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Mini-Review

Role of carotenoid cleavage dioxygenase 1 (CCD1) in apocarotenoid biogenesis revisited

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Oxidative tailoring of C_{40} carotenoids by double bond-specific cleavage enzymes (carotenoid cleavage dioxygenases, CCDs) gives rise to various apocarotenoids. AtCCD1 generating C_{13} and C_{14} apocarotenoids and orthologous enzymes in other plants are the only CCDs acting in the cytosol, while the hitherto presumed C_{40} substrate is localized in the plastid. A new model for CCD1 action arising from a RNAi-mediated *CCD1* gene silencing study in mycorrhizal hairy roots of *Medicago truncatula* may solve this contradiction. This approach unexpectedly resulted in the accumulation of C_{27} apocarotenoids but not C_{40} carotenoids suggesting C_{27} as the main substrates for CCD1 in planta. It further implies a consecutive two-step cleavage process, in which another CCD performs the primary cleavage of C_{40} to C_{27} in the plastid followed by C_{27} export and further cleavage by CCD1 in the cytosol. We compare the specificities and subcellular locations of the various CCDs and propose the plastidial CCD7 to be the first player in mycorrhizal apocarotenoid biogenesis.

Carotenoids are isoprenoids (mostly C_{40}) synthesized by bacteria, algae, fungi and plants. Coordinated tailoring by specific enzymes is thought to be the main principle for generation of defined carotenoid cleavage products (apocarotenoids), which have important functions in their own right.[1] These enzymes, called carotenoid cleavage dioxygenases (CCDs), exhibit a high degree of regio- and stereospecificity for certain double bond positions as opposed to their frequent promiscuity towards substrates. In Arabidopsis, the CCD family consists of nine members forming the basis for CCD classification in plants. Five of them exhibit specificity for 9-cis-epoxycarotenoids and are designated nine-cis-epoxycarotenoid dioxygenases (NCEDs). They are involved in ABA biosynthesis.[1-3] The remaining 4 CCDs (AtCCD1, AtCCD4, AtCCD7, AtCCD8) cleave a variety of trans-carotenoid substrates.[1,4] The majority of CCDs/NCEDs has been shown to reside in plastids. The only exception is AtCCD1 and orthologous enzymes in other plants, which act in the cytosol to generate C_{13} and C_{14} apocarotenoids.[1,4] This contribution will compare current knowledge on enzymatic carotenoid cleavage pathways with recently obtained new insights resulting from silencing the expression of a *CCD1* gene in plant roots colonized by arbuscular mycorrhizal (AM) fungi.[5]

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Known Pathways of Carotenoid Cleavage Leading to C_{15} and C_{18} Apocarotenoids

The phytohormone ABA is the best-studied member of plant apocarotenoids. Single-step cleavage of the 11,12 double bond of 9-cis violaxanthin and 9'-cis neoxanthin by NCEDs results in formation of C_{15} xanthoxin inside plastids (Fig. 1A).[2] The next steps of xanthoxin conversion to abscisic aldehyde and abscisic acid are known to take place in the cytosol. Thus, considering the plastidial location of NCEDs, a transport of xanthoxin into the cytosol must be postulated but the mechanisms by which this occurs are still unknown.[6]

In addition to ABA, another carotenoid-derived phytohormone exists, whose long-sought chemical nature was recently identified as strigolactone.[7-9] Mutants in its biosynthesis or its perception display a striking increase in shoot branching.[7] One CCD involved is CCD7 converting C_{40} trans-carotenoids to C_{27} apocarotenoids (Fig. 1B).[10,11] A second cleavage activity is contributed by CCD8. Several lines of evidence argue for a consecutive action of these CCDs with CCD8 converting C_{27} to C_{18} and C_9 (Fig. 1B).[7,11] Both CCDs have transit peptides indicative of their action in plastids.[4] The C_{18} reaction product of CCD8 and strigolactone precursor subsequently undergoes still uncharacterized steps of export from the plastid, further metabolization and transport to the shoot (Fig. 1B).

Strigolactones were previously known as germination stimulants for parasitic weeds and as signaling molecules to promote hyphal branching of AM fungi.[12,13] Most recent data might point to additional roles of strigolactones in roots. A C_{18} β-apo-13-carotenone called "D'orenone" blocks the growth of root hairs by interfering with PIN2-mediated auxin transport.[14] The synthetic compound "D'orenone" is structurally identical to the proposed C_{18} apocarotenoid precursor of strigolactone biosynthesis (Fig. 1B). The effects observed might therefore be strigolactone-related.

