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Characterisation of CaaX-prenyltransferases in *Catharanthus roseus*: relationships with the expression of genes involved in the early stages of monoterpenoid biosynthetic pathway

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Abstract

CaaX-prenyltransferases (CaaX-PTases), including protein farnesyltransferase (PFT) and type I protein geranylgeranyltransferase (PGGT-I), catalyse a post-translational prenylation of proteins. In *Catharanthus roseus* dedifferentiated cell culture, both CaaX-PTase activities are required for the expression of genes involved in monoterpenoid biosynthesis, including the first two genes of the 2-*C*-methyl-erythritol 4-phosphate (MEP) pathway, *Crdxs* and *Crdxr*. In this work, we investigated the possibility that this functional relationship is also necessary in differentiated tissues.

In planta, the organ-specific distribution of *Crdxs* transcripts was correlated with the presence of the transcripts of the β-subunit of PFT (*Crftb*), PGGT-I (*Crggt_Ib*) and of the common α-subunit (*Crfta*/*ggt_Ia*). Furthermore, in plant organs, *Crdxs* expression follows the pattern of CaaX-PTases activities. In young leaves, *Crftb* and *Crdxs* transcripts were localised to the same vascular bundles albeit in the external conducting phloem (ecp) and in the internal phloem parenchyma (ipp), respectively. Although *Crftb* transcripts were not detected in ipp, low transcript abundance in this tissue might hinder its detection. Alternatively, the possibility that farnesylated proteins, or other intermediates, required for *Crdxs* expression might translocate from ecp to ipp is discussed. Finally, we showed using prenyltransferase inhibitor that protein prenylation is also essential for the proper expression of MEP pathway genes in hairy roots.

Keywords: CaaX-prenyltransferases; Protein farnesyltransferase; Type I protein geranylgeranyltransferase; *dxs*; *dxr*; MEP pathway

1. Introduction

Protein prenylation consists in the post-translational addition of prenyl moieties derived from the mevalonate pathway. This reaction is catalysed by three different protein prenyltransferases distributed in two functional classes: CaaX-prenyltransferases (CaaX-PTases) and Rab geranyl-geranyltransferase (EC 2.5.1.60). CaaX-PTases include two enzymes, protein farnesyltransferase (PFT, EC 2.5.1.58) and type I protein geranylgeranyltransferase (PGGT-I, EC 2.5.1.59), that differ in their isoprenoid substrates and protein targets. PFT and PGGT-I catalyse the covalent attachment of farnesyl and geranylgeranyl moieties, respectively, to the cysteine residue located in the C-terminal tetrapeptide CaaX of protein substrates, where "C" is a cystein residue, "a" are aliphatic amino acids and "X" is the residue which determines CaaX-PTase nature. Indeed, "X" is preferably serine, methionine, cysteine, alanine or glutamine for PFT, whereas it is specifically a leucine for PGGT-I [1]. CaaX-PTases are heterodimeric enzymes that share a common α-subunit and that differ by their distinct β-subunits conferring both isoprenoid and protein substrate specificities [2]. In mammalian, yeast and plant systems, protein prenylation is an important post-translational modification involved in many aspects of cell biology. In plants, it is required in cell cycle progression [3], in

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hormonal signalling such as in response to abscissic acid [4] and in membrane trafficking regulators (reviewed in [5–7]).

Recently, in Madagascar periwinkle (*Catharanthus roseus*) cell culture, we have shown that both PFT and PGGT-I activities are required for the biosynthesis of the monoterpenoid precursor that fuels the monoterpenoid indole alkaloid (MIA) pathway [8]. Interference RNA suppression of CaaX-PTase β-subunit gene expression and inhibitor-mediated suppression of CaaX-PTases activities revealed that both farnesylated and geranylgeranylated proteins are part of the hormonal signalling that regulates the expression of genes involved in the early stages of monoterpenoid biosynthetic pathway (ESMB genes). Indeed, these experiments revealed that both prenylated proteins are necessary for the expression of 1-deoxy-D-xylulose 5-phosphate synthase gene (*Crdxs*) and 1-deoxy-D-xylulose 5-phosphate reductoisomerase gene (*Crdxr*) which encode the first two enzymes of the 2-*C*-methyl-erythritol 4-phosphate (MEP) pathway, as well as for the expression of the gene encoding geraniol 10-hydroxylase (*Crg10h*), an enzyme involved in first committed step in the biosynthesis of secoiridoid monoterpenoids (Fig. 1). The MEP pathway

