Plant Mol Biol (2006) 62:683–695
DOI 10.1007/s11103-006-9051-9

Enhanced flux through the methylerythritol 4-phosphate pathway in *Arabidopsis* plants overexpressing deoxyxylulose 5-phosphate reductoisomerase

Lorenzo Carretero-Paulet · Albert Cairó · Patricia Botella-Pavía · Oscar Besumbes · Narciso Campos · Albert Boronat · Manuel Rodríguez-Concepción

Received: 11 April 2006 / Accepted: 7 July 2006 / Published online: 29 August 2006

Abstract The methylerythritol 4-phosphate (MEP) pathway synthesizes the precursors for an astonishing diversity of plastid isoprenoids, including the major photosynthetic pigments chlorophylls and carotenoids. Since the identification of the first two enzymes of the pathway, deoxyxylulose 5-phoshate (DXP) synthase (DXS) and DXP reductoisomerase (DXR), they both were proposed as potential control points. Increased DXS activity has been shown to up-regulate the production of plastid isoprenoids in all systems tested, but the relative contribution of DXR to the supply of isoprenoid precursors is less clear. In this work, we have generated transgenic *Arabidopsis thaliana* plants with altered DXS and DXR enzyme levels, as estimated from their resistance to clomazone and fosmidomycin, respectively. The down-regulation of DXR resulted in variegation, reduced pigmentation and defects in chloroplast development, whereas DXR-overexpressing lines showed an increased accumulation of MEP-derived plastid isoprenoids such as chlorophylls, carotenoids, and taxadiene in transgenic plants engineered to produce this non-native isoprenoid. Changes in DXR levels in transgenic plants did not result in changes in DXS gene expression or enzyme accumulation, confirming that the observed effects on plastid isoprenoid levels in DXR-overexpressing lines were not an indirect consequence of altering DXS levels. The results indicate that the biosynthesis of MEP (the first committed intermediate of the pathway) limits the production of downstream isoprenoids in *Arabidopsis* chloroplasts, supporting a role for DXR in the control of the metabolic flux through the MEP pathway.

Keywords *Arabidopsis* · Carotenoids · Deoxyxylulose 5-phosphate reductoisomerase (DXR) · Isoprenoid biosynthesis · Methylerythritol 4-phosphate (MEP) pathway · Taxadiene

L. Carretero-Paulet · A. Cairó · P. Botella-Pavía · O. Besumbes · N. Campos · A. Boronat · M. Rodríguez-Concepción (✉)
Departament de Bioquímica i Biologia Molecular, Facultat de Biología, Universitat de Barcelona, Av Diagonal 645, 08028 Barcelona, Spain
e-mail: mrodrigu@sun.bq.ub.es

L. Carretero-Paulet
Laboratori de Genètica Molecular i Vegetal, CSIC-IRTA, Jordi Girona 18-36, 08034 Barcelona, Spain

Introduction

Plants produce an astonishing diversity of isoprenoids, a functionally and structurally diverse group of compounds synthesized from the C5 precursors isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP) (Chappell 1995; Croteau et al. 2000). Addition of IPP units to DMAPP leads to the synthesis of prenyl diphosphates of increasing size such as geranyl diphosphate (GPP, C10), farnesyl diphosphate (FPP, C15) and geranylgeranyl diphosphate (GGPP, C20), which are the starting points for multiple branches leading to the final isoprenoid products (Fig. 1). Unlike most organisms, plants have two separated pathways for IPP and DMAPP biosynthesis: the mevalonic acid (MVA) pathway, which produces cytosolic IPP, and the plastid-localized methylerythritol 4-phosphate (MEP) pathway (Lichtenthaler 1999; Eisenreich et al. 2001; Rodríguez-Concepción and Boronat 2002). Despite plastidial isoprenoids including