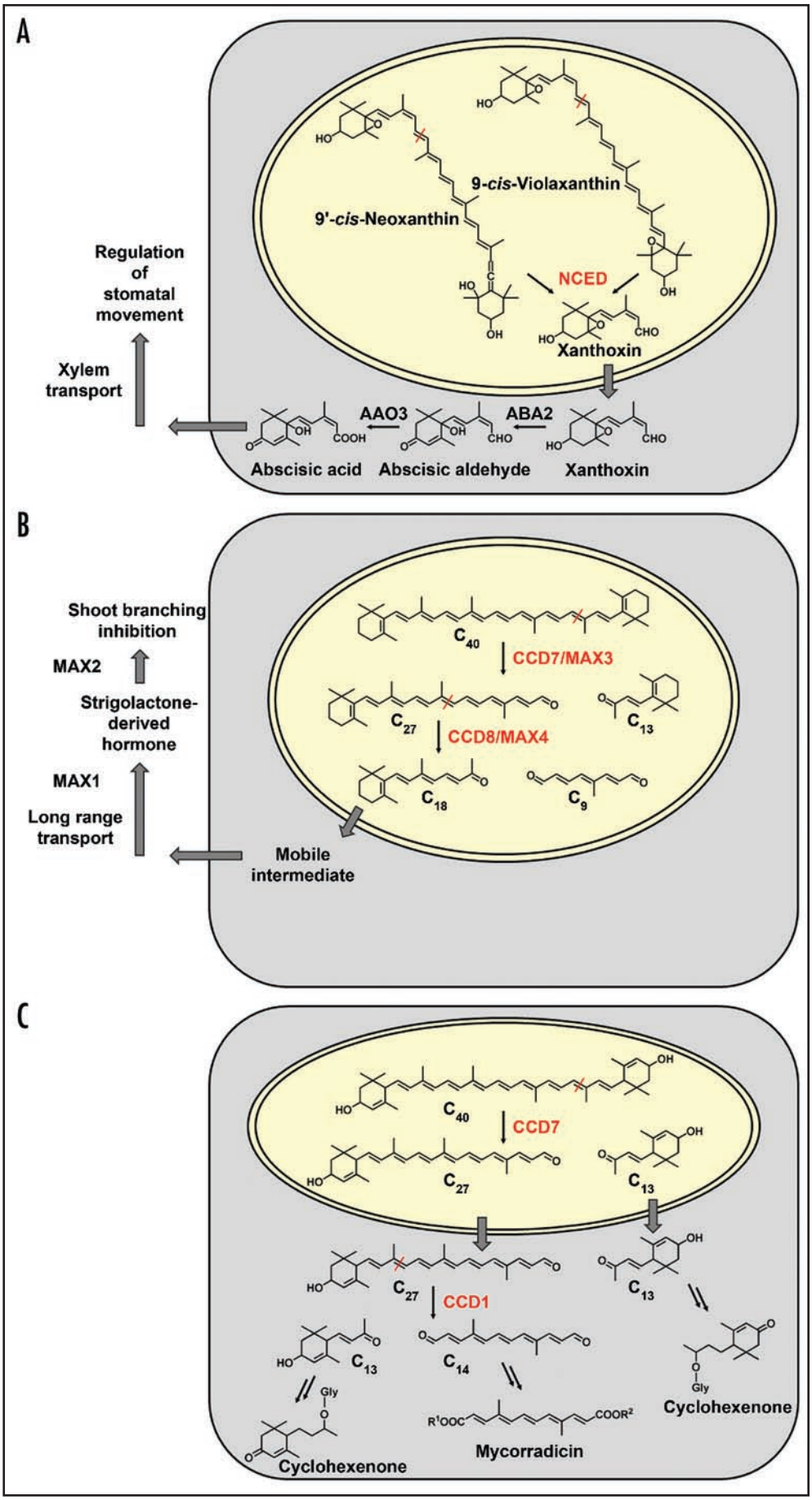

Figure 1. Comparison of substrates, enzymes and their compartmentation in three carotenoid cleavage pathways. (A) ABA biosynthesis involves cleavage of cis-carotenoid substrates by NCEDs in the plastid, C_{15} xanthoxin export to the cytosol followed by further metabolization steps and transport.[6] (B) Strigolactone biosynthesis is assumed to start from β-carotene and to proceed via two consecutive cleavage steps (CCD7 and CCD8) inside the plastid as exemplified by the MAX3 and MAX4 proteins of Arabidopsis.[4] The C_{18} cleavage product of CCD8 or a derivative of it is predicted to serve as mobile strigolactone precursor undergoing export to the cytosol, further modification steps, transport and eventually perception as a regulator of shoot branching.[7] (C) Proposed organization of local C_{13} and C_{14} apocarotenoid biosynthesis in a mycorrhizal root cell. Lactucaxanthin as the tentatively proposed C_{40} carotenoid precursor containing two α-ionone rings is tailored by two consecutive cleavage steps in the plastid (CCD7) and subsequently, following export of the C_{27} intermediate, in the cytosol (CCD1). The C_{27} intermediate has only been detected upon silencing *CCD1* expression.[5] Additional modification steps in the cytosol lead to the various C_{13} cyclohexenone and C_{14} mycorradicin derivatives accumulating in mycorrhizal roots. Abbreviations: MAX, more axillary branching; Gly, glycoside.

