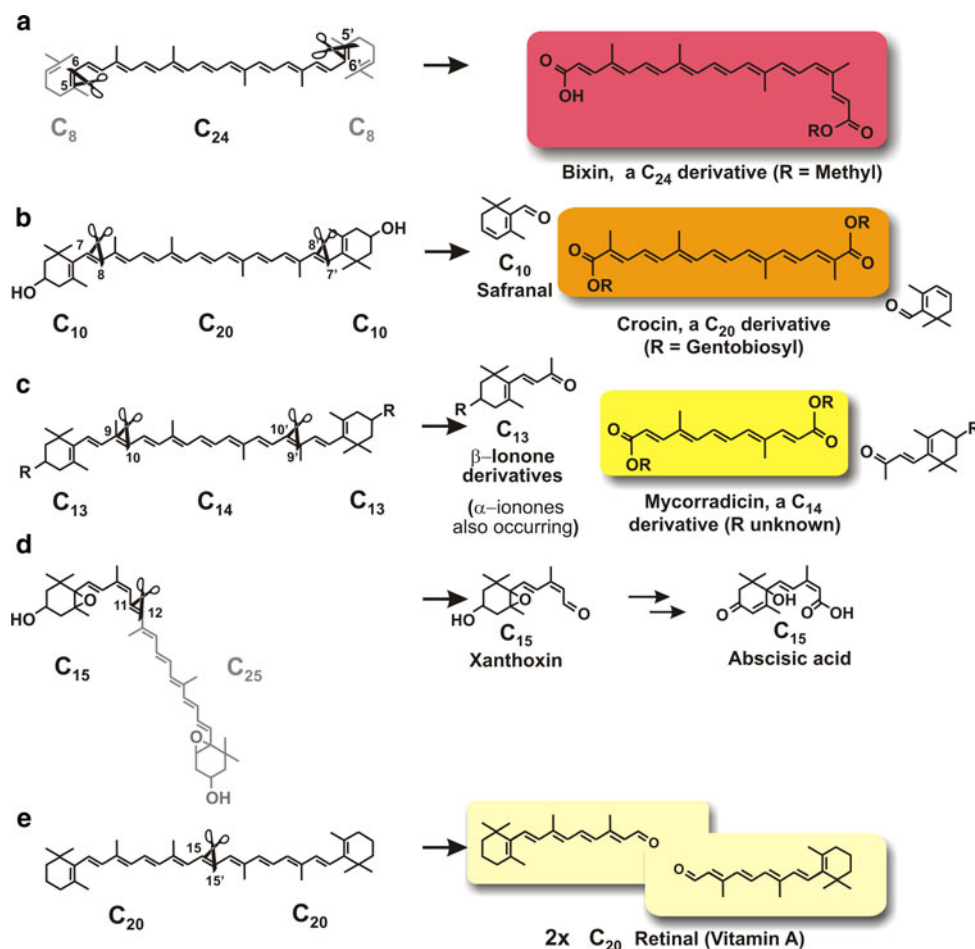
apocarotenoids is performed by regiospecific oxidative enzymes targeting different double bonds of the carotenoid polyene chain (Fig. 1). These carotenoid cleavage oxygenases (CCOs) can act via a single cleavage of C_{40} carotenoid substrates or via several sequential cleavage events, leading to a multitude of products with various functions and human uses.

Two different apocarotenoid pigments of commercial value are formed by plant reproductive tissues. The first is bixin, a polyene derived from the central part of C_{40} lycopene after an enzymatic cleavage of the 5–6 and 5′–6′ double bonds (Fig. 1a). Bixin is the monomethylester of the C_{24} dicarboxylic acid norbixin. It is obtained from the seeds of *Bixa orellana* (the annatto bush native to Central and South America) and is used as a food colorant or lipstick ingredient, covering a range of yellow to red colors. Engineering bixin biosynthesis into *Escherichia coli* via transfer of three biosynthetic genes from *B. orellana* has been reported (Bouvier et al. 2003a).

Both coloring and seasoning food are important features of saffron contained in the stigmas of *Crocus sativus*. The color originates from a C_{20} polyene derivative, tailored from C_{40} zeaxanthin, via cleavage of the 7–8 and 7′–8′

double bonds (Fig. 1b). The C_{20} dicarboxylic acid crocetin occurs as digentiobiosyl ester termed crocin. The compound, which is mainly responsible for saffron's flavor, is the glucoside picrocrocin of the C_{10} cyclohexene cleavage product safranal. The volatile safranal can be released from the glucoside to add to saffron's aroma. The molecular basis of saffron biogenesis has been investigated (Bouvier et al. 2003b; Rubio et al. 2008; Moraga et al. 2009; Ahrazem et al. 2010).

Dual excentric cleavage of the 9–10 and 9′–10′ double bonds of plant C_{40} carotenoids also results in colored moieties along with aroma/scent-bearing ones (Fig. 1c). However, derivatives of the colored central C_{14} cleavage product accumulate only in roots colonized by arbuscular mycorrhizal fungi (see below). On this basis, the C_{14} dicarboxylic acid has been termed mycorradicin (Klingner et al. 1995), but later studies have shown mycorradicin to be present also in nonmycorrhizal roots of some species albeit in very low concentrations (Fester et al. 2002). In all other cases, C_{14} polyene cleavage products are usually undetectable and the metabolic fate of the primary C_{14} dialdehyde cleavage product remains unknown. On the contrary, derivatives of the C_{13} cyclohexene moieties are frequently found

in many flowers and fruits as well as in mycorrhizal roots (Bouvier et al. 2005; Auldridge et al. 2006b; Strack and Fester 2006). They contribute to rose scent (Eugster and Märki-Fischer 1991; Huang et al. 2009a), tomato aroma (Simkin et al. 2004a) and wine bouquet (Mendes-Pinto 2009) to name just a few. Again, glycosylation is common, apparently serving as a general storage principle of aroma compounds (Maicas and Mateo 2005).

The best-studied plant apocarotenoid is the hormone abscisic acid (ABA, C_{15}). The apocarotenoid nature of plant ABA formed via a so-called indirect pathway through cleavage of C_{40} carotenoids is now well established (Schwartz et al. 2003; Fig. 1d). A direct pathway via C_{15} farnesyl diphosphate, which has earlier also been considered for plants, operates only in certain fungi (Nambara and Marion-Poll 2005). Carotenoid cleavage toward ABA biogenesis is unique among apocarotenoid biosynthetic routes in that only *cis*-isomers of C_{40} carotenoid substrates (9-*cis* violaxanthin and 9-*cis* neoxanthin) are accepted by a specific type of cleavage enzyme. A single cleavage results in the formation of the C_{15} ABA precursor xanthoxin and of a C_{25} cleavage product. The metabolic fate of the latter is unknown. Neoxanthin isomers appear to be the major route for stress-induced ABA accumulation in Arabidopsis as concluded from the study of the *aba4* mutant (North et al. 2007). However, this study also demonstrated that ABA4 does not carry out the isomerization of *trans*-violaxanthin to the *cis*-isomer, and that an independent enzyme must be responsible for isomerization, which remains to be identified. With this exception, most other steps of ABA biosynthesis as well as its catabolism have been studied extensively, frequently using viviparous mutants compromised in seed dormancy and stomata closure regulation (reviewed by Nambara and Marion-Poll 2005). Perception and transduction of the ABA signal is less well understood as illustrated by the description of several different putative ABA receptors in the last few years. However, finally converging studies on ABA receptor function have recently considerably improved our understanding of ABA signaling (commented in summary by Sheard and Zheng 2009).

Outside the plant kingdom, in fruit flies and mammals, including humans, central cleavage of C_{40} carotenoids at the 15–15′ double bond is the dominating cleavage type (Fig. 1e; von Lintig and Vogt 2004), but excentric cleavage being commonplace in plants does also occur (Kiefer et al. 2001). This is explained by the many essential functions of C_{20} retinal-derived products, including the vitamin A function for color vision or retinoic acid-mediated signaling in these organisms. Retinoids serving as photosensory pigment and as regulators are also found in eubacteria and fungi. A number of fungal and cyanobacterial genes and their protein products, involved in biosynthesis, modification and cleavage reactions of carotenoids (both central and

excentric cleavage), have recently been characterized (Prado-Cabrero et al. 2007; Scherzinger and Al-Babili 2008; Alder et al. 2009; Estrada et al. 2009). Other fungal apocarotenoids with more clearly defined functions in sexual communication are C_{18} trisporic acid derivatives (Schimek and Wöstemeyer 2009).

