ARTICLE ADDENDUM

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Triple subcellular targeting of isopentenyl diphosphate isomerases encoded by a single gene

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Isopentenyl diphosphate isomerase (IDI) is a key enzyme of the isoprenoid pathway, catalyzing the interconversion of isopentenyl diphosphate and dimethylallyl diphosphate, the universal precursors of all isoprenoids. In plants, several subcellular compartments, including cytosol/ER, peroxisomes, mitochondria and plastids, are involved in isoprenoid biosynthesis. Here, we report on the unique triple targeting of two *Catharanthus roseus* IDI isoforms encoded by a single gene (*CrIDI1*). The triple localization of CrIDI1 in mitochondria, plastids and peroxisomes is explained by alternative transcription initiation of *CrIDI1*, by the specificity of a bifunctional N-terminal mitochondria/plastid transit peptide and by the presence of a C-terminal peroxisomal targeting signal. Moreover, bimolecular fluorescence complementation assays revealed self-interactions suggesting that the IDI likely acts as a multimer in vivo.

Keywords: isopentenyl diphosphate isomerase, isoprenoid, alkaloid, triple targeting, subcellular localization, *Catharanthus roseus*

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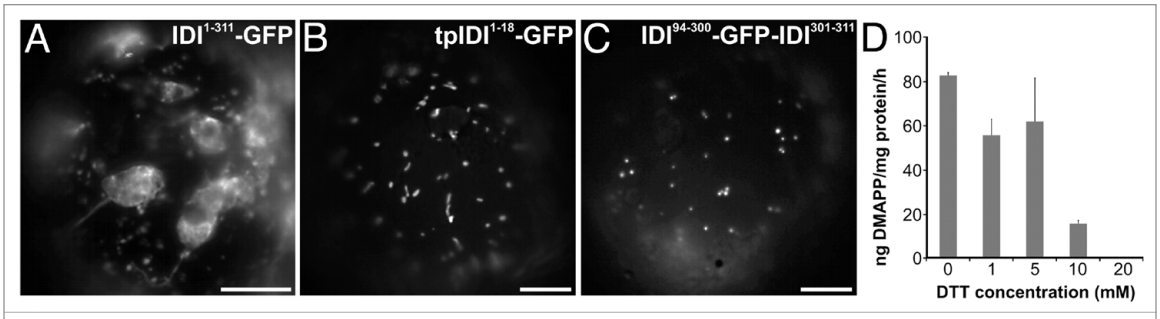
All isoprenoids originate from the two fundamental building blocks: isopentenyl diphosphate (IPP) and its allylic isomer dimethylallyl diphosphate (DMAPP). Sequential condensations of these C_5 units lead to the formation of increasing chain-length polyprenyl diphosphates, which are the precursors of a variety of isoprenoid end products.[1] IPP and DMAPP may be synthesized via two different biosynthetic pathways. In fungi and mammals, the C_5 units come from the mevalonic acid (MVA) pathway, while in most eubacteria they are produced through the methyl erythritol phosphate (MEP) pathway. By contrast, plants harbor both a cytosolic/peroxisomal MVA pathway[2] and a plastidial MEP pathway.[1]

Interconversion of IPP and DMAPP is governed by IPP isomerase (IDI; EC 5.3.3.2). Based on cofactor requirement, two types of IDIs have been characterized.[3] Type I IDIs correspond to zinc metalloproteins needing Mg^{2+} for activity and are widely distributed in different kingdoms of life such as in fungi, mammals and plants, whereas type II IDIs are restricted to archaea and some bacteria. While fungi possess a single Type I IDI gene copy, mammals and plant genomes typically contain two duplicated copies (*IDI1* and *IDI2*).[4,5] Both genes are differentially expressed and encode distinct protein isoforms displaying a complex subcellular distribution. In mammals, each gene produces a single isoform localized exclusively to peroxisomes in a tissue-dependant manner with a widely distributed *IDI1* and a skeletal muscle-specific *IDI2*.[5] The situation is more complex in plants. In Arabidopsis, both genes (*AtIDI1*, At5g16440 and *AtIDI2*, At3g02780) are expressed in all plant organs, both producing long and short transcripts encoding protein isoforms that differ in length at their N-terminal ends. The long AtIDI1 isoform (AtIDI1L) is targeted to plastids while the long AtIDI2 isoform (AtIDI2L) is sorted to mitochondria.[6,7] Both short



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Organism	Accession number	PTS1	Localization	Reference
Arabidopsis thaliana	At5g16440 (AtIDI1)	HKL*	Peroxisome	8
Arabidopsis thaliana	At3g02780 (AtIDI2)	HKL*	Peroxisome	8
Catharanthus roseus	EU135981 (CrIDI1)	HKLI*	Peroxisome	9
Homo sapiens	AAK29357 (IDI1)	YRM*	Peroxisome	5
Homo sapiens	NP_150286 (IDI2)	HRV*	Peroxisome	5
Mesocricetus auratus	AAC53283 (IDI1)	HRM*	Peroxisome	10
Saccharomyces cerevisiae	NP_015208	HRML*	ND	-
Physcomitrella patens	XP_001757553	RKL*	ND	-
Chlorella variabilis	EFN53784	HKIMA*	ND	-
Dictyostelium discoïdeum	XP_635929	HRY*	ND	-