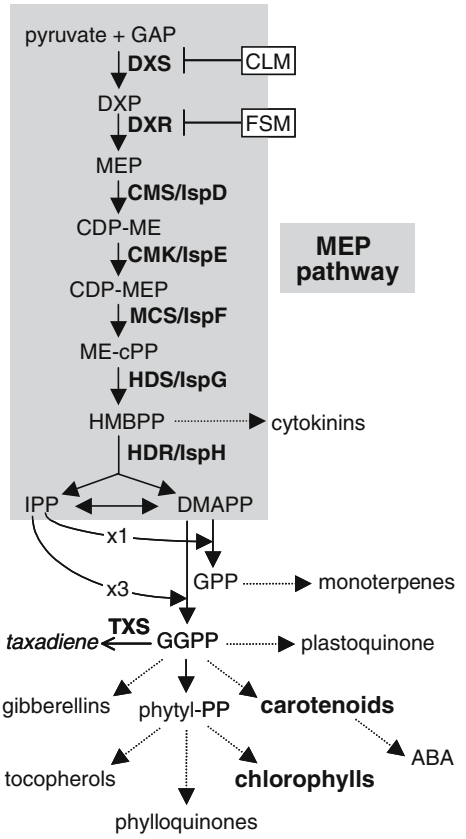

Fig. 1 The pathway for isoprenoid biosynthesis in plastids. GAP, glyceraldehyde 3-phosphate; DXP, deoxyxylulose 5-phosphate; MEP, methylerythritol 4-phosphate; CDP-ME, 4-diphosphocytidyl-methylerythritol; CDP-MEP, CDP-ME 2-phosphate; ME-cPP, methylerythritol 2,4-cyclodiphosphate; HBMPP, hydroxymethylbutenyl diphosphate; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; GPP, geranyl diphosphate; GGPP, geranylgeranyl diphosphate; ABA, abscisic acid. The MEP pathway enzymes are indicated in bold (the names of the bacterial homologues are also indicated): DXS, DXP synthase; DXR, DXP reductoisomerase; CMS, CDP-ME synthase; CMK, CDP-ME kinase; MCS, ME-cPP synthase; HDS, HMBPP synthase; HDR, HMBPP reductase. The step catalyzed by taxadiene synthase (TXS) is shown, as well as the steps inhibited by clomazone (CLM) and fosmidomycin (FSM)

the major photosynthetic pigments (chlorophylls and carotenoids) as well as other key photosynthesis-related compounds (plastoquinones, phylloquinones, and tocopherols), hormones (gibberellins and abscisic acid), and monoterpenes are synthesized from MEP-derived precursors (Fig. 1), the MEP pathway was only recently discovered and fully elucidated (Rodríguez-Concepción and Boronat 2002; Rohdich et al. 2003).

The initial reaction of the MEP pathway, catalyzed by deoxyxylulose 5-phosphate (DXP) synthase (DXS), involves the condensation of (hydroxyethyl)thiamin derived from pyruvate with the C1 aldehyde group of glyceraldehyde 3-phosphate (GAP) to produce DXP. In the second step, an intramolecular rearrangement and reduction of DXP by the enzyme DXP reductoisomerase (DXR) yields MEP, the first committed precursor of plastid isoprenoids. After conversion of MEP into hydroxymethylbutenyl diphosphate (HMBPP) in four enzymatic steps (Fig. 1), the enzyme HMBPP reductase (HDR) converts HMBPP into IPP and DMAPP in the last step of the MEP pathway (reviewed in Rodríguez-Concepción and Boronat 2002; Rohdich et al. 2003). Despite the impressive progress in the elucidation of the MEP pathway, relatively little is currently known on the contribution of the corresponding enzymes to control the flux of intermediates through the pathway and the supply of IPP and DMAPP for the synthesis of plastid isoprenoid end-products. Since the identification of the first two enzymes of the pathway (DXS and DXR), they both were tentatively proposed as potential control points. The role of these enzymes in plants has been often investigated using model systems in which high IPP and DMAPP levels are required to support an increased production of plastid isoprenoids in response to external stimuli or developmental cues. Thus, analysis of *DXS* expression patterns during accumulation of different plastidial isoprenoid end products suggested that increased DXS levels might be required to supply their precursors (Mandel et al. 1996; Bouvier et al. 1998; Lange et al. 1998; Chaded et al. 2000; Estévez et al. 2000; Lois et al. 2000; Veau et al. 2000; Walter et al. 2000; Walter et al. 2002; Burlat et al. 2004; Botella-Pavía et al. 2004). Transgenic *Arabidopsis* and tomato plants in which DXS activity had been altered confirmed the regulatory role of DXS in controlling flux through the MEP pathway (Estévez et al. 2001; Enfissi et al. 2005). In the case of DXR, a positive correlation between enhanced plastid isoprenoid biosynthesis and transcript accumulation has been observed in some plant systems (Walter et al. 2000; Veau et al. 2000; Carretero-Paulet et al. 2002; Hans et al. 2004; Hsieh and Goodman 2005a; Mayrhofer et al. 2005; Bede et al. 2006) but not in others (Rodríguez-Concepción et al. 2001; Dudareva et al. 2005). Overexpression of DXR in peppermint led to increased monoterpene levels in leukoplasts of the non-photosynthetic secretory cells of glandular trichomes (Mahmoud and Croteau 2001), but it is possible that the production of MEP from DXP might also limit the biosynthesis of other isoprenoids in other plastid types. In this work we have modified DXR activity levels in transgenic *Arabidopsis* plants to characterize its relative contribution to the production of primary (chlorophylls and carotenoids) and secondary (taxadiene) isoprenoid end-products in chloroplasts. The results demonstrate that DXR, together with other enzymes