This review aims to collect recent molecular data on apocarotenoid biosynthesis to improve our understanding of carotenoid metabolism and the multiple biological functions derived from it. It will highlight the recent breakthrough identification of the long-sought carotenoid-derived shoot branching inhibitor hormone as a strigolactone derivative. It will further discuss implications from the results of several knockdown approaches of apocarotenoid biosynthetic genes on novel functions of apocarotenoids in the arbuscular mycorrhizal symbiosis. The use of the experimental system of AM-induced apocarotenoid biosynthesis has also provided new insights into the importance of subcellular compartmentation of enzymes and intermediates in a highly regulated carotenoid catabolism potentially applicable to scent and aroma compound biogenesis. The key players in the process of a double bond-specific oxidative tailoring of carotenoids are the members of a family of evolutionarily conserved CCOs present in eubacteria, algae, fungi, animals and plants.

Carotenoid tailoring enzymes

All enzymes committed to carotenoid cleavage are members of an ancient, highly heterogeneous superfamily of polyene chain oxygenases distributed across all taxa (Ryle and Hausinger 2002). Lipoxygenases and peroxidases can also cleave carotenoids as co-substrates, but whether this activity serves specific biological roles is unclear. The common characteristic of all the committed carotenoid cleavage enzymes is a non-heme iron cofactor requirement. However, apart from four strictly conserved histidine residues involved in iron and oxygen binding and a few glutamate and aspartates fixing them, there is little amino acid sequence similarity recognizable between the various clades of CCOs (Kloer and Schulz 2006). Any of the four histidines is essential for activity as has been experimentally confirmed for mouse β-carotene-15–15′-monooxygenase (Poliakov et al. 2005) and cyanobacterial NSC1 (Marasco et al. 2006). Although the longstanding controversy about a mono- or di-oxygenase reaction type appears not to be finally settled (Kloer and Schulz 2006), the plant enzymes are usually considered to act as dioxygenases and are therefore called carotenoid cleavage dioxygenases (CCDs). A monooxygenase mechanism has been proposed for mammalian 15–15′ carotenoid oxygenases (Poliakov et al. 2005) and recently also for some related enzymes

cleaving stilbenes (Marasco and Schmidt-Dannert 2008). The conflicting results in terms of incorporating either two labeled oxygen atoms from O_2 (dioxygenase) or one labeled oxygen atom from O_2 and one from water (mono-oxygenase) have been explained by exchange of label between water and the primary cleavage product, thus arguing for a uniform dioxygenase mechanism in all cases (Schmidt et al. 2006; Auldridge et al. 2006b).
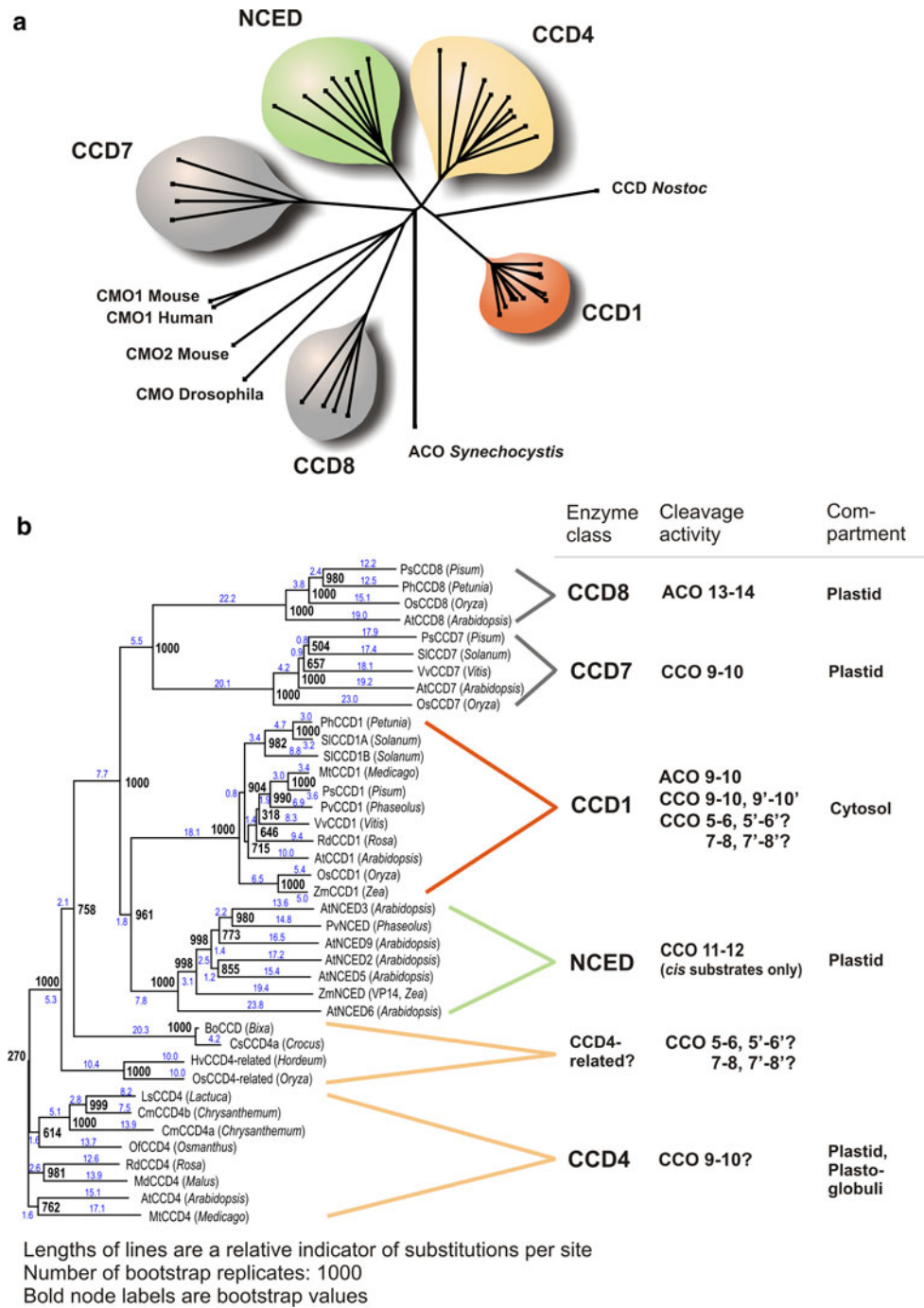
Assembling 40 different CCO sequences from various organisms illustrates the evolution of various enzyme clades with biochemically distinct functions (Fig. 2a). The cyanobacterial apocarotenoid-15–15′-oxygenase (apocarotenoid cleavage oxygenase, ACO) from *Synechocystis* sp. PCC 6803 has been used as an outgroup. This enzyme has been crystallized, and its three-dimensional structure has been determined (Kloer et al. 2005). Its substrate specificity is unique in that it cleaves only all-*trans* apocarotenoids of different chain lengths, but not C_{40} *β*-carotene to yield all-*trans* C_{20} retinal or its derivatives (Ruch et al. 2005). The mammalian and insect enzymes cleaving C_{40} carotenoids including *β*-carotene at the 15–15′ double bond form a different clade. Five plant CCO classes marked in color have been characterized (Fig. 2a). Their evolutionary distance, as far as can be judged from sequence alignments, their cleavage specificities and the cellular compartments where they reside are listed in Fig. 2b. The first CCO from any organism was cloned from a maize viviparous mutant (*vp14*) defective in ABA biosynthesis (Tan et al. 1997; Schwartz et al. 1997). The enzyme specifically cleaves the 11–12 double bond of nine-*cis*-epoxycarotenoid substrates giving rise to the term NCED (Fig. 2b). Cloning and sequence information of the *vp14 NCED* gene of maize formed the basis for the molecular identification of the first insect 15–15′ carotenoid oxygenase and subsequent clonings of mammalian CMO1 and CMO2 enzymes (von Lintig and Vogt 2000; von Lintig et al. 2001; Redmond et al. 2001; Hessel et al. 2007; see Fig. 2a). In *Arabidopsis thaliana*, the plant NCED family comprises five members all targeted to plastids and involved in ABA biosynthesis, yet with somewhat differential localization in stroma and thylakoid membranes. The *AtNCED* isogenes also differ in their organ and stress-induced expression (Tan et al. 2003).