C_{13} and C_{14} Apocarotenoid Biogenesis via CCD1: Single-Step or Stepwise Cleavage and the Importance of Compartmentation

Strigolactones exert their signaling functions in low amounts and can act in early stages of the AM symbiosis. Conversely, two other classes of apocarotenoids with unknown functions accumulate in large amounts in mycorrhizal roots and at later stages of the AM interaction.[15,16] These AM-induced colorless C_{13} cyclohexenone and yellow C_{14} linear polyene derivatives have been identified independently but probably originate from a common precursor (Fig. 1C).[15-17] Both types of compounds accumulate locally in cells harbouring arbuscules, which are the symbiotic organs of the AM symbiosis mediating nutrient exchange between plant and fungus.[18] To identify a function for these apocarotenoids in the symbiosis, both a *CCD1* gene and an AM-induced MEP pathway isogene were targeted by gene silencing approaches.[5,19]

CCD1 is, next to the NCEDs, the best-studied CCD due to its involvement in C_{13} apocarotenoid-based flower scent as well as fruit and wine aroma biosynthesis.[20-22] Recombinant CCD1 enzymes from several plants have been shown to preferentially catalyze a single-step symmetrical cleavage at the 9,10 and the 9',10' double bonds of various C_{40} carotenoids.[1,4,22,23] Cleavage activity on 5,6 (5',6') double bonds in vitro has also been reported.[24] However, being able to convert a substrate in vitro does not mean that this activity must be the main in vivo functional role of the enzyme. Indeed, in planta studies with *CCD1* mutants or gene silencing transgenics have raised doubts in an exclusive role of CCD1 in C_{13} apocarotenoid generation.[4,20,21] Strong suppression of *CCD1* transcript accumulation resulted in near 50% C_{13} apocarotenoid levels compared to wild type plants implicating additional players in C_{13} apocarotenoid biogenesis.[20,21]

Given this background knowledge of presumed single-step symmetrical CCD1 action on C_{40} carotenoid substrates we performed a *CCD1* RNAi knock-down approach in hairy roots of *Medicago truncatula*. Surprisingly, HPLC analyses of mycorrhizal hairy roots clearly indicated a differential reduction of C_{13} and C_{14} apocarotenoids. C_{14} mycorradicin derivatives were strongly reduced (3–6% residual amounts relative to mycorrhizal empty vector controls) but C_{13} cycohexenone derivatives exhibited an actual decrease to only 30–47% of empy vector controls.[5] This result is incompatible with the assumption of a symmetrical cleavage action of CCD1 in planta. Moreover, a striking color change to yellow-orange was observed in mycorrhizal RNAi roots. Analysis of the corresponding chromophore indicated that its chemical nature is a C_{27} apocarotenoid. This suggests that C_{27} but not C_{40} derivatives as previously thought[17] are the main substrates for CCD1 in mycorrhizal roots. Based on these data a new scheme of carotenoid cleavage and CCD1 action in mycorrhizal roots is proposed, in which CCD1 catalyzes only the second of at least two carotenoid cleavage steps from C_{40} carotenoids to the C_{13}/C_{14} apocarotenoid end-products (Fig. 1C). As in the case of strigolactone biosynthesis a consecutive action of two CCDs on C_{40} carotenoids and on the primary cleavage product, respectively, is predicted. Interestingly, the two pathways have a C_{27} intermediate in common (Fig. 1B and C). In strigolactone biosynthesis the generation of C_{27} apocarotenoids is due to CCD7 activity and it is tempting to speculate that CCD7 is the first cleavage enzyme in AM-induced C_{13}/C_{14} apocarotenoid biosynthesis as well. Preliminary evidence for such an involvement has been obtained.[5] CCD7 might thus constitute a crosspoint where the two pathways meet but subsequently branch into different directions towards CCD8 or CCD1, respectively (Fig. 1B and C). This is supported by the wide substrate specificity of CCD7,[10] as opposed to the narrow specificity reported for CCD8.[25]

However, compartmentation of the second cleavage step in both pathways is different. The C_{27} compound tentatively identified in mycorrhizal *CCD1*-RNAi roots was a 3-hydroxy-α-apo-10'-carotenoic acid glycosylated by two hexose moieties at the 3-hydroxyl position.[5] Glycosylation is a modification usually carried out by cytosolic enzymes. Therefore, the C_{27} compound is most likely exported from the plastid to the cytosol, where it is further cleaved by CCD1. In this new view of carotenoid cleavage and its compartmentation (Fig. 1A–C) the cytosolic location of CCD1 finally makes sense.

In conclusion, we have presented here a new scheme of C_{13} and C_{14} apocarotenoid biogenesis and of the role of CCD1 in this process, which illustrates both the importance of consecutive cleavage steps and the decisive role of compartmentation. The pivotal question is now, whether this scheme is also applicable to the biosynthesis of C_{13} apocarotenoids (also referred to as "C_{13} norisoprenoids"[22]) involved in flower scent, fruit aroma and wine bouquet.

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