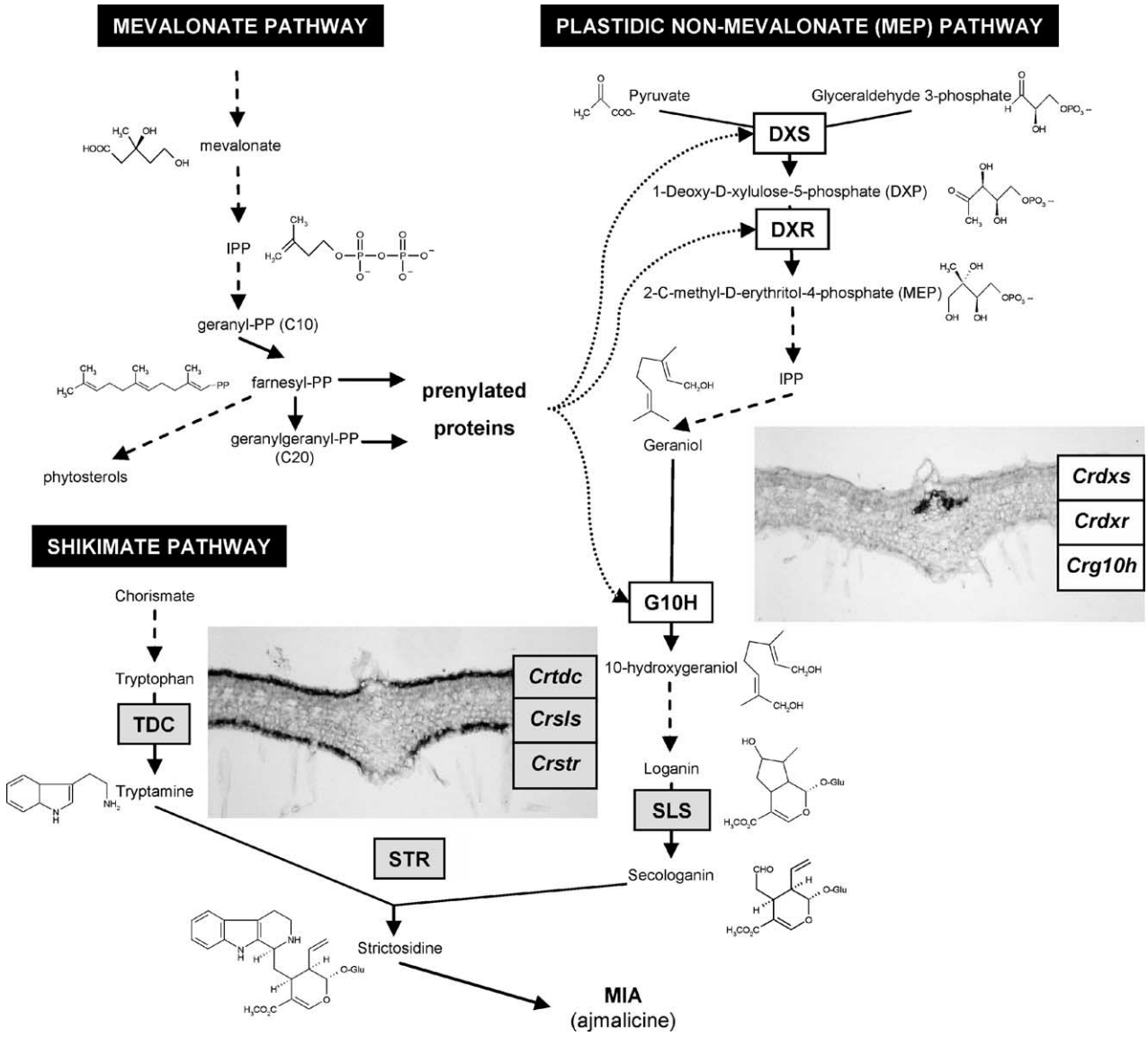

Fig. 1. Mevalonate pathway leading to phytosterols and prenyl substrates of protein prenyltransferases and biosynthetic pathway of monoterpenoid indole alkaloids (MIA) in *C. roseus* aerial organs showing the multicellular organisation of MIA gene expression (adapted from [15]). In dedifferentiated *C. roseus* C20D cells, prenylated proteins are required for the expression of the first two genes of the MEP pathway, *1-deoxy-D-xylulose 5-phosphate synthase* (*Crdxs*) and *1-deoxy-D-xylulose 5-phosphate reductoisomerase* (*Crdxr*) and for *geraniol 10-hydroxylase* (*Crg10h*), which encodes the first monoterpenoid dedicated enzyme, whereas prenylated protein are not necessary for the expression of *secologanin synthase* (*Crsls*), *tryptophane decarboxylase* (*Crtdc*) and *strictosidine synthase* (*Crstr*) involved in later MIA biosynthetic steps [8]. In *C. roseus* aerial organs, the group of prenylated protein-dependent gene is expressed in internal phloem parenchyma [15] whereas the three prenylated protein-independent genes are expressed in epidermis [16,17]. IPP, isopentenyl diphosphate. Black arrows and dashed arrows represent respectively unique or multiple enzyme reactions.