A class of plant CCD completely different in amino acid sequence, subcellular localization, double bond cleavage specificity and expression of corresponding genes is CCD1. CCD1 has been shown to be located to the cytosol in contrast to all other CCDs (Bouvier et al. 2003b; Simkin et al. 2004a; Auldridge et al. 2006a). Focusing on the cleavage of C_{40} carotenoids, many studies have demonstrated dual excentric cleavage of 9–10 and 9′–10′ double bonds for CCD1. (Schwartz et al. 2001; Mathieu et al. 2005; Ohmiya et al. 2005; Ibdah et al. 2006; Kato et al. 2006; Garcia-Limones et al. 2008; Huang et al. 2009a; Fig. 2b). Recom-

binant CCD1 enzymes can also cleave 5–6/5′–6′ and 7–8/7′–8′ double bonds under certain buffer conditions and on specific substrates (Vogel et al. 2008; Ilg et al. 2009), but whether this activity is also relevant *in planta* remains to be elucidated. A revised view of the *in planta* role of CCD1 concluded from an RNAi approach will be presented below. The 9–10 and/or 9′–10′ cleavage activity of CCD1 is required for the formation of C_{13} apocarotenoid scent and aroma compounds (Simkin et al. 2004a, b; Mathieu et al. 2005; Ibdah et al. 2006; Kato et al. 2006; Garcia-Limones et al. 2008; Huang et al. 2009a). CCD1 enzymes can accept a wide range of C_{40} carotenoid substrates in vitro (Vogel et al. 2008; Ilg et al. 2009; Huang et al. 2009a). *Solanum lycopersicum* produces two CCD1 isoforms, but the difference between them appears to rest on the differential expression of the corresponding genes rather than on differential enzyme characteristics (Simkin et al. 2004a).

The CCD4-type enzymes are the worst-characterized CCD family despite some recent reports on their properties. In *Chrysanthemum morifolium* (*Cm*), loss of CCD4 activity results in yellow petals indicating that the normal white-petal phenotype is brought about by carotenoid degradation (Ohmiya et al. 2006, 2009; Ohmiya 2009). *CmCCD4a* gene expression was limited to petals suggesting a specific role of this CmCCD4 isoform in flowers, whereas *CmCCD4b* transcript levels were high in leaves and stems (Ohmiya et al. 2006). A comparison of recombinant CCD4-type proteins from four different plant families reported surprising differences in substrate acceptance. *C. morifolium* (*Cm*) and *Malus domestica* (*Md*) recombinant CCD4 enzymes converted a C_{40} carotenoid (*β*-carotene), but *Rosa damascena* (*Rd*), *Osmanthus fragrans* (*Os*) and *A. thaliana* (*At*) CCD4 proteins did not. Conversely, RdCCD4 and AtCCD4 converted a C_{30} apocarotenoid in vitro, while the other three proteins did not or only with very low efficiency (Huang et al. 2009b). Sequence alignments do not reveal any particular structural subclasses within these CCD4 sequences (Fig. 2b) and thus a subclassification of the CCD4 enzymes investigated is not possible at this point. Moreover, a CCD4-type sequence from *C. sativus* and a partial sequence from *B. orellana* cluster outside of the main group of CCD4 enzymes indicating additional complexity within this class. The latter sequences are therefore termed CCD4-related in this article (Fig. 2b). Analysis of the plastoglobule proteome of *A. thaliana* has identified the AtCCD4 (but not any of the AtNCEDs) to be located in this compartment (Ytterberg et al. 2006) and CCD4 from *Crocus* has been attributed to plastoglobules as well (Rubio et al. 2008). The various CCD4-type proteins may thus differ in many respects including their subplastidial location. A common feature identified now in several reports is a 9–10 or 9–10/9′–10′ cleavage activity (Rubio et al. 2008; Huang et al. 2009b). A 7–8/7′–8′-specific activity previously reported

Fig. 2 Features of carotenoid tailoring enzymes. **a** Amino acid sequence similarity tree of carotenoid cleavage oxygenases (CCOs) from various organisms. The five clades of plant CCOs called CCDs or NCEDs are highlighted in *color*. Mammalian and insect enzymes are termed CMOs. The apocarotenoid cleavage oxygenase (ACO) of *Synechocystis* is used as an outgroup. The tree has been generated from clustalW alignments using the programs Distances and Splits of the HUSAR software package. **b** More detailed HUSAR-based unrooted dendrogram of plant CCOs including information on cleavage activity on C_{40} carotenoids (CCOs) or apocarotenoid substrates (ACOs), specificity for certain double bonds and subcellular location. The dendrogram has been generated from clustalW alignments by the neighbor-joining method using ClusTree for displaying results

Lengths of lines are a relative indicator of substitutions per site
Number of bootstrap replicates: 1000
Bold node labels are bootstrap values

for an incomplete CCD4-type sequence fom *C. sativus* (Bouvier et al. 2003b) has not been confirmed. Therefore, CCD enzymes with confirmed specificity for the 7–8/7′–8′ double bonds still need to be identified. Similarly, there is no confirmation yet for an enzyme with 5–6/5′–6′ specificity, which has been described from *B. orellana* (Bouvier et al. 2003a).

The cleavage specificities and functions of the CCD7 and the CCD8 classes of enzymes are less controversial (Fig. 2b). *CCD7* and *CCD8* genes have been identified from shoot branching mutants (Sorefan et al. 2003; Booker et al. 2004). The specific role of both enzymes defined so far is in the concerted production of an apocarotenoid shoot branching inhibitor hormone. The recent discovery of its structural identity will be highlighted in "Strigolactones: from rhizosphere signals to shoot branching inhibitor hormones". The complementarity of the action of CCD7 and CCD8 rests on a sequential cleavage of a C_{40} carotenoid (most likely β-carotene) to a C_{18} apocarotenoid via a C_{27} intermediate. The first cleavage $C_{40} \rightarrow C_{27}$ is carried out by

CCD7 in agreement with an experimentally demonstrated single 9–10, but not double 9–10/9′–10′ cleavage activity of AtCCD7 to yield a C_{27} 10′-apo-β-carotenal (Schwartz et al. 2004). Coexpression of both AtCCD7 and AtCCD8 in a β-carotene-producing *E. coli* strain resulted in the production of a C_{18} 13-apo-β-carotenone. AtCCD8 expression alone in the same *E. coli* strain did not give any apocarotenoid products (Schwartz et al. 2004). This result has been explained by two sequential cleavage reactions $C_{40} \rightarrow C_{27} \rightarrow C_{18} + C_9$. The secondary cleavage is carried out via the action of CCD8 on the 13–14 double bond of a C_{27} apocarotenoid substrate (Fig. 2b). That this is the most likely sequence of cleavage events is corroborated by characterization of recombinant CCD8 proteins from rice, pea and Arabidopsis. Only the C_{27} aldehyde β-apo-10′ carotenal and its corresponding alcohol were converted in vitro, but not C_{40} carotenoids (Alder et al. 2008). This enzyme is thus an exclusive ACO similar to that from *Synechocystis*. However, in the study mentioned and in an independent one on Arabidopsis (Auldridge et al. 2006a), *CCD8* expressed in carotenoid-producing *E. coli* strains reduced carotenoid content to some extent indicating that CCD8 might also act directly upon C_{40} carotenoids. Then again, such in vivo experiments in *E. coli* may not represent the situation *in planta*, where different physiological conditions, compartmentation and/or metabolic channeling may ensure a proper sequence of cleavage events. The plastidial location of both CCD7 and CCD8 has been proven (Booker et al. 2004; Auldridge et al. 2006a).

Strigolactones: from rhizosphere signals to shoot branching inhibitor hormones

The old science joke that a few month in the laboratory can save a few hours in the library, sometimes quoted as Westheimer's discovery, may also be applicable to the discovery story of the long-elusive structure of the apocarotenoid shoot branching inhibitor hormone synthesized via CCD7 and CCD8 activities. After a long struggle for its structure, it was eventually identified as being a strigolactone derivative, a compound already known (Gomez-Roldan et al. 2008; Umehara et al. 2008). The description and structural elucidation of the first strigolactones date back to the 1960s and 1970s (Cook et al. 1966, 1972). They have been extensively characterized as seed germination stimulants of parasitic weeds, which are exuded from plant roots into the rhizosphere (Bouwmeester et al. 2003). Subsequently, they were identified as hyphal branching signals for arbuscular mycorrhizal soil fungi (Akiyama et al. 2005). Whether strigolactones are essential for establishing the AM symbiosis has frequently been discussed, but their indispensibility has never been proven

(Humphrey and Beale 2006; Akiyama 2007; Garcia-Garrido et al. 2009).

The strigolactone branching signal for AM fungi was initially described as a sesquiterpene in 2005 (Akiyama et al. 2005); at about the same time, the first evidence appeared that strigolactones are not classical sesquiterpene lactones synthesized directly, but are rather generated via an indirect pathway through carotenoid cleavage in analogy to ABA (Matusova et al. 2005). The key arguments for the apocarotenoid nature of strigolactones were presented from experiments using carotenoid mutants and inhibitors of isoprenoid pathways in maize, sorghum and cowpea (Matusova et al. 2005) and subsequently confirmed in tomato (Lopez-Raez et al. 2008).