Figure 1. Subcellular localization of IDIs. (**A**) Dual plastid and mitochondrial targeting of the long isoform of CrIDI1 (residues 1 to 311) fused to GFP (IDI^{1-311}-GFP). (**B**) Mitochondrial targeting of the first 18 residues of the CrIDI1 transit peptide fused to GFP ($tpIDI^{1-18}$-GFP). (**C**) Peroxisomal targeting of the CrIDI1 short isoform bearing an internally fused GFP (IDI^{94-300}-GFP-$IDI^{301-311}$). (**D**) Effect of DTT on CrIDI1 activity as judged by incubation of IPP with CrIDI1 at different DTT concentrations. IPP isomerized to DMAPP was detected as isoprene gas following its acidification with phosphoric acid as previously described.[15] Error bars signify standard deviation of two replicates. (**E**) Sequences of PTS1 and putative PTS1 in IDIs from different organisms. The tripeptide of PTS1 is underlined. The asterisk indicates the end of the polypeptide chain. Peroxisomal localization is indicated when experimentally validated. Accession numbers are from GenPept and from Arabidopsis Genome Initiative (for AtIDI1 and AtIDI2). ND, not determined; Bars, 10 μm.

isoforms (AtIDI1S and AtIDI2S), devoid of a N-terminal transit peptide (TP), are targeted to peroxisomes demonstrating the occurrence of a dual targeting of both gene products (plastid/peroxisome and mitochondria/peroxisome).[8]

Our recent work[9] showed that the situation is somehow different in Madagascar periwinkle (*Catharanthus roseus*), a medicinal plant widely used to study the architecture of the isoprenoid biosynthetic pathways leading to the formation of monoterpene indole alkaloids. Indeed, in *C. roseus*, *CrIDI1* is highly expressed in all organs as long and short transcripts, while *CrIDI2* is barely transcribed only in mature leaves.[9] Furthermore, *CrIDI1* gene products display an original triple targeting (**Fig. 1A–C**). Expression of green fluorescent protein fusions revealed that the CrIDI1 long isoform exhibits a N-terminal TP targeting the pseudo-mature protein to both mitochondria and plastids at an apparent similar efficiency (**Fig. 1A**). This dual targeting can be explained by TP specificities. Indeed, the entire 77-residue long TP needed for plastid targeting contains a specific sequence composed of the first 18-residues that is absent in the Arabidopsis orthologs and sufficient to direct proteins to mitochondria (**Fig. 1B**). In addition, the short CrIDI1 isoform, devoid of the TP, is directed to the peroxisome by a Peroxisomal Targeting Signal 1 (PTS1) located at the C-terminal end of the protein (**Fig. 1C**). CrIDI1 displays the same PTS1 (HKL) as its Arabidopsis orthologs, but differs in the presence of an additional isoleucine residue that does not alter the peroxisomal import. It is interesting to notice that even if PTS1 are present on the C-terminus extremity of both long and short isoforms in Arabidopsis and *C. roseus*, the peroxisomal sorting is observed only for short isoforms. This highlights the importance of alternative transcription initiation events that probably allow countering competition between N-term located plastid and/or mitochondrion targeting sequence(s) and C-term located PTS1. Similar PTS1 are present in the IDI1 and IDI2 mammalian isoforms targeted to peroxisomes via the PTS1-dependent import pathway.[5,10] Although it was a question of debate for many years, it is now clear that peroxisomes are key actors of isoprenoid metabolism in higher plants[11] and mammals.[12] This may be extended to other taxa on the basis of the wide distribution of putative PTS1-containing IDI in fungi, amoeba, mosses and alga (**Fig. 1E**).

Another result of our study[9] showed self-interactions of CrIDI1. Unlike previous results based on gel exclusion chromatography stating that IDIs are monomeric proteins,[13,14] bimolecular fluorescence complementation approaches established that CrIDI1 isoforms are capable of self-interactions within plastids, mitochondria and peroxisomes. Self-interactions were also confirmed by migration of the recombinant enzyme on native PAGE. In gel, slow migrating bands corresponding to high molecular complexes dissociate in

the presence of 10 mM of dithiothreitol (DTT). Although low DTT concentrations had no significant effect on CrIDI1 activity in vitro, we observed that concentrations of 10 mM DTT or higher strongly inhibited enzyme activity (**Fig. 1D**), suggesting that self-association of monomers might be important for CrIDI1 activity.

In conclusion, CrIDI1 gene products are targeted to plastids, mitochondria and peroxisomes to form self-interacting complexes that allow the isomerization of IPP and DMAPP synthesized from both the MEP and MVA pathways. Such a triple subcellular targeting of the products of a single gene seems to be a mechanism rarely described so far. Moreover, our results suggest the occurrence of distinct targeting mechanisms of isoprenoid biosynthetic enzymes in plants producing high amounts of specialized isoprenoids (e.g., *C. roseus*) and plants mainly producing housekeeping isoprenoid metabolites (e.g., *A. thaliana*).

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