From the other research community working on the shoot branching inhibitor hormone in search of its structure, only the apocarotenoid nature was established from studies of CCD7 and CCD8 mutants in different experimental systems as described above (Sorefan et al. 2003; Booker et al. 2004; Schwartz et al. 2004). Its biogenesis in roots and transport into shoots to regulate shoot branching phenotypes was well established from grafting studies (reviewed by Mouchel and Leyser 2007). Eventually, the two research directions united in experiments applying a synthetic strigolactone (GR24) to shoot branching *ccd7* and *ccd8* mutants of pea, rice and Arabidopsis, proving the ability of strigolactones or a compound derived from them to at least partially restore wild-type branching phenotypes and bud outgrowth (Gomez-Roldan et al. 2008; Umehara et al. 2008). The strigolactone discovery story is also covered in several recent reviews from various perspectives (Leyser 2008; Chandler 2009; Dun et al. 2009; Tsuchiya and McCourt 2009).

Apart from the two carotenoid cleavage steps, little is known about the strigolactone biosynthetic pathway (Fig. 3). A series of branching and dwarf mutants from *A. thaliana*, pea (*Pisum sativum*), rice (*Oryza sativa*) and petunia (*Petunia hybrida*) affected in strigolactone biosynthesis or perception are available (Stirnberg et al. 2002; Sorefan et al. 2003; Ishikawa et al. 2005; Johnson et al. 2006; Simons et al. 2007; Drummond et al. 2009). Four *A. thaliana max* (*more axillary branching*) mutants and eight *d* (*dwarf*) or *htd* (*high tillering dwarf*) rice mutants have been characterized at the molecular level as have been several pea *rms* (*ramosus*) and petunia *dad* (*decreased apical dominance*) mutants. The *max3*, *rms5*, *d17/htd1* and *dad3* mutants have been shown to be orthologous and encode defective *CCD7* genes. Similarly, *max4*, *rms1*, *d10* and *dad1* are compromized in the *CCD8* gene function. Another *A. thaliana* branching mutant (*max1*) has led to the identification of an oxidative modification step downstream of CCD7/CCD8 catalyzed by a cytochrome P450 enzyme (CYP711A1-related), but neither its substrate nor its product

is known (Booker et al. 2005; Fig. 3). The *max2* mutant is not a biosynthesis mutant, but rather defective in strigolactone signal perception or transduction. *MAX2* encodes an F-box leucine-rich repeat protein, which appears to act locally at the node in branching suppression (Stirnberg et al. 2007). Pea *rms4* and rice *d3* can also not be complemented by exogenous strigolactones and seem to encode functions orthologous to *MAX2*. Recent additions to mutant characterizations have only given rough ideas about additional biosynthetic or perception steps. An iron-containing rice protein (DWARF27, D27) was shown to be required for strigolactone biosynthesis and its mutation causes a phenotype similar to the *d10* (*ccd8*) mutant (Lin et al. 2009). The D27 amino acid sequence does not display similarity to any CCD or to any protein of known function. A D27–GFP fusion was targeted to chloroplasts suggesting a plastidial location and an involvement in the early steps. However, the *D27* expression profiles determined thus far do not support a concerted action with CCD7 and CCD8, since transcript levels in roots were low but elevated in panicles and axillary buds (Lin et al. 2009). There are genes putatively orthologous to D27 in many other plant genomes, indicating a conserved, but still unknown function. Another rice branching mutant (*d14*) cannot be rescued by exogenous strigolactones. It encodes a protein of the α/β hydrolase superfamily suggesting a function downstream of strigolactone biosynthesis either in signaling or in conversion of strigolactones to a bioactive form (Arite et al. 2009). Two other rice mutants, which are allelic to *d14,* are *dwarf88* (*d88*) and *htd2*. The corresponding genes have been described to encode esterase/lipase-like functions (Gao et al. 2009; Liu et al. 2009; Fig. 3). The *d14* mutant contains elevated levels of the strigolactone *epi*-isomer $2'$-*epi*-5-deoxystrigol (Arite et al. 2009). Moreover, *ccd8* (*rms1, d10, dad1*) mutants of pea, rice and petunia contain elevated transcript levels of the mutated genes or other pathway genes (Foo et al. 2005; Snowden et al. 2005; Arite et al. 2007) and a *ccd7* mutant exhibits comparable alterations (Drummond et al. 2009), indicating extensive feedback control of strigolactone levels and strigolactone biosynthesis. The topic of feedback upregulation of the strigolactone pathway and the idea that D14 might function as a strigolactone receptor are covered in more detail in a recent review (Beveridge and Kyozuka 2010).

As expected for a genuine phytohormone, strigolactones may have additional regulatory functions and may interact with other plant hormones. In roots, strigolactones might also be involved in the control of root hair formation. This is suggested by experimental results from applying a yellowish golden compound called "d'orenone" to roots of *A. thaliana*, which resulted in a block of root hair growth by interfering with PIN2-mediated auxin transport (Schlicht et al. 2008). "D'orenone" is a synthetic C_{18} β-apo-13-caro-
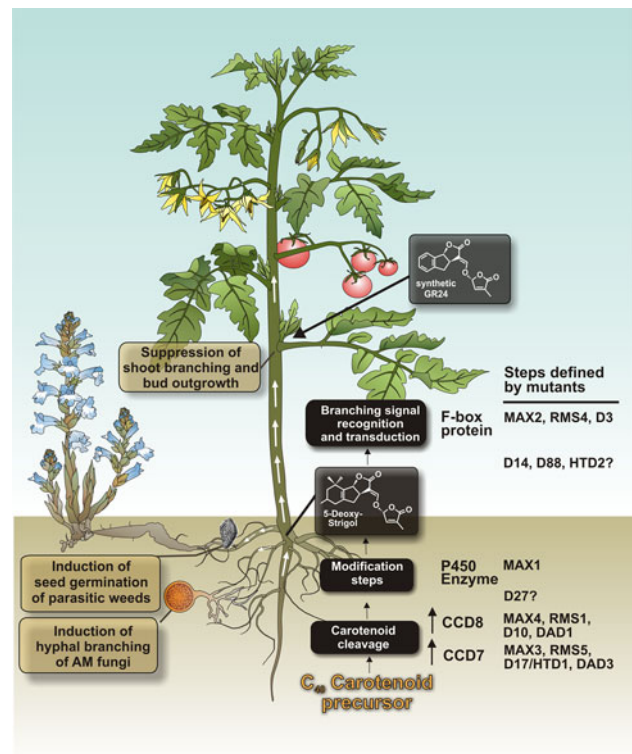

Fig. 3 Multiple functions of strigolactones in the rhizosphere and in shoots. Strigolactones in root exudates induce hyphal branching of AM fungi to promote the AM symbiotic interaction. Concomitantly, strigolactone signals are abused by parasitic weeds to detect the presence of host roots and initiate their seed germination. In addition, strigolactones are transported to shoots, where they (or their metabolites) are recognized as branching inhibitor signals, which are transduced to regulate shoot branching together with other hormones. Application of a synthetic strigolactone (GR24) to axillary buds can inhibit bud outgrowth. Known biosynthetic and hormone recognition steps defined by mutants are listed on the *right*

tenone, structurally identical to the enzyme reaction product of recombinant CCD8 (Alder et al. 2008). The observed alterations might have therefore been brought about at least in part by strigolactone-mediated signaling. In support of this idea, application of high concentrations of GR24 led to the inhibition of root hair elongation and asymmetric root growth in tomato. These effects were again suggested to be mediated by auxin-efflux carriers (Koltai et al. 2010). A connection among strigolactone signaling, auxin transport and auxin action has also been implicated in other studies (Beveridge et al. 2009; Chandler 2009; Hayward et al. 2009). Both strigolactones and auxins may therefore jointly and coordinatedly control axillary branching and other developmental processes.

Beyond such interactions of strigolactones with known hormones, additional carotenoid-derived signaling independent of ABA or strigolactones seems to occur. The *BYPASS1* (*BPS*) gene of *A. thaliana* controls the production of a root-derived graft-transmissible signal necessary for

root and shoot development (Van Norman et al. 2004). Current knowledge indicates that this signal is carotenoid derived, is not produced via the action of a single CCD alone and is neither strigolactone nor ABA (Van Norman and Sieburth 2007). Virus-induced gene silencing of a putative *BPS* ortholog in *Nicotiana benthamiana* caused pleiotropic detrimental effects on plant development (Kang et al. 2008). Therefore, sequential carotenoid cleavage reactions might have additional importance in controlling plant growth and development. Outside of the plant kingdom, C_{18} trisporic acid apocarotenoid derivatives of fungi are involved in the recognition between mating partners. A β-carotene oxygenase gene (*TSP3*) has been identified from *Rhizopus oryzae* (Burmester et al. 2007). Unfortunately, only the decoloration of a β-carotene-producing bacterial strain has been described so far as a functional proof for recombinant TSP3, but no cleavage products or cleavage specificity have yet been identified. Interestingly, a second CCO (TSP4) can be identified in fungal genomes and it is tempting to speculate that it might act in concert with TSP3 to produce fungal C_{18} apocarotenoid derivatives comparable to the concerted action of CCD7 and CCD8 in plants.

While the discovery stories of strigolactone functions proceeded from the initially known role in parasitic weed germination through the mycorrhizal interaction-promoting function to the identity as novel phytohormones, nature's order of functional attribution may have been the other way round. The exploitation of strigolactones by *Striga* and *Orobanche* parasitic weeds as seed germination signals can now be regarded as highjacking of an existing essential plant developmental signal that plants cannot escape via mutations. This may be a strategy comparable to microbial defense of plants by selecting pathogen-associated molecular patterns (PAMPs) as targets for early pathogen recognition. AM fungi, whose earliest relationship with plants predates the evolution of roots (Brundrett 2002), probably also adopted strigolactones as a signal during their coevolution with roots, but at what time this might have occurred is a matter of speculation.

Abundant apocarotenoid accumulation in mycorrhizal roots

Strigolactones exert their regulatory functions in plant shoot architecture and in regulating rhizosphere interactions in very low concentrations and act in early stages of the AM interaction. Conversely, other classes of apocarotenoids with still unknown functions accumulate in large amounts in mycorrhizal roots at the later stages of AM symbiosis. Two different structural types of these AM-induced abundant apocarotenoids can be clearly distinguished: (1) colorless C_{13} cyclohexenone derivatives,

which are structurally related to the C_{13} apocarotenoids occurring in flowers and fruits (see Fig. 1c), but always contain an α-ionone ring structure (Maier et al. 1995; Strack and Fester 2006; Walter et al. 2007) and (2) yellow C_{14} polyene derivatives called mycorradicins based on their initial identification from AM-colonized maize roots (Klingner et al. 1995). In roots strongly colonized by the AM fungi *Glomus intradices* and *Glomus mosseae*, the mycorradicin concentrations are often sufficiently high to recognize a yellow or yellowish coloration of roots with the naked eye (Klingner et al. 1995; Walter et al. 2000, 2007). These two types of abundant AM-induced apocarotenoids have been identified independently, but they probably originate from a common C_{40} carotenoid precursor (Walter et al. 2000; Fig. 1c).

Both C_{13} and C_{14} apocarotenoids accumulate locally in root cells harboring fungal arbuscules, and biosynthetic enzymes are localized to arbusculated cells (Fester et al. 2002; Hans et al. 2004; Walter et al. 2007). Arbuscules are highly branched nutrient-exchange organs of AM fungi formed in root cells of the inner cortex. They are homologous to haustoria of pathogenic fungi, yet arbuscules are different in that they can deliver mineral nutrients, mainly phosphate, to the root. Before delivery, these nutrients are collected in the soil by fungal hyphae, transported and passed on to the plant pertinent to the beneficial nature of the interaction. In return, the fungus receives carbohydrates from the plant. The ability to deliver phosphate to the plant appears to be mandatory for arbuscules to survive in the root cortex. A mutation in an AM-specifically regulated plant phosphate transporter of *Medicago truncatula* (MtPT4) resulting in an interruption of a continuous fungus–plant phosphate flux causes premature death of arbuscules and a very low frequency of symbiotic structures altogether (Javot et al. 2007).

During studying the C_{13}/C_{14} apocarotenoid biosynthetic pathway in mycorrhizal roots, initial emphasis has been placed on the early steps of isoprenoid biosynthesis, providing isopentenyl diphosphate building blocks via the plastidial methylerythritole phosphate (MEP) pathway. The first step of the MEP pathway catalyzed by 1-deoxy-D-xylulose 5-phosphate synthase (DXS) has been shown to be encoded by differentially regulated genes. Only the *DXS2* isogene is regulated by mycorrhization and its transcript levels are upregulated concomitantly with C_{13} and C_{14} apocarotenoid accumulation (Walter et al. 2000, 2002). Therefore, this isogene emerged as a potential target for manipulation of AM-induced C_{13}/C_{14} apocarotenoid biosynthesis (Walter et al. 2007), since general manipulation of the carotenoid biosynthetic pathway is likely to result in unspecific, detrimental and pleiotropic effects. Strong knockdown of *MtDXS2* gene expression in mycorrhizal hairy roots of *M. truncatula* resulted in equally strong

suppression of both C_{13} and C_{14} apocarotenoid accumulation, proving a role of MtDXS2 in their synthesis (Floss et al. 2008a). Nonmycorrhizal plants suppressed in *MtDXS2* expression did not show any phenotypical alterations. Mycorrhizal functions were negatively affected in mycorrhizal roots depleted in C_{13} and C_{14} apocarotenoids as indicated by an intriguing abolishment of expression of many plant mycorrhizal marker genes. This abolishment included also the AM-specifically regulated phosphate transporter gene *MtPT4*. Moreover, apocarotenoid-depleted mycorrhizal roots contained more phosphate than empty vector-transformed mycorrhizal roots in agreement with a potential impairment of fungus–plant phosphate flux at an impaired arbuscule–root cell interface. However, these phenomena occurred only in roots strongly silenced for *DXS2* expression and below about 5–10% residual levels of apocarotenoids. At higher levels of residual apocarotenoids (10–80%), there was no effect on the markers and the relationship of apocarotenoid concentration to AM marker gene repression was not linear (Floss et al. 2008a). This might be explained by indirect effects or a situation, in which apocarotenoid levels need to go below a certain threshold level before alterations of AM marker transcription become effective. Furthermore, it could not be excluded that the absence of another DXS2-dependent isoprenoid product is responsise for or contributes to the observed effects on mycorrhizal parameters.

Role of CCD1 in mycorrhizal roots and beyond

These still inconclusive results led to the targeting of a later step in the pathway, specifically a carotenoid cleavage step, for an additional knockdown approach. Previous predictions (Walter et al. 2007) and experimental evidence for upregulation in mycorrhizal maize roots (Sun et al. 2008) clearly pointed to CCD1 as a potential player in AM-induced C_{13}/C_{14} apocarotenoid biosynthesis and to represent the elusive CCD predicted in the C_{13}/C_{14} biosynthetic pathway. Moreover, reported cleavage specificities of recombinant CCD1 from *Arabidopsis* and other plants (Schwartz et al. 2001; Simkin et al. 2004a, b; Mathieu et al. 2005; Ibdah et al. 2006) were in agreement with a predicted dual excentric cleavage of both the 9–10 and the 9′–10′ double bond of a common C_{40} carotenoid or C_{40} xanthophyll precursor to yield the AM-induced C_{13} cyclohexenone and C_{14} mycorradicin end products (Walter et al. 2007). The knockdown approach on *CCD1* expression was carried out in the same *M. truncatula* hairy root experimental system as the previous *DXS2* suppression experiments.

This *MtCCD1* knockdown approach delivered unexpected results. Comparative HPLC analyses of mycorrhizal hairy roots clearly indicated a differential reduction of C_{13}

and C_{14} apocarotenoids in *MtCCD1*-repressed mycorrhizal roots and not an equal reduction, as was found with the *MtDXS2* gene target. C_{14} mycorradicin derivatives were strongly reduced in roots silenced for *MtCCD1* expression (about 5% residual amounts relative to mycorrhizal empty vector controls), whereas the C_{13} cyclohexenone derivatives in the same root samples exhibited a statistically significant decrease to only about 50% of empty vector controls (Floss et al. 2008b). This means that only one of the two C_{13} compounds predicted to be produced by CCD1 is generated by CCD1 action, but the other must be generated by another enzyme. Concomitantly, a conspicuous color change to yellow-orange was observed in the transgenic roots with suppressed *MtCCD1* transcript accumulation, indicating the formation of (a) novel chromophore(s). At least seven new compounds were recognized in the mycorrhizal RNAi roots exhibiting UV spectra resembling those of C_{27} apocarotenoids. Two major new products could be separated after alkaline hydrolysis and one of them was identified as a C_{27} apocarotenoic carboxylic acid (3-hydroxy-α-apo-10′-carotenoic acid) glycosylated at the 3-hydroxyl position (Floss et al. 2008b). This suggests that C_{27} apocarotenoids and not C_{40} carotenoids are the main substrates of CCD1 *in planta* in mycorrhizal roots. A second CCD must be involved in the cleavage of a still elusive C_{40} carotenoid precursor to C_{27} intermediates in mycorrhizal roots. Since C_{27} intermediates have never been observed in wild-type mycorrhizal roots, they are expected to be instantly cleaved by CCD1 in the wild-type situation. A sequential two-step cleavage of C_{40} carotenoids thus appears to govern AM-induced C_{13} and C_{14} biosynthesis in the order $C_{40} \rightarrow C_{27} + C_{13}$ followed by a $C_{27} \rightarrow C_{14} + C_{13}$ conversion (Fig. 4a).

The discussion of a sequential cleavage mechanism for C_{13} and C_{14} apocarotenoid formation must incorporate the known locations of CCD1 and other plants CCDs. As mentioned above, CCD1 has been shown in several studies to be the only CCD located in the cytosol (see Fig. 2b). The contradiction concerning the proposed C_{40} substrates being inside the plastid and the cleavage enzyme being outside has been addressed, but has tentatively been explained by limited access of CCD1 to outer envelope carotenoids (Simkin et al. 2004a). Assuming now a two-step process allows to propose a plastidial cleavage enzyme to carry out the primary cleavage inside the plastid and to assume an export of the cleavage product to the cytosol (Fig. 4a). Therefore, the proposed mechanism of a C_{27} intermediate exported from the plastid to become a CCD1 substrate eventually reconciles the earlier contradictions on how CCD1 can access its presumed substrate.

C_{27} apocarotenoids have rarely been found in nature, perhaps because of CCD1 activities in many plant tissues. One of the few examples where abundant C_{27} apocarotenoids
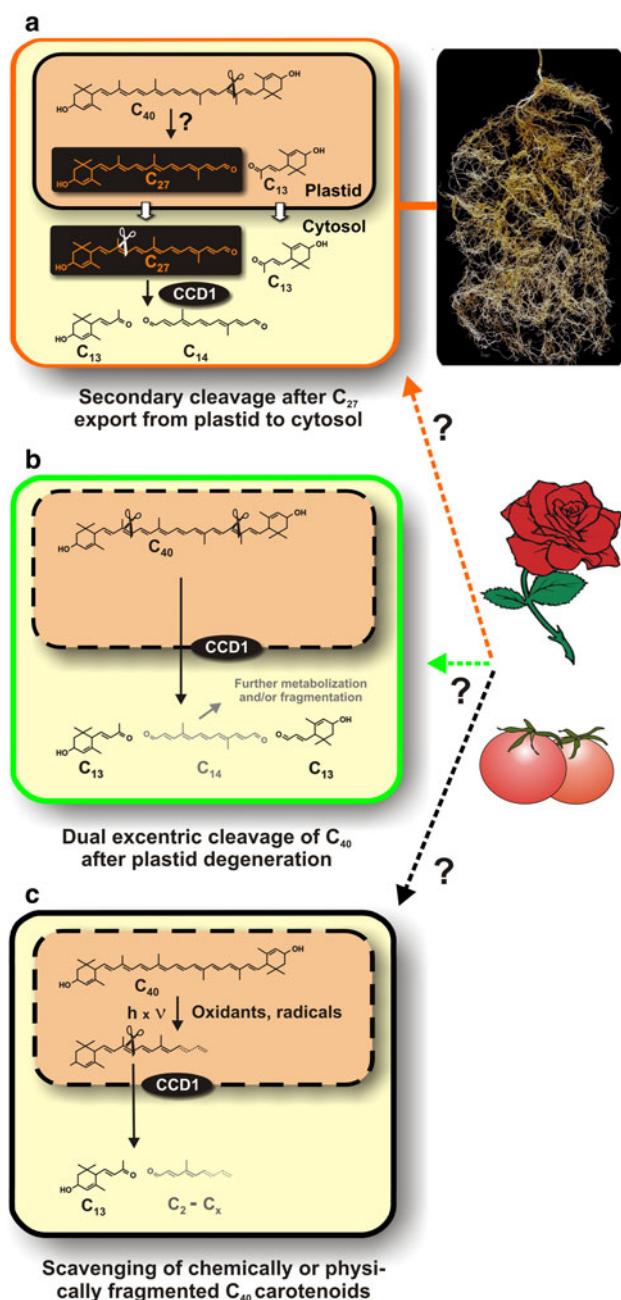
Fig. 4 Newly defined role of CCD1 in a sequential, compartmented C_{40} carotenoid cleavage mechanism (**a**) or potential alternative scenarios for CCD1 action (**b**, **c**). Accumulation of C_{27} apocarotenoids in roots with suppressed CCD1 activity (associated with strongly enhanced yellow-orange coloration of roots shown on the *upper right image*) suggests mechanism (**a**) for mycorrhizal roots. Model (**b**) proposes access of cytosolic CCD1 to C_{40} carotenoid substrates following plastid degeneration and/or via enhanced permeability of the plastidial membrane. Model (**c**) involves scavenging of C_{40} carotenoids damaged or degraded by chemical or physical means and transformation by CCD1 to bioactive C_{13} apocarotenoids. In flowers, such as roses, developing strong C_{13} apocarotenoid scents or in fruits, such as tomato, producing C_{13} aroma volatiles, the mechanisms of CCD1 action are still unknown and indicated by differently *colored dashed lines* and *question marks*

have been identified is *Boronia megastigma*. The flowers of this small shrub native to southwestern Australia are collected for their flavor and fragrance based on various C_{13} apocarotenoid constituents (Cooper et al. 2003). A palmitic ester of a C_{27} 3-hydroxy-10′-apocaroten-10′oic acid and other C_{27} apocarotenoids have been tentatively identified recently from this system (Cooper et al. 2009). Based on C_{27} identification in rose petals, such a two-step sequential cleavage mechanism has been proposed for C_{13} scent and aroma compound biogenesis many years ago (Eugster and Märki-Fischer 1991), but this mechanistic proposal has never been substantiated by enzymatic data and fell into oblivion in the wake of many studies showing dual 9–10 and 9′–10′ cleavage activity of recombinant CCD1 proteins on various C_{40} carotenoids in vitro. However, conversion of a substrate in vitro by a recombinant enzyme does not mean that this activity must constitute the main biological function of the enzyme *in planta*. The results from the mycorrhizal root system suggest CCD1 to preferentially act as an apocarotenoid oxygenase (ACO) *in planta* cleaving C_{27} and perhaps other apocarotenoids.

The question thus arises, whether a two-step sequential cleavage mechanism involving CCD1 as a second player also applies to the many instances of C_{13} apocarotenoid formation in flowers and fruits leading to important scent and aroma constituents. This topic has already been briefly discussed (Floss and Walter 2009). Two studies using antisense or cosuppression strategies to downregulate *CCD1* genes in tomato or petunia, respectively, both came up with unexpectedly high residual levels of C_{13} compounds despite strong reduction in *CCD1* transcript abundance (Simkin et al. 2004a, b; Auldridge et al. 2006a). These results are reminiscent of the data from the mycorrhizal root system, but they have previously been explained by additional activities redundant to CCD1, rather than by CCD1 being the second of two sequential players as suggested above. The CCD1-depleted tissues of tomato and petunia have not yet been analyzed for the potential accumulation of C_{27} intermediates. Information on whether or not C_{27} compounds accumulate under these conditions could be helpful to decide about the applicability of the sequential cleavage scheme (Fig. 4a) to systems other than mycorrhizal roots or about other modes of C_{13} volatile generation.

Such potential other modes of C_{13} generation in flowers and fruits are depicted in Fig. 4b, c. A dual excentric cleavage of C_{40} carotenoids as observed in vitro is also conceivable *in planta*, but only if CCD1 gets access to its substrates normally protected from CCD1 cleavage by the plastidial membrane. This may be brought about by regular degeneration of plastids and release of C_{40} or by damage to or destruction of plastidial membranes (Fig. 4b). A third

scenario of potential CCD1 action is a function as a scavenger of carotenoid catabolites. Carotenoids, which have suffered structural damage or fragmentation from chemical reactions or from exposure to UV light, radicals or other destructive physical means, might still be amenable to transformation into bioactive C_{13} apocarotenoids via CCD1 action (Fig. 4c). In these scenarios, the metabolism of the C_{14} cleavage product, which is only traceable in mycorrhizal roots, and the metabolic fate of any hypothetical remnants of damaged carotenoids are unknown. A scavenger function on cytosolic apocarotenoids as by-products of photodestructive processes has been proposed for the cyanobacterial NosCCD (Scherzinger and Al-Babili 2008). This NosCCD has earlier been described as NSC1 (Marasco et al. 2006). It has some evolutionary relationship with plant CCD1 enzymes (Fig. 2a) and shares several features with its plant relative including 9–10 cleavage specificity, a cytosolic localization and the ability to cleave monocyclic C_{40} carotenoids or apocarotenoids at a higher rate than bicyclic C_{40} carotenoids (Schmidt et al. 2006; Scherzinger and Al-Babili 2008). Therefore, plant CCD1 enzymes and the NosCCD might have evolved into ACOs without losing their ability to cleave C_{40} carotenoids. Their main role *in planta* may then be cleavage of apocarotenoid substrates provided by plastidial primary cleavage companions or of substrates taken from a multitude of carotenoid debris as a leftover of physical or chemical destruction of carotenoids.

If the proposed two-step, differentially compartmented mechanism of carotenoid cleavage toward C_{13} apocarotenoids (Fig. 4a) is valid beyond the mycorrhizal root system, potential candidates for performing the first cleavage step can be evaluated. Both CCD7 and CCD4 fulfill the condition of being plastidial enzymes and having 9–10 cleavage activity proposed. As discussed above, there is evidence that CCD7 catalyzes a single 9–10 cleavage only and does not continue to cleave the 9′–10′-double bond as well (Schwartz et al. 2004). This is in agreement with the established role of CCD7 in generating a C_{27} intermediate in strigolactone biosynthesis. CCD4 has recently been shown to also exhibit 9–10/9′–10′ activity that might include a single cleavage only, but the latter has not explicitly been shown yet (Rubio et al. 2008; Huang et al. 2009b). *CCD4* transcripts have been identified in flowers. Only transcripts of *C. sativus*, *CsCCD4a* and *CsCCD4*b, accumulated in a coordinated manner along with C_{13} *β*-ionone volatile release in stigma development, whereas *CsCCD1* transcripts did not (Rubio et al. 2008). *CCD7* transcripts are abundant in roots and stems (Drummond et al. 2009; Vogel et al. 2010) in agreement with their role in strigolactone formation, but surprisingly there were strongly elevated levels in green tomato fruits but not in flowers (Vogel et al. 2010). Similarly, rice *CCD7* (*D10*) is expressed in panicles as well as in buds and the shoot apex (Arite et al. 2007). To

our knowledge, none of the *ccd7* or *ccd4* mutants or knockdown transgenics has yet been analyzed for alterations in C_{13} scent and/or aroma compound production. Such data might be helpful to further decide about the pathways of C_{13} generation. Therefore, both CCD4 and CCD7 are currently candidates to deliver C_{27} intermediates for CCD1 in a potentially widely distributed two-step two-compartment cleavage mechanism toward C_{13} apocarotenoids.

The mycorrhizal roots strongly depleted by the *MtCCD1* knockdown approach for CCD1 activity and C_{14} mycorradicin, but only to a limited extent on C_{13} cyclohexenone derivatives, were also evaluated for mycorrhizal parameters. Apart from one minor alteration, there was no change in plant marker gene expression, representing symbiotic activity of the AM–plant interaction (Floss et al. 2008b). Thus, C_{14} mycorradicin reduction alone without concomitant reduction in C_{13} cyclohexenone derivatives does not appear to significantly impair the functioning of the AM symbiosis as did the strong reduction in both C_{13} and C_{14} apocarotenoids in the *MtDXS2* knockdown approach (Floss et al. 2008a). This is in agreement with other arguments previously collected for a role of C_{13} rather than C_{14} apocarotenoids in the AM symbiosis (Walter et al. 2007). Although the results of the *MtCCD1* knockdown approach did not contribute to the elucidation of C_{13} and C_{14} apocarotenoid function in the AM symbiosis in the expected way, they strengthen the case for a role of C_{13} cyclohexenone derivatives rather than C_{14} mycorradicins.

A model for novel C_{13} apocarotenoid functions in the AM symbiosis

Fungal arbuscules, the functional entities of the AM symbiosis, are short-lived organs that emerge from hyphal branchings inside root cells and develop into highly branched mature, functional structures for mineral nutrient delivery, but also rapidly senesce subsequently displaying collapsing branches. They eventually end up as clumpy remnants of disintegrated arbuscules, which disappear and allow the same root cell to host another arbuscule. This arbuscule generation cycle takes about 7–10 days as determined from monocot roots (Alexander et al. 1988). It appears to be cell-autonomous and not to be synchronized across the root system. Arbuscule deterioration and turnover has been studied at the ultrastructural levels in the 1970s (Cox and Sanders 1974; Kinden and Brown 1976), but has not received much attention since.

Pertaining to the topic of this review, it has been shown that the deposition of both AM-induced C_{13} and C_{14} apocarotenoids is located on cells hosting arbuscules (Fester et al. 2002). At the ultrastructural level, it could be demonstrated that an apocarotenoid-synthesizing MEP pathway enzyme

(1-deoxy-D-xylulose 5-phosphate reductoisomerase, DXR) is closely associated with arbuscule branches (Hans et al. 2004). Most importantly, DXR accumulation accompanies the process of arbuscule senescence and degradation. The highest DXR abundance was found at the early and intermediate degenerating stages (Hans et al. 2004; Lohse et al. 2006). Another hint for a potential function of C_{13} apocarotenoids in the arbuscule turnover process came from the confocal analyses of arbuscules in roots strongly depleted in C_{13} apocarotenoids by the above-mentioned *DXS2* knockdown experiments. Arbuscule morphology appeared to be unaltered, but there was a significant shift in the distribution of the various stages of arbuscule development and degradation. More degenerating and dead arbuscules appeared at the expense of mature ones (Floss et al. 2008a; Fig. 5). A similar shift toward more degenerating and less mature arbuscules was found in the *MtCCD1* knockdown approach (Floss et al. 2008b; Fig. 5), indicating that it is not caused by any DXS2-regulated isoprenoid factor, but must be apocarotenoid dependent. This phenomenon, which might be called "arbuscule overaging" constituted the only parameter, which appeared to be correlated with the levels of residual C_{13} apocarotenoids in an approximately linear fashion, i.e., stronger effects in the *DXS2* knockdown experiments, including elevated numbers of dead arbuscules (Fig. 5). In contrast, the symbiotic marker gene transcript levels were unaffected over a wide range of C_{13} apocarotenoid depletion, and the *MtPT4* marker transcripts even exhibited an upward trend at intermediate levels of pathway interference with the *MtDXS2* target (Floss et al. 2008a). These shifts in the relative distribution of arbuscule stages can presently be interpreted in two ways: Either the lifespan of individual arbuscules is reduced and overall arbuscule degradation is enhanced upon the lack of C_{13} apocarotenoids as supporters of mature stages or the lifespan is extended as a consequence of reduced normal degradation. The upward trends in both *MtPT4* transcript and total arbuscule abundance at intermediate stages of apocarotenoid pathway interference (Floss et al. 2008a, b) argue for the second interpretation. As a consequence of reduced normal arbuscule degradation, the root may become clogged with too many older arbuscules, resulting directly or indirectly in a shut down of plant AM marker gene expression.

The results from the two knockdown approaches thus suggest that arbuscule degradation and turnover is not simply a developmental process of the symbiosis, but appears to be regulated by the plant, and its impairment by molecular genetic means can impair the functionality of the symbiosis. The potential mode of action of C_{13} apocarotenoids may be related to their moderately, but not strongly allelopathic, growth-inhibiting, fungitoxic and antimicrobial properties (Macias et al. 1998, 2004; Park et al. 2004). This
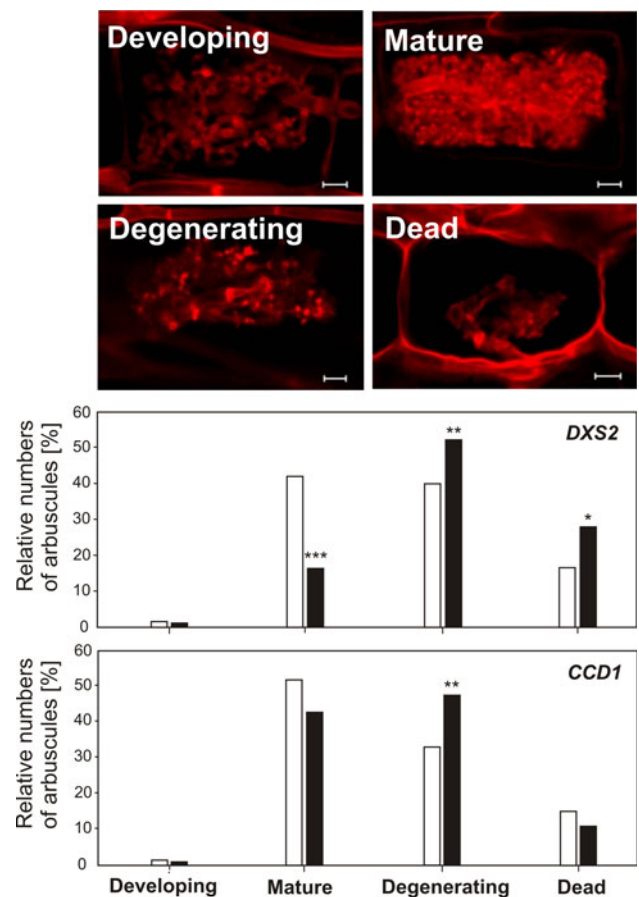

Fig. 5 Distribution of developmental stages of arbuscules in mycorrhizal roots of *M. truncatula* knocked down in *DXS2* or *CCD1* expression and strongly (*DXS2*) or moderately (*CCD1*) depleted in C_{13} cyclohexenone apocarotenoids (*black columns*). *Empty columns* represent empty vector controls. Individual arbuscules were classified into four developmental stages as shown on top (redrawn from Floss et al. 2008a, b). *Asterisks* indicate statistical significance of alterations (*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$)

may allow for subtle defensive measures against a fungal symbiont rather than brute force as required for killing a pathogen via more toxic phytoalexins. In agreement with this view, hypersensitive cell death processes appear to be absent from arbuscule-degrading cells (Javot et al. 2007). A mere signaling function of C_{13} apocarotenoids in the arbuscule turnover process is unlikely due to the high abundance of the compounds produced. C_{14} mycorradicin forms a complex with C_{13} apocarotenoid moieties and perhaps other compounds probably through ester bonds, resulting in a polymer-like mixture of compounds difficult to handle and separate on chromatographic systems in its native state. Only upon alkaline hydrolysis or derivatization, the complex disintegrates to some extent. Whether this complex or mycorradicin itself fulfills any function in the arbuscule turnover process remains to be elucidated.

Fig. 6 Common role of CCD7 in C_{18}-derived strigolactone and AM-induced C_{13}/C_{14} apocarotenoid formation. Current data are compatible with CCD7 catalyzing a central step in apocarotenoid biosynthesis providing C_{27} precursors to both CCD8 for C_{18} apocarotenoid and strigolactone formation, and to CCD1 for synthesis of AM-induced C_{13} and C_{14} apocarotenoids in mycorrhizal roots. Tentatively predicted C_{40} carotenoid precursors are in *brackets*. Isopentenyl diphosphate (IPP) precursors are provided by the MEP pathway through AM-induced DXS2 for C_{13}/C_{14} apocarotenoids and presumably via DXS1 for strigolactone formation
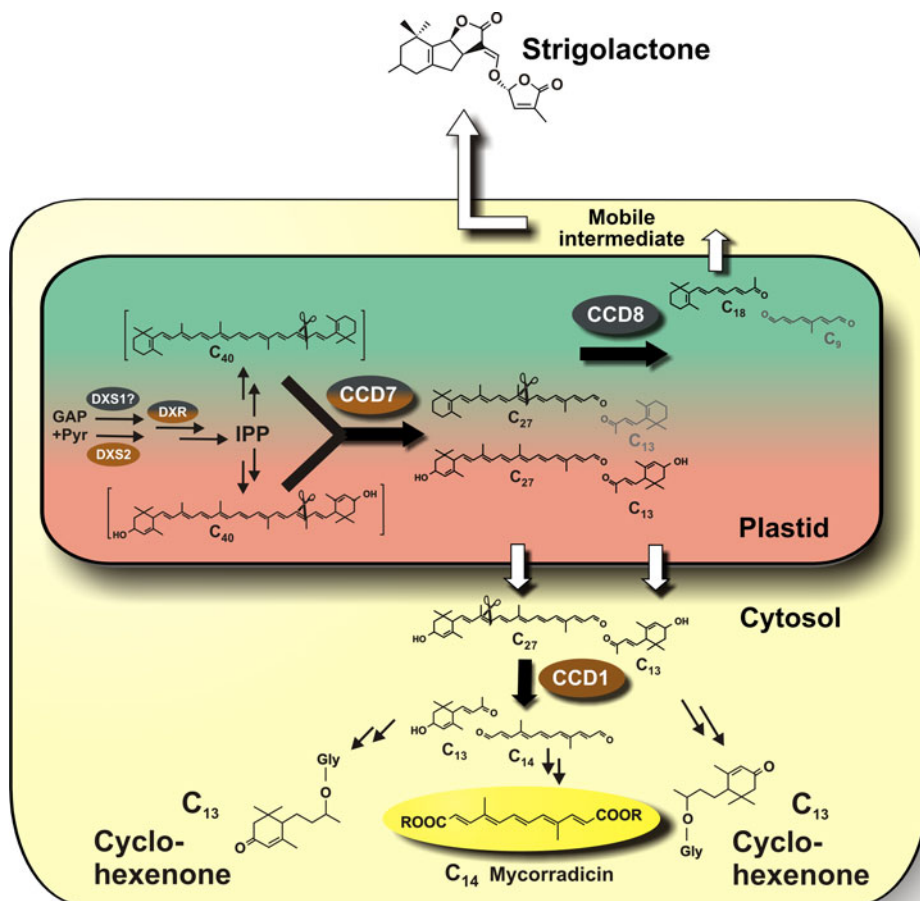
Emerging crosspoints in apocarotenoid biosynthesis and their potential impact

Following up the question on candidates for the first one of two players in C_{13} apocarotenoid biosynthesis, the first experimental results now point to CCD7 for the mycorrhizal root system. The analysis of several lines of transgenic tomatoes severely downregulated in *CCD7* expression has clearly shown that both C_{13} and C_{14} apocarotenoid levels are strongly reduced (Vogel et al. 2010). The level of C_{13}/C_{14} apocarotenoid reduction followed the level of *CCD7* transcript reduction when several transgenic lines were compared. This reduction is accompanied by reduced levels of strigolactones, reduced levels of parasitic weed seed germination capacity and increased branching (Vogel et al. 2010). To exclude that the reduction in C_{13}/C_{14} apocarotenoids is due to reduced mycorrhizal colonization, several parameters of root colonization were determined. The frequency of mycorrhizal structures was unchanged, but arbuscule abundance was reduced to about 60% of wild-type roots (Vogel et al. 2010). Ongoing further analyses indicate that the distribution of arbuscule stages is altered toward more degenerating and dead arbuscules in a similar

way, as observed in the other knockdown approaches of the pathway (Fig. 5; Floss et al. 2008a, b). Comparable results of strong reduction of both C_{13} cyclohexenone and C_{14} mycorradicin apocarotenoid derivatives have been obtained from mycorrhizal roots of the pea *rms5*/*ccd7* mutant including the altered distribution of arbuscule stages. On the contrary, preliminary analyses of a pea *ccd8* mutant show mycorrhizal colonization without such a shift in arbuscule stages (M. H. Walter, unpublished data). These results establish CCD7 as a control point not only for strigolactone, but also for AM-induced C_{13} and C_{14} apocarotenoid biosynthesis (Fig. 6). On the other hand, precursor supply from the MEP pathway may be supported differentially by the two DXS isozymes, since no branching phenotype has been observed in the *MtDXS2* knockdown approach (Floss et al. 2008a). CCD7 thus appears to catalyze a highly conserved key biosynthetic step in the biosynthesis of various apocarotenoids, after which the pathways branch off in different directions and into different compartments (Fig. 6).

Interdependence between root colonization by AM fungi and strigolactone-mediated germination of parasitic weeds has been observed in several systems. Mycorrhizal plants

exhibited less infection by *Striga* parasites than those without colonization (Lendzemo et al. 2007, 2009). Thus far, this has been interpreted as a consequence of mycorrhizal autoregulation of subsequent mycorrhization events involving reduced production and secretion of strigolactones, which eventually leads to reduced *Striga* seed germination (Bouwmeester et al. 2007; Lendzemo et al. 2007; Garcia-Garrido et al. 2009). Identification of CCD7 as a common step in both strigolactone and C_{13} cyclohexenone formation now allows the proposal of a potential alternative mechanism. Abundant C_{13} cyclohexenone production in colonized roots might generate a metabolic sink for strigolactone precursors, thereby reducing strigolactone production and secretion. Such a potential biogenetic interdependence of little-abundant strigolactones and highly abundant AM-induced C_{13} cylohexenones (and C_{14} mycorradicin) should therefore be considered when designing strategies against *Striga* infestations based on mycorrhization.

Conclusions and perspectives

Five years on from the prospect for novel apocarotenoid functions (Bouvier et al. 2005), there is progress on many fronts. Not only have strigolactones joined the family and their multiple functions and interactions with other hormones are beginning to be unraveled, but also novel roles of apocarotenoids are emerging. One of them appears to be a function of C_{13} cyclohexenone apocarotenoids in maintaining the functionality of the AM symbiosis by regulating the turnover of arbuscules. This may be related to the moderately fungitoxic and antimicrobial properties of these compounds, which could also complement their known roles in flowers and fruits as attractors of pollinators and seed dispersers. Several potential mechanisms of their biogenesis can now be further investigated having great application potential for the food and fragrance industries. On the biochemical side, there is still only a single three-dimensional structure of a cyanobacterial CCO available, which accepts apocarotenoids only as substrates and is therefore classified as an ACO. Its cleavage mechanism may therefore not be readily applicable to all CCOs, and additional structures are needed to learn about the principles of double bond-specific cleavage. In addition, the issues preferentially addressed in this review being sequential cleavage reactions and CCOs being ACOs by design (CCD8, cyanobacterial ACO) or by substrate access (CCD1), as well as compartmentation and transport between compartments, still await further clarification.

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